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Review

Screening for cystic fibrosis

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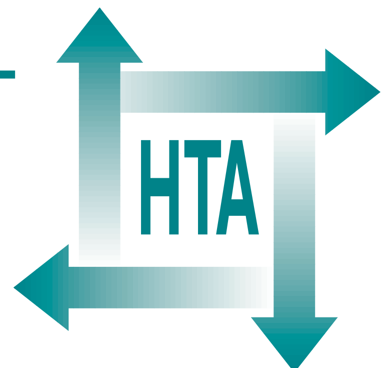

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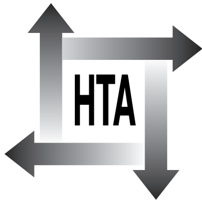

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Screening for cystic fibrosis

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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. The editors wish to emphasise that funding and publication of this research by the NHS should not be taken as implicit support for the recommendations for policy contained herein. In particular, policy options in the area of screening will be considered by the National Screening Committee. This Committee, chaired by the Chief Medical Officer, will take into account the views expressed here, further available evidence and other relevant considerations.

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Glossary and list of abbreviations

Technical terms and abbreviations are used throughout this report. The meaning is usually clear from the context but a glossary is provided for the non-specialist reader. In some cases usage differs in the literature but the term has a constant meaning throughout this review.

Glossary

Carrier Individual unaffected by CF but having a mutation in one of their CFTR genes.

Cascade screening Systematic identification and testing of members in a proband's family.

Case-finding Actively trying to diagnose probands for cascade screening.

cDNA Complementary DNA, made from an RNA template and used in gene therapy.

CF transmembrane conductance regulator The gene or its protein product which is mutated in CF.

Compound heterozygote Individual affected CF, having different mutations on each CFTR gene.

Chorionic villus sampling Invasive procedure to obtain placental tissue for prenatal diagnosis.

ΔF508 Three base pair deletion, the most common mutation in the CFTR gene.

Detection rate Proportion of affected individuals with positive results.

False-positive rate Proportion of unaffected individuals with positive results.

Heterozygous Having different CFTR alleles.

Homozygous Having identical CFTR alleles.

Negative predictive value Probability that an individual with a negative result is unaffected.

Obligate carrier Person who from pedigree analysis must have passed on an affected gene.

Polymerase chain reaction Method of amplifying small amounts of DNA.

Positive predictive value Probability that an individual with a positive result is affected.

Proband Affected individual through whom attention is drawn to a pedigree.

Quantitative pilocarpine iontophoresis test Sweat test used in the diagnosis of CF.

List of abbreviations

ARMS	amplification refractory mutation system
AVV	adeno-associated virus
CBAVD	congenital bilateral absence of the vas deferens
CF	cystic fibrosis
CFGAC	CF Genetic Analysis Consortium
CFTR	cystic fibrosis transmembrane conductance regulator
CPX	8-cyclophenyl-1,3-dipropylxanthine
CVS	chorionic villus sampling
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GP	general practitioner
ICSI	intracytoplasmic sperm injection
IRT	immunoreactive trypsinogen
mRNA	messenger ribonucleic acid
MSD	membrane-spanning domain
NBD	nucleotide-binding domain
NIH	National Institutes of Health (USA)
OLA	oligonucleotide ligation assay
PCR	polymerase chain reaction
PI	pancreatic insufficient
PS	pancreatic sufficient
RCT	randomised controlled trial
RD	regulatory domain
rhDNase	recombinant human deoxyribonuclease
SPQ	halide sensitive fluorophore 6-methoxy-N-(3-sulphopropyl)-quinolium
UTP	uridine triphosphate

Executive summary

Background

Cystic fibrosis (CF) is a common serious inherited disorder associated with considerable morbidity and high case-fatality. Two recent developments have implications for screening policy, the discovery of the gene responsible for the condition and the continuing improvement in life expectancy.

Aim

To provide the information needed to help decide whether screening should become routine and, if so, which strategy to adopt.

Methods

The review is based on a literature search of electronic reference databases of published and ‘grey’ literature together with handsearching of the most recent publications.

Results

Treatment

CF is a disorder in which the exocrine glands of the epithelia produce abnormally thick secretions of mucus and elevated sweat electrolytes. It is characterised by progressive respiratory and gastrointestinal problems, and is associated with impaired fertility. There is substantial variability in severity, with some patients symptomatic at birth, while others may not present for months or even years.

