

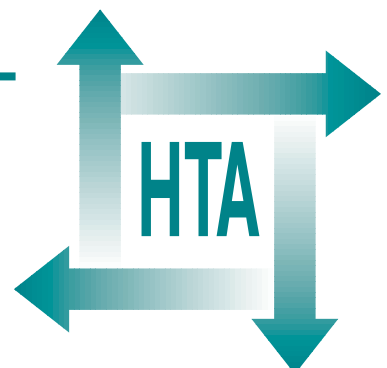
Neonatal screening for inborn errors of metabolism: cost, yield and outcome

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Health Technology Assessment
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This report is one of a series covering acute care, diagnostics and imaging, methodology, pharmaceuticals, population screening, and primary and community care. It was identified as a priority by the Population Screening Panel (see inside back cover).

The views expressed in this publication are those of the authors and not necessarily those of the Standing Group, the Commissioning Board, the Panel members or the Department of Health.

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Contents

List of abbreviations	i	Congenital adrenal hyperplasia	57
Executive summary	iii	Muscular dystrophy	62
1 Background	1	Biotinidase deficiency	63
The application	1	9 Disorders of organic acid and fatty acid metabolism detectable by tandem MS	67
Structure of this report	2	Methylmalonic, propionic and isovaleric acidaemias	67
2 Inborn errors of metabolism	3	Other defects of branched-chain acyl-CoA metabolism	69
General overview	3	Medium-chain acyl-CoA dehydrogenase deficiency	70
Inheritance	3	Defects of long-chain fatty acid catabolism ...	74
Family size and prenatal diagnosis	4	Short-chain acyl-CoA dehydrogenase deficiency	76
Genetic and phenotypic heterogeneity	4	Glutaryl-CoA dehydrogenase deficiency	76
Population genetics	5	Glutaric aciduria type II	78
Levels of diagnosis	5	Other possible screens	79
3 Neonatal screening – general topics	7	Disorders excluded	79
Historical development	7	10 Quality of the literature on individual diseases	81
Organisation in the UK	7	Expected incidence	81
Criteria for screening: the Wilson and Jungner principles	10	Natural history of clinically-detected disease	82
Measures of screening performance	10	Response to treatment	82
4 Laboratory techniques	15	11 The psychological costs and benefits of neonatal screening	83
Introduction	15	Introduction	83
Amino acid screens	15	The direct psychological impact of neonatal screening	84
Other individual screens	16	Neonatal screening: guidelines for good practice	95
Tandem mass-spectrometry	16	Main findings and recommendations	100
DNA analysis	19	Areas for further research	101
5 The literature search	21	12 Economics of neonatal metabolic screening – a general introduction to the literature review	103
Search methodology	21	Introduction	103
Literature identified	22	Organisation of the economic review	103
Nature of the literature	23	General economic issues of neonatal screening	103
6 Phenylketonuria and congenital hypothyroidism	27	Methods	109
Phenylketonuria	27	13 Economic evaluations of neonatal metabolic screening for phenylketonuria ...	111
Congenital hypothyroidism	31	Introduction	111
7 Other disorders of amino acid metabolism	37	Research findings	111
Tyrosinaemia type I	37		
Homocystinuria (cystathionine β -synthase deficiency)	40		
Maple syrup urine disease	43		
Urea cycle disorders	45		
8 Diseases with existing stand-alone screens..	49		
Galactosaemia	49		
Cystic fibrosis	51		

General discussion	118	I8 Economic evaluation of neonatal screening..	141
Summary	118	Introduction	141
I4 Economic evaluations of neonatal metabolic screening for congenital hypothyroidism, cystic fibrosis and Duchenne muscular dystrophy	119	Decision-tree model of neonatal screening ..	141
Congenital hypothyroidism	119	Data requirements for neonatal screening model	142
Cystic fibrosis	122	Method of data collection	143
Duchenne muscular dystrophy	124	Results of data collection	144
I5 Economic evaluations of neonatal screening programmes and of alternative technologies for neonatal screening	127	Results of the economic evaluation	148
Neonatal screening programmes	127	Sensitivity analysis	157
Alternative technologies for neonatal screening	130	Discussion	159
I6 Economics of neonatal metabolic screening: the results of the literature review	133	Conclusions	162
Quality of the studies	133	I9 Overview and recommendations	163
Discussion	134	The current UK screening programme	163
I7 Criteria for neonatal screening: evaluation from evidence in the literature	137	Introducing additional screens	164
Wilson and Jungner revisited	137	Pilot study of tandem MS based screening ...	165
‘A suitable test’: literature evidence on screening test performance	140	Organisational issues	167
		Acknowledgements	171
		References	173
		Appendix 1 Laboratory questionnaire	193
		Appendix 2 Description of undiscounted treatment cost calculations	201
		Health Technology Assessment reports published to date	203



List of abbreviations

ACTH	adrenocorticotrophin
CF	cystic fibrosis*
CFTR	cystic fibrosis transmembrane regulator
CI	confidence interval
CK	creatine kinase
CT	computed tomography
DELFLIA	dissociation-enhanced lanthanide fluorescence immunoassay*
DOCA	deoxycorticosterone
ELISA	enzyme-linked immunoassay*
EQAS	External Quality Assessment Scheme
FEV	forced expiratory volume
IRT	immunoreactive trypsin/trypsinogen
MRC	Medical Research Council
MRI	magnetic resonance imaging
MS	mass-spectrometer/spectrometry
NTBC	2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione
17-OHP	17-hydroxyprogesterone*
OPCS	Office of Population Censuses and Surveys
PAH	phenylalanine hydroxylase
PKU	phenylketonuria
PPV	positive predictive value
QALY	quality-adjusted life year
RCT	randomised controlled trial
RIA	radio-immunoassay*
TSH	thyrotropin

* Used only in figures and tables



Executive summary

Objectives

- To systematically review the literature on inborn errors of metabolism, neonatal screening technology and screening programmes in order to analyse the costs and benefits of introducing screening based on tandem mass spectrometry (tandem MS) for a wide range of disorders of amino acid and organic acid metabolism in the UK.
- To evaluate screening for cystic fibrosis, Duchenne muscular dystrophy and other disorders which are tested on an individual basis.

How the research was conducted

Systematic searches were carried out of the literature on inborn errors of metabolism, neonatal screening programmes, tandem MS-based neonatal screening technology, economic evaluations of neonatal screening programmes and psychological aspects of neonatal screening. Background material on the biology of inherited metabolic disease, the basic philosophy, and the history and current status of the UK screening programme was also collected. Relevant papers in the grey literature and recent publications were identified by hand-searching. Each paper was graded. For each disease an aggregate grade for the state of knowledge in six key areas was awarded.

Additional data were prospectively collected on activity and costs in UK neonatal screening laboratories, and expert clinical opinion on current treatment modalities and outcomes. These data were used to construct a decision-analysis model of neonatal screening technologies, comparing tandem MS with the existing phenylketonuria screening methods. This model determined the cost per additional case identified and, for each disease, the additional treatment costs per case, and the cost per life-year saved. All costs and benefits were discounted at 6% per annum. One-way sensitivity analysis was performed showing the effect of varying the discount rate, the incidence rate of each disorder, the number of neonates screened and the cost of tandem MS, on the cost per life-year gained.

Research findings

The UK screening programmes for phenylketonuria and congenital hypothyroidism have largely achieved the expected objectives and are cost-effective. Current concerns are the difficulty of maintaining adequate coverage, perceived organisational weaknesses, and a lack of overview.

For many of the organic acid disorders it was necessary to rely on data obtained from clinically-diagnosed cases. Many of these diseases can be treated very effectively and a sensitive screening test was available for most of the diseases.

Except for cystic fibrosis, there have been no randomised controlled trials of the overall effectiveness of neonatal screening.

Despite the anxiety generated by the screening process, there is strong parental support for screening. The effects of diagnosis through screening on subsequent reproductive behaviour is less clear.

Conflicts exist between current concepts and the traditional principles of screening. The availability of effective treatment is not an absolute prerequisite: early diagnosis is of value to the family concerned and, to the extent that it leads to increased use of prenatal diagnosis, may help to reduce the overall burden of disease. Neonatal screening is also of value in diseases which present early but with non-specific symptoms. Indeed, almost all of the diseases considered could merit neonatal screening.

The majority of economic evaluations failed to incorporate the health benefits from screening, and therefore failed to address the value of the information which the screening programmes provided to parents.

The marginal cost of changing from present technology to tandem MS would be approximately £0.60 per baby at a workload of 100,000 samples a year, and £0.87 at 50,000 samples per year. The ability to screen for a wider range of diseases would lead to the identification of some 20 additional cases per 100,000 infants screened, giving a laboratory cost per additional diagnosis of £3000 at an annual workload of 100,000 babies per year.

This compares with average, approximate laboratory costs of £6000 for diagnosing a case of phenylketonuria and £4000 for congenital hypothyroidism, and costs including specimen collection of £27,000 and £15,000, respectively.

The overall marginal costs of screening for additional disorders will include the additional costs of earlier treatment of all patients and the additional lifetime costs of treatment of those patients who would have died in the absence of screening, e.g. for the fatty acid oxidation defects. For a population with 100,000 births per year, short-term costs are estimated at £18,000 per year with long-term costs rising eventually to £174,000 per year. There are likely to be substantial cost-savings to set against these treatment costs.

The health benefits of diagnosis by neonatal screening range from prevention of mental retardation, severe neurological disease, or physical deformity, to avoidance of sudden death. The model only included the mortality health benefits and did not incorporate a measure of quality of life or the non-health benefits of screening. The results can be viewed as conservative estimates of the total benefits of screening for each disease. The data on the treatment efficacy and life expectancy with treatment are largely based on clinical opinion, and may therefore be open to challenge. However, there are so few cases of each of these diseases that it is unrealistic to look for an alternative source of data. The estimated treatment cost per life-year saved ranged from £8339 for tyrosinaemia type I to £31 for medium-chain acyl-CoA dehydrogenase deficiency.

The case for screening for cystic fibrosis has been examined in some detail. The cost is small relative

to the total cost of the disease, there are recognised short-term benefits and emerging evidence of long-term benefits from very early treatment.

Main recommendations

- The existing programmes for phenylketonuria and congenital hypothyroidism should be continued, but consideration should be given to strengthening the organisation by the establishment of a national multidisciplinary forum to give guidance on performance criteria, organisational matters and monitor the impact of introducing new screens.
- The Welsh scheme for Duchenne muscular dystrophy should be continued on a research basis and the findings used to inform decisions on introducing screening elsewhere.
- Performance data should be collected from the current UK screening programmes for cystic fibrosis. Expansion of screening for this disease should be encouraged.
- There appears to be a strong case for introducing tandem MS-based screening. Screening should be limited to clearly-defined diseases where specificity is known to be adequate and there are satisfactory confirmatory tests. Given the technical complexity of the method, the large number of diseases covered, and limited experience of applying tandem MS-based screening to UK populations, a 3-year pilot study is proposed as detailed in the main text of the report.

Chapter I

Background

The application

This review was commissioned under the Health Technology Assessment programme of the NHS Executive. The primary aim was to consider “neonatal screening for inborn errors of metabolism: to assess the burden of disease, the evidence for effective intervention and identify further questions for research”. The subject was further defined as follows.

- A. The priority area** All infants in the UK are screened for phenylketonuria and hypothyroidism by analysis of blood spots collected routinely on ‘Guthrie cards’. This policy has been adopted because the consequences of these disorders are minimised by early diagnosis and treatment. There exist a range of other disorders which could be detected through abnormal concentrations of metabolites in Guthrie spots, including fatty acid oxidation defects, organic acidaemias, urea cycle defects and amino acidopathies. Recent technological developments could allow these disorders to be detected from a single dried blood spot.
- B. Focus of the assessment** A systematic review of the literature was required to establish, where possible, the cost-effectiveness of screening for inborn errors of metabolism. In so doing the study should review:
- the natural history of untreated cases
 - the burden of inborn errors of intermediary metabolism
 - the proportion identifiable by existing and emerging tests
 - sensitivity and specificity of the tests
 - effectiveness of existing interventions for these conditions
 - the number of cases presenting before 10 days (the point at which about 75% of Guthrie cards have been tested).
- C.** The review should provide information which can be used to inform local protocols and should identify where further research is needed.
- D.** The review should provide advice to purchasers on the costs and benefits of introducing a

screening programme and the marginal costs and benefits of modifications to either the range of inborn errors included or in the design and implementation of the programme.

- E.** The review will need to gather and use information on: costs (including direct and indirect costs); effectiveness and psychological and social impact, including acceptability to patients.

The authors’ modified the review as initially proposed to make disorders detectable by tandem mass-spectrometry (tandem MS) an explicit focus and reinterpreted the aims of the study slightly by:

- (a) considering neonatal screening as a whole, rather than just inborn errors of intermediary metabolism. Haemoglobinopathies were excluded as these will be covered in separate health technology assessments;
- (b) including an investigation of the current costs of screening by existing methodologies in the UK and unpublished data on the incidence of various disorders in areas of the UK, where known;
- (c) collecting data on sensitivity and specificity of detection for a range of disorders and on laboratory costs by direct contact with centres in the UK and USA, where screening based on tandem MS is being developed.

In addition to this study, a second review is being undertaken at St George’s Hospital Medical School, London, under Professor Carol Seymour. Though clearly overlapping in major areas there are differences in approach in the two projects and, after discussion, it was decided that the St George’s group would concentrate on a broad systematic review of the literature, while this group would focus on the decision analysis model and the psychological study. The two studies have been conducted separately, with separate reports, but data on cost and performance collected from UK screening laboratories and laboratories in the USA using tandem MS for neonatal screening have been shared.

Two further reviews on neonatal screening for **haemoglobinopathies** are being undertaken by

Dr AE Ades, Institute of Child Health, London, and Dr SC Davies, Central Middlesex Hospital, London.

The review of screening for **cystic fibrosis** being undertaken by Professor H Cuckle, University of Leeds, will include a section on neonatal screening.

Structure of this report

Discussions within the group convinced us of the need for a general overview of the scientific and organisational background to neonatal metabolic screening, as a prelude to the systematic review of the literature on specific topics. Some of the basic features of inborn errors of metabolism, particularly as they relate to genetic diversity and their impact on the family, are summarised in chapter 2. The principles that underlie screening practices and some of the controversy which surrounds them are discussed briefly in chapter 3.

Neonatal metabolic screening is a sequential process and, for most of the diseases under

consideration, there has been no formal evaluation of the effectiveness of the programme as a whole. A 'causal pathway' approach has therefore been followed¹ (see chapter 5). In its generalised form this approach attempts to estimate:

- (a) prevalence of the condition
- (b) accuracy of the screening test
- (c) uptake of the screening test
- (d) effectiveness of early treatment
- (e) uptake of treatment following the screening test.

An additional consideration for this review is the clinical significance of the biochemical abnormality concerned. This and items (a), (b), and (d) above are assessed for individual diseases in chapters 6–9, although it has been necessary to limit the amount of material included. In addition, because the screening programme must be viewed as a whole and adding new diseases will have implications for its general organisation, the historical development of neonatal screening and its current organisation in the UK are described in chapter 3, which also deals with coverage (uptake) and how screening performance is measured.

Chapter 2

Inborn errors of metabolism

General overview

Inborn errors of metabolism are permanent and inherited biochemical disorders, also sometimes known as inherited metabolic diseases. Nearly all the conditions described in this review are inherited metabolic diseases, the most important exception being congenital hypothyroidism (see page 31), in which approximately 90% of cases are sporadic with no discernible genetic component.

With the growth of biochemical genetics and the discovery of the underlying chemical abnormalities in an ever-increasing number of diseases, it has become difficult to draw the boundaries of inherited metabolic disease.

As far as there is a useful distinction, it lies in the function of the affected gene-product: in general, **an inborn error of metabolism is caused by lack of a functional enzyme, transmembrane transporter, or similar protein, which then results in blockage of the corresponding metabolic pathway.** There may be accumulation of metabolites prior to the metabolic block, and/or deficiency in the ultimate product(s) of the pathway. Both may provide a means of therapeutic intervention, either by restricting the supply of precursors to the pathway or by supplying a missing product, or both. Thus approximately half of all inborn errors of metabolism can be treated biochemically, although the success of such treatment is variable (see chapters 6–9). In contrast, diseases caused by inherited abnormalities of structural proteins are seldom amenable to effective treatment.

Taken individually, inborn errors of metabolism are rare. However, their collective importance is growing and they occupy a significant place in the practice of paediatrics. The reasons for this include:

- the large and still-increasing number of conditions which have been identified
- a general improvement in diagnostic services for symptomatic patients, resulting in a greater proportion of affected individuals being identified and at an earlier stage in the disease
- dramatic improvements in the effectiveness of treatment for many conditions over the past

decade, giving increased survival and a better quality of life

- improvements in carrier detection and the wider availability of first-trimester prenatal diagnosis which have led to the genetic implications of prompt and correct diagnosis of inherited metabolic disease becoming much more important.

An extremely brief summary of the biology of inherited metabolic disease follows. For general reading there are the recent books edited by Holton² and Fernandes and colleagues.³ More detailed biochemical information and much more background information on genetics will be found in the latest edition of Scriver and colleagues.⁴

Inheritance

The genes that code the primary structures of nearly every protein in the human body are carried in the cell nucleus as DNA. The inherited disorders covered in this review all arise from some abnormality (mutation) of this nuclear DNA which prevents the production of the biologically-active form of the protein concerned. This, in turn, results in the metabolic block which gives rise to the disease.

For the majority of proteins the coding DNA is present in one of the **autosomal chromosomes.** These chromosomes are paired in somatic (non-germ) cells so that each gene is present as two copies, one derived from each parent. Cells will usually function normally even if one of a pair of genes has been disabled by mutation. The affected person will, however, be a **carrier** for the corresponding disorder, which will show Mendelian recessive genetics. Thus, two carrier parents (each with a mutation on only one of the chromosome pair) will produce, **on average**, one affected offspring, two carriers, and one with no mutation for every four children born.

Some inherited diseases, Duchenne muscular dystrophy being the best-known example, are due to defects in genes carried on the sex-determining X-chromosome and are known as **X-linked conditions.** Females have two X-chromosomes but males have only one, that being derived from the mother.

Half the male offspring of a woman carrying an X-linked disease will be affected irrespective of the father's genetic status. Half the female offspring will be carriers and the disease may recur in subsequent generations and also in the mother's relatives through the female line. Thus, in contrast to autosomally-inherited conditions, X-linked disorders have genetic importance for the wider family. Because of the mode of inheritance, there is considerable natural selection against X-linked diseases which cause early death, so that up to a third of patients present with diseases caused by new mutations and have no family history.

- **Practical implication** There is a high risk (between 16% and 25%) of recurrence of inherited metabolic disease in subsequent offspring and, in some instances, a moderate risk in the wider family.

Family size and prenatal diagnosis

The birth of a child with an inherited disease has a profound effect on the family, in many cases markedly influencing future reproductive patterns. Even a disease which may be regarded by the professionals as being eminently treatable may place a considerable burden on the family and discourage further childbearing. In a small and self-selected group, 15 from 38 parents (39%) with children with phenylketonuria reported that the risk of subsequent children with phenylketonuria had limited the size of their family, even though few would have wished to avail themselves of prenatal diagnosis.⁵ A comparison of the number of children in 87 families with phenylketonuria with the distribution in the general population also indicated that the birth of the first affected child exerted a 40% deterrent effect on having further children,⁶ although this may have been exaggerated by discounting children born to either parent following divorce or separation. An alternative approach, considering the birth orders of affected and unaffected siblings within these families, suggested a deterrent effect of 25%. A similar tendency to smaller family size, with the affected child being the last, has been reported for cystic fibrosis in East Anglia.⁷ The impact of neonatal screening on reproductive decision-making is discussed further later (see page 93).

The recurrence risk for autosomal recessive and completely recessive X-linked conditions in any one child is 0.25 (see page 3). For most diseases it is now possible to offer prenatal diagnosis, in many instances during the first trimester.⁸ Using statistics from the UK Office of Population Censuses and

Surveys (OPCS) for family size distribution and assuming 100% ascertainment of affected children shortly after birth, it can be calculated that prenatal diagnosis and termination of affected pregnancies has the potential to achieve approximately a 20% reduction of affected births.⁷ This figure reduces progressively for disorders that present (or are diagnosed) after the neonatal period, so that for late-presenting conditions, such as Duchenne muscular dystrophy, it is not uncommon to find one or more affected younger siblings following the diagnosis of the index case. It is in these conditions that early diagnosis by neonatal screening would make the greatest difference to the effectiveness of prenatal diagnosis in reducing the number of affected babies born. Early diagnosis also enables those families who may not wish to have prenatal diagnosis to make other informed choices about any future childbearing.

- **Practical implication** Early diagnosis of an inherited disorder is of value *per se*, irrespective of whether the condition is treatable.

Genetic and phenotypic heterogeneity

The biochemical nature of an inborn error of metabolism is determined by the normal function of the affected protein. As an example, phenylketonuria is by definition due to a deficiency of phenylalanine hydroxylase activity. However, the gene coding for the amino acid sequence of phenylalanine hydroxylase is approximately 90,000 nucleotide bases long and different mutations have occurred *de novo* many times in the history of man. Over 200 different mutations have been identified in phenylketonuric individuals. Though there are trends in the geographical distribution of some of the more common mutations which allow us to chart prehistoric population movements, there is considerable heterogeneity, making it impossible to use DNA analysis as a primary diagnostic tool in this disorder. This is the situation for the majority of inborn errors of metabolism. Some conditions are much more homogeneous at the DNA level, however, and for these DNA analysis may be a useful adjunct to the neonatal screening protocol.

While some mutations are inconsequential, others prevent the synthesis of the affected protein or produce a protein which is unstable or has little or no enzyme activity and result in metabolic disease. The **clinical severity** of the disease and the ease with which it can be biochemically controlled may depend markedly on the properties of the altered

protein. Even a small percentage of residual activity may be sufficient to maintain normal metabolic function, or at least result in a much milder course. For example, in phenylketonuria it is now possible to predict to a limited extent, from a knowledge of the mutations present, how readily a patient will respond to dietary control.

In addition to these direct examples of the effect of genetic heterogeneity on disease severity, there are numerous examples of differing clinical severity in sibling pairs or of unrelated patients with identical mutations at the DNA level. This may be ascribed to the effects of other as yet unidentified genes, so that these are an extreme example of the **polygenic disorder** continuum. In other instances, particularly for disorders that tend to produce intermittent acute metabolic crises, there are clear **triggering factors**, particularly intercurrent infection, and the clinical progress of an affected individual depends critically on the degree of exposure to such metabolic stress.

Population genetics

The frequency of affected births in a population with random (non-consanguineous) matings may be calculated from the frequency of carriers using the formula:

$$\text{frequency of affected births} = 0.25 \times (\text{frequency of heterozygotes})^2$$

As an example, if the overall frequency of heterozygotes[†] for a disease in a particular population is 0.02 (1 in 50), then it will occur with a frequency of 0.0001 (1 in 10,000 births). It is sometimes possible to estimate the birth incidence of a particular condition by determining the carrier frequency in the general population (see, for example, page 70). For autosomally-inherited conditions, the large number of carriers in the general population, even for diseases that are comparatively rare, has implications for the use of DNA-based screening (see page 19). This is not a problem when screening for X-linked disease.

In groups which have grown from a small number of founders and have kept a separate identity, for either geographical, religious, or other reasons, individual disorders may be unusually common and the spectrum of clinical features, for example

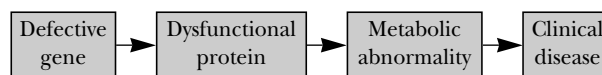
severity, may differ significantly from those found in more mixed populations. Within the UK, 'travelers' groups form such an example. Even within the general UK population there are geographical variations; for example, a three-fold difference in incidence of phenylketonuria between East Anglia and south-western Scotland (see page 27). Differences of even greater magnitude have been shown in medium-chain acyl-CoA dehydrogenase deficiency (see page 70).

A further important factor in determining the frequency of inherited metabolic disease in a population is the prevalence of consanguineous marriage, since this increases the risk of affected offspring for all autosomally-inherited disease (although not X-linked disorders). In Birmingham, where 70% of marriages in families of Pakistani descent are consanguineous, this group has a 16-fold increase in the overall incidence of recessive diseases compared to the indigenous population.⁹ Thus, despite a lower gene frequency, phenylketonuria is as common in children of Pakistani descent as in white children. There is a particularly high incidence of tyrosinaemia in Pakistani children, which must be partly explained by a higher gene frequency.¹⁰

- **Practical implications** There are likely to be significant geographical differences in the incidence of individual inborn errors of metabolism, and in the relative frequencies of different clinical sub-types. In areas where there is a high rate of consanguineous marriage there will be a higher incidence of inherited metabolic disease overall.

Levels of diagnosis

A chain of causation leads from DNA mutation to clinical disease.



As already discussed, dysfunction at the protein level may be caused by one of a number of genetic mutations. To a lesser degree, a particular metabolic abnormality may result from dysfunction of any of a number of proteins. Methylmalonic acidaemia (see page 67) is a particularly complex example but there are many others.

[†] Some studies express the frequency of a mutation to the number of alleles rather than the number of heterozygous individuals. For rare conditions, the allele frequency is very close to half the heterozygote frequency.

The correlation between metabolic abnormality and clinical presentation is often relatively weak: a condition may vary greatly from patient to patient, both in severity and in the presenting symptoms. Thus, metabolite analysis is the most general method for approaching a diagnosis and is especially useful when group methods such as chromatography or tandem MS allow many different compounds to be examined

simultaneously. Only rarely, and in particular populations, is DNA analysis an appropriate tool for primary diagnosis, though it may be useful as a supplementary or confirmatory test where the function of the affected protein is difficult to assay directly.

The laboratory techniques available for neonatal screening are discussed further in chapter 4.

Chapter 3

Neonatal screening – general topics

Historical development

Neonatal screening developed following the successful dietary control of phenylketonuria by Bickel and co-workers in 1954.¹¹ In the discussion of their results they wrote, “Our present studies suggest that treatment does not benefit older children of low grade intelligence. ... it is reasonable to presume that the best results of dietetic treatment of phenylketonuria will be obtained if treatment is started in infancy and particularly in the neonatal period.” This prediction was justified by subsequent studies at several centres, and pilot schemes of whole-population neonatal screening using the ferric chloride test on urine were started both in the UK and abroad.

In 1960, at the instigation of the Ministry of Health, the UK Medical Research Council (MRC) convened a conference on phenylketonuria. The subsequent report¹² provided detailed guidance on the early detection and subsequent treatment of the disease. The conference recommended “that local authorities should continue to maintain and, if possible, expand their present programme of routine screening tests” using the ferric chloride (Phenistix®) test strip because of its “convenience, quickness and relative reliability”. By May 1962, 131 of 145 local health authorities in England and Wales were screening routinely, and a further five were planning schemes.¹²

The development of neonatal screening for phenylketonuria in the USA diverged from the UK model, largely due to the efforts of Robert Guthrie who developed a bacterial inhibition assay¹³ for phenylalanine in dried blood spots (Guthrie spots) collected on a filter paper card (Guthrie card). Guthrie’s efforts were stimulated by the belated diagnosis of phenylketonuria in a niece and he worked tirelessly to promote screening throughout the USA and abroad.¹⁴ The Guthrie test was introduced into Scotland by JS Stevenson in 1964, covering the whole of that country by 1968. In Merseyside, Guthrie’s assay was applied to urine dried on filter paper: taking blood from healthy neonates was considered unacceptable. In the rest of the UK, screening programmes mostly remained locally-organised and based on the Phenistix test. However, as time progressed, the superiority of

the Guthrie approach became obvious and in 1969, after some 4 years of deliberation, it was recommended in Department of Health circular, HM(69)72, that blood-based screening be adopted.¹⁵ Thus began our modern neonatal screening programme.

The results of the screening programme for phenylketonuria are summarised in chapter 6. Screening for congenital hypothyroidism using assay of thyrotropin as a single-step screen was recommended on a national basis in 1980.¹⁶ This screen and its results are also reviewed in chapter 6.

Whole population screening for sickle cell disease has been introduced in selected areas where a high gene-frequency is expected. This screen is the focus of two separate health technology assessments and is not discussed here.

Organisation in the UK

The introduction of blood-based screening in England and Wales in 1969–70 marked a shift from locally-based screening programmes, which had relied entirely on the individual health visitors both to perform the test and to interpret the (qualitative) result, to laboratory-based testing. It also allowed more centralised checking to ensure that every baby was tested. At this time, midwifery and other community-based medical services were run by local authorities through their Medical Officers of Health, and hospital-based services were managed through Regional Hospital Boards, Hospital Management Committees, and Boards of Governors. In the Department of Health circular,¹⁵ collection of blood samples and record-keeping were made the responsibility of the Medical Officer of Health and his staff, laboratory services and treatment facilities being organised through the Regional Hospital Boards. This divided responsibility for different aspects of the programme remains and, indeed, has become more pronounced with the fragmentation of services into individual NHS Trusts.

In the early years, some oversight of phenylketonuria screening was exercised by the Steering

Group of the MRC/DoH Phenylketonuria Register. More recently, the UK Screening Laboratory Directors Audit and Advisory Group has formed a focus for evaluating various aspects of the programme but, since the disappearance of Regional Scientific Officers, has had no formal connection with the administrative apparatus.

Information and consent

Unlike many of the early state screening programmes in the USA, screening in the UK has always been voluntary, though there is no clear policy on the question of whether parental consent should be implicit or explicit. There are no nationally-produced information pamphlets for parents or professionals, although many screening schemes have produced their own.

Nature of sample

In the original Guthrie method, blood from a heel-prick is allowed to drop on to filter paper cards to form a series of discrete spots. The card is then dried and transported, usually by public mail, to the screening laboratory. At the laboratory small disks are punched from the dried blood spots and used for assay. This remains the most commonly-used method and, in the UK, a standard Guthrie card is produced by HMSO and may be purchased through NHS supplies.

As an alternative, the blood sample may be collected in a small heparinised capillary tube and the plasma then separated in the laboratory. This method is more laborious and prone to problems of leakage in the post but gives better results for several tests. It is used in only a few UK screening programmes.

Age at sampling

The 1969 Department of Health circular¹⁵ recommended that the screening sample should be collected between the infant's sixth and fourteenth days of life, preferably as early as possible during this period. Most screening programmes have chosen the sixth day but a few wait until day 11,^{17,18} when sampling may be performed by health visitors rather than midwives. The delay in sampling is to ensure sufficient dietary intake of phenylalanine to give an unequivocal result on the screening test: at least 48 hours on full milk feeds was recommended.¹⁵ With the increased use of total parenteral nutrition for long periods, the requirement for milk feeding has become a problem. In a number of cases involving babies in special care or neonatal surgery

units, diagnosis has been unacceptably delayed,¹⁹ some for this reason. The optimum timing of sampling is discussed later (see page 167).

Laboratory services

The Department of Health circular¹⁵ instructed that laboratory examination of blood specimens should be centralised to the greatest possible extent, on a regional or even supra-regional basis. "Concentration of this scale will ensure the most efficient use of staff and, equally important, the reliable interpretation of results. Designated laboratories should have full facilities for carrying out biochemical confirmatory tests and should be associated with the specialised service responsible for treatment." Not all Regions complied fully with these suggestions and, in 1979, there were 39 laboratories involved in screening for phenylketonuria, only 13 of which screened > 25,000 births a year. There were 11 laboratories in the South East Thames Region.¹⁸ The introduction of an external quality assessment scheme in 1980 revealed gross inadequacies, and a number of laboratories with low workloads ceased screening. Nevertheless, there were still 26 laboratories measuring phenylalanine for screening purposes in 1988,¹⁷ a figure that had changed little by 1991 (see chapter 6). In at least three areas, testing for phenylketonuria and congenital hypothyroidism is performed at separate laboratories.

Referral and treatment

Referring to phenylketonuria, the 1969 Department of Health circular¹⁵ stated "... it is essential that all infants found to have a positive test ... should be referred immediately to the consultant paediatric service. Specialist advisory centres, with the necessary facilities for the biochemical control of treatment, psychometric testing and expert dietary advice could with advantage be associated with the designated laboratory." As with the establishment of screening laboratories, the degree of implementation varied from Health Region to Region. The effect on the quality of treatment provided has not been adequately documented but it is worth noting that, in the survey by Smith and colleagues,¹⁷ the Region with the worst performance in terms of delay in starting treatment for classical phenylketonuria had six screening laboratories and no co-ordinated arrangements for clinical referral; in one city, there were five patients with phenylketonuria, each under a different consultant paediatrician. (Unpublished, privileged information.)

No specific recommendations were made for the organisation of treatment for hypothyroidism.¹⁶

Coverage

The duty to ensure that every baby is offered screening was clearly laid on the Medical Officer of Health of the local authority concerned by the Department of Health circular.¹⁵ However, since then there have been numerous changes in NHS organisation, without corresponding explicit transfer of responsibility. Following the 1974 reorganisation it was assumed that responsibility had passed to the District Medical Officer or a member of his staff. The documents introducing screening for congenital hypothyroidism in 1981 refer specifically to the District Community Physician as the agent for this process, negative results being checked against lists of births to ensure that all babies have been tested.¹⁶ In practice, the actual process of checking that every baby has been tested is generally carried out by the staff of Child Health Records sections. The national blood test card (HMR 101/6) was redesigned in 1985, with the addition of flimsy copies for negative reporting "to facilitate the identification of neonates who have missed being tested and hence to ensure the effective coverage of the screening programme".²⁰

Accurate coverage data are hard to ascertain. Manual checking of a one in eight sample showed that, in Scotland, coverage in 1991 was 99.86% (95% confidence interval (CI) 99.78–99.94)²¹ but there are numerous examples, both published^{22–26} and unpublished, to indicate that elsewhere there are widespread inadequacies in current arrangements for ensuring that all babies are being tested. Where screening progress is linked by computer to Child Health Records, coverage is virtually complete. (A Green: personal communication, 1996.) This is a major focus of an on-going National Audit of Neonatal Screening led by Dr A Streetley (UMDS, St Thomas's Hospital, London).

Some indication of the effects of incomplete coverage have come from an audit of missed cases conducted in 1991 for the UK Screening Directors Advisory and Audit Group by Dr A Green (Birmingham). Eight cases of congenital hypothyroidism were known to have been missed on screening over a 10-year period caused by the specimen not having been taken or having failed to reach the laboratory. This corresponds to one baby in 250 (0.4%) having missed screening but, for reasons described in chapter 6, this probably underestimates the number missed by a factor of nearly two. The corresponding figure for phenylketonuria is three cases over the period 1974–88, during which time 1195 cases of

phenylketonuria (classical and atypical) were diagnosed by screening.¹⁷ This suggests that, on average, only one in 400 babies (0.25%) have missed screening but, in view of the data in *Table 1*, it seems more likely that some missed cases have never been diagnosed.

TABLE 1 Coverage of neonatal screening: selected examples

Area covered	Unscreened (%)
Riverside ²⁷	6.5
Merseyside Region ²⁵	2.6*
Wandsworth ²³	2.0
North Hertfordshire ²⁷	0.3
Scotland ²¹	0.1
Birmingham City (1995–6, unpublished)	0.1
* Individual areas ranged from 0 to 3.8.	

Range of diseases: international comparisons

Once neonatal blood samples were routinely available, it became relatively simple to develop new screening tests and by 1980 Guthrie was able to list over 20 blood-spot screening tests for inherited metabolic disease.²⁸ Relatively few of these achieved widespread acceptance, however. The current status of screening for individual conditions is summarised in the appropriate sections of chapters 6–9.

In the UK, only screening for phenylketonuria and congenital hypothyroidism are provided on a national basis; the extent of screening for other diseases is summarised in *Table 2*. The range of diseases covered in the UK is rather restricted compared with many other Western countries. In (West) Germany, for example, 99% of the newborn population is screened for galactosaemia, 50% for biotinidase deficiency, 32% for homocystinuria and 28% for maple syrup urine disease.²⁹ In New Zealand, screening covers phenylketonuria, congenital hypothyroidism, cystic fibrosis, galactosaemia, congenital adrenal hyperplasia, maple syrup urine disease and biotinidase deficiency.³⁰ In the USA, only three out of 51 state screening programmes are restricted to phenylketonuria, congenital hypothyroidism and (usually) sickle cell disease: the majority screen additionally for galactosaemia and at least one other disorder.³¹ A commercially-operated 'supplementary' screening programme in Pittsburgh, endorsed

TABLE 2 Additional diseases tested for in neonatal blood specimen in the UK (data obtained May 1993)³²

Disorder	Annual number screened	Percentage of UK births	Number of laboratories
Amino acids by chromatography ^a	268,000	34	9
Homocystinuria (specific screen)	95,000	12	2
Tyrosinaemia (specific screen)	25,000	3	1
Galactosaemia (specific screen) ^b	70,000	9	1
Cystic fibrosis	130,000	16	4
Duchenne muscular dystrophy ^c	20,000	3	1
Hyperlipidaemias ^c	4000	1	1

^a See Table 7.
^b Galactosaemia may be diagnosed incidentally with some phenylketonuria screening methods; see page 15.
^c Pilot studies.

In addition, residual blood spots are used for anonymous HIV surveillance in Scotland and several English regions.

by the Blue Cross/Blue Shield organisations, offers specific assays for nine additional disorders and covers approximately 20 other diseases by group tests with tandem MS (Table 3).

TABLE 3 Pittsburgh supplementary screening programme (results 9/85–3/96)

Disease	Infants screened*	Cases found*
Adenosine deaminase deficiency	237,557	0
Arginase deficiency	377,659	0
Biotinidase deficiency	485,104	7
Congenital adrenal hyperplasia	485,104	31
Cystic fibrosis	435,243	73
Galactosaemia (all three types)	486,992	
Classical		10
Epimerase deficiency		4
Erythrocyte glucose-6-phosphate dehydrogenase deficiency	420,876	2413
Muscular dystrophy (Duchenne & Becker)	614,520	60
Glutathione synthase deficiency	420,608	0
Organic acid and fatty acid disorders [†]	211,067	31
Amino acid disorders [†]	212,955	25

* Quoted by Sweetman³³ and updated by Dr Ed Naylor, Pittsburgh; personal communication, July 1996.
[†] By tandem MS, further details in Tables 9 and 10.

Criteria for screening: the Wilson and Jungner principles

To a large extent, the conservatism of the UK programme has been justified by reference to the principles for screening formulated by Wilson and Jungner in 1968³⁴ (see box opposite) and since restated in various forms.^{35–37} The wording in the box in bold type follows the original; the remaining text is taken from subsequent paragraphs in which Wilson and Jungner expand on these principles. Further discussion of this appears later in chapter 17.

These principles have been expanded by others. Of particular relevance to the discussion on page 12 is a principle introduced by Haggard – “...the incidental harm done by screening, and by the information (correct or otherwise) that it gives, should be small in relation to the total benefits from the screening–assessment–treatment system”.³⁸ The incidental harm done by a screening programme is very much linked up to its analytical performance and particularly to what are loosely called ‘false-positive results’.

Measures of screening performance

Formal definitions

Several formal definitions have been developed to assist in the assessment of screening test performance. The relationship between test result and presence or absence of the disease gives rise to four categories:

	Test result	
	Positive	Negative
Disease present	True-positive	False-negative
Disease absent	False-positive	True-negative

These are then used to calculate the **sensitivity**:

$$\frac{\text{True-positives}}{\text{True-positives} + \text{False-negatives}}$$

and the **specificity**.

$$\frac{\text{True-negatives}}{\text{True-negatives} + \text{False-positives}}$$

Sensitivity is the proportion of all cases found by the screening programme. **Specificity** is the proportion of individuals without the disease that is correctly identified by the screening programme.

(Unfortunately, both terms are also used in the analytical literature to define assay performance: sensitivity in terms of the minimum detectable concentration; specificity to measure cross-reactivity or other types of interference.) In neonatal screening, where both true- and false-positive rates are often very small, the **false-positive rate** is sometimes approximated as a ratio per head of the population screened: a specificity of 0.9999 becomes a false-positive rate of 1 in 10,000.

A further measure of assay performance is the **positive predictive value** (PPV):

$$\frac{\text{True-positives}}{\text{True-positives} + \text{False-positives}}$$

Unlike the previous parameters, this is related to the frequency of the disease in the population under consideration. It has the advantage of comparing the benefits of a method in terms of cases detected with the harm it does by producing spurious positive results and is thus

PRINCIPLES OF EARLY DISEASE DETECTION – WILSON AND JUNGNER, 1968³⁴

- The condition sought should be an important health problem** to the individual and/or the community. Phenylketonuria is extremely uncommon but warrants screening on account of the very serious consequences if it is not discovered and treated very early in life. ... certain individually mild conditions, but having serious consequences for the community if not discovered early and treated, will justify screening on these grounds. An example ... might be the finding and control of overweight in a population.
- There should be an accepted treatment for patients with recognised disease.**
This [raises] two [further] questions:
 - Does treatment at the pre-symptomatic border-line stage of a disease affect its course and prognosis?
 - Does treatment of the developed clinical condition at an earlier stage than normal affect its course and prognosis?
 It is axiomatic that case-finding should only be undertaken when the prospects for treating the condition are at least reasonable.
- Facilities for diagnosis and treatment should be available.**
- There should be a recognised latent or early symptomatic stage.**
- There should be a suitable test or examination.**
The screening test (which of its nature should be easy and quick to perform) is allowed to possess a higher margin of error and may be less valid than a diagnostic test. ... In case-finding work a fairly high false-positive rate is acceptable but the false-negative rate should be very low, since missed cases may lead to individual disasters.
- The test should be acceptable to the population** [to be tested].
- The natural history of the condition, including development from latent to declared disease, should be adequately understood.**
- There should be an agreed policy on whom to treat as patients.**
- The costs of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.**
- Case-finding should be a continuous process and not 'a once and for all' project.**

of direct relevance to Haggard’s principle described above.

Interpretation problems

Definition of false-negative and false-positive, even in purely biochemical terms, is not always easy. As already described (page 4), there is great genetic variability in individual disorders so that a screening test designed primarily for detecting the ‘classical’ clinical form of an inborn error of metabolism may well miss the milder variants. Equally, patients with minor biochemical variants of no clinical significance may well be considered as ‘false-positives’ as shown in *Figure 1* below.

This problem of defining what is to be screened for is particularly marked for cystic fibrosis (see page 55).

Sensitivity

Many different factors contribute to the overall false-negative rate for a screening programme (*Table 4*) and it is important to consider all of these in assessing performance.

Specificity

A major ambiguity in discussing specificity is the definition of a false-positive result. The draft Newborn Screening Lexicon (International Society for Neonatal Screening, 1996) defines a positive test as “a test result which indicates that the infant from which the specimen is taken has a **greater likelihood than the population frequency** of having the condition screened for”, and uses this as the basis for defining a positive result. Although from the laboratory standpoint this is correct analytical terminology, to other health professionals and the general population it implies a result that indicates that the baby **probably** has the disease concerned. Most neonatal screens are multi-step processes and false-positive results at different stages and for different reasons have such different implications that it is important to develop terms that differentiate between them and which will be clear to all parties involved in the screening process.

A schematic view of a neonatal screen based on a single analyte is given in *Figure 2*. It follows that

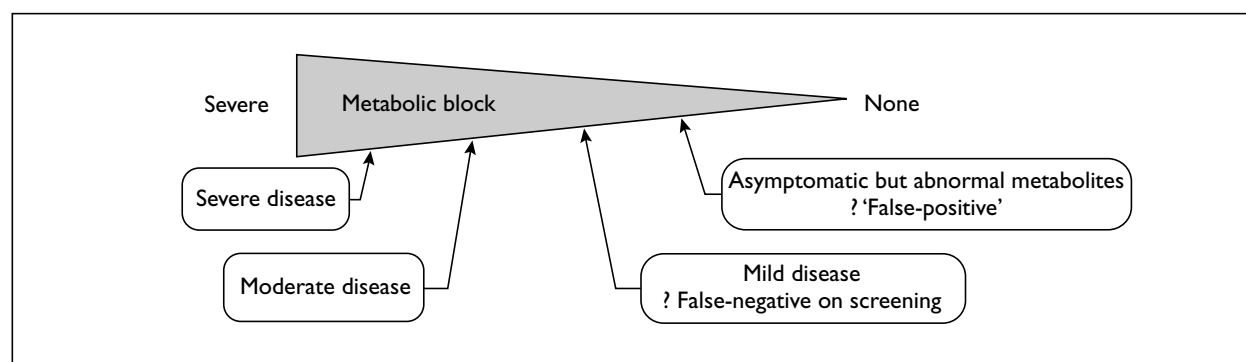


FIGURE 1 Because of genetic variability, patients with minor biochemical variants of no clinical significance may well be considered false-positives

TABLE 4 Contributors to the false-negative rate

Component	Possible remedy
Specimen not taken or loss in transit undetected (see page 9)	Better checking systems in child health departments
Contaminated specimen, mislabelled specimen, denatured specimen (heat, alcohol, etc.)	Improve training and systems in midwifery/nursing and laboratory services
Inadequate substrate intake to produce metabolite accumulation	Test at a later age
Genetic heterogeneity (mild variants, clinically affected)	Improve sensitivity of the screening test; lower cut-off
Poor discrimination between normal and affected ranges	Use an analyte with better discrimination
Poor performance of laboratory assay	Improve method or instrumentation
Laboratory error	Improve laboratory staff or systems

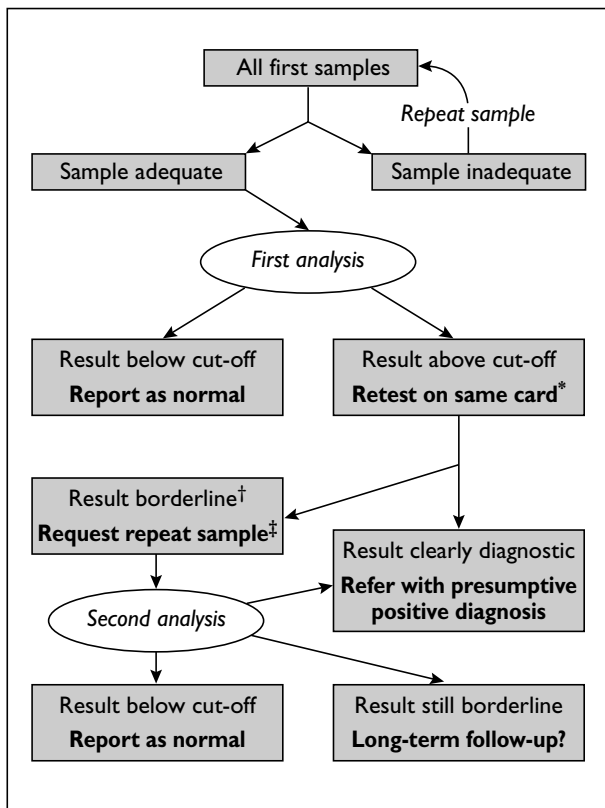


FIGURE 2 Schematic of a screening protocol based on a single analyte, the concentration of which is increased in the condition sought (*, using either the original method or an alternative; †, 'borderline' refers primarily to clinical interpretation; ‡, usually after an interval of 1–2 weeks)

suggested in the appendix to the Department of Health circular on screening for congenital hypothyroidism.¹⁶ Other protocols (e.g. for cystic fibrosis screening, see chapter 8) may be more complicated but in most screens the analytical results obtained on the first blood specimen are subjected to a triage system. Even in screens where there is good discrimination between normal and affected ranges, a number of specimens give results which, though formally 'positive' (in that they lie outside the accepted normal range), are

not clearly diagnostic. Such results are usually termed borderline, intermediate, or equivocal and usually attributed to 'biochemical immaturity'. The normal procedure is to obtain a repeat specimen. The great majority of these repeat specimens give normal results, i.e. the PPV of an intermediate result on the first specimen is low. Depending on the screen in question, the technical details of its implementation, and the way the results are interpreted, such intermediate or equivocal results may greatly outnumber true-positive diagnoses (see data from the laboratory surveys on screens for phenylketonuria and congenital hypothyroidism, chapter 18).

The steps which may follow an abnormal screening result, in order of increasing impact on the family, are as follows.

- **Retesting within the laboratory** on the same sample either for the same analyte and/or a different one. This has no impact on the screened baby's family unless the initial blood specimen is insufficient and a repeat specimen is required.
- **Requesting a repeat blood specimen.** This invariably raises some anxiety although the majority of such requests are due to inadequate first specimens or borderline abnormalities which turn out to be insignificant (Table 5). It is important that these requests are not understood by the parents to indicate a positive diagnosis.
- **Follow-up testing** involving a specimen of liquid blood or urine specimen, or some direct procedure such as a sweat test. For many disorders, a presumptive positive diagnosis can be made without requiring follow-up testing.
- **Clinical referral** with a presumptive positive diagnosis. In general, laboratory-generated false-positives at this stage should be very rare (Table 6).

TABLE 5 Contributors to the repeat sampling rate

Component	Possible remedies
Sample collected too early, or not clearly labelled. Insufficient blood, contaminated specimen or faulty collection technique	Improve training and systems in midwifery/nursing services
Leakage (liquid specimen) or loss in transit	Check systems
Borderline result – probably 'biochemical immaturity'	Test at a later age
Poor performance of laboratory assay	Improve method or instrumentation
Poor discrimination between affected and normal ranges	Alternative analyte
Mild variants or other disease	

TABLE 6 Contributors to the false-positive rate[†]

Component	Possible remedies
Genetic heterogeneity (mild variants, not clinically affected)	Refine limits for pathogenesis if possible. Adjust cut-off for the screening test
Contaminated specimen, mislabelled specimen	Improve training and systems in midwifery/nursing services
Laboratory error	Improve laboratory staff or systems
Misinformation or misunderstanding	Better systems for conveying information

[†] In much of the screening literature the term false-positive is used rather loosely. In this context, it refers to any situation which leads to parents being told that their baby has a disease when in fact it does not have a clinically significant abnormality. Ideally, a screening programme should not generate a 'positive' result until a clear presumptive diagnosis has been established. The term should not be used for borderline results.

Comparing performance

In comparing the performance of screening schemes in different places it is important to remember that the populations may be genetically different, not only in the most prevalent sub-types of the disorder being screened for, but also in non-pathogenic polymorphisms which may affect the false-positive rate. An example is the Northern Ireland experience of screening for cystic fibrosis (see chapter 8). A further confounding factor in such comparisons is that in many parts of the world the specimen is taken at an earlier age than in the UK, and this often reduces the difference between the affected and normal ranges. In the

Nord-Pas de Calais (France) screening service, a marked and unexpected change in specificity of phenylketonuria screening was experienced on changing collection from day 5 of life to day 3.³⁹

Given these regional differences and problems of definition, it is clearly inappropriate to apply meta-analytical methods to neonatal metabolic screening. The best that can be achieved from a study of literature is a likely range for key parameters, giving greatest weight to results obtained from populations most closely related to those in question.

Chapter 4

Laboratory techniques

Introduction

The options available in designing a neonatal screening programme are very much determined by analytical techniques. Some techniques dictate highly specific single-disease screens, while others are capable of detecting a much wider spectrum of conditions or allow two or more single-disease screens to be performed simultaneously, with a saving in manpower costs. The major techniques in current use or at an advanced stage of development are briefly surveyed here. Further information on screening tests and definitive diagnosis may be found in standard texts.^{40,41}

“The technical issues are relevant. A crucial attribute of a screening test is its sensitivity – its ability to minimise the frequency of false-negative results”.⁴²

Amino acid screens

There are a variety of methods available for screening for phenylketonuria and some of these will detect other conditions (*Table 7*). Guthrie and chromatographic methods are generally regarded as semi-quantitative and have low capital costs. The fluorometric and enzymatic methods are quantitative down into the normal range. The fluorometric method requires special equipment but has low reagent costs. The enzymatic assay may be run on standard multipurpose clinical chemistry analysers but reagent costs are relatively high.

Although chromatography will detect other amino acid disorders, these are relatively rare in most parts of the UK (see chapter 7).

TABLE 7 Amino acid screens

Primary disease	Technique	Comments
Phenylketonuria (chapter 6)	Guthrie test	Specific for phenylalanine*
	Fluorometry	Specific for phenylalanine*
	Enzymatic	Specific for phenylalanine*
	Chromatography (paper or thin layer) with ninhydrin detection	Also detects other abnormalities, particularly: maple syrup urine disease citrullinaemia tyrosinaemia type I (sensitivity and specificity low) homocystinuria (sensitivity low without special stain) <i>plus</i> a number of other rarities – not always particularly sensitive
	Tandem MS	See page 16
Homocystinuria [†]	Specific Guthrie assay or chromatography with special stain	North-west Region and Northern Ireland
Maple syrup urine disease [†]	Specific Guthrie assay	Not used in UK because of rarity
Tyrosinaemia type I [†]	Several methods, see chapter 7	

* Increased phenylalanine may indicate a disorder of bipterin metabolism (see chapter 6) or a disorder producing hepatic dysfunction, e.g. galactosaemia.

† Also detected by chromatographic methods used for phenylketonuria and by tandem MS.

Other individual screens

These are summarised in *Table 8*. As noted, four of these screens can use immunoassay techniques with similar detection probes. Some commercially available systems, DELFIA® in particular, have the potential to perform more than one assay simultaneously on a single blood spot, with substantial savings in manpower. Although a demonstration that combines primary screens for congenital hypothyroidism, congenital adrenal hyperplasia, Duchenne muscular dystrophy, and cystic fibrosis was reported in 1992,⁴³ this system is still not commercially available. The screens for galactosaemia and biotinidase deficiency are essentially stand-alone assays but recently-developed instrumentation allows these and the enzymatic assay of phenylalanine to be automated using a single analytical system.

Tandem mass-spectrometry

Principle of the method

Mass spectrometry is a technology whereby molecules are broken down into smaller charged fragments by a process of collision. The profile of fragments is known as a spectrum and is characteristic for each individual parent molecule. The analysis of complex mixtures by tandem MS has been

available since the early 1980s but it is only in recent years that its application to neonatal screening has begun to be explored.⁴⁴

In tandem MS there are two mass spectrometers 'in tandem' (*Figure 3*). In the collision cell, ionised molecules of the same type (e.g. amino acids) will lose a common fragment. The two mass spectrometers are set up to detect the mass of the parent molecules of interest **either** by detecting ions which have lost the mass of a characteristic neutral fragment (as in amino acid analysis) **or** by monitoring a common ionised fragment which has been lost by all the parent ions of interest (as in acylcarnitine analysis). Deuterated internal standards are used for quantitation. The specificity of the method is dependent on the weight of the particular fragments lost from the original (parent) molecule. There is no preliminary chromatographic separation so the method is rapid. Positional isomers of the same compound (i.e. different positions of individual atoms giving rise either to stereo isomerism or geometrical isomerism) have the same molecular weight and will not be differentiated.

The preferred type of sample introduction is by electrospray ionisation, as this allows a 'continuous flow' approach and hence use of an automated sampler.⁴⁵ Unattended

TABLE 8 Other individual screens

Primary disease	Technique	Comments
Galactosaemia (three types)	Bacterial inhibition or Paigen assay, or enzymatic determination of galactose and galactose-1-phosphate	Specific for galactosaemia and related disorders. Used in Scotland and Eire*
Biotinidase deficiency	Biotinidase assay	Specific. Km variants have produced problems
Congenital adrenal hyperplasia [†]	Immunoassay of 17- α -hydroxy-progesterone	Pre-extraction increases specificity
Duchenne muscular dystrophy [†]	Creatine kinase: activity or immunoassay	Must be combined with DNA studies to provide confirmation
Congenital hypothyroidism [†]	Thyroxine determination	Used mainly in the USA. Primary and secondary hypothyroidism are detected but requires thyrotropin assay as a second stage. Largely obsolete
	Thyrotropin (TSH) immunoassay	Specific for primary hypothyroidism. Several methods using different detection labels, all with adequate performance
Cystic fibrosis [†]	Immunoreactive trypsin (IRT) assay	Several methods using different detection labels. Two-stage or three-stage screens possible. DNA analysis increasingly being used as the second stage

*With quantitative methods for phenylalanine (page 15) up to 70% of cases of galactosaemia may also be detected.
[†]These assays may now be combined, using dissociation-enhanced lanthanide fluorescence immunoassay (DELFLIA) techniques.

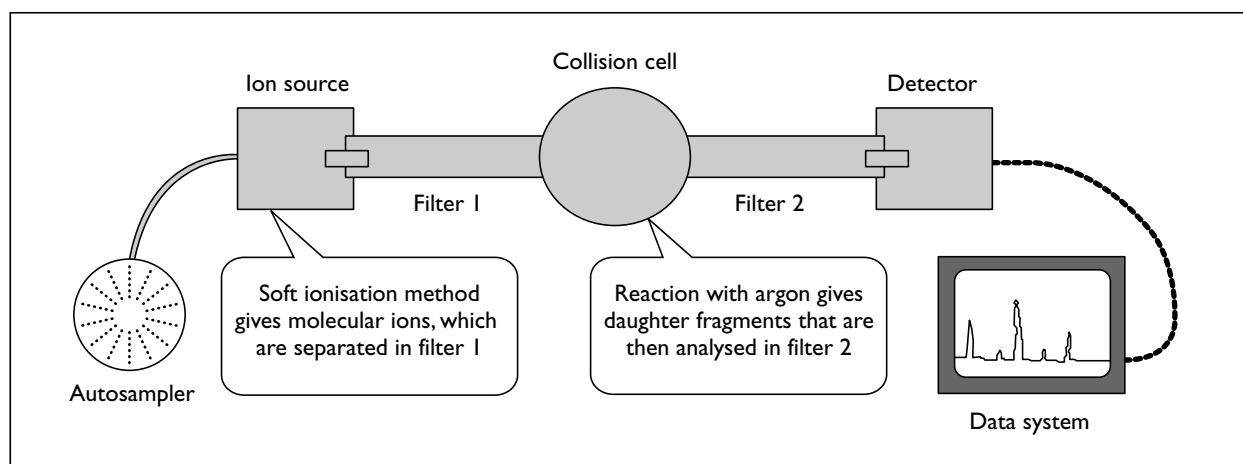


FIGURE 3 Schematic of tandem mass-spectrometer (electrospray tandem MS for rapid profiling of specific classes of metabolites) *Acylcarnitine* butyl esters all give a strong fragment at m/z 99 [Tune filter 2 to m/z 99 and scan filter 1 to obtain m/z of parent molecular ions].

Amino acid butyl esters all lose a neutral fragment of 102 amu – 119 for basic amino acids [Scan both filters together but with filter 2 lagging by 102 (or 119) amu].

introduction of 1000 samples over a period of 21 h 40 min with automatic data collection has been demonstrated.⁴⁶

By automatic programming of the instrument, different groups of compounds from the same sample (e.g. amino acids, acylcarnitines) can be analysed over a period of less than 1 minute. Alternatively, programming in the 'specific reaction monitoring' mode restricts analysis to specific compounds, thereby omitting detection of disorders of unknown or uncertain significance.

Analytical validation

Practical experience of neonatal screening using tandem MS is still limited. Workers at the Duke University Medical School Mass Spectrometry Facility, sometimes in collaboration with workers from Pittsburgh, have published a series of papers formally validating the method for diagnosis of specific disorders: phenylketonuria,⁴⁷ maple syrup urine disease,⁴⁸ medium-chain acyl-CoA dehydrogenase deficiency,⁴⁹ and homocystinuria.⁵⁰ The effect of various forms of sample abuse (prolonged storage, incomplete filling of circle, double application of blood, surface abrasion, deliberate contamination with water, hand lotion, isopropyl alcohol) on the absolute and relative concentrations of analytes has also been studied.⁵¹ By using selected metabolite ratios rather than absolute quantities it is possible to compensate for sample imperfections, particularly the common situations where there has been insufficient blood to fill the required circle or the blood has failed to penetrate the card completely. This should reduce the rate of repeat sampling due to inadequate initial samples and be a marked operational advantage.

The only programme which has so far processed a large numbers of samples is that operated by NeoGen Screening Inc., Pittsburgh, PA, USA (see *Table 9*). This laboratory has now analysed over 200,000 samples, largely using manual methods of sample preparation and data analysis. Improved data analysis software is currently being developed, by both NeoGen and instrument manufacturers, Micromass UK Ltd.

TABLE 9 Results of Pittsburgh tandem MS screening programme

Method and disease	Number diagnosed
Organic acid and fatty acid disorders (acylcarnitine scan)	
Methylmalonic acidaemias	1
Propionic acidaemia	2
Isovaleric acidaemia	0
Glutaric acidaemia type I	5
Short-chain acyl-CoA dehydrogenase deficiency	2
Medium-chain acyl-CoA dehydrogenase deficiency	16
3-methylcrotonyl-CoA carboxylase deficiency	4
HMG-CoA lyase deficiency	1
Total screened	211,067
Amino acid disorders (amino acid scan)	
Phenylketonuria	14
Hyperphenylalaninaemia	6
Tyrosinaemia	1
Homocystinuria	2
Maple syrup urine disease	2
Total screened	212,955
Overall yield for tandem MS	1/3803

Screening by tandem MS in the UK

There are currently two locations (Institute of Child Health, Great Ormond Street, London; Institute of Child Health, Royal Victoria Infirmary, Newcastle-upon-Tyne) where tandem MS is under development for the specific application of neonatal screening/inherited metabolic disorders. An outline of work conducted to date follows. Both instruments (MS–MS) are the VG Quatro model, manufactured by Micromass UK Ltd.

Royal Victoria Infirmary, Newcastle-upon-Tyne (Professor K Bartlett)

A blind, retrospective study to validate the methodology for a wide range of inherited metabolic disorders has been undertaken. This was completed using approximately 500 specimens (blood spots) in collaboration with colleagues in Manchester and Sheffield (approximately 5% of specimens were positive). As this was a retrospective study, several specimens were several years old. This study has confirmed the work in the USA by Chace and colleagues,^{47–51} that is, it is a sensitive technique with no false-negatives. It has also provided some information about the reliability of retrospective diagnosis on stored blood spots.

It has clearly not been possible to validate specificity (i.e. false-positives/impact on repeat rates in a real screening situation) on such a small sample number and, to address this, a larger retrospective study (30,000 specimens) using normal blood spots from other screening laboratories has been undertaken with satisfactory results. A satisfactory automated ‘front end’ sample preparation/liquid handling system for large-scale use has been developed.

A prospective study has not been undertaken, although one is planned.

Institute of Child Health, Great Ormond Street, London (Drs AW Johnson and PT Clayton)

A tandem MS unit for neonatal and metabolite screening has been set up with grant monies and industrial partners. Work completed to date includes the following.

- (a) **Validation of analysis for diagnosis of known disorders** – the group has assayed over 4500 blood spots from a wide range of diseases, including the fat oxidation defects, organic acidurias, amino acidopathies, bile acid defects, biliary atresia and peroxisomal disease. A particular area of interest has been the development of conjugated bile acid analysis in blood spots.

- (b) **Assessment of false-positive/false-negative results** – with confirmed diseases there have been no false-negatives detected, that is, the method is sensitive. In sick children, a pattern suggestive of mild glutaric aciduria type II but which appears to relate to fasting, hypoxia, respiratory chain disorders or agonal changes rather than to a permanent metabolic defect has occasionally been seen. Otherwise, false-positives are minimal.
- (c) **Retrospective analysis of blood spots** – the system has been highly successful in retrospective diagnosis from the original Guthrie cards of children who have died, where there has been a suspicion of a metabolic cause of death at post-mortem.

Further work planned includes full automation of the process of derivatisation to provide cost-effective large-scale screening. A prospective screening evaluation has not yet been undertaken but is planned. For ethical reasons, this will probably, to a large extent, use anonymous specimens, which will limit the value of the exercise.

Birmingham Children's Hospital

Work will commence in 1997 at a third UK location (West Midlands Regional Screening Laboratory, Department of Clinical Chemistry, Birmingham Children's Hospital NHS Trust) on a large-scale prospective study.

Disorders detectable

The application of tandem MS to the diagnosis of amino acid disorders draws heavily on experience accumulated over the past 30 years using other techniques of amino acid analysis. The use of acylcarnitines for diagnosis of organic acid disorders is, however, a new development and experience is still somewhat limited, especially for the neonatal period. However, acylcarnitine analysis using tandem MS has proved a reliable diagnostic tool in symptomatic patients with a variety of disorders.^{52,53} A summary of the main diseases detectable is presented in *Table 10*. Details for individual disorders are provided in chapters 6–9.

It has been reported recently that tandem MS may be used to assay bile acid taurine conjugates and conjugated bilirubin in dried blood spots.^{54,55} Potentially these two assays may be used to screen for biliary atresia and other disorders producing neonatal liver dysfunction,⁵⁶ and for a variety of inherited peroxisomal and bile acid disorders (see chapter 9). Given the preliminary nature of these reports, these screens are not included in

TABLE 10 Main diseases detectable by tandem MS

Primary disease/screen	Technique	Diseases detected
Amino acid scan	Tandem MS	Phenylketonuria (see also note on galactosaemia) Maple syrup urine disease Argininosuccinic aciduria Tyrosinaemia type I (low specificity, confirmatory test needed) Homocystinuria (by hypermethioninaemia) 5-oxoprolinuria
Urea cycle scan	Tandem MS	Ornithine carbamoyltransferase deficiency and other urea cycle defect by secondary increase in glutamine Citrullinaemia Hyperornithinaemias
Acylcarnitine scan	Tandem MS	Propionic acidaemia Methylmalonic acidaemia (but possibly not mildest forms) Isovaleric acidaemia Medium-chain acyl-CoA dehydrogenase deficiency Defects of long-chain fatty acid catabolism Defects of branched-chain fatty acyl-CoA catabolism Glutaric acidaemia type I Glutaric acidaemia type 2 and related disorders

the model described in chapter 18; however, once they have been properly validated they could be added to an existing tandem MS-based programme at little extra cost.

DNA analysis

Techniques for the detection of abnormalities of DNA structure are developing very rapidly and those for individual discrete mutations can now be automated for large-scale application. However, compared to other methods used in large-scale screening, DNA analysis is still expensive and time-consuming; further development is needed if these assays are to become realistic for large-scale use. More importantly, most inherited metabolic disorders are extremely heterogeneous at the DNA level (see page 4), so that analysis for any one mutation will only detect a small proportion of cases. Important exceptions are medium-chain acyl-CoA dehydrogenase deficiency and cystic fibrosis, where common mutations (A985G and Δ F508, respectively) are found in the UK population. Even for these diseases, screening for these mutations using homozygosity as the only diagnostic criterion would detect only about 85% and 67% of cases, respectively. An even greater problem would arise from the detection

of unaffected heterozygous carriers for these mutations:

- these vastly outnumber patients with the disorder (see page 5)
- there is an increased risk of the carriers having siblings affected by the disease in question
- investigation and counselling of the parents would be a major undertaking.

For further discussion of this subject, see the section on cystic fibrosis in chapter 8.

Although whole population neonatal screening by DNA analysis is impractical at present, the technique can be used in a selective manner for disorders where the primary screen is lacking in specificity. Screening programmes for cystic fibrosis and muscular dystrophy are increasingly using DNA analysis as a second-tier screen. Scanning the entire exonic region (those sections of the gene coding for protein structure as opposed to the non-expressed intervening sections) of a gene is now becoming a feasible proposition in many disorders, enabling DNA analysis to be used as a second-stage investigation even when there is no predominant disease-causing mutation in the population concerned.

Chapter 5

The literature search

Search methodology

The methodology for the literature review has been filed with the NHS Centre for Reviews and Dissemination.

Searches were conducted for diseases where there are **established screens**.

- Biotinidase deficiency
- Congenital adrenal hyperplasia
- Congenital hypothyroidism
- Cystic fibrosis
- Duchenne muscular dystrophy
- Galactosaemia
- Homocystinuria
- Maple syrup urine disease
- Medium-chain acyl-CoA dehydrogenase deficiency
- Phenylketonuria
- Tyrosinaemia (type I)

An additional search was made for screening using tandem MS.

Disease-specific searches were made for disorders that could be included in tandem MS screens but for which there is, at present, no screening-based information available.