Modern treatment with physiotherapy, antibiotics and enzyme supplements delays disease progression and survival rates are now predicted to exceed 40 years. Newer treatments, including anti-inflammatory agents and gene replacement therapies, may eventually lead to even greater longevity. However, research is still in its earliest stages and success is not guaranteed.

Genetics

It has been known since the 1940s that CF was an autosomal Mendelian recessive disorder and, in 1989, the transmembrane conductance regulator (CFTR) gene situated at 7q31, was shown to be responsible for the condition. The gene spans over 250 kb and comprises 27 exons; the mRNA transcript is 5 kb long and codes for a protein which controls the electrochemical balance of chloride secretion and sodium absorption.

To date over 800 mutations in the CFTR gene have been identified, although not all are disease causing. The most common mutation in the UK is the three base pair deletion, ΔF508, which accounts for 75% of carriers; three commercial multiple-mutation assays are available that can detect about 86% of carriers in Scotland, Wales and the North of England, or 80% elsewhere. Different proportions apply to Asians (35%), Ashkenazi Jews (95%) and Blacks (41%).

The UK birth prevalence is 1 in 2400, which implies a carrier frequency of 1 in 24. A carrier couple have a one in four risk that each of their children has CF; this is reduced to under one in 50,000 if neither parent has a detectable mutation. When only one parent is a carrier the risk is about 1 in 500.

Genetic screening

The aim of genetic screening for CF is to reduce the birth prevalence of the disorder. This is principally achieved by identifying carrier couples who can have prenatal diagnosis and selective termination of pregnancy. Other options are to: avoid pregnancy; change partners; have artificial insemination using donor sperm or egg; and have pre-implantation diagnosis to select unaffected zygotes.

Carrier couples can be identified directly during pregnancy or when it is being planned, or indirectly by determining the carrier status of everyone of reproductive age in the population. A third approach is systematic ‘cascade’ testing within CF families.

Antenatal and pre-conceptional genetic screening

There have been 11 published studies reporting the results of antenatal screening pilot projects. The combined results on over 40,000 tests

demonstrate the feasibility of the method, and show the acceptability of screening (uptake 74%) and invasive prenatal diagnosis in carrier couples (uptake 89%).

Pre-conceptional CF screening has been tried at a family planning clinic setting with high uptake. Pre-nuptial testing is already available for orthodox Ashkenazi Jews. Pre-implantation diagnosis is currently being carried out at six licensed UK centres, although worldwide less than 100 procedures for CF have been performed.

Other genetic screening

Four general population screening studies have been carried out in general practice with a total of almost 11,000 patients. Uptake was only 8% when invited by letter but 48% when approached opportunistically in the clinic. Uptake was also low when screening was offered to school students (41%, 42% and 70% in three studies), in the workplace (21%) and as the result of a general community-wide campaign (8%).

Usually probands are told that close relatives can be screened but only one-third of first- and one-tenth of second-degree relatives are tested. There have been three studies of the more active cascade screening approach. Uptake was higher and a large proportion of those tested were carriers. However, mathematical models have shown that under 15% of carriers in the population would be detectable this way.

There is also experience of screening in selective groups such as those already having invasive prenatal diagnosis unrelated to CF, and in assisted reproduction units for infertile men and sperm donors.

Neonatal screening

This aims to bring forward the diagnosis of CF and so improve prognosis. The detailed experience of neonatal CF screening has been reported for 20 programmes including six in the UK. Protocols vary: single or repeat testing; foetal blood spots or meconium; immunoreactive trypsinogen (IRT) or DNA. In total more than five million neonates were screened with a low false-positive rate (0.5 per 1000), acceptable detection rate (90%), and favourable positive predictive value (33%).

The ability of screening to alter long-term prognosis has not been conclusively proven. Two randomised trials of screening, five case–control studies, a study of sib-pairs and a trial of prophylactic versus symptomatic treatment of early disease all provide relevant information. However, this is either predominantly short term or subject to strong statistical bias. Nevertheless there is some circumstantial evidence favouring a benefit.