- Argininosuccinic aciduria
- Branched-chain ketothiolase deficiency
- Carnitine palmitoyltransferase deficiency type II
- Citrullinaemia
- Glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency)
- 3-hydroxy-3-methylglutaryl-CoA dehydrogenase deficiency
- Hyperornithinaemia
- Isovaleric acidaemia
- Long-chain acyl-CoA dehydrogenase deficiency
- Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency
- β -methylcrotonyl-CoA carboxylase deficiency
- Methylmalonic acidaemias
- Multiple acyl-CoA dehydrogenation defects (glutaric aciduria type II)
- Propionic acidaemia
- Short-chain acyl-CoA dehydrogenase deficiency

Data sources

All the above aspects of the review were searched across four core biomedical databases; Medline, Embase, *Biological Abstracts* and *Science Citation Index* (the last two through BIDS). General databases, such as the National Library of Medicine Book and Reports Catalogue, the British Library's Inside Information service and Uncover were used to identify books or recently published articles, and the Index of Scientific and Technical Proceedings (BIDS) was searched for conference papers. In addition, several databases (HealthPlan, DHSS-Data, HELMIS and Social Science Citation Index) were specifically searched for (a) economic issues of neonatal screening, and (b) psychological and ethical issues of screenable diseases and of administering screening tests. Economic issues were also examined through the EconLit and IBIS (British Library Politics and Economics service) databases, while PsychLit was used to explore the psychosocial issues. Finally the new 'evidence-based' databases of the Centre for Reviews and Dissemination (DARE and NEED) and the Cochrane Collaboration (the Cochrane Library) were used to verify the comprehensiveness of the search coverage.

Periods searched

The respective emphases of the review were reflected in the periods covered by the search strategies. Reviews and randomised controlled trials (RCTs) of neonatal screening were searched for over the period 1966–95 on Medline, using the Cochrane Collaboration's specialist search strategies. The yield of RCTs was particularly poor, probably due to their acknowledged limited applicability for diagnostic procedures. A comprehensive search for tandem MS was conducted back to 1986. The psychological and economics searches, however, cover the literature back to the beginning of each database (e.g. Medline – 1966; PsychLit – 1967).

In addition, supportive information was compiled for a disease either if **it has an established screen** or it is **a candidate disease for tandem MS**. Diseases were categorised according to both the incidence of the disease and the volume of the screening literature into 5-year, 10-year (screening only) and 10-year (all references) categories (see *Table 11*). The specific information derived from retrieved

TABLE 11 Disease literature categories

Diseases covered for 5 years (1991–95)	Diseases covered for 10 years – screening only (1986–95)	Diseases covered for 10 years – all references (1986–95)
Biotinidase deficiency	Congenital hypothyroidism	Maple syrup urine disease
Congenital adrenal hyperplasia	Phenylketonuria	Argininosuccinic aciduria
Cystic fibrosis		Citrullinaemia
Duchenne muscular dystrophy		Hyperornithinaemia
Galactosaemia		Methylmalonic acidaemias
Homocystinuria		Propionic acidaemia
Tyrosinaemia		3-hydroxy-3-methylglutaryl-CoA lyase deficiency
		Branched-chain ketothiolase deficiency
		β -methylcrotonyl-CoA carboxylase deficiency
		Medium chain acyl-CoA dehydrogenase deficiency
		Long-chain acyl-CoA dehydrogenase deficiency
		Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency
		Carnitine palmitoyltransferase type 2 deficiency
		Short-chain acyl-CoA dehydrogenase deficiency
		Glutaryl-CoA dehydrogenase deficiency
		Glutaric aciduria type II

references relates to incidence, natural history, costs (treated and untreated), screening and treatment of the disease or sensitivity, specificity and cost of screening.

Some older literature was retrieved through the 4600 page 1995 edition of ‘Scriber’⁴ and monographs from early screening symposia,^{57,58} while abstracts of recent symposia of the International Society for Newborn Screening and the Society for the Study of Inborn Errors of Metabolism were scanned for very recent developments.

Search strategy

A very sensitive strategy was used to ensure retrieval of all relevant references. For example, the Medical Subject Heading on Medline ‘Neonatal screening’ was found by investigation to be inadequate for retrieval of all required items. The combination ‘Mass screening’ AND ‘Infant – newborn’ was used to pick up incorrectly indexed items. Each disease index term was used in conjunction with a diagnosis subheading and variations on newborn, neonate and infant to identify terms where the screening concept was unindexed. These principles were translated to other databases as appropriate.

Reference management

Retrieved references were loaded to a Reference Manager[®] software package and coded for topic,

sub-topic and reviewer. Lists comprising bibliographical references and abstracts (if available) were generated by topic for initial inspection by the expert reviewer. Articles were categorised for definite inclusion or exclusion and, in cases of doubt, the full article was obtained. Photocopies of articles for definite or potential inclusion were subsequently evaluated by the expert reviewers.

Inter-rater agreement

An assessment of inter-rater agreement⁵⁹ to compare the ability of the two observers to classify articles according to the defined inclusion and exclusion criteria, was performed on a sample of 316 articles. The results are presented in *Table 12*.

While no strict definitions of the resulting kappa statistic are possible, guidelines for evaluating observed values of kappa have been established by Landis and Koch.⁶⁰ Using these guidelines, it was possible to interpret a kappa value of 0.775 as indicating ‘substantial’ agreement between the two observers.

Literature identified

The yield of literature from the systematic searches by disease/topic is shown in *Table 13*.

TABLE 12 Inter-rater agreement results

		Observer 1			
Observer 2	Included	Excluded	Undecided	TOTAL	
Included	25	23	2	50	
Excluded	6	165	2	173	
Undecided	0	7	86	93	
TOTAL	31	195	90	316	
<i>kappa value, 0.775; CI, 0.71–0.84.</i>					

Nature of the literature

Neonatal metabolic screening is a sequential process and we have adapted the 'causal pathway' methodology for estimating effectiveness suggested by the NHS Centre for Reviews and Dissemination,¹ breaking down the assessment for each disease or group of diseases into five components. These are:

- (i) the birth frequency of the disease
- (ii) natural history of the untreated condition

TABLE 13 Literature identified by systematic searching

Disorder/technique	Literature category (number of articles)					
	Total number of articles identified	Selected for review	Not relevant	Prevalence	Neonatal screening	Treatment
Phenylketonuria	316	153	163	23	89	41
Congenital hypothyroidism	255	104	151	17	51	13
Tyrosinaemia type I	138	83	155	6	6	27
Homocystinuria	242	122	120	17	25	38
Maple syrup urine disease	257	43	214	1	3	7
Argininosuccinic aciduria, citrullinaemia, hyperornithinaemia	14	4	10	1	1	0
Galactosaemia	434	134	300	24	69	32
Cystic fibrosis	1110	128	982	16	64	13
Congenital adrenal hyperplasia	485	190	295	27	68	51
Muscular dystrophy	374	28	346	2	16	3
Biotinidase deficiency	165	37	128	8	16	11
Methylmalonic acidaemia	64	26	38	1	0	1
Propionic acidaemia	37	20	17	0	1	3
Isovaleric acidaemia	20	2	18	0	0	0
Other branched chain acyl-CoA metabolism	46	13	33	1	2	3
Medium-chain acyl-CoA dehydrogenase deficiency	333	12	321	2	2	1
Defects of long-chain fatty acid catabolism	57	10	47	0	1	1
Short-chain acyl-CoA dehydrogenase deficiency	11	1	10	0	0	0
Glutaryl-CoA dehydrogenase deficiency	11	9	3	0	0	1
Glutaric aciduria type II	15	9	6	0	0	0
Tandem MS	402	28	374	N/A	8	N/A
<i>N/A, not applicable</i>						

- (iii) the performance (accuracy) of the screening test (as defined in chapter 3)
- (iv) the uptake (coverage) of the screening test
- (v) the effectiveness of early treatment.

Items (i)–(iii) and (v) are assessed for individual diseases in chapters 6–9. Item (iv), coverage, will be essentially the same for all disorders and has been discussed in chapter 3. Evaluation of the uptake of treatment has not been formally attempted as no pertinent data were encountered. For the existing metabolic screening programmes (see chapter 6), the initial uptake of treatment has not been a significant problem but there may be problems of compliance in later years.

For each of the disorders (or groups of disorders) surveyed in chapters 6–9, the available key information is summarised, together with an assessment of the quality of the evidence, under the following headings, where appropriate.

Expected incidence (cases per 100,000) average within the UK.

- I** Data obtained from whole-population screening or comprehensive national surveys of clinically detected cases.
- Ia** As **I** but more limited in geographical coverage or methodology.
- II** Extrapolated from class **I** data for non-UK (but broadly similar) populations.
- III** Estimated from number of cases clinically diagnosed in UK (compared with similar disorders of established incidence).

Proportion of cases likely to be clinically affected to a significant degree (%).

- I** Data from retrospective screening studies in UK or similar population.
- II** Data from systematic studies other than whole population screening.
- III** Estimated from the known clinical features of the condition as described for individual cases or short series.

Effectiveness of treatment – classified as high, medium, low, or nil. The hierarchy of evidence¹ follows standard guidelines.

- I** Well-designed RCTs.
- II-1** Well-designed controlled trials with pseudo-randomisation or no randomisation.
- II-2** Well-designed cohort studies
 - (a) prospective with concurrent controls
 - (b) prospective with historical control
 - (c) retrospective with concurrent controls.
- II-3** Well-designed case-control (retrospective) studies.

- III** Large differences from comparisons between times and/or places with and without intervention (in some circumstances these may be equivalent to level **I** or **II**).
- IV** Opinions of respected authorities based on clinical experience, descriptive studies and reports of expert committees.

For the vast majority of inherited metabolic diseases there have been no controlled trials of treatment but some type **III** studies produce especially convincing evidence of efficacy of treatment.

- (a) For some disorders which produce acute metabolic crises, there is an immediate life-saving response to metabolic ‘first aid’ which is sufficiently self-evident to require no further proof. This response may be unduly reassuring, however, as in some such diseases (e.g. galactosaemia) there are additional, chronic processes for which, despite treatment, the outcome is poor.
- (b) For a few disorders which exert their effects through well-understood biochemical processes and respond well to rational treatment, there is no evidence as yet of disease progression under such treatment (e.g. biotinidase deficiency).
- (c) For large studies, in which the natural history of a condition before the introduction of screening is compared with that experienced subsequently and where the clinical outcome can be related to the degree of biochemical correction that has been achieved (e.g. phenylketonuria).

Overall sensitivity (%) of screening process

for severe or ‘classical’ disease, assuming 100% coverage.

- I** Data obtained from screening programmes in UK population or similar.
- II** Data from systematic studies other than from whole population screening.
- III** Estimated from the known biochemistry of the condition.

Repeat specimen rate (per 100,000) – this is for dried blood or liquid specimens similar to those taken on days 6–14.

- I** Data obtained from screening programmes in UK population or similar.
- II** Data from systematic studies other than whole population screening.
- III** Estimated from the known biochemistry of the condition.

Follow-up testing if required (nature and number per 100,000). This heading is only used if the investigation required is an integral part of the screening process, otherwise it is not included. Tests required to confirm diagnosis after clinical referral are not included.

- I** Data obtained from screening programmes in UK population or similar.
- II** Data from systematic studies other than whole population screening.
- III** Estimated from the known biochemistry of the condition.

False-positive rate at clinical referral (%). This is the proportion of patients referred at the end on the screening process who turn out to be unaffected and is $(1 - \text{PPV})$ expressed as a percentage.

- I** Data obtained from screening programmes in UK population or similar.
- II** Data from systematic studies other than whole population screening.
- III** Estimated from the known biochemistry of the condition.

Chapter 6

Phenylketonuria and congenital hypothyroidism

Phenylketonuria

Phenylketonuria was first described by Fölling in 1934⁶¹ and is caused by defective hydroxylation of phenylalanine to tyrosine in the liver owing to a deficiency of the enzyme phenylalanine hydroxylase (PAH). This results in accumulation of phenylalanine in blood and tissues. As a consequence, there is excessive production of phenylpyruvic, phenylacetic and phenyllactic acids (phenylketones) in tissues, resulting in high levels in the urine. Phenylketonuria is an autosomal recessive disorder and is genetically very heterogeneous.⁴²

A group of diseases caused by defects in bipterin metabolism were originally referred to collectively as **malignant phenylketonurias**. They may be detected when screening for phenylketonuria.

Prevalence

The average incidence of phenylketonuria (classical and atypical combined) in the UK is 11 cases for 100,000 births (*Table 14*).¹⁷ There is a wide geographical variation, with a particularly high incidence in Ireland of 1:4500.⁶² Phenylketonuria is rare among American blacks and Ashkenazic Jews.^{63a}

Natural history of the untreated condition

The severe form of the disease caused by complete deficiency of PAH is usually referred to as **classical**

TABLE 14 Birth incidence of phenylketonuria in the British Isles^{17,18,62}

	Incidence per 100,000	Mild cases ^a (% of total)
England	10.2	20
Wales	9.9	3.8
Scotland	12.7	13
N. Ireland	24.9	16
UK average	11.0	18
Eire	22	–

^a Phenylalanine below 900 $\mu\text{mol/l}$.

phenylketonuria. If untreated, this usually leads to severe mental handicap associated with seizures and other neurological abnormalities (*Table 15*)^{63b} although a few individuals with the biochemical features of the severe disease only experience mild to moderate handicap. A baby with phenylketonuria usually appears normal for the first few months but by 6–12 months shows delayed mental development, with profound handicap evident by about 2 years.⁶⁴ There is generally no further loss of abilities in adult life.⁶⁵

TABLE 15 Clinical features of untreated classical phenylketonuria*

Finding	Incidence (%)
Agitated behaviour	32–90
EEG abnormalities	80
Muscular hypertonicity	75
Microcephaly	68
Hyperactive reflexes	66
Blond hair, blue eyes	62
Inability to talk	63
Hyperkinesia	50
Inability to walk (and usually incontinent)	35
Tremors	30
Eczema	19–34
Seizures	26

* Data from Knox (1972).⁶⁸

Milder forms of the disease also exist (atypical or benign persistent) with lower plasma phenylalanine concentrations than in untreated classical phenylketonuria.⁶⁶ There may be slight to moderate mental impairment, depending partly on the average plasma phenylalanine concentration.⁶⁷

Treatment and outcome

Treatment in infancy and childhood

In the early 1950s, Bickel and colleagues⁶⁹ demonstrated that dietary phenylalanine

restriction improved behaviour in a 2-year-old girl with phenylketonuria. Dietary therapy using synthetic amino acid mixtures as a substitute for natural protein is now well established as the treatment for phenylketonuria, and the commercially-produced products currently available are much more palatable than those of the early years. Dietary treatment needs careful attention to ensure appropriate vitamins and trace elements are provided and plasma phenylalanine concentrations must be closely monitored. A multidisciplinary team with experience of phenylketonuria treatment is essential for optimal management.⁷⁰

There have been numerous reports on the effectiveness of treatment of phenylketonuria. Early experience was reviewed by Knox,⁶⁸ who compiled data from several studies covering a total of 392 untreated patients with phenylketonuria and 158 patients with phenylketonuria who started treatment at various ages from birth to 2 years. Although some of the late-treated groups showed substantial gains in IQ on treatment, only those starting treatment during the first few weeks of life achieved IQ levels approaching normal. Other studies have confirmed that for good outcome, treatment must be started early in life. Treatment begun after 2 months has a poor outcome;⁷¹ better results are obtained if dietary treatment is commenced before 30 days.⁷²

Because of the dramatic improvement achieved by early treatment, there has never been a controlled trial: attention of later systematic studies focused on the questions of how strict dietary control must be and for how long must it be continued. From US data it is now clear that strict dietary treatment needs to be continued until at least 10 years of age.⁷³ In the UK detailed study has been made possible by the existence of the MRC–DoH Phenylketonuria Register, in which details of all patients with phenylketonuria born in the UK between 1964 and 1980 were collected. The main conclusions from this study are summarised in the box below. Similar findings have been made elsewhere, particularly by the US PKU Collaborative Study,^{74–77} although there are inconsistencies, both within and between studies, over the degree of dietary control that is necessary. A limitation of all these studies is that they have assumed a linear relationship between blood phenylalanine concentration and intellectual impairment, and it is not possible to distinguish between the effects of continuous moderately-increased phenylalanine levels and those of the more occasional extreme excursion.

Although most treated patients with phenylketonuria have IQs within the normal range, the general shift to lower values has significant consequences. In the UK group aged 14–18 years (born 1964–71), 27% individuals had IQs below two standard deviations of the population norm

MRC–DoH Phenylketonuria Register – major findings

Smith *et al*, 1988. *Behaviour disturbance in eight year old children with early treated phenylketonuria*.⁸⁹

- Increase in behavioural problems, particularly in hyperactivity.

Smith, *et al*, 1990. *Intelligence and quality of dietary treatment in phenylketonuria*.⁷⁹

Intelligence at 4 years of age of 808 children with phenylketonuria:

- was, on average, below that of the general population by 0.5 of one standard deviation of the normal population norms for the cohort born 1972–80; the cohort born 1964–71 fared worse
- decreased by 7–10 IQ points for each 4 weeks that treatment was delayed after birth
- decreased by 7–10 IQ points for each 5 months that the plasma phenylalanine concentration fell below 120 $\mu\text{mol/l}$ during the first 2 years of life
- decreased by 7–10 IQ points for each 300 $\mu\text{mol/l}$ increase in time-averaged plasma phenylalanine concentration.

Smith *et al*, 1991. *Effect on intelligence of relaxing the low phenylalanine diet in phenylketonuria*.⁹⁰

Intelligence from 4 to 14 years of age of 559 children with phenylketonuria:

- decreased by 4–6 IQ points for each 300 $\mu\text{mol/l}$ increase in time-averaged plasma phenylalanine concentration between 4 and 8 years of age
- was less clearly affected by phenylalanine concentration after 8 years of age.

Beasley *et al*, 1994. *Outcome of treatment in young adults with phenylketonuria detected by routine neonatal screening between 1964 and 1971*.⁷⁸

- IQ changes between 14 and 18 years of age are not clearly related to degree of phenylalanine control.

Costello *et al*, 1994. *Intelligence in mild atypical phenylketonuria*.⁶⁷

- Milder atypical forms of phenylketonuria are associated with significant intellectual impairment and should be treated.

(against 2% in the control population), 8% had attended schools for children with educational difficulties, and 10% had received remedial education in a normal school.⁷⁸ However, it must be noted that this group began treatment at a time when experience was relatively limited and many of the dietary products available today had not been introduced. Smith and colleagues,⁷⁹ reviewing UK patients born between 1964 and 1980 noted that “fewer than 10% of subjects received the ‘best treatment’ according to the criteria derived from our study”. Recommendations for dietary management have recently been updated by an MRC working party in the light of the Register findings.⁸⁰

Late effects of phenylketonuria

Despite adequate biochemical control during childhood minor neurological deficits have been found in adolescents and adults who have relaxed or discontinued diet.^{81,82} Acute neurological illness has occurred in a few patients.^{83–85} It is unclear to what extent these problems relate to damage sustained during childhood or reflect more recent insults. Some performance-related tasks are executed less well when phenylalanine levels are high.^{86,87} White matter changes can be demonstrated using magnetic resonance imaging (MRI) of the brain in almost all adult patients with phenylketonuria, but are probably related more to increased water content than to demyelination.⁸⁸ Concerns about the possible consequences of discontinuation of dietary treatment has led the several groups, including the recent MRC working group,⁸⁰ to recommend that dietary restriction be continued throughout life. This recommendation has major social and financial implications but is based on as yet incomplete information. In a recent review, Walters⁸⁸ concludes that, while it seems prudent to advise patients to continue on a low phenylalanine diet, the necessity of doing so should not be overemphasised. Until matters become clearer, adult patients with phenylketonuria should be followed-up carefully.

Treatment during pregnancy

There have been many reports of abnormalities in children born to mothers with uncontrolled phenylketonuria. Abnormalities include mental retardation, microcephaly, intrauterine growth delay and congenital heart malformation.⁹¹ There is now good evidence to show that the incidence of such abnormalities can be reduced if the mother has appropriate dietary management before conception and throughout pregnancy.^{92–94} It is very important that maternal phenylketonuria is managed otherwise the advantage of neonatal screening in one generation could be lost in the next.

Other benefits of early diagnosis

Phenylketonuria is generally considered to be a successfully ‘treatable’ disorder. Thus, although DNA-based first trimester prenatal diagnosis is available it is rarely requested.⁵

Neonatal screening

Universal screening for phenylketonuria is provided across the UK (*Table 16*). Methods using measures of blood phenylalanine replaced the urine Phenistix[®] test in the late 1960s.¹⁸

Enzymatic⁹⁹ and tandem MS⁴⁷ methods are available. Both are in use in large-scale programmes abroad but not currently in the UK.

Laboratory performance

A two-stage screening protocol is usually used, requiring a second blood sample from babies showing intermediate phenylalanine concentrations in the first.

- (a) **Specificity** In the original guidance on neonatal screening for phenylketonuria¹⁵ the upper limit for a normal result was set at 6 mg/100 ml (360 $\mu\text{mol/l}$). Subsequently, the MRC–DoH Phenylketonuria Register requested notification of all babies with phenylalanine levels persistently above 240 $\mu\text{mol/l}$ and this became the *de facto* upper limit for a ‘normal’ result. Since

TABLE 16 Screening methods for phenylketonuria in the UK^{18,95}

Method ^a	Number of laboratories		Babies tested (%)	
	1978–79	1991	1978–79	1991
Guthrie microbiological assay ⁹⁶	23	9	56	45
Fluorometry ⁹⁷	4	5	16	19
Chromatography ⁹⁸	12	11	28	36

^a See page 15.

both the Guthrie test and paper or thin-layer chromatography are semi-quantitative methods, the classification of a borderline test result as normal or abnormal tends to be somewhat imprecise and the proportion of initial blood samples classified as abnormal varies greatly from laboratory to laboratory (chapter 18). There are no comprehensive national data on the false-positive rate for second blood samples but the authors' personal experience indicates that the main problem is deciding what level of phenylalanine justifies a diagnosis of non-classical phenylketonuric (?benign) hyperphenylalaninaemia.

- (b) **Sensitivity** Infants with phenylketonuria have near normal concentrations of phenylalanine at birth but, once protein feeding is established, this rises rapidly to give concentrations usually well in excess of the 240 $\mu\text{mol}/\text{l}$ cut-off point. Sensitivity of screening at 6 days or later is high, with few clearly-documented cases of infants with classical phenylketonuria giving a false-negative result at this age.¹⁰⁰

Between 1974 and 1988, ten cases of phenylketonuria out of a total of 3,800,800 are known to have been missed by the UK programme, three because the sample was not taken or was lost, and seven on laboratory testing.¹⁷ Data collected in 1991 for the UK Screening Directors Advisory and Audit Group by Dr A Green (Birmingham) showed that between 1971 and 1990 inclusive, seven cases were missed because of failure to collect a specimen and five because of test failure (laboratory or administration). This compares with data from the French programme,¹⁰¹ which showed that in all cases of missed phenylketonuria (seven up to 1989) technical or human error were at fault: four of the seven were because no screening was performed (i.e. no sample or sample lost in transit). The sensitivity of the laboratory process is thus > 99.5%.

In the USA, early blood sampling (usually before 48 hours of life) is a cause of false-negative screening results,¹⁰² because the child with phenylketonuria has not received sufficient protein feeds to attain abnormal phenylalanine levels. It has been suggested that using a more quantitative assay for phenylalanine such as tandem MS would help to overcome this problem.⁵¹

A comparison of results from several countries is shown in *Table 17*.

Timeliness in the UK programme

An audit of timeliness was performed by Smith and colleagues using Phenylketonuria Register

TABLE 17 Sensitivity and specificity of screening for phenylketonuria: international comparisons

	Sensitivity (%)	Specificity (%)	PPV of first test (%)
The Netherlands ¹⁰³	98	99.99	49.5
Sweden ¹⁰⁴	100	99.98	21.2
France ¹⁰⁵	99.2	99.94	11.5
Wisconsin, USA ¹⁰⁶	100	99.9	6.9 ^a
UK 1979–88 ¹⁷	99.8	99.95 ^{b,d}	20 ^{c,d}

^a 25% for follow-up test; ^b median; range for individual laboratories 98.3–100; ^c median; range for individual laboratories 0.59–100; ^d calculated from data in chapter 18.

data for 1984–88.¹⁷ Only those babies whose initial test results were > 1200 $\mu\text{mol}/\text{l}$, that is, those diagnosed immediately as having classical phenylketonuria, were considered and, for England, the results were grouped by the then-existing NHS Regions. The median age at starting treatment varied from 9.5 days to 17 days, with ranges of 1–17 and 8–47 days, respectively, and the proportion of patients starting treatment after 20 days of age varied between 0% and 27%. The conclusion from this study was that better organisation was required in some Regions.

Differential diagnosis of hyperphenylalaninaemia

Increased blood phenylalanine in the neonatal period can be caused by:

- complete or nearly complete deficiency of PAH (classical phenylketonuria)
- partial deficiency of PAH ('atypical' or 'benign persistent' hyperphenylalaninaemia)
- transient hyperphenylalaninaemia (immaturity of enzyme systems)
- phenylalanine overload associated with some types of total parental nutrition
- liver disease, including galactosaemia (see chapter 8)
- Biopterin defects (see below).

Biopterin defects

Between 1% and 3% of all cases of persistent hyperphenylalaninaemia are due to deficiency of tetrahydrobiopterin caused by inborn errors of biopterin metabolism,⁴² of which there are currently four clearly defined types. Tetrahydrobiopterin is a co-factor for phenylalanine hydroxylation and is also required for the production of important neurotransmitters (dopamine, noradrenaline and

5-hydroxytryptamine). Thus, infants with tetrahydrobiopterin deficiency deteriorate neurologically in spite of dietary phenylalanine restriction, and without specific treatment will usually die in early childhood. Treatment includes dietary restriction of phenylalanine, with the addition of biopterin and/or dopa/5-hydroxytryptophan.

Not all patients with tetrahydrobiopterin deficiency show increased blood phenylalanine concentrations on neonatal screening but the majority can be detected in this way. Diagnosis of biopterin defects requires measurement of biopterin and dihydrobiopteridine reductase activity, both of which can be performed on the neonatal screening sample. This should be standard procedure on all babies with persistently increased blood phenylalanine concentrations.

Data from the MRC–DoH Phenylketonuria Register¹⁷ showed eight diagnoses of biopterin co-factor defects over the period 1974–88. Two of these patients were missed on initial screening, presumably because of low or borderline phenylalanine concentrations. Three of the remaining patients were diagnosed by routine testing for biopterin after a positive initial screening test and the other three after failure to respond to conventional treatment for phenylketonuria.

Overall case for screening

Phenylketonuria was the first disorder for which neonatal screening was introduced. It has been analysed and discussed many times. It fulfils the criteria¹⁰⁷ in that early intervention prevents mental handicap, effective therapy is available and a suitable screening test exists. Cost–benefit analysis has been performed on several programmes (see chapter 13).

Current status

Screening for phenylketonuria is practiced in all developed countries where there is an appreciable incidence. In the UK, analytical performance is monitored as part of the UK External Quality Assessment Scheme for Neonatal Screening. Limited data on cases detected is still being collected by the DoH Phenylketonuria Register, although the MRC no longer has a formal interest.

The data for phenylketonuria are summarised in *Table 18*.

Congenital hypothyroidism

Congenital hypothyroidism is defined as defective function of the thyroid gland from birth. **Primary hypothyroidism** is caused by a defect at the level of the thyroid gland itself, either **thyroid agenesis** (athyreosis, absence of the gland), **ectopic thyroid** (failure to migrate in the embryo with subsequent lack of normal development) or **dyshormonogenesis** caused by an inborn error of one of the several steps in the synthesis of the principal thyroid hormone, thyroxine. Primary congenital hypothyroidism may also arise due to suppression of the foetal thyroid by maternal medication (inorganic iodide or anti-thyroid drugs). **Secondary hypothyroidism** is the result of defects on the hypothalamic-pituitary axis leading to very low levels or complete absence of circulating thyrotropin (thyroid stimulating hormone). The thyroid gland itself is normal.

Formerly, children with severe congenital hypothyroidism were referred to as **cretins**, a corruption of the French word for Christian, on account of their ‘good’ docile behaviour as babies.

TABLE 18 Summary table for phenylketonuria

		Classification*
Expected incidence (cases per 100,000)	10–25	I
Proportion likely to be clinically affected	> 90%	II
Effectiveness of treatment	High	II-2
Overall sensitivity of screening process	> 98%	I
Repeat specimen rate (per 100,000)	See chapter 17	I
False-positive rate at clinical referral	< 1%	I

* see page 24.

Prevalence

Data collected over a period of 3 years by the MRC Register of Children with Congenital Hypothyroidism, covering England, Wales and Northern Ireland, showed an overall incidence of persistent primary hypothyroidism of 1 in 3937 births.¹⁹ This is similar to the incidences reported from other European countries and the USA.¹⁰⁸ As found in the USA,¹⁰⁹ the incidence in black children in the UK is relatively low but in children of Asiatic mothers (i.e. of Indian, Pakistani or Bangladeshi origin) it may possibly be higher.¹⁹ Overall, the ratio of boys to girls was 1:2.3.¹⁹

Secondary hypothyroidism is much less common, with incidences ranging from 1 in 135,000 births to 1 in 25,000 births reported in systematic studies.^{110–114} There are no reliable UK data.

Natural history of the untreated condition

The developing foetus synthesises substantial amounts of thyroxine from about 20 weeks gestation and transplacental passage of maternal hormone is insufficient to maintain adequate levels later in the pregnancy. Thus babies with congenital hypothyroidism are likely to be clinically affected at birth, although to a very variable degree. A severely-affected baby may show characteristic coarse facies and have a low hair line, enlarged protruding tongue, cold mottled skin, umbilical hernia, large posterior fontanelle, persistent jaundice, feeding difficulties, constipation and generally sluggish behaviour. However, this full-blown picture is relatively rare at birth and the majority of cases are not recognised clinically in the neonatal period.^{110,115,116} If the condition is left untreated, growth will be severely retarded and there will be mild to severe (IQ < 40) mental retardation. There are also neurological abnormalities in the more severe cases, with spasticity, lack of coordination, jerky movement and tremors.¹¹⁷ In general, patients with thyroid agenesis are the most severely affected.¹¹⁸

Secondary hypothyroidism often presents with additional features (hypoglycaemia, diabetes insipidus, microgenitalia, midface deformities) that lead to clinical diagnosis soon after birth although some children present later in infancy with hypothyroidism as the main feature.

Treatment and outcome

For primary hypothyroidism, treatment with oral thyroxine begun in infancy will lead to resumption of growth and eventually result in normal stature. The delay in mental development is only partially correctable and the longer the delay in starting

treatment, the worse the eventual outcome.^{117,118} Treatment for secondary hypothyroidism requires replacement of other affected hormones as well as thyroxine.

Since the advent of neonatal screening much detailed information on outcome in early-treated cases of primary hypothyroidism has become available. The UK series of patients born between 1982 and 1984 and diagnosed by neonatal screening has been extensively reported.^{119–124} Growth is essentially normal, although head circumference remained slightly increased.¹²¹ At the age of 10 years, children with low plasma thyroxine levels (< 40 nmol/l) before treatment had, as a group, an IQ deficit of 10 points, whereas those with thyroxine levels > 40 nmol/l had normal IQs.^{123,124} These findings have largely been supported by major studies in other countries,^{125–132} with additional reports of subtle speech and motor deficits,^{133,134} and persistent mild hearing loss.¹¹⁹ It is apparent that the prenatal effects of hypothyroidism, most severe in complete agenesis, are to some extent irreversible. A recent study in the USA found that children with hearing impairment (20% of the total) had started treatment on average at 22 days of age, compared with 14 days for the remainder.¹³⁵

Other benefits of early diagnosis

The majority of cases of congenital hypothyroidism are sporadic with a low risk of recurrence in subsequent pregnancies. The dysmorphogeneses are usually inherited as Mendelian recessive traits. Prenatal diagnosis is not generally undertaken.

Neonatal screening

Methods

Screening based on radioimmunoassay of thyroxine in cord blood or a later heel-prick dried blood specimen was introduced in Canada and much of the USA in the late 1970s.¹¹⁰ This detected both primary and secondary hypothyroidism but specificity was extremely low, which meant that up to 20% of babies had to be re-tested by assay of thyrotropin.

In Europe screening was introduced later¹³⁶ and mostly used thyrotropin, thus limiting detection to primary hypothyroidism. A variety of immunoassay methods are available for thyrotropin in dried blood spots and most have adequate analytical performance. The possibility of combining screening for congenital hypothyroidism with other screens is referred to in chapter 4.

A generalised flow diagram of neonatal screening using thyrotropin is presented in *Figure 4*. Most

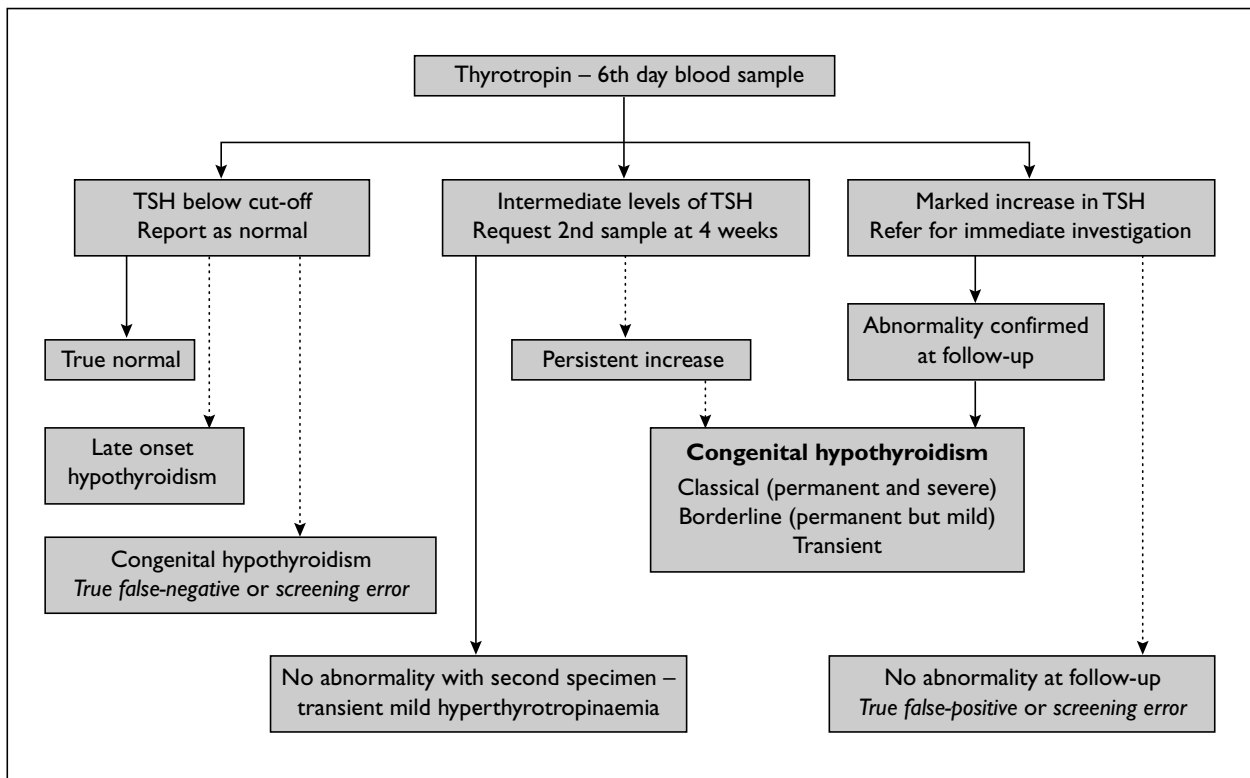


FIGURE 4 Screening for congenital primary hypothyroidism (dotted lines represent minor flows)

laboratories distinguish three categories of result from the initial screening: normal, intermediate or equivocal, and clearly abnormal. In general, a second blood sample from babies with intermediate results is requested but those with a marked increase in thyrotropin (usually confirmed on a second spot from the initial Guthrie card) are referred immediately for clinical examination and further diagnostic tests.

Performance

Much of the earlier literature on screening performance, particularly from the USA, is based on thyroxine–thyrotropin two-stage screens or thyroxine–thyrotropin simultaneous screens, rather than the single-stage thyrotropin screen used in most of the UK. The two-stage screen gives rise to a number of additional problems;¹³⁷ these are not discussed further here.

Hypothyroidism is a very variable condition, both in severity and over time, and this causes difficulties in applying the usual concepts of false-positive and false-negative when evaluating screening performance. Four aspects have caused particular problems.

(a) **Clinically undetected cases** The incidence of hypothyroidism diagnosed through neonatal screening is approximately double that

previously experienced from clinical ascertainment.¹³⁸ It might be argued that the additional cases diagnosed by screening would have developed normally without any treatment and should be considered as false-positives. Evidence to the contrary comes from a Swedish study, in which dried blood spots that had been stored for 5 years were screened retrospectively.¹¹⁵ Of 31 babies who had increased thyrotropin in the 5-day blood sample and were followed-up, 15 had been diagnosed clinically (median age 5 months) and seven were still biochemically hypothyroidic but undiagnosed and not on treatment by 5 years of age. Some of them might later have presented with juvenile hypothyroidism.¹⁹ Of these seven patients, six were given Griffith tests and had low scores ($p < 0.05$ compared to normal individuals) for motor and performance. Thus, in this series, 71% of those who would have been labelled positive by neonatal screening had a persistent and clinically-significant disorder.

(b) **Transient hypothyroidism** A proportion of cases detected by neonatal screening are transient. Of the 31 Swedish cases referred to above, nine (29%) were euthyroid (biochemically normal) on follow-up at 5 years of age.¹¹⁵ In the 422 infants entered on the MRC Register, 29 transient cases were detected.¹⁹ In another programme, a significant number

of cases of transient hypothyroidism resulting from postnatal exposure to iodine in anti-septics have been detected.¹³⁹ In the absence of any systematic collection of long-term follow-up data, it is unclear to what extent this is currently a problem in the UK.

- (c) **Late developing cases** Some cases of infantile hypothyroidism develop only after the neonatal screening sample has been taken. Data from the Northwest Regional Screening Program in the USA, where the first sample is usually taken within 48 hours of birth and a second sample at 6 weeks of age, indicate that a second thyrotropin screen at this age could contribute a further 5% of cases to those detected on the first-sample thyrotropin-based screen.¹¹² This proportion is probably higher than would be found in the UK, where the sample is taken at 6 days or later. False-negative results due to normal thyrotropin levels in the neonatal period have also been reported in some cases of dyshormonogenesis.^{19,139,140} In monozygotic twins, the hypothyroidism of one twin will be compensated *in utero* by the other, leading to the diagnosis being missed in the neonatal period.^{141,142} A preliminary report suggests that late-developing hypothyroidism may also be frequent in babies with very low birth-weight.¹⁴³
- (d) **Borderline hypothyroidism** The recognition that a significant proportion of infants with only moderately-increased thyrotropin on initial screening will not have a clinically-significant condition has led Holtzman¹⁴⁴ to urge caution in following-up babies with very marginal increases. The trend in Japan is, however, to pay increasing attention to marginally-increased thyrotropin results, which are claimed to indicate mild but persistent thyroid disorder.¹⁴⁵ The clinical significance of such mild abnormalities is currently unclear.

Laboratory performance

Given the biological variability of congenital hypothyroidism, with transient and late-developing cases and a wide variation in severity, it is perhaps unrealistic to try to define performance formally in terms of specificity and sensitivity. However, data from missed cases give some indication of the effectiveness of the programme. The French National Screening Association has been collecting data for France since the inception of hypothyroidism screening.¹⁴⁶ During the period 1978–87, 50 cases (3% of total) were ‘missed’, corresponding to one false-negative case per 118,000 tests. Of these, 23 were most probably true false-negatives, classified as biochemically normal on screening and,

in several of these, it was possible to confirm the original normal result using stored sample. A total of 15 cases were missed because of failures in the collection or transmission of specimens to the laboratory and 12 because of errors in the screening laboratory.

No comparable published data exist for the UK. The MRC Register of Congenital Hypothyroidism reported four missed cases (0.8%) for the period 1982–84, of whom two were genuine false-negatives with dyshormonogenesis.¹⁹ A further three cases with normal thyrotropin levels on screening were reported by Grant and colleagues¹⁴⁷ and one was reported by Pharoah and Madden.²⁴ Other late-developing cases are known to the authors. More recently, data has been collected by Dr A Green for the UK Screening Laboratory Directors Advisory and Audit Group. These show that between 1982 and 1991, four cases were missed because of laboratory error. Eight cases are known to have been missed owing to failure to collect a sample or to loss of sample in transmission.

The specificity of the initial test varies from one screening laboratory to another, even when the same assay is used (see chapter 17). A retest rate of 0.084% was reported from Merseyside,²⁴ corresponding to a specificity of 99.9%.

Timeliness

The British Paediatric Association has suggested a series of outcome measures for child health.¹⁴⁸ One is, “The ages at which all children with congenital hypothyroidism begin their treatment. Failure to start treatment before age one month should be treated as a sentinel event.” Performance to this standard was to be monitored at district level within the NHS; however, no comprehensive recent data have yet been published. In 1982–84, the median age at referral of babies with clear congenital hypothyroidism in the UK was 17 days, with 5th and 95th percentiles of 10 and 43 days.¹⁹ Regional performance varied from a median of 14 (10–58) days to 30 (18–116) days. Slightly more recent data (1983–88) from Merseyside showed 90% on treatment within 21 days, the latest treatment being started at 26 days after birth.²⁴

Overall case for screening

Despite the interpretational problems outlined above, congenital hypothyroidism fulfils the criteria for screening and, in practice, the programme works well. The resource implications under UK conditions have been considered by Smith and Morris,¹⁴⁹ and their assessment was updated by the

Joint Standing Sub-Committee on Screening in Medical Care in 1980.¹⁶ Further details are given in chapter 14.

Current status

In the UK, screening for hypothyroidism using the single-stage thyrotropin strategy was officially recommended by the Department of Health in 1981.¹⁶ The status of the UK programme, for the period 1982–84 was reviewed by Grant and colleagues.¹⁹ Currently, only the East Anglian region, which had been screening for some time prior to 1981, uses the thyroxine–thyrotropin two-stage protocol. Analytical performance of all NHS

screening laboratories is monitored as part of the UK External Quality Assessment Scheme (EQAS), there are annual European circulations and a number of laboratories participate in other overseas schemes.

Elsewhere, screening for congenital hypothyroidism is practiced throughout the developed world and is increasingly being adopted as a cost-effective way of reducing the burden of mental subnormality in developing countries.

A summary of the data for congenital hypothyroidism is given in *Table 19*.

TABLE 19 Summary table for congenital hypothyroidism

		Classification*
Expected incidence (cases per 100,000)	25	I
Proportion likely to be clinically affected	70%	I
Effectiveness of treatment	High	II-2
Overall sensitivity of screening process	> 95%	I
Repeat specimen rate (per 100,000)	See chapter 17	I
False-positive rate at clinical referral	< 1% †	I and II
* see page 24.		
† Approximately 30% of cases may be transient.		

Chapter 7

Other disorders of amino acid metabolism

Tyrosinaemia type I

Tyrosinaemia type I is caused by a deficiency of fumarylacetoacetase, the last specific enzyme in tyrosine degradation, cleaving fumarylacetoacetate to fumaric and acetoacetic acids.¹⁵⁰ Fumarylacetoacetase occurs mainly in the liver and kidneys,¹⁵¹ and the liver and kidney disease characteristic of this disorder is thought to be caused the accumulation of toxic metabolites, fumarylacetoacetate and maleyl-acetoacetate. These primary metabolites are converted to succinylacetoacetate and then to succinylacetone which is excreted in large amounts in urine. The excretion of succinylacetone, together with deficient fumarylacetoacetase activity in liver tissue and/or skin fibroblasts, forms the basis of diagnosis. Tyrosinaemia types II and III are rare and are not covered in this review.

Prevalence

Tyrosinaemia type I occurs world-wide, but the frequency is variable (*Table 20*). In the Saguenay-Lac-St-Jean region of northeastern Quebec, Canada, it is particularly common with an incidence of 1:1846.¹⁵² In most other countries the frequency is closer to 1 in 100,000.¹⁵⁰

TABLE 20 Incidence of tyrosinaemia type I

Country	Incidence (per 100,000)
Quebec, Canada ¹⁵²	54
Scandinavia ¹⁵³	1.0
Norway ¹⁵⁴	2.0
UK: Birmingham City ¹⁵⁵	4.7
West Midlands ¹⁵⁵	0.95

Natural history of the untreated condition

Patients with tyrosinaemia type I may present as liver failure in infancy (acute form) or show a more protracted course resulting in hepatocellular carcinoma (subacute and chronic forms). Both are progressive and, unless treated, invariably fatal.

Acute forms

The majority of children with tyrosinaemia type I (77%) fall into this category.¹⁵⁶ Symptoms appear

during the first weeks or months of life, with vomiting and diarrhoea, failure to thrive and lethargy. There are signs of severe liver disease with hypoproteinaemia and hyperbilirubinaemia. Defective coagulation capacity and hypoglycaemia may be significant features. Serum tyrosine and methionine concentrations are elevated, as is α -fetoprotein. Tyrosine metabolites are excreted in urine in large amounts. There may be signs of hypophosphataemic rickets. Death usually occurs from liver failure within a few months.¹⁵⁴

Neurological crises similar to those seen in acute porphyria can occur in up to 50% of cases and may be the presenting feature.^{157,158} They are thought to be due to inhibition of heme synthesis at the porphobilinogen synthetase level by succinylacetone and possibly by fumarylacetoacetate. Biochemically, these crises are accompanied by a rise in serum δ -amino-laevulinic acid.^{157,159,160} Mortality rates of up to 70%, resulting from respiratory paralysis, have been reported.¹⁵⁷

Chronic form

This form is milder, with patients presenting with hepatomegaly and/or rickets either early in childhood or at school age. Characteristic features are chronic liver disease, renal tubular dysfunction and hypophosphataemia with vitamin D-resistant rickets. Death occurs from liver failure or hepatoma usually during the first decade. Most hepatomas occur in children over 2 years of age.¹⁶¹

Treatment and outcome

Dietary restriction

For many years a low tyrosine, low phenylalanine (and often low methionine) diet, intended to reduce fumarylacetoacetate and succinylacetone levels was the only treatment for tyrosinaemia type I.¹⁶² Dietary restriction ameliorates the renal tubular defects and may be life-saving in the acute phase, but does not prevent progressive liver damage and development of hepatoma.¹⁶³ An international survey of patients with tyrosinaemia type I examined the probability of survival on dietary treatment and the causes of death in 108 patients.¹⁵⁶ Liver failure and recurrent bleeding (67%), hepatocellular carcinoma (17%), and the porphyria-like syndrome with respiratory failure (10%) were the most common causes of

death. Study of survival probability after the onset of symptoms showed that survival varied with the age at presentation; the earlier the symptoms developed, the poorer the outlook. Onset of symptoms before 2 months of age was associated with a 1-year survival probability of 38%. The corresponding figures for presentation at 2–6 months and after 6 months were 74% and 96%, respectively. Thus, although dietary treatment can slow the progression of the disease it does not prevent it.

Liver transplantation

More recently, liver transplantation before malignancy develops has proved to be a successful treatment for tyrosinaemia type I.^{164–167} It has the potential to cure the hepatic and neurological components of the disease,^{158,159,168} and averts the risk of hepatic malignancy.

Since the transplant retains the genotype of the donor and produces normal fumarylacetoacetase, there is no further need for dietary restriction. Children with metabolic disease generally show better survival after transplant than patients in whom the transplant was for liver failure from other causes. One-year survival rates of 95% have been reported, with a 4-year survival rate of 88%.¹⁶⁹

Although liver transplantation corrects the liver disease, the genetic defect is retained in the kidneys, and toxic metabolites continue to be produced.^{154,168} Although liver transplantation may stabilise the progression of renal disease,^{167,170,171} the possibility of deterioration of kidney function post transplant is still of concern.^{158,172} Frequent monitoring of renal function in all patients and combined liver–kidney transplantation in children with deteriorating renal disease has been suggested.¹⁵⁸

Liver transplantation should be performed as early as possible to avoid the development of hepatocarcinoma or irreversible extra-hepatic disease and the high morbidity and mortality resulting from neurological crises that can occur in the first months of life.¹⁵⁷ Good survival rates for very young patients with metabolic disease have been reported.¹⁷³ The successful development of reduction hepatectomy extends the age and size range of transplantation to very young babies with comparable results to whole graft transplantation.¹⁷⁴

While the effect of liver transplantation on disease progression is striking, there are negative aspects: lifelong immuno-suppressive therapy is required with the inevitable side-effects, including nephrotoxicity, increase in viral infection with cytomegalovirus and

Epstein–Barr virus, and the potential development of lymphoproliferative disease.^{167,174,175}

NTBC treatment

The second enzyme of tyrosine metabolism, 4-hydroxyphenylpyruvate dioxygenase, is inhibited by 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), which thus prevents the formation of the toxic metabolites in tyrosinaemia type I.¹⁷⁶ Oral administration of NTBC leads to normalisation of liver and renal functions, and prevents neurological crises.^{176,177} Successful treatment with NTBC in two patients with life-threatening neurological crises not amenable to tyrosine and phenylalanine restricted diets have been reported,^{178,179} and it has been used successfully in at least four infants with acute liver failure.^{176,180,181} The 1-year success rate (improvement in biochemical parameters) in 15 children in whom NTBC therapy was started early (2–4.5 months) was 87%.¹⁷⁷ However, NTBC offers no cure in patients with hepatoma.

The long-term outlook is still uncertain, as it is not known whether NTBC will prevent or merely delay the onset of malignancy and whether there are any long-term adverse effects of the drug itself. Dietary restriction is usually continued in order to avoid the long-term ocular complications of hypertyrosinaemia as seen in tyrosinaemia type II. Currently, about 120 patients worldwide are being treated with NTBC in a multicentre trial.

NTBC versus liver transplantation

Patients with severe acute liver failure who do not respond to NTBC should be considered for urgent transplantation (*Table 21*). Patients who have already established hepatocellular carcinomas at diagnosis are also candidates for liver transplantation. The cost of a liver transplant is approximately £50,000, with £4000–£5000 required annually thereafter for follow-up treatment. The cost of NTBC treatment may range from £10,000–£40,000 per year depending on individual dose requirements, with a further £5000–£10,000 per year spent on dietary management. (PJ McKiernan, Birmingham: personal communication, 1996.)

Other benefits of early diagnosis

Despite the advances in treatment, tyrosinaemia type I remains a very unpleasant disease. Prenatal diagnosis is possible by determination of succinylacetone in cultured amniotic fluid supernatant¹⁸² and by assay of fumarylacetoacetate hydrolase in cultured amniotic fluid cells or in a chorionic villus sample.¹⁸³ Earlier diagnosis, particularly in the milder late-onset cases, will increase the number

TABLE 21 NTBC versus liver transplantation – the current position

	Transplantation	NTBC
Long-term diet	No	Yes
Effective in severe liver disease	Yes	Yes
Effective in patients with hepatoma	Yes	No
Long-term increased risk of hepatoma	No	?
Long-term adverse effects	Yes	?

of families able to make appropriate decisions about future child-bearing.

Neonatal screening

Screening for tyrosinaemia type I has been undertaken in Quebec, Canada, since 1971 and there is substantial early experience from other centres.¹⁸⁴ These programmes were based on detection of increased tyrosine concentrations in blood. This is a secondary abnormality resulting from the liver damage and screening had a high false-positive and false-negative rate, particularly before the introduction of lower-protein formula feeds for infants in the mid-1970s. Transitory tyrosinaemia of the neonate caused by immaturity of liver enzymes is relatively common, particularly in infants of low birth weight.

Following the discovery of the basic defect in tyrosinaemia type I, alternative approaches based on the measurement of succinylacetone became available. These rely on the inhibitory effect of succinylacetone on porphobilinogen synthase (δ -aminolevulinic acid dehydratase). In the original method, the eluted blood sample was boiled to complete the conversion of succinylacetoacetate to succinylacetone, and the porphobilinogen synthase was then supplied in the form of haemolysed normal erythrocytes.^{185,186} Introduction of this assay into the neonatal screening programme as a secondary test reduced the rate of false-positives to 0%. A simpler variant dispenses with the heat treatment and uses the porphobilinogen synthase in the original blood sample.¹⁸⁷ It was reported as a 'sensitive, specific and cheap' method. However, when evaluated in the South-East Thames Region, this method was found to be unsuitable, because the limited stability of porphobilinogen synthase in blood spots resulted in a high false-positive rate.¹⁸⁸

An alternative screening approach developed in Quebec is based on determination of fumarylacetoacetase immunoreactive material

in dried blood spots as the primary screen.¹⁸⁶ However, this is a genetically homogeneous population, in which the common tyrosinaemia type I mutation results in complete absence of immunologically-detectable enzyme protein,¹⁸⁹ and this approach is not appropriate in other areas with more mixed populations.

At present all screening programmes for tyrosinaemia type I use tyrosine as the initial indicator.¹⁵⁰ Hypertyrosinaemia can be detected by existing neonatal screening programmes for phenylketonuria which use chromatographic methods. There is also a microbiological (Guthrie) assay and a more quantitative continuous flow fluorometric assay. Tyrosine will also be measured accurately in tandem MS-based screening programmes. In dried blood spots, this method showed a recovery of 93% and a precision (standard deviation) of 6% at concentration range of approximately 300 $\mu\text{mol/l}$, and would thus be suitable as the primary screen.^{47,51}

A recent retrospective study¹⁵⁵ reported the detection of tyrosinaemia type I in the City of Birmingham as an additional product of the phenylketonuria screening programme. This programme uses plasma amino acid chromatography¹⁹⁰ as the first-line test: chromatograms were assessed visually and no estimate of the tyrosine concentration at the cut-off level was given. The initial positive rate for increased tyrosine in the first blood sample (6–10 days) was 0.31%. On repeat testing at 6 weeks of age, no false-positives were found (diagnostic specificity, 100%). Overall diagnostic sensitivity was 71%, one of the two missed cases having had normal plasma tyrosine at the initial screen. The disadvantages of this two-stage tyrosine approach are the relatively late diagnosis and a high repeat sample requirement combined with the very low PPV (0.32%) of the initial positive result. A second-stage screen using the initial blood sample removes the main disadvantage of a low initial PPV and would allow a lower initial cut-off, with an increase in sensitivity. The Quebec programme currently uses an initial cut-off of 248 $\mu\text{mol/l}$ which, with an average age at screening of 4 days, produces an initial positive rate of 2.4%. No false-negative results have been obtained at that cut-off level but this level of sensitivity would not necessarily be obtained in populations in which other types of mutation predominated.

Overall case for screening

The long-term outlook for patients with tyrosinaemia type I is improving. Whichever treatment route is ultimately taken, early diagnosis through neonatal screening followed by treatment with

NTBC will preserve hepatic and renal function and prevent porphyria-like attacks.

Current status

A few of the screening programmes in the UK can detect tyrosinaemia either with normal amino acid chromatography (34% of births) or with a more specific method (3%).³² The only indication of performance under these conditions comes in the recent report from Birmingham.¹⁵⁵ Several overseas programmes include tyrosinaemia type I screening, but with varying levels of success.¹⁵⁰

A summary of the data for tyrosinaemia type I is given in *Table 22*.

Homocystinuria (cystathionine β -synthase deficiency)

The most common cause of homocystinuria is a deficiency of cystathionine β -synthase activity, which is essential for the conversion of methionine to cysteine and the eventual conversion of the sulphur in dietary methionine to inorganic sulphate. Biochemically, this defect results in elevated levels of homocystine, methionine, and cysteine–homocysteine disulphide in blood and/or urine.¹⁹¹

Homocystine is rapidly cleared by the kidneys and is, therefore, most easily detected in urine; this gives the disease its name. There are several other rare metabolic disorders that can also cause homocystinuria¹⁹¹ but these are not discussed in this review.

Cystathionine β -synthase deficiency is inherited as an autosomal recessive condition.

Prevalence

The only data on the prevalence of cystathionine β -synthase deficiency come from neonatal screening (*Table 23*). These figures are underestimates, since the pyridoxine-responsive type is not often detected using current methods of neonatal screening (see below).

Natural history of the untreated condition

Patients appear normal at birth but progressively develop a variety of clinical and pathological abnormalities involving the eye, central nervous system, skeletal and vascular systems (*Table 24*). Ectopia lentis (dislocation of the lens) affected 97% of all patients by 38 years of age. The most common abnormality of the central nervous system is mental retardation, presenting as developmental delay in the first and

TABLE 22 Summary table for tyrosinaemia type I

		Classification*
Expected incidence (cases per 100,000)	I (to 5 in places)	Ia
Proportion likely to be clinically affected	100	III
Effectiveness of treatment	Medium	III
Overall sensitivity of screening process	> 90%	I
Repeat specimen rate (per 100,000)	> 2 †	Ia
False-positive rate at clinical referral	< 5%	III
* see page 24.		
† Using a second-tier test for succinylacetone on the initial blood spot.		

TABLE 23 Cystathionine β -synthase detected by screening in the British Isles

Place	Number screened	Number detected	Incidence
NW Health Region, England	1,257,179	10	1:126,000
Scotland	1,012,500	1	1:1,000,000
Northern Ireland	549,219	7	1:78,000
Republic of Ireland	1,341,272	23	1:58,000
Data collected by Mudd et al ¹⁹²			

TABLE 24 Major clinical abnormalities in untreated cystathionine β -synthase deficiency

System /organ	Frequent	Less frequent
Eye	Ectopia lentis Myopia	Glaucoma Optic atrophy Retinal degeneration Retinal detachment Corneal abnormalities, cataracts
Skeletal system	Osteoporosis Biconcave vertebrae Scoliosis Elongated long bones Pes cavus High arched palate Abnormal epiphyses and metaphyses	Arachnodactyly Pectus excavatum or carinatum Genu valgum Kyphosis
Central nervous system	Mental retardation Psychiatric disturbances	Seizures, abnormal EEG Extrapyramidal signs
Vascular system	Vascular occlusions Malar flush Livido reticularis	

After Mudd et al¹⁹¹

second years of life. Behavioural problems are common. Thinning and lengthening of the long bones results in a marfanoid appearance by late childhood. Serious complications of thromboembolism have included optic atrophy secondary to occlusion of the optic artery, hemiparesis, cor pulmonale secondary to pulmonary artery occlusions, severe hypertension due to renal infarcts, seizures or focal neurological signs due to cerebral thrombosis.¹⁹¹

Approximately half of all patients respond to treatment with pyridoxine (see treatment section) and these patients usually have a milder or more slowly developing disease than patients who do not respond, even in the untreated state (Table 25).

Treatment and outcome

Patients can be classified according to whether their biochemical abnormalities are corrected by administration of vitamin B6 (pyridoxine), a precursor for pyridoxal phosphate, the co-factor of cystathionine β -synthase. In 529 cases, there were equal numbers (43.7% each) of pyridoxine-responsive and unresponsive types, with 12.7% intermediate (partial) response.¹⁹¹

Patients responding to pyridoxine

Treatment requires large doses of pyridoxine. Doses ranging from 250–1200 mg/day have been used.^{193,194} Pyridoxine treatment in responsive patients prevents thromboembolic events and may reduce the frequency of lens dislocation.¹⁹¹ Improvement in behaviour and IQ has been

TABLE 25 Clinical progression and pyridoxine responsiveness in untreated cystathionine β -synthase deficiency

	Responders	Non-responders
50% have ectopia lentis	by 10 years of age	by 5 years of age
IQ (median score)	78	56
Spinal osteoporosis at 12 years	25%	50%
25% have thromboembolism	by 20 years of age	by 15 years of age

After Mudd et al¹⁹¹

reported in late-treated responsive patients.¹⁹⁵ Folic acid depletion has been noted in a number of patients who are cystathionine β -synthase deficient,^{196,197} and therapy with folate in combination with vitamin B12 and pyridoxine may be required.

Side-effects of excessive pyridoxine dosage are few: sensory neuropathy with ataxia has been reported in otherwise normal adults ingesting large amounts of pyridoxine (> 1000 mg/day);^{198,199} however, 17 patients with homocystinuria receiving 200–600 mg/day showed no neurological disturbance.²⁰⁰

Patients not responding to pyridoxine

Patients not responsive to pyridoxine are treated by a diet restricted in methionine, with cystine supplementation. This requires the replacement

of most of the of natural dietary protein by an artificial amino acid mixture and, like the diet for phenylketonuria, is very restrictive in the types of food allowed.

Betaine

Methyl donors such as betaine reduce homocysteine concentrations by promoting remethylation to methionine. It is a useful adjunct to treatment of patients who are both pyridoxine-responsive^{201–203} and pyridoxine-non-responsive.²⁰⁴ Addition of betaine often makes it possible to relax protein restriction considerably while maintaining the same homocysteine concentrations in plasma.

Response to treatment

The response to treatment varies both with the severity of the biochemical lesion (particularly whether pyridoxine-responsive or non-responsive) and the age at which treatment started. The risk of complications can be substantially reduced if patients are started on treatment within 6 weeks of birth.²⁰⁵ Of 19 patients in one study, 14 started treatment with dietary restriction and vitamin supplementation in the newborn period, and none had developed ectopia lentis after a mean follow-up of 8.2 years, compared with a 70% lens dislocation rate in untreated patients with similar follow-up. Ectopia lentis developed and progressed in two patients treated late despite tight biochemical control.²⁰⁶ However, other studies have shown considerable benefit from treatment, even when instituted late: seizures are prevented, and there is significant improvement in IQ and in the incidence of thromboembolic events.^{203,204}

Complete control of homocysteine levels often proves difficult in older children because of the unpalatable and restrictive nature of the diet. Betaine is a great help in this respect. There are insufficient data to establish the true incidence of long-term complications using betaine, although it has been concluded that thromboembolism is uncommon, even in late-treated patients.²⁰⁷ A combination of betaine and low protein diet also prevents lens dislocation if started early. (JV Leonard: unpublished observations.)

Neonatal screening

Newborn screening for homocystinuria is usually based on detection of elevated methionine using a specific Guthrie bacterial inhibition assay²⁰⁸ or chromatography, with or without special staining. This mostly appears to detect those patients not responding to pyridoxine. In Manchester, all ten infants and, in New England, USA, all 12 patients who were identified on the basis of early

hypermethioninaemia were pyridoxine-unresponsive.¹⁹² The sole infant identified through screening in New England who was pyridoxine-responsive had a normal blood methionine concentration at 4 days of age but an increased concentration in a sample collected at 4 weeks. In an international survey,²⁰⁹ only 13% of the 55 patients detected by neonatal screening, were pyridoxine-responsive, with 78% non-responsive and 9% showing an intermediate response. These figures differed significantly from the data obtained for clinically-diagnosed patients. The pyridoxine-responsive form is being missed because of a slower rise and lower levels in blood methionine concentration in this form of disease.

At most centres, a Guthrie microbiological test for hypermethioninaemia is considered positive when the methionine level is ≥ 20 mg/l. Lowering the cut-off limit for positives has been suggested as a means of detecting more affected infants.²¹⁰ In New England, USA, using a methionine cut-off level of ≥ 20 mg/l, the frequency of homocystinuria detected by screening was 1:250,000. However, at the lower cut-off level of ≥ 10 mg/l adopted in 1988, the frequency rose to 1:130,000. Despite this lower cut-off level, the false-positive rate decreased from 0.02% to 0.007%. This possibly reflects changes in current practices of infant feeding, which have led progressively to lower protein intake.

A tandem MS method for the analysis of methionine has been reported.⁵⁰ Optimal results were obtained when the concentration of methionine was expressed relative to that of another amino acid in the same sample. The authors predict that the ratio of methionine:(leucine + isoleucine) determined by tandem MS will successfully detect the hypermethioninaemia associated with cystathionine β -synthase deficiency with very low rates of false-positives and false-negatives. In samples from neonates previously categorised by the Guthrie microbiological assay as true-positive, unaffected, or false-positive, tandem MS correctly identified all affected cases and reduced the false-positive rate to nil. Fewer false-negative samples would be expected due to the higher sensitivity of this method, but they are unlikely to be eliminated, because blood methionine concentration may not be elevated during the first few days of life.¹⁹¹

Current status

In 22 states in the USA, in most European countries and in Japan, screening is undertaken for homocystinuria by measuring blood spot methionine concentration. Blood spot screening

for homocystinuria was stopped in New Zealand in 1986 because its ability to detect the disease was very poor.²¹¹ In the UK, only two laboratories test specifically for hypermethioninaemia/homocystinuria,³² although centres which screen for phenylketonuria by chromatography may sometimes detect hypermethioninaemia.

Overall case for screening

Cystathionine β -synthase deficiency is a serious disease and is clinically silent until childhood, by which time the complications are largely irreversible. Even when symptoms are obvious, diagnosis may be delayed for many years.²¹² Treatment will either prevent or greatly slow the progression of the disease.

At present, neonatal screening misses most of the milder cases but more sensitive and specific methods for the detection of hypermethioninaemia by tandem MS are likely to increase the sensitivity of screening. Persistent hypermethioninaemia in the absence of homocystinuria or severe liver disease is very rare so that the false-positive rate with tandem MS is likely to be low.

The data for cystathionine β -synthase deficiency is summarised in *Table 26*.

Maple syrup urine disease

First described by Menkes in 1954,²¹³ maple syrup urine disease is caused by an inherited deficiency of the branched-chain 2-keto acid dehydrogenase, resulting in a marked increase in plasma and urinary concentration of branched-chain ketoacids and the corresponding branched-chain amino-acids, leucine, isoleucine and valine. The disorder is inherited in an autosomal recessive mode. The

characteristic smell of maple syrup is caused by accumulation of isoleucine metabolites.²¹⁴

Prevalence

In a review of over 9 million neonatal screening tests performed worldwide over a 12-year period, the combined incidence of classical and intermediate forms of disease was found to be 1:224000 births.²¹⁵ In the UK, there are small areas with higher incidence in immigrant populations with a high rate of consanguineous marriage.

Natural history of the untreated condition

There are four major variants of maple syrup urine disease – the severe (classical), intermediate, intermittent and thiamine-responsive forms. The classical disease is associated with very low levels of branched-chain 2-keto acid dehydrogenase activity (< 2% of normal). Patients appear normal at birth, with symptoms of lethargy and poor sucking developing at 4–7 days, followed by weight loss and neurological symptoms by 7–10 days. Progression of the disease is then rapid, with increasing lethargy, convulsions and irregular respiration. Severe ketoacidosis may lead rapidly to coma and death. Surviving infants who are not treated from an early age suffer from neurological damage including mental retardation, spasticity and blindness.

In the intermediate and intermittent forms, residual enzyme activity is higher – of the order of 3–30% of normal.²¹⁶ Onset of symptoms is generally delayed and less severe, and may be intermittent, arising during catabolic stress such as infection or a high intake of branched-chain amino acids as protein. A thiamine-responsive form²¹⁷ has been described but appears to be extremely rare, and is clinically less severe than the thiamine non-responsive forms.

TABLE 26 Summary table for cystathionine β -synthase deficiency

		Classification*
Expected incidence (cases per 100,000)	1.5	I ^a
Proportion likely to be clinically affected	> 99%	II
Effectiveness of treatment	Medium to high	II-2
Overall sensitivity of screening process	50% ^a	I
Repeat specimen rate (per 100,000)	10 ^b	II
False-positive rate at clinical referral	?10% (liver disease)	III

* See page 24.

^a Sensitivity would probably be higher using tandem MS.

^b The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh, USA, is approximately 35 per 100,000.

Treatment and outcome

Management of the severe forms of maple syrup urine disease involves supportive treatment of the acute neonatal illness, if necessary with peritoneal dialysis, haemodialysis, or haemofiltration to lower metabolite levels.²¹⁸ Supplementary treatment aims at reducing catabolism of body protein by a high energy intake, intravenous glucose with concomitant insulin²¹⁸ or intravenous lipids.^{219,220} Subsequent management requires reduction of dietary branched-chain amino acid intake largely by replacing natural dietary protein with a synthetic amino acid mixture.²²⁰ As in phenylketonuria, the dietary regime is extremely restrictive and careful monitoring of levels of the three individual branched-chain amino acids in blood is required. Additionally, metabolic derangement owing to intercurrent infections in later infancy and childhood may require emergency support with intravenous fluids as before.

Long-term outcome

The early experience of outcome of the severe form of maple syrup urine disease under dietary therapy was generally poor, with both mental retardation and cerebral palsy being common.^{220–224} In one study, almost half the patients died and the majority of survivors suffered severe mental delay.²¹⁶ However, children beginning treatment after 1980 at the same clinic all survived, 70% with IQ levels > 90 and none with major impairment. Developments in intensive care techniques, and particularly in haemodialysis and haemodiafiltration, are continuing to improve outcome. The key is prompt diagnosis. Early reports suggested a delay in diagnosis of more than 10–14 days was associated with neurological problems.^{220,222} Only a few patients treated after 14 days of age achieved normal intellect.^{220,223,225} A recent study has pinpointed this threshold to an earlier age with normal IQ in children diagnosed and treated within the first week (mean age 3.5 ± 3 days).²²⁶ Those treated later (mean age 10 ± 4 days) had below-normal IQ levels. In the same study children treated presymptotically had higher IQs than their affected siblings, who were treated when their disease was symptomatic.