Human and financial costs

Screening may result in psychological harm and, if invasive prenatal diagnosis is involved, there is an approximately 1% risk of foetal loss. The cost of antenatal screening is estimated to be between £46,000 and £53,000 per CF pregnancy detected, considerably less than the lifetime cost of treatment. Neonatal screening costs about £4400 per case detected or £6400 for those who would not otherwise have had an early diagnosis, and about £1500 and £2200, respectively, when combined with antenatal screening.

Conclusions

Evidence supports the following actions:

- antenatal genetic screening should be offered routinely
- pre-conceptional genetic screening should be made available for couples who request it
- genetic screening should be available for infertile men and for sperm donors
- testing should be undertaken in laboratories with an annual throughput of at least 5000 CF tests
- health authorities could consider introducing neonatal screening.

Recommendations for future research

- Re-analysis of the Wales and West Midlands neonatal screening trial.
- More research on psychological and medical consequences for carrier detection in neonatal screening.
- Neonatal screening programmes to undertake RCTs of specific early treatments.
- Innovative methods for presenting information on genetic screening.
- Audit procedures to ensure that parents give informed consent to neonatal screening.

Chapter 1
Background

Cystic fibrosis (CF) is a serious inherited disorder associated with considerable morbidity and reduced life expectancy. In the UK the birth prevalence is about 1 in 2400, equivalent to 300 new cases each year. CF is characterised by an excessive accumulation of thick mucus in the epithelium of the respiratory system and digestive tract. The main clinical features include progressive lung disease and pancreatic enzyme deficiency.

In recent years there have been two important developments which have implications for screening policy. First, the gene responsible for the condition has been identified and, second, the vast improvements in treatment have increased life expectancy substantially.

Genetics

CF is the most common serious single gene disorder in Caucasians. It is inherited as a recessive condition and, in the UK, about 4% of people are carriers. CF is now known to be caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7. To date over 800 different disease-causing mutations have been found in the CFTR gene and these vary in frequency according to geographical and ethnic background. In the UK, a single mutation (ΔF508) accounts for about three-quarters of defective CFTR genes in non-Jewish Caucasians; all the common mutations together account for over four-fifths. A different mutation is the main cause of CF in Ashkenazi Jews.

Life expectancy

Long-term prognosis for patients with CF has improved substantially over the years. This has been attributed to improved treatment regimes together with the provision of centralised special CF units. Over 90% of affected infants now survive beyond 1 year and the current median age of death has increased to 20–30 years. Moreover, the model-predicted life expectancy for children born in the 1990s now exceeds 40 years.

Routine screening

A simple DNA test could be used to routinely screen for the most common CFTR mutations, with the ultimate purpose of reducing birth prevalence. There are a number of possible strategies, including antenatal testing of apparently low-risk pregnancies, pre-conceptional testing in the general population, and systematic testing within the families of affected individuals.

Another screening strategy is the routine testing of neonates with the aim of bringing forward a clinical diagnosis and maximising the benefits of modern treatment. This could make use of both traditional biochemical screening methods and DNA testing.

Screening policy

In this document, structured reviews of the literature are used to obtain the information needed by planners to make policy decisions. Detailed information is presented and synthesised relating to natural history, genetics, prevalence, laboratory techniques and screening strategies. Statistical modelling techniques are used to explore the likely consequences of each strategy. In addition to screening efficiency, the human and financial costs including psychosocial aspects are considered. Finally, those areas in which information is lacking are highlighted, together with areas for future research.

Chapter 2
Search methods

In conducting this review, the guidelines produced by the NHS Centre for Reviews and Dissemination at the University of York were followed.

Electronic literature searches were conducted periodically using mainly MEDLINE and the Science Citation Index of the Bath Information and Data Services (BIDS). Other sources of information included CINAHL and Sociofile. Key words and phrases were used in conjunction with CF/cystic fibrosis when searching these databases. For example, to obtain references of a psychosocial nature the search strategy included stigma#, psycho#, anxiety, knowledge, attitude and perception. Various CF-related web sites have also been accessed, as has the Cochrane CF database. The organisation of publications was similar to that used in a previous review.[1] Because of the vast quantity of literature on CF it was decided that information on natural history and established treatments would be derived mainly from previous reviews. However, new and innovative research of significant importance, such as gene therapy, are briefly discussed. With regard to the relationship between genotype and clinical features, publications based on case reports were not included in meta-analyses. In order to achieve a high recall rate, a structured search strategy was employed in electronic searching. Over 2000 references were considered for inclusion in the report and, of these, 455 are directly quoted.

In addition to electronic methods, the journals in which relevant publications are most often found were handsearched regularly for missed and recent papers. These included *The Lancet, BMJ, Journal of Medical Genetics, Thorax* and *Archives of Disease in Childhood*. The Section of Paediatrics of the Royal Society of Medicine also hold regular 1-day meetings, the proceedings of which are published in the *Journal of the Royal Society of Medicine*. Papers from the following conferences are also published in *Pediatric Pulmonology*: International Congress on Pediatric Pulmonology (1993), IVth International Conference on Newborn Screening for Cystic Fibrosis (1990) and the Annual North American and International Cystic Fibrosis Conference. The European Society for Cystic Fibrosis (formerly known as the European Working Group for Cystic Fibrosis) also hold annual meetings, the proceedings of which are published in various journals. Abstracts from the 20th and 21st such conferences, together with the 1996 International Cystic Fibrosis Congress, have been searched for relevant material.

The Cystic Fibrosis Trust also produces two quarterly newsletters, the quarterly *CF News* and *Input*, of which we are in regular receipt. These cover a wide range of topics, including research funding and findings, information on clinical trials, helplines, support contacts and fund-raising ideas. We have also obtained the newsletters of the European Community Concerted Action for Cystic Fibrosis. Finally, we have contacted several groups for up-to-date information on a number of related aspects. While not everyone responded to our request, any information received has been incorporated into the review.

Chapter 3

Natural history

The first comprehensive description of CF was provided by Andersen in 1938 who called it 'cystic fibrosis of the pancreas'.[2] Also known as 'mucoviscidosis', CF was later identified more specifically as a disorder of the exocrine glands.[3] Characterisation of the disease was enhanced in the 1950s when the first diagnostic test based on elevated salt concentrations in sweat became available.[4] In the 1980s the basic biochemical defect underlying CF was discovered; this was the cyclic adenosine monophosphate (cyclic AMP) chloride channel sited in the apical membrane of epithelial cells.[5,6] In 1989, understanding of the disorder at a molecular level was advanced with the discovery of the gene responsible for the condition and its most common mutation.[7–9] This major discovery now forms the basis of much research into potential treatments for CF, including the possibility of finding a more specific treatment using gene therapy (see chapter 4). If this is successful, the outlook for people with CF will change dramatically.

Clinical features

CF is a disorder in which the exocrine glands of the epithelia produce abnormally thick secretions of mucus and elevated sweat electrolytes. It is characterised by progressive respiratory and gastrointestinal problems, including liver disease and diabetes mellitus, and is associated with impaired fertility in both sexes but particularly in males.

Respiratory problems

In the normal respiratory tract, bacterial colonisation, infection and lung damage are prevented by the combined efforts of the endogenous mucus layer and the cilia lining the airways, which trap and expel inhaled particles. In CF, the infected mucus secretions are highly viscous; this results in poor airway clearance and provides an ideal habitat for bacterial colonisation and subsequent lung infection. The principal pathogens include *Haemophilus influenzae* and *Staphylococcus aureus*, and in later years, *Pseudomonas aeruginosa* and *Burkholderia cepacia*.[10] Tissue damage which is secondary to recurrent lung infections is mediated by a severe inflammatory response. Chronic lung infections and inflammation eventually result in destruction of the bronchial passages and, together with plugging of the airways, leads to respiratory failure.

Studies on foetal epithelial lung tissue have shown that there is consistent expression of CFTR mRNA throughout foetal development.[11,12] Despite this, however, most neonates with CF have virtually normal lungs.[13] This implies either that abnormal levels of the CFTR protein in the lungs of those with CF do not have a major effect on the development of the foetal respiratory tissues or that any defect is compensated for while *in utero*.[14–16] Tissue damage may only ensue with the biochemical switch from chloride secretion to sodium absorption which occurs when the lungs become filled with air and are subjected to airborne pathogens.[12]

Although neonates with CF appear to have normal and uninfected lungs, bronchoscopic studies have shown that bacterial infections associated with a marked inflammatory response frequently occur in untreated infants under 3 months of age.[10,17] Occasionally, these inflammatory responses have been seen in the absence of positive bacterial cultures, inferring the possible involvement of other intrinsic factors in the inflammatory response such as the biochemical defect itself.[10] Furthermore, histopathological changes have been observed in foetal lung (and other) tissue in the second trimester, which supports the notion that some primary lung changes may precede postnatal lung infection.[18] This may be of relevance for presymptomatic treatment in CF patients.