Reviews of management of patients with maple syrup urine disease^{218–222,225–227} suggest that normal somatic growth and normal psychomotor development can be achieved if there is very early diagnosis and treatment, and meticulous attention to controlling catabolism during even minor illness.

Neonatal screening

The majority of centres screening for maple syrup urine disease measure leucine in filter paper blood

spots using a specific bacterial inhibition assay.^{215,228,229} The prompt rise in blood leucine levels after milk feeding makes maple syrup urine disease screening highly sensitive. No cases of the classical or intermediate forms are known to have been missed in more than 9 million neonates screened using this method.²¹⁵ A more recent evaluation of screening in the USA reports six cases of maple syrup urine disease which were missed, three because of a technical error and three because of delayed sample collection. The false-positive rate is reported at 0.04%.²²⁸ Patients with milder forms of the intermediate disease with lower leucine concentrations and patients with the intermittent disease may be missed.^{215,230} Centres which screen for phenylketonuria by amino acid chromatography can pick up maple syrup urine disease as an 'add on' by detection of elevated branched-chain amino acid levels.

Tandem MS has been used successfully to detect maple syrup urine disease by measurement of leucine and isoleucine in blood spots.⁴⁸ The leucine (plus isoleucine):phenylalanine ratio was found to be a good indicator of the branched-chain amino acid status. On comparison with the bacterial inhibition assay, tandem MS accurately detected all six cases of maple syrup urine disease and reduced the 22 false-positive results to nil.⁴⁸

Overall case for screening

Patients with the severe form of the disease need to be diagnosed and treated within the first week of life to ensure a good outcome. Thus, the timing of sample collection in the UK is not ideal for maple syrup urine disease screening. The cost of treatment is high, similar to that in phenylketonuria but with a need to continue stricter control into adult life. In addition, the incidence of maple syrup urine disease is low in most of the UK so that, as a stand-alone programme, screening for maple syrup urine disease is unlikely to be cost-effective.

Current status

Newborn screening for maple syrup urine disease is performed in 25 states of the USA²³¹ and in about 11 countries worldwide.²²⁸ At least five other countries have initiated screening programmes and then stopped them.²²⁸ In the UK, only programmes using chromatographic methods for phenylketonuria screening will detect maple syrup urine disease. In some areas, for example, Bradford, there is a significant yield.

The data for maple syrup urine disease are summarised in *Table 27*.

TABLE 27 Summary table for maple syrup urine disease

		Classification*
Expected incidence (cases per 100,000)	0.5	II
Proportion likely to be clinically affected	> 99%	I
Effectiveness of treatment	Medium	III
Overall sensitivity of screening process	80% ^a	I
Repeat specimen rate (per 100,000)	40 ^{b,c}	II
False-positive rate at clinical referral	< 5%	III

* See page 24.
^a Intermittent forms may be missed.
^b Using bacterial inhibition test; lower with tandem MS.
^c The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh, USA, is approximately 35 per 100,000.

Urea cycle disorders

The urea cycle is the metabolic pathway which brings about detoxification of ammonia by synthesis of arginine and of urea. The complete pathway is confined to the liver.

Five diseases, each with considerable genetic and phenotype variability have been described, each representing a defect in one of the enzymes of the urea cycle:

- (i) carbamoyl phosphate synthase deficiency
- (ii) ornithine carbamoyltransferase (transcarbamylase) deficiency
- (iii) argininosuccinate synthase deficiency/citrullinaemia
- (iv) argininosuccinate lyase deficiency/argininosuccinic aciduria
- (v) arginase deficiency/argininaemia.

In addition two other disorders, **lysinuric protein intolerance** and **hyperornithinaemia, homocitrullinuria, hyperammonaemia syndrome** also impede the urea cycle and cause hyperammonaemia.

Non-specific biochemical characteristics of the urea cycle defects are increased plasma ammonium and glutamate/glutamine. Alanine may also be increased in (i) and (ii). Specific increases in citrulline, argininosuccinic acid and arginine occur in (iii), (iv) and (v) respectively. Except in (i), accumulation of carbamoyl phosphate leads to synthesis and excessive urinary excretion of pyrimidines, in particular, orotic acid.

Ornithine carbamoyltransferase deficiency is inherited as an X-linked disorder. The other diseases are inherited as autosomal recessive traits.

Prevalence

No reliable data are available. Ornithine carbamoyltransferase deficiency is the most frequently diagnosed of this group of diseases, followed by argininosuccinate synthase and lyase deficiencies.

Natural history of the untreated conditions

The phenotypes found in carbamoyl phosphate synthase, ornithine carbamoyltransferase, argininosuccinic acid synthase, and argininosuccinase deficiencies show many common features and will be discussed as a group distinct from arginase deficiency which has quite a different phenotype.

There is a broad spectrum of clinical presentation in patients with one of the diseases (i) – (iv) listed above. Severity is variable, each disorder having a neonatal acute form which usually proves rapidly fatal, and a spectrum of milder variants with chronic presentation later in infancy and childhood.

Neonatal presentation

The acute neonatal presentation occurs in full-term infants with no particular obstetric risk factors, who appear normal for the first 24–48 hours. The symptoms of hyperammonaemia – lethargy, poor feeding and vomiting, hypotonia or spasticity, convulsion and coma – develop within hours or days. Pulmonary and gastric haemorrhages occur, particularly in deficiencies of carbamoyl phosphate synthase or ornithine carbamoyltransferase. Most patients who survive the first few days of life untreated probably have significant level of residual enzyme activity. If untreated, most patients die in the neonatal period.

Later onset forms

Chronic presentation, with non-specific developmental delay, is often associated with a history of episodic vomiting, lethargy, irritability or headaches. These episodes are characteristically associated with high-protein meals or a catabolic state due to minor infections. Severe episodes may result in seizures, or even periods of coma. Some late onset patients remain healthy with no evidence of developmental delay until presenting in infancy or later childhood as an acute illness, often associated with an infection. If undiagnosed, these may be fatal.

Ornithine carbamoyltransferase deficiency may remain unsuspected throughout infancy and early childhood, and can present as a rapidly progressive encephalopathy with fatal outcome.

Arginase deficiency is usually associated with irritability and mild developmental delay in infancy. Later progressive spasticity of the lower limbs, tremor, ataxia, choreoathetosis, seizures and severe mental retardation develop.

Treatment and outcome

Ammonia is highly toxic and severe hyperammonaemia characteristic of the neonatal forms must be corrected promptly if irreversible neurological damage is to be avoided. Haemodialysis works well for rapid removal of ammonia.²³² Haemofiltration is also effective. Intravenous arginine given in neonatal arginininosuccinate synthase and lyase deficiencies promotes more efficient urinary excretion of waste products in the form of citrulline and argininosuccinate, respectively.²³³ Nitrogen may also be trapped and excreted as hippuric acid and as phenylacetylglutamine; the production of these compounds is increased by giving, respectively, sodium benzoate and sodium phenylacetate or sodium phenylbutyrate intravenously.²³⁴

Following treatment of the acute episode, and for the milder or late onset forms, the mainstay of long-term therapy is dietary protein restriction, with continued oral administration of sodium benzoate, phenylacetate, or phenylbutyrate and, particularly for argininosuccinate synthase and lyase deficiencies, supplementary arginine.

In arginase deficiency, control of plasma arginine levels appears to prevent the clinical manifestations and, again, addition of benzoate and phenylacetate to the treatment protocol aids the prognosis.²³³

In spite of very vigorous treatment, the neonatal onset forms of carbamoyl phosphate synthase

deficiency and ornithine carbamoyltransferase deficiency are associated with a high mortality and morbidity. Survival may be as low as 40%.²³⁵ Survivors show severe mental retardation with a very high incidence of cerebral palsy.²³⁶ Life-threatening hyperammonaemic crises may recur unpredictably and the long-term outlook is poor. Patients treated from birth show a slightly better prognosis; of 11 such patients three had normal development, six had borderline normal development, and two were moderately impaired.²³⁷

Patients with the milder varieties of these two disorders, and those with argininosuccinate synthase and argininosuccinate lyase deficiencies have higher (90%) survival rates, and may show normal development if promptly recognised and effectively treated. These patients, however, are susceptible to recurrent hyperammonaemic crises particularly during infectious illness.

Other benefits of early diagnosis

As even the late onset forms of urea cycle disorders have a rather poor prognosis, early detection may lead to some families wanting prenatal diagnosis. All five inborn errors of urea genesis can be diagnosed prenatally. The techniques for doing so vary widely and include measurement of an abnormal metabolite in amniotic fluid, analysis of DNA from chorionic villi or amniocytes, and enzyme analysis of *in utero* liver biopsy samples.²³⁴

Neonatal screening

In symptomatic patients, blood ammonia is increased and there is usually an increased plasma glutamine concentration. Except in carbamoyl phosphate synthase deficiency, there is overproduction of orotic acid, which is usually detected by excessive excretion in urine. Deficiencies of argininosuccinate synthase, argininosuccinate lyase and arginase are recognised by amino acid chromatography by the detection of elevated levels of citrulline, argininosuccinic acid and arginine, respectively.

Neonatal screening, where it has been performed, has been for specific diseases. The Massachusetts, USA, screening programme has been successful in identifying late onset argininosuccinate lyase deficiency using paper chromatography of urine obtained at 3–4 weeks of age. A frequency of 1 in 70,000 was found; this is probably an underestimate because of the loss of an unknown number of patients who may have died prior to screening. The Quebec, Canada, urine screening programme using the same method found a similar incidence for this disease. In this study, urinary citrulline was

measured and an incidence for argininosuccinate synthase deficiency of 1 in 250,000 was found. Specific Guthrie bacterial inhibition assays have been proposed for argininosuccinate synthase, argininosuccinate lyase and arginase deficiencies.²³⁸⁻²⁴¹ The effectiveness of these tests in the neonatal period has not been established.

Tandem MS screening has the ability to detect increased citrulline and arginine in blood spots; this should lead to the diagnosis of argininosuccinate synthase and arginase deficiencies, respectively, and possibly to argininosuccinate lyase deficiency, where the increase in citrulline concentration is less marked. Urea cycle disorders usually produce increased blood glutamine concentrations but there has been no systematic collection of data to show how sensitive and specific this would be as a basis for detection. There are also analytical problems due to spontaneous conversion of glutamine to glutamic and pyroglutamic acids. A study to

investigate the potential of high glutamine/ glutamic acid concentrations as a screening test on neonatal dried blood spots is needed. Orotic acid could be used as a second-line test.

Overall case for screening

Both neonatal and later onset forms of these diseases are life-threatening and, unless severe hyperammonaemia can be prevented, mental handicap results. Infants with the neonatal form of the disease need to be identified and treated within the first week of life; screening at 6–10 days is too late. Patients with the chronic or later onset forms of these diseases would benefit from identification and prospective treatment to prevent hyperammonaemia. Thus, if methods of sufficient specificity were available, neonatal screening as an add-on test would be justified.

A summary of the data for urea cycle disorders is shown in *Table 28*.

TABLE 28 Summary table for urea cycle disorders

		Classification*
Expected incidence (cases per 100,000)	2.5	III
Proportion likely to be clinically affected	> 95%	III
Effectiveness of treatment	High to low, depending on type	IV
Overall sensitivity of screening process	–	
Follow-up testing	–	
False-positive rate at clinical referral	–	
* See page 24.		

Chapter 8

Diseases with existing stand-alone screens

Galactosaemia

Galactosaemia is an inborn error of carbohydrate metabolism caused by an inability to metabolise galactose to glucose because of a deficiency of the enzyme galactose-1-phosphate uridylyltransferase. The result is accumulation of galactose and galactose-1-phosphate in blood and tissues. Two other, rarer, disorders are also grouped under the general title of galactosaemia: **galactose kinase deficiency** leads to cataracts but not the other symptoms of galactose-1-phosphate uridylyltransferase deficiency; **uridine diphosphate-galactose epimerase deficiency** is clinically similar to galactose-1-phosphate uridylyltransferase deficiency.²⁴² Neither of these is covered in detail here. All three forms of galactosaemia are autosomal recessive disorders.

Prevalence

The reported incidence of galactosaemia caused by galactose-1-phosphate uridylyltransferase deficiency varies considerably among different populations, ranging from 1:23,500 in Ireland to 1:101,000 in Sweden.²⁴² Systematic registration of diagnosed cases gave an estimate of incidence in the UK of 1:44,000 and, in the Republic of Ireland, 1:23,500.²⁴³

Natural history of the untreated condition

Affected babies often appear normal during the immediate newborn period, though a few have cataracts at birth. Symptoms develop within days of beginning milk feeding and include jaundice, poor feeding, vomiting, liver disease and neurological symptoms.^{244,245} If untreated, 20–30% of these infants will die as a result of Gram-negative sepsis, to which affected babies are particularly susceptible.^{246–248} Others may die in the neonatal period of acute liver failure. Patients who survive the neonatal period suffer from chronic liver disease, with cirrhosis, cataracts and severe mental retardation if untreated.^{249,250}

Several variants of galactose-1-phosphate uridylyltransferase deficiency exist in which patients have different amounts of normal enzyme activity.²⁴⁴ The most frequent is the Duarte-classical galactosaemia compound heterozygote, where patients have about 25% of normal enzyme activity and are usually

asymptomatic,^{251,252} but occasionally present with symptoms of classical galactosaemia.²⁵³

Treatment and outcome

Elimination of dietary galactose is the mainstay of therapy. Avoiding galactose in breast milk, cow's milk, or milk-based infant feeding formulae results in a dramatic and almost complete reversal of the acute neonatal presenting features. Feeding improves, vomiting ceases, weight gain ensues, liver abnormalities clear and cataracts regress. Complete elimination of galactose is required, and specific commercially-produced formulae or soya-based milks are available for use in infancy. There is no evidence that diet can be relaxed at any particular age and current policy is to treat throughout life.²⁵⁴

Although removal of galactose from the diet prevents the acute disease in the neonatal period it does not avert long-term complications. Intellectual impairment, growth failure and ovarian dysfunction occur, irrespective of when the diet is commenced or strictness of dietary control.²⁵⁵ Some 70% of patients aged > 6 years and on restricted diet have diminished intellectual capacity.^{252,256} In Manchester, out of seven patients aged 15–27 years treated adequately since the neonatal period, five patients had IQs in the low 60s.²⁵⁷ Delayed onset of speech, or other speech abnormalities were reported in two-thirds of patients over 3 years of age.^{252,258} Studies of large groups of patients have shown no significant relationship between IQ and the age at which therapy was initiated;^{252,258} these studies also report a decline of IQ with age, suggesting progression of the disease in spite of dietary therapy. A high incidence (88%) of speech and language disorders has been reported in a smaller study of eight children detected by screening and all treated before 16 days of age.²⁵⁹

Between 80% and 90% of female patients have hypergonadotropic hypogonadism or primary ovarian failure.^{258,260} It is likely that, by the third decade of life, almost all females with galactosaemia will demonstrate evidence of primary ovarian disease or premature menarche. Men with galactosaemia do not appear to have gonadal damage.²⁶⁰

The poor long-term outcome in spite of a galactose restricted diet, with no clear indication of how and

when the underlying damage occurs has stimulated further studies to investigate the pathogenesis of the disease. Abnormal glycoprotein glycosylation may be implicated but so far this discovery has not lead to any improvement in treatment.²⁶¹

Prenatal diagnosis

Prenatal diagnosis for galactosaemia may be carried out by a galactose-1-phosphate uridytransferase assay in cultured amniotic fluid cells^{262,263} or in chorionic villus biopsies²⁶⁴ and by galactitol estimation in amniotic fluid supernatant.²⁶⁵ The long-term outcome of patients treated on a galactose-restricted diet is recognised to be unsatisfactory, so that prenatal diagnosis with view to terminating the affected pregnancy may be considered justifiable.

Neonatal screening

The first mass screening test for galactosaemia in dried blood spots was the *E. coli* metabolite inhibition assay described by Guthrie in 1963.²⁶⁶ Since then a number of different methods have been employed, which are either based on metabolite accumulation (galactose/galactose-1-phosphate),²⁶⁷⁻²⁶⁹ or measurement of galactose-1-phosphate uridytransferase activity. The methods currently in use, and their advantages and disadvantages are summarised in *Table 29*.

If the newborn child with galactose-1-phosphate uridytransferase deficiency has not yet been exposed to sufficient galactose via milk feeds, a false-negative result may be obtained using methods which measure metabolites rather than enzymes. Methods measuring enzyme deficiency are more specific. However, a false-negative result may occur in an affected infant who has been transfused before the screening blood sample

was collected.²⁷²⁻²⁷⁴ Another common problem is low transferase activity resulting in a positive result in variants such as the Duarte/galactosaemia compound heterozygotes. Clinically these infants are not severely affected and probably do not require treatment.²⁷⁵

Most centres screen primarily for either the metabolites or the enzyme activity, then back this up with the other test as a second-line screening, (although some centres measure both side-by-side on every sample). This combination helps to keep false-positives and false-negatives to a minimum.

Selective screening

Some babies with galactosaemia have raised blood phenylalanine concentrations by the sixth or seventh day of life caused by liver dysfunction and it has been shown that they can be detected in the course of screening for phenylketonuria. In a prospective study in the Trent (UK) Region, 16 out of 17 patients with galactosaemia with no family history of the disease, who were diagnosed between 1979 and 1996, were picked up through the phenylketonuria screen.²⁷⁶ (RJ Pollitt: personal communication, 1996.) The primary screen was the automated fluorometric method and all samples with increased phenylalanine concentrations were examined for galactose using thin-layer chromatography. Only two diagnoses had already been established clinically, on days 6 and 9, before the screening result. Four babies died, two before and two on the day of diagnosis (all between 8 and 14 days of age).

A retrospective study of patients with galactosaemia in the West Midlands Region covering a period of 10 years (A Green: personal data) showed that of

TABLE 29 Blood spot screening methods for galactosaemia

Method (metabolite)	Advantages	Disadvantages*	Study
Guthrie microbiological inhibition (galactose)	Detects galactose-1-phosphate uridytransferase and galactokinase deficiency	<i>E. coli</i> mutant unstable. Antibiotic interference gives false-positive result	Guthrie, 1964 ²⁶⁶
Paigen microbiological (galactose + galactose-1-phosphate)	Detects all three galactose disorders		Paigen <i>et al</i> , 1982 ²⁶⁹
Galactose dehydrogenase and alkaline phosphatase (galactose + galactose-1-phosphate)	Detects all three galactose disorders		Fujimura <i>et al</i> , 1981 ²⁶⁷ Hill <i>et al</i> , 1983 ²⁷¹
Beutler spot test (galactose-1-phosphate uridy transferase)	Specific for galactose-1-phosphate. Detects low activity variants	High number of false-positives. Sensitive to enzyme denaturation (heat and humidity)	Beutler & Baluda, 1966 ²⁷⁰
* See also text.			

16 patients in whom the phenylketonuria screening results could be traced, seven patients had elevated phenylalanine and tyrosine. In six of these patients, the result was available before clinical diagnosis. One child had elevated tyrosine, again available before clinical diagnosis – the phenylalanine level was not recorded. In four patients the phenylalanine concentration was normal, and another four patients were diagnosed and treated before the neonatal screening sample was taken. In this case, screening for phenylketonuria was performed either by Guthrie microbiological assay or by Scriver thin-layer chromatography.

Overall case for screening

A study of British patients with galactosaemia, comparing infants detected by screening with a non-screened group,²⁴³ found that 71% in the non-screened group had started treatment by 15 days of age. Although the comparative figure in the screened group was 93%, eight out of 14 infants detected by screening had a family history or clinical features of galactosaemia. The case for universal neonatal screening in the UK was not supported, and the authors suggested that the emphasis should be put on clinical diagnosis and the importance of awareness amongst paediatricians.

However, diagnostic delays do occur, even in infants displaying very early symptoms of the disease. A study from Germany²⁵² showed that, of 27 infants who were diagnosed as a result of a positive screening test, clinical symptoms of galactosaemia had been present from day 3 onwards in 24 cases. In the cases of the two patients from the Trent Region who died before the positive screening test result became available, both had been examined *post mortem* and death had been ascribed to other causes. (RJ Pollitt: personal data.)

The screening test sample in Britain is taken too late for optimum screening for galactosaemia. Nevertheless, galactosaemia screening could prevent significant short-term morbidity and detect a few cases who would otherwise die undiagnosed (with important advantages for the family). The inability of screening to otherwise improve long-term outlook significantly for the majority of patients lessens its value considerably.

The current position

In the USA, more than 40 states have universal newborn screening for galactosaemia. Many Western European countries also screen universally. Newborn screening for galactosaemia is practiced in the Republic of Ireland and in Scotland, but has not been introduced elsewhere in the UK. Screening was introduced but stopped in Norway.

A summary of the data for galactosaemia is presented in *Table 30*.

Cystic fibrosis

Cystic fibrosis is defined as a defect in transmembrane chloride transport, typically characterised by pancreatic insufficiency and recurrent pulmonary infections leading to severe respiratory problems. Until very recently, definitive diagnosis has relied on the 'sweat test' to demonstrate impaired chloride transport but the disease can now be identified by measuring nasal transepithelial voltage or by detecting mutations of the cystic fibrosis transmembrane regulator (CFTR) gene. Less severe defects of CFTR may manifest themselves in adult life as male infertility due to congenital absence of the vas deferens²⁷⁷ or chronic pulmonary disease.^{278,279} This review concentrates on the severe or moderately severe forms of the disease which present in childhood.

TABLE 30 Summary table for galactosaemia

		Classification*
Expected incidence (cases per 100,000)	2.5	I
Proportion likely to be clinically affected	> 95%	I
Effectiveness of treatment	Low	II-2c
Overall sensitivity of screening process	> 90%	I
Repeat specimen rate (per 100,000)	?	
False-positive rate at clinical referral	< 5%	I

* See page 24.

Prevalence

Overall, the prevalence of cystic fibrosis in the UK is relatively constant at about 1 in 2500 births²⁸⁰ but it is particularly common in Northern Ireland with a prevalence rate of 1 in 1850 live births.²⁸¹ The most common mutation in the UK is $\Delta F508$. It contributes approximately 85% of total cystic fibrosis-causing mutations in South-West England, East Anglia, Central England and Northern Scotland, but only 63–65% in the West Midlands and 56–59% in Northern Ireland.²⁸² The next most common mutation is G551D, contributing nearly 8% of total mutations in Northern Scotland but < 1% in Central England. The balance is made up of a large number of relatively rare mutations.

The prevalence of milder forms of the CFTR defect has yet to be determined – see page 55.

Natural history of the untreated disorder

The pathological process starts *in utero*: meconium ileus (neonatal intestinal obstruction) occurs in about 15% of patients. Approximately 70% of infants detected by neonatal screening are showing some symptoms by 6 weeks of age.⁷ Without treatment, many babies with cystic fibrosis suffer from severe malabsorption due to lack of pancreatic enzymes and die of malnutrition in childhood. However, some patients have adequate pancreatic function, at least in the initial stages. Evidence of airway inflammation is present as early as 4 weeks of age and is found in all subjects examined during the first year of life even in the absence of detectable infection.²⁸³ Respiratory problems may be present very early, even in the immediate postnatal period, and a number of infants die of respiratory infection in the first few months.²⁸⁴ Eventually chronic lung-disease becomes established in all cases. The median survival up to the mid-1950s was 2.5 years.²⁸⁵ Historically, there has often been a considerable delay between the onset of the rather non-specific symptoms and correct diagnosis. In a West Midlands and Wales study involving babies born in the period 1985–90, 27% of unscreened patients were reported as having been diagnosed after 1 year of age,²⁸⁶ a figure that has now increased following further clinical diagnoses.²⁸⁷

Treatment and outcome

The basic aims of treatment are:

- to improve nutrition by dietary manipulation and the provision of pancreatic enzyme supplements

- to reduce the impact bacterial colonisation of the lungs by regular and frequent physiotherapy and continuous or intermittent use of antibiotics.

Progression of the disease is slowed dramatically but not halted. The median life expectancy has now risen to over 30 years. In many areas, treatment is concentrated at specialist centres. A recent report from such a centre,²⁸⁸ specialising in teenagers and adults, gives a good picture of the range and scope of services required.

In most parts of the UK, cystic fibrosis is now treated vigorously from the time it is diagnosed. However, the value of early diagnosis through neonatal screening has been hotly debated.^{289–292}

Other benefits of early diagnosis

Cystic fibrosis is inherited as an autosomal recessive condition. It is sufficiently common for ‘cascade screening’ of relatives of newly-detected cases to determine carrier status to be considered worthwhile. Despite the improvements in treatment, cystic fibrosis remains an extremely unpleasant disease and there is significant demand for first trimester prenatal diagnosis for at-risk pregnancies.

Neonatal screening

Screening by immunoreactive trypsin

For the first few weeks of life, babies with cystic fibrosis show increased concentrations of trypsinogen-like species (IRT) in plasma, although later they will show subnormal levels. An assay for IRT in dried blood spots was first reported in 1979²⁹³ and has since been used as the basis for many neonatal screening programmes. A variety of reagents and protocols have been applied; these are reviewed by Heeley and Bangert.²⁹⁴

In the first 2 weeks of life, the diagnostic specificity of increased IRT for cystic fibrosis is low, with a PPV usually below 10%. If the cut-off is adjusted to give a sensitivity of > 90%, approximately one baby in 200 must be followed up with further tests. Most programmes use a two-tier IRT screen: babies with results above the 99.5th percentile on the first sample have a second sample taken at about 4 weeks of age. Beyond 40 days of age, discrimination becomes very poor.²⁹⁵ Persistently-increased IRT in the 4-week blood sample has a PPV ranging in different centres from 25% to 86% (Travert,²⁹⁶ summarised by Heeley and Bangert²⁹⁴) and, at this stage, babies are referred for a sweat test.

Until recently, the two-stage IRT-based screen has been the standard method. Although it has

generally proved 'acceptable', experience has been mixed. In the first 4 years of operation, the screening programme in Northern Ireland found it necessary to obtain a second sample in 5% of babies screened, the PPV of the second test was only 25%, and sensitivity was only 71%.⁴⁶ Despite these problems it was decided to continue with screening. A pilot scheme of the French national screening programme was terminated due to a higher-than-expected incidence of false-negative results and the adverse psychological effects on families of infants with high results on the initial test.²⁹⁷ Even at its best, the two-stage IRT-IRT protocol requires a large number of second heel-prick samples, and a significant number of unaffected babies are subjected to a sweat test. The latter, in particular, generates a great deal of parental anxiety²⁹⁸ and is technically difficult in young babies.

Babies with meconium ileus give unreliable results on neonatal screening. Part of the problem is that, because of surgical intervention and lack of enteral nutrition, there is a fall in plasma IRT that takes some days to recover, but there may be other factors. Some immunoassays are more badly affected than others because of differing antibody specificities. Additionally, sample from such babies are frequently not taken at the specified time. Thus, babies with meconium ileus are usually excluded when calculating screening performance data.

IRT-DNA screens

Alternative screening strategies have become possible following the discovery of the CFTR gene and the development of simple tests on dried blood spots for the more common mutations. The widely-adopted protocol replaces the second-stage IRT assay by DNA analysis for the $\Delta F508$ mutation, thus avoiding the need for a second blood sample (*Figure 5*). First suggested by Bowling and colleagues in 1990²⁹⁹ and initiated by Ranieri and colleagues,^{300,301} it is now used in several other programmes.^{289,302-308}

This **two-stage** IRT-DNA screen leads directly to diagnosis in homozygous $\Delta F508$ patients but all babies found to be heterozygous for $\Delta F508$ are subjected to a sweat test, a marked increase in numbers over IRT-IRT screening. A **three-stage** IRT-DNA-IRT protocol, which has been used in the Trent neonatal screening programme since April 1994, differs from the two-stage protocol in that a second blood sample is obtained from babies who are found to be heterozygous for $\Delta F508$, and such babies are only referred for sweat testing if the IRT level is still raised at 27 days of age. The comparative performance of these three methods in representative centres is summarised in *Table 31*.

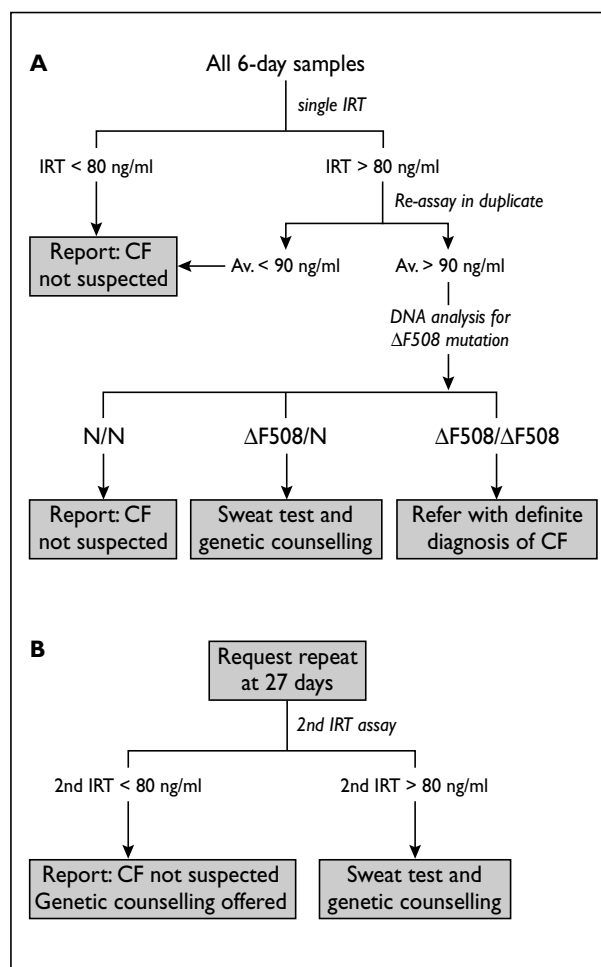


FIGURE 5 DNA-based screening protocols for cystic fibrosis (CF): A, Two-stage protocol; B, Alternative to immediate sweat test for $\Delta F508/N$ babies (3-stage protocol). N = absence of $\Delta F508$ mutation

The three-stage protocol on trial in the Trent programme produced a 20-fold reduction in second samples and a four-fold reduction in negative sweat tests compared to the two-stage IRT-IRT screen.

The applicability of both DNA-based protocols depends crucially on the proportion of cystic fibrosis-producing mutations that can be incorporated. As shown in *Table 32*, a 30% reduction in the frequency of $\Delta F508$ among the disease-causing mutations produces a six-fold increase in the number of babies with cystic fibrosis without this mutation on either chromosome, and halves the number of cases that could be diagnosed directly by gene analysis alone. In most geographical areas, the opportunity to improve performance of DNA-based screens by incorporating other mutations is very limited: only in Scotland and parts of Wales does the frequency of the second most common mutation, G551D, exceed 5%.²⁸² In such geographical areas, a very wide panel of

TABLE 31 Comparison of three screening protocols for cystic fibrosis

	Cut-off percentile	27-day blood sample*	Sweat test negative*	Sweat test positive (%)
Two-stage IRT-RT				
East Anglia (to 1988) ²⁹⁶	99.65	35	0.6	82
Northampton (to 1988) ²⁹⁶	99.66	34	1.6	63
Trent region (UK) 8/89-3/94	99.41	59	0.8	73
New South Wales, Australia ³⁰⁸	99.29	71	3.3	48
Two-stage IRT-DNA				
Calculated from above Trent data	99.41	0	3	43
Victoria, Australia ²⁸⁹	99.12	0	5.3	18
New South Wales, Australia ³⁰⁸	98.96	0	5.4	36
South Australia ³⁰⁰	98.87	0	7	9
Wisconsin, USA ³⁰⁴	97.60	0	9.4	5
Three-stage IRT-DNA-IRT				
Trent region (UK) 4/94-10/95	99.47	2.8	0.2	89

* Number per 10,000 screened.
Patients with meconium ileus are excluded from these figures.

TABLE 32 Proportion of patients with cystic fibrosis with different $\Delta F508$ genotypes

	$\Delta F508$ frequency [†]	Patients with cystic fibrosis		
		$\Delta F508/\Delta F508$	$\Delta F508/?$??/?
Trent region	0.82	0.67	0.30	0.03
West Midlands*	0.67	0.45	0.44	0.11
Northern Ireland*	0.58	0.34	0.49	0.18

* Illustrative data taken from Schwarz et al.²⁸²
[†] Proportion of all mutations causing cystic fibrosis.

mutation probes would have to be used to give acceptable sensitivity. There is an additional problem of equity in areas with a significant ethnic diversity, as some non-white populations have a significant incidence, a low frequency of $\Delta F508$, and experience delayed diagnosis because of the belief that cystic fibrosis is predominantly a 'white disease'.³⁰⁹

Unaffected $\Delta F508$ heterozygotes

The inadvertent discovery of $\Delta F508$ heterozygosity in unaffected babies is an unavoidable by-product of the DNA-based protocols. It is usual to inform families of this and arrange appropriate counselling. Both parents need to have their genotype established and there may be a call for relatives to be genotyped as well, a process referred to by geneticists as 'cascade screening'. Whilst elective pre-reproduction screening of healthy adults for

their $\Delta F508$ heterozygote status is being practised in a number of areas in the UK (see review by Professor H Cuckle: to be published) the inadvertent discovery of heterozygosity in a newborn baby is more problematical. There is an increased risk that future siblings will have cystic fibrosis and the parents must be informed of this. Unfortunately, if only one of the parents carries the $\Delta F508$ mutation, and the other relatively-common mutations have been excluded in the partner, they are still left with a higher than normal risk of having an affected child. These limitations are accepted by adults participating voluntarily in the programmes of cystic fibrosis carrier detection but the situation in neonatal screening is somewhat different since the parents are presented with this unsolicited information. With the stress of coping with a newborn baby, they are not in an ideal position to deal with it. Full exon-scanning of the CFTR gene could provide more definite information in these cases and would make counselling easier (although see below).

The difficulty of dealing appropriately with inadvertent detection of carriers is a clear incentive to minimise the number of samples being analysed for the $\Delta F508$ mutation. In fact, many programmes set a lower cut-off than previously when the DNA step was introduced, arguing that now a second sample was not required it would be possible to increase the sensitivity of the screen without sacrificing its specificity. In fact, as can be seen from *Table 31*, lowering the cut-off point for the first sample leads to a disproportionate increase in babies who are heterozygous for the $\Delta F508$

mutation but give negative sweat tests. This is clearly a disadvantage.

Milder phenotypes of CFTR deficiency

The increasing information coming from genetic studies has led to problems in the definition of cystic fibrosis. The limitations of the sweat test as the ultimate diagnostic arbiter have long been recognised, yet all current neonatal screening protocols rely on it for the ultimate test. Some mutations, particularly R117H and 3849 + 10kb C→T when present as compound heterozygotes with ΔF508 or similar severe mutation, may be associated with a cystic fibrosis-like phenotype but normal sweat test.^{278,279,310} The 3849 + 10kb C→T mutation was identified in 13 of 23 patients with chronic obstructive lung disease characterised by persistent infection with *Staphylococcus aureus* and/or *Pseudomonas aeruginosa* but with normal or borderline sweat tests.²⁷⁸ These mutations have also been found, though less commonly, in cystic fibrosis patients with abnormal sweat tests. Mild cystic fibrosis mutations, including ΔF508/R117H compound heterozygosity, may also be associated with isolated congenital absence of the vas deferens but no other cystic-fibrosis-like symptoms.³¹¹ Thus, on the one hand, the sweat test is no longer an adequate diagnostic tool and may need to be replaced by more comprehensive genetic analysis; on the other, such analysis is likely to reveal conditions that are unlikely to manifest before adulthood and which we would normally not wish to diagnose in the neonatal period. The position is further complicated by the rather loose correlation between disease expression and genotype, at least as indicated simply in terms of a pair of allelic mutations.

We do not know the incidence of these milder forms of CFTR abnormality. In a very recent study of hypertrypsinaemic ΔF508 heterozygous babies with a normal result on the sweat test (the group marked 'sweat test and genetic counselling' in the upper part of *Figure 5*), 15 of 23 babies had abnormal pancreatic function and, of these, three were identified as ΔF508/R117H compound heterozygotes.³¹² This result may suggest a higher number of patients with mild disorder than previously imagined. Those who are at risk of developing chronic suppurative lung disease would almost certainly benefit from long-term follow-up and some form of pre-emptive treatment. Clearly, further research on the UK population is needed.

Overall case for screening

There have been a few attempts at systematic evaluation of the effects of neonatal screening for cystic fibrosis on the clinical progress of the disease.

These are listed in *Table 33*. The strengths and weaknesses of the three main screening protocols for cystic fibrosis are shown in the box below.

- Wilken & Chalmers, 1985;³¹³ Waters *et al*, 1996³¹⁴ – A comparison of cases detected by neonatal screening with a cohort born in the immediately preceding 3-year period. Two-tiered IRT screen.
- Bowling *et al*, 1988³¹⁵ – A comparison of cases detected by neonatal screening with a cohort born in the immediately preceding period. Two-tiered IRT screen.
- Mastella *et al*, 1988³¹⁶ – A comparison of screened babies with those born in surrounding regions and not screened. There is a possible bias in this study in that treatment facilities in the screened and unscreened regions were not necessarily equivalent.

Summary: Strengths and Weaknesses of the Three Main Cystic Fibrosis Screening Protocols

Two-stage IRT-IRT

Strengths	fairly cheap; simple protocol restricted to the more severe forms of CFTR deficiency mainly independent of genetic composition of the population
Weaknesses	a high requirement for second blood samples diagnosis is always later than 4 weeks of age

Two-stage IRT-DNA

Strengths	no need for second blood sample rapid diagnosis of many cases without recourse to sweat test
Weaknesses	slightly more expensive and complicated than IRT-IRT protocol inadvertent detection of ??unaffected carriers increased number of negative sweat tests dependent on prevalence of ΔF508: will miss non-ΔF508 cystic fibrosis

Three-stage IRT-DNA-IRT versus two-stage IRT-IRT

Strengths	greatly reduces number of second blood samples required rapid diagnosis of many cases without recourse to sweat test reduced number of negative sweat tests
Weaknesses	slightly more expensive and complicated than IRT-IRT protocol inadvertent detection of ??unaffected carriers dependent on prevalence of ΔF508: will miss non-ΔF508 cystic fibrosis

TABLE 33 Systematic studies of clinical effects of neonatal screening for cystic fibrosis

Type of study ¹	Reports	Place
II-2b	Wilcken & Chalmers, 1985 ³¹³	New South Wales, Australia
II-2b	Bowling <i>et al</i> , 1988 ³¹⁵	Queensland, Australia
III	Mastella <i>et al</i> , 1988 ³¹⁶	NE Italy
I	Rock <i>et al</i> , 1990; ²⁹⁵ Farrell & Mischler, 1992 ²⁸⁵	Wisconsin, USA
I	Chatfield <i>et al</i> , 1991; ²⁸⁶ Bradley <i>et al</i> , 1995 ²⁸⁷	Wales and West Midlands, UK
II-2a	Dankert Roelse & te Meerman, 1995 ³¹⁸	Groningen, The Netherlands
II-2c & III	Littlewood <i>et al</i> , 1995 ³²⁰	Leeds, UK

- Rock *et al*, 1990;²⁹⁵ Farrell & Mischler, 1992²⁸⁵ – All babies were screened but in 50% the results were stored coded and not reported. Codes were broken following clinical diagnosis or at 4 years of age, whichever was the sooner. Initially (15/4/85 to 30/6/91) this study used a single-phase IRT screen with referral for sweat testing for babies with results > 99.8th percentile. From 1/7/91, a two-tiered IRT–DNA screen was used.³⁰⁴ Wisconsin has now adopted cystic fibrosis screening as a routine programme.¹⁰⁶
- Chatfield *et al*, 1991;²⁸⁶ Bradley *et al*, 1995²⁸⁷ – Screening ran from 1985 to 1989 and used a two-tiered IRT screen.³¹⁷ Babies in Wales and the West Midlands were screened on alternate 2-week periods, thus producing a screened and control cohort for each area.
- Dankert Roelse & te Meerman, 1995;³¹⁸ – Screening was based on a determining the **albumin** content of meconium (a test of low sensitivity and specificity, long superseded) between March 1973 and March 1979. Three groups were compared:
 - (a) the screened group
 - (b) non-screened patients born during the same period
 - (c) a group born during the 6 years immediately after the screening programme ended.
 There were a number of possible biases related to the design of this programme, although the investigators did not think that these could explain the results obtained.^{318,319}
- Littlewood *et al*, 1995³²⁰ Comparison of screened (on meconium) and non-screened patients treated at the same clinic.

Short-term benefits of early treatment

The Wales–West Midlands²⁸⁶ and New South Wales, Australia,³¹³ studies both showed a significant reduction in number of days of hospital stay during the first 1 or 2 years of life for the screened group

(19.2 versus 27 and 4.8 versus 27.7 days per patient, respectively). Other published studies have not found this effect although in Queensland, Australia, there was a marked reduction in the number of treated chest infections with screening. Rapid correction of nutritional deficits usually follows diagnosis^{313,321} but, in the medium term, there is no significant difference in growth between screened and non-screened patients. It is clear that experiences on all these points have differed widely, reflecting in part considerable variation in the pattern of medical care in various parts of the world. Evaluation is made more difficult by rapid improvements in management. Prophylactic treatment following early diagnosis can have a marked effect: a randomised trial of continuous flucloxacillin from the neonatal period showed reduced infection with *S. aureus*, and fewer and shorter hospital admissions, particularly in the second year of life.³²² Nevertheless, some authors see reduction of diagnostic delay as the most important proven benefit of neonatal screening for cystic fibrosis (Phelan³¹⁹). There is strong parental support for neonatal screening on these grounds (see page 89).

Long-term benefits of very early treatment

Evidence of long-term benefits of vigorous early treatment is even more difficult to assess. Recent reports from The Netherlands³¹⁸ and Italy³¹⁶ demonstrated increased survival and much better lung-function in patients detected neonatally by screening compared with the non-screened group born during the same period. A similar cohort born from 1970 onwards in the Leeds area showed less chronic *S. aureus* and *P. aeruginosa* infections than a non-screened group treated in the same clinic.³²⁰ However, it is difficult to extrapolate from these very early results to the current situation as the outlook for patients with cystic fibrosis, whether screened or not, is now so much more favourable.

More recent studies have yet to be completed. The Wisconsin trial reported no differences in pulmonary function between screened and non-screened patients in 1992, at which stage the oldest screened patients were just over 6 years of age.²⁸⁵ However, patients detected by neonatal screening between 1985 and 1990 in the West Midlands and Wales study²⁸⁶ have now been followed up to 8 years of age and, according to unpublished data submitted to the Welsh Office in support of neonatal screening for cystic fibrosis, are showing better Shwachman and Crispin Norman scores for general health and chest X-ray appearance (statistically significant) than the non-screened control group.²⁸⁷ Data from the New South Wales study, available at present only in abstract form,³¹⁴ also show improved pulmonary function in the screened group, with an average 10% difference in FEV₁ (a measure of forced expiratory volume) between screened and non-screened groups at 5 years of age and a mean difference in Shwachman score (of chest X-ray) of 5.3 (95% CI, 1.9–8.8) by 10 years of age. The screened group also showed significantly better height and weight. These studies thus support the intuitively attractive hypothesis that controlling lung infection during the period of active growth and modelling in early infancy has a lasting beneficial effect.

Overview

At present the case for routine neonatal screening for cystic fibrosis rests on two types of benefit. As outlined above there are **immediate** health gains for the affected infant and, in some medical settings, there may be significant short-term cost savings. Screening also leads to considerable streamlining of diagnosis which has clear advantages to the parents as outlined on pages 86–89. Of greater interest are the **long-term** effects of very early treatment on the progression of the disease. Favourable evidence is beginning to emerge. The Medical Advisory Committee of the Cystic Fibrosis Trust (UK) believes that cystic fibrosis already conforms to the World Health Organisation (Wilson and Jungner³⁴) criteria for screening for genetic disease³²³ but it may be a few years before this case is conclusive. Present indications are that neonatal screening **followed by vigorous treatment** will move the survival curve significantly to the right, giving a longer period of good quality life before the onset of severely debilitating respiratory disease.

While at this stage, neonatal screening for cystic fibrosis may be seen as part of a general move towards earlier and more vigorous treatment of this disease, in the future it may have a more specific role: considerable effort is currently being directed

towards gene therapy and other fundamental approaches to treatment that will need to be applied early to achieve maximum benefit.

Cost-effectiveness

The literature on the economics of neonatal screening for cystic fibrosis, is sparse and rather inconclusive (see chapter 14). Detection by neonatal screening constitutes only a very small proportion of the lifetime health costs of a cystic fibrosis patient²⁸⁵ and, given the uncertainties surrounding the long-term effects of very early treatment, its impact on the costs of long-term treatment are hard to estimate. The mean annual cost of an adult attending a specialist centre in 1991 was £8241, with a range from £2792 to £19,955 depending on the stage of the disease.³²⁴ Thus, a relatively small change in long-term progression could have a marked (positive or negative) impact on the cost–benefit balance of the screening programme. Equally, a relatively small increase in the number of terminations of affected pregnancies, resulting either from earlier diagnosis of the index case in a family or as a by-product of a more structured service induced by the introduction of neonatal screening,²⁸⁹ would result in positive cost-saving.

Current status

Neonatal screening for cystic fibrosis is performed throughout Australia and New Zealand, in four state programmes and privately elsewhere in the USA, and in several parts of Europe. In the UK, East Anglia and Trent Regions, Northern Ireland and Northamptonshire have all been screening for some years. Screening has just restarted in Wales on a permanent basis (as opposed to the previous randomised Wales–West Midlands trial) and has recently begun in the City of Leeds.

The data for cystic fibrosis are presented in summary form in *Table 34*.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia describes a group of disorders caused by an enzymatic defect in the steroid biosynthetic pathway, most commonly (approximately 90% of all cases) a deficiency of 21-hydroxylase. This results in decreased levels of glucocorticoids and mineralocorticoids in body fluids, which prompts excessive adrenocorticotrophin (ACTH) secretion by the pituitary and subsequent hyperplasia of the adrenal gland. Large quantities of cortisol precursors are channelled into androgen production, affecting the normal

TABLE 34 Summary table for cystic fibrosis

		Classification*
Expected incidence in UK (cases per 100,000)	40	I
Proportion likely to be clinically affected (%)	100% by definition	
Effectiveness of treatment	Medium	Various
Overall sensitivity of screening process	0.85–0.90	I
Repeat specimen rate, unaffected (per 100,000)	0–550	I
Follow-up testing (nature and number per 100,000 screened)	Sweat test; 46–80 according to strategy	I
False-positive rate at clinical referral	Not applicable	
* See page 24.		

process of sexual differentiation and development, and of growth.³²⁵ Other types of congenital adrenal hyperplasia are caused by deficiency of the enzymes 11- β -hydroxylase (approximately 5% of all cases), 17- α -hydroxylase and/or 17,20-lyase, and the extremely rare 3- β -hydroxysteroid dehydrogenase. All these forms are transmitted in an autosomal recessive manner.

Prevalence

A very high incidence of salt-losing congenital adrenal hyperplasia is found in Yupik Eskimos (1:272) and in Reunion Island (1:4110).³²⁶ Elsewhere the incidence appears to be relatively uniform – between 1 in 11,000 and 1 in 23,000 (Table 35). The combined worldwide incidence of classical congenital adrenal

hyperplasia (salt-wasting and simple virilising forms) is reported as 1:15,300 live births (6.5 per 100,000).³²⁶

Natural history of the untreated disorder

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency can be divided into three categories depending on clinical symptoms on presentation; the salt-losing, simple virilising and late onset forms.

Salt-losing form

This is associated with the most severe or complete deficiency of the 21-hydroxylase enzyme resulting in very low or absent secretion of cortisol. There is an accumulation of the cortisol precursor 17-hydroxyprogesterone producing a salt-losing effect. The adrenal cortex cannot secrete sufficient aldosterone to respond to the salt loss, resulting in an acute adrenal crisis, usually between the fourth and fifteenth day of life but sometimes as late as 6–12 weeks of age.³²⁵ Clinical symptoms include excessive vomiting, diarrhoea and loss of weight due to dehydration; biochemical signs include hyperkalaemia and hyponatraemia. Unless recognised and treated rapidly, the adrenal crisis leads to cardiovascular collapse and death. Salt losers account for 66% of all patients with 21-hydroxylase deficiency congenital adrenal hyperplasia.

Excess androgen production causes varying degrees of masculinisation of the female external genitalia. In extreme cases, the ambiguity may result in incorrect sex assignment. In male infants, the absence of ambiguous genitalia makes the diagnosis more difficult but the biochemical markers are the same.

TABLE 35 Prevalence of congenital adrenal hyperplasia from screening studies

Country	Prevalence	Study
Scotland	1:18,401	Wallace <i>et al</i> , 1986 ³²⁷
France (mainland)	1:20,670	Dhondt <i>et al</i> , 1993 ²⁹⁷
Sweden	1:12,800	Larsson <i>et al</i> , 1988 ³²⁸
Italy	1:11,116	Cicognani, 1992 ³²⁹
Portugal	1:14,300	Vaz Osorio & Vilarinho, 1989 ³³⁰
USA	1:15,305	Pang & Clark, 1993 ³³¹
Canada	1:16,666	Pang & Clark, 1993 ³³¹
New Zealand	1:23,344	Cutfield & Webster, 1995 ³³²
Japan	1:20,570	Suwa <i>et al</i> , 1988 ³³³

Simple virilising form

The 21-hydroxylase deficiency is not complete and cortisol secretion may approach normal levels.³³⁴ The salt-losing effect of 17-hydroxyprogesterone is counteracted by increased activity of the renin-angiotensin-aldosterone system.³²⁵ However, the high ACTH secretion results in abnormally high secretion of adrenocortical androgens resulting in masculinisation of the female foetus. In the male foetus, testicular androgens carry out the normal masculinisation of the external genitalia and the secretion of excess adrenal androgens has no effect. Consequently, females with milder degrees of sexual ambiguity and most affected males may go undiagnosed until early to mid-childhood, when excessive secretion of adrenal androgens in both sexes results in precocious puberty.

Late onset form

This is associated with a mild degree of deficiency of the 21-hydroxylase enzyme. There are no signs of ambiguous genitalia at birth. Symptoms occur in females at puberty with acne, hirsutism, primary or secondary amenorrhoea, polycystic ovarian disease and infertility.³³¹

In all types of congenital adrenal hyperplasia, the secretion of adrenal androgens in infancy and early childhood creates an anabolic condition that results in rapid osseous development. In mid-childhood, bone age and height age are markedly advanced. However, premature closure of the epiphyseal plates then results in permanent short stature.

Treatment and outcome

Life-threatening, salt-losing crises need to be recognised and treated promptly with intravenous saline and glucose, and glucocorticoid and mineralocorticoid replacement. Long term, the tendency to salt loss is managed with 9 α -fluorohydrocortisone (fludrocortisone), sometimes supplemented with DOCA (deoxycorticosterone).

The mainstay of treatment in all forms of congenital adrenal hyperplasia is cortisol replacement to correct the cortisol deficiency and reverse the abnormal hormonal pattern resulting from excessive ACTH secretion, so that androgen secretion is suppressed and virilisation controlled. Hydrocortisone is given in the early years, replaced in the later years with dexamethasone and prednisolone because of their longer action. Glucocorticoid replacement must be increased during periods of stress.

Female patients with prenatal masculinisation require surgical correction early in life.

Long-term outcome

The most widely-used long-term measure of efficacy of treatment is somatic growth (bone age and height age).³³⁵ Both under- and overtreatment with glucocorticoids affect bone development and the attainment of expected height.^{336,337} Even with treatment, the majority of patients have short stature.^{338,339}

Treatment compliance in adult males may be poor,³⁴⁰ since there are no major health problems on stopping treatment even in patients who had presented initially with salt loss. Steroid studies showed that testosterone levels were within the normal limits and, based on sperm count and reproductive history, all patients appeared fertile. The occurrence of testicular tumours which regress with dexamethasone replacement has been reported.³⁴¹

A study of outcome in adult females showed the average age of pubarche to be 3 years earlier, and menarche 3 years later than normal.³⁴² More recently, a study of 80 adult female patients with congenital adrenal hyperplasia showed problems with psychosexual development and low fertility.³²⁵

A recent report from the South-West of England³⁴³ describes learning difficulties in patients with congenital adrenal hyperplasia. A study of 63 salt-losing patients found learning difficulties in 30% of boys and 21% of girls aged 6–19 years. The histories of these patients was significantly worse than those of patients who did not have learning difficulties in respect of illness at presentation, hypoglycaemic episodes, and growth in the first year.

Neonatal screening

Neonatal screening is performed by immunometric assay of 17-hydroxyprogesterone concentrations in Guthrie card blood spots. A total of 28 programmes for congenital adrenal hyperplasia screening have been conducted in 12 countries. Details of newborn screening programmes for congenital adrenal hyperplasia worldwide are shown in *Table 36*.³³¹

There are considerable variations in the cut-off levels among the programmes, ranging from > 12 nmol/l to > 150 nmol/l in full-term, and up to 600 nmol/l in low birth weight or premature infants. This is due partly to the different antibodies and reagents used in the assay systems. The requirement for repeat samples also varies greatly.

Values for 17-hydroxyprogesterone obtained in screening are greater than true serum

TABLE 36 Newborn screening for congenital adrenal hyperplasia³³¹

Country	Method	17-OHP cut-off (nmol/l)	Age at screening sample collection (days)	Age at result (days)	Repeat sample rate (%)
Canada	RIA	40/70 ⁺	2–4	4–9	2.50
France	RIA	71–108*	3–5	11–20	0.30
Germany	DELFI A	20	3–10	6–12	0.02
Italy	RIA	30–40*	2–15	7–25	0.21
Japan	ELISA	12–90*	4–7	8–12	0.04
New Zealand	RIA	50/70 ⁺	2–5	7–14	0.23
Portugal	RIA	90	5–10	20	0.01
Scotland	RIA	140	5–10	10	0.04
Spain	RIA	60/84 ⁺	5–7	4–12	0.79
Sweden	RIA	150/600 ⁺	4	11	0.03
Switzerland	DELFI A	30/60 ⁺	4	7	0.17
USA	RIA	60–255*	3	6	0.20

RIA, radio-immunoassay; ELISA, enzyme-linked immunoassay; DELFI A, dissociation-enhanced lanthanide fluorescence immunoassay; 17-OHP, 17-hydroxyprogesterone.
* Different regional programme cut-off level.
⁺ Cut-off level adjusted depending on gestational age and/or birth weight.

determinations by specific tests,³³¹ indicating the presence of cross-reacting substances in whole blood samples. In addition, 17-hydroxyprogesterone levels may be higher in infants who are premature,^{344,345} of low birth weight³⁴⁶ or acutely ill.³⁴⁷ In one study,²⁹⁷ 71% of premature babies and 66% of low birth weight babies had elevated levels of 17-hydroxyprogesterone in the screening sample. These are probably the major contributing factors to the variable and, in some programmes, high false-positive rates. Attempts to overcome this have included:

- (i) adjustment of cut-off limits³⁴⁸
- (ii) improved (more specific) 17-hydroxyprogesterone assays³⁴⁹
- (iii) confirmatory second test to reduce the need for a second blood sample.³⁴⁹

The sensitivity of neonatal screening for congenital adrenal hyperplasia is reported to be fairly high. In a review of 4.5 million babies screened worldwide,³²⁶ only 14 (4.7% of total cases) were known to have been missed. Seven were salt losers and seven were non salt losers. The seven non salt losers had a mild or late onset form of 21-hydroxylase deficiency congenital adrenal hyperplasia.

A review of 409 infants detected by neonatal screening showed that 68% were not clinically suspected to have congenital adrenal hyperplasia at the time the screening result was obtained.³³¹ The remaining 32% were suspected on clinical grounds: these included positive family history, positive prenatal diagnosis, or clinical signs such as ambiguous genitalia. The majority of clinically-suspected cases were females.

Neonatal screening for congenital adrenal hyperplasia in the UK

Only one centre (Scotland) in the UK has undertaken a pilot study to screen for congenital adrenal hyperplasia.³⁵⁰ In this study, 120,000 neonates were screened, and seven cases of 21-hydroxylase deficiency detected, five male, two female. All seven patients were salt losers. In four, the screening result was available before clinical suspicion of abnormality and, in two, the infants were in the early stages of adrenal (salt-losing) crisis. In this retrospective study, only 30% of positive males were clinically suspected to have 21-hydroxylase deficiency by the time that the result from a neonatal screening programme would have become available.

Screening versus clinical ascertainment

Comparisons of incidence data from screening and from clinical ascertainment has demonstrated a higher incidence by newborn screening in most cases (*Table 37*). However, recent unpublished data collected over the period 1980–96 (presented by Dr S Pang at the 3rd International Congress on Neonatal Screening, Boston, USA, October 1996) showed that, in Europe, the incidence by case survey was not significantly different from that by screening (1 in 15,000 versus 1 in 12,000). This contrasts with North America and Japan, where differences were significant.

Other benefits of early diagnosis

Early diagnosis in an affected infant allows prenatal diagnosis in a subsequent pregnancy. Prenatal diagnosis of 21-hydroxylase deficiency congenital adrenal hyperplasia is possible both on chorionic villus samples in the first trimester, and on amniotic fluid cells in the second trimester.³³¹

Prenatal treatment for congenital adrenal hyperplasia is available, and is aimed at the prevention of external genital virilisation of affected female foetuses, thereby avoiding the need for corrective genital surgery later. Dexamethasone given to the mother during the first trimester and continued till late in the second trimester, or throughout gestation, prevented virilisation in approximately 31% of affected cases and reduced the degree of virilisation in 42%, while 26% did not benefit from the treatment.³³¹ Treatment is more effective when given as early as the fifth week of gestation, that is, before the more critical period of sexual differentiation at 8–13 weeks,³³¹ and usually before the chorionic villus sample is taken for prenatal diagnosis. Dexamethasone should be given in high risk pregnancies and, if prenatal diagnosis shows the foetus to be a male or an unaffected female, the treatment should be discontinued.

Overall case for screening

Congenital adrenal hyperplasia leads to a variety of problems from early death, gender misassignment, learning difficulties secondary to early neurological

damage, through to premature puberty and short stature. Many patients present clinically in the first two weeks of life, before any screening result would be available. However, the reported discrepancies in some countries between the incidence found by screening and that of clinically-diagnosed disease suggest that screening has a valuable role to play.

Reviews of worldwide congenital adrenal hyperplasia screening programmes³³¹ suggest that screening can be effective and reliable. In addition, screening can provide early diagnosis in two-thirds of cases before clinical diagnosis, and may possibly speed the diagnosis in some clinically-suspected cases.

In the UK, there is still controversy regarding the need for congenital adrenal hyperplasia screening. The prevalence of congenital adrenal hyperplasia in the UK appears to be variable. In a retrospective survey in the West Midlands,³⁵¹ a prevalence of 1:6188 was reported, much higher than the figure of 1:17,000 quoted from Scotland.³⁵⁰

A retrospective analysis³⁵² of 117 patients with congenital adrenal hyperplasia in the Birmingham (UK) area born between 1958 and 1985 showed that:

- (i) 90% of diagnoses in salt losers were made within the first month of life
- (ii) the ratio of salt-losing males to females was 1:1 for children born between 1970 and 1985, whereas before 1970 the ratio had been 2:3 indicating a loss (death) of boys
- (iii) the detected incidence of affected (salt-losing and non-salt-losing) males was higher in babies born after 1970
- (iv) the four females incorrectly gender-assigned at birth, and all females in whom diagnosis was made late, were born before 1970
- (v) the two patients with congenital adrenal hyperplasia who had died, because of salt-losing crises as babies, were both born before 1970.

The authors concluded that improved clinical awareness in recent years had led to early diagnosis

TABLE 37 Incidence of congenital adrenal hyperplasia by newborn screening and by clinical ascertainment (case survey)³³¹

Country	Number screened	Number affected	Incidence by screening	Incidence by case survey
France	270,060	21	1:12,860	1:23,000
USA	1,806,039	118	1:15,305	1:15,000–1:40,000
Japan	2,523,948	132	1:19,121	1:43,674

Only studies yielding > 20 cases by screening have been included.

and that in countries like the UK, where a rapid diagnosis is readily available, neonatal screening for congenital adrenal hyperplasia was not necessary. A similar view has been expressed in a report from Wales.³⁵³

A survey of the data for congenital adrenal hyperplasia is presented in *Table 38*.

Muscular dystrophy

This review covers Duchenne muscular dystrophy and its milder variant, Becker dystrophy.³⁵⁴ Both are X-linked conditions (see chapter 2) caused by defects in the gene coding for a muscle protein known as dystrophin. The conditions are almost entirely confined to males.

Prevalence

Duchenne muscular dystrophy has a fairly uniform incidence of between 1 in 3500 and 1 in 4500 newborn males. The prevalence is not affected by consanguinity. Becker dystrophy is ten-fold less frequent. There is considerable selection pressure against the Duchenne muscular dystrophy and about one-third of cases are due to new mutations.

Natural history

Though delay in walking is often found, the first clear symptoms occur on average early in the fourth year of life. Diagnosis usually occurs much later, for example, the average age at diagnosis in Merseyside is currently 4.5 years (range, 3 months to 8.5 years).³⁵⁵ There is progressive loss of muscle strength, the legs being most affected initially. In the untreated state, the ability to walk unaided is usually lost by 9–10 years of age. Progression is

inexorable and, despite palliation by physiotherapy and orthopaedic operations, death usually occurs in the patient's early twenties in Duchenne muscular dystrophy, later in Becker variants.

Mild signs are seen in about 8% of female carriers of the Duchenne muscular dystrophy gene.

Benefits of early diagnosis

The benefits of early diagnosis relate mainly to the family as a whole rather than to the patient. Given advance warning, parents can make appropriate plans, including physical alterations to their house, to cope with an increasingly-disabled child. Prompt diagnosis also increases the effectiveness of prenatal diagnosis in preventing the birth of further affected children to the mother or her female relatives. The psychological benefits of early diagnosis are discussed in chapter 11.

Neonatal screening

The primary screen is creatine kinase (CK) activity, which is usually measured by a bioluminescence test or a fluorescence assay. For babies with increased levels this may then followed-up by assay on a second dried blood sample or on liquid blood. Depending on the exact cut-off level and probably other factors, the reported rates for repeat sampling range between 0.02–0.8%, typically 0.2%.³⁵⁶ In the Welsh programme, transient increase in CK was found in 18 of 97,945 male babies tested (0.02%).^{357,358} Because of the long delay before the development of clear clinical symptoms, it is difficult to estimate the false-negative rate. Several individual cases have been reported.^{359,360} Results from seven studies involving 1.25 million newborn boys have recently been summarised.³⁵⁹

TABLE 38 Summary table for congenital adrenal hyperplasia

		Classification*
Expected incidence in UK (cases per 100,000)	5–10	Ia
Proportion likely to be clinically affected	95% ^a	III
Effectiveness of treatment	Moderate	III
Overall sensitivity of screening process	95%	I
Repeat specimen rate, unaffected (per 100,000)	10–250	I
Follow-up testing	_ ^b	
False-positive rate at clinical referral	Unclear ^b	

* See page 24.
^a Ignoring mild cases presenting in adults.
^b Further investigations may be needed for differential diagnosis in seriously ill or preterm neonates.

One of the main problems with early screening programmes for Duchenne muscular dystrophy was differentiating true- from false-positive results, particularly important given the time before the development of unequivocal clinical signs. Molecular analysis of the Duchenne muscular dystrophy gene and direct measurement of dystrophin in muscle biopsy have now made absolute confirmation possible with little delay.

Perhaps more than for any other screen, it is important that the results are disclosed to parents in an appropriate manner. The Welsh screening programme has a clear disclosure protocol "to maximise parental choice and minimise distress".³⁶¹ The disclosure of a positive initial test and request for a second sample (venous blood) is delayed until the baby is at least 6 weeks old. This encounter involves a nominated paediatrician and the family health visitor. The result of this second test is then available within 24 hours.

Overall case for screening

Neonatal screening for Duchenne muscular dystrophy has long been contentious, given the rather limited range of benefits accruing from early diagnosis. However, a number of studies have demonstrated general approval for universal screening of male babies. In the on-going Welsh study, only 5–6% of parents declined to have their male babies tested (a finding in agreement with a preliminary questionnaire survey³⁶²); a further 5% of screening cards were not marked with a preference either way by the midwife taking the sample.³⁵⁸ This study is of particular relevance to the UK and it is examining all aspects of the programme, with particular emphasis on

the impact of positive results on the families concerned.^{358,361,363} There is increasing acceptance of the programme by relevant health professionals, with 79% in favour of screening and only 1.5% wishing to see it discontinued.^{361,364}

Current status

There are, worldwide, currently six large-scale programmes offering neonatal screening for Duchenne muscular dystrophy. The Welsh programme was instituted on 1 July 1990 on a research basis and is still on-going.

The data for muscular dystrophy are summarised in Table 39.

Biotinidase deficiency

Biotin is a vitamin which acts as a co-factor to four carboxylases. Before it can act it must be covalently bound to the enzyme proteins, a function carried out by another enzyme, holocarboxylase synthase. During the process of cell protein turnover, biotin which is bound to the carboxylases is normally released by a further enzyme, biotinidase, and can then be recycled. In **biotinidase deficiency**, this recycling cannot occur, dietary intake of biotin becomes insufficient, and biotin deficiency ensues.³⁶⁵ This in turn means that production of enzymatically-active carboxylases can not take place, leading to **multiple carboxylase deficiency**. Multiple carboxylase deficiency caused by **holocarboxylase synthase deficiency** is relatively rare and is not dealt with in this review. It may be detectable neonatally by tandem MS but there is no direct experience of this.

TABLE 39 Summary table for Duchenne muscular dystrophy

		Classification*
Expected incidence (cases per 100,000)	22–28	I
Proportion likely to be clinically affected (%)	100%	
Effectiveness of treatment	Nil	
Overall sensitivity of screening process	? > 90%	I
Repeat specimen rate, unaffected (per 100,000)	18–300	I
Follow-up testing (nature and number per 100,000 screened)	DNA and CK on the second blood sample	
False-positive rate at clinical referral	12%, 1 in 31,000 babies tested ^a	I

* See page 24.
^a 3% is due to other neuromuscular disease; half the remainder are attributable to Germany, where high plasma CK activity is occasionally found as a normal variant.

Prevalence

Biotinidase deficiency has been found in many countries. There have been neonatal screening programmes in 14. A survey in 1990, covering 8,532,617 newborn babies, showed an overall incidence for total or partial deficiency of 1 in 60,089.³⁶⁶ In the UK, we have data only from a pilot study in Scotland, which screened 102,393 babies without finding a case.³⁶⁷

Natural history of the untreated condition

The onset of biotin deficiency due to biotinidase deficiency is usually insidious and occurs from a few months of age onwards, although patients with symptoms from birth have been reported. There are often dermatological signs, including seborrhoeic dermatitis and hair loss. Optic atrophy and keratoconjunctivitis are fairly common findings. Neurological symptoms include hypotonia, ataxia and seizures. In a large series, seizures were the presenting feature in 38%.³⁶⁸ These are resistant to conventional anticonvulsant medication in about 50% of cases. There may be a mild acidosis. Without appropriate treatment, many early onset patients die.

Severe late onset biotinidase deficiency has been reported in only one patient. A 10-year-old girl experienced sudden loss of vision due to optic atrophy and progressively developed spastic paraparesis and a predominantly motor-type neuropathy over the next 5 years.³⁶⁹ Hair loss during pregnancy has also been reported in a case of partial deficiency.³⁷⁰

Diagnosis in the early stages requires specific assay of biotinidase in plasma. In the later stages of biotin deficiency, there are usually characteristic organic acid abnormalities in urine but by that time the patient may have experienced irreversible damage.

Treatment and outcome

Oral biotin supplements rapidly correct the dermatological symptoms, and seizures usually stop within 24 hours. Some patients, mainly those treated later, experience continued neurological problems³⁶⁸ and some show severe hearing loss and visual impairment.³⁷¹ Patients detected by

neonatal screening and treated thereafter remain symptom-free and have normal development.³⁷²

Neonatal screening

The determination of biotinidase in dried blood spots presents no technical problems: a variety of methods are available using commercially-available substrates and either visual or instrumental assessment of results. There are two problems:

- (i) pre-term infants tend to show low activity and will need to be retested on a later sample³⁷³
- (ii) about half of the cases detected by neonatal screening have relatively high residual activity. It is believed that most of these will not experience biotin deficiency if left untreated. Recently a method for differentiating the few patients with high residual activity who are at risk of developing symptoms has been described.³⁷⁴ Unfortunately, this requires a liquid blood sample.

The repeat rate (second dried blood sample) in the Scottish study was 0.02%,³⁶⁷ which is in line with general experience.

Overall case for screening

The serious effects of late-treated or untreated biotinidase deficiency and the success, ease, and cheapness of treatment make it a good candidate for neonatal screening. With automation, screening for biotinidase deficiency is relatively cheap: a recent article from New Zealand³⁰ puts the cost at £0.43 per baby; the report from Scotland in 1989³⁶⁷ at £0.08, but no details of these calculations were given. However, the relatively low incidence, particularly if it is accepted that about half the cases detected will not need treatment, militates against it as a stand-alone screen.

Current status

Though screening for biotinidase deficiency continues elsewhere, notably in Australia, New Zealand, and parts of Europe and the USA, it is not included in any of the current UK programmes.

The available data for biotinidase deficiency is summarised in *Table 40*.

TABLE 40 Summary table for biotinidase deficiency

		Classification*
Expected incidence (cases per 100,000)	1.4	II
Proportion likely to be clinically affected (%)	50	I & II
Effectiveness of treatment	High	III
Overall sensitivity of screening process	> 95%	I
Repeat specimen rate (per 100,000)	50	I
Follow-up testing (nature and number per 100,000 screened)	Liquid blood or skin biopsy (0.7) ^a	I
False-positive rate at clinical referral	< 10%	III
* See page 24.		
^a To determine whether patients with Km mutants are likely to be clinically affected.		

Chapter 9

Disorders of organic acid and fatty acid metabolism detectable by tandem MS

Methylmalonic, propionic and isovaleric acidaemias

Propionic and methylmalonic acidaemias are the result of inherited defects in the catabolism of propionyl-CoA; this is formed from the catabolism of the amino acids isoleucine, valine, threonine and methionine, and from cholesterol and odd carbon fatty acids. Deficiency of propionyl-CoA carboxylase results in the disease known as **propionic acidaemia**, which is characterised by accumulation of propionic acid and the production of a wide range of abnormal metabolites, in particular, 3-hydroxypropionate, methylcitrate and propionyl glycine. Secondary inhibition of other enzyme systems may lead to hyperammonaemia, ketosis and inhibition of glycine catabolism.³⁷⁵

There are several types of **methylmalonic acidaemia**.³⁷⁶ Most reported cases are caused by a defect in the methylmalonyl-CoA mutase apoenzyme or in one of the several steps in the processing of hydroxocobalamin to deoxyadenosyl cobalamin, a co-factor in the mutase reaction. These cobalamin (vitamin B12) variants include forms with combined methylmalonic acidaemia and homocystinuria. Methylmalonic acidaemia gives rise to a range of secondary effects similar to those of propionic acidaemia – hyperammonaemia, ketosis, and inhibition of glycine catabolism – and, in the older literature, the two disorders are often referred to collectively as **ketotic hyperglycinaemia**.

Isovaleric acidaemia is a defect of leucine catabolism caused by deficient activity of the enzyme isovaleryl-CoA dehydrogenase. This leads to intra-

cellular accumulation of isovaleryl-CoA, with the appearance of the characteristic metabolite isovalerylglycine in body fluids. Patients with this disorder have a characteristic unpleasant odour, especially during acute episodes.

Prevalence

The incidence of methylmalonic acidaemia found by the Massachusetts urine-based neonatal screening programme was 1:48,000³⁷⁷ but, with less-severe cases included, may be as high as 1:25,000.³⁷⁸ In the Massachusetts screening programme, only one patient with propionic acidaemia was found amongst 331,143 infants screened. However, in a paper describing 326 patients with inherited metabolic disease, 21 cases of propionic acidaemia were diagnosed compared with 31 of methylmalonic acidaemia,³⁷⁹ suggesting that the incidence of propionic acidaemia may be only a little lower than methylmalonic acidaemia, in the range of 1:35,000–1:70,000.

Data from the West Midlands Region over a 5-year period suggest that the incidence for organic acidaemias collectively is at least 1:15,000 live births and, for methylmalonic acidaemia, about 1:50,000.³⁸⁰

Natural history of the untreated conditions

In all three disorders, symptoms are generally non-specific, with variable clinical expression both in severity and age of onset (*Table 41*).

Neonatal period

Affected babies are usually healthy at birth and become ill shortly after starting milk feeds in the

TABLE 41 Presentation of methylmalonic, propionic and isovaleric acidaemias

Onset	Symptoms
Neonatal	Acute life-threatening illness with hypotonia metabolic acidosis, vomiting, lethargy, coma
In infancy	Failure to thrive and developmental regression Episodic illness associated with infection or stress Reye syndrome-like illness Convulsions associated with hypoglycaemia Renal tubular acidosis

first few days of life.³⁷⁶ They present with a severe illness of rapid onset with poor feeding/vomiting, jitteriness, convulsions and lethargy. Hypotonia is a constant feature. Untreated, this condition progresses to metabolic acidosis and early death.

Infancy

The acute presentation is frequently precipitated by infection or some other form of stress, and may be manifested as a Reye syndrome-like encephalopathy. A more chronic presentation with failure to thrive and developmental regression, sometimes punctuated by attacks of metabolic acidosis, may occur.

All three disorders have severe variants, presenting in the first week of life, and later onset forms but, in general, there is a gradation in severity, with propionic acidaemia being particularly resistant to treatment and isovaleric acidaemia the least severe.^{381,382}

Treatment and outcome

Initial treatment of the patient with an acute presentation is supportive, and involves stopping all protein intake, and correction of the acidosis and other secondary metabolic abnormalities.³⁸³ Removal of abnormal metabolites by peritoneal dialysis, haemofiltration, or exchange transfusion may be necessary. Once diagnosis has been established, more specific therapeutic measures can be instituted.

Dietary

It is important to promote anabolism as soon as possible by gradual introduction of a small amount of dietary protein. Long-term management involves restriction of dietary protein, sometimes in combination with an artificial amino acid supplement, especially in severe forms of methylmalonic acidaemia and propionic acidaemia, omitting those amino acid precursors associated with the particular disorder.

Co-factor therapy

Vitamin B12 in large doses may overcome the metabolic defect in the cobalamin defective types of methylmalonic acidaemia sufficiently to produce good results with a fairly relaxed dietary regime.

Substrate removal

It is possible in some instances to reduce harmful intracellular accumulation of abnormal metabolic intermediates by stimulating alternative pathways for removal. Glycine is used in isovaleric acidaemia, either intravenously during acute ketoacidotic episodes, or orally on a long-term basis. Patients with organic acidaemias frequently have low plasma

and tissue concentrations of free carnitine. Carnitine conjugates with a number of acyl moieties including isovaleryl and propionyl groups and supplemental L-carnitine is used for the treatment of patients with these organic acidaemias.^{384,385} Their value as part of long-term therapy and effect on the outcome is still controversial.³⁸⁶

Liver transplantation

The poor outcome for conventional treatment of the early onset forms of propionic acidaemia has led to other treatment possibilities being explored, in particular, liver transplantation. Potentially, this treatment allows the patient to return to an unrestricted normal diet. So far, only a few successful treatments have been reported.^{387,388}

Outcome

The outcome in patients with the severe forms of propionic and methylmalonic acidaemias is not good. Almost all have some morbidity and disability. In one study of propionic acidaemia, all the patients presenting in the newborn period were severely handicapped and all but one had died before reaching the age of 6 years.³⁸⁹ Acute episodes are associated with permanent irreversible neurological damage. The neuropathologic findings are non-specific and include white matter dysmyelination and cerebral atrophy. The basal ganglia are particularly vulnerable.^{390,391} A movement disorder is a common complication in propionic acidaemia.³⁸⁹ A recent study of methylmalonic acidaemia also demonstrated a disappointing outcome for patients who presented in the newborn period.³⁹² Tubulointerstitial nephritis is a constant finding in methylmalonic^{393–395} but not in propionic acidaemia. The earliest changes are found in the renal tubular function, and glomerular failure develops later.³⁹⁶ Other complications include acute pancreatitis^{397,398} and cardiomyopathy.³⁹⁹ Some patients have stroke-like events that may cause severe and permanent neurological deficit.⁴⁰⁰ In both diseases, patients who presented later in infancy or who were co-factor responsive did better.

In patients with isovaleric acidaemia presenting acutely in the newborn period, death has occurred within a few weeks in about half of such cases, with sepsis, leucopenia and pancytopenia contributing to the high mortality. However, early diagnosis and effective treatment provide a relatively good prognosis with survival into adult life.

Neonatal screening

Newborn screening has been performed on 3–4 week urine samples in Massachusetts, USA,

Quebec, Canada, and Australia⁴⁰¹ by paper or thin-layer chromatography or, more recently, in Quebec by gas-chromatography MS.⁴⁰² More sensitive and specific methodology for the detection of organic acidaemias using dried blood spots has become available with tandem MS (chapter 4). Propionylcarnitine and isovalerylcarnitine can readily be quantitated, giving diagnosis of propionic acidaemia and isovaleric acidaemia. Methylmalonylcarnitine is not readily detected in methylmalonic acidaemia blood spots, however, and diagnosis of this disease relies on detecting the secondary accumulation of propionylcarnitine. From preliminary investigations it seems likely that sensitivity for the milder forms of methylmalonic acidaemia may be limited (K Bartlett and M Porfarzam, Newcastle-upon-Tyne: Personal communication, September 1996), and that severely-ill babies and those tested very early may show non-specific increases in propionylcarnitine (M Rashed, Saudi Arabia; Personal communication, October 1996).

Overall case for screening

Neonatal screening is unlikely to be of benefit to patients with the neonatal onset forms of methylmalonic acidaemia, propionic acidaemia, or isovaleric acidaemia. However, the later onset variants are much more amenable to treatment, and significant morbidity and mortality arising prior to diagnosis could be avoided by screening. For late onset forms, the availability of prenatal diagnosis is another reason why early diagnosis can be important.

A summary of the data available on methylmalonic, propionic and isovaleric acidaemias is given in *Table 42*.

Other defects of branched-chain acyl-CoA metabolism

This section deals briefly with three disorders which are fairly rare but which can be detected by acylcarnitine analysis in dried blood spots. Both **3-hydroxy-3-methylglutaryl-CoA lyase deficiency** and isolated **β -methylcrotonyl-CoA carboxylase deficiency** affect the pathway of leucine catabolism; **2-methylacetoacetyl-CoA thiolase** is on the pathway of isoleucine catabolism. **3-hydroxy-3-methylglutaryl-CoA lyase** is also involved in ketone body (acetoacetic and 3-hydroxybutyric acid) production and utilisation, respectively, and are thus important in the physiological response to prolonged fasting. There is confusing nomenclature surrounding 2-methylacetoacetyl-CoA thiolase: it is also referred to as mitochondrial acetoacetyl-CoA thiolase, K^+ -stimulated acetoacetyl-CoA thiolase, or simply as β -ketothiolase. Its deficiency causes 2-methylacetoacetic aciduria.

Prevalence

There are no data on prevalence of these disorders in the UK. Even collectively they are relatively uncommon, probably occurring less than 1 in 100,000 births in the general population.

Natural history of the untreated conditions

3-hydroxy-3-methylglutaryl-CoA lyase deficiency

More than 40 cases of this disorder have been reported in the literature (reviewed by Sweetman and Williams).⁴⁰³ Acute neonatal presentation occurred in about 30% of patients reported with this condition. The others mostly presented from 3–11 months. In both cases, attacks were characterised by lethargy, hypotonia, vomiting, metabolic acidosis, hypoglycaemia and hepatomegaly, with

TABLE 42 Summary table for methylmalonic, propionic and isovaleric acidaemias

		Classification*
Expected incidence (cases per 100,000)	3	II & III
Proportion likely to be clinically affected	100% ^a	III
Effectiveness of treatment	Low to high	IV
Overall sensitivity of screening process	> 90% (except for mild methylmalonic acidaemia)	III
Repeat specimen rate (per 100,000)	b	
False-positive rate at clinical referral	< 5%	III

* See page 24.
^a Mild cases on methylmalonic acidaemia are unlikely to be detected by neonatal screening.
^b The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000.

raised transaminases and hyperammonaemia. In about 20% of cases the attack was fatal. A diagnosis of Reye syndrome was frequently considered. Several patients have shown macrocephaly, a feature seen in some other organic acid disorders.

β -methylcrotonyl-CoA carboxylase deficiency

This is a less severe disorder than 3-hydroxy-3-methylglutaryl-CoA lyase deficiency, usually presenting in the second or third year of life with a Reye-like illness following infection. Persistent hypotonia may be the initial symptom. A number of asymptomatic patients have been reported.⁴⁰³

2-methylacetoacetyl-CoA thiolase deficiency

This has a similar type of clinical presentation to the previous two disorders but a very wide range of severity. There is a tendency to bloody diarrhoea and haematemesis during acute attacks. Some surviving patients have been badly damaged neurologically.

Treatment and outcome

Acute attacks are treated by intravenous glucose and correction of acidosis. For all these disorders, long-term treatment consists of restriction of dietary protein and the avoidance of prolonged fasting. A fat-restricted diet is also advisable in 2-methylacetoacetyl-CoA thiolase deficiency and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. The degree of dietary restriction required varies greatly from patient to patient. Oral supplements of L-carnitine are sometimes used. Emergency support with intravenous glucose may be necessary during severe intercurrent illness in childhood.

There is insufficient data to predict long-term outlook in these disorders in any detail: by analogy with medium-chain acyl-CoA dehydrogenase deficiency, the prognosis is probably good provided that acute episodes can be avoided.

Neonatal screening

Abnormal plasma acylcarnitines have been reported in all three of these disorders,^{45,404–407} even in asymptomatic patients.⁴⁰⁸ There has been no systematic study of neonatal blood spots, and sensitivity for the milder variants may be low. However, four cases of β -methylcrotonyl-CoA carboxylase deficiency and one of 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (combined incidence; 1 in 43,000 babies tested) have been diagnosed by the Pittsburgh programme (see *Table 43*).

Confirmation of diagnosis is readily achieved by organic acid analysis on a random urine sample.

Overall case for screening

Though the presence of asymptomatic cases raises similar problems to those discussed below for medium-chain acyl-CoA dehydrogenase deficiency, the potentially serious effects of these conditions makes them suitable for neonatal screening.

Medium-chain acyl-CoA dehydrogenase deficiency

The main energy-producing pathway for the oxidation of fatty acids is situated in the cell mitochondria and consists of a repeated sequence of four reactions which result in the shortening of the fatty acid chain by two carbon atoms at each pass. This is known as the β -oxidation spiral, and medium-chain acyl-CoA dehydrogenase is a component enzyme of this pathway, dealing with straight-chain intermediates of chain length C₁₂ to C₆. For further details of the underlying biochemistry of this disorder, see chapter 45 of the 1995 edition of Scriver *et al.*⁴

TABLE 43 Summary table for other disorders of branched-chain acyl-CoA metabolism

	Classification*	
Expected incidence (cases per 100,000)	I	III
Proportion likely to be clinically affected	80%	III
Effectiveness of treatment	Medium to high	IV
Overall sensitivity of screening process	90%	III
Repeat specimen rate (per 100,000)	†	
False-positive rate at clinical referral	< 5%	III

* See page 24.
† The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000.

Prevalence

Medium-chain acyl-CoA dehydrogenase deficiency is mainly a disease of North-Western Europe and countries colonised from this region.⁴⁰⁹ This founder effect (see chapter 2) is caused by a 985A→G mutation on the medium-chain acyl-CoA dehydrogenase gene, which contributes nearly 90% of all medium-chain acyl-CoA dehydrogenase deficiency alleles in the UK. Within the UK, the distribution of the 985A→G mutation is non-uniform. In Shropshire, Herefordshire and Worcestershire (combined), one in 52 of the newborn population is heterozygous for this mutation; in the Trent Region, one in 83.⁴¹⁰ These correspond to birth incidences of affected patients of one in 8850 and one in 22,200 respectively (*Table 44*). A smaller survey in Western Scotland found two heterozygotes in 552 healthy babies, suggesting a much lower birth incidence of homozygous, medium-chain acyl-CoA dehydrogenase deficiency.⁴¹¹ It is possible that other mutations may be locally frequent, as has been found in Pennsylvania, USA,⁴¹² in which case, particularly in Scotland, the estimated incidence of medium-chain acyl-CoA dehydrogenase deficiency could be too low.

The difficulties in diagnosing medium-chain acyl-CoA dehydrogenase deficiency clinically on the one hand, and the presence of individuals with asymptomatic medium-chain acyl-CoA dehydrogenase deficiency on the other, might be expected to lead to a discrepancy between the incidence predicted from the gene frequency (using the formula in chapter 2) and the number of patients diagnosed. This was found to be the case in the West Midlands Region, although not in Trent (*Table 44*).

The use of gene frequency data for three rather 'Olde English' counties to calculate the frequency of medium-chain acyl-CoA dehydrogenase deficiency for the whole of the West Midlands may have introduced some distortion, but it is hard to escape the conclusion that medium-chain acyl-CoA dehydrogenase deficiency is significantly underdiagnosed in the West Midlands. Possible explanations advanced for the discrepancy have included a lower clinical awareness of medium-chain acyl-CoA dehydrogenase deficiency and its presenting features, failure to detect characteristic metabolites in urine specimens collected during an asymptomatic period, and a large number of totally asymptomatic cases.⁴¹⁰ The case for screening hinges on whether these missing cases were clinically unaffected or simply undiagnosed. The Trent data⁴¹⁰ suggest that even when ascertainment is fairly complete, most medium-chain acyl-CoA dehydrogenase deficient children are symptomatic: in all of the 19 Trent families diagnosed between 1987 and 1994, a child had either died or experienced an acute life-threatening episode.

Natural history of the untreated condition

There is a degree of overlap between some of the chain-length specific enzymes of the β -oxidation spiral and there is another parallel pathway of limited capacity operating in peroxisomes. Therefore deficiency of medium-chain acyl-CoA dehydrogenase results only in a partial blockage of fatty acid oxidation and individuals with medium-chain acyl-CoA dehydrogenase deficiency retain considerable capacity to oxidise fatty acids. Because of this, the disorder generally only becomes manifest when

TABLE 44 Calculated and observed frequencies of medium-chain acyl-CoA dehydrogenase deficiency in two English health regions⁴¹⁰

	West Midlands	Trent
Total tested	5014	5157
G985 heterozygotes detected	96	62
G985 heterozygote frequency* (95% CIs)	1 in 52 (44–65)	1 in 83 (67–111)
Calculated† frequency of medium-chain acyl-CoA dehydrogenase deficiency	11.3 in 100,000 (7.3–15.9)	4.5 in 100,000 (2.5–6.9)
Expected cases 1987–94 (range from calculated frequencies)	63 (14–89)	23 (13–35)
Diagnosed cases 1987–94	10	22§
Number of families‡	7	19

* Difference significant ($z = 7.98; p < 0.01$)
 § Including a further case inadvertently omitted from the report by Seddon et al.⁴¹⁰
 † Calculated on the basis of 90% of the disease-producing mutations being 985A→G.
 ‡ In the 25 families genotyped, 94% of mutations were 985A→G.

demands on fatty acid oxidation are particularly high. Some affected babies show symptoms shortly after birth.⁴¹³ In older patients, symptoms usually follow a prolonged fast, although increased energy demands due to vigorous exercise or infection may also help to precipitate the crisis. Typically, attacks occur between 3 months and 3 years of age, with an average of 12 months, and follow an infectious illness during which there has been reduced food intake, often aggravated by vomiting. The latest onset reported so far occurred at 30 years of age and followed strenuous exercise in the cold, coupled with inadequate food intake.⁴¹⁴

The severity of metabolic crises in medium-chain acyl-CoA dehydrogenase deficiency is very variable. At its mildest, it may take the form of an isolated self-limited hypoglycaemic episode. More usually attacks are progressive and severe. They are initially accompanied by lethargy, vomiting and encephalopathy. In about half, these symptoms are accompanied by hepatomegaly and biochemical signs, such as hyperammonaemia which are suggestive of Reye syndrome. Seizures, apnoea and cardiac arrest may then ensue. In other cases, death has occurred very suddenly, often overnight, and thus may have been ascribed to Sudden Infant Death Syndrome. Individuals who are not subjected to a severe metabolic stress during childhood may remain completely asymptomatic, though late presentation at 30 years with rhabdomyolysis and acute encephalopathy has been described.⁴¹⁴

The clinical characteristics of a collection of patients largely from the USA⁴¹⁵ are summarised in *Table 45*. Another review⁴¹⁶ of 65 published cases, reports both clinical and biochemical findings. Both these studies included material collected over a number of years. The current position in Great Britain and Northern Ireland has been assessed through a survey (JV Leonard and RJ Pollitt: unpublished data) of cases diagnosed between March 1994 and March 1996, which was carried out through the British Paediatric Association Surveillance Scheme.⁴¹⁷ Data were collected on age at diagnosis, number of attacks, outcome (alive/dead, neurologically impaired/normal) and relevant family history. During this period, 56 new cases were diagnosed and five siblings had died previously in circumstances suggestive of medium-chain acyl-CoA dehydrogenase deficiency. Of 42 cases presenting acutely, 35 (83%) were diagnosed on first presentation, which compares favourably with the earlier USA experience,⁴¹⁵ where on average 35% of patients had more than one episode of illness before diagnosis. Of these 42 acute presentations, six were fatal. Overall, 13 (27%) of the 49 affected families had experienced at least one death. Five of

TABLE 45 Clinical characteristics (% affected) of 120 cases of medium-chain acyl-CoA dehydrogenase deficiency⁴¹⁵

Initial symptoms/signs	% affected
Probable infection prior to onset	85
Lethargy	84
Vomiting	66
Encephalopathy	49
Respiratory arrest	48
Hepatomegaly	44
Seizures	43
Apnoea	37
Cardiac arrest	36
Sudden death	18
Time from onset to diagnosis (years)	0–13 (mean, 1.8)
Previous undiagnosed sibling deaths*	14 in 56 families
Survivors	
Developmental/behavioural disability	32
Persistent seizures	14
Muscle weakness [†]	16
* Persistent muscular weakness is a feature of patients whose diagnosis has been delayed, with more pre-diagnosis clinical events and hospitalisations than the group without weakness.	
[†] Ascribed causes: Sudden Infant Death Syndrome (11); Reye syndrome (1); adrenal insufficiency (2).	

the surviving 36 patients (14%) were described as neurologically damaged. Although the majority of surviving patients were described as neurologically normal, this is only an immediate assessment, usually in a young child. Other studies^{415,418} have suggested that there is significant intellectual handicap in up to one-third of surviving patients.

Diagnosis of medium-chain acyl-CoA dehydrogenase deficiency relies initially on the recognition of hypoketotic hypoglycaemia and initiation of appropriate specialised laboratory investigations. Episodes may be unclassified or diagnosed as febrile convulsions and deaths due to the disorder may be classified as Sudden Infant Death Syndrome or Reye syndrome⁴¹⁵ or, in certain circumstances, ascribed to an 'anaesthetic accident'. It is generally believed that a substantial proportion of cases of medium-chain acyl-CoA dehydrogenase deficiency are never diagnosed. This view is supported by surveys of gene frequency in France,⁴¹⁹ Switzerland,⁴²⁰ England⁴¹⁰ and elsewhere, and anecdotally by numerous case reports where older siblings

of a newly-diagnosed case have shown marked symptoms or died without the diagnosis being made. Additionally, there have been a few reports of adults with medium-chain acyl-CoA dehydrogenase deficiency who have remained completely asymptomatic, and more of older siblings of newly-diagnosed cases who had thus far escaped any clinical consequences. The balance between clinically affected and asymptomatic patients is important in considering the case for screening.

Treatment and outcome

The acute attack must be treated immediately with large doses of glucose, given intravenously and maintained until metabolic homeostasis is re-established. Complications such as apnoea, cardiac arrest, or cerebral oedema are treated in the standard manner.

Long-term management is directed primarily towards preventing fasting stress. In infancy, meals should be frequent and the overnight feed should be discontinued later than normal. Diet can be essentially normal but avoiding very high fat intake. The main danger is a reduced food intake during intercurrent illness. In infancy and early childhood, it is advisable to admit to hospital during any prolonged illness. The need for these precautions decreases with age as the length of fast that can be tolerated before liver glycogen deposits are depleted increases. By adulthood a fast of 24 hours or more should normally be uneventful.

In the USA, in particular, patients with medium-chain acyl-CoA dehydrogenase deficiency are often given supplementary carnitine orally. There is as yet little evidence to support this practice and it is less common in the UK.

The outlook for patients who have been diagnosed is believed to be good, although it is not possible to correct neurological damage caused by previous episodes. No formal collection of UK data has been attempted but the consensus is that in almost all cases it is possible to prevent further attacks of metabolic decompensation.

Other benefits of early diagnosis

Prenatal diagnosis of medium-chain acyl-CoA dehydrogenase deficiency is possible in the first trimester but, given the good outlook once the disorder has been diagnosed, termination of affected pregnancies is seldom considered. However, given the significant mortality during the first few days of life,^{413,421,422} prenatal diagnosis may be useful as an indicator that special precautions (intravenous glucose drip, regular biochemical monitoring) are necessary during this period.⁴²²

Neonatal screening

Laboratory diagnosis of medium-chain acyl-CoA dehydrogenase deficiency is based on finding a variety of metabolites in abnormal quantity or abnormal ratios in blood or urine.⁴²³ Possible screening methods have been developed based on hexanoylglycine,⁴²⁴ cis-4-decenoic acid,⁴²⁴ and octanoylcarnitine.^{49,425} Analysis for octanoylcarnitine using tandem MS has been developed to a stage where it is applicable to large numbers of specimens, and criteria have been established to discriminate medium-chain acyl-CoA dehydrogenase deficiency from other potential causes of increased blood octanoylcarnitine concentrations.⁴⁹ Results from screening 80,371 neonates in Pennsylvania have been reported.⁴¹² This programme continues and has now screened over 200,000 babies and detected 16 cases (*Table 9*). No formal data for repeat sample requirements have been published for this screen but, on a visit to the Pittsburgh laboratory by one of the authors (RJP), it was noted that over a 3-month period, with 20,027 babies screened, only seven repeat samples owing to non-diagnostic abnormalities were required for **all** tandem MS-based screens (acylcarnitines and amino acids, *Table 10*).

Confirmation of diagnosis may be made from the initial blood spot by DNA analysis for the G985 mutation: approximately 80% of cases will be homozygous. For the remainder, confirmation would rest on demonstration of increased cis-4-decenoic acid in the initial blood spot or a characteristic metabolite pattern in a urine sample.

Overall case for screening

Medium-chain acyl-CoA dehydrogenase deficiency fulfils all the classical requirements for a screening programme. The main concern is that a proportion of biochemically-affected individuals will never experience any ill-effects and there is no way of predicting which individuals these will be. Thus, all babies with medium-chain acyl-CoA dehydrogenase deficiency detected by neonatal screening must be treated as at risk. Fortunately, the treatment is not onerous but the need to carefully monitor intercurrent illnesses for the first few years of life is bound to provoke parental anxiety.

Current status

Whole population neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency is at present performed only as part of the NeoGen Inc. supplementary scheme in Pittsburgh, USA. A pilot study of tandem MS screening, which includes medium-chain acyl-CoA dehydrogenase deficiency, is planned at the Institute of Child Health, London.

TABLE 46 Summary table for medium-chain acyl-CoA dehydrogenase deficiency

		Classification*
Expected incidence (cases per 100,000)	5–10	Ia
Proportion likely to be clinically affected	70%	III
Effectiveness of treatment	High	III
Overall sensitivity of screening process	> 95%	II, III
Repeat specimen rate (per 100,000)	†	
False-positive rate at clinical referral	< 5%	III

* See page 24.
† The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000.

The available data for medium-chain acyl-CoA dehydrogenase deficiency are summarised in Table 46.

Defects of long-chain fatty acid catabolism

Several disorders, all of which all affect the oxidation of long-chain fatty acids are included here. These disorders comprise defects in the transport of long-chain fatty acids (C₁₂ to C₂₀) into the mitochondria and their subsequent degradation in long-chain section of the β -oxidation spiral. The individual enzymes involved, listed in sequence of action, are:

- carnitine palmitoyltransferase type I
- carnitine–acylcarnitine translocase
- carnitine palmitoyltransferase type II
- very long-chain acyl-CoA dehydrogenase (Until very recently, this enzyme has been confused with long-chain acyl-CoA dehydrogenase. All patients previously described as having long-chain acyl-CoA dehydrogenase probably had very long-chain acyl-CoA dehydrogenase deficiency.)
- long-chain enoyl-CoA hydratase – mitochondrial trifunctional enzyme (Isolated deficiencies of these enzymes have not yet been identified, although combined defects with long-chain 3-hydroxyacyl-CoA dehydrogenase (trifunctional enzyme deficiency) is known.)
- long-chain 3-hydroxyacyl-CoA dehydrogenase – mitochondrial trifunctional enzyme
- long-chain thiolase – mitochondrial trifunctional enzyme (Isolated deficiencies of these enzymes have not yet been identified, although combined defects with long-chain 3-hydroxyacyl-CoA dehydrogenase (trifunctional enzyme deficiency) are known).

Systemic carnitine deficiency caused by defective plasma membrane carnitine transporter also affects long-chain fatty acid oxidation. Carnitine palmitoyltransferase type I deficiency cannot be detected at present by neonatal screening and will not be considered further.

For additional information on all these disorders and their associated biochemistry, see recent reviews.^{426,427}

Prevalence

No secure data are available on prevalence. Most of these disorders have only been fully characterised within the last decade, their clinical presentations are varied and non-specific, and laboratory diagnosis is often difficult even in specialised centres. Cases are being diagnosed in rapidly-increasing numbers – in order of frequency, medium-chain acyl-CoA dehydrogenase deficiency > long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency > very long-chain acyl-CoA dehydrogenase deficiency > systemic carnitine deficiency > carnitine palmitoyltransferase type II severe deficiency. The best guess is that the early-presenting forms of these disorders may have a combined frequency of between 40% and 80% of that for medium-chain acyl-CoA dehydrogenase deficiency.

Natural history of the untreated condition

There is a wide range of severity and clinical presentation for all these disorders but they share a general pattern. Severe cases of carnitine palmitoyltransferase type II deficiency may produce congenital defects and tend to be rapidly fatal. For others, the range of initial symptoms is similar to that seen in medium-chain acyl-CoA dehydrogenase deficiency (Table 45) but with earlier onset, greater severity, and additional features. Neonatally,

there is a tendency towards cardiac arrest, particularly in carnitine–acylcarnitine translocase deficiency, and cardiac symptoms, particularly cardiomyopathy, are common. Reye-like hypoglycaemic episodes are a less consistent feature than in medium-chain acyl-CoA dehydrogenase deficiency. While chronic presentations are rare in medium-chain acyl-CoA dehydrogenase deficiency, long-chain defects may produce failure to thrive, diarrhoea, recurrent vomiting, hypotonia or cardiomyopathy without any indication of acute metabolic decompensation. Surviving patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency often develop severe peripheral neuropathy and an unusual type of retinopathy.⁴²⁸ There is also a maternal effect in this disorder: pregnant women carrying an affected foetus tend to develop fatty liver of pregnancy and other life-threatening complications, which resolve promptly after delivery.

Mild cases of carnitine palmitoyltransferase type II deficiency and, more rarely, very long-chain acyl-CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency can present as myopathy with rhabdomyolysis in the teens or adulthood.

Treatment and outcome

As in medium-chain acyl-CoA dehydrogenase deficiency, it is important to avoid metabolic stresses that would (in normal individuals) stimulate fatty acid catabolism. However, in most cases this is not sufficient to prevent chronic problems developing, particularly cardiomyopathy. A carbohydrate-rich diet is usually prescribed, with restriction of long-chain fats (the normal form of fat in food) and their replacement by medium-chain triglycerides. Although rather unusual and, in its strictest form requiring special culinary manoeuvres, this diet has proved highly effective in a number of patients. The myopathy and neuropathy of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency has recently been shown to respond to prednisone.⁴²⁹ Presently, very limited experience suggests that carnitine-acylcarnitine translocase is essentially untreatable, at least in its more severe forms.

The disorders of long-chain fatty acid oxidation have only quite recently been characterised and there is no long-term experience of outcome. By analogy with medium-chain acyl-CoA dehydrogenase deficiency, it is expected that the susceptibility to acute episodes will decrease with age. Myopathy may tend to get worse, though ample intake of medium-chain triglyceride may prevent this.

Systemic carnitine deficiency stands out from the other defects in this section in that it is very easily treated by large oral doses of L-carnitine.

Other benefits of early diagnosis

Prenatal diagnosis is available for all these disorders. Given their relative severity and the risk to the mother in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, there is a good case for offering prenatal diagnosis to most affected families with a view to termination of affected pregnancies.

Neonatal screening

There is no experience of whole-population screening for any of the disorders of long-chain fatty acid oxidation. There is, however, rapidly-accumulating experience in the diagnosis of symptomatic cases of these disorders by acyl-carnitine profiling.^{52,430} In a number of cases it has been possible to recover the stored Guthrie card and demonstrate that the metabolic defect would have been detectable neonatally.

It seems likely that mild cases of carnitine palmitoyltransferase type II deficiency will not be detected.

The diagnostic acyl-carnitine patterns in defects of long-chain fatty acid oxidation are less clearly defined than those in the other groups of organic acid disorders, and there may be confusing non-specific abnormalities in children seriously ill from other causes. The acyl-carnitine profile will give an indication of which disease(s) to consider first but confirmation/differential diagnosis will always be necessary. In general, confirmation of presumptive diagnoses is more difficult in these disorders than in medium-chain acyl-CoA dehydrogenase deficiency. Support for presumptive diagnoses may be obtained by analysing monocarboxylic acids in the dried blood spot⁴³¹ but, in general, more invasive investigations are required. For this group of disorders, the risk of catastrophe in the neonatal period is so great that, in most cases, immediate hospital-based investigation using lymphocytes from a venous blood sample will be justified. In Europe, approximately 90% of disease-causing mutations in the long-chain 3-hydroxyacyl-CoA dehydrogenase gene are C→G 1528.⁴³² This may be detected in dried blood samples, giving an immediate confirmation in homozygous cases.

Overall case for screening

Collectively these diseases are relatively common. They are life-threatening or produce severe chronic disability. Treatment is reasonably effective. Case-finding by neonatal screening would seem to be justified even though sensitivity has yet to be

determined. For screening to be practicable, specificity would need to be high.

Current status

While sample introduction by electrospray is now being phased in, much of the earlier work in the Pittsburgh supplementary screening programme used methods that are relatively insensitive to long-chain acylcarnitines and would thus not reliably detect these disorders. Thus, experience of large-scale screening for long-chain defects is still lacking.

A summary of the available data for defects of long-chain fatty acid catabolism is given in *Table 47*.

Short-chain acyl-CoA dehydrogenase deficiency

This disorder has been described in about a dozen patients who have had variable clinical expression.⁴³³ It results in the excretion of excessive ethylmalonic acid in urine, reflecting intramitochondrial accumulation of butyryl-CoA. A polymorphism of the short-chain acyl-CoA dehydrogenase gene (G→A 625), which is present in the homozygous state in 4–7% of the normal population, seems to interact with other genetic defects to produce a more severe phenotype: ethylmalonic aciduria is found in patients with other metabolic disorders affecting energy metabolism when the G→A 625 polymorphism is present.^{434,435}

Short-chain acyl-CoA dehydrogenase deficiency can be detected by the presence of butyrylcarnitine in dried blood spots⁴⁵ and could thus be included in the tandem MS screen. However, the uncertainty surrounding this condition and the lack of any

accepted treatment makes it unsuitable for routine neonatal screening. It can be specifically excluded if selected reaction monitoring is used (see chapter 4). A prospective research study without clinical intervention is needed but could be difficult to conduct without causing great parental anxiety.

Glutaryl-CoA dehydrogenase deficiency

Glutaryl-CoA dehydrogenase is a mitochondrial enzyme involved in the catabolic pathways of lysine and tryptophan. Its deficiency leads to accumulation of glutaric acids in tissues and body fluids. Glutaryl-CoA dehydrogenase deficiency is often referred to as glutaric acidemia type I or glutaric aciduria type I, though not all patients show a glutaric acidemia or aciduria.⁴⁰⁸

Prevalence

No definitive data are available. The disease is widespread among a variety of racial groups and is probably very much underdiagnosed. For patients who do not excrete excessive glutaric acid, the only methods of diagnosis are acylcarnitine assay in plasma or assay of glutaryl-CoA dehydrogenation in fibroblasts or lymphocytes. Neither assay has been widely available. The figure of 1 in 40,000 births adopted here is a conservative estimate.

Natural history of the untreated condition

Over 100 cases of glutaryl-CoA dehydrogenase deficiency have been reported in some detail and the natural history, although not the underlying pathology, is fairly well understood.^{408,436–439} Typically glutaryl-CoA dehydrogenase deficiency presents

TABLE 47 Summary table for defects of long-chain fatty acid catabolism

		Classification*
Expected incidence (cases per 100,000)	3	III
Proportion likely to be clinically affected	> 95%	III
Effectiveness of treatment	High to medium †	IV
Overall sensitivity of screening process	> 95%	III
Repeat specimen rate (per 100,000)	‡	
False-positive rate at clinical referral	? < 10%	III

* See page 24.
† Except for severe neonatal presentations.
‡ The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000 but the sensitivity for long-chain acyl-carnitines may have been limited.

acutely in a child who has previously appeared well or shown only minor neurological symptoms. Macrocephaly from birth or excessive increase in head circumference in infancy is shown by approximately 70% of patients. If subjected to neuroimaging (MRI or CT scans), many show delayed myelination and fronto-temporal atrophy. In general, acute attacks are triggered by intestinal or upper respiratory tract infection, with average onset time at 12 months (range, 3–37 months). As patients mature their susceptibility to further attacks seems to decrease. Following an acute episode, patients usually show usually an irreversible loss of motor skills, with severe dystonia and dyskinesias. Intellect may be well preserved. Untreated, patients may have further acute attacks, leading eventually to death, but those surviving may later be labelled as having cerebral palsy or even as having had poliomyelitis. Approximately 20% of patients show a more insidious onset with sub-acute motor delay and progressive dystonic cerebral palsy.⁴³⁷ Asymptomatic adults are known, even from families where a sibling may have been profoundly affected.⁴⁴⁰

Treatment and outcome

Dietary restriction of lysine and tryptophan will reduce the flux through the glutaryl-CoA dehydrogenase-requiring pathway but, once an acute attack has occurred, the results of such treatment have been extremely modest with either no response or a limited recovery of some functions.⁴⁴¹ Better results have been achieved when treatment has started in the presymptomatic phase. Approaches have differed: some have used long-term dietary restriction, riboflavin, and/or vigabatrin but clear evidence of efficacy is lacking. Probably the most important component of treatment is vigorous intervention during acute infectious illness in order to reduce catabolic stress,⁴⁴² although continuous carnitine supplementation is also supported by

many workers. Thus, in an on-going multicentre study, 20 from 21 patients diagnosed presymptomatically and treated by crisis intervention and carnitine (eight without any dietary restriction), have developed normally well beyond the mean age of onset in untreated children. The remaining patient has other problems probably not related to the glutaryl-CoA dehydrogenase deficiency.⁴³⁷

Other benefits of early diagnosis

Glutaryl-CoA dehydrogenase deficiency has long been considered a devastating and untreatable disorder and, despite the encouraging preliminary results described above, there will probably continue to be a role for early genetic counselling and probably prenatal diagnosis in the management of affected families.

Neonatal screening

Glutaryl-CoA dehydrogenase deficiency can be detected by finding increased glutarylcarnitine in blood.^{45,405,430} The Pittsburgh programme has detected five patients using neonatal dried blood spot samples. There has, however, been no formal assessment of sensitivity. Differential diagnosis must include glutaric aciduria type II. Initial confirmation may be obtained from organic acid analysis in a random urine sample but, ultimately, the diagnosis should be established by glutaryl-CoA decarboxylation assay using fibroblasts or lymphocytes.

Overall case for screening

Glutaryl-CoA dehydrogenase deficiency is relatively common, extremely serious, and there are indications that presymptomatic treatment can largely prevent disease progression.

The available data for glutaryl-CoA dehydrogenase deficiency are given in *Table 48*.

TABLE 48 Summary table for glutaryl-CoA dehydrogenase deficiency

		Classification*
Expected incidence (cases per 100,000)	2	III
Proportion likely to be clinically affected	80%	III
Effectiveness of treatment	? Moderate	IV
Overall sensitivity of screening process	> 95%	III
Repeat specimen rate (per 100,000)	†	
False-positive rate at clinical referral	? < 5%	III

* See page 24.
† The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000.

Glutaric aciduria type II

The term glutaric aciduria type II is applied to a group of conditions in which the dehydrogenation of many different acyl-CoA esters is impaired because of some defect in the electron-transfer chain which accepts reducing equivalents generated during the dehydrogenation reactions. These disorders are also known as multiple acyl-CoA dehydrogenase deficiencies or, more correctly, multiple acyl-CoA dehydrogenase deficiencies, since the dehydrogenases themselves are fully functional if assayed in isolation. The best-characterised metabolic defects involve either electron-transfer flavoprotein or electron-transfer flavoprotein dehydrogenase. The acyl-CoA dehydrogenases involved include isovaleryl-CoA dehydrogenase (see page 67) and glutaryl-CoA dehydrogenase (see page 76), as well as the short, medium, and very-long-chain acyl-CoA dehydrogenases (see pages 76, 70 and 74), and the pattern of accumulating metabolites is very complex. Milder variants show particularly metabolites derived from short- and medium-chain length fatty acyl-CoA esters; these are sometimes referred to as ethylmalonic-adipic acidurias.

Prevalence

Relatively rare. No reliable data are available.

Natural history of the untreated condition

There is a wide range of clinical severity.⁴⁴³ The most severely affected patients show multiple congenital abnormalities and usually die within a few days of birth. Patients with slightly less severe forms present in the first week or so of life with acidosis and profound metabolic decompensation combining many of the features seen in isovaleric acidemia (including the sweaty-feet odour) with those of defects of straight-chain fatty acids. Those surviving the neonatal period usually die in the course of further

episodes of metabolic decompensation or fatty infiltration of the heart. Milder cases may present later in infancy with an acute Reye-like episode, reminiscent of those seen in medium-chain acyl-CoA dehydrogenase deficiency, or may follow a more chronic course with poor feeding, hypotonia and general failure to thrive. A patient who developed dystonic posturing as seen in glutaryl-CoA dehydrogenase deficiency has also been reported. As with the long-chain fatty acid oxidation defects, patients with very mild forms develop myopathy in their teenage years or later.

Treatment and outcome

There is no effective treatment for the severe forms. Milder variants will benefit from a diet high in carbohydrate and low in fat and protein. As with the individual dehydrogenase deficiencies, steps must be taken to reduce catabolism during intercurrent illness. Some patients with mild variants respond well to high-dosage riboflavin supplementation and may take an essentially normal diet.

Other benefits of early diagnosis

Genetic counselling and prenatal diagnosis are appropriate in the management of families with all but the mildest variants.

Neonatal screening

Severe and moderate forms of glutaric aciduria type II are detectable by screening for abnormal acylcarnitines. No information is available as to sensitivity for very mild variants.

Overall case for screening

Except for the provision of genetic information, there is little benefit in diagnosing early-presenting cases. Moderately severe and milder cases can benefit considerably from early diagnosis.

The available data for glutaric aciduria type II are given in *Table 49*.

TABLE 49 Summary table for glutaric aciduria type II

		Classification*
Expected incidence (cases per 100,000)	2	III
Proportion likely to be clinically affected	> 95%	III
Effectiveness of treatment	High to low, depending on type	IV
Overall sensitivity of screening process	? > 90%	III
Repeat specimen rate (per 100,000)	†	
False-positive rate at clinical referral (%)	< 5%	III

* See page 24.
 † The repeat specimen rate for tandem MS screening (all disorders) in Pittsburgh is approximately 35 per 100,000.

Other possible screens

Peroxisomal disorders

Peroxisomal disorders form a large and complicated group of disorders which have only been recognised biochemically for some 15 years.⁴⁴⁴ A major group of these disorders, including Zellweger syndrome, are characterised by loss of many peroxisomal functions, including those of β -oxidation of bile acids. Another group of patients show more specific disorders of peroxisomal β -oxidation. Some patients of this type are diagnosed at birth on account of characteristic morphological abnormalities, whereas the milder forms may not be diagnosed for months or years. These are multi-organ diseases and are progressive and largely incurable.

Preliminary observations suggest that patients in both these groups may be diagnosed by demonstration of abnormal bile acid taurine conjugates in their neonatal dried blood spots.^{54,5} In view of the lack of useful treatment, the main advantages of early diagnosis would be the genetic information it would provide to the family as a whole, and the avoidance of a protracted period of clinical investigation prior to diagnosis.

Biliary atresia

Biliary atresia results from a progressive fibrotic obstruction of the ducts of part or all of the extra-hepatic biliary tree. This process occurs in the perinatal period but the underlying cause is not understood. Without surgery, most children with biliary atresia die within 2 years of birth. Early surgery (Kasai or similar procedure) gives a 90%

chance of re-establishing bile drainage. The longer surgery is delayed, the worse the outlook. Ideally, the operation should be performed before 8 weeks of age.⁴⁴⁵ In the UK, 46% of affected children are operated on after this age at present.⁴⁴⁶ There is a good case for screening for this condition, provided that a suitable test can be found.⁵⁶ Over the period March 1993 to February 1995, 90 cases were confirmed in the UK, giving an incidence of 1 in 18,000 live births.⁴⁴⁶

At present, there is no accepted screening method for early detection of biliary atresia but quantitation of conjugated bilirubin or bile acid conjugates in dried blood spots by negative MS may provide a viable approach.^{54,55} This method uses the same instrumentation and derivatised extract as the amino acid- and acyl-carnitine based screens (see chapter 4), but would require some resetting of the mass spectrometer and would increase the analysis time. This method of screening would not be specific for biliary atresia and would detect other forms of liver disease (*Table 50*). A careful follow-up protocol would need to be devised.

Disorders excluded

In the past, metabolic disorders that have turned out to be benign have been included in neonatal screening programmes, histidinaemia being the best-known example.^{98,387,448,449} A number of other disorders of amino acid metabolism, some of which may be detected by chromatography or tandem MS profiling, are believed to be harmless, or at least of uncertain status. These include hyperlysinaemia,

TABLE 50 Causes of conjugated hyperbilirubinaemia in the neonate

Diagnostic group	Frequency per 100,000 births
Extrahepatic biliary atresia	7
Idiopathic neonatal hepatitis	12.5
Intra-hepatic cholestasis with or without paucity of bile ducts	1.4
α_1 -antitrypsin deficiency (minority of cases)	2.5
Bacterial sepsis	< 1
Cytomegalovirus hepatitis	< 1
Rubella or herpes hepatitis	< 1
Hypothyroidism (minority of cases)	< 1
Galactosaemia (proportion uncertain)	2.5 ?
<i>Data from Schiff & Schiffer,⁴⁴⁷ slightly modified.</i>	

sarcosinaemia, hyperprolinaemia type 1 and cystathioninuria.⁴ These have all been excluded from this review.

Two other disorders leading to abnormal blood-amino acid profiles may sometimes be detected neonatally but are not reviewed in detail here. **Non-ketotic hyperglycinaemia** is a serious neurological disease, usually presenting in the neonatal period and without effective treatment.⁴⁵⁰ Plasma glycine levels are usually increased 2–8 times normal but, in the neonatal period, the large normal range, depending greatly on nutritional status, leads to poor sensitivity and specificity. Lack of specificity is a particular problem as definitive diagnosis is based on measurement of glycine in cerebrospinal fluid. This disorder is thus unsuitable for including in a neonatal screening programme.

Disorders of pyruvate metabolism may sometimes be detected through the associated hyperalaninaemia. This is a complex group of disorders, including both primary defects of pyruvate metabolism and the mitochondrial respiratory chain defects. There have been only occasional instances of such disorders being diagnosed by chromatographic screening of amino acids and there are no data on sensitivity or specificity.

Other disorders have been screened for on a pilot basis but have not achieved significant acceptance. These include α_1 -antitrypsin deficiency^{451–453} and familial hypercholesterolaemia,⁴⁵⁴ in both cases lack of support is largely due to uncertainty as to how to manage the cases detected. Other diseases, such as adenosine deaminase deficiency and glutathione synthase deficiency, are extremely rare and, when they have been screened for on an individual basis, it is either because of a special clinical interest or, almost, as a hobby. Screening for erythrocyte glucose-6-phosphate dehydrogenase deficiency is performed in some countries with a high incidence, particularly where exposure to anti-malarial drugs is likely to put affected individuals at special risk. It is not offered on a population-wide basis in the UK. None of these disorders is dealt with in this assessment.

The review of the literature also revealed a number of screening programmes based on urine, most of which have been discontinued. Because our brief was to study additions to the existing UK national programme, which is based on blood, they are not considered further.

Chapter 10

Quality of the literature on individual diseases

For the most part the quality of the literature uncovered in this study has fallen far short what one would normally require as the basis for a systematic review.¹ The gradings given are summarised in *Table 51*. The quality standards referred are those described in chapter 5.

Expected incidence

Only when a disease has already been screened for systematically, either in terms of affected cases

or gene frequency, **in the population under consideration**, is it possible to provide a firm estimate of the true incidence (given quality grade I). There are major theoretical problems in extrapolating data obtained from one geographical population to others, even within the UK (see chapter 2). Estimates based on international surveys have therefore been graded II. For many diseases, it has only been possible to base estimates on the frequency with which the disease is diagnosed clinically (III). However, unless a disease has a prolonged course and a highly suggestive clinical

TABLE 51 Strength of literature evidence on individual diseases

Type of information	Incidence	Proportion affected	Effectiveness of treatment	Sensitivity of screening	Repeat sample rate	False-positive rate
Range of scores	I-III	I-III	I-IV	I-III	I-III	I-III
Disease or disease group						
Phenylketonuria	I	II	II-2	I	I	I
Congenital hypothyroidism	I	I	II-2	I	I	I-II
Tyrosinaemia type I	Ia	III	III	I	Ia	III
Homocystinuria	Ia	II	II-2	I	II	III
Maple syrup urine disease	II	I	III	I	II	III
Urea cycle disorders	III	III	IV	a	a	a
Galactosaemia	I	I	II-2	I	b	I
Cystic fibrosis	I	c	d	I	I	I
Congenital adrenal hyperplasia	Ia	III	III	I	I	–
Duchenne muscular dystrophy	I	c	–	I	I	I
Biotinidase deficiency	II	I-II	III	I	I	III
Methylmalonic, propionic and isovaleric acidurias	II-III	III	IV	III	e	III
Other defects of branched-chain acyl-CoA metabolism	III	III	IV	III	e	III
Medium-chain acyl-CoA dehydrogenase deficiency	Ia	III	III	II-II	e	III
Defects of long-chain fatty acid catabolism	III	III	IV	III	e	III
Glutaryl-CoA dehydrogenase deficiency	III	III	IV	III	e	III
Glutaric aciduria type II	III	III	IV	III	e	III

a,Varies with the individual disease.

b,Several methods in use. Repeat rate very dependent on screening method.

c,By definition of the disease all patients are clinically affected.

d,A range of types of evidence.

e,Unpublished data only.

presentation, when case ascertainment may be nearly complete, it is likely that incidence derived from surveys of clinically-diagnosed cases will be an underestimate. Family histories of newly-diagnosed patients often reveal that one or more siblings have died in circumstances compatible with them having had the same disorder as the newly-diagnosed patient but with the death(s) being ascribed to some other cause. There must, therefore, be many similar cases – perhaps the only affected child in a family – which escape even retrospective diagnosis (see, for example, pages 70–71).

Natural history of clinically-detected disease

For nearly all the diseases covered in chapters 6–9, information on the natural history of the untreated condition is based on clinically-presenting cases (quality III). Only very rarely has it been possible to relate this directly to the patient population revealed by systematic neonatal screening. A disease population diagnosed by biochemical screening (quality I studies) may differ significantly from carefully collected clinically-diagnosed populations (quality II studies); in the example of cystathionine β -synthase deficiency, this is because the milder pyridoxine-responsive variants tend to be missed by screening but, in the example of congenital hypothyroidism, it is because of the existence of transient and late-developing forms of the disease and, also, because clinical diagnosis tends to be less sensitive than biochemical diagnosis. Thus, for the most part, we have been forced to extrapolate from the known biochemistry of a disease and the reported range of clinical severity to estimate the likely characteristics of the population detected by screening. In few diseases, cystic fibrosis, medium-chain acyl-CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, the degree of genetic homogeneity of the clinically-defined disease would soon alert us to any significant differences between the clinically-diagnosed and screening-diagnosed populations.

Response to treatment

The literature on treatment is also lacking in systematic studies. Except for specific components

of treatment in relatively common disorders, cystic fibrosis being the main example, there have been no controlled trials. For many of the organic acid disorders, initial interventions are life-saving and the **short-term** difference in reported outcome with or without treatment appear sufficiently striking to provide “evidence equivalent to well-designed studies of level I or level II”.¹ However, the medium-term effectiveness of treatment, as actually experienced in a major metabolic treatment centre, may be less good than indicated in the literature because of reporting bias – failure tends not to be well-publicised.

Long-term outcome is more difficult to assess, particularly for the rarer diseases, and is often less good than short-term. The example of galactosaemia shows how a problematic long-term outcome can be generally ignored for some years after it is first demonstrated. In general, long-term studies compare treated populations with unaffected controls and, when this is done carefully and on a sufficient scale, and the natural history of the untreated condition is well-documented, it can provide good quality (equivalent to grade I) evidence of the effectiveness of treatment and of its limitations. The data from the UK Phenylketonuria and Hypothyroidism Registers fall into this category. In a few instances, particularly cystic fibrosis and galactosaemia, the effects of early diagnosis have been investigated specifically by comparing screened and unscreened populations (quality II-2) but, unless the control group has been carefully chosen, there tend to be confounding factors, particularly the different qualities of treatment available for the two populations being compared.

Unexpected late complications may arise on prolonged survival, for example, renal failure in methylmalonic acidaemia. However, radical therapies such as organ transplant, gene therapy, and novel drugs, such as NTBC (for tyrosinaemia type I), are still being developed and even relatively simple improvements in treatment, such as nocturnal nasogastric feeding, may result in marked improvement in outcome. Information on both these topics tend to come from quality III or IV studies.

Chapter 11

The psychological costs and benefits of neonatal screening

Introduction

The psychological issues surrounding neonatal screening are assessed in this chapter as an attempt to consider intangible costs and benefits. The focus is on the direct psychological impact of neonatal screening on infants and their parents, and an analysis of methods whereby any negative effects can be improved through appropriate changes in medical practice. The acceptability of neonatal screening to parents is also assessed, to determine the demand for early diagnosis of genetic disease within the general population.

The nature of the literature

The review of the psychological literature on the direct psychological impact of neonatal screening on infants and their parents was subject to the systematic searching strategy common to the other areas in this document and described in chapter 5. However, at present neither the Cochrane Collaboration nor the Centre for Reviews and Dissemination at York have produced a comparable rating system for grading evidence from psychological studies. As a result, the review was conducted in the traditional narrative manner, drawing upon the systematic literature search. Psychological research on the direct impact of neonatal screening is not uniform in its focus nor does there appear to be a coherent, chronological trend. Furthermore, material on psychological issues is found scattered throughout the general literature on neonatal screening.

Criteria for exclusion/inclusion

It was decided to exclude papers that focused on outdated procedures with the emphasis on the impact of those procedures. Similarly excluded were papers emphasising diseases that were no longer subject to screening for scientific reasons. However, if the focus of a paper meeting these criteria was on psychological impact *per se*, then it was judged on the basis of its broader contribution to psychological evidence. Hence, papers included were those which focused on procedures and diseases that are currently subject to screening, and which also had a significant contribution to make towards explaining the direct psychological impact

of screening on infants and their families. From the papers identified in the original search, 57 from 758 met the relevant criteria. The study aims of the literature identified are summarised in a table at the beginning of each section of this chapter.

Organisation of the literature

There has been a limited focus in the literature on the psychological implications of established screening programmes, such as those for phenylketonuria and congenital hypothyroidism. There has been greater focus on the psychological issues surrounding more controversial screening programmes, such as those for cystic fibrosis and Duchenne muscular dystrophy. This may reflect the fact that there is as yet no known cure for these diseases (although treatments may be available to alleviate symptoms or slow the progression of the disease) and, hence, the benefits of early detection are less clear-cut. Other inborn errors of metabolism, such as galactosaemia and homocystinuria, which are potentially screenable in the neonatal period, are hardly considered at all.

Organisation of the review

The review is organised in two main parts. The first deals with the direct psychological impact of screening on both infants and their parents, examining the short- and long-term effects of the medical interventions involved in the testing process, and of parents' experiences of the diagnosis of genetic disease through screening. The psychological effects of screening are compared with the impact of diagnosis through symptom presentation. The acceptability of screening to parents is also considered. The psychological implications of diagnostic errors in neonatal screening are discussed. Finally, the effects of neonatal screening on reproductive decision-making are examined, in order to investigate the effects of early diagnosis on the frequency of genetic disease within individual families as well as in the population as a whole.

The second part of the review outlines the way in which the impact of diagnosis through screening could be managed, providing some guidelines of 'good practice' with regard to the actual process

of neonatal screening. This includes a discussion of the psychological impact of the current practices involved in neonatal screening, including the period between screening and diagnosis, the provision of information to parents before screening, and the issues surrounding the obtaining of informed consent. The review therefore highlights timely and problematic questions about the development of science in medical practice, the provision of meaningful information to parents and the ethics of informed consent.

The direct psychological impact of neonatal screening

This part of the review explores the direct psychological impact of the screening process on both infants and their parents. It begins with a consideration of the psychological effects of the medical procedures involved in screening, assessing infants' and parents' experiences of blood sampling via the heel-prick test. This is followed by a discussion of the effects of diagnosis through neonatal screening on parents in comparison with symptom-based diagnosis. While research in this area has tended to focus on the psychological impact on parents of false-positive test results (see page 90 below), a limited amount of research has also focused on responses to screening in the parents of affected children. A third area of discussion focuses on parental support for neonatal screening among parents of screened and clinically-diagnosed

children. Finally, there is an examination of the effects of neonatal screening on reproductive decision-making, identifying parental attitudes and behaviour in relation to prenatal diagnosis, termination of pregnancies and restrictions in family size through the use of contraception.

Medical procedures involved in neonatal screening: pain and heel-prick blood sampling

One potential psychological effect of neonatal screening relates to infants' and parents' experiences of the medical procedures involved in obtaining blood samples for screening. Blood samples are obtained through the heel-prick test, where the baby's heel is pricked manually and then gently squeezed until enough blood is obtained for the test.^{455,456} In many cases, more than one prick may be needed,⁴⁵⁷ especially if the procedure is performed by someone inexperienced.⁴⁵⁶ In Britain, it is usually performed by the midwife a few days after birth when mother and baby have returned home.⁴⁵⁸ The infant's mother or father are normally present, which minimises problems relating to separation anxiety and its effect on the parent-child bond.⁴⁵⁹

Infant pain responses to the heel-prick

A number of recent studies have found that the heel-prick test is a painful procedure for infants (Table 52). This is seen in behavioural responses such as the immediate withdrawal of the affected foot followed by crying and vigorous gross motor

TABLE 52 Pain and the heel-prick test: a summary of study aims

Study	Aim of study
Infant pain response to heel-prick	
Field & Goldson, 1984 ⁴⁶⁹ (USA)	To monitor behavioural state, heart rate, and respiration in term and pre-term neonates during heel-prick procedures.
Owens & Todt, 1984 ⁴⁶⁵ (USA)	To observe the neonatal reaction to a heel prick.
Franck, 1986 ⁴⁵⁵ (USA)	To quantitatively describe behaviour of infants caused by pain.
Grunau & Craig, 1987 ⁴⁶² (Canada)	To observe the facial movement and vocalisation to discomfort and pain as a function of four sleep/waking states.
Campos <i>et al</i> , 1989 ⁴⁶⁰ (USA)	A prospective analysis to investigate the risk factors for emotional/behavioural problems in adolescents and parental stress and symptoms.
Johnston <i>et al</i> , 1993 ⁴⁶³ (Canada)	To assess the behavioural responses of infants to pain stimuli across a range of developmental ages.
McIntosh <i>et al</i> , 1993 ⁴⁶⁴ (Scotland)	To investigate the variability of commonly-monitored physiological parameters in pre-term infants subjected to the pain of a heel-prick, and to evaluate the procedures established to reduce the physiological disturbance.
Campos, 1994 ⁴⁶¹ (USA)	To investigate the effect of two comforting interventions (rocking and pacifiers) for heel-prick pain experienced by infants.
Takai-Kawalami <i>et al</i> , 1995 ⁴⁷⁰ (Japan)	To investigate the cortisol and behavioural responses to stress in newborn infants.

continued

TABLE 52 contd Pain and the heel-prick test: a summary of study aims

Study	Aim of study
Pain alleviation	
Harpin & Rutter, 1983 ⁴⁵⁶ (UK)	A discussion of heel-prick methods to reduce pain.
Field & Goldson, 1984 ⁴⁶⁹ (USA)	See above.
Owens & Todt, 1984 ⁴⁶⁵ (USA)	See above.
Franck, 1986 ⁴⁵⁵ (USA)	See above.
Grunau & Craig, 1987 ⁴⁶² (Canada)	See above.
Campos <i>et al</i> , 1989 ⁴⁶⁰ (USA)	See above.
Johnston <i>et al</i> , 1993 ⁴⁶³ (Canada)	See above.
McIntosh <i>et al</i> , 1993 ⁴⁶⁴ (Scotland)	See above.
Campos, 1994 ⁴⁶¹ (USA)	See above.
Blass & Shah, 1995 ⁴⁶⁶ (USA)	To investigate the effect of sucrose in reducing pain in neonates.
Bucher <i>et al</i> , 1995 ⁴⁶⁸ (Switzerland)	To observe whether sucrose reduces pain reaction to heel lancing in pre-term infants – a placebo-controlled, randomised and masked study.
Haouari <i>et al</i> , 1995 ⁴⁶⁷ (UK)	To investigate the use of sucrose to reduce pain in neonates exposed to heel-prick blood sampling – an RCT.
Takai-Kawalami <i>et al</i> , 1995 ⁴⁷⁰ (Japan)	See above.
Other effects of the heel-prick	
Harpin & Rutter, 1983 ⁴⁵⁶ (UK)	See above.
Polichroniadis, 1989 ⁴⁵⁷ (UK)	To investigate parents' attitudes to neonatal biochemical screening and to assess the amount of anxiety or concern as a result of the screening process.

activity, such as facial grimacing and movement of extremities.^{455,460–463,465,470} There is also an increase in heart-rate⁴⁶⁵ and respiration rate.⁴⁶⁹

Pain alleviation

Despite this pain response, newborn infants are rarely given analgesics or anaesthetics while the heel-prick procedure is being carried out. This is based on the traditional view that neonates only respond reflexively to painful stimuli and are not capable of perceiving pain. Following studies which refute this view,^{455,460–465} researchers have attempted to investigate methods whereby the pain of the heel-prick test can successfully be reduced (as measured through decreases in the frequency of crying, heartbeat rate, respiration rate or cortisol levels). These methods have been found to include:

- the oral administration of sucrose solution to full-term^{466,467} and pre-term⁴⁶⁸ infants
- the use of pacifiers (or dummies) for both full-term^{461,469} and pre-term infants⁴⁶⁹
- the presentation of heart-beat sounds⁴⁷⁰
- the use of soothing rocking movements after completion of the test.⁴⁶¹

Other researchers found that spring-loaded rather than manual lances reduced the pain experienced

by neonates^{456,464} and have consequently called for their widespread use in the UK.

Other effects of the heel-prick test

There has been little investigation of the long-term effects of the heel-prick procedure on infants or the effects which the distress caused has on the infants' parents. While some researchers have commented on the distress of mothers in relation to their babies' pain response to the heel-prick test,⁴⁵⁶ there has been little systematic study of this phenomenon. Polichroniadis⁴⁵⁷ reports that the initial test involved in neonatal screening causes parents little concern, as it merges into the many other checks which their babies experience in the neonatal period. However, no data are presented to support these findings.

Summary

Research indicates that new-born infants do experience pain in response to the heel-prick test as seen through their behavioural and physiological responses. Evidence suggests that this pain can be reduced effectively through the use of new lancing techniques and can be alleviated through drugs or physical comforting strategies. While there is no systematic evidence that the pain of heel-prick procedures causes long-term psychological effects, the short-term pain

experienced by infants should be kept to a minimum.

Diagnosis through neonatal screening: a comparison with the traditional diagnosis of genetic disease

A second psychological implication of neonatal screening relates to parents' short- and long-term experiences of having their child's condition diagnosed through screening. Concern about this has focused on the fact that the early detection of disease means diagnosis through screening occurs before the presence of symptoms. Being told that one's child has a serious genetic disease is likely to be a great shock to parents, for whom there may have been no warning signs. The shock of diagnosis may be harmful for parents and cause problems in

their relationship with the affected child. Alternatively, early diagnosis of a problem may be helpful for parents, allowing them to prepare emotionally and practically for the development of disease in their child, or preventing the worrying delays in obtaining a diagnosis for a child's persistent symptoms. These issues are explored below; parents' experiences of diagnosis through screening are compared with those of parents whose children are diagnosed through the traditional identification of clinical symptoms (*Table 53*).

Psychological implications of traditional clinical diagnosis

Research indicates that the clinical diagnosis of serious genetic disease in a child is emotionally traumatic for parents. Parental reactions involve

TABLE 53 A comparison with traditional diagnosis of genetic disease: a summary of study aims

Study	Aim of study
Psychological implications of traditional clinical diagnosis	
Venters, 1981 ⁴⁷³ (USA)	To investigate families' initial and long-term reactions to chronic and severe childhood illness in the case of cystic fibrosis, and to study coping strategies.
Firth, 1983 ⁴⁷⁶ (UK)	The views and experiences of parents of children with Duchenne muscular dystrophy.
Firth & Wilkinson, 1983 ⁴⁷⁷ (UK)	To assess parental views on neonatal screening for Duchenne muscular dystrophy.
Wilcken <i>et al</i> , 1983 ⁴⁷⁴ (Australia)	To investigate diagnostic delays in cystic fibrosis and the case for neonatal screening.
Holtzman, 1984 ⁴⁷⁸ (USA)	A discussion of the issues surrounding routine neonatal screening for cystic fibrosis.
Accurso <i>et al</i> , 1988 ⁴⁷⁹ (USA)	A summary of the evidence in support of neonatal screening for cystic fibrosis.
Aljader <i>et al</i> , 1990 ⁴⁸⁰ (UK)	To investigate the attitudes towards neonatal screening and prenatal screening of parents of cystic fibrosis children.
Boland & Thompson, 1990 ⁴⁷⁵ (Australia)	To assess the effects of neonatal screening for cystic fibrosis on maternal behaviour.
Helton <i>et al</i> , 1991 ⁴⁷² (USA)	To investigate parental attitudes and emotional response to the diagnosis of cystic fibrosis.
The psychological implications of diagnosis through screening: initial reactions and long-term implications for the parent-child relationship	
Benke, 1975 ⁴⁸¹ (USA)	An overview of the general issues surrounding neonatal screening.
Zellweger & Antonik, 1975 ⁴⁸² (USA)	Neonatal screening of male newborn infants for Duchenne muscular dystrophy.
Bodegard <i>et al</i> , 1983 ⁴⁸³ (Sweden)	To investigate the psychological impact on parents whose neonate receives a false-positive screening test result.
Holtzman, 1984 ⁴⁷⁸ (USA)	See above.
Messer, 1985 ⁴⁸⁴ (Austria)	The issue of parental management of a phenylketonuric child written from personal experience.
Mischler <i>et al</i> , 1987 ⁴⁸⁵ (USA)	To discuss the psychological reactions of patients and their parents to a false-positive screening test result for cystic fibrosis.
Aljader <i>et al</i> , 1990 ⁴⁸⁰ (UK)	See above.
Boland & Thompson, 1990 ⁴⁷⁵ (Australia)	See above.
Helton <i>et al</i> , 1991 ⁴⁷² (USA)	See above.
Tluczek <i>et al</i> , 1991 ⁴⁸⁶ (USA)	To discuss the psychological impact of false-positive results in neonatal screening for cystic fibrosis.
Tluczek <i>et al</i> , 1992 ²⁹⁸ (USA)	Parental reactions to a false-positive test result from neonatal screen for cystic fibrosis.
Bradley <i>et al</i> , 1993 ³⁵⁸ (UK)	An assessment of the acceptability of Duchenne muscular dystrophy neonatal screening of newborn boys.

feelings of shock, denial, anger, guilt, worry and sadness.^{471–473} In addition to this, evidence suggests that psychological problems for parents may be created by misdiagnosis and diagnostic delay of clinical diagnosis. This has been found to occur particularly in relation to cystic fibrosis and Duchenne muscular dystrophy.