The basic objective of the inflammatory reaction, to effectively eradicate foreign material from the lungs, is not achieved in patients with CF. Instead, an overwhelming response brings about large quantities of highly viscous mucus which, in combination with poor mucociliary clearance[19] and possible increased bacterial adherence,[20] further enhances colony establishment. High concentrations of DNA released from disintegrating neutrophils and glycoproteins contribute to increased mucus viscosity.[21–24] Tissue damage also occurs in the presence of chronic infection due to high concentrations of neutrophil elastase[25–27]

and other bacterial proteinases which, in turn, stimulate further mucus production.[28,29]

Bronchial mucus hypersecretion and stasis are early changes. Early bacterial infection results in chronic bronchial and lung inflammation. Chronic bronchitis and bronchiolitis eventually progress to widespread obstruction and bronchiectasis. Airway obstruction, caused by abnormal secretion, inflammatory exudate and epithelial debris, cause further hyperinflation or collapse. Chronic hypoxia is a major factor causing pulmonary hypertension and cor pulmonale. Pneumothorax and haemoptysis are common complications in those with advanced disease.

Gastrointestinal problems

Severe gastrointestinal disease is the initial pathological feature in CF. Studies of foetal tissue support the importance of CFTR mRNA during foetal development in the pancreas, liver and small intestine. The CFTR protein is present throughout the epithelium of the gastrointestinal tract.[30]

Approximately 10–18% of neonates present with intestinal obstruction caused by meconium ileus.[31,32] Neonatal complications of meconium ileus, which affect a large proportion of CF infants, include peritonitis, volvulus and atresia. During the first month of life, mortality is higher in infants with meconium ileus compared with those without it. Thereafter the clinical course is similar in both groups,[33] although those with meconium ileus may have a higher risk of developing liver complications.[34]

Oesophageal problems include frequent gastro-oesophageal reflux, peptic oesophagitis or oesophageal varices. Approximately 25% of CF patients aged 5 years or more have gastro-oesophageal reflux.[35] In the small intestine, increased expression of viscous mucin leads to obstruction of the goblet cells, Brunner cells and even the lumen. Clinical problems include rectal prolapse, distal intestinal obstruction syndrome, intussusception and volvulus. More recently, fibrosing colonopathy leading to colonic strictures has been observed as a rare complication of high lipase pancreatic enzyme treatment.

With more CF individuals now surviving into adulthood the frequency of cancer is also increasing. Although the overall risk of all types of cancer combined is similar to that in the general population, CF patients are approximately six times more likely to develop digestive tract cancers.[36,37]

Many of the clinical manifestations related to the gastrointestinal tract are due to malabsorption, the main cause of which is insufficient pancreatic enzyme and bicarbonate activity. Pancreatic dysfunction is apparent before birth as is indicated by histological and biochemical evidence. Abnormal levels of immunoreactive trypsinogen (IRT) and chymotrypsin are found in the amniotic fluid.[38] This is thought to be caused by either inspissated pancreatic fluid or increased duodenal mucus obstructing the passage of pancreatic enzymes through the gut.[39] At birth, pancreatic dysfunction and, thus, CF are indicated by increased levels of IRT in the blood; leakage of pancreatic enzymes into the circulation may be the result of blocked fibrous pancreatic ducts. In the absence of pancreatic enzyme secretion, protein and fat maldigestion occurs, leading to bulky, frequent malodorous stools with an abnormally high fat (steatorrhoea) and nutrient content.[40]