Misdiagnosis and delays in the diagnosis of cystic fibrosis

Diagnostic delays involved in cystic fibrosis have been found by a number of studies.^{473,474} Wilcken and colleagues⁴⁷⁴ explored the delay between the first presentation of symptoms and the diagnosis of cystic fibrosis among infants diagnosed before and after the establishment of a neonatal screening programme in Australia. The mean period between the first occurrence of symptoms and diagnosis was 2.6 years for the clinically-diagnosed infants, with 39% of these infants being diagnosed after more than 12 months. By comparison, the testing of the screened babies generally took place between 3 and 6 weeks, with a mean age of diagnosis of 37 days. Other studies indicate that diagnostic delay is often accompanied by misdiagnosis. In a study carried out in Colorado, USA, 86% of the parents of cystic fibrosis children diagnosed clinically ($n = 30$) had experienced incomplete or incorrect diagnosis before receiving the final diagnosis.⁴⁷²

Psychological effects of delays in the diagnosis of cystic fibrosis

The delays involved in the diagnosis of cystic fibrosis have been identified as an area of concern for both psychological and medical reasons. Research shows that diagnostic delays result in long-term anxiety and worry for parents,^{471,475} as well as a sense of guilt that they should have noticed their child's symptoms earlier or that they should have done something to help their child at an earlier point.⁴⁷² In addition, stress and anxiety have been found to result from parents' relationships with the medical professionals involved in the diagnosis of their children.

In some cases, parents reported that their concerns about their child's health and development were labelled as the result of their own psychological problems, thus dismissing their concerns and anxiety.⁴⁷² Similarly, in an Australian study of cystic fibrosis, Boland and Thompson⁴⁷⁵ found that 75% of the mothers of non-screened children and 61% of those of symptomatic-screened children (that is, those who had been screened following the presentation of symptoms) had experienced great degrees of medical scepticism. These included a reluctance to believe their child was sick,

patronising reassurances and expressions of indignation at parents' repeated requests for a second, third or fourth medical opinion. This caused the mothers to feel great distress and resulted in increased bitterness and cynicism towards the medical profession.

Delays in the diagnosis of Duchenne muscular dystrophy

Similar findings have emerged from studies of Duchenne muscular dystrophy. In a study of 53 families, Firth and Wilkinson⁴⁷⁷ found that the average age at diagnosis of the disease was 5.9 years, and the average delay between first symptoms and final diagnosis was 2.5 years. Again, the long delays involved great feelings of concern for parents as they watched their child's symptoms (such as mobility problems and slight developmental delay) worsen in spite of their doctors' reassurances.^{476,477} Many parents felt very bitter about their experiences and felt that the diagnosis should have been made much earlier.

Parents' experiences of diagnostic delay and misdiagnosis have been found to result in their widespread support for neonatal screening. Of the parents of boys with Duchenne muscular dystrophy,⁴⁷⁷ 75% were in favour of neonatal screening. A major reason for this support was that it would avoid the anxiety involved in diagnostic delay.^{476,477} Similar results have been found in relation to parental support of neonatal screening for cystic fibrosis.^{471,472,479} Parental support of neonatal screening is discussed in more detail below (page 89). These results have been used as support for the introduction of neonatal screening programmes for cystic fibrosis and muscular dystrophy. Despite this, however, there have been objections to such programmes, based on the lack of treatment available for these diseases. Definitive evidence that presymptomatic treatment of cystic fibrosis leads to an improved outcome is only just beginning to emerge (see chapter 8), although prior to this there was a widespread belief in its effectiveness.^{474,478}

Psychological implications of diagnosis through screening

The outcome of research on the psychological implications of diagnosis is the suggestion that neonatal screening may be beneficial to parents because it removes the stress of delayed diagnosis. However, it may be that diagnosing a disorder early in infancy creates other sources of psychological stress for parents. Neonatal screening programmes have enabled researchers to explore these psychological consequences, either through studies of the

parents of screened children or studies comparing groups of parents of both clinically-diagnosed and screened children. These researchers have assessed:

- parents' psychological responses to diagnosis through neonatal screening
- the short- and long-term effects of diagnosis via screening on the parent–child bond
- parental attitudes towards screening in terms of their support for screening programmes generally.

Psychological responses to diagnosis through screening

Research has demonstrated that parents' experience of the initial diagnosis of a metabolic disease, such as cystic fibrosis,^{298,471,472,486} phenylketonuria^{484,487} and congenital hypothyroidism,^{483,488} through neonatal screening is emotionally traumatic. Reactions to the diagnosis include crying, feelings of concern, shock, anger, confusion and depression, as well as a sense of disbelief that one's child is ill. This disbelief originates in the fact that the child may have no observable symptoms, because the diagnosis is obtained so early in the child's life. Parents' reactions to diagnosis through screening can thus be seen to be similar to those of parents whose children have been diagnosed clinically (see page 86 above). Research assessing the reactions of parents of children diagnosed through screening or through more traditional means has found no differences in anxiety or depression levels between these two groups,^{471,472} although statistical comparisons were only made in one of these studies.⁴⁷¹ It therefore seems that parents' experience initial emotional difficulties when their children are diagnosed as having a genetic disorder, regardless of how this diagnosis has been obtained. The initial shock of diagnosis when no symptoms have been observed does not appear to create more distress than when a parent is aware that there may be a problem with their child's health, as observed through persistent symptoms.

Psychological effects of screening on parent–infant bonding

It has been suggested that neonatal screening may have deleterious effects on the parent–infant bond either immediately after diagnosis or in the longer term. For example, it has been suggested that screening may cause mothers who had considered their child to be healthy before diagnosis to over-protect the child because of their anxiety about the development of the symptoms involved in the disease.⁴⁷⁵ Alternatively, diagnosis through screening may result in parents distancing themselves from their child, in an attempt to protect themselves from

the difficult emotions engendered by having a child with a serious genetic disease.⁴⁷¹ Concerns about these issues have been raised, particularly in relation to the appropriateness of screening for disorders which could not be treated, such as cystic fibrosis and Duchenne muscular dystrophy. It has been suggested that the early detection of these disorders may have caused more harm than good because of the effect of the constant expectation of the development of an incurable disease on both patient and family.⁴⁸² This has led some writers to assert that infants should not be screened for these disorders, so as to allow them and their parents to enjoy their lives for as long as possible before the disease develops.^{481,482}

To date, there is little evidence to support the view that screening has negative effects on the parent–child relationship. However, the studies which have been carried out differ greatly in methodology, making it difficult to generalise from their findings or to compare one study with another.

In a small-scale study carried out in Wales and the West Midlands, parents of children were asked about their feelings about their child immediately after the diagnosis of cystic fibrosis. Of 29 families, four reported temporarily rejecting their infants, particularly if there were undue delays in the diagnostic procedure, and 16 reported feeling over-protective and more attached to their infants.⁴⁷¹ However, as no comparisons were drawn between infants diagnosed clinically or those diagnosed through neonatal screening, these data fail to provide an insight into any additional psychological effects suffered by parents attributable to diagnosis through neonatal screening for cystic fibrosis.

In a study of 57 parents receiving a false-positive diagnoses of cystic fibrosis in their children, 73% felt no differently about seeing their baby immediately after the diagnosis. Furthermore, 98% felt comfortable touching and holding their babies.⁴⁸⁵ Here then it seems that the diagnosis of a disease through screening did not result in emotional withdrawal or distancing. The psychological effects of finding out that such a diagnosis was a false-positive are explored in more detail below (page 90).

In an Australian study of cystic fibrosis, Boland and Thompson⁴⁷⁵ compared the attitudes and behaviours of the mothers of 29 children diagnosed through newborn screening and 29 children diagnosed through the identification of clinical symptoms. In this study, over-protectiveness was measured through three subscales of the Parental Attitude Research Inventory which assessed 'fostering dependency', 'intrusiveness' and 'excluding

outside influences'. Here, over-protectiveness was defined as a negative quality in mothers and as a quality often found in relationships between sick children and parents.⁴⁸⁹ The results of the study indicated that there were no significant differences in levels of over-protectiveness between mothers of children diagnosed through newborn screening and those diagnosed through clinical symptoms. The considerable length of time involved in obtaining a final diagnosis through clinical symptoms was not found to increase mothers' over-protectiveness but resulted in great personal distress stemming from medical scepticism (see page 86 above).

In Helton and colleagues in-depth interview study⁴⁷² of the impact of the diagnosis of cystic fibrosis on the parent-child relationship, almost all the newborn-screening parents reported that they had attempted to maintain 'normal' relationships with their children. They had tried to maintain similar behavioural expectations and exert the same levels of discipline in relation to their child as they would to a child who was not suffering from a chronic illness. However, 35% reported feeling more protective of their children than they would be of a child without cystic fibrosis, with much of this protectiveness focusing on the child's health and physical symptoms. Rather than distancing themselves from their children, 96% of the parents of screened children reported that the diagnosis made them feel emotionally closer to their children, with 38% reporting a desire to optimise the child's life through ensuring that the child experienced a wide range of opportunities, relationships and events. Similarly, 27% asserted that family relationships were enhanced as a result of the diagnosis, because of the way in which it had resulted in a reordering of values and priorities.

Changes in the mother-child relationship was also assessed in a pilot study for neonatal screening for Duchenne muscular dystrophy carried out in Wales.³⁵⁸ Of nine boys diagnosed through the screening programme, only one of the families experienced above-average levels of trauma, which consequently altered the relationship between mother and child. Here, the mother was observed to handle her baby differently and visitors were no longer encouraged to hold the baby. Bradley and colleagues³⁵⁸ report that while the changes in the treatment of the baby lasted for several weeks, the baby was at no physical or psychological risk and the mother-baby bond was not threatened. It is likely that this situation arose because this family claimed they were not given information about the screening programme and asserted that they would have refused if they had been informed. In addi-

tion, the anxiety experienced by this family was also likely to have been increased by the subsequent identification of muscular dystrophy in an older son in whom signs of clinical abnormality had been recognised before the birth of his younger brother.

A comparison of studies of the impact of screening on the parent-infant bond indicates that screening does not appear to have negative effects on this relationship. Thus, parents of screened children seem no more likely to over-protect their children than those diagnosed clinically, but they are also unlikely to distance themselves from their children as a result of the stress of the diagnosis.^{472,475} However, the different methodologies employed in these studies makes it difficult to compare one with another and, hence, to generalise from them. The studies vary in the way in which parent-child relationships are assessed, with methodologies including standardised questionnaires,⁴⁷⁵ observation of parental behaviour³⁵⁸ and the analysis of parental reports.⁴⁷² In addition, the studies use different measures of 'healthy' or 'normal' parent-child relationships. For example, Helton and colleagues' study⁴⁷² does not see protectiveness in terms of deleterious 'over-protectiveness', as in Boland and Thompson's study,⁴⁷⁵ but as involving a greater 'caring' attitude towards affected children arising reasonably from concerns about their ill-health.

Summary

Research evidence indicates that the shock and anxiety caused by a positive diagnosis through neonatal screening are considerable but that these emotional reactions are no greater or less than those experienced by parents whose child has been clinically diagnosed. There appear to be few differences between these groups of parents in terms of negative effects on the parent-child relationship, either in terms of 'over-protectiveness' or emotional withdrawal and distancing. However, the methodological differences between studies measuring these factors make it difficult to reach firm conclusions.

Parental support for screening

Research indicates that the diagnosis of genetic disorder through neonatal screening is emotionally traumatic for parents but that there are few effects on their relationships with the affected infant (see page 87 above). A further area of research within the psychology of neonatal screening relates to the acceptability of screening to parents in terms of their support of screening programmes (*Table 54*). This is important in assessing the implementation of new programmes or for the extension of existing programmes.

TABLE 54 Parental support for screening: a summary of study aims

Study	Aim of study
Parental support for screening	
Bodegard et al, 1983 ⁴⁸³ (Sweden)	To investigate the psychological impact on parents whose neonate receives a false-positive screening test result.
Firth, 1983 ⁴⁷⁶ (UK)	The views and experiences of parents of a Duchenne muscular dystrophy child.
Firth & Wilkinson, 1983 ⁴⁷⁷ (UK)	To assess parental views on neonatal screening for Duchenne muscular dystrophy.
Aljader et al, 1990 ⁴⁸⁰ (UK)	To investigate the attitudes towards neonatal screening and prenatal screening of parents of cystic fibrosis children.
Boland & Thompson, 1990 ⁴⁷⁵ (Australia)	To assess the effects of neonatal screening for cystic fibrosis on maternal behaviour.
Smith et al, 1990 ³⁶² (Wales)	To investigate the attitudes of mothers to neonatal screening for Duchenne muscular dystrophy.
Helton et al, 1991 ⁴⁷² (USA)	To investigate parental attitudes and emotional response to the diagnosis of cystic fibrosis.

There is considerable evidence to suggest that the majority of parents support diagnosis via screening in relation to cystic fibrosis,^{471,472,475} Duchenne muscular dystrophy,^{358,476,477} and congenital hypothyroidism.⁴⁸³ This is even the case for parents whose children have been wrongly diagnosed with false-positive test results (see below).^{471,483} Mothers of newborn babies in the general population have also been found to be in favour of screening newborn boys for Duchenne muscular dystrophy.³⁶²

Parental support for neonatal screening relates to many different medical and psychological factors which parents believe are advantageous to themselves and their children. Medical advantages include parents' beliefs that early diagnosis may lead to an effective treatment for their child's disorder or that it will at least reduce the seriousness of the effects of the disorder.^{471,472} Psychological advantages include the reduction of the anxiety surrounding diagnostic delay and misdiagnosis (see page 86 above). In addition, parents have been found to support screening from a practical and ethical standpoint. On a practical level, parents feel that screening would mean that they would have more time to make plans about the appropriate finances or housing necessary for the care of a disabled child, such as one suffering from Duchenne muscular dystrophy.⁴⁷⁶ On an ethical level, parents feel that they have a right to be informed as soon as possible about their child's disorder.⁴⁷⁶ A smaller number of parents also value screening for its role in future reproductive planning⁴⁷¹ although, as discussed below (page 93) few parents actually

change their reproductive plans as the result of neonatal screening.⁴⁷²

Psychological implications of false diagnoses

A potential psychological cost of neonatal screening relates to the occurrence of false diagnoses. A significant amount of research has focused on the psychological implications of receiving a false-positive or false-negative diagnosis, either looking at specific disorders such as congenital hypothyroidism^{483,488,490,491} or cystic fibrosis^{298,486,492} or at inborn errors of metabolism more generally.⁴⁹³ In comparison, there is a lack of research on the psychological implications of false-negative results, although some anecdotal reports have been published.

False-positive results

Research carried out on the psychological effects of false-positive diagnoses draws on studies of the parents of children who have recovered from a life-threatening illness experienced early in their lives.⁴⁹⁴⁻⁴⁹⁶ Parents such as these have been found to develop a continuing or recurrent fear about their child's particular vulnerability to injury or accident, manifested by increased visits to doctors and 'over-reaction' to minor complaints. This can lead to parental over-protectiveness resulting in separation difficulties and psychological problems in the child.⁴⁹⁵

The psychological effects of false-positives in neonatal screening were first observed in relation to the identification of a 'PKU anxiety syndrome', involving acute and chronic anxiety in parents whose infants had been falsely diagnosed[†] with

[†] False-positive 'diagnoses' were common in some early screening programmes in the USA, where the initial screen was centralised in state laboratories but follow-up, including repeat samples owing to antibiotic interference or borderline results, relied on local physicians. In UK experience, false-positive diagnoses for phenylketonuria are extremely rare.

phenylketonuria.⁴⁹⁷ Although no data was provided, the report suggested that this anxiety related to parents' beliefs that their infants were or would become mentally retarded. These fears persisted, despite the fact that repeated negative test results had been obtained and explained to the parents.

Since Rothenberg and Sills' observations,⁴⁹⁷ a number of empirical studies have been carried out to assess the psychological impact of false-positive results (*Table 55*). These studies have focused on:

- parents' reactions to the initial (false) diagnosis of genetic disorder

- parental experiences of receiving the results of repeat tests, showing the first test to be false
- the longer-term effects of these experiences on parents' feelings and relationship with their child.

Psychological implications of the initial (false) diagnosis: emotional reactions and the parent–child bond

Investigations of parental reactions in the period between receiving a false-positive diagnosis and a disconfirming repeat test[†] have revealed similar results to studies of parental reactions to true-positive diagnoses. Thus, the majority of parents

TABLE 55 Psychological implications of false diagnoses: a summary of study aims

Study	Aim of study
False-positive results	
Green & Solnit, 1964 ⁴⁹⁵ (Indiana, USA)	A discussion of the reactions to the threatened loss of a child.
Rothenberg & Sills, 1968 ⁴⁹⁷ (USA)	The 'phenylketonuria anxiety syndrome' as a result of false-positive results.
Levy, 1980 ⁴⁹⁶ (USA)	To assess parents' perspectives of their children's health status and their use of paediatric services.
Bodegard <i>et al</i> , 1983 ⁴⁸³ (Sweden)	To investigate the psychological impact on parents whose neonate receives a false-positive screening test result.
Dankert-Roelse <i>et al</i> , 1983 ⁴⁹² (The Netherlands)	False-positive test results in cystic fibrosis screening.
Sorenson <i>et al</i> , 1984 ⁴⁹³ (USA)	To investigate parents' understanding and reaction to the need for a repeat neonatal screening test.
Green, 1986 ⁴⁹⁴ (Indiana, USA)	A discussion of vulnerable child syndrome and its variants.
Tymstra, 1986 ⁴⁸⁸ (The Netherlands)	To establish parents' experiences of false-positive screening test results for congenital hypothyroidism.
Fyro & Bodegard, 1987 ⁴⁹¹ (Sweden)	To investigate psychological reactions of parents to false-positive screening test results of congenital hypothyroidism over a 4-year follow-up period.
Fyro, 1988 ⁴⁹⁰ (Sweden)	To establish life-stress scores in parents receiving a false-positive screening test result.
Fyro & Bodegard, 1988 ⁴⁹⁸ (Sweden)	To investigate psychological reactions of parents to false-positive screening test result in a newly-introduced neonatal screening programme for congenital hypothyroidism.
Heyerdahl, 1988 ⁴⁹⁹ (Norway)	An overview of the psychological problems related to neonatal screening.
Rock <i>et al</i> , 1990 ²⁹⁵ (USA)	To investigate refinements of cystic fibrosis screening methodology necessary to achieve an acceptable sensitivity and specificity: an RCT.
Kerem & Lynch, 1991 ⁵⁰⁰ (Canada)	A discussion of the ethical and social issues associated with cystic fibrosis screening.
Tluczek <i>et al</i> , 1991 ⁴⁸⁶ (USA)	To discuss the psychological impact of false-positive results in neonatal screening for cystic fibrosis.
Wilfond <i>et al</i> , 1991 ⁵⁰¹	A discussion of mutation analysis for cystic fibrosis newborn screening.
Tluczek <i>et al</i> , 1992 ²⁹⁸ (USA)	Parental reactions to a false-positive test result from neonatal screen for cystic fibrosis.
Bradley <i>et al</i> , 1993 ³⁵⁸ (UK)	To assess the acceptability of Duchenne muscular dystrophy neonatal screening of newborn boys.
Balnaves <i>et al</i> , 1995 ²⁸⁹ (Australia)	A presentation of the newborn screening results of an Australian state.
False-negatives	
Heyerdahl, 1988 ⁴⁹⁹ (Norway)	An overview of the psychological problems related to neonatal screening.
Kerem & Lynch, 1991 ⁵⁰⁰ (Canada)	A discussion of the ethical and social issues associated with cystic fibrosis screening.
Farrell & Mischler, 1992 ²⁸⁵ (USA)	An overview of neonatal screening for cystic fibrosis and the main issues regarding efficacy, risks and cost.

[†] It is unusual in UK practice for parents to be given a positive diagnosis which is then retracted – see chapter 3.

report strong emotional reactions to the initial diagnosis of disorder in their child.^{298,483,488} However, as in true-positive diagnoses, parents' relationships with their children are not found to be affected by the identification of a disorder. Parents report no adverse changes in their parenting behaviours or feelings towards their child in the interval between the first (false diagnosis) and second (true diagnosis) test.²⁹⁸ Despite this, a number of studies have found some parents to have lingering concerns about their children's health.^{298,488,493} These concerns have been related to the provision of information about the screening process (see below),^{298,486,488} as well as to the occurrence of health problems in infants prior to the test, such as birth asphyxia and other related problems.²⁹⁸ It is therefore suggested that parents' concerns about their child's test results may relate to their child's predisposition to health problems.

Long-term implications of false-positive results

The long-term effects of false diagnoses through neonatal screening are unclear. Fyro and Bodegard's research on congenital hypothyroidism^{491,498} suggests that there are serious negative effects of false-positives 6–12 months after the screening,⁴⁸³ which may actually worsen over time. Although the majority of parents were found to cope well with the experience of a false-positive test 6–12 months later, 18 families from 102 were judged to have failed to have successfully 'integrated' the experience, in accordance with a hypothetical psychodynamic model. These parents were found to have persistent concerns about their child's health, despite the fact that a negative test result had been obtained and explained to them. Similarly, at a 4-year follow-up, the majority of these parents had still failed to integrate the experience of the false diagnosis and, moreover, many parents who were defined as having successfully integrated the experience at the first follow-up were no longer coping as well. These results have been used to suggest that false-positive results constitute a major psychological risk for parents, who therefore need extensive support and counselling to avoid adverse effects.^{491,498}

As Heyerdahl⁴⁹⁹ points out, however, Fyro and Bodegard's research⁴⁹⁸ has a number of methodological limitations which make its findings questionable. The problems in psychological adaptation found in the research are not compared to a control group of any kind. Thus, no reference can be made to levels of parental anxiety about their children's health in the general population, for example. This criticism can also be made of other studies reporting lingering parental concern about their child's health following false-positive

diagnoses,^{298,488} which are again based only on groups of parents who have experienced a false-positive result. In addition, Fyro and Bodegard's research⁴⁹⁸ is based on a hypothetical psychoanalytical model of integration or adaptation (defined as the process of coping with a traumatic life experience leading to anxiety-free knowledge⁴⁸³), which is used to interpret parents' statements. Such a model is not based on any criteria of reliability or validity, so that no evidence is provided that this is a valuable method of measuring parental adaptation to the experience of a false-positive test result. Criticisms can also be made about the small sample sizes used in many of the studies (for example, Fyro and Bodegard⁴⁹⁸). Further research is therefore required with the use of adequate controls and improved methodology before firm conclusions can be made about the long-term effects of false-positive diagnoses.

Information provision and the communication of test results

Research suggests that parental anxiety following false-positive diagnosis may relate to the type, amount and method of information provided to parents about the screening process. In Bodegard and colleagues' study,⁴⁸³ parents who had adapted poorly to the false diagnosis at 6–12 months, and who had persistent concerns about their children's health, were those who asserted that they had not been provided with information about the testing process. In other studies, parents have been found to attribute their anxiety following a false-positive to the lack of information provided by doctors and midwives, as well as to the delays involved in obtaining the results of the repeat tests when reassurance was given.^{488,492} In addition to this, a study investigating the provision of information to parents about the need to obtain a second blood sample to confirm or disconfirm an initial positive found parents' concerns about their babies' health were related to the type of information provided.⁴⁹³ Parents who had been given a specific reason for a retest, such as that an abnormal or ambiguous result had been obtained, were less likely to be concerned about their children's health than parents who had been given more general reasons, such as that the first results had been lost or that a laboratory error had occurred.

Thluczek and colleagues^{298,486} found a significant correlation between parental knowledge about neonatal screening and the method of information provision. Parents who were informed about the results of a disconfirming sweat test for cystic fibrosis over the telephone were more likely to

misunderstand the meaning of the test result than those informed during a face-to-face encounter with medical personnel. All of the parents who had lingering concerns about their child's health following the test results had been contacted by telephone. The provision of accurate information to parents about the precise procedures which are taking place is therefore critical as well as a face-to-face method of information provision. In addition, it is recommended that the delay period between first and final diagnosis remains as short as possible. These points will be returned to later.

Technological advances in neonatal screening: reducing parental anxiety

It is important to note that the rate of false-positives and false-negatives in neonatal screening varies in relation to the disease tested for and the method used in screening. The psychological implications of false screening results are therefore likely to change as technological advances are made. This can be seen in relation to screening for cystic fibrosis, for example. Prior to the discovery of the cystic fibrosis gene in 1989,⁵⁰⁰ the most effective way to screen for cystic fibrosis was through the assessment of IRT levels in blood samples taken 5–6 days after birth.²⁸⁹ After a positive first and second test, a 'sweat test' was carried out and definitive diagnosis was achieved through the analysis of sweat electrolytes. The ability to detect abnormalities in the CFTR gene using the screening blood sample has allowed the development of alternative protocols that do not require a second blood sample. However, these approaches increase the number of sweat tests required and also raise difficult issues over counselling families with unaffected heterozygous babies (see chapter 8).

False-negative results

Although there have been a number of studies investigating the psychological effects of false-positive diagnoses, to date there have been no studies of the implications of false-negative results. It has been suggested, however, that false-negatives may create a false sense of security for doctors so that the symptoms of a disorder are not identified in a child because of the results of screening. There is thus the potential for reduced alertness to the clinical signs associated with inborn errors of metabolism.^{285,499} This delay in diagnosis may result in the child receiving inappropriate treatment. There may also be psychological costs in terms of parents receiving false reassurances about their child's health when they themselves observe symptoms.⁵⁰⁰ Parents such as these are likely to experience the same anxiety and stress associated with delays

involved in traditional diagnostic techniques (see page 86 above). There is, as yet, no systematic evidence to support these suggestions.

Summary

In summary, the false diagnoses involved in neonatal screening can create psychological problems for parents. These problems relate not only to the emotionally traumatic experience of receiving a (false) positive diagnosis of a genetic disorder but, in the longer term, to the persistent fear or concern that the initial diagnosis was actually correct. Whilst the majority of parents appear to adapt to the experience, a significant proportion may experience some psychological difficulties. The provision of adequate information about the processes involved in neonatal screening may ease the anxiety which some parents feel, as may the method whereby this information is presented to parents. The delay in reporting an accurate diagnosis should be kept to a minimum.

The impact of neonatal screening on reproductive decision-making

A further implication of neonatal screening is its effects on parents' subsequent reproductive decision-making and the future birth of children affected by screened disorders. One of the arguments presented in support of neonatal screening is that early diagnosis and appropriate genetic counselling for parents will prevent the subsequent birth of affected siblings.^{502–504} Neonatal screening will therefore reduce the frequency of genetic disease within individual families, as well as the population as a whole. The influence of neonatal screening on reproductive decision-making may be particularly important in relation to disorders which do not manifest themselves immediately after birth, such as cystic fibrosis or Duchenne muscular dystrophy. If diagnosis relies on the presence of clinical symptoms rather than on screening, a number of similarly-affected children may be born within a particular family before the disorder is identified.

Although there has been extensive research on the attitudes and behaviour of parents of affected children towards prenatal screening and the termination of subsequent affected pregnancies,^{505–508} there has been less research on the influence of neonatal screening on these factors. Research has focused on parental attitudes towards prenatal diagnosis and the termination of affected pregnancies,^{471,472} as well as on parents' actual reproductive behaviour following the birth of an affected child (*Table 56*).^{289,472,509}

TABLE 56 The impact of neonatal screening on reproductive decision-making: a summary of study aims

Study	Aim of study
Parental attitudes towards prenatal screening and termination of affected pregnancies	
Aljader et al, 1990 ⁴⁸⁰ (UK)	To investigate the attitudes towards neonatal screening and prenatal screening of parents of cystic fibrosis children.
Helton et al, 1991 ⁴⁷² (USA)	Parental attitudes toward neonatal screening for cystic fibrosis.
Neonatal screening and reproductive behaviour	
Dankert-Roelse et al, 1983 ⁴⁹² (The Netherlands)	False-positive test results in cystic fibrosis screening.
Helton et al, 1991 ⁴⁷² (USA)	See above.
Hildes et al, 1993 ⁵¹¹ (Canada)	A pilot neonatal screening programme for Duchenne muscular dystrophy: a summary of the reproductive outcome after genetic counselling of families on the pilot programme.
Balnaves et al, 1995 ²⁸⁹ (Australia)	Presentation of the newborn screening results of the state of Victoria, Australia.

Parents' intentions towards future reproduction may diverge from their actual reproductive behaviour. This reflects the fact that reproduction is not always preceded by deliberation. Thus, as with couples in the general population, many of the subsequent pregnancies of parents of children with genetic disease are unplanned. In addition, pregnancy in parents of children with a genetic disease may relate to the need to compensate for a dead or 'defective' child or to show that they are capable of having a 'normal' child.⁵¹⁰

Research on the effect of neonatal screening on reproductive decision-making focuses on reproductive behaviour following screening, subsequent genetic counselling and prenatal screening. It aims to assess the potential long-term 'benefit' of reducing the extent of genetic disease within the population as well as within individual families. Within this context, there has been little research examining parents' experiences of reproductive decision-making following neonatal screening. Apart from studies which point out that reproduction is not mentioned as a benefit of screening by parents, there has been little analysis of whether the information which neonatal screening, subsequent genetic counselling and prenatal diagnosis provides is beneficial or detrimental to parents in coping with the diagnosis and long-term care of a child with a genetic disease.

Parental attitudes towards prenatal diagnosis and termination of affected pregnancies

Studies of attitudes towards prenatal diagnosis and termination of affected pregnancies have shown that there is some support for these options amongst parents of screened children. In 1990, Aljader and colleagues⁴⁸⁰ found that 61% of all couples whose infants had been diagnosed through neonatal screening and 36% of those

whose infants had been diagnosed clinically stated that they would terminate a foetus with cystic fibrosis identified through prenatal diagnosis. No reason is given for why support is higher amongst the parents of screened children. Of the 20 parents who wished to have further children, just over 50% (n = 11) would opt for prenatal diagnosis with a view to the termination of a foetus with cystic fibrosis, 25% (n = 5) were against screening and 20% (n = 4) couples were uncertain of their views.

However, other research indicates that parents of children with cystic fibrosis tend not to support the termination of pregnancies, even though they may opt for prenatal diagnosis in future pregnancies. Thus, in a group of 40 families interviewed about reproductive decision-making, Helton and colleagues⁴⁷² found that 61% would not consider the termination of a pregnancy if cystic fibrosis was diagnosed and 22% were undecided. Although prenatal diagnosis was preferred by 44% of the group, this information was mainly required in order to make financial and insurance decisions as well as to reduce anxiety about the possibility of the disorder during pregnancy. Helton and colleagues found that only 6% of the parents of screened children and 17% of the parents of children diagnosed traditionally spontaneously mentioned family planning as a benefit of newborn screening. They concluded that neonatal screening may not be particularly helpful to parents in making reproductive decisions and that parents may opt for prenatal testing of future pregnancies for psychological and practical rather than reproductive reasons. Similarly, in Aljader and colleagues' study,⁴⁸⁰ only two families supported screening because the information would be considered helpful with regard to future reproductive decisions.⁴⁷¹

Neonatal screening and reproductive behaviour

Research has not yet clarified the effect of neonatal screening on actual reproductive behaviours; different studies indicate great differences in findings. These studies have a number of methodological problems, including a lack of analysis of the reproductive behaviour of control groups and a failure to analyse the number of previous children in a family – which is likely to affect decisions about future children. Helton and colleagues⁴⁷² found that 30% of a group of 55 parents of children with cystic fibrosis identified through neonatal screening had subsequent pregnancies but only two families had used prenatal testing and only one had chosen to terminate the pregnancy on the basis of the test. A further study of children with cystic fibrosis identified through neonatal screening found that, following genetic counselling, 77% of parents decided against further high-risk pregnancies, but that there were no statistically significant differences between the number of subsequent children born to these parents than to mothers of equal age and parity in the same period, based on population statistics.⁴⁹²

Similar results were found in parents of children diagnosed as having Duchenne muscular dystrophy through screening⁵¹¹ in Manitoba, Canada, where early diagnosis of Duchenne muscular dystrophy was not found to result in the widespread uptake of prenatal diagnosis. Similarly, in a study of phenylketonuria families, about a quarter of parents interviewed 1 year after the birth of their affected child stated that they intended to have no more children, with 52% of these parents ($n = 35$) indicating that that this was because of their child's illness. However, at a follow-up 5 years later, 36% of parents had had another child. It was concluded that parents of children with phenylketonuria (all of whom had been diagnosed through screening) had not changed their reproductive plans as a result of the disorder (but see also chapter 2).

It has been suggested that the low uptake of prenatal screening in pregnancies following neonatal diagnosis of Duchenne muscular dystrophy may relate to the fact that the diagnosed infants had relatively mild symptoms at the time of the subsequent pregnancy. There may therefore be a failure to recognise the seriousness of the disorder, as well as a possible denial by some parents of the disorder's existence in their sons.⁵¹¹ This may also have been the case in Helton and colleagues' study⁴⁷² of cystic fibrosis in which interviews were carried out between 4 days and 5 years (median, 10 months) after neonatal diagnosis; symptoms may not yet have developed in all of the affected infants.

Other studies have shown that neonatal screening does influence reproductive behaviour. In a study of cystic fibrosis families, Balnaves and colleagues²⁸⁹ found a 2.25-fold increase in the uptake of prenatal diagnosis by families with a first affected child over a period before and after neonatal screening had been introduced. All but one of these families had chosen to terminate the affected pregnancies. Those families who did not opt for prenatal diagnoses were observed to do so for mainly religious reasons or because of a lack of understanding about the nature of the disease and the availability of the test.

Summary

The limited evidence surrounding reproductive decision-making following neonatal screening suggests that parental support of neonatal screening does not generally refer to the effect the results may have on their reproductive plans. Although some parents may support prenatal screening because of the reproductive choices it provides, findings surrounding the actual uptake of prenatal screening in subsequent pregnancies are inconclusive.

Neonatal screening: guidelines for good practice

The way in which the negative psychological impact of diagnosis through screening can be improved by providing some guidelines of 'good practice' in relation to the procedures involved in neonatal screening is outlined below. The discussion of the psychological impact of current practices involved in neonatal screening includes the provision of information to parents before screening, obtaining informed consent for screening, and the time involved in the screening process.

Provision of information and communication in the screening process: relationships between parents and health professionals

There is some evidence to suggest that the psychological impact of diagnosis through neonatal screening is associated with the type and amount of information provided to parents about the screening process and its results. This research indicates that it is extremely important for parents to be aware that their children have been tested for inborn errors of metabolism and other genetic disorders; hence, they should be told why a blood sample is being taken, what disorders are being tested for, and what the results will mean for the child. Much of this evidence has already been

discussed above. It is examined here in order to outline possible improvements that could be made in the practices of neonatal screening, which would consequently reduce the potentially negative psychological impact of screening (Table 57).

Parents' knowledge of neonatal screening

There is substantial evidence to suggest that parents have limited knowledge of neonatal screening in terms of the disorders which are tested for, the effects of the disorders, and the treatments available. In a study carried out in Cambridge,⁵¹² although most women said that the Guthrie test had been fully explained to them, many were unable to identify the disorders screened for. While older and better educated women were more likely to identify the diseases correctly, only 51 of the 150 best educated women correctly identified hypothyroidism and 105 identified phenylketonuria. Similarly, in a study of 201 mothers of new-born babies, only 68% were aware that their babies had been screened for genetic diseases and only 38 had heard of congenital hypothyroidism and 36 of phenylketonuria.³⁶² Finally, in Tluczek and colleagues' study^{298,486} of the psychological effects of false-positives (see above), 59% of parents knew that their infants had been

tested for phenylketonuria but the majority were unaware of testing for hypothyroidism (66%) or cystic fibrosis (73%). In fact, 27% of the sample were unaware that any testing had taken place at all. These results suggest that an effort should be made to ensure parents are fully informed about neonatal screening, in terms of both the screening being carried out *per se* and the diseases being screened for. The results also have implications for implied consent as discussed below.

The relationship between information provision and psychological reactions to screening

As already discussed (page 90), parents' knowledge about screening may influence their psychological reaction and adaptation to a positive diagnosis.^{483,488,492,493} In addition, there is a limited amount of research which indicates that the type of information provided as well as the method of information provision are important in reducing parental anxiety. A British study found that parents were not provided with accurate information about the need for further blood test.⁴⁵⁷ The midwives only gave parents the correct reason for a retest if an inadequate sample had been taken. When ambiguous results were obtained, the parents were not told this but were told that it was a matter of

TABLE 57 Relationship between parents and health professionals: a summary of study aims

Study	Aim of study
Parents' knowledge of neonatal screening	
Smith <i>et al</i> , 1990 ³⁶² (Wales)	To investigate the attitudes of mothers to neonatal screening for Duchenne muscular dystrophy.
Tluczek <i>et al</i> , 1991 ⁴⁸⁶ (USA)	To discuss the psychological impact of false-positive results in neonatal screening for cystic fibrosis.
Tluczek <i>et al</i> , 1992 ²⁹⁸ (USA)	Parental reactions to a false-positive test result from neonatal screening for cystic fibrosis.
Relationship between information provision and psychological reactions to screening	
Bodegard <i>et al</i> , 1983 ⁴⁸³ (Sweden)	To investigate the psychological impact on parents whose neonate receives a false-positive screening test result.
Dankert-Roelse <i>et al</i> , 1983 ⁴⁹² (The Netherlands)	False-positive test results in cystic fibrosis screening.
Sorenson <i>et al</i> , 1984 ⁴⁹³ (USA)	To investigate parents' understanding and reaction to the need for a repeat neonatal screening test.
Tymstra, 1986 ⁴⁸⁸ (Netherlands)	To establish parents experiences of false-positive screening test results for congenital hypothyroidism.
Polichroniadis, 1989 ⁴⁵⁷ (UK)	To investigate parents' attitudes to neonatal biochemical screening, and to assess the amount of anxiety or concern as a result of the screening process
Methods of information provision	
Bodegard <i>et al</i> , 1983 ⁴⁸³ (Sweden)	See above.
Polichroniadis, 1989 ⁴⁵⁷ (UK)	See above.
Marteau, 1990 ⁵¹³ (UK)	An overview of the psychological costs of screening and how they could be reduced.
Tluczek <i>et al</i> , 1992 ²⁹⁸ (USA)	See above.

routine or were given no reason. This study is problematical, however, because few data are provided.

Sorenson and colleagues⁴⁹³ found that parents' satisfaction with the screening process was related to the type of information they were given about the need for a repeat blood test. Those who were told that the first test indicated an abnormality or potential problem were less likely to want to talk to someone about the screening results and more satisfied with the process as a whole than those who were not provided with any specific reason for a repeat blood test being carried out (that is, they were told the repeat test was a matter of routine or that the results had been lost by the laboratory). Both Polichroniadis⁴⁵⁷ and Sorenson and colleagues⁴⁹³ recommend that parents be provided with more information about the testing process so that they can "put the repeat test and its results in perspective".⁴⁹³

Methods of information provision

Other studies have indicated ways in which parent's understandings of neonatal screening can be improved. Tluczek and colleagues²⁹⁸ found that parents' understanding of the results of screening for cystic fibrosis was better if the parents were personally informed in a one-to-one interview rather than by telephone. These parents were less likely to misunderstand the different stages of the testing process and the meaning these stages had in relation to diagnosis. Polichroniadis⁴⁵⁷ found that parents were often informed about the need for a second blood test by a midwife visiting the home. The midwife was often not familiar with the family, and some families felt that a warning letter might have been helpful before the midwife's visit, although no data are provided to support this finding.

The large numbers of individual families involved in neonatal screening programmes may make it difficult to ensure that every parent receives adequate information. Some researchers point out that parents are liable to forget the information they are given by health professionals,⁴⁸³ particularly in the context of the stresses involved in the arrival of a newborn baby. It may be that written information may be more beneficial to parents and medical personnel, thus reducing the workload of midwives, for instance, as well as providing parents with a permanent reminder of what their child has been tested for and what the implications of the testing process are for their child. This method has been recommended by a number of researchers.^{457,513}

Marteau,⁵¹³ considering screening more generally, advocates that patients (or in the case of neonatal screening, the infant's parents) should be given detailed, accurate information in a sensitive manner before the test is carried out. This should include what is being tested for, how the test will be carried out, when and how the results will be available, the likelihood of a repeat test, and the meaning of the results (both positive and negative). She also emphasises that stress may be reduced by informing patients that repeat tests are not unusual, and that a positive result on a first test is not the same as diagnosis of the disease. Given the high rate of false-positive results in relation to tests for cystic fibrosis, for example, this is particularly important in neonatal screening.

Summary

Research suggests that parents have little knowledge about neonatal screening in terms of the diseases tested for and the process involved in the screening process. A proportion of parents may actually be unaware that their child has been screened. The provision of information has also been related to parents' emotional reactions to neonatal screening, so that parental anxiety and dissatisfaction with screening has been found to be greater in those who feel they have not received adequate information. Finally, the method by which information is transmitted may influence parental reactions. It is suggested therefore that parents should be provided with information about why a blood sample is being taken, what disorders are being tested for, and what the results will mean for the child. Information about false-positive and false-negative test results should also be provided in order to reduce the lingering concerns which these can create for parents.

Obtaining informed consent

There is substantial literature based around the ethics of informed consent for neonatal and other forms of genetic screening (*Table 58*). Most of this literature is North American and, hence, is concerned with a different medical and legal system than in the UK. The American literature is reviewed here briefly in order to outline the potential ethical issues surrounding informed consent.

Informed consent in North American screening programmes

Mandatory screening for phenylketonuria was introduced in the 1960s in the majority of North American states. This was related to health professionals' strong beliefs in the accuracy, sensitivity and specificity of the test, and in the availability of a safe and effective treatment. These beliefs were

TABLE 58 Obtaining informed consent: a summary of study aims

Study	Aim of study
Informed consent in North America screening programme	
Faden et al, 1982 ⁵¹⁹ (USA)	The necessity of parental consent as public policy for neonatal screening.
Rowley, 1984 ⁵¹⁰ (USA)	An overview of the issues surrounding genetic screening.
Motulsky, 1989 ⁵¹⁸ (USA)	An overview of the modalities of diagnosis and management in genetic disease followed by a discussion of societal issues.
Andrews, 1991 ⁵¹⁵ (USA)	Legal aspects of legal aspects of genetic information.
Naylor, 1991 ⁵²⁰ (USA)	New technologies in newborn screening.
Edwards & Hall, 1992 ⁵¹⁶ (UK)	The importance of ensuring parents understand the nature of the screening process.
Thuczek et al, 1992 ²⁹⁸ (USA)	Parental reactions to a false-positive test result from neonatal screening for cystic fibrosis.
Fost, 1993 ⁵¹⁴ (USA)	A review of the main ethical issues associated with new genetic technologies.
Knoppers, 1994 ⁵¹⁷	The need for parental consent in the case of neonatal screening.
Informed consent in British screening programme	
Polichroniadis, 1989 ⁴⁵⁷ (UK)	To investigate parents' attitudes to neonatal biochemical screening, and to assess the amount of anxiety or concern as a result of the screening process.
Statham et al, 1993 ⁵¹² (UK)	Parental consent for neonatal screening (letter).
Kent, 1996 ⁵²¹ (UK)	To illustrate how the work of cognitive and social psychologists can provide insights which are both relevant and valuable to the process of attaining consent.
Informed consent, parental knowledge of screening and attitudes towards consent	
Faden et al, 1982 ⁵¹⁹ (USA)	See above.
Holtzman, 1984 ¹⁴⁴ (USA)	The issues surrounding routine neonatal screening for cystic fibrosis.

later challenged on the grounds that insufficient research had then been carried out.⁵¹⁴ However, there were problems with this study, which did not apply to the UK experience because it was in response to inadequate implementation of procedures. In more recent years, there have been discussions about the ethics of legislation making neonatal screening compulsory versus the practice of informed consent.^{510,514-518} Arguments for informed consent have focused on the moral principles surrounding the rights of the individual to decide what is done to one's body (in this case, the parents' right to decide what is done to their child's body). Alternatively, it has been argued that it is a parents' duty to protect their children from the negative effects of genetic disease and that the stakes are too high and time too short to make screening voluntary. Here, parental refusal to have their child tested is regarded as medical neglect of the child.

As a result of these debates, phenylketonuria screening is no longer compulsory in many American states and participation is, theoretically, left to the discretion of the parents.⁵¹⁷ The North American practice of carrying out heel-prick tests in the hospital nursery means that blood samples are taken for screening without the parents' presence or awareness.⁴⁶⁶ In this situation, parents have little chance of making an informed choice. These reports are backed up by the lack of knowledge which parents have about neonatal screening (see page 96).

Thuczek and colleagues²⁹⁸ also note that 45% of parents interviewed in their study of false-positive diagnoses were unaware that they could refuse neonatal screening (generally on religious grounds), or that the test for cystic fibrosis could be refused on any grounds (29%).

Informed consent in British health care

At a general level, informed consent has become a central issue within British health care over the past two decades. This relates to changes in the medical ethos from one of parentalism to one of autonomy and patients' rights to self-determination. Informed consent plays an important part in this notion of autonomy as part of the shift of power away from the doctor to the patient.⁵²¹ However, as in North America, British research suggests that informed consent may not always be obtained in relation to neonatal screening and that parents are not provided with enough information to make a truly informed choice.^{457,512} Statham and colleagues⁵¹² concluded that:

"...most new mothers do not know what the Guthrie test is for: a considerable number incorrectly believe that it will detect more disorders than is the case. These results clearly challenge any notion that women are giving informed consent for their babies to be tested, even though they believe themselves to have been informed."

The small amount of research into informed consent which has been carried out suggests that parents do not have the knowledge required to make an informed choice, and that greater efforts are required to ensure adequate information is provided to parents.

Informed consent, parental knowledge of screening and attitudes towards consent

Research findings on the provision of information to parents about neonatal screening indicate that parents' knowledge about screening is increased by attempts to obtain informed consent by health professionals, and suggests that this may therefore help to reduce parents' anxiety about their child's health.

One of the objections raised by health professionals in relation to informed consent is that parents are unable to understand the reasons for screening and giving information in order to obtain consent will not increase this understanding. However, there is evidence to suggest that mothers' knowledge of neonatal screening can be increased through the process of obtaining informed consent. Holtzman⁴⁷⁸ reported that women who were given information about the diseases screened for, their effects and treatments, and details of the possibility of false test results in order to obtain their consent had significantly more knowledge about these issues than women who had not been given this information. These findings are important in relation to research indicating that parental anxiety is reduced through the provision of information about screening (see page 96). It may be that the process involved in providing information necessary to obtain informed consent would be helpful in reducing the effects of diagnosis through screening.

Parental attitudes to consent have also been assessed. In a study in Maryland, USA, Faden and colleagues⁵¹⁹ found that 46% of women preferred the screening test performed without their consent. This related to the women's beliefs that the test was simple and not dangerous, or that consent might worry parents unnecessarily about the test. Women who felt that their permission should be sought said that this was because they wanted to know everything that would be done to their babies. Less than 5% said that they wanted to be asked for permission to carry out the test because they felt they should have a 'right' to refuse the test.

However, even if screening was compulsory, most women (79%) asserted that they should be told that the test had been performed. These results suggest that attitudes towards autonomy and patients' rights within the general population may not match current healthcare philosophy.

Summary

While there have been extensive debates about the ethical principles of informed consent in relation to North American neonatal screening programmes, there has been little British research in this area. There have been moves towards the philosophy of patient autonomy resulting, in the USA, in the recommendation of informed consent rather than mandatory neonatal screening. However, there is evidence to suggest that this consent is rarely obtained. In addition, parents are provided with little information about screening, so that they have limited knowledge about the process involved and hence, are unable to make a truly informed choice about whether to have their child screened for genetic disease. Obtaining informed consent from parents through the provision of comprehensive information about screening may reduce parental anxiety.

Delay between screening and diagnosis

As discussed earlier (page 86), one of the disadvantages of traditional clinical diagnosis is the anxiety and stress caused by long delays in obtaining a diagnosis, and the effects this has on parents' attitudes towards doctors and other medical personnel.^{471,472,475,476} Parents' experiences of these delays lead to their support for neonatal screening for disorders such as cystic fibrosis and Duchenne muscular dystrophy. However, there may be delays in the screening process which are also stressful for parents, even though these will not be on the same scale as in traditional diagnosis. Such delays may be the inevitable consequence of the time involved in taking a blood sample, carrying out the necessary tests, repeating the blood test if an abnormal result is obtained and, in the case of cystic fibrosis, taking a sweat test.

As yet, there have been no major studies of the delays involved in the diagnosis of inborn errors of metabolism via neonatal screening or of their psychological implications for parents (*Table 59*). A small number of studies have indicated that some parents may experience undue delays in obtaining the results* of neonatal screening and that this causes

* A recent audit of the UK screening programme revealed that in at least 20% of the Health Districts surveyed there were no arrangements for routinely reporting normal screening test results to parents.

TABLE 59 Delays between screening and diagnosis: a summary of study aims

Study	Aim of study
Delays between screening and diagnosis	
Dankert-Roelse <i>et al</i> , 1983 ⁴⁹² (The Netherlands)	False-positive test results in cystic fibrosis screening.
Firth & Wilkinson, 1983 ⁴⁷⁷ (UK)	To assess parental views on neonatal screening for Duchenne muscular dystrophy.
Sorenson <i>et al</i> , 1984 ⁴⁹³ (USA)	To investigate parents' understanding and reaction to the need for a repeat neonatal screening test.
Tymstra, 1986 ⁴⁸⁸ (The Netherlands)	To establish parents' experiences of false-positive screening test results for congenital hypothyroidism.
Aljader <i>et al</i> , 1990 ⁴⁸⁰ (UK)	To investigate the attitudes towards neonatal screening and prenatal screening of parents of children with cystic fibrosis.
Boland & Thompson, 1990 ⁴⁷⁵ (Australia)	To assess the effects of neonatal screening for cystic fibrosis on maternal behaviour.
Helton <i>et al</i> , 1991 ⁴⁷² (USA)	To investigate parental attitudes and emotional response to the diagnosis of cystic fibrosis.

anxiety and distress.⁴⁷¹ Other studies have indicated that parents experience delays in relation to false test results and that these parents attribute their anxiety to the time taken to obtain the results of repeat tests (see page 90).^{488,492} These parents felt that their anxiety could have been reduced if the time between receiving an initially positive result and receiving reassurance that that result had been a false-positive were shorter. These preliminary findings suggest that efforts should be made to limit the time involved in the screening process, although this should obviously not be done at the expense of the quality of the testing process.

Main findings and recommendations

The systematic review of the research surrounding the psychological impact of neonatal screening indicates that the psychological benefits of neonatal screening outweigh the costs. This can be seen to be the case for the psychological effects of the medical procedures involved in screening, as well as for those involved in the early diagnosis of genetic disease through screening in comparison to traditional clinical diagnosis. However, there are problems involved with both the technology of the screening process and doctor–patient and inter-professional communication which need to be resolved. These problems relate to the incidence of repeat testing through neonatal screening and to the provision of information to parents about the screening process. Recommendations for the reduction of these problems are made.

Although infants have been found to experience pain from the taking of blood samples for neonatal

screening, there is little research on the potential long-term effects of this on the infants or of the anxiety which it causes parents. However, it seems likely that this pain can be successfully reduced by the use of automatic lancing techniques (such as spring-loaded lances) and by pain-alleviation methods.

Research indicates that although the diagnosis of genetic illness is distressing for parents, there are no differences in parental reactions to diagnosis by neonatal screening or by traditional clinical diagnosis. In addition, there appear to be no long-term disadvantages of neonatal screening in terms of its effects on the parent–child bond compared with traditional diagnosis. An advantage of neonatal screening is that it avoids the delay involved in the diagnosis of cystic fibrosis, Duchenne muscular dystrophy, and many of the other disorders described in this review. Parents support neonatal screening because it reduces this delay, thus decreasing anxiety and avoiding the distress and bitterness they experienced in response to medical scepticism. Parents' widespread support of neonatal screening also relates to the psychological, financial and practical benefits which parents view as outcomes of screening for themselves and their children.

Unnecessary parental anxiety results from repeat testing of genetic disease through neonatal screening. This anxiety stems from an initially positive diagnosis and may persist even after the diagnosis has been overturned by repeat testing. As far as possible, the screening process should be designed to produce a presumptive-positive diagnosis, without recourse to a second blood sample or other follow-up testing.

More research is required on the psychological effects of false-negatives. It has been suggested that neonatal screening reduces medical alertness to the symptoms of the genetic disorders in question (see also recommendations in chapter 19).

The relationship between reproductive decision-making and neonatal screening is currently unclear. Although parents whose children have been diagnosed through neonatal screening may support prenatal testing and report their intentions of terminating subsequent affected pregnancies, there is conflicting evidence as to the extent to which they actually change their reproductive behaviour, and no data on whether this differs from that following clinically-based diagnosis of the same disease. Thus, the suggestion that neonatal screening will significantly reduce the incidence of genetic disease within the population as a whole is not supported at this stage of research. Few of the reported findings relate to the UK population and more research is needed in this area.

There is evidence to suggest that parental anxiety and distress following diagnosis through screening

relates to the type and amount of information provided to parents as well as to the actual methods by which information is conveyed to them. A consideration of the best approach to this, in the light of the greatly increased number of diseases likely to be covered by neonatal screening in the future, is included in the recommended pilot study (see chapter 19).

Areas for further research

Further psychological research, associated with neonatal screening, should focus on the following areas.

- The relationship between neonatal screening and subsequent reproductive decision-making.
- The ways in which effective and compassionate communication can provide relevant and accurate information and thus reduce parental anxiety (inter-professional and doctor/health professional–parent communication).
- What constitutes informed consent and the most effective ways of providing information to enable parents to make informed choices.

Chapter 12

Economics of neonatal metabolic screening: a general introduction to the literature review

Introduction

Since the mid-1960s, neonatal screening programmes for metabolic disorders have been established throughout the developed world. The specific conditions screened for and the technique(s) adopted for the conduct of such screening programmes varies both within and between countries. Despite the scale of activity, little systematic work has been carried out to compare the costs and benefits associated with the different screening programmes nor screening technologies.

The objective of this part of the systematic review is to identify the published economic evaluations of neonatal screening programmes, to critically review the 'state of knowledge' for neonatal screening by specific disease, and to summarise the 'state of knowledge' in this field.

Organisation of the economic review

The first part of this chapter provides a general introduction to economic evaluations of healthcare technologies, setting out the types of economic evaluation that can be undertaken and discussing the strengths and weaknesses of each. Important methodological considerations such as discounting and sensitivity analysis are described. The objective is to familiarise the reader with the terminology used in the papers identified by the review and in the review process itself.

In the second part of this chapter, the systematic search process and the number of articles identified at each stage of this process are described, together with the methods for rating the quality of each paper and the evidence in each area.

The relevant papers are reviewed in chapters 13–15 under the headings given below. A grade for the overall evidence on the cost-effectiveness of screening for the four specific diseases is presented.

- Economic evaluations of neonatal metabolic screening for phenylketonuria

- Economic evaluations of neonatal metabolic screening for congenital hypothyroidism, cystic fibrosis, and Duchenne muscular dystrophy
- Economic evaluations of neonatal metabolic screening programmes and alternative technologies for neonatal screening

Finally, the discussion of the results of the literature review presented in chapter 16 highlights the methodological limitations of the existing work and indicates the key issues for any subsequent economic evaluations of neonatal screening.

General economic issues of neonatal screening

In 1968, Wilson and Jungner, in their report for WHO,³⁴ listed the criteria that should be met when considering a disease suitable for early detection by screening. One of the principles refers to the cost of case finding; it states:

“...the cost of case finding (including confirmatory diagnostic tests and treatment) should be economically balanced in relation to possible expenditure on medical care as a whole.”³⁴

Wilson and Jungner³⁴ discuss the opportunities for cost saving on medical care through the introduction of screening. They define the medical aim of screening as to “improve the health of a population by the early detection and treatment of illness”, and the immediate economic aim to be the time saving of highly trained people due to the use of “technicians, and perhaps automated methods, as a first line in disease detection”. However, they appreciated that, although screening programmes may reduce physicians' time, it is unlikely that the total amount of diagnostic and therapeutic work will be reduced by the introduction of a screening programme. Hence, the real economic benefit of being able to prevent and/or treat a disease earlier than if detected symptomatically, is the lengthening of the productive life of the at-risk population, thereby resulting in an improvement in the overall economy.³⁴ Although not an unusual approach to the evaluation of healthcare procedures at the

time it was written, this statement would not be acceptable today.

This focus on the costs is contrary to the general approach to evaluating healthcare interventions.⁵²² As a general principle, the costs of an intervention should be balanced by the value of the health benefits of that intervention. Any improvement in the productivity of individuals is considered to be an indirect benefit of the intervention. The health benefits are the primary focus of the intervention, and it is the value of these benefits, which may include but will not be limited to increased productivity, which need to be taken into account.

If the Wilson and Jungner criteria were to be used as the sole arbiter for evaluating screening, then screening would be evaluated in a manner that was systematically different from the approach adopted for all other healthcare interventions. This difference would lead to a systematic underestimation of the benefits of screening and, thus, bias the allocation of resources in the healthcare budget away from screening.

Economic perspective

Economics is a useful analytical tool for assessing whether resources are being used in the most effective manner to achieve the maximum gain to society; that is, in the case of neonatal screening, whether “the organisation and routine used provides maximum benefit in relation to invested resources”.¹⁰⁴

Health economics has a well-established taxonomy of the costs and benefits that can be used in the evaluation of healthcare procedures, and a range of evaluative techniques for addressing different questions that may arise.

Types of costs

The taxonomy of costs used by economists in the evaluation of healthcare interventions consists of direct costs, indirect costs, and intangible costs.

- **Direct costs** are the organising and operating costs within the health sector that are directly attributable to the intervention being considered.⁵²³ In addition, out-of-pocket expenses incurred by patients and their families in relation to the intervention are considered direct costs.
- **Indirect costs** are those which result from the intervention but which are not a part of the intervention itself, for example, the value of lost production caused by time away from work. It has been argued that short-term losses in

production are often compensated for when an individual returns to work and that, for long-term absences, the individual will be replaced by a previously unemployed worker.⁵²² However, to date, lost production has been viewed as a desirable component for costs in economic evaluations.

- **Intangible costs** are the negative effects of an intervention which cannot be given a financial value, often described as ‘pain and suffering’. These costs are considered more fully in the review of the literature on the psychological aspects of neonatal screening.

Types of benefits

There are three main categories of consequences or benefits; direct, indirect and impact on health status.

- **Direct benefits** include the savings in health resources as a result of an improvement in a patient’s health state, as well as savings in expenditure of leisure time input.⁵²³
- **Indirect benefits** include the benefits in improved productivity as a result of the patient or family member returning to work.
- **Impact on health status** may be expressed in terms of survival, clinical morbidity, quality of life or some combination of these.⁵²²

Types of economic evaluations

Cost-minimisation analysis

- **Definition** A comparison of costs only, owing to the consequences of the two interventions of interest being identical (that is, no measurement of consequences performed).

Cost-minimisation analysis^{522,523} is adopted where the consequences of two interventions are either known or proven to be identical. In neonatal screening such an evaluation may be considered appropriate for comparing two techniques of screening for the same disorder, as the benefits will be the avoided costs associated with the care and treatment required by an ‘affected’ individual who present symptomatically later in life. For example, phenylketonuria can be screened for using the Guthrie microbiological assay, chromatography or fluorometry, all of which have different costs associated with them but achieve the same benefit of avoiding individuals with phenylketonuria who are mentally retarded.

However, no cost-minimisation analyses have been identified in the literature. One reason for this may be that each screening test has different sensitivity and specificity rates, which may mean that the

benefits of screening for a particular disorder do alter according to the technique used; that is, those screens with the highest specificity and sensitivity rates will result in the minimum number of false-positive and false-negative diagnoses and, hence, increase the benefit to both the neonates and their families.

Cost-effectiveness analysis

- **Definition** Relates the difference in costs between two interventions to the difference in effects, measured in natural units.

As there are few situations where the consequences of two interventions are identical, it is often more appropriate to perform a comparison on the difference in costs with the difference in effects of two interventions. Cost-effectiveness analysis⁵²²⁻⁵²⁴ is appropriate where it is possible to measure the difference in effect in terms of a change in one main parameter expressed in natural units, such as life-years saved.

The use of cost-effectiveness analysis in the area of neonatal screening for metabolic disorders is very limited. It would be appropriate to compare two methods of screening for the same condition. To date, analyses have concentrated on whether to screen rather than on how to screen.

Cost-utility analysis

- **Definition** Values changes in health status relative to one another in order to produce an overall index of health gain (e.g. quality-adjusted life year (QALY)).

Unlike cost-effectiveness analysis, cost-utility analysis^{522,523} incorporates a trade-off between length and quality of life. This is done by valuing changes in health status relative to one another to obtain an overall index of health such as the QALY. Drummond⁵²² describes cost-utility analysis as more demanding than other types of economic evaluation because “not only do the different impacts in length and quality of life need to be measured, but also a set of values for health status need to be obtained in order to compare the states with one another”.

Cost-utility analysis may be an appropriate analysis for assessing neonatal screening programmes as an important aspect is the evaluation of the quality of life of an ‘affected’ individual without the existence of a screening programme, against the quality of life of an ‘affected’ individual with the existence of a screening programme. However, it is the most difficult type of evaluation to implement⁵²² and,

hence, only one paper using this technique⁵²⁵ was identified in the systematic literature review.

Cost-benefit analysis

- **Definition** Values both costs and consequences in the same (usually monetary) units and assumes each programme is being compared with an alternative with no costs and no benefits (i.e. the ‘do-nothing scenario’).

Cost-benefit analysis⁵²²⁻⁵²⁴ values both costs and benefits in the same, usually monetary, units and evaluates them under the assumption that each programme is being compared with the ‘do-nothing’ alternative; for example, in neonatal screening the intervention would be compared with the ‘no screening’ alternative. However, it is often difficult to quantify all the costs and all the benefits associated with a particular programme; hence, in practice, cost-benefit analyses are often limited to the evaluation of those costs and benefits that can easily be expressed in monetary terms.

Attempts have been made to overcome the challenge of expressing all the consequences associated with a particular programme in monetary terms by using a ‘willingness-to-pay’ assessment.^{526,527} As part of a neonatal screening evaluation, parents may be asked what they would be willing to pay to prevent their child suffering from the effects of a particular disorder, say phenylketonuria. The parents asked may be the parents of ‘affected’ children or a sample from the parent population in general, viewing a hypothetical situation. The ethics of such an approach are questionable.⁵²⁷

Most studies identified in the literature claimed to have used cost-benefit analysis to evaluate neonatal screening.^{104,144,149,528-541} However, the extent of the costs and benefits incorporated in the analyses varied considerably across studies from the inclusion of direct costs and costs avoided only, to the inclusion of all quantifiable costs and benefits. All of these studies failed to incorporate a measure of health benefit into their analyses (that is, quality of life or life-years gained).

Incremental analysis

- **Definition** A comparison of the additional costs one programme imposes over another with the additional benefits imposed.

Incremental analysis^{522,523} is a useful type of analysis for evaluating expanding neonatal screening programmes as it provides a comparison of the incremental costs and benefits of introducing a new screen to an existing neonatal screening

programme. For example, incremental analysis can be used to evaluate the extra costs and benefits that would result from incorporating a screen for cystic fibrosis into an already-established phenylketonuria screening programme. A number of studies^{104,144,531,536,537,540} adopted this type of analysis for evaluations similar to the one described above.

Incremental analysis can also be used to compare the additional costs one screening technique imposes over another with the additional benefits imposed. For example, the additional costs and benefits associated with screening for phenylketonuria using fluorometry rather than the more traditional Guthrie method.

In such comparisons there exists a trade-off between the technology used and the amount of labour required. In the example used above, the Guthrie microbiological assay method requires minimal capital equipment but is more labour intensive than the fluorometric method, which has a high capital equipment cost. Only a limited number of the studies identified^{285,304,525} undertook a comparison of the different techniques available for screening for a specific disorder.

Importance of data collection and data quality

The quality of data used in an analysis is as important as the analysis itself. Ideally, relevant data should be obtained through RCTs. However, this is not usually a viable option when evaluating neonatal screening for treatable metabolic disorders as it would be unethical to screen one group of neonates and not the other. Hence, the most reliable costing data for the screening process would be obtained prospectively, direct from laboratories and hospitals, for example, while consequence data would be obtained retrospectively from published literature and official statistical reports.

Identifying and quantifying the costs of neonatal screening programmes

Neonatal screening costs are summarised in *Table 60*.

Specimen-collection costs

Although the specimen-collection cost appears negligible at an individual level (e.g. £5.04 per newborn blood specimen collected), this cost becomes significant when multiplied by the whole population screened by the laboratory (e.g. £50,400 for 10,000 neonates screened).

The costs of specimen collection are likely to be stable in relation to the number of metabolic disorders screened for by the laboratory. At the margin, screening for more disorders increases the risk of having an insufficient sample but, in general, increasing the number of diseases screened for will lead to a significant reduction in the specimen-collection cost apportioned to each disease.

The most reliable source for the collection of such data is from the health visitor, midwife and/or the neonatal screening laboratories.

Laboratory analysis costs

The identification costs are those of the screening laboratory for detecting those neonates suffering from certain disorders. These costs include the capital equipment, consumable and labour costs, plus the cost of referral of detected cases, notification and possible counselling of parents. Given the significant variation in the processes used by different screening centres, the most robust method of obtaining this type of data is primary data collection from the neonatal screening laboratories.

Follow-up costs

Once a presumptive diagnosis has been arrived at by the screening laboratory, the best practice for following-up the 'affected' child has not been

TABLE 60 A summary of neonatal screening costs

Costs	Description
Specimen-collection costs	e.g. midwife's time; equipment (filter paper, swab, stylette, etc.); postage to laboratory
Laboratory analysis costs	e.g. capital and consumable equipment; manpower; administration; overheads
Follow-up	patient identification; communication of result and referral to paediatrician; diagnostic tests; laboratory tests
Treatment costs	e.g. treatment (dietary, for example) (for metabolic diseases with no known 'cure', this may include prenatal tests and abortion)
Misclassification costs	costs associated with false-positive and false-negative results at the identification stage

settled. The number of hospital/clinic visits and tests performed during follow-up are not well established. For the purposes of evaluation, reliable information on follow-up is best obtained locally from hospitals, clinics and/or dieticians.

Treatment costs

Unlike the other categories of cost, treatment costs for metabolic disorders are less clearly defined. Although the treatment for such metabolic disorders as phenylketonuria are well established, the duration of the treatment is a subject of controversy; in reported evaluations, the duration of treatment for phenylketonuria ranges from 6 years to lifetime. Similarly, the number of hospital/clinic visits and tests performed during follow-up are not well established. Reliable information on treatment costs is obtainable locally from hospitals, clinics and/or dieticians.

In addition to treating the affected child, the cost of maternal phenylketonuria; that is, the reintroduction of treatment (special diet) together with extra tests and clinic visits for phenylketonuric females during pregnancy,⁵³⁰ should also be taken in to account.

Misclassification costs

Most screening tests have imperfect sensitivity and specificity, thus leading to the misclassification of 'unaffected' neonates as 'affected' and vice versa. The consequences of such events reach further than the monetary costs of classification error (for example, unnecessary tests and treatment, legal settlements) and include inconvenience, worry and personal suffering to the family as well as the loss of public confidence in the screening programme (see page 90).⁵⁴² It is important, therefore, to acknowledge this issue in evaluations of any neonatal screening programme although, for established screening programmes such as phenylketonuria or congenital hypothyroidism, a misclassification event is rare in the UK.

Identifying and quantifying the benefits (avoided costs) of neonatal screening programmes

The benefits of neonatal screening are summarised in *Table 61*.

Health status

The effect of early diagnosis through screening varies, in terms of improvements in physical and mental health status, according to the disorder in question. Health status is usually expressed in terms of quality of life or life-years gained. Alternatively, the willingness-to-pay approach discussed above can be applied to quantify health gain. Measurements of health benefit have not readily been incorporated into neonatal screening evaluations and the application of the willingness-to-pay approach to health issues is still at the experimental stage.

Laboratory analysis

In unscreened individuals suffering from a metabolic disorder, the disorder will present symptomatically during childhood, at which point medical costs to confirm and treat the disorder will arise.

Laboratory analysis costs include the medical tests performed to identify the disorder affecting the particular individual. It is not possible to calculate such costs by undertaking an RCT as this would be unethical. Such information must therefore be obtained from the published literature describing the history of the disease, unless circumstance should happen to provide an opportunity for primary data collection.

Care

The amount and type of care required by affected individuals depends upon the degree of retardation and/or physical disability and the life expectancy of the affected individual (which also depends on the degree of retardation). In order to calculate the scale of this type of benefit, data from the literature

TABLE 61 A summary of benefits of neonatal screening

Health benefits/costs avoided	Description
Health status	Improvements in the physical and mental health status of individuals with the metabolic disorder
Laboratory analysis	e.g. analysis and detection costs
Care	e.g. institutional, foster, parental care
Loss of productivity	e.g. the 'affected' individual, parent-carer
Other	e.g. improved quality of life, psychological suffering of an 'affected' individual and family

may be used to estimate the number of affected individuals and the range of health states if their condition was not identified pre-symptomatically. Given the radical changes in the type of care provided to people with physical and mental disabilities over the past decade, data from the literature on the costs of such care are mostly outdated. Costs must be obtained from primary sources or from up-to-date publications such as Netten and Dennett.⁵⁴³

Loss of productivity

It is difficult to calculate the loss of productivity avoided by early detection of a metabolic disorder. A value can be estimated, based on the average wage earned by 'normal' individuals of the same age and sex acquired from national statistical surveys. Most of the reported analyses which attempt to quantify loss of productivity, calculate values for both the 'affected' individual and the parent-carer.

Other benefits

Other benefits of neonatal screening programmes may include an 'affected' individual's improved quality of life, a reduction in the psychological suffering experienced by both the 'affected' individual and their family, a better understanding of the frequency and variability of the disease state,⁵⁴⁴ and the stimulation of interdisciplinary cooperation between dietitians, physicians, social workers, nurses, psychologists.⁵⁴⁴ Some of these benefits are discussed in more detail in chapter 11.

Differential timing of costs and benefits

All the costs and consequences accruing from a particular programme rarely occur at the same point in time. This is particularly apparent in screening programmes where most of the cost will be incurred immediately but the majority of consequences will not be realised until future years. For this reason, it is important, when performing an economic evaluation, to quantify present and future costs (and, similarly, present and future benefits) in comparable units by adjusting for differential timing;⁵²² that is, by weighting future costs and benefits by a discount factor to make them comparable to present costs and benefits.

By discounting future costs and benefits to the present value, there is the assumption that costs and benefits encountered in the future are relatively less significant in present value terms than costs and benefits occurring now.⁵²⁸ For example, the discounted present value of £1000, assuming a 7% discount rate, would be valued at only £508 in 10 years' time.

The choice of discount rate is important. If a high discount rate is incorporated into an evaluation, this would cause a programme with high costs now and benefits in the future to be economically less appealing than a programme with similar costs and benefits all experienced now.⁵²⁸ Likewise, a low discount rate would give more weight to costs and benefits in the future.

The value of discount rate that should be used in economic evaluations is questionable. Some economists believe that the discount rate should reflect the average rate of growth of the gross national product, whereas others believe the rate of inflation or rate of interest to be more suitable.⁵²⁸ In a number of studies identified for this review,^{104,144,149,528,529,531,533,534} the authors used several discount rates leaving the reader to choose the most suitable discount rate for their particular situation. In the UK, the Treasury recommends a discount rate of 6% per annum for all publicly-funded programmes.⁵⁴⁵

The importance of discounting in the context of neonatal screening varies enormously from one metabolic disorder to the next. With some disorders the time lag between diagnosis through screening and asymptomatic presentation is so small that discounting becomes irrelevant. For other disorders, symptomatic presentation may occur many years after screening would have identified the affected individual. For these cases, appropriate discounting is much more important.

Sensitivity analysis

In economic evaluations, as in many aspects of life, it is impossible to identify and quantify everything with certainty. Imperfect information means that many of the benefits and a fair proportion of the costs cannot be quantified with certainty. In order to take account of this uncertainty, it is useful to test the impact of varying the values of key variables on the result of the analysis. The objective of this process of sensitivity analysis is to increase the level of confidence in the results obtained.

Sensitivity analysis can take a number of different forms,⁵⁴⁶ the most widely-used being simple sensitivity analysis.⁵⁴⁷ Here, one or more of the components in the evaluation are varied across a plausible range; for example, the blood specimen collection cost of a neonatal screening programme may be varied from the minimum reported to the maximum reported, in order to identify the effect this has on the cost-effectiveness of screening.

Simple sensitivity analysis is split into one-way analysis and multi-way analysis. In one-way analysis,

“each uncertain component of the evaluation is varied individually, while the others retain their base-case specification, in order to establish the separate effect of each component on the results of the analysis”.⁵⁴⁶ In multi-way analysis, uncertain components are varied one or more at a time to investigate the effect on the outcome, including interactions between important factors.

Sensitivity analysis is important, especially when assessing neonatal screening programmes, because of uncertainties about prevalence, mortality and morbidity probabilities, life expectancy, duration of treatment, specificity and sensitivity, for example.

Generalisability of results

The results of many economic evaluations are not generalisable as they are specific to certain populations or organisations. Evaluations of neonatal screening are no exception, with variations in results existing across studies due to many factors. The main reasons for lack of generalisability are as follows.

- The incidence rate of individual metabolic disorders differs from country to country; for example, the incidence rate of phenylketonuria in Japan is one case detected in 80,000 neonates screened,⁵³⁵ compared with Belgium where the incidence rate of phenylketonuria is one case detected in 10,000 neonates screened.⁵³⁴ Similar variations exist for other metabolic disorders. Incidence rates also differ between ethnic groups; for example, sickle cell disease has a high incidence rate in African-Americans.
- The results of economic evaluations are influenced by both the methodology used and the sociobiological background in each country.⁵³⁵
- The size of the neonate population tested by the screening laboratories and the number of disorders screened for affects the resource costs of the screening programme. This is because basic personnel, overheads and equipment expenses are necessary, regardless of the number of specimens tested, leading to an inverse relationship between the cost per specimen and the number of specimens tested by a single laboratory.
- The tests used and their effectiveness vary from country to country because of variations in the population genetics and structural differences in the screening programmes (e.g. different blood sampling times).

Ideally, economic evaluations should attempt to explore these issues through appropriate sensitivity analysis.

Methods

Searching strategy – study identification

Literature on the economics of neonatal screening was identified by using a clearly-defined systematic strategy to search the following databases.

- Medline
- Health Plan
- Biological Abstracts
- Embase
- Science Citation Index
- IBSS
- EconLit

The total number of articles identified, excluding duplicates, was 343.

Inclusion criteria – study selection

The titles, and abstracts where available, of all the articles identified by the literature searches were downloaded and assessed by applying the following inclusion and exclusion criteria.

Inclusion criteria

- Any English language article which gives costs with or without outcomes data for neonatal screening programmes or technologies.

Exclusion criteria

- Foreign language article.
- Disorders not relevant to the review (e.g. sickle cell anaemia).
- No costs quoted in the results section of the abstract.

As a result, 65 articles were selected for full review.

Preliminary review

A preliminary review of all the articles obtained was conducted to confirm their relevance to the literature review. This was carried out by categorising each of the articles into one of the following subject areas:

- general literature on the economics of neonatal screening
- the costs and outcomes of specific neonatal screening programmes
- the costs and outcomes of neonatal screening programmes
- the economics of alternative technologies for neonatal screening technologies
- not relevant.

The number of articles identified in each category are recorded in *Table 62*.

TABLE 62 Articles identified in each category of the preliminary review

Category	Number of articles
General literature on the economics of neonatal screening	7
The costs and outcomes of specific neonatal screening programmes	20
The economics of screening programmes	6
The economics of alternative technologies for neonatal screening technologies	3
Not relevant	26

TABLE 63 Classification of quality of evidence

(I)	Evidence obtained from a well-designed RCT
(II-1a)	Evidence obtained from a well-designed controlled trial with pseudo-randomisation
(II-1b)	Evidence obtained from a well-designed controlled trial with no randomisation
(II-2a)	Evidence obtained from a well-designed cohort (prospective study) with concurrent controls
(II-2b)	Evidence obtained from a well-designed cohort (prospective study) with historical controls
(II-2c)	Evidence obtained from a well-designed cohort (retrospective study) with concurrent controls
(II-3)	Evidence obtained from a well-designed case control (retrospective) study
(III)	Evidence obtained through large differences in comparisons between times and/or places with and without intervention (in some circumstances these may be equivalent to level I or II)
(IV)	Evidence obtained from the opinions of respected authorities based on clinical experience, descriptive/published studies and/or reports of expert committees
(V)	Evidence inadequate owing to problems of methodology; e.g. discount rate, sensitivity analysis; evaluation of costs and benefits; lack of detail

On completion of the ‘study identification’ process of the review, 34 of the original 343 articles identified from the databases (excluding duplicates) were judged relevant, having satisfied the inclusion criteria at both the abstract and preliminary review stages.

Assessment of the quality of evidence

Studies reporting an economic evaluation of neonatal screening have been graded according to their study design, using a rating scale based on work by the US Preventive Task Force¹⁰⁷ and the UK Centre for Reviews and Dissemination.¹ A description of these quality of evidence classifications is provided in *Table 63*. Papers of a descriptive nature containing information on the economics of neonatal screening (e.g. letters and editorials) have not been graded.

Assessment of the strength of recommendation

In the case where several studies have conducted an economic evaluation on the same screen or screening programme, the group of studies have been assigned a collective strength of recommendation grade based on a judgement of the overall weight of evidence (*Table 64*).

TABLE 64 Classification of strength of recommendation

A	Good evidence to support an ‘intervention’
B	Fair evidence to support an ‘intervention’
C	Poor evidence to support an ‘intervention’
D	Fair evidence to reject the use of an ‘intervention’
E	Good evidence to support the rejection of an ‘intervention’

Chapter 13

Economic evaluations of neonatal metabolic screening for phenylketonuria

Introduction

Over the last few decades, phenylketonuria screening programmes have been established throughout the developed world. As a result, a number of economic evaluations have been conducted to assess the cost-effectiveness, from a societal perspective, of screening neonates for this particular metabolic disorder.

The evaluations of phenylketonuria screening published to date are reviewed below and assigned

a grade with respect to study quality and thoroughness of conduct (*Table 65*).

Research findings

Cunningham, 1969

One of the earliest reported analyses of phenylketonuria screening was published by Cunningham in 1969.⁵⁴⁸ The study quantifies the costs of such a screening programme by obtaining relevant information from all participating laboratories

TABLE 65 Neonatal screening for phenylketonuria: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2b/IV	Bush <i>et al</i> , 1973 ⁵²⁵ (USA)	Is it worthwhile screening for phenylketonuria and what is the most efficient method? Major focus: to validate the use of a health status index.
II-2b	Barden <i>et al</i> , 1984 ⁵²⁹ (USA)	Benefit–cost analysis of screening programme to detect neonatals with phenylketonuria in Wisconsin.
II-2b	Hisashige, 1994 ⁵³⁵ (Japan)	To evaluate the efficiency of the mass screening system for phenylketonuria in Japan.
II-2b/c	Dhondt <i>et al</i> , 1991 ⁵³³ (France)	To compare the costs of identification and care of patients with expenditure on the care of untreated patients (phenylketonuria and congenital hypothyroidism).
II-2c	Alm <i>et al</i> , 1982 ¹⁰⁴ (Sweden)	A benefit–cost analysis to optimise the routines for neonatal metabolic screening.
V	Cunningham, 1969 ⁵⁴⁸ (USA)	To review the phenylketonuria programme in California, with emphasis on the role of testing laboratories.
V	Webb <i>et al</i> , 1973 ⁵⁴⁹ (Canada)	To evaluate the Ontario phenylketonuria screening experience in order to address the question ‘Is screening worth it?’
V	Steiner & Smith, 1973 ⁵³⁹ (USA)	To investigate the appropriateness of applying cost–benefit analysis to a phenylketonuria screening programme.
V	Veale, 1980 ⁵⁴¹ (New Zealand)	To highlight problems of phenylketonuria screening and to address difficulties that might arise from carelessly applying what is technically feasible without considering whether it is economically appropriate or ethical.
V	Goss, 1983 ⁵³⁴ (Belgium)	To estimate using cost–benefit analysis whether or not the expense generated by phenylketonuria screening is justifiable.
V	Cockburn <i>et al</i> , 1992 ⁵³⁰ (Scotland)	To address the issue of maternal phenylketonuria.
Strength of recommendation: A (see <i>Table 64</i>)		

in California and calculating a mean charge to parents per neonate screened. These charges are then compared with the estimated cost of running the screening programme. A 'rough' estimate of the total cost of detection and treatment of an unscreened individual with phenylketonuria is calculated, assuming a minimum of 30 years' life expectancy – the effect of relaxing this assumption is not investigated.

The study provides information on the prices charged per neonate screened in such a screening programme. However, these are not the real costs encountered and, hence, together with the failure to subject the 'rough' estimate of costs avoided because of the screening programme to a sensitivity analysis, provide no real evidence on the cost-effectiveness of neonatal screening for phenylketonuria. The study also fails to address the issue of discounting.

Bush and colleagues, 1973

The most sophisticated evaluation of phenylketonuria screening to date, was conducted by Bush and colleagues⁵²⁵ in an attempt to answer the question, "Is it worthwhile to screen for phenylketonuria?" However, unlike Cunningham⁵⁴⁸ and more recent studies, the main emphasis was the validation of the use of a health status index, by measuring the impact of phenylketonuria screening upon survival and quality of life.

The data for this study were obtained from the US National Phenylketonuria Collaborative Study reports, treatment centres and laboratory visits, published literature, and fiscal and patient data from the state. In addition, a team of 11 nationally-recognised phenylketonuria consultants were assembled to derive realistic estimates of the probability of various forms of phenylketonuria occurring, levels of retardation, levels of social function and mortality. Judgements were also made on the percentage of patients with phenylketonuria who would develop mental retardation with or without treatment depending on the disease form. This information was used to calculate an estimate of the expected number of years of full function capacity given to detected and treated patients with phenylketonuria, and a cost per output ratio was calculated using a discount rate of 4% (between \$2896 and \$3372 per function-year). A benefit–cost ratio was also calculated as part of the study (benefit–cost ratio = 1.33), which led to the conclusion that a phenylketonuria programme benefits society financially. Sensitivity to variations in inputs were tested for both ratios.

The method adopted by Bush and colleagues⁵²⁵ makes allowances for differences in mortality between patients with severe and mild mental retardation, and also recognises that not all cases of mental retardation are necessarily institutionalised for the same period; a fact often overlooked in reported phenylketonuria screening evaluations.

The analysis of phenylketonuria screening performed by Bush and colleagues⁵²⁵ did not incorporate the 'contribution' that an treated individual with phenylketonuria makes to society through the wages he earns. They argue that "such an analysis exaggerates the benefit of health programs with unrealistic economic imputation and that it is discriminatory and contradicts accepted social goals if it is applied systematically". This is a controversial issue which most evaluations reported to date ignore in their analyses, including changes in productive output.

Webb and colleagues, 1973

Another study was published in the same year by Webb and colleagues.⁵⁴⁹ Because of changes in the Ontario, Canada, phenylketonuria screening programme over the period of this study, they were unable to calculate accurate cost and benefit values. Their calculated estimates exclude the cost of the hospital's involvement in the programme (such as staff time and mailing charges to laboratories) and, as in most reported studies, no attempt has been made to put a price for the prevention of retardation to the child and his family. The study provides a useful description of the costs and benefits of screening but fails to conduct an actual analysis, hence, fails to address the issue of discounting and test the sensitivity of cost estimates.

Steiner and Smith, 1973

The study by Steiner and Smith⁵³⁹ consists of a retrospective and a prospective approach to assess the cost-effectiveness of phenylketonuria screening. The retrospective approach estimates the direct costs of the current population with phenylketonuria by collecting information from three mental institutions and calculating the cost per patient per year and multiplying this by the number of years an individual with phenylketonuria is institutionalised. The indirect costs are estimated using the median incomes of the population segmented by age and sex as a measure of the loss of income resulting from institutionalisation owing to phenylketonuria, based on the assumption that 1% of the population who are mentally handicapped suffer from phenylketonuria. The total costs (direct plus indirect costs) are compared with the estimated programme costs of detecting these

individuals through screening and maintaining them at a self-supporting status in society. This resulted in a benefit–cost ratio of 1.66.

The prospective study calculates the costs of screening, detecting and treating all neonates within a particular year. It evaluates these costs against the future savings (cost to society) of preventing the direct medical cost of individuals successfully diagnosed and treated plus the indirect costs of future economic productivity of these infants. However, although the authors discount the indirect benefits at the 4% level, they fail to discount the direct benefits; this results in an inconsistency in their methodology. If the direct benefits had been discounted to the present value, the cost–benefit ratio of 1.37 would have been reduced to an estimate closer to 1.⁵⁵⁰ The authors assumed that the average time for which an individual with phenylketonuria was institutionalised was 30 years; however, when they relaxed this assumption and, instead, assumed full life expectancy, the benefit–cost ratio was calculated as 2.60. This suggests that, if the life expectancy is less than 30 years, the benefit–cost ratio may well be less than 1 and, hence, phenylketonuria screening would not be beneficial.

In both approaches, Steiner and Smith⁵³⁹ showed phenylketonuria screening programmes to be beneficial. However, no measure of health status was incorporated into either of their analyses. They claimed that in their study the “costs of detection and control programs were maximised, while the direct and indirect costs (benefits) were minimised”, hence evaluating the most pessimistic scenario. However, because of inconsistencies in their use of discounting, the estimates of benefits calculated may in fact be optimistic.

Veale, 1980

Veale⁵⁴¹ adopts the prospective approach described above to examine the costs and benefits of phenylketonuria screening in New Zealand. The study uses data from the University Department of Community Health to estimate the cost of phenylketonuria screening and 10 years' treatment. In addition, the analysis attempts to incorporate a measure of mortality by using life-tables to depict the mortality experience of the 'normal' individual compared with individuals suffering from severe mental retardation (*Figure 6*). A life-table for treated cases of phenylketonuria should have been used to enable corrections to be made for expected mortality.

Different mortality experiences are assumed for different lengths of time for untreated individuals

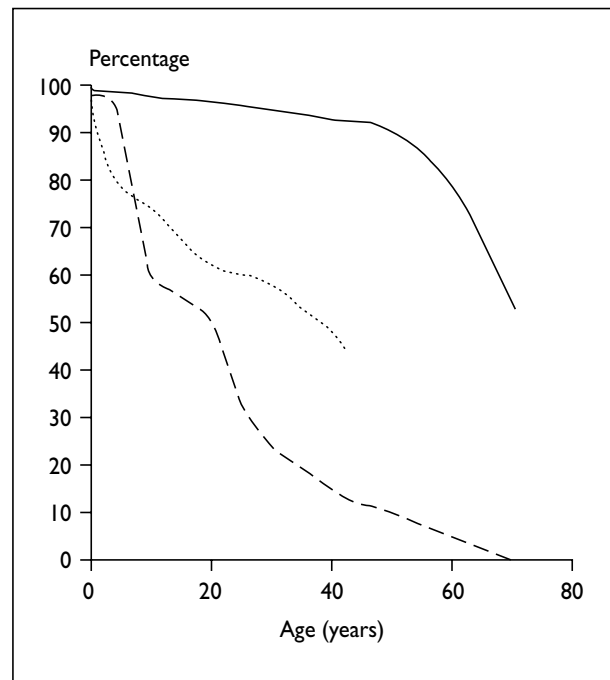


FIGURE 6 Life-tables for normal and severely retarded populations plus age distribution of 155 cases of untreated phenylketonuria (—, normal; ·····, severely retarded; ---, PKU).⁵⁴¹ Reprinted with permission

with phenylketonuria, and the cost of institutional care per annum is estimated to enable a series of benefit–cost ratios to be calculated for various scenarios. For example, assuming that all the cases detected in 1 year require 10 years institutional care, the benefit–cost ratio is approximately equal to 1 (i.e. the break-even point).

The results from the study showed that assuming averted institutional care to be the only benefit of phenylketonuria screening and considering only the first 10 years of life then the benefit–cost ratio could be less than 1 (*Table 66*). The author goes on to discuss the issue of lost productivity of an individual with phenylketonuria; however, this is not incorporated into the analysis.

Alm and colleagues, 1982

The prospective approach is also adopted by Alm and colleagues¹⁰⁴ in their attempt to optimise the routines for neonatal metabolic screening. The study assesses the whole Swedish neonatal screening programme, calculating a series of benefit–cost ratios for combinations of different screening tests, recall routines, and varying degrees of coverage.

The study used the results of Swedish neonatal metabolic screening programme for 1965–79, together with statistical reports, to inform the economic analysis. Only those costs and benefits that could be provided with a monetary value were

TABLE 66 Averted hospital costs of varying periods, the ratio is the benefit–cost ratio⁵⁴¹

Phenylketonuria screening in New Zealand Averted hospital costs (10% discount rate)*		
		Benefit–cost ratio
10 years' care	\$123,778	1.0
Mortality to 70 years	\$175,037	1.4
Mortality to 50 years	\$163,548	1.31
First 10 years (mortality to 70 years)	\$107,636	0.86
First 10 years (mortality to 50 years)	\$100,779	0.80
Reprinted with permission.		
* Averted hospital; costs based on 2.6 cases per annum; cost of blood spot collection, screening and treatment estimated to be \$47,625 per case or \$2.28 per neonate screened per annum; cost of institutional care approximately \$7000 per annum; calculation of the benefit–cost ratio only incorporates the first 10 years of life.		

included in the evaluation and the consumer price index was used to enable these costs and benefits to be adjusted to the 1979 fiscal year. A number of assumptions were made by the authors concerning dietary treatment and outcome/development of 'affected' individuals with and without a screening programme (*Table 67*) resulting in a benefit–cost ratio of 1.79. The authors made no attempt to incorporate a measure of health status into their analysis of phenylketonuria screening.

The effect on this ratio of varying the duration of dietary treatment was addressed in the form of a sensitivity analysis, along with the blood phenylalanine recall level, population coverage, discount rate and life expectancy, the effect of which are presented in the discussion.

Goss, 1983

The aim of the study conducted by Goss⁵³⁴ was to estimate whether the cost of neonatal screening for phenylketonuria is justifiable. In this study, the most important category of benefits is considered

to be "the 'saving' of a number of human lives each year". Hence, an estimate of the value of human life to render the benefits comparable to the costs is used. The approach adopted by Goss⁵³⁴ uses adult life-values to express mortality; that is, he assumes individuals who are mentally-handicapped as a result of phenylketonuria to be institutionalised at the age of 5 years, to have a lifespan of 30 years and an adult life-value of zero. However, rather than expressing the results as a cost per adult life-year saved, Goss loses accuracy by estimating the economic value of the average individual of different ages to enable the benefits (that is, the number of adult life-years saved plus other social benefits) to be expressed in monetary terms and, hence, a benefit–cost ratio to be evaluated. By increasing the discount rate adopted from 4.18% to 7%, the resulting benefit–cost ratio is reduced from 6.1 to 3.6. However, using either discount rate, a substantial benefit from phenylketonuria screening programmes is apparent.

Barden and colleagues, 1984

Barden and colleagues⁵²⁹ conducted a comprehensive cost–benefit analysis of screening for phenylketonuria. Data for the study were obtained from the State Laboratory of Hygiene for Phenylketonuria Testing, Waisman Center, Madison, Wisconsin, USA, and from US statistical abstracts. In the absence of life-tables for individuals with phenylketonuria, survival rates derived from a study by Fisch and colleagues, on the age at death of institutionalised individuals with phenylketonuria, are used to quantify the benefits (costs saved) resulting from phenylketonuria screening. Although these figures reflect past mortality rates, it is probable that they fail to reflect improved life-expectancy owing to improvements in medical care, hence resulting in an underestimate of the benefit–cost ratio.

A number of assumptions were made in the analysis about the severity of unscreened individuals with phenylketonuria (i.e. percentage severely retarded, moderately retarded and mildly retarded), the length of residential institutional care, and the percentage of mother-carers who, in the absence of a sibling with phenylketonuria, would normally choose to work (*Table 68*). The study fails to

TABLE 67 Assumptions used for the benefit–cost calculations for phenylketonuria¹⁰⁴

With screening		Without screening	
Dietary treatment	Outcome/development	Dietary treatment	Outcome/development
Treated 0–18 years and females during pregnancy	97% normal, 3% mentally retarded	10% detected and treated 0–18 years	Treated: 97% normal development Untreated: 98.5% retarded

TABLE 68 Assumptions concerning the degree of retardation and the level of care required by an untreated individual with phenylketonuria⁵²⁹

Assumption	Benefit–cost ratio
Option 1	
(a) 64% of individuals with phenylketonuria were profoundly or severely retarded and institutionalised from age 5 years for life	
(b) 36% of individuals with phenylketonuria were moderately or mildly retarded; 18% require foster care from age 5 to 20 years; 36% require adult care and services from age 20 years for life.	3.48
Option 2	
(a) 64% of individuals with phenylketonuria profoundly or severely retarded; 32% are institutionalised from age 5 years for life; 32% require foster care from age 5 to 20 years; 32% require adult care and services from age 20 years for life.	
(b) 36% of individuals with phenylketonuria moderately (20%) or mildly (16%) retarded; 18% require foster care from age 5 to 20 years; 30% require adult care and services from age 20 years for life.	2.67

incorporate a measure of health status and makes no attempt to include the costs encountered as a result of maternal phenylketonuria, despite devoting a section to its discussion.

The study used a 7% discount rate but also investigated the effect on the benefit–cost ratio of using a 4% and a 10% discount rate. The benefit–cost ratios calculated lay between 2.77 and 4.57, indicating, in agreement with previous studies, that phenylketonuria screening is beneficial to society.

Dhondt and colleagues, 1991

The study by Dhondt and colleagues⁵³³ compared the cost of identification and care of individuals with phenylketonuria with the expenditure on care of untreated, retarded patients with phenylketonuria. The data for the study were collected from the Nord-Pas-de-Calais regional screening centre, interviews with patients' families and the French national statistical agency. However, the analysis only takes account of the monetary benefits, merely acknowledging that some benefits are not easily quantified (e.g. the "increase in the well-being of parents and of 'affected' children"). Using a discount rate of 4.5%, the study resulted in an extremely favourable benefit–cost ratio of 6.6. Increasing the discount rate to 7% and 10% decreased the benefit–cost ratio to 4.7 and 3.3, respectively.

Cockburn and colleagues, 1992

As part of an article discussing maternal phenylketonuria, Cockburn and colleagues⁵³⁰ briefly reported the findings of a cost–benefit analysis carried out in the west of Scotland. The paper lacks information on how the costs and benefits in the analysis were calculated, merely stating that by "making many assumptions about job

opportunities and health costs per individual identified by screening a benefit of £174,124 at 1988 costs clearly indicates that neonatal screening is effective". However, detailed information about the additional costs of management in maternal phenylketonuria is supplied (i.e. clinic visits, blood tests, ultrasound scans, treatment and dietary management). The issue of maternal phenylketonuria is discussed in many of the articles discussed above but the costs involved are excluded from most economic evaluations. Inclusion of maternal phenylketonuria costs would add an extra £9000 per female with phenylketonuria to the costs of a screening programme.

Hisashige, 1994

The most recently published analysis of phenylketonuria screening was conducted by Hisashige.⁵³⁵ The analysis investigates the efficiency of mass screening for phenylketonuria in Japan by evaluating the costs and benefits accruing from such a programme. Cost data for the analysis were obtained from main laboratory centres and compared to averted cost (benefit) data obtained from the Ministry of Health and Welfare and the Ministry of Education. The benefits were quantified based on the percentage of unscreened individuals with phenylketonuria with varying degrees of mental retardation (i.e. most severely 'affected', severely 'affected', moderately 'affected', and mildly 'affected' and 'normal'). This information was obtained from the published literature on the outcomes of metabolic disorders in Japan prior to screening. Despite the low incidence rate of phenylketonuria in Japan (one case detected in 80,500 neonates screened), the study results are consistent with the results of other studies discussed above. The benefit–cost ratio obtained was 2.52, varying from 1.7 to 3.0 as the incidence rate was reduced

to one in 140,000 and increased to one in 60,000, respectively. A discount rate of 7% was used.

Discussion of study results

The majority of these studies obtained favourable results with benefit–cost ratios exceeding 1 (*Table 69*). Veale⁵⁴¹ was the only exception to this, obtaining inconclusive benefit–cost ratios ranging from 0.80 to 1.40. The benefit–cost ratios reported by Veale⁵⁴¹ to be less than 1 may be explained by the fact that only the first 10 years of life were included in these calculations.

In the studies that did find phenylketonuria screening programmes to be beneficial to society, the value of the benefit–cost ratio obtained varies considerably, ranging from 1.33 to 6.60.^{525,533}

By displaying the incidence of phenylketonuria and the chosen discount rate alongside the results obtained by the different studies, it becomes apparent that the highest benefit–cost ratio is encountered by the study with the highest incidence rate and the lowest discount rate.⁵³³ Certain studies have examined this relationship,^{104,535} along with other factors, by the use of sensitivity analysis and their findings are discussed below.

More recent studies tend to show neonatal screening for phenylketonuria to be more cost-effective than older studies (resulting in benefit–cost ratios > 2). This may be explained by improvements in technical and operational expertise, as well as developments such as automation of sample

preparation, leading to increased efficiency of screening programmes. However, the data from the literature do not offer a clear explanation.

Note that the results of different studies cannot be compared directly because of differences in both methodological and socio-biological backgrounds.

Discussion of sensitivity analyses findings

Having calculated a benefit–cost ratio, the majority of evaluations^{104,525,529,533–535,541} tested the ‘robustness’ of their findings by subjecting their results to a simple one-way sensitivity analysis; that is, by varying a number of uncertain components of the evaluation individually to establish the effect each component had on the benefit–cost ratio.⁵⁴⁶ However, many of the studies only varied one of the following components to establish their effect upon the benefit–cost ratio: the discount rate,⁵³³ the incidence rate⁵³⁵ or the life expectancy of an individual with phenylketonuria.^{539,541} Thus, they failed to address other areas of uncertainty, such as estimates of costs, life expectancy, treatment duration and coverage of the screening programme.

1. **Discount rate** An important factor affecting the benefit–cost ratio is the discount rate. Of the studies that investigated the influence of the discount rate on the benefit–cost ratio, it was discovered that the ratio decreases as the discount rate is increased (*Table 70*). For example, Barden and colleagues⁵²⁹ varied the discount rate from 4% to 10%, resulting in a

TABLE 69 Phenylketonuria: summary of study results

Study	Discount rate (%)	Incidence	Benefit–cost ratio
Bush <i>et al</i> , 1973 ⁵²⁵ (USA)	4.00	–	1.33 [mean output of functional capacity = 47.3]
Alm <i>et al</i> , 1982 ¹⁰⁴ (Sweden)	6.00	1:31,000	1.79
Barden <i>et al</i> , 1984 ⁵²⁹ (USA)	7.00	1:17,000	3.48
Dhondt <i>et al</i> , 1991 ⁵³³ (France)	4.50	1:15,937	6.60
Hisashige, 1994 ⁵³⁵ (Japan)	7.00	1:80,500	2.52
Steiner & Smith, 1973 ⁵³⁹ (USA)	4.00	1:46,714	1.66 (retrospective) 1.37 (prospective)
Veale, 1980 ⁵⁴¹ (New Zealand)	10.00	–	0.80–1.40
Goss, 1983 ⁵³⁴ (Belgium)	4.18	1:10,000	6.10
Cunningham, 1969 ⁵⁴⁸ (USA)	–	1:16,000	Costs & benefits NOT evaluated
Webb <i>et al</i> , 1973 ⁵⁴⁹ (Canada)	–	1:16,700	Costs & benefits NOT evaluated
Cockburn <i>et al</i> , 1992 ⁵³⁰ (Scotland)	–	–	Net benefit = £174,124

TABLE 70 The effect of different discount rates on the benefit–cost ratio for phenylketonuria

Study	Discount rate (%)	Benefit–cost ratio
Alm <i>et al</i> , 1982 ¹⁰⁴	6.00	1.79
	8.00	1.08
Goss, 1983 ⁵³⁴	4.18	3.50
	7.00	2.20
Barden <i>et al</i> , 1984 ⁵²⁹	4.00	4.57
	7.00	3.48
	10.00	2.77
Dhondt <i>et al</i> , 1991 ⁵³³	4.50	6.60
	7.00	3.30

decline in the benefit–cost ratio from 4.57 to 2.77, respectively.

- Incidence** Another factor influencing the benefit–cost ratio is the incidence of phenylketonuria within a population. Two of the published studies^{104,535} investigated the change of the benefit–cost ratio by sensitivity analysis for incidence, concluding that the benefit–cost ratio increased with incidence (*Table 71*).
- Life expectancy** The life expectancy of an untreated individual with phenylketonuria varies depending on the degree of mental disability and hence affects the number of years an individual requires care. Three studies^{104,539,541} investigated this issue using sensitivity analysis and, as would be expected, discovered that the benefit–cost ratio increased as the number of years institutionalised increased.

However, as can be seen from *Table 72*, the results from Veale⁵⁴¹ and Alm and colleagues¹⁰⁴ show only a slight effect on the benefit–cost ratio, whereas Steiner and Smith⁵³⁹ found the benefit–cost ratio doubled as the number of

TABLE 71 The effect of different incidence rates of phenylketonuria on the benefit–cost ratio

Study	Incidence	Benefit–cost ratio
Hisashige, 1994 ⁵³⁵	1:140,000	1.70
	1:120,000	1.90
	1:100,000	2.20
	1:80,000	2.50
	1:60,000	3.00
Alm <i>et al</i> , 1982 ¹⁰⁴	1:31,000	1.79
	1:65,000	1.00

Note: the incidence of phenylketonuria in Japan is lower than in many other countries.

TABLE 72 The effect of the number of years institutionalised on the benefit–cost ratio for phenylketonuria

Study	Period institutionalised (years)	Benefit–cost ratio
Steiner & Smith, 1973 ⁵³⁹	30	1.37
	70.8	2.60
Veale, 1980 ⁵⁴¹	50	1.31
	70	1.40
Alm <i>et al</i> , 1982 ¹⁰⁴	30	1.53
	50	1.71
	70	1.79

institutionalised years was approximately doubled. The reason is probably because of the inconsistency in the methodology adopted by Steiner and Smith,⁵³⁹ that is, the discounting of indirect but not direct benefits. The shift towards care in the community in the UK means that evolutions based on institutionalised care are no longer applicable in the UK context.

- Other influential factors** Other possible influential factors of the benefit–cost ratio were investigated by Alm and colleagues.¹⁰⁴ In their analysis of phenylketonuria screening, the authors investigated the effect of varying the phenylalanine recall level, the neonate population coverage and treatment period of detected phenylketonuria cases had upon the benefit–cost ratio. Unfortunately, the authors failed to investigate the effect that varying two or more components at the same time had upon the outcome, that is, a multi-way simple sensitivity analysis was not performed.⁵⁴⁶

Figure 7 shows how the benefit–cost ratio is affected by varying the blood phenylalanine recall level. The benefit–cost ratio reaches a maximum of 1.79 when the borderline level of 0.5 mmol/l is adopted. A marked decline in the benefit–cost ratio occurs at higher cut-off levels because of the increase in false-negatives. As would be expected, reducing the neonatal population coverage of the screening programme from 100% to 95%, reduces the benefit–cost ratio from 1.76 to 1.67 (*Figure 8*). This shows that population coverage contributes to the effectiveness of a phenylketonuria screening programme but is not as important as the phenylalanine recall level.

Alm and colleagues also investigated the uncertainty surrounding the period of treatment for a case of phenylketonuria detected by screening. They found that by reducing the

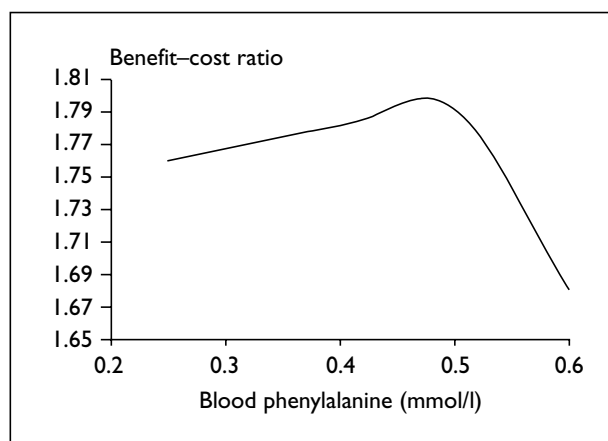


FIGURE 7 The effect of different phenylalanine thresholds (Guthrie test) for phenylketonuria screening on the benefit-cost ratio.¹⁰⁴ Reprinted with permission

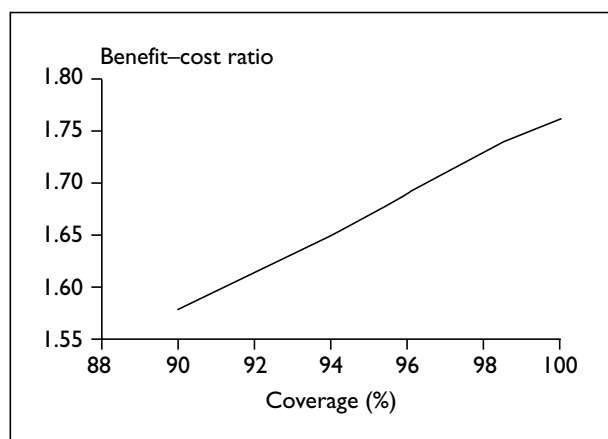


FIGURE 8 The effect of the coverage of the neonatal phenylketonuria screening programme on the benefit-cost ratio.¹⁰⁴ Reprinted with permission

treatment period from 18 years to 10 years, the benefit-cost ratio increased from 1.76 to 1.98. It would have been interesting if they had investigated the rate of decline in the benefit-cost ratio of an extended treatment period, to discover how rapidly it approached 1.

General discussion

A review of the literature on the economics of phenylketonuria screening revealed a lack of clearly defined and conducted evaluations. In the studies identified and discussed above, there was a lack of consistency in the costings methodology adopted, source/detail of data collection and the types of assumptions made. All the studies failed to reflect the emotional stress and personal care required from the families of an untreated child with phenylketonuria – that is, they neglected values like the reduction of suffering and handicap.

A category of costs often not incorporated in analyses is the additional treatment required by females with phenylketonuria during pregnancy, in order to prevent or minimise foetal damage. Cockburn and colleagues⁵³⁰ do include in their analysis the additional costs of management in maternal phenylketonuria ignoring the “possible deterioration of the older phenylketonuric individual and the physical, mental and intellectual handicaps of the child born to phenylketonuric mothers”. They acknowledge that “in spite of the diet, dangers, dilemmas and difficulties there is enormous reward for the families concerned when a healthy baby is born” and, hence, that it is a factor that should have been incorporated in the analysis; however, it is difficult to evaluate.

For a study to be classified as Quality Grade I, it has to be built around an RCT. The literature reviewed here compares screening with not screening for phenylketonuria, an area where RCTs are unlikely to receive ethical approval. However, five of the studies^{104,527,529,533,535} were allocated a Quality Grade II, indicating that the economic evaluations had been conducted as part of a well-designed cohort or case-controlled analytical trial. The UK Centre for Reviews and Dissemination guidelines¹ for systematic reviews allows that, in certain circumstances, Grade II or III may be equivalent to Grade I.¹ Thus, the evidence for phenylketonuria presented by some of these studies may be regarded as Grade I. The remaining studies were classified as Quality Grade V; they either suffered methodological problems,^{534,539,541,548,549} and/or lacked detail on the analysis performed.⁵³⁰

Summary

“Screening programmes for the detection of phenylketonuria in newborns are now so widespread and the benefits to society at large so generally accepted that any developed country without phenylketonuria screening program might be regarded as not having achieved the best possible standard of preventive medical care.”⁵⁴¹ This quote expresses the support for phenylketonuria screening, and the evaluations published to date provide the evidence of the programmes’ cost-effectiveness.

Despite a limited number of clearly-defined and well-conducted economic evaluations, the strength of the evidence provided through the identified literature was classified as Grade A, leading to the conclusion that good evidence exists to support a phenylketonuria screening programme.

Chapter 14

Economic evaluations of neonatal metabolic screening for congenital hypothyroidism, cystic fibrosis and Duchenne muscular dystrophy

Congenital hypothyroidism

In June 1981, the Department of Health and Social Security in Great Britain, issued a Health Notice¹⁶ stating that screening for early detection of congenital hypothyroidism should be incorporated into priorities for preventive medicine. Since this time, and earlier in some cases, congenital hypothyroid screening has been practiced in Great Britain, mainly by integration into the existing framework for phenylketonuria screening.

The literature identified on the economics of screening for congenital hypothyroidism evaluates the costs and benefits of such a screening compared to the 'no screening' alternative. Studies evaluating the incremental costs and benefits of introducing congenital hypothyroidism screening to established phenylketonuria screening programmes are reviewed in chapter 15.

Research findings

The aims of the relevant studies are summarised in *Table 73*.

Smith and Morris, 1979

Smith and Morris¹⁴⁹ undertook a literature review to establish the relationship between the IQ attained by infants with congenital hypothyroidism and the age at which therapy is initiated. The results of the literature search led to the conclusion that the "expected IQ of the hypothyroid patient is inversely related to the age at which therapy is initiated". However, the authors were cautious about the strength of such a conclusion, stressing that:

- the literature may fail to represent a cross-section of cases (for example, publishing only positive results)
- the origin of data may be inappropriate to present-day UK practice.

TABLE 73 Neonatal screening for congenital hypothyroidism: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2b	Barden & Kessel, 1984 ⁵²⁸ (USA)	Benefit–cost analysis of screening programme in Wisconsin to detect neonatals with congenital hypothyroidism.
II-2b/c	Dhondt <i>et al</i> , 1991 ⁵³³ (France)	A comparison of the costs of identification and care of patients, and the expenditure of the care of untreated patients with phenylketonuria and congenital hypothyroidism.
III	Smith & Morris, 1979 ¹⁴⁹ (England)	To perform a literature review to obtain published data with which to demonstrate the implications of a congenital hypothyroidism screening programme.
III	Layde <i>et al</i> , 1979 ⁵³⁸ (USA)	To analyse the economic consequences of a congenital hypothyroidism screening programme in terms of the costs of instituting such a programme nationwide in the USA and averted costs stemming from reduced institutionalisation and special education.
IV	La Franchi <i>et al</i> , 1979 ⁵⁵² (USA)	To present the results of the states of Oregon, Montana, Alaska and Idaho, regional programme of screening for neonatal hypothyroidism.
IV	Varma, 1979 ⁵⁵³ (Canada)	To discuss the results of hypothyroidism screening pilot projects set up in Quebec, Pittsburgh and Toronto.
Strength of recommendation: B (see <i>Table 64</i>)		

The benefits of screening for congenital hypothyroidism are also assessed by Smith and Morris¹⁴⁹ and include the consequent cost-savings to the community of special education, care and loss of productivity calculated from official statistics (that is, using average costs) together with the probabilities of an individual with congenital hypothyroidism being in care at various ages – derived from various UK prevalence studies. The weaknesses in the quantification of benefits are that only the benefits of avoiding a severely mentally-handicapped individual (i.e. not the moderate or mild cases) are assessed; only the average costs are used, resulting in a possibility of underestimation of total costs; the reduced burden imposed on the family of avoiding a mentally-handicapped dependent are ignored, and no measure of health status is incorporated.

The costs are quantified assuming that congenital hypothyroid screening is introduced using blood spots already collected as part of phenylketonuria screening programmes. The quantification of costs ignores the minimal costs of initiating treatment at an earlier age than unscreened cases and assumes the costs of identification of false-positive results to be negligible. Discount rates of 5% and 10% are adopted in the evaluation of the costs and benefits of screening.

Having only included the benefits accruing to an individual with severe mental retardation as a result of undetected congenital hypothyroidism, Smith and Morris¹⁴⁹ investigated the effect of including the estimated cost-saving of educating those with a mild mental handicap. This increased the benefit–cost ratio from 0.82 to 1.03 (10% discount rate) and from 1.70 to 2.00 (5% discount rate), thus resulting in a more favourable result than was at first calculated.

La Franchi and colleagues, 1979

Also published in the same year were the results of a study of screening for congenital hypothyroidism by La Franchi and colleagues.⁵⁵² This study attempted to extract the costs associated with screening for congenital hypothyroidism from the total cost of a screening programme for six metabolic diseases. The authors assumed that one-third of the costs encountered resulted from screening for congenital hypothyroidism. However, the effect of this assumption on the study findings was not investigated.

The averted costs/health benefits of a congenital hypothyroidism screening programme were not

quantified by the authors. Their reason for this was that although the average cost of institutional care for an untreated case may be obtained from the federal General Accounting Office, the number of neonates detected by the screening programme who would actually require institutionalisation was not obtainable. Hence, an evaluation of the costs and benefits was not performed.

Varma, 1979

The analysis by Varma⁵⁵³ uses cost and benefit data published in a report by the committee of the American Thyroid Association. From this data, together with estimates of the percentage of infants with congenital hypothyroidism requiring institutional care and their life expectancy, the authors are able to estimate the net saving of introducing a congenital hypothyroidism screening programme of between \$350,000 to \$500,000 for each ten cases detected. The calculated net saving is very approximate and the result of a number of assumption. No discount rate is adopted and no sensitivity analysis performed to address the uncertainties.

Barden and Kessel, 1984

In 1984, Barden and Kessel⁵²⁸ published a benefit–cost analysis of a screening programme to detect neonates with congenital hypothyroidism. The authors performed a very thorough and comprehensive analysis, quantifying the detection and treatment costs using data from the State Laboratory of Hygiene for hypothyroidism screening, and comparing these to the projected benefits resulting from the prevention of a mentally-retarded individual associated with this disorder (data obtained from *Statistical Abstracts* of the USA and the National Center for Health Statistics monthly *Vital Statistics Report*). However, they made no attempt to incorporate a measure of health improvement into their analysis. The authors assumed that 15% of individuals with undetected congenital hypothyroidism would be severely mentally retarded, 25% would be moderately retarded, 20% would be mildly retarded and 20% would have sub-optimum intelligence.

Both the costs and benefits were quantified in monetary terms and discounted at the 4%, 7% and 10% levels. The resulting net benefit values for early detection and treatment of an individual with congenital hypothyroidism were calculated. Converting the net benefit values to benefit–cost ratios (2.25–5.17) one can conclude that a congenital hypothyroidism screening programme is beneficial to both the ‘affected’ individual and to society as a whole.

TABLE 74 Summary of congenital hypothyroidism study results

Study	Discount rate (%)	Incidence	Benefit–cost ratio
Barden <i>et al</i> , 1984 ⁵²⁸ (USA)	7.00	1:6500	3.26
Dhondt <i>et al</i> , 1991 ⁵³³ (France)	4.50	1:4041	13.80
Smith & Morris, 1979 ¹⁴⁹ (England)	10.00	1:4700	0.82
Layde <i>et al</i> , 1979 ⁵³⁸ (USA)	7.50	1:6000	8.90
La Franchi <i>et al</i> , 1979 ⁵⁵² (USA)	–	1:5000	Costs & benefits NOT evaluated
Varma, 1979 ⁵⁵³ (Canada)	–	1:5750	Costs & benefits NOT evaluated

Dhondt and colleagues, 1991

Dhondt and colleagues⁵³³ investigated the cost of identification and care of patients screened for congenital hypothyroidism compared with the expenditure on care for patients with the untreated condition. Data were obtained from the Nord-Pas-de-Calais regional screening centre and the French National Statistical Agency, with future costs and benefits discounted at the 4.5%, 7% and 10% level. However, the analysis was limited to the averted costs (benefits) that were easily quantifiable in monetary terms and failed to address the effect of congenital hypothyroidism screening upon the health status of the ‘affected’ individual. The resulting benefit–cost ratio was extremely high, lying between 6.9 and 13.8.

Study results

All except one of the studies discussed above,¹⁴⁹ assessing the costs of implementing a congenital hypothyroidism programme and the likely benefits accruing from it, concluded that such a screening programme is beneficial. A summary of all the study results are presented in *Table 74*. A large variation in the benefit–cost ratio is apparent across studies, from 0.82 to 13.8.^{149,533}

The result obtained by Smith and Morris¹⁴⁹ using a 10% discount rate suggests that such a programme yields more costs than benefits; however, by varying one or two assumptions (e.g. the discount rate and severity of mental retardation), the result was altered to a more positive outcome.

Sensitivity analyses findings

Having obtained a beneficial result, most studies failed to perform a sensitivity analysis to assess the ‘robustness’ of their results. The three studies that did undertake a sensitivity analysis^{149,528,536} all adopted the simple, one-way sensitivity analysis approach,⁵⁴⁶ and all assessed the effect of varying the discount rate. None of the studies investigated areas of uncertainty such as estimated average

costs and benefits, treatment period of infants in whom congenital hypothyroidism was detected, and neonate population coverage.

Discount rate

By examining the effect of varying the discount rate, it becomes apparent that the benefit–cost ratio decreases as the discount rate is increased but the effect is less dramatic than for the phenylketonuria analyses (*Table 75*).

TABLE 75 The effect of different discount rates on the benefit–cost ratio for congenital hypothyroidism

Study	Discount rate (%)	Benefit–cost ratio
Smith & Morris, 1979 ¹⁴⁹	5.00	1.70
	10.00	0.82
Barden <i>et al</i> , 1984 ⁵²⁸	4.00	5.17
	7.00	3.26
	10.00	2.25
Dhondt <i>et al</i> , 1991 ⁵³³	4.50	13.80
	7.00	6.90

General discussion

A review of the literature on the economics of congenital hypothyroidism screening revealed a limited number of clearly defined and conducted economic evaluations. The six studies that were identified, were graded according to their quality. As was the case in the phenylketonuria economic literature review, none of the studies could be assigned a Quality Grade I because of the inappropriateness of an RCT for assessing the effectiveness of congenital hypothyroidism screening programmes. Two studies^{528,533} were allocated a Quality Grade II-2 for being part of a well-designed trial. Smith and Morris¹⁴⁹ and Layde and colleagues⁵³⁸ were both assigned a Quality Grade III because their evaluations of congenital hypothyroidism were conducted using descriptive/published

studies and reports of expert committees. As with the literature on phenylketonuria screening, literature fulfilling the criteria for Quality Grade I is highly unlikely to be available because of the ethical difficulties involved in conducting RCTs in this area. Therefore, literature of Grade II and Grade III may be thought of as equivalent to Grade I and II, respectively.¹ The remaining two articles^{552,553} were given a Quality Grade IV since they failed both to present their calculations clearly and to evaluate the costs and benefits of such a screening programme.

Summary

The main weakness of all the studies identified, and discussed above, was their failure to incorporate a measure of health gain (e.g. quality of life or life-years gained), limiting themselves to an evaluation of the costs and costs-avoided as a result of screening. All the studies showed screening for congenital hypothyroidism to be cost-effective but the extent of the cost-effectiveness ranged dramatically across countries and populations. The studies were collectively assigned a strength of recommendation Grade B; that is, fair evidence to support a congenital hypothyroidism screening programme.

Cystic fibrosis

Few evaluations of neonatal screening for cystic fibrosis have been reported in the literature. This is partly because of the uncertainty which has surrounded the question of benefit to both the patient with cystic fibrosis and their family and to society, and also because it is a less widely-practised screen.⁵³² As a result, there are few data on the diagnosis and management costs for patients with cystic fibrosis compared with, for example, patients with phenylketonuria.

Of the four analyses reporting evaluations of cystic fibrosis screening, two assess the difference in costs between screening and non-screening,^{532,554} while the other two perform a comparison of two screening protocols,^{285,304} viz. one-tiered screening – IRT assay, and two-tiered screening – IRT–DNA (see page 52).

Research findings

The aims of the relevant studies are summarised in *Table 76*.

Pauly, 1983

Pauly⁵⁵⁴ discusses the economics of cystic fibrosis and performs an assessment of the costs and costs-avoided as a result of screening. The author uses the assumption that a child with cystic fibrosis can be ‘cured’ or detected via prenatal screening and replaced with a healthy child, and uses ‘forgone earnings’ to estimate cost of illness, providing an underestimate owing to the failure to incorporate the positive effect of reduced pain and discomfort. The author assumes, with no justification, that 25% of children with cystic fibrosis will be hospitalised, that all such children will have the same mean non-hospital expense as all adults, and that mean hospital costs for hospitalised children are the same as for adults.

The costs and benefits of cystic fibrosis screening were quantified but not evaluated based on the assumption that an effective treatment existed for cystic fibrosis in 1983. This was an unrealistic assumption, since effective treatment for cystic fibrosis has only been established in more recent years. The study makes no attempt to incorporate a measure of the health benefit experienced by the individual (and their family) as a result of early diagnosis.

TABLE 76 Neonatal screening for cystic fibrosis: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2b	Farrell & Mischler, 1992 ²⁸⁵ (USA)	To analyse the costs of the Wisconsin screening programme in order to address a number of medical and social concerns, e.g. the financial uncertainty surrounding cystic fibrosis screening.
IV	Pauly, 1983 ⁵⁵⁴ (USA)	To investigate some of the economic aspects of research and care for patients with cystic fibrosis.
IV	Dauphinais, 1992 ⁵³² (USA)	To compare the costs of diagnosis by screening with the costs of diagnosis by signs and symptoms.
IV	Gregg <i>et al</i> , 1993 ³⁰⁴ (USA)	To compare two cystic fibrosis screening protocols.
Strength of recommendation: C (see <i>Table 64</i>)		

Dauphinais, 1992

An analysis by Dauphinais⁵³² compared the costs related to diagnosing cystic fibrosis by IRT screening to the costs associated with symptomatic diagnoses (i.e. relying on signs and symptoms). In the study, the medical costs of the non-screened patients with cystic fibrosis were collected from hospital billing and medical records, physicians' offices, and through personal communication with parents of affected children.

In the study, only the costs generated before diagnosis for non-screened patients were considered and the cost of sweat tests on children with cystic fibrosis, whether screened or not screened, were excluded. The total cost of screening was calculated based on labour and material costs. The analysis performed failed to incorporate costs associated with misdiagnoses of screening – that is, the additional costs of false-negative and false-positive results. The costs and benefits were evaluated by the author but failed to incorporate a measure of health benefit, a discount rate and no sensitivity analysis was performed.

Farrell and Mischler, 1992

Farrell and Mischler²⁸⁵ performed an evaluation of cystic fibrosis screening by using relevant data on costs and medical care collected in detailed records, and based on the actual experience of cystic fibrosis screening over a 5-year period in Wisconsin, USA. The analysis is mainly limited to direct medical costs, excluding physician fees for medical visits, family travel time, time lost from work and the costs of family stress. Farrell and Mischler do not incorporate discounting into their analysis. However, this may not be crucial as cystic fibrosis will present symptomatically very quickly and, therefore, the time lag between costs and benefits is likely to be small.

The study assesses the cost-effectiveness of both the one-tiered and two-tiered screening strategies, and identifies the main difference in cost between the two protocols to be the number of sweat tests that need to be performed and the number of false-positive cases.

Farrell and Mischler²⁸⁵ concluded that “neonatal screening *per se* contributes a relatively small additional cost to the overall cost of diagnosis and management of cystic fibrosis”. However, if better treatment could be developed or evidence produced to show early diagnosis and therapy in childhood prevents progressive lung disease over a patient's lifetime, the benefit from screening may be more substantial.

Gregg and colleagues, 1993

The most recently reported study by Gregg and colleagues,³⁰⁴ performed a comparison of the two screening protocols for detecting cystic fibrosis in individuals. Two protocols are described in detail with the differences highlighted, but the authors are very vague as to how the costs of each approach were calculated; no breakdown is provided of how the costs quoted (e.g. cost per sample tested, cost per sweat test performed) were calculated.

The authors concluded with a comparison of the direct costs for the IRT–DNA approach and the direct costs for the single-tiered IRT approach. The indirect costs were not included and no sensitivity analysis was performed to investigate how this might affect the results. There was no attempt to evaluate the costs and benefits of a cystic fibrosis screening programme.

Study results

The results from the four studies identified and discussed above are summarised in *Table 77*.

TABLE 77 Summary of cystic fibrosis study results

Study	Discount rate (%)	Incidence	Benefit–cost ratio
Dauphinais, 1992 ⁵³² (USA)		1:2700	2.32
Farrell & Mischler, 1992 ²⁸⁵ (USA)			Cost \$7244 per case detected (one-tiered IRT testing) Cost \$7043 per case detected (two-tiered IRT–DNA testing)
Pauly, 1983 ⁵⁵⁴ (USA)	6.00		Costs & benefits not evaluated
Gregg <i>et al</i> , 1993 ³⁰⁴ (USA)		1:3431	Cost \$10,187 per case detected (one-tiered IRT testing) Cost \$11,374 per case detected (two-tiered IRT–DNA testing)

Although both Pauly⁵⁵⁴ and Dauphinais⁵³² identified the costs and benefits associated with cystic fibrosis screening, only the latter evaluated the costs and benefits resulting in a favourable benefit–cost ratio of 2.32.

Both the remaining studies^{285,304} compared different approaches for cystic fibrosis screening rather than performing an economic analysis to evaluate the costs of screening compared to no screening.

Sensitivity analyses findings

All the authors of the studies discussed above failed to perform sensitivity analyses on their results; that is, they failed to investigate how the results may be affected by varying certain components of the analysis/calculations.

General discussion

Neonatal screening for cystic fibrosis has not been widely accepted because, until recently, it had not been definitely established that it improved the long-term outcome (see chapter 8). A major advantage of screening for an affected family is that the parents will be offered prompt genetic counselling with regard to their ‘carrier status’, and other relatives can be offered carrier testing (often called cascade screening).⁵⁵⁵

Four studies were identified as having performed some type of economic evaluation on cystic fibrosis screening. Of these four evaluations, only two made a comparison to the ‘do-nothing’ scenario, the other two concentrated on assessing the difference in costs and benefits of two different screening methods. Given that there is uncertainty as to the type and scale of the benefits from cystic fibrosis screening, such evaluations should include a comparison with the no-screening alternative. A common weakness of all the studies identified and discussed above is their failure to incorporate a measure of health benefit.

The identified studies were each assigned a grade according to their quality, that is, a grade determined by the study design, source of the data and appropriateness of costing methodology adopted. The Farrell and Mischler study²⁸⁵ was assigned a Quality Grade of II-2, since a well-defined evaluation was performed. The Dauphinais study⁵³² was assigned a Quality Grade of IV because of methodological problems, such as limited to direct costs and no sensitivity analysis. Similarly, the studies by Pauly⁵⁵⁴ and Gregg and colleagues³⁰⁴ were also assigned a Quality Grade of IV owing to the lack of an economic evaluation and a clear breakdown of costing calculations, respectively.

Summary

Dauphinais⁵³² identifies the most significant cost associated with screening as the failure to detect an ‘affected’ individual, that is, a false-negative screen. The costs incurred in this way include the medical costs when the ‘affected’ individual does present symptomatically plus the unmeasurable costs of parental anxiety. The main benefit identified by Dauphinais⁵³² is the benefit to the family of carrier testing and prenatal screening to avoid repeat cases. A Grade C was assigned for the strength of overall recommendation for cystic fibrosis screening assessed from the studies identified.

Duchenne muscular dystrophy

Duchenne muscular dystrophy is presently an incurable disease. The benefits of early diagnosis relate mainly to the family as a whole rather than to the individual patient. Given early warning, parents can make preparations for the future care of a child. In addition, early diagnosis provides information on the possible birth of further affected children to the mother or her female relatives, allowing appropriate reproductive decisions to be taken.⁵⁵⁶

A screening programme for Duchenne muscular dystrophy will often consist of a neonatal component for identifying affected individuals, and a prenatal component for identifying mothers at risk of giving birth to affected individuals. Evaluations of the costs and benefits associated with neonatal screening programmes should include prenatal screening and intervention, as well as the neonatal component.

Rosenberg and colleagues, 1993

Only one article was identified in the literature on Duchenne muscular dystrophy in which the cost-effectiveness of neonatal screening for the disease was investigated (*Table 78*). In this study, Rosenberg and colleagues⁵⁵⁷ quantify the total direct costs to avert a case of Duchenne muscular dystrophy, and compare these costs to similar costs for neonatal screening for other metabolic disorders. The study uses data obtained from laboratories and from the Manitoba Health Services Commission, Canada, to estimate the costs of the neonatal screening component.

The prenatal screening component costs rely on the probability of an identified carrier conceiving an ‘affected’ boy in subsequent pregnancies. The probability that the family obtaining a positive result will lead to the termination of the ‘affected’ pregnancy also has an impact on the cost.

TABLE 78 Neonatal screening for Duchenne muscular dystrophy: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2b	Rosenberg <i>et al</i> , 1993 ⁵⁵⁷ (Canada)	To estimate and analyse the total direct costs to avert a case of Duchenne muscular dystrophy and to compare these to similar costs for neonatal screening for metabolic disorders.
Strength of recommendation: C (see Table 64)		

The analysis calculates the incremental costs of averting one case of Duchenne muscular dystrophy by establishing and running a new screening programme compared with an 'add-on' screen in an already existing programme.

The analysis focuses on the direct costs associated with neonatal screening for Duchenne muscular dystrophy, and fails to take full account of the indirect and the intangible (reduced sorrow, anxiety and frustration) costs and benefits. The benefits of screening for Duchenne muscular dystrophy are described but not quantified and, hence, there is no evaluation of the costs and benefits. The study is purely a cost analysis, comparing the costs of Duchenne muscular dystrophy screening to the costs of other metabolic screening programmes. However, the authors do perform a thorough sensitivity analysis on their results by varying the component costs, CK test sensitivity, efficacy and compliance.

Study results

Rosenberg and colleagues⁵⁵⁷ calculate both the total and incremental costs to avert a case of Duchenne muscular dystrophy by neonatal screening, prenatal diagnosis and intervention (Table 79). A comparison is made between the laboratory screening costs per Duchenne muscular dystrophy case detected and the laboratory cost of detecting other metabolic disorders. The conclusion from this comparison is that the screening cost per case detected is approximately equal to that for galactosaemia, greater than for biotinidase deficiency and lower than for maple syrup urine disease.

Sensitivity analyses findings

Rosenberg and colleagues⁵⁵⁷ tested the 'robustness' of their results by subjecting them to a simple

one-way sensitivity analysis; that is, they varied the component costs, the CK test sensitivity, the efficacy and the compliance, in order to establish how a change in one of these components affected the study results. However, the authors failed to investigate the effect of varying the discount and incidence rates.

- Component costs** The effect of doubling the various component costs on the total discounted costs of detecting a case of Duchenne muscular dystrophy are shown in Table 80. The results show the most significant variation to be associated with the reagent costs; that is, doubling the reagent costs increases the total cost by 28%, whereas halving the reagent costs decreases the total cost by 14%.
- CK test sensitivity** The sensitivity of the CK test is uncertain; hence, it is important to vary the sensitivity to establish what effect this will have on the total cost of averting a case of Duchenne muscular dystrophy. From Table 81, it can be concluded that as the sensitivity of the CK test decreases, the total cost of averting a case of Duchenne muscular dystrophy increases.
- Efficacy** The proportion of preventable cases of Duchenne muscular dystrophy was assumed to be 15% in the original analysis. When this assumption was relaxed to 12.5% and 10%, the results were, respectively, a 15% reduction in cost and a 23% increase in cost.
- Percentage of carrier families who comply with advice** The compliance of carrier families with genetic counselling advice is uncertain. The authors calculated that if the percentage of families who followed advice decreased from 100% to 90%, then the number of years of screening required to avoid one case of

TABLE 79 Summary of Duchenne muscular dystrophy study results

Study	Discount rate (%)	Incidence	Benefit–cost ratio
Rosenberg <i>et al</i> , 1993 ⁵⁵⁷ (Canada)	5.00	1:4000 live born males	\$172,064 (total cost) \$83,103 (incremental cost)

TABLE 80 Effect of component costs on total discounted costs for Duchenne muscular dystrophy

Component costs	Effect on total discounted cost (%)
Reagent costs x 2	+28
Capital outlays x 2	+8
Genetic analysis x 2	+4
Prenatal diagnosis x 2	+2
Reagent costs x 0.5	-14

TABLE 81 Effect of CK test sensitivity on total discounted costs for Duchenne muscular dystrophy

CK test sensitivity (%)	Years of screening to avoid a case	Effect on total discounted cost
90	3.55	10% increase
80	4.0	23% increase
75	4.26	30% increase

Duchenne muscular dystrophy would increase from 3.2 years to 4.26 years, leading to a 30% increase in costs. Similarly, 50% or 25% compliance rates would increase the total cost by 86% and 221%, respectively.

General discussion

The analysis of Duchenne muscular dystrophy screening conducted by Rosenberg and colleagues⁵⁵⁷ fails to quantify the benefits accruing from such a programme; that is, the costs avoided owing to the prevention of repeat cases. The analysis concentrates on comparing the total direct costs per

case averted/repeat case prevented with the incremental costs.

The majority of metabolic screening programmes use mass population screening of neonates to identify individuals with a specific condition so that they can receive effective treatment. The objectives of Duchenne muscular dystrophy screening are distinctly different. The objectives of mass screening of neonates for Duchenne muscular dystrophy are:

- to identify affected individuals pre-symptomatically in order to allow the family to prepare appropriately for the long-term care of the affected child
- to identify women at risk of giving birth to affected individuals (carriers), leading to improved decision-making with an expectation of a reduction in number of 'affected' children.

The study by Rosenberg and colleagues⁵⁵⁷ was assigned a Quality Grade of II-2. Although the authors did not evaluate the costs and benefits of a Duchenne muscular dystrophy screening programme, they did perform a well-designed and thorough cost analysis.

Summary

Only one study was identified that evaluated the cost-effectiveness of Duchenne muscular dystrophy neonatal screening. However, this analysis was purely a cost analysis and did not quantify the health benefit to society of introducing such a screening programme. The overall strength of recommendation for Duchenne muscular dystrophy screening was assigned a Grade C classification – that is, the evidence to support the introduction of such a programme is poor.

Chapter 15

Economic evaluations of neonatal screening programmes and of alternative technologies for neonatal screening

Neonatal screening programmes

Nowadays, most of metabolic disorders are screened for as part of a screening programme rather than as an individual screen. It is, therefore, important to assess the cost-effectiveness of screening programmes as a whole, as well as by individual disorders screened. It is also important to assess the incremental costs and benefits resulting from the introduction of new screens to already established screening programmes.

Published studies assessing the costs and benefits accruing from a screening programme as a whole are limited in number. The studies that have been published are discussed below and are quality graded (*Table 82*) according to the classifications defined earlier (see page 110).

van Pelt and Levy, 1974

The Massachusetts Department of Public Health conducted an economic analysis in 1974⁵⁴⁰ to assess the costs of neonatal screening for a number of metabolic disorders in relation to realised and

potential benefits. The analysis quantifies the total cost of screening using reliable data obtained from surveying all hospitals with obstetric and neonatal units in the state. However, the estimated savings from the prevention of mental retardation and other complications is only based on the avoided costs of institutional care. Other benefits, such as cost saving on special education, lost productivity and improvement in quality of life, are not incorporated. The study fails to adjust for costs and benefits occurring at different times, and performs no sensitivity on the estimates used.

Alm and colleagues, 1982

With the aim of optimising the routines for neonatal metabolic screening, Alm and colleagues¹⁰⁴ carried out a cost-benefit analysis “to investigate what changes in screening routines might increase the benefit obtained with available resources and what further increase in investments might improve the benefit-cost ratio of the programme”. The analysis was based on the results of the Swedish neonatal screening programme, together with published data/statistics where available. Only

TABLE 82 Neonatal screening for neonatal screening programmes: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2c	Alm <i>et al</i> , 1982 ¹⁰⁴ (Sweden)	A benefit-cost analysis to optimise the routines for neonatal metabolic screening.
II-2	Dagenais <i>et al</i> , 1985 ⁵³¹ (Quebec, Canada)	To evaluate the profitability of the Quebec Network to society.
V	van Pelt & Levy, 1974 ⁵⁴⁰ (USA)	A cost-benefit analysis of newborn screening for metabolic disorders.
V	Holtzman, 1983 ¹⁴⁴ (USA)	To assess the costs and benefits of neonatal screening plus identify the pitfalls and consider how to overcome them.
V	Laberge, 1980 ⁵³⁷ (Quebec, Canada)	To study the organisation and cost-benefits of mass screening programmes – in particular, the Quebec network.
V	Kitagawa & Owada, 1983 ⁵³⁶ (Japan)	To report the results of cost-benefit analysis of newborn mass-screening for metabolic disorders, including congenital hypothyroidism.
Strength of recommendation: B (see <i>Table 64</i>)		

costs and benefits that could be expressed in monetary terms were included in the evaluation, and assumptions were made concerning the treatment (e.g. duration) and the outcomes (e.g. the degree of mental retardation).

The study performed an incremental analysis by first undertaking a cost–benefit assessment of phenylketonuria screening and then adding the other disorders screened for in the Swedish programme one at a time to establish the effect on the benefit–cost ratio. Many of the assumptions made in the analysis were relaxed in the sensitivity analysis but only with regards to phenylketonuria screening (see chapter 13).

Holtzman, 1983

A study conducted by Holtzman¹⁴⁴ assessed the cost-effectiveness of screening for both phenylketonuria and congenital hypothyroidism in a single programme. The analysis relies on cost data collected from the Maryland, USA, newborn screening programme and estimates, from the available data, figures for the costs avoided (benefits) as a result of screening (no measure of the effect upon quality of life as a result of introducing such a screening programme has been incorporated into the benefit calculations).

There is no description in the report of how the costs and benefits were quantified, although it is stated that a 6% discount rate was adopted. The author comments on the benefit–cost ratio of screening for maple syrup urine disease and galactosaemia, both as separate screens and as part of established phenylketonuria and congenital hypothyroid screening programmes. However, no actual analysis is performed on the incremental costs and benefits of expanding the existing screening programme to include these additional screens; hence, the author only speculates that the additional costs would be relatively small.

Kitagawa and Owada, 1983

Kitagawa and Owada⁵³⁶ evaluate the costs and benefits of neonatal mass-screening to detect phenylketonuria, maple syrup urine disease, histidinaemia, homocystinuria, galactosaemia and congenital hypothyroidism. Although tables of costs and benefits are provided in the published report, the source of the information is not given. No attempt is made to incorporate indirect costs and benefits associated with loss of production, and no allowance is made for differential timing of costs and benefits. In the analysis, assumptions are made concerning the duration of treatment and life-expectancy of screened individuals and the

degree of retardation suffered by the unscreened ‘affected’ individuals. No sensitivity analysis was performed to test these assumptions.

Laberge, 1980

Both Laberge⁵³⁷ and Dagenais and colleagues⁵³¹ (see below) assess the costs and benefits of the Quebec Network (Canada) neonatal mass screening programme for metabolic disorders, in an attempt to evaluate the profitability of the Network to society.

Laberge⁵³⁷ investigated the effect of adding congenital hypothyroid screening to the already established Network screening for phenylketonuria. The author evaluated the benefit–cost ratio for the introduction of congenital hypothyroidism screening both with and without the Network. However, he failed to state the source of the data used in the analysis and provided an inadequate description of how the costs and benefits were quantified. Assumptions were made regarding the IQs of early-diagnosed and treated individuals; however, the effect that varying these assumptions had on the results is not investigated by the use of sensitivity analysis.

Dagenais and colleagues, 1985

Dagenais and colleagues⁵³¹ made an assessment of the Quebec Network programme of neonatal mass screening included the screens of phenylketonuria, congenital hypothyroidism, Tay–Sachs disease and tyrosinaemia type I. The authors recognised the difficulties involved in quantifying the less tangible benefits and, hence, the study is restricted to the more obvious benefits, establishing a lower bound on the benefits of the Network and hence a lower bound of the profitability/net social benefit of the Network. As with most other studies, assumptions were made concerning the life expectancy, degree of retardation of screened and unscreened individuals. The detail of the costs and benefits used in the analysis are very explicit. The authors considered the costs and benefits associated with the separate screens and then calculated the net benefit associated with the Network as a whole. The costs associated with the Network system are not calculated; instead, an estimate is achieved by doubling the direct costs to allow for the indirect costs.

Study results

The summary of the study results presented in *Table 83* shows neonatal screening programmes to be beneficial. In particular, it portrays the change in the benefit–cost ratio as the number of disorders screened for increases. For example, the study by

TABLE 83 Summary of neonatal screening study results

Study	Discount rate (%)	Benefit–cost ratio	
Alm <i>et al</i> , 1982 ¹⁰⁴ (Sweden)	6.00	Phenylketonuria	1.76
		Phenylketonuria + galactosaemia	1.90
		Phenylketonuria + galactosaemia + tyrosinaemia	1.80
		Phenylketonuria + galactosaemia + tyrosinaemia + homocystinuria	1.78
		Phenylketonuria + galactosaemia + tyrosinaemia + homocystinuria + histidinaemia	1.74
van Pelt & Levy, 1974 ⁵⁴⁰		Phenylketonuria + galactosaemia + maple syrup urine disease + other urine-based screens	1.79
Laberge, 1980 ⁵³⁷ (Quebec, Canada)		Congenital hypothyroidism (without the Network)	1.80
		Congenital hypothyroidism (with the Network, i.e. with phenylketonuria)	3.90
Holtzman, 1983 ¹⁴⁴ (USA)	6.00	Phenylketonuria + congenital hypothyroidism	2.47
Kitagawa & Owada, 1983 ⁵³⁶ (Japan)		Congenital hypothyroidism	11.7
		Congenital hypothyroidism + phenylketonuria + galactosaemia + tyrosinaemia + homocystinuria + histidinaemia	5.13
Dagenais <i>et al</i> , 1985 ⁵³¹ (Quebec, Canada)	6.00	Phenylketonuria + congenital hypothyroidism + Tay–Sachs disease and tyrosinaemia	1.67

Alm and colleagues¹⁰⁴ shows that the benefit–cost ratio increases when galactosaemia screening is added to an existing phenylketonuria screening programme but the ratio decreases as more screens are introduced.

Sensitivity analyses findings

Many of the studies demonstrated a poor understanding of the diseases concerned (see chapters 6–9). Few studies investigated the ‘robustness’ of their conclusions by subjecting their results to sensitivity analyses.

However, two studies^{144,531} did investigate the effect of varying the discount rate on the net benefits. As would be expected, both studies concluded that the net benefit decreases as the discount rate is increased (Table 84).

General discussion

A review of the literature on the economics of neonatal screening programmes revealed a lack of well-defined and conducted evaluations. All the authors of the studies identified concluded that neonatal screening programmes were beneficial; however, the number and type of screens performed were not consistent across the studies making result comparisons difficult. All of the studies identified concentrated on evaluating the costs and the costs avoided, the authors failing to incorporate a measure of health gain into their evaluations of screening.

TABLE 84 The effect of different discount rates on the net benefit value of neonatal screening programmes

Study	Discount rate (%)	Net benefit
Holtzman, 1983 ¹⁴⁴	6.00	\$755,750 (Benefit–cost ratio = 2.47)
	10.00	\$115,750 (Benefit–cost ratio = 1.22)
Dagenais <i>et al</i> , 1985 ⁵³¹	4.00	\$53.2 million
	6.00	\$29.8 million
	10.00	\$2.6 million
	10.90	\$0 million – break-even point

Each of the studies was assigned a quality grade classification. The studies by Alm and colleagues¹⁰⁴ and Dagenais and colleagues⁵³¹ were allocated a Quality Grade of II-2 because of the well-designed and comprehensive economic evaluations. The remaining studies^{144,536,537,540} were all assigned a Quality Grade of V, owing to problems of methodology or inadequate descriptions of calculations.

Summary

The studies identified show neonatal screening programmes to be beneficial; however, as it becomes possible to screen for more disorders, economic evaluations, similar to those described above, need to be extended to investigate the effect on the cost-effectiveness and to incorporate a measure of health benefit. From these studies, the overall strength of

recommendation for neonatal screening laboratories to screen for a number of disorders from the same blood sample, was classified as Grade B.

Alternative technologies for neonatal screening

The literature reviewed so far has concentrated on either the evaluation of the costs and benefits associated with neonatal screening for a specific disorder in comparison to the 'no screen' alternative or the incremental costs and benefits owing to an expanding neonatal screening programme. However, once a screening programme has been proven to be beneficial to society, it would be useful to perform an analysis comparing the different methods of screening for a specific disorder. For example, in phenylketonuria screening, possible screening techniques include the Guthrie assay, fluorometry, enzymatic analysis and chromatography.

Only three of the studies identified performed an analysis to compare alternative technologies (*Table 85*).^{285,304,525} These studies were discussed earlier and their findings are revisited below.

Research findings

Bush and colleagues, 1973

The cost-utility evaluation of phenylketonuria screening performed by Bush and colleagues⁵²⁵

has been discussed earlier (page 112). The authors also performed a comparison of alternative phenylketonuria screening methods/technologies; that is, Guthrie microbiological assay, paper chromatography, automated fluorometry, ethnic screening, and 'private' programme. The total costs and outputs identified are summarised in *Table 86* (i.e. the estimated number of years of full capacity bestowed upon detected and treated cases of phenylketonuria, based on expert judgement of the percentage of individuals with phenylketonuria who would develop mental retardation with or without treatment) for these alternative screening methods, together with the cost per output/function year. By expressing the final results in terms of cost per output/function year, Bush and colleagues⁵²⁵ were the only study to incorporate a measure of health benefit into their analyses.

- **Paper chromatography** The use of paper chromatography as an alternative to the Guthrie assay, results in identical blood collection and reporting costs (i.e. hospital costs) but lower laboratory, follow-up and treatment costs. Besides reduced costs, Bush and colleagues⁵²⁵ discovered the paper chromatography method of screening for phenylketonuria resulted in lower ranges of abnormality than the Guthrie method, and also enabled additional aminoaciduria disorders to be detected.

TABLE 85 Neonatal screening using alternative technologies: quality of evidence and summary of study aims

Quality of evidence	Study	Aim of study
II-2b/IV	Bush et al, 1973 ⁵²⁵ (USA)	Is it worthwhile screening for phenylketonuria? What is the most efficient way to screen for phenylketonuria? Major focus of the study: to validate the use of a health status index.
II-2b	Farrell & Mischler, 1992 ²⁸⁵ (USA)	To analysis the costs of the Wisconsin screening programme in order to address a number of medical and social concerns, such as the financial uncertainty surrounding cystic fibrosis screening.
V	Gregg et al, 1993 ³⁰⁴ (USA)	To compare two cystic fibrosis screening protocols.

TABLE 86 Comparison of costs and outputs for alternative phenylketonuria programmes⁵²⁴

Cost or output per annual cohort	Guthrie assay	Paper chromatography	Automated fluorometry	Ethnic screening	Private programme
Total costs	\$836,387	\$690,831	\$721,187	\$589,450	\$1,301,386
Output (function-years)	288.8	287.3	288.8	288.8	231.2
Cost/output ratio*	\$2896	\$2405	\$2497	\$2041	\$5629
* cost per function-year					

However, they also found that by using paper chromatography, the output would only be 99% of that using the Guthrie method. Despite the results of their analysis, from which it would be concluded that the Guthrie method was more expensive in terms of cost per function-year, the authors identified a need for a thorough analysis of the sensitivity of both tests to be examined before conclusions were drawn about the allocation of resources to the most productive procedure.

- **Automated fluorometry** Bush and colleagues⁵²⁵ found that the laboratory costs associated with automated fluorometry were approximately 60% of those associated with the Guthrie method. All other costs and output were reported to be the same. The authors concluded that automated fluorometry for phenylketonuria screening would result in a 15% saving over the Guthrie procedure.
- **Ethnic screening** Bush and colleagues⁵²⁵ suggested that limiting phenylketonuria screening to the white Gentiles and Puerto Ricans would result in a significant reduction in phenylketonuria screening programme costs and have little effect on the output. The authors estimated a cost saving of 30%. However, the problems faced by the introduction of an ethnic screening programme would include the feasibility of such a screening programme (i.e. the identification and selection of 'at risk' neonates) and its political acceptability to the community.
- **Private programme** Without a state screening programme, a number of separate laboratories could offer a service on an individual neonate basis. A private programme like this would result in higher collection and testing costs and also a lower output level, as coverage of the neonatal population would be significantly reduced. By comparing the private screening programme with the centralised screening method (using the Guthrie procedure), the authors estimated that the cost per function-year would be approximately doubled.

Farrell and Mischler, 1992

Farrell and Mischler²⁸⁵ conducted an analysis to compare two screening methods for cystic fibrosis – the one-tiered IRT assay method and the two-tiered IRT–DNA method. The authors performed a costing comparison for the two protocols, concluding that the one-tiered IRT approach is slightly more expensive than the two-tiered IRT–DNA approach owing to a difference in

the number of sweat tests required and the number of false-positive cases identified.

Gregg and colleagues, 1993

Gregg and colleagues³⁰⁴ also undertook a comparison of both the above screening protocols for detecting neonates with cystic fibrosis. The report provided no breakdown of the calculations performed to obtain the costs of each screening method. Excluding indirect costs, the authors concluded that costs for the two-tiered method exceeded those for the one-tiered method by about 10%.

Study results

The results of the three studies are summarised in *Table 87*.

General discussion

Few technology comparison studies were identified through the literature search. Of the three studies identified and discussed above, only one⁵²⁵ evaluated the costs and consequences of different screening technologies and attempted to incorporate a measure of health gain, resulting in an economic analysis of neonatal screening being conducted ahead of its time. Both the remaining studies performed a cost comparison of the two protocols for cystic fibrosis screening but failed to incorporate consequences into their analyses. All the studies identified in this section were carried out in the USA and, hence, their results may not be completely applicable to the UK.

The studies identified were assigned grades according to their quality, as discussed earlier.

TABLE 87 Summary of results of studies of alternative technologies

Study	Incidence	Results
Bush <i>et al</i> , 1973 ⁵²⁵ (USA)		(see <i>Table 86</i>)
Farrell & Mischler, 1992 ²⁸⁵ (USA)		Cost \$7244 per case detected (one-tiered IRT testing) Cost \$7043 per case detected (two-tiered IRT–DNA testing)
Gregg <i>et al</i> , 1993 ³⁰⁴ (USA)	1:3431	Cost \$10,187 per case detected (one-tiered IRT testing) Cost \$11,374 per case detected (two-tiered IRT–DNA testing)

Summary

A review of the literature on the economics of alternative technologies for neonatal screening programmes revealed the lack of clearly defined and conducted evaluations. With present-day

advances in technology (i.e. automated DELFIA and tandem MS), it is important that more technology assessments are performed to identify the most effective approaches to neonatal screening for metabolic disorders of metabolism.

Chapter 16

Economics of neonatal metabolic screening: the results of the literature review

As a result of systematically searching the literature databases listed on page 109, together with the application of a well-defined inclusion/exclusion criteria, 43 studies were judged as relevant for the review. Of these, 70% focused on the economics of specific neonatal screens, 14% on the economics of neonatal screening programmes and 7% on the economics of neonatal screening technologies. The studies selected for review covered neonatal screening for four main disorders (i.e. phenylketonuria, congenital hypothyroidism, cystic fibrosis and Duchenne muscular dystrophy) and a number of 'add-on' screens (e.g. galactosaemia, tyrosinaemia, and homocystinuria).

Quality of the studies

Most of the studies identified suffered from problems of methodology (Grade V), with the remainder either classified as well-designed cohort studies (Grade II-2) or based on the opinions of respected authorities, descriptive/published studies and/or reports of expert committees (Grade IV) (see *Table 88*).

Studies classified as Quality of Evidence Grade V included studies which used neonatal screening charges instead of the real costs associated with the screening process, suffered from inconsistencies in their application of discounting costs and consequences, lacked an explanation for not adopting discounting and/or quantifying the costs and consequences, but failed to evaluate them.

Studies that failed to clearly state the assumptions made to achieve their final outcome (i.e. cost per screen, total cost, etc.) were also classified as Quality of Evidence Grade V.

Most studies classified as Quality of Evidence Grade II-2 collected the cost of screening and treatment data prospectively from screening laboratories and dieticians, and evaluated these against historical data, extracted from the literature, on the costs averted as a result of screening.

Strength of recommendations

Although a large proportion of the studies suffered from problems of methodology, for congenital hypothyroidism, phenylketonuria and multiple screens, the strength of recommendation for implementation of screening programmes was judged as good (A) to fair (B) (see *Table 89*). For disorders such as cystic fibrosis and Duchenne muscular dystrophy, the strength of recommendation was classified as poor (C) owing to a lack of well-conducted studies identified in the literature.

Country of origin

Of the 43 studies identified, only two were conducted in the UK (*Table 90*). Most studies included in the review were performed in the USA and, hence, are not directly relevant to neonatal screening in the UK. International comparisons in neonatal screening may be invalid for a number of reasons:

- differences in the prevalence of disorders between countries

TABLE 88 Summary of quality of evidence

Quality of evidence	Description	Number of articles
(II-2a)	Evidence obtained from a well-designed cohort (prospective study) with concurrent controls	1
(II-2b)	Evidence obtained from a well-designed cohort (prospective study) with historical controls	5
(II-2c)	Evidence obtained from a well-designed cohort (retrospective study) with concurrent controls	3
(IV)	Evidence obtained from the opinions of respected authorities based on clinical experience, descriptive/published studies and/or reports of expert committees	2
(V)	Evidence inadequate owing to problems of methodology; e.g. discount rate, sensitivity analysis; evaluation of costs and benefits; lack of detail	15

TABLE 89 Summary of strength of recommendations

Screens	Strength of recommendation*	Evidence to support the intervention
Phenylketonuria	A	Good
Congenital hypothyroidism; neonatal screening programmes	B	Fair
Cystic fibrosis; Duchenne muscular dystrophy	C	Poor
* see Table 64		

TABLE 90 Country of origin of the studies identified

Country	Number of studies
USA	12
Sweden	1
France	1
Japan	2
Canada	6
New Zealand	1
Scotland	1
Belgium	1
England	1

- different test thresholds leading to different specificity and sensitivity rates
- screening at different times; for example, neonates are screened earlier in the USA (day 2/3) than in the UK (day 6/7)
- structural differences in the healthcare systems leading to different costs of provision.

Of the two UK-based studies one, from 1979, is a literature review of screening for congenital hypothyroidism;¹⁴⁹ the other, from 1992, is a retrospective study of screening for phenylketonuria in Scotland.⁵³⁰ Neither study provides data which could be used as the basis for examining the probable costs and benefits of expanding the existing UK neonatal screening programmes. Given this, a full prospective evaluation of neonatal screening in the UK, incorporating the significant developments in the methodology of economic evaluations in health care, would represent a major improvement in the knowledge base for neonatal screening.

Discussion

Work to date has been heavily influenced by Wilson and Jungner's ninth principle.³⁴ This puts the focus on the costs of the screening programme being compared with the costs of care for affected individuals if the screening programme did not exist. This approach treats screening as a special case in health care by excluding the value of the health benefits from the evaluation.

The range of techniques for evaluating healthcare technologies were briefly described earlier. Over the past 5 years, significant work has been carried out looking specifically at the best approach to evaluating screening programmes, based on the argument that they are a special case in health care, providing information that may lead to the possibility of improved health rather than to an improvement in health directly.⁵²⁶

Cost-effectiveness analysis, that is, cost per true case detected, is only appropriate if the only outcome of interest is the true-positive. Cost-effectiveness may be an appropriate way to consider at alternative ways of screening for a single condition but is unlikely to be appropriate for evaluating screening programmes or extensions to screening programmes, as it assumes that the outcomes are the same for all cases detected.

Cost-utility analysis may overcome this difficulty, in allowing the benefits of the different screening programmes to be expressed in a common unit (QALY). However, work by Donaldson and colleagues⁵⁵⁸ (among others) has established that there are some benefits from screening, specifically the value of the knowledge and the option value which comes with that knowledge, and this will not be included in a QALY measure.

Cost-benefit analysis requires that all relevant benefits are given a monetary value. It is normally proposed that these valuations are obtained through the 'willingness-to-pay approach. This essentially consists of asking individuals how much they are willing to pay to achieve a certain outcome, that is, how much do they value that outcome. It is assumed that in arriving at a valuation, individuals will incorporate all the aspects (both positive and negative) of the outcome that are important to them. There are a number of criticisms of this approach, the most frequently cited one being that willingness-to-pay is strongly related to ability-to-pay, and may therefore produce inequitable solutions.

Another, frequently-cited criticism is that, in societies where individuals receive health care free at the point of consumption, this type of question is not meaningful and an individual's responses cannot be relied upon to reflect true values. Research into the role of willingness to pay in the area of health care is in its infancy but results of willingness-to-pay studies have been used in other areas of public expenditure, particularly transport.⁵⁵⁹ On this basis it would seem sensible and consistent to at least explore the potential of this approach to economic evaluation.

Whatever technique is adopted by future evaluations of neonatal screening, it must be one that incorporates at the very least the health benefits resulting from the screening programme in to the evaluation. Ideally, it should incorporate the non-health benefits as well.

Although a number of the studies reviewed did carry out some sensitivity analysis, none of them went further than one-way analysis. More extensive, multi-way sensitivity analysis in future evaluations would add greatly to the generalisability of such work.

Many of the studies identified do not set out the derivation of the estimated costs they use. This makes it difficult to compare studies or to attempt

to reproduce a particular study's results. Ideally, the resources used by a screen need to be identified, and then costs attached to these resources for the purposes of the evaluation. This approach allows others to calculate what it would cost to provide an identical service using local costs, thereby increasing the value of such work.

There are a large number of candidate diseases for neonatal screening but economic evaluations have been reported for only a very small proportion. The majority of these evaluations have been of stand-alone screens. This reflects the state of knowledge about many of the candidate diseases, and the state of development in screening technology.

Technological developments such as tandem MS and DELFIA mean that many more candidate diseases can technically be screened for, and far fewer of these screens will be stand-alone. As a result, there will be pressure to screen for many more conditions. More data on long-term outcomes for children with these conditions and efficacy of treatment will be required if robust economic evaluations are to be undertaken. Evaluations of these screens must examine the incremental costs and benefits on a disease-by-disease basis, and not take an 'all or nothing' approach which could lead to a non-optimum screening strategy.

Chapter 17

Criteria for neonatal screening: evaluation from evidence in the literature

The literature survey revealed many inherited metabolic diseases which could be screened for and, indeed, have been screened for at various times and in various places. As outlined in chapter 9, not all have been followed-up in detail but there is still a formidable list of conditions for which practical techniques are available and which could well be suitable for whole population neonatal screening.

The most widely quoted set of criteria for judging such candidate screens are the 'Principles of Early Disease Detection' formulated by Wilson and Jungner in 1968.³⁴ Most of these are still considered applicable to screening activities of all types but some are being seen increasingly as inappropriate for neonatal screening,^{30,486} particularly for heritable diseases which have a high risk of recurrence in subsequent siblings. In the Wilson and Jungner principles, the focus was the individual being screened whereas, in neonatal screening, the beneficiary is the family – the parents being the sentient 'customers', having almost as much to gain from the screening process as the baby itself.

Wilson and Jungner revisited

How far do the diseases under review meet the original Wilson and Jungner principles? In the following discussion, where there appears to be conflict between the principles and currently accepted practice, suggestions as to how the principles should be modified for this specific application are interpolated in boxes. A slightly fuller version of the principles is presented in chapter 3.

Principle 1 The condition sought should be an important health problem

It is clear in their discussion of this principle that, for Wilson and Jungner, importance as a health problem was to be judged by **incidence** as well as the **consequence for the affected individual**: "Phenylketonuria is extremely uncommon but warrants screening on account of the very serious consequences if it is not discovered and treated very early in life".

Incidence – Phenylketonuria is often regarded as the paradigm of neonatal screening tests and, in the UK, the population frequency needed to justify screening has tended to be judged using phenylketonuria as a yardstick. National perspectives may vary considerably as is exemplified by the comments made at an international meeting in 1979 by RWE Watts (UK).³⁶ "This [minimum incidence of the disease in the target population] was provisionally set at 1 in 10,000 by analogy with the approximate overall incidence of phenylketonuria in Europe and North America. Discussions at the present meeting suggested that a lower incidence should be accepted, provided other criteria are met, to include galactosaemia and maple syrup urine disease, which have incidences of 1 in 50,000 and 1 in 200,000 respectively." Many of the diseases discussed in this review are exceedingly uncommon but, when they can be grouped together with more common disorders and screened for at little additional cost, this rarity is no longer a bar.

Consequences for the affected individual – With the exception of short-chain acyl-CoA dehydrogenase deficiency, the diseases reviewed in chapters 6–9 are serious or potentially serious health problems to the individuals concerned. Biochemical abnormalities of dubious clinical significance have been excluded (see chapter 9). The only philosophical difficulty arises with diseases such as medium-chain acyl-CoA dehydrogenase deficiency, where all affected individuals are at risk but there is no way of knowing in advance which will suffer serious consequences.

The matter of incidence should be considered as part of the economic evaluation under Principle 9.

Principle 2 There should be an accepted treatment for patients with recognised disease

In nearly all the diseases reviewed, specific treatment is given to the patients once they have been diagnosed, irrespective of whether this was clinically or by screening. The main exceptions are Duchenne muscular dystrophy and the very severe variants of

some organic acid, fatty acid oxidation, and urea cycle disorders. Such treatment is often successful in the short term although, for some conditions, the long-term results are still disappointing or uncertain. Wilson and Jungner³⁴ request consideration of whether treatment "...at an earlier stage than normal affects...course and prognosis" – most paediatricians will embark on treatment if it makes the patient better for the moment.

There are other benefits from diagnosis. It has very important genetic implications for the family, enabling informed choices to be made in its plans for the future (chapter 2). If diagnosis is significantly delayed beyond the neonatal period, or even missed altogether, further pregnancies may be undertaken without parents appreciating the risk involved. Families place great value on prompt diagnosis for its own sake (chapter 11). In the absence of screening there is often a considerable delay from the appearance of the first symptoms and a diagnosis being made. Hall and Michel⁵⁶ ask, "Why does early diagnosis of serious disease seem so important to parents even when it makes little or no difference to outcome? ...Avoidable delays in diagnosis and referral feed the natural sense of anger and betrayal felt by the parents of a sick child...they want to know about the child's problems as soon as possible. It seems that the longer the parents' shared life with a child they believe to be normal, the more devastating is the discovery of a serious illness or disorder." Prompt diagnosis makes a major contribution to the parents' perception of the effectiveness of their child's medical care. Thus the **lack of effective treatment** is not an absolute contraindication to neonatal screening, as evidenced by the acceptance of screening for Duchenne muscular dystrophy both among parents and professionals (see chapter 8). As a corollary to this, **a diagnosis is of value to the family even if the affected child has already died.**

Effective treatment is not an absolute prerequisite for neonatal screening to be of value to the families concerned.

Principle 3 Facilities for diagnosis and treatment should be available

The facilities for diagnosis and treatment of the diseases under consideration are already available in most areas of the UK: arrangements to ensure a rapid and 'seamless' response to an abnormal screening test result will need to be made prior to introducing new screens. The importance of a coordinated service has been stressed by Laberge.⁵⁶⁰

"Newborn genetic screening programs should integrate follow-up procedures into their system since it provides and validates the benefit to the newborn. Such follow-up should include referral to effective medical intervention and to other support services and resources." The facilities required and their organisation are discussed further in chapter 19.

Principle 4 There should be a recognised latent or early symptomatic stage

The specification that for this review we should ascertain for the conditions concerned "...the number of cases presenting before 10 days (the point at which about 75% of Guthrie cards have been tested)" (chapter 1) reflects this principle. It rests on the assumption that in the neonatal period diagnosis of inherited metabolic disease normally follows closely on symptomatic presentation. In fact, symptoms are often non-specific and, in an ill neonate, investigations for metabolic disease are frequently delayed until more common and easily detectable causes have been excluded (see, for example, the New Zealand experience with screening for maple syrup urine disease³⁰). A plausible explanation for the symptoms may be found without the underlying disorder being uncovered. Not infrequently the family history of a child diagnosed with an inherited metabolic disease reveals a sibling death which had been ascribed to some other cause but which could equally have been due to the disorder in question. Galactosaemia presents a good example of this, as in untreated neonates the terminal event is often liver failure or *E. coli* septicaemia (see chapter 8). In the series of 47 families reported by Donnell and colleagues,²⁴⁶ there had been 13 infant deaths, mostly ascribed to these causes, before the diagnosis of galactosaemia in a later child. Universal screening, irrespective of clinical state, may well be the most sensitive and cost-effective approach to diagnosis. As a corollary to this, and almost reversing the principle, if a tandem MS were introduced as a screening test, arrangements should be made for symptomatic neonates to be screened from an early sample as well as at the appointed time.

Neonatal screening is also of value in diseases which present early but with non-specific symptoms.

Principle 5 There should be a suitable test or examination

Wilson and Jungner³⁴ remark "...the screening test...is allowed to possess a higher margin of error and may be less valid than a diagnostic test. ...In

case-finding work a fairly high false-positive rate is acceptable but the false-negative rate should be very low, since missed cases may lead to individual disasters.” The psychological literature shows that false-positive results in neonatal screening create considerable stress and anxiety to parents (see chapter 11); however, different centres appear to place considerably different emphases on the relative importance of minimising the number of ‘false-positive’ second heel-pricks on normal babies on the one hand and avoiding (a very much smaller number of) false-negatives on the other. What constitutes a **suitable test** and ways of optimising screening test performance are discussed below.

Principle 6 The test should be acceptable to the population

The literature shows that there is general parental support for neonatal screening using heel-prick blood samples (see chapter 11). However, the problem of acceptability has become more complicated in recent years and there has been an increasing tendency to include legal, ethical and ‘consumerist’ considerations in the evaluation of screening programmes.⁵⁶⁰ The Wilson and Jungner principle assumes that the **subject to be screened** can make an informed choice but in neonatal screening it is the attitudes of the parents that have to be considered. Some parents refuse the test, which raises difficult legal and ethical questions. It has been argued that screening is part of routine neonatal medical care and that it is doubtful whether parents have the (moral or legal) right to refuse screening for preventable disease on behalf of the child.⁵¹⁷ In theory, the newborn baby could be made a ward of court but in practice, unless there is a high prior risk, this is unlikely to be put to the test. The opposite view is that informed consent is a paramount prerequisite and that there should be prenatal counselling on the issues surrounding neonatal screening.⁵⁶¹ The screens for Duchenne muscular dystrophy and cystic fibrosis are usually cited in favour of this stance. The literature on informed consent in the UK is inconclusive but shows that parents generally have a very poor knowledge of the details of the programme (see chapter 11). Practical issues relating to information provision are discussed later in chapter 19.

Principle 7 The natural history of the condition, including development from latent to declared disease, should be adequately understood

The literature on this aspect is fairly comprehensive for all the diseases reviewed (except short-chain acyl-CoA dehydrogenase deficiency).

Principle 8 There should be an agreed policy on whom to treat as patients

There is general agreement in the literature on this matter. In a few diseases, problems arise over patients with very mild biochemical variants – of phenylketonuria, for example, where it is not possible to draw a firm boundary. In these patients, a modified less-demanding treatment regime is usually adopted.

Principle 9 The costs of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole

As described earlier, published economic evaluations of neonatal screening programmes to date have concentrated on comparing the costs of screening and treating the identified patients with the costs of managing cases who present symptomatically. This also reflects the historical development of neonatal screening programmes; public funding of screening for phenylketonuria being justified (particularly in the USA) on the basis that there would be a net saving to the public purse. However, this approach to the economic evaluation of neonatal screening is very limited, taking no account of the health and information benefits that are attributable to the screening programme. To this extent, neonatal screening has been treated as something of a special case in the field of economic evaluation of health care. Normally, the costs of an intervention are set against the healthcare benefits from that intervention, not merely the costs avoided as a result of the intervention. The issues of what should be included in future economic evaluations have been discussed in chapter 12.

Principle 10 Case-finding should be a continuous process and not “a once and for all” project

The current neonatal screening programme is a permanent service and the proposed extensions would be permanent additions.

The conclusions from this discussion is that any serious inherited metabolic disease is a candidate for neonatal screening: whether it is acceptable will depend on the performance of the screening test and the balance between cost and resulting health benefits.

‘A suitable test’: literature evidence on screening test performance

The essential requirement for a suitable test is best summarised by Haggard³⁸ – “...the incidental harm done by screening, and by the information (correct or otherwise) that it gives, should be small in relation to the total benefits from the screening–assessment–treatment system”. The incidental harm done in the course of screening depends very much on the technical performance of the screen concerned, in particular, on the numbers of repeat samples, follow-up tests, and false-positives on clinical referral. False-negative results detract from the value of a screen by reducing the yield and theoretically may increase the impact of the eventual diagnosis.

False-positive results – As described earlier (chapter 3), there are different types or degrees of ‘false-positive’ result. Some screening protocols require a relatively high proportion of repeat blood samples, while others, such as the two-stage IRT–DNA protocol for cystic fibrosis, have virtually no requirement for repeat blood samples but refer a high proportion of non-affected patients for sweat-testing. In addition, even in screening for the same disease, there are large inter-laboratory variations in the number of repeat blood samples requested because of ‘intermediate’ results (see next chapter). This may partly reflect differences in precision of the analytical methods used, though even laboratories using the same commercial assay kits show considerable differences in repeat rates. Not all laboratories use the standard UK screening card produced by HMSO and differing degrees of uniformity of the blood collection paper, or even differences in the general quality of the blood spots themselves, may also contribute to differences in repeat rates. However, a major factor leading to differing repeat rates is the relative priority given by different centres to minimising the number of second heel-prick tests on normal babies on the one hand and avoiding false-negatives on the other.

To retain a sense of proportion when discussing repeat sampling rates it should be borne in mind that the most common reason for a repeat sample request is insufficient blood on the initial card. This produces a repeat sampling rate of approximately 1% in many areas.

False-negative results – The significance of a false-negative screening result is different for different diseases. A timely clinical diagnosis of medium-chain acyl-CoA dehydrogenase deficiency, for example, may redeem the situation, but for phenylketonuria the symptoms are irreversible and a missed case is a disaster. Where screening tests lack sensitivity it will often be milder variants that are missed, for example, in cystathionine β -synthase deficiency the pyridoxine-responsive form. It may be necessary to accept a screening test with low sensitivity as better than no test at all but it is important that the limitations are made clear.

Designing a suitable test – There appears to be no general consensus on the relative importance that should be given to false-positive and false-negative results; the impact of these will differ greatly from disease to disease and with the screening method used. The most suitable test is the one that minimises both types of error simultaneously, and improving analytical precision by using tandem MS rather than the Guthrie microbiological assay, for example, should have that effect. However, in many diseases the basic biochemistry is the limiting factor and it is not possible to increase specificity without sacrificing sensitivity. In these cases, it may be possible to progress by combining an initial low specificity screen with a higher specificity diagnostic test using the same blood sample, as in the Quebec tyrosinaemia type I protocol (see chapter 7). This allows a low cut-off point to be set on the initial screening test, giving high sensitivity, while at the same time having a low false-positive rate for the overall procedure.

The organisational measures required to minimise the negative impact of any extension to neonatal screening are considered in chapter 19.

Chapter 18

Economic evaluation of neonatal screening

Introduction

All regions of the UK have neonatal screening programmes for the disorders of phenylketonuria and congenital hypothyroidism, with many regions also screening for additional disorders.

This chapter of the report describes a model covering neonatal screening for inborn errors of metabolism. The model can be used to analyse the costs and benefits of introducing tandem MS, focusing on the additional diseases that could be screened for as a result.

The construction of the decision model involved the identification of pathways and subsequent choice nodes and chance nodes appearing in the neonatal screening process. The data requirements, and the appropriate sources for the data necessary to populate the model to examine neonatal screening, were identified; this process is described below.

Decision-tree model of neonatal screening

A simple model of neonatal screening for inborn errors of metabolism is illustrated in *Figure 9*.

The process starts with the collection of a blood sample from the infant and the delivery of the blood sample to the neonatal screening laboratory. An assumption is made that once the blood sample has been collected, it is forwarded to the screening laboratory through the standard postal service. In practice, a number of methods of delivery exist and the model proves a simplification of reality. This simplification is not expected to impact heavily upon the cost of the screening programme.

The next node of the model, the first decision node, requires a choice of conditions to screen for (in addition to phenylketonuria and congenital hypothyroidism, which are already established screens across the whole of the UK).

A second decision is the choice of technology for screening. For each 'condition-technology' combination, a subsequent chance node exists to determine the number of neonates testing positive and the number of neonates testing negative through the primary screen. In addition, it is possible that the blood sample collected is inadequate or the primary test result is indecisive and, hence, a repeat sample is necessary before proceeding any further through the model.

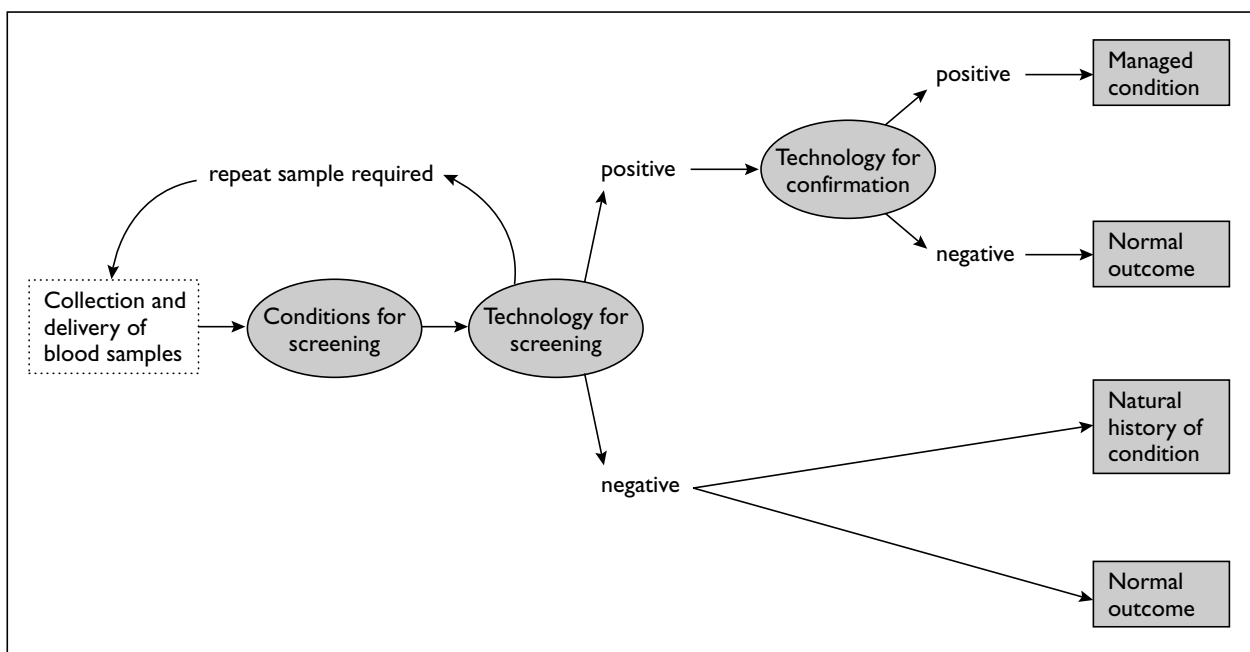


FIGURE 9 A decision-tree model of neonatal screening for in-born errors of metabolism

The majority of the blood samples that test negative, result in a normal outcome (true-negative) and arrive at the appropriate terminal node of a ‘normal life’ outcome where no further managed decisions are necessary. However, a subset of blood samples testing negative are given a false-negative diagnosis and arrive at the terminal node showing the outcome to be one of a ‘natural history of the condition’.

For blood samples that receive a positive result on the primary screen, a decision is required covering the ‘choice of confirmation technology’. This second ‘condition–technology’ combination gives both the number of true-positive cases identified and the number of false-positive cases identified from the initial screen. The confirmation test ensures that any false-positive diagnoses resulting from the primary screen can be eliminated before the results are acted upon. Neonates with a true-positive diagnosis reach the terminal node showing the outcome of a ‘managed condition’, while the neonates with a false-positive diagnosis reach the terminal node of a ‘normal life’ outcome, where no further managed decisions are necessary.

The model identifies the number of neonates reaching each outcome, a cost per true case identified, a cost per life-year saved and a total cost per cohort of neonates specified for each disorder using both existing technology and tandem MS. This model can provide information which allows comparisons to be made between existing screening methods and tandem MS, and also allows the identification of the incremental costs encountered should the screening programme be extended to incorporate screening for more disorders. The model does not incorporate a measure of the quality of the life-years saved as a result of screening.

A summary of the initial assumptions built into the model is presented in *Table 91*.

Outputs

The model developed for neonatal screening has been outlined above. The five main outputs of the model are:

- the total cost of screening a cohort of ‘*x*’ neonates for ‘*y*’ disorders
- the cost per true case identified by the screening programme
- the total cost of screening a cohort of ‘*x*’ neonates for ‘*y*’ disorders and the management of identified cases
- the cost per true case identified by the screening programme including additional treatment costs
- the cost per life-year saved by the screening programme.

In addition, the model can be used to calculate both the incremental costs of replacing existing screening technologies with tandem MS and the marginal costs and consequences of extending existing neonatal screening programmes (i.e. phenylketonuria and congenital hypothyroidism) through the introduction of additional screens.

Data requirements for neonatal screening model

At each node in the model described above, two types of data input are required – probability data and cost data.

To populate the model, data are required on the number of neonates entering the neonatal screening programme in the UK or region of the UK. Having entered this information, cost data associated with the collection of the blood sample from the neonate and the delivery of this sample to the laboratory are also required.

The first decision node requires data on conditions screened for in the UK at present. It also requires

TABLE 91 A summary of the initial assumptions built into the model

All initial blood samples collected by the midwife/health visitor. As this coincides with their routine ‘house-visits’, no travel costs are included in specimen collection calculations.
All repeat blood samples (where required) collected by the midwife/health visitor as a ‘house-visit’ additional to their routine rounds.
All blood samples collected from the neonate sent to the laboratory by the standard postal service (one sample per envelope).
100% of the neonate population are covered by the screening programme.
6% discount rate.
All capital equipment has a life expectancy of 10 years and a scrap value, after this time, of 10% of the original cost.

information on additional disorders for which it may be possible to screen, given future knowledge and technological advances.

The second decision node requires data on technologies used in the UK to screen for specific disorders and data on costs, including costs of capital equipment, consumables used, employees involved in the programme and accommodation maintenance/overheads of screening programmes.

For each screening technology chosen, data on the probability of obtaining a positive/negative result from the primary screen are required. Data on the number of repeat samples necessary are also required, together with the associated costs.

Sensitivity and specificity values for the different screening technologies are required since they are key determinants of the relative cost-effectiveness (measured as the cost per true case identified) of screening for each disorder. Variations in the sensitivity and specificity of the primary screening technology affect:

- the number of repeat tests required
- the number of neonates wrongly diagnosed as positive (false-positives) and, hence, entered into the treatment/management protocol
- the number of neonates wrongly diagnosed as negative (false-negatives) and, hence, follow the 'natural history of the disorder' outcome.

Similar data are required for the decision node on the choice of confirmation technology; that is, data on the costs associated with the confirmation test and data on the probability of obtaining a positive or negative result.

By inputting the data identified above, for each screening 'technology–disease' combination, the model is able to allocate a cohort of neonates across the model's three terminal nodes; viz:

- 'normal life'
- 'managed condition'
- 'natural history of condition'.

For each of the disorders considered by the model, a description of the terminal node states is necessary (*Table 92*). An outcome scenario for both the 'managed condition' and the 'natural history of the condition' are required for each of the disorders in question. To produce such scenarios, data are required on the treatment/management protocol for individuals with these conditions. No description is required for the 'normal life' outcome state as it is assumed the number of cases in this terminal node remain constant, irrespective of the screening technology adopted.

Method of data collection

Data for the model were obtained from a number of different sources using a variety of methods.

Laboratory questionnaire

A laboratory questionnaire (see Appendix 1) was used to:

- identify the present state of neonatal screening in the UK
- to collect primary data on the screening process.

The questionnaire was developed in collaboration with the St. George's Hospital group and was used on a pilot basis in four laboratories. It was designed as a postal questionnaire and focused on extracting data from the laboratories on the costs they incurred in running a neonatal screening programme.

The questionnaire was split into four sections.

- Activity
- Manpower
- Specific disorders
- Research and development

The questionnaire included questions on the disorders screened for, the number of neonates screened per annum, the employees involved and the accommodation. Laboratories were also asked specific questions about the disorders screened for,

TABLE 92 Definitions of the terminal nodes of the model

Managed condition	Neonates correctly diagnosed as positive for a specific disorder are 'referred' and enter the treatment/management protocol (where such a protocol exists).
Normal condition	Neonates correctly diagnosed as negative for a specific disorder live a normal life.
Natural history of condition	Neonates wrongly diagnosed as negative for a specific disorder present symptomatically later in life and either follow the natural history of the condition or are treated with reduced benefits.

the technology used, the cost of capital equipment and consumables, and the number of repeat tests performed per annum. The questionnaire was sent to 28 laboratories around the UK and the results are presented below.

Laboratory visits

Data on tandem MS were obtained from visits (in both the USA and the UK) to laboratory centres with experience of tandem MS as a neonatal screening method. The information collected from the laboratory visits included the range of disorders it was possible to screen for, the specificity of the particular screening technology, the consumables and the amount of employee support needed to run the programme.

Literature review

Additional information on the incidence of the disorders in question, and the sensitivity and specificity of the different primary and confirmatory tests, were extracted from the literature where possible. The systematic literature review, performed as part of this report, also provided information on the 'natural history' and the appropriate treatment for the disorders in question.

Miscellaneous

The remainder of the data required to complete the model, were collected from a number of different sources. Data on the specimen collection costs were obtained in collaboration with a midwife who outlined the equipment used and the time required. An assumption was made that the initial sample is collected as part of a routine 'house visit' and that, if a repeat blood sample is required, it is necessary to make another 'house visit' in addition to the routine rounds.

Having obtained a list of the employees involved in the screening programme from the questionnaire, the corresponding salary costs including on-costs, were acquired from the Management Accountancy Finance Department within the University of Sheffield.

The questionnaire used identified the floor space required for the laboratory to run a neonatal screening programme. This measurement was combined with the maintenance cost per square metre obtained from the Management Accountancy Finance Department within the University of Sheffield to calculate the annual overheads of the laboratory (e.g. lighting and electricity).

Equipment costs of tandem MS along with maintenance and running costs were obtained

direct from the manufacturers (Micromass UK Ltd and Perkin-Elmer Ltd).

Dietary treatment costs for each of the disorders under investigation were estimated in collaboration with a dietitian. Additional information on the treatment/management protocol of the 'affected' individuals was obtained either from the literature or through paediatricians actively involved in the treatment of children with the disorders in question.

Avoided mortality resulting from the early treatment of each disease and estimates of the life-expectancy of individuals treated early for each disease were, where possible, obtained from the literature and supported by clinical opinion. The data covering avoided mortality and life expectancy were used to estimate the number of life-years gained as a result of screening.

Results of data collection

The questionnaire was distributed to 28 neonatal screening laboratories around the UK. Of these, 14 completed and returned the questionnaire, two refused, and the remainder failed to respond.

Information obtained from responding laboratories, showed that most of the responding laboratories screened between 10,000 and 50,000 neonates in 1995–96 (*Table 93*). The mean number of neonates screened by the 14 responding laboratories in the UK in 1995/6 is calculated to be 38,492 neonates. If the two smallest laboratories and the largest laboratory are disregarded as outliers, the mean is reduced only slightly to 37,531 neonates.

TABLE 93 Number of neonates screened by neonatal screening laboratories in the UK

Number of neonates	Number of laboratories
< 10,000	2*
10,000–30,000	5
30,000–50,000	2
50,000–80,000	4
80,000–100,000	0
> 100,000	1*
* outliers	

The disorders currently screened for by the responding laboratories, either as a primary screen or as a by-product of the primary screen for phenylketonuria are summarised in *Table 94*, together with the technique used.

The remainder of the data collected are summarised in the tables that follow. These are divided into four categories:

- laboratory questionnaire
- tandem MS
- incidence
- treatment.

Data are standardised for a cohort of 100,000 neonates screened per annum to ensure a sensible/ manageable number of cases detected.

TABLE 94 The disorders screened for by responding laboratories

Disorders and methods	Number of laboratories screening for disorders listed	
	Primary purpose of screen	By-product of phenylketonuria screen
Phenylketonuria*		
<i>Methods</i>		
Guthrie	6	
Fluorometry	4	
Chromatography	5	
Congenital hypothyroidism		
<i>Methods</i>		
Delfia	7	
RIA	4	
Other	1	
Cystic fibrosis		
<i>Methods</i>		
IRT-Delfia	2	
RIA-trypsinogen	1	
Galactosaemia		4
Tyrosinaemia	1	5
Homocystinuria	1	1
Maple syrup urine disease		3
Other amino acid disorders		1
Thalassaemia	1	
Sickle cell disease	1	

* In screening for phenylketonuria, one laboratory used both Guthrie and chromatography screening methods.

Where possible the median and the range of data collected have been included in the summary tables. In a number of cases the observed range of costs, especially those collected through the laboratory questionnaire, are wide. This is partly related to:

- the variable size of neonatal screening laboratories across the country
- the organisation of the screening programme
- the amount of detail laboratory's chose to disclose in their questionnaire response
- how laboratories apportioned resources shared with other services.

Data from laboratory questionnaire

The costs of specimen collection, receipt and result reporting are summarised in *Tables 95* to *97*. Using data from the responding laboratories, together with estimates derived in collaboration with a midwife, the total cost of specimen collection, receipt and result reporting for 100,000 neonates was calculated to be £504,452, that is, £5.04 per specimen collected. The cost per repeat specimen collected was calculated to be three times more expensive than the first specimen collected, that is, £15.29. In all of the following calculations half of

TABLE 95 Specimen collection costs (estimated in collaboration with a health visitor)

	First sample	Second sample*
Consumable costs	£1.00	£1.00
Travel costs per sample taken	£0.00	£3.00
Health visitor's time per sample† (minutes)	15	45
Health visitor's salary costs (including on-costs)	£3.75	£11.29

* If a second sample is required from a neonate, the health visitor has to make a 'special' journey whereas for the first sample the specimen is collected as part of the health visitor's 'routine' calls.

† Health visitor's time per second sample includes travel time.

TABLE 96 Specimen receipt and result reporting costs (calculated from questionnaire)

	Median	Range
Number of laboratories	14	
Laboratory maintenance costs	£1077	£510–£4892
Employee costs	£5734	£2658–£12,784

TABLE 97 Total cost and cost per specimen collected, receipt and result reporting* (calculated from questionnaire)

	Median	Range
Total cost for 100,000 first specimen collection	£504,452	£476,218–£531,661
Cost per first specimen collection	£5.04	£4.76–£5.32
Cost per second (repeat) specimen collection	£15.29	
* Specimen collection, receipt and result-reporting costs divided equally between phenylketonuria and congenital hypothyroidism for all calculations that follow.		

the specimen collection costs were assigned to phenylketonuria screening and the remaining half to congenital hypothyroidism screening.

Laboratory data for phenylketonuria screening are summarised in *Tables 98* to *101*. The total cost of screening 100,000 neonates, depending on the technology used, ranged from a median of £41,619 (£293,845) for chromatography to £108,388 (£360,922) for fluorometry; that is, from £0.42 (£2.94) to £1.08 (£3.61) per neonate screened, respectively. The cost per true case identified ranged from a median of £2903 (£20,495) to £8481 (£25,152); the figures in parentheses include specimen collection costs.

The laboratory data on screening for congenital hypothyroidism are presented in *Tables 102* to *105*. Three methods of screening were identified among the responding laboratories, and the total costs of screening 100,000 neonates ranged from a median

of £100,595 (£352,821) to £101,388 (£353,614); that is, approximately £1.00 (£3.55) per neonate screened, regardless of the technology adopted. The cost per true case identified was calculated to be approximately £4250 (£14,860). Again, the figures in parentheses include specimen collection costs.

Laboratory data on screening for cystic fibrosis are presented in *Tables 106* to *109*. Three of the responding laboratories screened for cystic fibrosis and two techniques were identified. The total cost of screening 100,000 neonates for cystic fibrosis ranged from a mean (median) of £150,686 for the IRT Delfia method to £166,121 for RIA trypsinogen + DNA method; that is, £1.51 and £1.66 per neonate screened, respectively. The cost per true case identified ranged from £4305 to £4746.

The laboratory data on screening for tyrosinaemia and homocystinuria are summarised in *Tables 110* to *113*. Only one responding laboratory undertook stand-alone screens for these disorders. The total cost of screening 100,000 neonates for tyrosinaemia and homocystinuria was calculated to be £32,650 and £31,029, respectively; that is, £0.33 and £0.31 per neonate screened.

Data for tandem MS

The costs for screening using tandem MS are summarised in *Tables 114* and *115*. Using data obtained from manufacturers, together with discussions with the Institute of Child Health, the total cost of screening 100,000 neonates using tandem MS was estimated to be £120,382 (£372,608 including specimen collection costs); that is, £1.20 per neonate screened (£3.73 including specimen collection costs).

TABLE 98 Testing and analysis data by screening technology for a cohort of 100,000 for phenylketonuria (calculated from questionnaire)

	Guthrie		Fluorometry		Chromatography	
	Median	Range	Median	Range	Median	Range
Number of laboratories	6		4		5	
Repeat samples (%)	0.07	0.00–1.70	0.04	0.02–0.50	0.03	0.03–0.09
Laboratory maintenance costs	£2036	£784–£9783	£2494	£1965–£3396	£1515	£917–£6650
Employee costs	£9201	£4418–£21,441	£15,509	£6310–£23,380	£7743	£1609–£15,931
Capital costs*	£255	£55–£722	£2151	£1154–£6972	£58	£22–£181
Consumable costs	£2830	£381–£5271	£1372	£568–£5547	£3457	£1750–£4081
* Capital costs discounted at 6.0% level with a scrap value of 10.0% of original cost						

Incidence data

A list of the possible disorders detectable using tandem MS is given in *Table 116*, together with the estimated frequency of the disorders in the UK and the sensitivity of screening (extracted from the summary tables presented earlier for each specific disorder).

TABLE 99 Data on confirmation, referral and advice for positive cases for a cohort of 100,000 neonates for phenylketonuria (calculated from questionnaire)

	Median	Range
Employee costs	£1534	£262–£9813

TABLE 100 Number of cases of phenylketonuria identified (calculated using incidence and sensitivity rate extracted from the literature)

	Guthrie		Fluorometry		Chromatography	
Number of true cases identified per 100,000 neonates screened	Phenylketonuria	10.8	Phenylketonuria	10.8	Phenylketonuria	10.8
	Galactosaemia	1.0	Galactosaemia	2.0	Galactosaemia	1.5
			Homocystinuria	1.0		
			Tyrosinaemia	0.9		
			Maple syrup urine disease	0.4		

Galactosaemia is a 'by-product' of phenylketonuria screening, regardless of technology adopted but sensitivity varies.

TABLE 101 Total cost and cost per neonate screened for phenylketonuria (calculated from questionnaire)

	Guthrie		Fluorometry		Chromatography	
	Median (£)	Range (£)	Median (£)	Range (£)	Median (£)	Range (£)
Total cost of screening 100,000 neonates	63,798	54,696–144,828	108,388	50,886–136,386	41,619	30,828–92,568
Total cost including specimen collection	316,025	306,922–397,054	306,922	303,112–388,612	293,845	283,054–344,794
Cost per neonate screened	0.64	0.55–1.45	1.08	0.51–1.36	0.42	0.31–0.93
Cost/neonate screened including specimen collection	3.16	3.07–3.97	3.61	3.03–3.89	2.94	2.83–3.45
Cost/true case identified	5416	4643–12,294	8481	3982–10,672	2903	2150–6456
Cost/true case identified including specimen collection	26,827	26,055–33,706	25,152	23,718–30,408	20,495	19,742–24,049

TABLE 102 Testing and analysis data by screening technology for a cohort of 100,000 neonates screened for congenital hypothyroidism (calculated from questionnaire)

	Wallac DELFIA–TSH		Radio-immunometric assay		MAIAclone
	Median	Range	Median	Range	
Number of laboratories	7		4		1
Repeat samples (%)	0.12	0.03–0.89	0.05	0.02–0.44	missing
Laboratory maintenance costs	£1609	£978–£3023	£2000	£784–£2272	£1077
Employee costs	£7052	£2509–£16,362	£11,053	£5667–£12,875	£27,636
Capital costs*	£1365	£860–£5680	£1540	£594–£12,505	£7838
Consumable costs	£12,675	£8490–£26,061	£7655	£6333–£8978	£6645

* Discounted at 6.0% level with a scrap value of 10.0% of original cost.

TABLE 103 Confirmation, referral and advice data for positive cases of congenital hypothyroidism (calculated from questionnaire)

	Median	Range
Employee costs	£3583	£1226–£7221

TABLE 104 Number of cases of congenital hypothyroidism identified (calculated from incidence and sensitivity rate extracted from the literature)

	Wallac DELFIA-TSH	RIA
Number of true congenital hypothyroidism cases identified per 100,000 neonates screened	23.75	23.75

Data on treatment

Dietary treatment costs

Treatment for disorders detected, following neonatal screening, can be categorised into four groups, with the exception of tyrosinaemia which requires special treatment (NTBC) and a liver transplant. These treatment categories are listed in *Table 117* along with the corresponding costs that would be incurred in years 1 and 2 of each category. It is assumed that the cost of treatment for year 2 will apply in subsequent years.

Additional treatment costs will be incurred as a result of expanding the neonatal screening programme – treatment starting at an earlier age; decreased mortality resulting in more patients requiring treatment.

Table 118 displays treatment category, additional treatment period (that is, the period between asymptomatic and symptomatic diagnosis), the

reduction in early deaths as a result of screening and the average life expectancy of the individuals with the disease condition, enabling the total additional treatment cost and the total treatment cost per life-year saved to be calculated (see *Table 124* on page 156). These latter costs have not been calculated for phenylketonuria, (or galactosaemia), as these disorders are already identified through existing screening programmes and no additional treatment costs are therefore incurred by the introduction of new screening technology.

Results of the economic evaluation

The number of cases identified and the total screening (and treatment) costs associated with screening for phenylketonuria (including ‘by-product’ disorders, i.e. amino acids) using tandem MS compared to present technologies are summarised in *Table 119*. The table also includes information on the effect upon the number of cases identified and the total cost incurred as the screening programme, using tandem MS, is expanded to include the acylcarnitine disorders and the urea cycle disorders.

The marginal costs (i.e. the difference in cost) associated with replacing existing technologies with tandem MS screening for phenylketonuria, including ‘by-product’ disorders (i.e. amino acids) are shown in *Table 120*. It is evident from the information in the table, that there would be no benefit from replacing chromatography with tandem MS, as chromatography has the potential to detect all the amino acids at a lower cost. However, the replacement of the Guthrie and

TABLE 105 Total cost and cost per neonate screened for congenital hypothyroidism (calculated from questionnaire)

	Wallac DELFIA-TSH		RIA	
	Median (£)	Range (£)	Median (£)	Range (£)
Total cost of screening 100,000 neonates	101,388	95,943–195,390	100,595	90,059–111,130
Total cost including specimen collection	353,614	348,170–447,616	352,821	342,285–363,356
Cost per neonate screened	1.01	0.96–1.95	1.01	0.90–1.11
Cost/neonate screened including specimen collection	3.54	3.48–4.48	3.53	3.42–3.63
Cost/true case identified	4269	4040–8227	4236	3792–4679
Cost/true case identified including specimen collection	14,889	14,660–18,847	14,856	14,412–15,299

TABLE 106 Testing and analysis data by screening technology for cystic fibrosis for a cohort of 100,000 neonates (calculated from questionnaire)

	IRT-DELFINA		RIA trypsinogen + DNA
	Mean	Range	
No. of laboratories	2		1
Repeat samples (%)	0.84	0.67–1.01	0.09
Laboratory maintenance costs	£843	£707–£978	£2000
Employee costs	£11,242	£6121–£16,362	£11,728
Capital costs*	£1260	£890–£1631	£1016
Consumable costs	£21,937	£11,235–£32,639	£16,844

* Discounted at 6.0% level with a scrap value of 10.0% of original cost

TABLE 107 Data for confirmation, referral and advice for positive cases of cystic fibrosis (calculated from questionnaire)

	Mean	Range
Employee costs	£3583	£2490–£5753

TABLE 108 Number of cases of cystic fibrosis identified (calculated using incidence and sensitivity rate extracted from the literature)

	IRT- DELFINA	RIA trypsinogen + DNA
Number of true cases of cystic fibrosis identified per 100,000 neonates screened	35.00	35.00

fluorometry methods with tandem MS would result in marginal discounted screening and treatment costs per additional case identified of approximately £26,849 and £19,300, respectively.

In *Table 121*, the effect of expanding tandem MS screening, to include the acylcarnitine disorders and urea cycle disorders, upon the marginal screening and treatment cost per true additional case identified is shown. The discounted cost per life-year saved is also reported.

This demonstrates that when the acylcarnitine disorders are incorporated into the tandem MS screening programme, the marginal costs are lower than when both the acylcarnitine disorders and the urea cycle disorder are included. For example, on

TABLE 109 Total cost and cost per neonate screened for cystic fibrosis (calculated from questionnaire)

	IRT-DELFINA		RIA trypsinogen + DNA
	Mean	Range	
Total cost of screening 100,000 neonates	£166,121	£153,274– £217,812	£150,686
Cost per neonate screened	£1.66	£1.53–£2.18	£1.51
Cost per true case identified	£4746	£4379–£6223	£4305
Cost per 'new' case*	£6979	£6439–£9151	£6330

All figures exclude specimen collection costs
* Without immediate family history (80%) or meconium ileus (85%)

TABLE 110 Testing and analysis data for tyrosinaemia and homocystinuria (calculated from questionnaire)

	Tyrosinaemia	Homocystinuria
No. of laboratories	1	1
Repeat samples (%)	0.15	0.05
Laboratory maintenance costs	£978	£978
Employee costs	£4418	£4418
Capital costs*	£277	£274
Consumable costs	£1792	£1792

* Discounted at 6.0% level with a scrap value of 10.0% of original cost

TABLE 111 Data on confirmation, referral and advice for positive cases of tyrosinaemia and homocystinuria (calculated from questionnaire)

	Tyrosinaemia	Homocystinuria
Employee costs	£118	£118

TABLE 112 Number of cases of tyrosinaemia and homocystinuria identified (calculated using incidence and sensitivity rate extracted from the literature)

	Tyrosinaemia	Homocystinuria
Number of true cases identified per 100,000 neonates screened	0.90	1.05

TABLE 113 Total cost and cost per neonate screened for tyrosinaemia and homocystinuria (calculated from questionnaire)

	Tyrosinaemia	Homocystinuria
Total cost of screening 100,000 neonates	£32,650	£31,029
Cost per neonate screened	£0.33	£0.31
Cost per true case identified	£36,278	£29,551

TABLE 114 Testing, analysis, confirmation, referral and advise for positive cases identified using tandem MS (calculated from data obtained directly from the manufacturers and in collaboration with Institute of Child Health)

	Median	Range (n = 3)
Laboratory maintenance costs	£16,000	£14,625–£26,000
Testing and analysis employee costs	£49,735	£35,471–£64,000
Confirmation employee costs	£14,777	£14,000–£15,554
Capital costs*	£29,262	£25,231–£30,951
Consumable costs	£8000	£6000–£10,000
* Discounted at 6.0% level with a scrap value of 10.0% of original cost		

TABLE 116 Estimated frequencies for screenable disorders in the UK and the sensitivity of the screening used (obtained from the literature and data from the Institute of Child Health, London)

Disease	Number per 100,000	Clinically affected	Detected by screening
Phenylketonuria	11 ^a	> 90%	> 98%
Tyrosinaemia type I	1 ^b	100%	> 90%
Homocystinuria	1.5 ^a	> 99%	70% ^c
Maple syrup urine disease	0.5 ^b	> 99%	80% ^c
Citrullinaemia and other urea cycle (including severe ornithine carbamoyltransferase deficiency)	2.5	> 95%	?
Galactosaemia	2.5	> 95%	80% ^d
Methylmalonic acidaemias	1.5 ^b	100%	> 80% ^c
Propionic acidaemia	0.8 ^b	100%	100%
Isovaleric acidaemia	0.7	100%	100%

^a Marked regional differences in incidence; accurate data available; ^b pockets of much higher incidence, especially in groups of Asian origin; ^c mild or intermittent forms may be missed; ^d detection by secondary increase in phenylalanine.

continued

TABLE 115 Total cost and cost per neonate screened using tandem MS (calculated from above data)

	Median	Range
Total laboratory cost of screening 100,000 neonates	£120,382	£104,600–£135,231
Total cost including specimen collection	£372,608	£356,826–£387,457
Cost per neonate screened	£1.20	£1.05–£1.35
Cost/neonate screened including specimen collection	£3.73	£3.57–£3.87

inclusion of the acylcarnitine disorders the marginal discounted screening and treatment cost per additional true case identified is estimated to be £2063 compared with £3246 on addition of the acylcarnitine disorders and the urea cycle disorders (*Table 121*).

Table 122 shows the additional discounted screening cost per additional true case identified when replacing present screening technologies with each of the three possible tandem MS screening protocols. The analysis shows that installing tandem MS to screen for the amino acid disorders only (1), would result in high marginal costs compared to tandem MS screening protocols (2) and (3), when it replaces Guthrie tests or fluorometry, and it has

TABLE 116 contd Estimated frequencies for screenable disorders in the UK and the sensitivity of the screening used (obtained from the literature and data from the Institute of Child Health, London)

Disease	Number per 100,000	Clinically affected	Detected by screening
Other disorders of branched-chain acyl-CoA catabolism	1	80%	90%
Medium-chain acyl-CoA dehydrogenase deficiency	8.3 ^e	70%	> 95%
Long-chain acyl-CoA dehydrogenase deficiency ^f	0.8	> 95%	90%
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency & related disorders	1.8	> 95%	> 95%
Carnitine palmitoyltransferase type II deficiency ^f	0.4	> 95%	> 95%
Glutaryl-CoA dehydrogenase deficiency	2	80%	> 95%
Glutaric acidaemia type II	2	> 95%	> 90%
Total detectable by tandem MS	38.3	(> 33.6)	(> 33.1)

^e Regional differences in heterozygote frequency, accurate estimates for a few areas; ^f excluding adult onset forms.

TABLE 117 Costs of treatment associated with each treatment category

Treatment category	Costs (£)	
	0–1 year	1–2 years
1. Total elemental diet	2727	5185
2. Low protein diet + supplements	1453	2163
3. No diet/emergency regime	70	70
4. General dietetic advice/co-factor treatment, e.g. riboflavin, pyridoxine, carnitine, biotin	500	500
Treatment costs for tyrosinaemia: NTBC treatment and diet, then liver transplant by 2 years	£12,727 year 1 and £15,185 in year 2 until transplant (£50,000) then £5000 p.a. following transplant	

Source: A MacDonald, Birmingham Children's Hospital, September 1996. Private communication.
All costs include products and dietetic time.
The cost of treatment increases after the first year as the infant's body mass increases.

TABLE 118 Treatment costs and outcomes by disease (estimated in collaboration with neonatal screening directors and paediatric metabolic physicians)

	Treatment category (see Table 117)	Additional treatment (years)*	Reduction in early deaths (%)	Life expectancy	Additional treatment cost per 100,000 neonates	Additional treatment cost per 100,000 neonates (discounted at 6% level)
Amino acids						
Tyrosinaemia	(5)	0.40	15	30	£33,313	£14,466
Homocystinuria (responsive)	(4)	5.00	10	60	£1575	£820
Homocystinuria (non-responsive)	(1)	2.50	20	40	£14,556	£11,919
Maple syrup urine disease	(1)	0.10	20	40	£16,483	£6155

* Additional treatment period is the period between symptomatic and asymptomatic diagnosis. Where disorders only require treatment in the short term, the treatment period is assumed to be 5 years. An explanation of additional treatment cost calculations is presented in Appendix 2.

continued

TABLE 118 contd Treatment costs and outcomes by disease (estimated in collaboration with neonatal screening directors and paediatric metabolic physicians)

	Treatment category (see Table 117)	Additional treatment (years)*	Reduction in early deaths (%)	Life expectancy	Additional treatment cost per 100,000 neonates	Additional treatment cost per 100,000 neonates (discounted at 6% level)
Acylcarnitines						
Propionic acidaemia (moderate) [†]	(2)	1.00	25	40	£9017	£3632
Methylmalonic acidaemia (moderate) [†]	(2)	1.00	25	30	£7711	£3774
Isovaleric acidaemia	(4)	0.50	20	50	£3640	£1247
Defects of branched-chain acyl-CoA	(3)	0.20	25	50	£88	£77
Medium-chain acyl-CoA dehydrogenase	(3)	1.00	25	60	£1104	£1003
Defects of long-chain fatty acid (moderate) [†]	(4)	0.50	50	50	£18,900	£3219
Glutaryl-CoA dehydrogenase deficiency	(4)	1.00	60	50	£28,880	£9397
Glutaric aciduria type II	(4)	0.30	20	50	£9216	£3063
Urea cycle						
Urea cycle disorders (moderate)	(2)	0.50	30	40	£32,814	£12,620
Urea cycle disorders (severe)	(2)	0.10	40	10	£10,569	£7775
* Additional treatment period is the period between symptomatic and asymptomatic diagnosis. Where disorders only require treatment in the short term, the treatment period is assumed to be 5 years. An explanation of additional treatment cost calculations is presented in Appendix 2.						
[†] Severe forms of these disorders are regarded as untreatable and are omitted from these tables.						

TABLE 119 Total costs of present technologies and three tandem MS screening strategies

	Number of cases identified	Total costs of screening (£)	Screening costs per true case identified (£)	Total screening and treatment costs (£)	Screening and treatment costs per true case identified (£)
Present technologies					
Guthrie	11.78	316,025	26,827	316,025	26,827
Fluorometry	12.78	360,614	28,217	360,614	28,217
Chromatography	14.63	293,845	20,085	327,205	22,365
Tandem MS					
Amino acids	15.13	372,608	24,627	405,968	26,832
Amino acids + acylcarnitines	32.85	372,608	11,344	431,380	13,132
Amino acids + acylcarnitines + urea cycle	35.22	372,608	10,579	451,775	12,827
(i) Specimen collection, receipt and result-reporting cost are divided equally between phenylketonuria and congenital hypothyroidism.					
(ii) All figures based on neonatal screening for a cohort of 100,000 neonates.					
(iii) All costs and benefits (life-years saved) discounted at the 6% level.					
(iv) Treatment costs exclude treatment for phenylketonuria and galactosaemia.					
(v) It is assumed that chromatography is used to detect all amino acids although, from the laboratory survey, it is evident that chromatography is often not used to its full potential.					

TABLE 120 Marginal costs in using tandem MS to screen for amino acids compared with present technologies

Present technologies	Marginal effect on number of cases identified	Marginal effect on costs of screening (£)	Marginal screening costs per additional true case identified (£)	Marginal effect on screening and treatment costs (£)	Marginal screening and treatment costs per additional true case identified (£)
Guthrie	3.35	56,583	16,890	89,943	26,849
Fluorometry	2.35	11,995	5104	45,354	19,300
Chromatography	0.50	78,764	157,527	78,764	157,526

(i) All figures based on neonatal screening for a cohort of 100,000 neonates.
(ii) All costs & benefits (life-years saved) discounted at the 6% level.
(iii) Treatment costs exclude treatment for phenylketonuria and galactosaemia.

TABLE 121 Marginal costs of expanding the neonatal screening programme using tandem MS compared with tandem MS screening for amino acids only

Present technologies	Marginal effect on number of cases identified	Marginal effect on costs of screening and treatment cost (£)	Marginal screening and treatment cost per additional true case identified (£)	Marginal screening and treatment cost per additional life-year saved (£)
Amino acids + acylcarnitines	17.72	25,412	1434	294
Amino acids + acylcarnitines + urea cycle	20.09	45,807	2280	483

(i) All figures based on neonatal screening for a cohort of 100,000 neonates.
(ii) All costs and benefits (life-years saved) discounted at the 6% level.
(iii) Treatment costs exclude treatment for phenylketonuria and galactosaemia.

TABLE 122 The marginal screening costs per additional true case identified of three possible tandem MS screening protocols compared with present technologies

	Cost of present technologies (£)		
	Guthrie	Fluorometry	Chromatography
Tandem MS screening protocols			
1. Amino acids	16,891	5104	157,527
2. Amino acids + acylcarnitine	2686	598	4324
3. Amino acids + urea cycle + acylcarnitine	2414	535	3825

(i) All figures based on neonatal screening for a cohort of 100,000 neonates.
(ii) All costs and benefits discounted at the 6% level.
(iii) Treatment costs exclude treatment for phenylketonuria and galactosaemia.

no 'real' advantage over chromatography (i.e. chromatography has the potential to screen for the same disorders at the lower cost to the laboratory of £293,845 compared with £372,608 (see *Table 119*).

An analysis of present technologies compared with tandem MS, which breaks down the marginal costs that would be incurred as a result of expanding the

phenylketonuria screening programme disease by disease, are reported in *Table 123*, with a discussion of the issues appearing later in this section.

The additional treatment costs associated with replacing present technology screening methods with tandem MS and expanding the neonatal screening programme by a single disorder at a

TABLE 123 The additional treatment costs associated with expanding the phenylketonuria screening programme using tandem MS

	Additional treatment cost per 100,000 neonates (£)	Additional treatment cost per 100,000 neonates (discounted at 6% level) (£)	Additional true cases identified	Total additional treatment cost per additional case identified (£)	Total additional treatment cost per additional case identified (discounted at 6% level) (£)	Additional treatment cost per additional life-year saved (£)	Additional treatment cost per additional life-year saved (discounted at 6% level) (£)	Priority ordering
Amino acids								
Tyrosinaemia	33,313	14,466	0.90	37,014	16,073	8225	8339	12
Homocystinuria	16,131	12,739	1.05	26,885	21,231	2068	4649	10
Maple syrup urine disease	16,483	6155	0.40	41,207	15,388	5093	5114	11
Urea cycle								
Urea cycle disorders	43,383	20,395	2.5	17,353	8158	2169	2188	7
Acylcarnitine								
Medium-chain acyl-CoA dehydrogenase	1104	1003	7.88	140	127	9	31	2
Propionic acidemia	9017	3632	0.40	22,542	9079	2254	2414	8
Methylmalonic acidemia	7711	3774	0.45	17,135	8396	2285	2437	9
Isovaleric acidemia	3640	1247	0.70	5200	1782	520	565	6
Branched-chain acyl-CoA metabolism	88	77	0.90	98	85	8	22	1
Long-chain fatty acid catabolism	18,900	7117	2.70	7000	1192	280	334	3
Glutaryl-CoA dehydrogenase	28,880	9397	1.90	15,200	4946	507	523	4
Glutaric aciduria type II	9216	3063153	1.80	5120	1702	512	540	5
(i) All figures based on neonatal screening for a cohort of 100,000 neonates.								
(ii) All costs and benefits discounted at the 6% level.								
(iii) Treatment costs exclude treatment for phenylketonuria and galactosaemia.								

time are also shown in *Table 123*. By calculating the additional (discounted) treatment cost per additional life-year saved, it allows the order of additional screens to be prioritised, that is from the lowest cost per additional life-year saved upwards.

It can be seen from this table that when adding disorders to the screening programme, regardless of the present technology, the order of priority would be:

- the acylcarnitine disorders (excluding propionic and methylmalonic acidemias)
- urea cycle disorders
- propionic and methylmalonic acidemias
- amino acid disorders.

Although the marginal (discounted) treatment cost per additional life-year saved associated with adding one disorder at a time allows a general

priority ordering of the additional screens to be considered, the economies of scale available by expanding the screening programme by a number of disorders at the same time are not addressed. Economies of scale occur when the long-run costs decrease as the output rises,⁵⁶² that is, the long-run cost of screening decreases as the number of disorders screened for increases.

Figures 10 to 12 demonstrate the total, marginal and average additional costs that will be incurred if tandem MS is introduced to the UK neonatal screening programme, and the programme is expanded sequentially according to the order of priority identified above (based on 100,000 neonates screened).

The figures show the marginal cost associated with the addition of the first disorder (i.e. branched-chain CoA metabolism) to be high relative to the addition of subsequent screens. This is because the

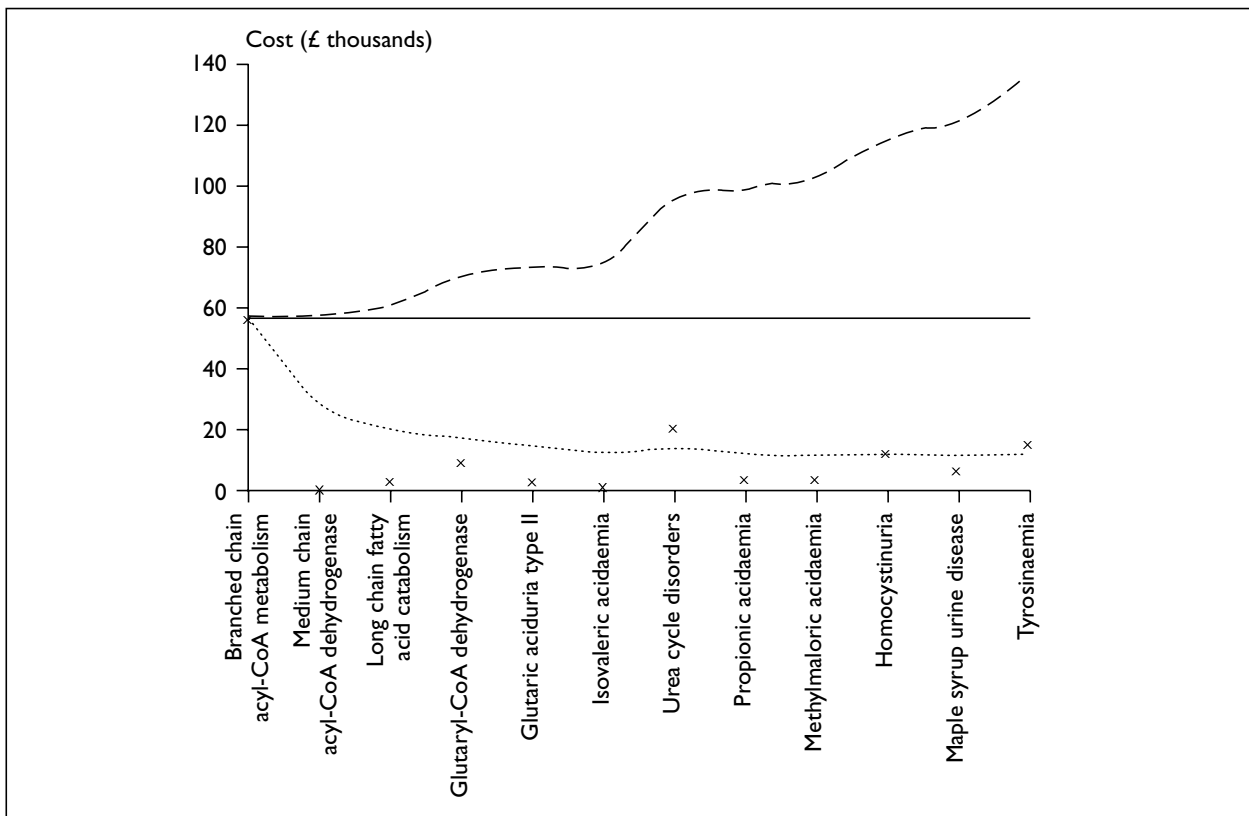


FIGURE 10 Total, marginal and average additional costs of expanding the UK neonatal screening programme using tandem MS compared with Guthrie method (—, fixed additional cost; ---, total additional cost; , average additional costs; x, marginal costs)

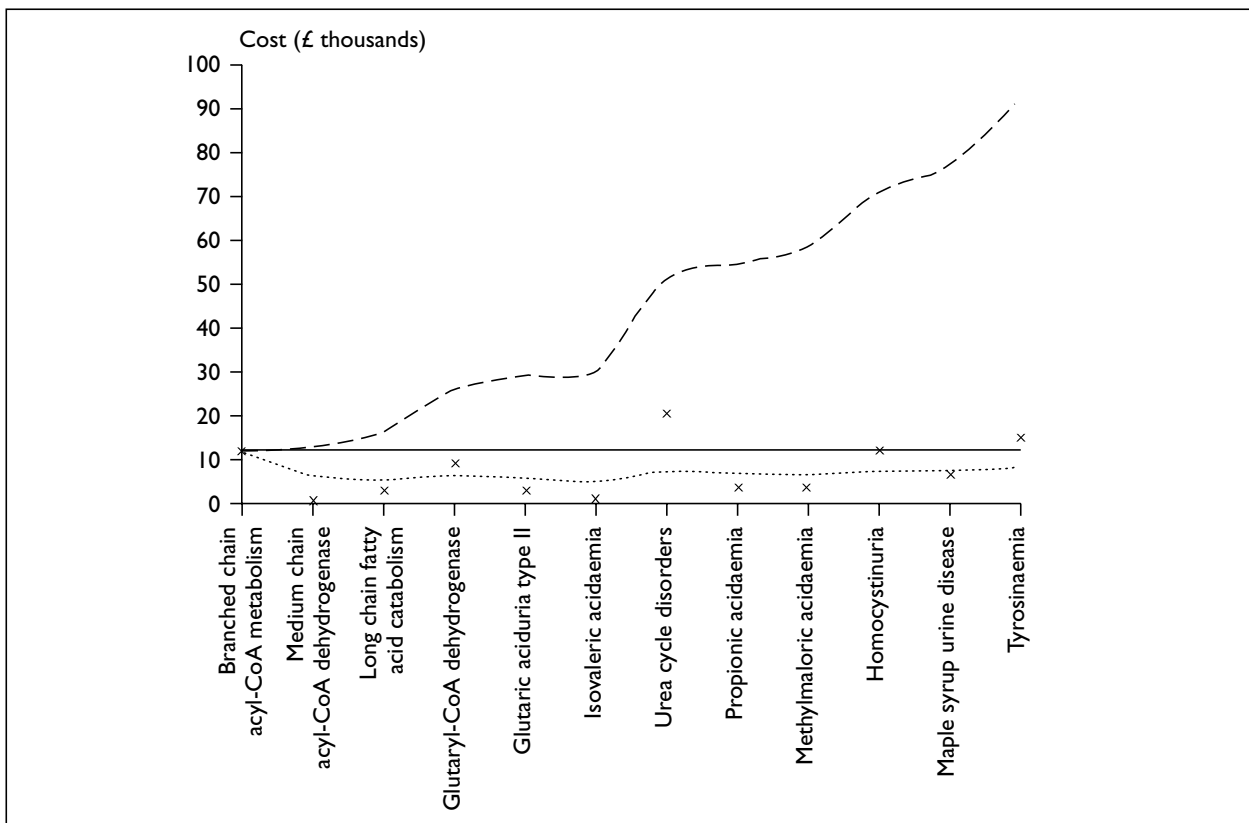


FIGURE 11 Total, marginal and average additional costs of expanding the UK neonatal screening programme using tandem MS compared with fluorometry method (—, fixed additional cost; ---, total additional cost; , average additional costs; x, marginal costs)

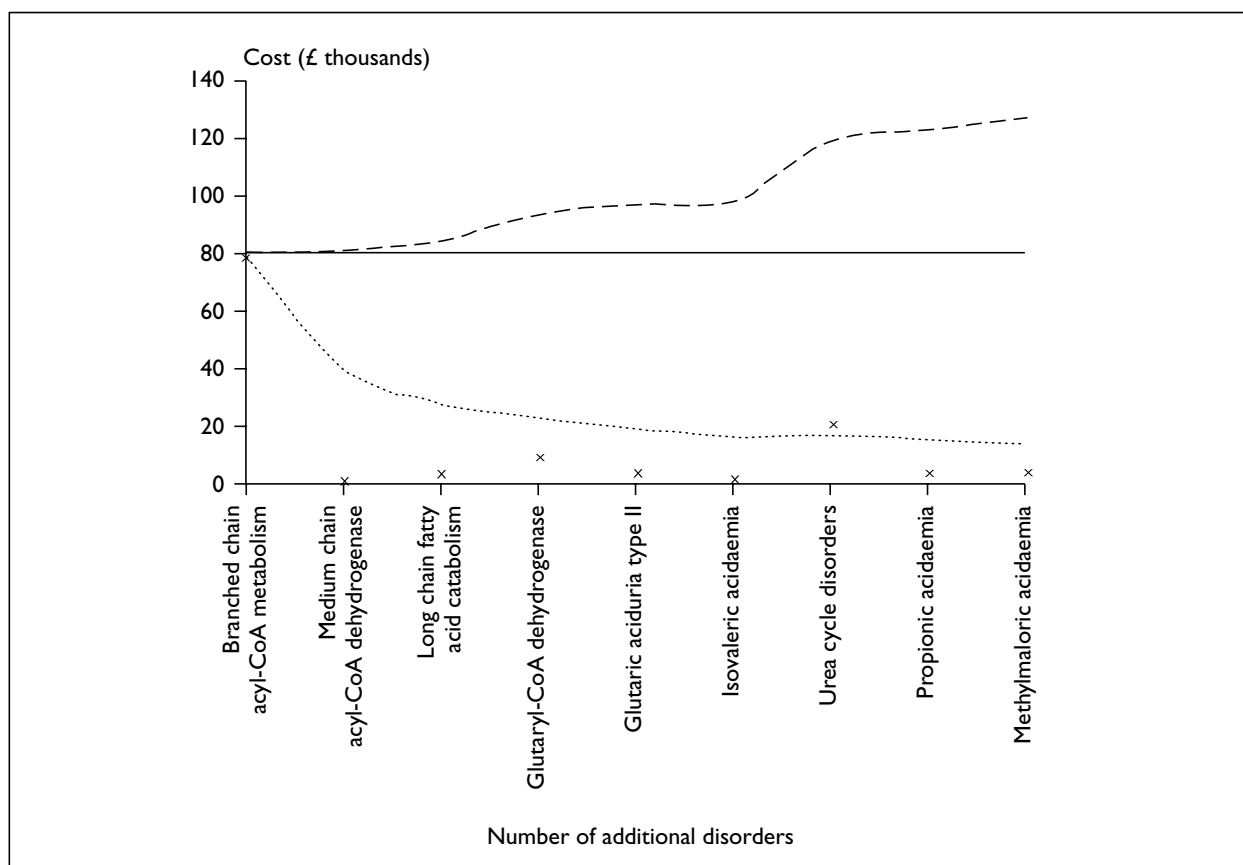


FIGURE 12 Total, marginal and average additional costs of expanding the UK neonatal screening programme using tandem MS compared with chromatography method (—, fixed additional cost; ---, total additional cost; ·····, average additional costs; x, marginal costs)

marginal cost includes the additional fixed cost of introducing tandem MS as well as the additional treatment cost whereas, with the addition of subsequent disorders, the associated marginal costs equal the additional treatment cost only. The marginal costs per additional discounted life-year saved are presented in *Table 124*.

The average costs equally divide the additional fixed costs of introducing tandem MS between the number of additional disorders incorporated into the screening programme and, hence, depict the economies of scale available by expanding the screening programme by a number of disorders at the same time.

TABLE 124 The marginal costs per life-year saved of expanding neonatal screening programme in accordance with the order of priority identified above

Priority order	Disorders	Marginal cost per life-year saved*	
1	Branched-chain acyl-CoA metabolism	Guthrie:	£15,873
		Fluorometry:	£3401
		Chromatography:	£22,209
2	Medium-chain acyl-CoA dehydrogenase		£31
3	Long-chain fatty acid catabolism		£334
4	Glutaryl-CoA dehydrogenase		£523
5	Glutaric aciduria type II		£540
6	Isovaleric acidaemia		£565
* All costs and benefits discounted at 6% level and based on 100,000 neonates screened.			

continued

TABLE 124 contd The marginal costs per life-year saved of expanding neonatal screening programme in accordance with the order of priority identified above

Priority order	Disorders	Marginal cost per life-year saved*
7	Urea cycle disorders	£2188
8	Propionic acidaemia	£2414
9	Methylmalonic acidaemia	£2437
10	Homocystinuria	£4649
11	Maple syrup urine disease	£5114
12	Tyrosinaemia	£8339

* All costs and benefits discounted at 6% level and based on 100,000 neonates screened.

The total additional cost of introducing tandem MS will increase gradually as additional disorders are added to the screening programme, with a more dramatic increase on the addition of the urea cycle disorders (see *Figures 10 to 12*).

From the figures, it can be concluded that the total additional cost of introducing tandem MS (to screen for all disorders sought in this report) compared to Guthrie tests, fluorometry or chromatography, will be approximately £135,000, £90,000 and £125,000 respectively, based on 100,000 neonates screened. It is estimated that 700,000 neonates are screened in the UK per annum; the introduction of tandem MS to screen for all the disorders under consideration (except tyrosinaemia) will therefore result in an additional cost of between £0.5 million and £1 million per annum (approximately), depending on the technology used at present.

Sensitivity analysis

A simple sensitivity analysis has been conducted to investigate the effect of varying a number of uncertain components incorporated in the economic analysis presented above. The sensitivity analysis investigates the effect upon the results of:

- (i) adopting alternative discount rates
- (ii) varying the costs associated with neonatal screening using tandem MS
- (iii) varying the percentage reduction in early deaths (and, hence, the number of life-years saved)
- (iv) varying the length of treatment for those disorders not treated for life.

It would usually be necessary to perform a sensitivity analysis to investigate how varying

the incidence rate estimates affects the marginal cost per additional life-year saved. However, due to the cost structure of this analysis, the marginal costs of expanding the neonatal screening programme are closely related to the resulting additional treatment costs. This means that variations in the incidence rate will have little effect upon the marginal cost per additional life-year saved.

Discount rate

In previous calculations, a discount rate of 6% has been used to adjust for the differential timings of the costs and benefits incurred. However, the value of the discount rate adopted will effect the value of the results obtained (see chapter 12). To investigate the size of this effect, the calculations presented in the results section above have been repeated using the discounted rates of 0% and 10%.

The additional treatment cost per additional life-year saved decreases as the discount rate increases, as shown in *Table 125*. A discussion on the value of discount rate that should be used in an economic evaluation was presented in the method section of the economic review (see chapter 12).

Tandem MS variable costs

Apart from the capital costs, which were obtained directly from the manufacturers, all other costs associated with replacing existing technologies by tandem MS for routine neonatal screening (i.e. variable costs such as consumable, maintenance, and employee costs per annum) were 'best estimates', derived in collaboration with experts in the field. For this reason, it was important to perform sensitivity analyses to establish how these results would vary if the variable costs associated with tandem MS screening were discovered to be more or less than those estimated.

TABLE 125 Additional treatment cost per additional life-year saved by discount rate

	Additional treatment cost per additional life-year saved by discount rate		
	0%	6%	10%
Amino acids			
Tyrosinaemia	£8225	£8339	£9825
Homocystinuria	£2068	£4649	£6660
Maple syrup urine disease	£5093	£5114	£5151
Urea cycle			
Urea cycle disorders	£2169	£2188	£2207
Acylcarnitine			
Medium-chain acyl-CoA dehydrogenase	£9	£31	£48
Propionic acidemia	£2254	£2414	£2556
Methylmalonic acidemia	£2285	£2437	£2571
Isovaleric acidemia	£520	£565	£605
Branched chain acyl-CoA metabolism	£8	£22	£32
Defects of long-chain fatty acid catabolism	£280	£334	£401
Glutaryl-CoA dehydrogenase	£507	£523	£538
Glutaric aciduria type II	£512	£540	£565

(i) Assumes number of confirmation staff required constant regardless of the number of disorders screened.
(ii) All based on neonatal screening for a cohort of 100,000 neonates.
(i) Specimen collection, receipt & result reporting costs are divided equally between phenylketonuria and congenital hypothyroidism.

The results of the sensitivity analysis are presented in *Table 126* and illustrate how laboratory cost per neonate screened (excluding specimen collection, receipt and result reporting) changes as the value of the variable costs are doubled and then halved. It also illustrates how the laboratory cost per neonate screened depends on the size of the cohort of neonates screened by a laboratory each year. It shows how the cost per neonate screened gradually increases as the number of neonates screened is first halved from 100,000 neonates per year to 50,000 neonates per annum and then to 25,000 neonates per year.

Avoided mortality rate

In the above analysis, life-years saved were calculated from 'best estimates' of avoided mortality and life expectancy of individuals identified and treated

early as a result of screening. Because of the presence of uncertainty, it was important to perform a sensitivity analysis to establish the effect upon the marginal cost per additional life-year saved of varying the percentage reduction in early deaths for each of the disorders in question.

The results of the sensitivity analysis are presented in *Table 127* and illustrate a slight decrease in the marginal cost per additional life-year saved, as the value of the percentage reduction in early deaths are increased. This is true for all except branched-chain acyl-CoA metabolism, where the marginal costs incorporate the additional fixed cost of introducing tandem MS compared with existing technologies and, hence, the effect of a change in avoided mortality rate is more dramatic.

TABLE 126 Cost per neonate screened as estimated costs of tandem MS are varied

	Cost per neonate screened (£) excluding specimen collection*		
	Estimated costs x 0.5	Original estimated costs	Estimated costs x 2
100,000 neonates	0.74	1.20	2.12
50,000 neonates	1.02	1.47	2.40
25,000 neonates	1.58	2.06	2.96

* All costs discounted at the 6% level.

TABLE 127 Marginal cost per additional life-year saved as avoided mortality rate is varied

Disorders	Marginal cost per additional life-year saved*		
	Estimated avoided mortality x 0.5	Original estimated avoided mortality	Estimated avoided mortality x 2
Branched-chain acyl-CoA metabolism	£6790–£44,449	£3401–£22,209	£1711–£11,124
Medium-chain acyl-CoA dehydrogenase	£49	£31	£23
Long-chain fatty acid catabolism	£366	£334	£319
Glutaryl-CoA dehydrogenase	£576	£523	£497
Glutaric aciduria type II	£587	£540	£516
Isovaleric acidemia	£645	£565	£562
Urea cycle disorders	£2305	£2188	£2130
Propionic acidemia	£2800	£2414	£2220
Methylmalonic acidemia	£2859	£2437	£2226
Homocystinuria	£7497	£4649	£3223
Maple syrup urine disease	£5204	£5114	£5068
Tyrosinaemia	£10,805	£8339	£7107

* All costs and benefits (life-years saved) discounted at the 6% level.

Treatment costs

The following disorders do not require lifetime treatment: branched-chain acyl-CoA metabolism, medium-chain acyl-CoA dehydrogenase and long-chain fatty acid catabolism. In the analysis above, a treatment period of 5 years was assumed; however, the effect on the additional treatment cost per life-years saved as the treatment period for these disorders is increased to 10 years and 15 years is shown in *Table 128*.

The table illustrates that the additional treatment cost per life-year saved will increase as the treatment period is extended, but not dramatically.

Discussion

It is essential to recognise that the results of the analysis are dependent upon the data used to populate the model, that is, the data on the structure and performance of the screening laboratories, on incidence of the diseases, sensitivity and specificity of the screening techniques, and on outcomes. In all cases, efforts were made to obtain the best data possible within the time and resource constraints of the study.

The responses to the laboratory questionnaire were such as to raise serious questions about the robustness of these data in the model. The

TABLE 128 Effect on the additional treatment cost per life-year saved as the treatment period is varied

	Additional treatment cost per additional life-year saved*		
	5 years	10 years	15 years
Branched-chain acyl-CoA metabolism	£22	£36	£46
Medium-chain acyl-CoA dehydrogenase	£31	£45	£55
Long-chain fatty acid catabolism	£334	£410	£446

* All costs and benefits discounted at the 6% level.

variations in response lead the authors to doubt as to whether the data requirements were interpreted uniformly in the different laboratories. Therefore, the model should be treated as **exploratory**. This exploration suggests that there is a *prima facie* case for introducing tandem MS into neonatal screening and thereby screening for a wider range of diseases than at present. However, these results should not be regarded as conclusive. The proposed pilot study (see chapter 19) will need to incorporate a detailed economic evaluation, with primary data collection on resource utilisation and the associated costs.

The decision-analysis model developed and described above, has focused on the incremental costs and benefits of introducing the new modern technology of tandem MS into the neonatal screening laboratory.

The analysis shows that installing tandem MS into neonatal screening laboratories to screen for the amino acids only would not be a cost-effective approach. That is, it would cost laboratories approximately an additional £30,000 and £24,000, respectively, per additional true case identified to replace Guthrie tests and fluorometry screening with tandem MS screening for the amino acids. Replacing chromatography with tandem MS screening for the amino acids only would result in offering the same service but at an additional cost to the laboratories of £79,000.

The incorporation of mortality benefits into the analysis made it possible to evaluate the additional treatment costs per additional life-year saved when replacing existing technologies with tandem MS and expanding the neonatal screening programme by a single disorder at a time. The results varied considerably, from an estimated £22 per additional life-year saved for branched-chain acyl-CoA metabolism to £8339 per additional life-year saved for tyrosinaemia (both costs and benefits discounted at the 6% level). By calculating the marginal costs per additional life-year saved, the case for expanding the screening programme by one disorder at a time, in terms of the marginal cost per additional life-year saved, is strongly supported for the acylcarnitine disorders and the urea cycle disorders (i.e. greater than £3000), but is less certain for maple syrup urine disease, homocystinuria and tyrosinaemia (i.e. £3000–£20,000).⁵⁶³

Taking these marginal costs to establish an order of screening priority, the model was used to investigate the marginal and average costs,

and mortality benefits of expanding the neonatal screening programme by more than one additional screen at a time. Despite these costs providing support for an expanding UK neonatal screening programme, if tandem MS is introduced the total additional cost of incorporating all the additional disorders based on 700,000 neonates screened in the UK per year would exceed £0.5 million per year, depending on the technology used at present (see *Figure 13*). However, there are likely to be substantial cost-savings to set against these additional costs, resulting from the reduction in severe neurological and mental handicap (for example, avoidance of special education and care) (see *Table 129*).

Although it was important to ensure a measure of health benefit was incorporated into the decision analysis, the improvement in quality of life, as well as the quantity of life (i.e. life-years saved), is a very important element of the health benefits that result from, in this case, early diagnosis through neonatal screening. In this analysis, no attempt was made to place a value on the quality of the life gained as a result of screening. The reason for this was the absence of reliable data identified in the literature to date upon which accurate estimates reflecting the true improvement in the quality of life could be based.

By offsetting the costs of screening against the mortality benefits, the model developed was able to provide conservative estimates of the cost-effectiveness of introducing tandem MS and expanding the neonatal screening programme. Using this approach has meant that where the case for screening for a particular disorder has been supported by the model (i.e. on the basis of mortality benefits alone), if the quality-of-life aspect was incorporated the case for screening may be strengthened further. For example, screening for phenylketonuria is of little benefit in terms of mortality but produces significant improvements in terms of quality of life.

The decision analysis performed in this report has concentrated on the additional treatment costs (incurred as a result of using tandem MS for screening) and the inclusion of additional screens to the neonatal screening programme. However, other additional costs that may be incurred as a result of early diagnosis, such as long-term monitoring costs, indirect costs borne by the family and non-health benefits of screening,⁵⁵⁸ are not considered by the model. This is owing to methodological problems relating to their identification and quantification.

TABLE 129 Additional disorders detectable by tandem MS; long-term clinical consequences prevented by early treatment

Disorder	Intellectual impairment	Neurological impairment	Other somatic problems	Notes
Tyrosinaemia type I	+	+	++*	* Liver failure, renal failure
Homocystinuria	++	–	++*	* Dislocated lens, thrombosis, skeletal deformity
Maple syrup urine disease	++	++	–	
Urea cycle disorders	++	++	–	
Methylmalonic acidaemia	++	++	–*	* Late renal problems even when treated
Propionic acidaemia	++	++	–	
Isovaleric acidaemia	++	+	+	
Other defects of branched-chain acyl-CoA oxidation	+	+	+	
Medium-chain acyl-CoA dehydrogenase deficiency	+	+	–	
Long-chain fatty acid oxidation defects	+	+	+†	* Retinitis pigmentosa, peripheral neuropathy † Myopathy/cardiomyopathy
Glutaric acidaemia type I	+	++*	–	* Dyskinesia, dystonia
Glutaric acidaemia type 2	+	+	+	* Myopathy/cardiomyopathy

(i) Intellectual and/or neurological impairment may result from episodes of acute metabolic disturbance rather than being primary consequences of the metabolic defect.

(ii) Intellectual impairment includes behavioural problems.

(iii) Except where noted, neurological impairment is mainly in the form of cerebral palsy or persistent fitting.

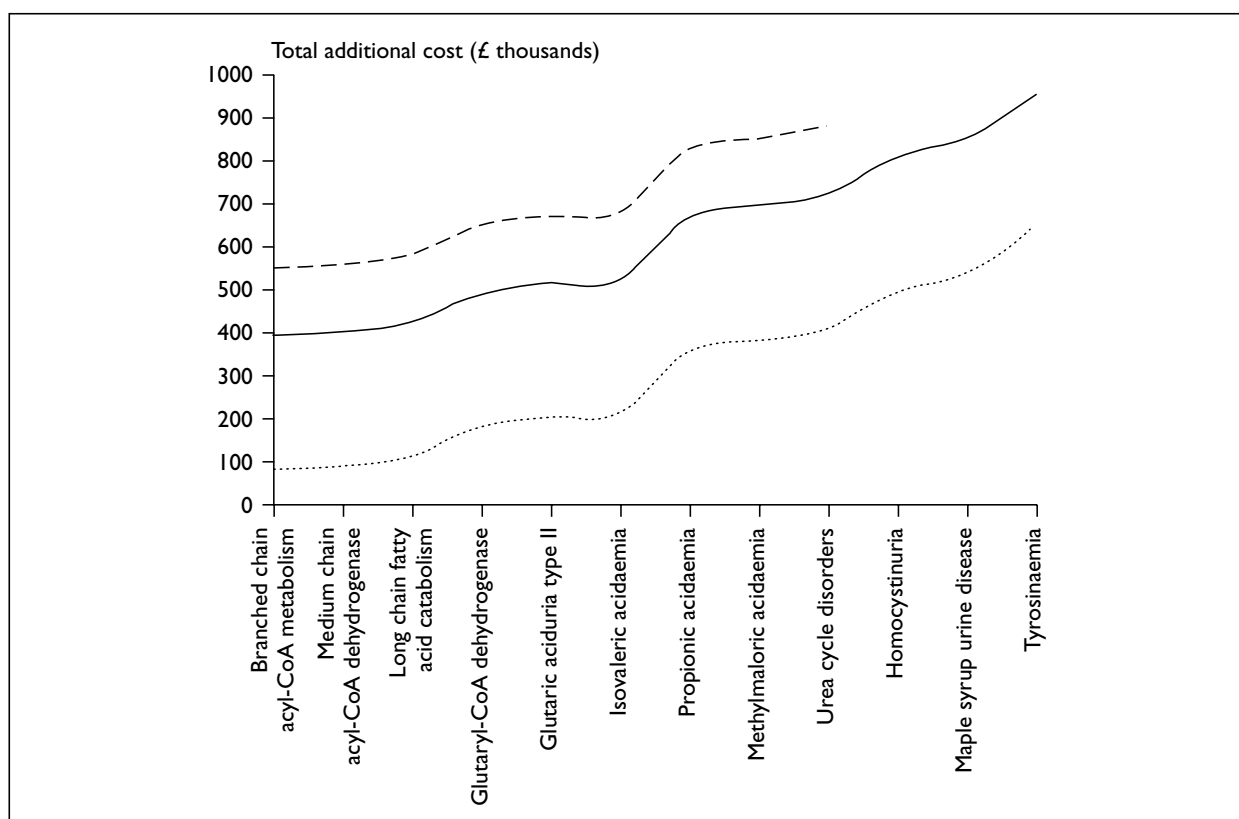


FIGURE 13 Increase in total additional cost of introducing tandem MS to expand the neonatal screening programme compared with existing technologies (—, Guthrie;, fluorometry; ---, chromatography)

In principle, it would have been desirable to have included all the costs and benefits identified above in the decision analysis. However, in practice it seemed more sensible to conduct a conservative analysis using the data available rather than to introduce unnecessary uncertainties by using unreliable estimates of the value for improvement in quality of life, or the indirect costs borne by the parents as a result of early diagnosis.

The main focus of the decision analysis model has been to assess whether there is a place for tandem MS technology in neonatal screening. The model did not therefore consider stand-alone screens, such as those for cystic fibrosis and Duchenne muscular dystrophy. The case for screening for these disorders has been presented in the disorder-specific chapters, and the published economic evaluations to date are appraised critically in the economic review.

Conclusions

The results of the economic evaluation support the view that the replacement of existing screening methods for phenylketonuria with tandem MS

should only be considered if laboratories plan to extend their neonatal screening programme.

From the decision analysis alone, there is support for including all of the disorders under consideration in the UK neonatal screening programme by the introduction of tandem MS. Based on 700,000 neonates screened per year in the UK, this would result in additional costs exceeding £0.5 million per year. However, there are likely to be substantial savings to set against the additional cost, as a result of the reduction in severe neurological and mental handicap.

It is recommended from this report that a number of laboratories be selected to pilot the use of tandem MS screening, primarily focusing on the subset of clearly-defined disorders; that is, where the specificity is adequate and a satisfactory confirmation test exists (the selection of this subset of disorders should be guided by the priority ordering identified by this analysis). As part of this pilot study, it will be important to collect routine data (including transition costs incurred by the laboratory of replacing present technology by tandem MS) to enable a full economic evaluation of neonatal screening using tandem MS to be conducted.

Chapter 19

Overview and recommendations

The current UK screening programme

Phenylketonuria and congenital hypothyroidism

Overall, the UK screening programmes for phenylketonuria and congenital hypothyroidism have fulfilled their original objectives. Severe mental retardation due to phenylketonuria has all but disappeared in the screened population and late-diagnosed hypothyroidism in infancy has been reduced by > 95%. Both programmes should be continued. Current concerns relate mainly to organisation, particularly inadequate coverage in some areas (see chapter 3), and lack of overview at either national or local level. Some of these shortcomings have been examined as part of a national audit. (A Streetly, C Corbett: in preparation.)

Analytical performance of screening laboratories is monitored by the UK EQAS for Neonatal Screening. However, it is clear from the results of the laboratory survey that there are still wide variations in the percentage of repeat specimens required. Given the anxiety that this provokes, an agreed standard for repeat rates should be established, based on the known biology of the disease in question and the analytical performance of the assay(s) used. This standard would attempt to balance the distress caused by repeat sampling against the potential damage caused by a very slight increase in the false-negative rate. This standard should be set by a broad inter-speciality professional group rather than by the laboratory directors in isolation. The existence of such a standard could be of great help in medico-legal disputes.

Recommendation

- Establish a broader forum for neonatal screening to bring together the UK Phenylketonuria and Congenital Hypothyroidism Registers, the Screening Laboratory Directors Audit and Advisory Group, UK EQAS, paediatricians and other interested professional bodies. This group would:
 - (a) determine and periodically update criteria for performance of screening laboratories, (e.g. acceptable resampling rates, timing, and coverage) and possibly continue some aspects of the current national audit

- (b) act as a focus for new initiatives (e.g. linkage of laboratory and child health systems computers)
- (c) monitor the overall impact of introducing new screening projects. The proposed tandem MS steering group (see later) would report to this forum
- (d) produce regular (biannual) reports for purchasers highlighting areas of progress and problems/failures
- (e) interact with the appropriate programme-specific advisory group of the National Screening Committee and the Faculty of Public Health Medicine Screening Group as required.

Current screens for other diseases

Local additions to the screening programme for phenylketonuria and congenital hypothyroidism are listed in *Table 2*. If tandem MS is adopted as a standard screening method, the use of chromatographic screening methods for amino acid disorders and the additional specific screens for homocystinuria and tyrosinaemia will be automatically superseded.

Whole-population neonatal screening for galactosaemia is currently only performed in Scotland. Given the limited advantages conferred by neonatal screening for this disease, particularly when the sample is taken on day 6 or later, and the fact that many cases can be detected by secondary screening of babies with raised blood phenylalanine concentrations, the case for a dedicated screen for galactosaemia is rather weak.

Cystic fibrosis is currently being screened for in at least five centres, covering about 16% of UK births. The psychological aspects of this screen have been extensively discussed in the international literature (see chapter 11), although no formal studies have been performed in the UK. There appears to be considerable public support for neonatal screening for cystic fibrosis, fostered particularly by the UK Cystic Fibrosis Trust, and there is increasing scientific evidence of beneficial long-term effects (see chapter 8). However, before expansion takes place (see below), there should be further consideration of the most appropriate screening protocol, particularly the place of DNA-based screening.

Duchenne muscular dystrophy screening in Wales is being conducted on a research/pilot basis with a high level of pre- and post-screening support. Current reports indicate that the programme is largely well-accepted but that further studies are needed to quantify its effects before such a relatively expensive screen is considered for general use.

Recommendations

- Current UK cystic fibrosis screening programmes should be continued. Information about their performance should be collected and used to inform decisions about future screening strategy for this disease (see recommendation below).
- The Welsh scheme for Duchenne muscular dystrophy should be continued, at least until sufficient data on the effects of early detection on family well-being and reproductive behaviour have been accumulated and analysed.
- Specific screening for galactosaemia is not recommended but all samples with increased phenylalanine should be screened for galactosaemia as a secondary test.

Introducing additional screens

Cystic fibrosis

The marginal cost of detecting a totally 'new' case of cystic fibrosis (i.e. one with no immediate family history and without meconium ileus) is approximately £6900. The benefits are:

- prompt diagnosis, avoiding prolonged worry to the family and possibly leading to more prenatal diagnoses
- savings in short-term healthcare costs
- immediate improvements in the health of an affected baby
- probably an increased life expectancy, with additional years of good quality life.

Taken together these benefits seem to justify the rather modest cost.

Recommendation

- Neonatal screening for cystic fibrosis should be encouraged.
 - (a) This should ideally be an add-on to existing screening programmes rather than a new initiative.
 - (b) The progress of new schemes should be monitored nationally.
 - (c) The best use of DNA-based protocols and most appropriate response to the inadvertent discovery of neonates who

are heterozygous for cystic fibrosis-producing mutations (but probably unaffected) should be reviewed in the light of experience from on-going projects for cystic fibrosis carrier 'screening' in adult volunteers.

Other stand-alone screens

At present galactosaemia, congenital adrenal hyperplasia, Duchenne muscular dystrophy, and biotinidase deficiency do not merit high priority as stand-alone screening programmes. Though all produce benefits of various kinds, the cost-benefit ratio is lower than for the other screens recommended here. From the clinical standpoint, the case for screening for biotinidase deficiency is particularly strong but the incidence is probably extremely low. An approximate estimate suggests that a cost per baby of £0.25 per test could bring this screen into the Wessex 'Recommended' category. This may be achievable by choice of method.

Recommendation

- Biotinidase deficiency should be included in the register of clinically-diagnosed patients suggested below. This may provide data relevant to the case for universal neonatal screening.

Tandem MS based screening

The decision analysis model of neonatal screening (chapter 18) shows that replacing the present screening methods for phenylketonuria by tandem MS and using this technique to screen for additional disorders of amino acid and organic acid metabolism would be highly cost-effective. Taking the average cost for screening for phenylketonuria as £0.60, the additional cost of introducing tandem MS would be approximately £0.60 per baby screened, and the cost per additional diagnosis would be approximately £3000. There would be a marked reduction in mortality from some disorders, particularly the fatty acid oxidation defects: for medium-chain acyl-CoA dehydrogenase deficiency the undiscounted total cost (detection by screening and costs of management) per life-year gained would be under £60. There would be some increase in treatment costs, partly due to treatment starting at an earlier age and partly due to decreased mortality resulting in more patients requiring treatment. These additional costs are estimated to be relatively modest, £18,000 per year for short-term costs, with long-term costs rising eventually to £174,000 per year for a population with 100,000 babies a year being screened. There would be marked health gains and, as with phenylketonuria, marked savings overall from the reduction in severe neurological and mental handicap.

Although sensitivity and specificity of tandem MS screening for several disorders has been well established, we should note that experience of large-scale prospective screening by this method is limited to one area in North America and uses blood spots taken at an earlier age than in the UK. Additional information should become available from current UK-based studies (see chapter 4) but, nevertheless, any large-scale introduction would have to be carefully monitored. Additionally, a change of this magnitude in a service which is, in some places, almost a cottage industry would raise important organisational issues and have consequences for more specialised clinical and laboratory services for inherited metabolic disease. These are discussed separately below.

Recommendations

- A large-scale pilot study of prospective neonatal screening using tandem MS should be initiated, with a view to subsequent introduction into general use. The aims and scope of this study are described in greater detail later.
- This study and subsequent more general introduction should be co-ordinated by a national steering group, which would probably have a life not exceeding 5 years.
- The pilot study should run for 3 years and encompass at least 2 years of prospective screening. At the end of the second year, provided that the initial experience is favourable, the general embargo on the purchase of tandem MS screening would be lifted, with a view to more general introduction after the end of the third year.
- Screening should be directed to a limited range of clearly-defined diseases, where specificity is known to be adequate (low requirement for repeat samples) and there are satisfactory

confirmatory tests. Decisions on these issues would be taken by the steering committee in the light of results of current UK research.

Preliminary proposals are presented in *Table 130*.

- Care should be taken to minimise potential adverse effects of introducing the new screen by providing accurate and appropriate information to all parties involved.
- A longer-term register should be established to record brief details of both screening-diagnosed and screening-missed cases. This could well be combined with other current register initiatives such as regional congenital abnormality registers, or a proposed Royal College of Paediatrics and Child Health Research Unit register of inherited metabolic disease.

Pilot study of tandem MS based screening

Aims

1. To provide a technical appraisal of large-scale screening by tandem MS, building on the work currently being performed in the UK on a research basis. This would encompass:
 - (a) technical validation of the method and assessment of automation
 - (b) trouble-shooting: hardware, computer software, chemical supplies, recording systems
 - (c) collection of data on technical robustness of the method, specimen throughput, optimum data processing methods, and overall long-term practicability – could our present estimate of up to 100,000 samples per year on a single instrument be too ambitious?
 - (d) collection of data on normal ranges, cut-off values and PPVs for the different metabolites

TABLE 130 Proposed allocation of diseases

All centres	Restricted to specific sites
Phenylketonuria	Tyrosinaemia type I
Maple syrup urine disease	Urea cycle defects
Methylmalonic acidemia	Biliary atresia/liver disease
Propionic acidemia	Homocystine assay for second-line test
Isovaleric acidemia	for cystathionine β -synthase deficiency and
Other branched-chain disorders	possibly homocysteine remethylation defects
Medium-chain acyl-CoA dehydrogenase deficiency	
Very long-chain acyl-CoA dehydrogenase deficiency	
Long-chain 3-hydroxyacyl acyl-CoA dehydrogenase deficiency	
Carnitine palmitoyltransferase deficiency	
Carnitine transporter defect	
Glutaryl-CoA dehydrogenase deficiency	
Glutaric aciduria type II	

- (e) development of an external quality assessment scheme.
- 2. To agree minimum criteria for definitive diagnosis of each disease.
- 3. Production of generic documentation and assessment of its effectiveness (see below).
- 4. To establish registration of screening-diagnosed and screening-missed cases, in order to determine sensitivity. In the long term, such data may indicate the need to modify screening methods for certain diseases.
- 5. To develop single-spot diagnoses of tyrosinaemia type I and cystathionine β -synthase deficiency (where there is a need for robust second-line tests), assess the diagnostic value of blood-spot glutamate–glutamine for urea cycle disorders, and evaluate possible screens for biliary atresia, taking into account existing work in progress.
- 6. To report on general practicability, including organisational and staffing requirements, and costs.

Scale and participation

A reasonable aim would be to cover a minimum of 500,000 births at four or five screening centres over a period of 2 years. The sites chosen would need:

- direct involvement in, or partnership with, an existing neonatal screening programme processing a minimum of 50,000 samples per year
- a strong, existing diagnostic service, particularly for organic acid/fatty acid disorders plus a clinical scientist of suitable standing to take charge of collaboration
- a well-defined catchment area with good links to all the paediatric centres therein
- a willingness to supply data to the steering group, including participation in external quality control/specimen sharing schemes, pooling data on normal ranges, and registering biochemical and clinical data on any cases detected
- a good record of collaboration in other national initiatives.

Organisation

The steering group

The steering group should include representative(s) from each pilot site, paediatricians with a specialist metabolic interest, community paediatricians and public health physicians, psychologists, midwives and clinical nurse-specialists. It would have links to the proposed national neonatal screening forum but would be accountable to the body funding the pilot study. The lead investigators at the individual pilot sites will need to make liaison arrangements with local purchasers, maternity services, paediatricians and others.

Timetable

Year 1: preliminary work

- Produce pamphlets for parents and professionals (see below).
- Agree minimum diagnostic criteria for each disease, both for presumptive positive and definitive diagnoses.
- Produce ‘immediate advice’ pamphlets for use with presumptive positive patients.
- Set up lines of communication to ensure prompt action for positive cases and reporting of missed diagnoses.
- Participate with other organisations in setting-up Register, for example, defining data to be collected.
- Agree range of diseases to be covered in initial phase and assign development projects (see below) in the light of information provided by the current UK research studies.
- Set preliminary thresholds for the different metabolites.

Year 2: prospective screening

- General screening – this should be targeted at the well-defined conditions for which there is believed to be a high degree of specificity and adequate follow-up tests (see below).
- Areas requiring development – sites with a special interest in the disorders concerned would be identified for the development of more difficult areas. Suggestions as to allocation are made in *Table 130*.
- Trial of documentation, particularly pamphlets for parents.
- Interim report – by the end of this year, sufficient data should have been assembled to produce a progress report indicating for which disorders methods are sufficiently robust to recommend general introduction. This should give sufficient notice to purchasers to allow pilot sites to move directly to routine service (where justified) without a break.

Year 3: Prospective screening continued

- Refine thresholds for action.
- Review working of external quality assessment scheme.
- Complete disease-specific development projects.
- Publish findings.

At the end of the pilot stage, technical advice and documentation would be in place to support new entrants should the general introduction of tandem MS based screening be recommended.

Funding the pilot phase

Laboratories would be invited to submit proposals to be included in the pilot phase, which would

need to be funded, at least in part, by a research/development grant. Funding would be conditional on full participation in the pilot phase projects. Specific additional funding would be needed to cover the disease-specific development studies.

Organisational issues

The authors attempt below to assess the impact of the developments proposed above organisational arrangements of the current screening programme. The 'conclusions' are in the nature of provisional guidelines, subject to change in the light of experience and having less force than the Recommendations of the previous section. They should be considered further by the steering group.

Provision of information

At present, there are a number of locally-produced information pamphlets describing the scope and arrangements for neonatal metabolic screening. There are doubts about how effective they are at imparting information (see chapter 11). With the increasing number of diseases being covered by the screening programme, the difficulty of providing appropriate information will also increase.

The principle of informed consent presents particular difficulties for neonatal screening. There is disagreement in the ethical and legal literature as to whether parents should have the right to refuse 'standard health care' which might benefit their child. On the other hand, they have a clear right to refuse screening which is being done primarily for the benefit of the family, as in Duchenne muscular dystrophy.

Screening will have limited sensitivity for a number of the diseases to be screened for, particularly mild forms of cystathionine β -synthase deficiency and methylmalonic acidaemia. This needs to be made clear at the outset in order to avoid litigation over missed cases.

Conclusions

- Generic information pamphlets for parents and professionals should be produced at a national level, with local amendments where necessary. The basic pamphlets:
 - (a) should describe the aims and general procedures of the neonatal metabolic screening programme, and the type of disorder that might be uncovered but should not go into detail except for a few of the more common and better-publicised conditions

- (b) must make it clear that, in the case of pamphlets both for parents and professionals, for some disorders the screening tests will not pick up every case. The pamphlet for professionals should go into greater detail on this point, and there may be a case for producing a special version for paediatricians
 - (c) should, where the screening programme includes disorders where detection is primarily of benefit to the family rather than the child, specifically Duchenne muscular dystrophy, be structured in a way that makes it easy for parents to refuse these tests without withdrawing from screens of direct benefit to the child. Details will need careful examination.
- Research is required to examine ways of reducing parental anxiety through more effective methods of inter-professional and doctor-patient communication. This could include the study of a variety of pamphlets to determine acceptability and effectiveness.

Specimen collection

At present, a number of UK screening centres use a liquid blood screening sample because plasma is better than dried whole blood for chromatographic screening. When tandem MS is adopted, chromatographic screening will no longer be required. Changing to standard Guthrie card sampling would have several practical advantages.

Specimen collection in the UK programme was delayed until the sixth day or later to maximise sensitivity for the phenylketonuria screen. This is rather late for disorders that present acutely in infancy (see, for example, maple syrup urine disease, chapter 7), and there may be a good case to move eventually to earlier collection. Such a move is likely to have effects on sensitivity and specificity of the existing screens, however, and will require adjustment to action thresholds. Screening for acute conditions also has implications for the method of specimen transport, often a major contributor to delay. In the short term, therefore, no recommendation is made to reduce the age of sample collection to below 5 days. As a separate measure, selective 'screening' of all sick neonates should be performed with minimum delay. This approach may lead to a cost-saving in intensive care bed occupancy. The interpretation of results from extremely sick infants is likely to be more difficult than with normal screening specimens, however, and such requests should be reported to the originating doctor as for other metabolic investigations rather than through the neonatal screening system.

Some of the conditions covered by tandem MS screening produce significant mortality in the

first few days of life. For the fatty acid oxidation disorders in particular, there may be minimal clinical indication that a metabolic disorder is responsible. Thus, a screening sample should be obtained *post mortem* from all babies who have died before the routine sample has been taken.

Conclusions

- Areas in which screening samples are currently taken later should consider moving to a sixth day collection when tandem MS screening is introduced. Samples should not be delayed because the baby is not on normal milk feeds.
- Early samples should be obtained for babies showing symptoms before the routine collection is due. Such samples should be processed urgently. A routine sample should be taken at 6 days.
- Samples should also be obtained from all babies dying before the routine sample has been taken.

Laboratory organisation

Scale of activity

The original recommendations¹⁵ on the appropriate scale for a screening laboratory have never been fully implemented. The need for substantial capital investment for tandem MS screening may force a re-examination of the situation, with the smallest laboratories ceasing to provide a neonatal screening service. However, perhaps more important than the initial capital outlay is the need for laboratories undertaking tandem MS screening to be staffed with scientists capable of running and maintaining the instrument, interpreting the results, and undertaking or organising the necessary confirmatory investigations. This implies at least one senior scientist with a major commitment to inherited metabolic disease and an understudy to cover for absences. Such staffing cannot be supported, either intellectually or financially, without a reasonable through-put of samples.

Conclusions

- Laboratories undertaking tandem MS screening should have an adequate workload to justify the capital investment and staffing required. A minimum of 50,000 samples per year is suggested.
- Neonatal screening and specialist diagnostic services for metabolic disease should be closely linked to provide the required levels of cross-cover at senior level.

Facilities for confirmation of diagnoses

The anxiety produced by false-positive diagnoses has been amply documented in the literature (see chapter 11). As far as possible, neonatal screening should be organised so as to produce a reasonably firm presumptive positive diagnosis using the first

blood sample or, failing this, on a repeat sample obtained by one month of age, at the latest.

Conclusions

- Neonatal screening should aim to provide a prompt and seamless service producing a presumptive positive diagnosis without the need for repeat testing or referral of the patient.
- Linkage between the screening laboratory and specialist services for diagnosing inherited metabolic disease should provide ready access to the full range of additional tests required for definitive diagnosis, including DNA analysis and those on any additional samples, such as urine.
- The costs of such further investigations should be included in the neonatal screening package to obviate the need for additional paperwork and possible delays.

The need for focus

The history of neonatal screening contains several examples where programmes have run into difficulties because of lack of attention to the basic principles of screening. In particular, the introduction of chromatography 30 years ago resulted in a number of very unsatisfactory programmes, with high rates of repeat sampling because of non-specific 'abnormalities' and many patients with non-diseases, such as histidinaemia, being subjected to investigation and sometimes treatment. The advent of tandem MS screening creates an analogous situation, although perhaps less hazardous because of greater clinical experience of inherited metabolic disease and better awareness of the pitfalls. Nevertheless, unless we are careful we will be lured into investigating non-specific findings of dubious significance. This is, perhaps, inevitable on occasion when investigating a symptomatic patient; it is unacceptable in the screening context.

Conclusions

- Screening by tandem MS should be performed in the multiple reaction monitoring (or MRM) mode, so that only compounds of known diagnostic significance are measured.
- Action limits for other analytes should be set so that repeat sample rates for individual diseases are kept to an agreed PPV, say > 25%.

Clinical referral and treatment

The acute nature of many of the conditions detectable by tandem MS will require a streamlined approach to referral and treatment. Good communication between the regional screening centres and local paediatricians will be essential, an argument against very large-scale centralisation. As many of the patients will still be asymptomatic,

emergency referral to a distant specialist hospital may be inappropriate, but the local paediatrician must be provided with clear advice about warning signs and management before specialist assessment.

The organisation of clinical services may also need to be reviewed. There are relatively few specialist metabolic paediatricians. For some of the 'new' acutely-presenting disorders, the mainstay of treatment is the avoidance of catabolic stress and, in more remote areas, it will be necessary to educate both the family practitioner and parents in emergency measures.

Conclusions

- The screening laboratory should have disease-specific documentation available so that it can be sent by fax machine to both the local paediatrician and the family doctor. It should include a description of initial management and sufficient background information to enable the doctor to respond authoritatively to parents' questions. Such documentation should, ideally, be produced at a national level, modified as necessary to suit local circumstances.
- Management arrangements within a screening region should be predetermined: it is not adequate for a newly-diagnosed baby with a metabolic disease to finish up in the routine 'take'.

Coverage and record-keeping

The difficulties with coverage in the existing programme have already been reviewed. The long-term solution seems to lie in better interfacing between Child Health computers and screening laboratories. Many of the current Child Health computing systems are configured with separate fields for the results (positive or negative) of individual screens. Although this may be appropriate for a laboratory computer, since the results will probably be entered in numerical form and linked to subsequent investigations, it is unsatisfactory in a Child Health Record – the child will become permanently labelled with a provisional diagnosis from the initial screening test, and this could re-surface years later. In any case, a different approach will be necessary when up to 20 diseases are covered by a screening programme.

Conclusions

- The interface of Child Health Record-keeping with neonatal screening should be kept under review.

- If it is considered necessary to enter diagnoses on computerised Child Health Records, then a mechanism is needed to ensure that such diagnoses are properly confirmed.

Impact on other laboratory services *Tandem MS in general metabolic investigations*

A number of existing clinical chemistry analyses can be performed more rapidly by tandem MS. Insofar as these relate mainly to the diagnosis and management of inherited metabolic disease, they could easily be transferred if screening and other paediatric clinical chemistry were closely integrated services. Examples already practical are:

- amino acid analysis for monitoring patients being treated on protein-restricted diets for phenylketonuria, homocystinuria or other diseases, or on long-term total parenteral nutrition
- assay of free carnitine and acylcarnitines, both for diagnosis and monitoring treatment
- bile acid analysis.

Tandem MS is not a replacement for qualitative examination of urinary amino acids or organic acids for investigating symptomatic patients, since it will miss some diagnostic abnormalities.

Effect on current work load

Tandem MS is likely to have a significant impact on the pattern of requests for some metabolic investigations, particularly urinary organic acids, by routinely diagnosing inherited disorders of amino acid and organic acid metabolism in a large proportion of affected patients shortly after birth. It will also lead to reduced demand for classical amino acid analysis. The effect will take some time to develop since clinical practice will not adjust immediately and, at first, there will also be many older patients who have not been screened. A number of disorders of organic acid metabolism do not produce abnormal acylcarnitine profiles and these will be missed by tandem MS screening as, probably, will milder forms of methylmalonic acidaemia and some others. Clinicians will need to be made aware of this. We may expect a reduced, or at least stabilised, demand for full organic acid profiles, with a more selected and largely non-neonatal group of patients being investigated.



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Appendix I

Laboratory questionnaire

CONFIDENTIAL

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NEONATAL SCREENING LABS QUESTIONNAIRE

This questionnaire is being carried out in complete confidentiality. This sheet will be detached on receipt to ensure confidentiality.

PLEASE ANSWER ALL THE QUESTIONS IN ALL FOUR SECTIONS A TO D.

Please return your completed questionnaire to:

Dr. G. M. Addison
Department of Biochemistry
Royal Manchester Children's Hospital
Pendlebury
Manchester
M27 1HA

Personal information

Name _____

Your job title _____

Your work address _____

Telephone Number _____

Fax. Number _____

**PLEASE DO NOT DETACH THIS PAGE, THIS WILL BE DONE BY THE RESEARCHERS
AT ST. GEORGE'S & SCHARR**

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NEONATAL SCREENING LABS QUESTIONNAIRE

Section A: ACTIVITY

- 1) What diseases are screened for by your laboratory? Please **include** both the primary disorders screened for and those disorders detected as by-products (*e.g. Galactosaemia from PKU screening*).

(Please tick and add to)

CODE*	DISORDER	AS PRIMARY PURPOSE OF SCREEN [#]	AS BY-PRODUCT
01	PHENYLKETONURIA		
02	CONGENITAL HYPOTHYROIDISM		
03			
04			
05			
08			
07			

* This code will be used as a disorder identifier throughout sections B and C.

Please tick the appropriate box.

- 2) How many neonates were screened by your laboratory in the Calendar/Financial year of 1995
(delete as appropriate)?
-

Section B: MANPOWER COSTS

- 3) Please list, in table a) the posts of **ALL** staff undertaking work on Neonatal Screening in your laboratory. (*e.g. M.L.S.O. 1, Clerical officer grade 2, etc.*)

Also indicate, in tables b), c) and d), how the time of **EACH** employee is divided between the 'specific' stages of Neonatal Screening process and the different screens performed by your laboratory.

(Please give the answers in whole time equivalents (WTE – e.g. PKU 0.5; CHT 0.5) including the proportion of staff from other sections for holiday and sick cover but excluding time for the HIV surveillance scheme.)

Table a) A list of all employees undertaking work on neonatal screening in your laboratory

POSTS (type & grade)	WTE

Table b) Specimen Receipt & Result Reporting**

POSTS (type & grade)	WTE

** Do NOT include time spent performing detailed checking against birth lists or similar records.

Table c) Testing and Analysis

POSTS (type & grade)	DIVISION OF TIME (WTE ⁺)							TOTAL WTE
	DISORDER CODE ^{##}							
	01	02	03	04	05	06	07	

⁺ Please give your answer in whole time equivalents – e.g. if an employee works 0.5 WTE and 50% of his time is spent on 'testing & analysing disorder 01' then WTE = 0.25.

^{##} The disorder codes are as defined in question 1).

Table d) Confirmation, Referral, Advise for positive cases***

POSTS (type & grade)	DIVISION OF TIME (WTE ⁺)							TOTAL WTE
	DISORDER CODE ^{##}							
	01	02	03	04	05	06	07	

*** Please ignore long term follow-up, teaching and training time

⁺ Please give your answer in whole time equivalents – e.g. if an employee works 0.5 WTE and 50% of his time is spent on 'confirmation, referral, advise for positive cases of disorder 01' then WTE = 0.25.

^{##} The disorder codes are as defined in question 1).

- 4) Which additional service components does your laboratory provide?
(e.g. liaison nurse, attendants at metabolic clinics, etc.)

- 5) What is the floor area of your laboratory devoted to each of the primary screens identified in question 1), **including** office and storage areas but **excluding** Guthrie card storage space? Where areas are shared with other procedures, please proportion the floor area accordingly?
(Please give the answer to the nearest m²)

DISORDER CODES		FLOOR AREA (m ²)
N/A	SPECIMEN RECEPTION (for all disorders collectively)	
01	PHENYLKETONURIA	
02	CONGENITAL HYPOTHYROIDISM	
03		
04		
05		
06		
07		

Section C

The questions in this section are presented as a form and relate to the primary disorders screened for by your laboratory as listed in question 1. Separate forms are attached for Phenylketonuria and Congenital Hypothyroidism. For each additional primary disorder screened for by your laboratory, please complete one of the **Disorder Unspecified** forms.

When completing the enclosed forms **please** include the costs of the primary screen **plus** 'by-product' disorders (e.g. Galactosaemia from PKU screen).

DISORDER CODE: **01: PHENYLKETONURIA (& by-products)**

- i) What technique do you use as your first line screen for phenylketonuria?
(Please tick or add to)

TECHNIQUE	please tick
Chromatography	
Fluorometry	

TECHNIQUE	please tick
Guthrie	

ii) Please list below the capital equipment required for screening and initial confirmation of Phenylketonuria (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to Phenylketonuria.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation of Phenylketonuria (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery)?

iv) How many repeat blood samples per year are required due to abnormal results?

v) Any further comments

DISORDER CODE: 02: CONGENITAL HYPOTHYROIDISM

i) a) What method do you use to screen for Congenital Hypothyroidism?

b) What manufacturer and kit do you use?

Manufacturer: _____

Kit: _____

ii) Please list below the capital equipment required for screening and initial confirmation of Congenital Hypothyroidism (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to Congenital Hypothyroidism.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation of Congenital Hypothyroidism (exclude costs of Guthrie cards, blood sample tubes, postage & stationery)?

iv) How many repeat blood samples per year are required due to abnormal results?

v) Any further comments

DISORDER UNSPECIFIED: **DISORDER CODE: 03**

i) What technique do you use as your first line screen for this disease?

ii) Please list below the capital equipment required for screening and initial confirmation of this disease (*i.e. before the patient is referred*).

If any of the equipment is shared between screens or other uses please state the **proportion of its use** allocated to this specific disease.

TYPE OF EQUIPMENT	CURRENT REPLACEMENT COST (if known)	PROPORTION OF USE ALLOCATED TO TESTING FOR THIS DISEASE

iii) What is your annual cost of consumables used for screening and initial confirmation for this specific disease (**exclude** costs of Guthrie cards, blood sample tubes, postage & stationery.)?

iv) How many repeat blood samples per year are required due to abnormal results?

v) What is the incidence for this disease in the population screened for by your laboratory (per 100,000 births)? (*Please state on how many years data this incidence is calculated*)

_____ per 100,000 births _____ years

vi) Any further comments

Appendix 2

Description of undiscounted treatment cost calculations

Tyrosinaemia type I

- Cost of early treatment: 0.4 years of NTBC and total elemental diet.
- Cost of treatment for additional lives saved: 2 years (minus 0.4 years above) of NTBC and total elemental diet followed by liver transplant and 28 years of subsequent treatment.

Homocystinuria (pyridoxine responsive)

- Cost of early treatment: 5 years of low protein diet plus supplements.
- Cost of treatment for additional lives saved: 60 years (minus 5 years above) of low protein diet plus supplements.

Homocystinuria (pyridoxine non-responsive)

- Cost of early treatment: 2.5 years of total elemental diet.
- Cost of treatment for additional lives saved: 8 years (minus 5 years above) of low protein diet plus supplements, followed by 32 years on general dietetic advice/co-factor treatment.

Maple syrup urine disease

- Cost of early treatment: 0.1 years of total elemental diet.
- Cost of treatment for additional lives saved: 40 years (minus 0.1 years above) of total elemental diet.

Urea cycle disorders (moderate)

- Cost of early treatment: 0.5 years of low protein diet plus supplements.
- Cost of treatment for additional lives saved: 40 years (minus the 0.5 years above) of low protein diet plus supplements.

Urea cycle disorders (severe)

- Cost of early treatment: 0.1 years of low protein diet plus supplements.
- Cost of treatment for additional lives saved: 10 years (minus the 0.1 years above) of low protein diet plus supplements.

Methylmalonic acidaemia (neonatal)

- Cost of early treatment: 1 year of low protein diet plus supplements.
- Cost of treatment for additional lives saved: 30 years (minus 1 year above) of low protein diet plus supplements.

Propionic acidaemia (neonatal)

- Cost of early treatment: 1 year of low protein diet plus supplements.
- Cost of treatment for additional lives saved: 40 years (minus 1 year above) of low protein diet plus supplements.

Isovaleric acidaemia

- Cost of early treatment: 0.5 years of general dietetic advice/co-factor treatment.
- Cost of treatment for additional lives saved: 50 years (minus 0.5 years above) of general dietetic advice/co-factor treatment.

Branched-chain acyl-CoA metabolism

- Cost of early treatment: 0.2 years of no diet/emergency regime.
- Cost of treatment for additional lives saved: 5 years (minus 0.2 years above) of no diet/emergency regime.

Medium-chain acyl-CoA dehydrogenase

- Cost of early treatment: 1 year of no diet/emergency regime.
- Cost of treatment for additional lives saved: 5 years (minus 1 year above) of no diet/emergency regime.

Long-chain defects

- Cost of early treatment: 0.5 years of general dietetic advice/co-factor treatment.
- Cost of treatment for additional lives saved: 5 years (minus 0.5 years above) of general dietetic advice/co-factor treatment followed by 45 years of general dietetic advice/co-factor treatment at a reduced cost of £250.

Glutaryl-CoA dehydrogenase deficiency

- Cost of early treatment: 1 year of general dietetic advice/co-factor treatment.

- Cost of treatment for additional lives saved: 50 years (minus 1 year above) of general dietetic advice/co-factor treatment.

Glutaric aciduria type II

- Cost of early treatment: 0.3 years of general dietetic advice/co-factor treatment.

- Cost of treatment for additional lives saved: 50 years (minus 0.3 years above) of general dietetic advice/co-factor treatment.

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