Approximately 62% of neonates diagnosed with CF through neonatal screening are already pancreatic insufficient (PI), insofar as they require dietary pancreatic enzyme supplements;[41] the remainder are pancreatic sufficient (PS). By 6 months the frequency of PI has risen to 79% and at 12 months only 8% remain pancreatic sufficient.[42] Most of those who are likely to become PI will do so before the age of 10 years.[43] Although the prevalence of PI in CF adults has not been clearly determined, a cross-sectional study suggests that the proportion exceeds 85%[44] and is probably closer to 95%. Further complications of pancreatic dysfunction include impaired glucose tolerance leading to diabetes mellitus. As with digestive tract cancer, the prevalence of diabetes is increasing because of improved survival of CF patients. In a recent study performed over a 5-year period, the average annual incidence was 3.8% and prevalence increased from 11% to 24% overall; in those aged over 20 years, the annual incidence was 9.3% and the prevalence rose from 25% to 53%.[45] In the longer term, diabetes is associated with microvascular complications such as retinopathy, nephropathy and neuropathy; reports of these conditions among CF patients with diabetes are also increasing.

CF-related liver disease is the second most common cause of mortality after lung disease. The incidence increases with age from about 0.3% before the age of 5 years to a peak of 8.7% in those aged 16–20 years.[46] The exact pathogenesis of the disease is unknown; however, recent evidence suggests that defective CFTR chloride channel function may cause abnormal bilary secretions resulting in mucus plugging of intrahepatic bile ducts.[47] This,

in combination with other factors such as increased levels of toxic bile acids and inflammatory cytokines, has been implicated in the development of portal hypertension and associated cirrhosis.[48,49]

Malnutrition

In some studies, the birth weight of CF infants has been reported as being below normal[50–52] but other studies have not found this.[53,54] In CF cases with meconium ileus, it has been suggested that birth weight is normal because of the high intestinal load;[55] however, this is based on a small number of cases and requires confirmation.

Because of the gastrointestinal problems associated with CF, affected individuals have a tendency to be undernourished. In 1993 in the USA, a survey of malnutrition was undertaken in more than 13,000 children with CF.[56] A large proportion had heights or weights below the normal 5th percentile for their age based on national statistics. The proportions were 47% for infants, 22% for those aged 1–10 years and 34% for 11–18 year-olds. A similar extent of malnutrition was seen for both height and weight considered separately.

Fertility

Over 95% of males with CF are infertile due to either absence or atrophy of the vas deferens, epididymis, and seminal vesicles.[57] Studies on foetal epididymal tissue have shown that CFTR mRNA is present at all stages of foetal development.[11] Abnormal secretions may affect the epididymis in one of two ways: either by preventing formation or by degeneration as a result of obstruction.[11,58] In addition to its developmental role, CFTR protein may also be independently involved in spermatogenesis. Histological examination of testicular tissues from infertile CF males has shown that spermatogenesis may be either normal or severely decreased with abnormal or normal sperm parameters.[59,60]

Females with CF do not have the equivalent tubal agenesis or atrophy. However, there can be impaired fertility caused by the presence of thick mucus in the genital tract.[57]

Age at diagnosis

There is substantial variability in the age at onset of symptoms in CF. In some patients symptoms are present at birth, while in others it may be months or even years before signs of CF become apparent. The most complete information on the distribution of age at diagnosis comes from the Canadian Patient Data Registry, 1970–89.[61] Since 1970, the 33 specialist CF centres throughout Canada have reported all cases to the Registry. By the end of 1989, a total of 3748 patients had been registered. The number diagnosed in the first year of life was 2115 (56%); in the second, 447 (12%) and in the third, 277 (9%). In the USA, the CF Foundation Registry includes almost 21,000 patients or 91% of all those under treatment in the country. In 1986 the reported median age at diagnosis was 8 months and by 1996 this had been reduced to 6 months.[62]

Survival

Time trends

Long-term prognosis for patients with CF has improved markedly over the years. Improved survival has been reported from Sweden,[63] Australia,[64] Japan,[65] Canada,[61] Denmark[66] and the USA.[67,68] There have been similar reports from different parts of the UK[69,70] but the best estimate is from the national UK CF Survey.[71–73] For example, over one-third of UK infants with CF born in 1968–70 died before the age of 10 years, whereas for those born in 1986–88 the proportion was under 5%.[73]

The improved mortality has been attributed to a number of factors,[74,75] including:

- improved treatment regimes such as daily physiotherapy
- aggressive anti-pseudomonal antibiotics
- more effective enzyme supplements
- better management of meconium ileus
- earlier diagnosis
- provision of centralised special CF units.

Although the first year of life remains a period with a particularly high mortality rate, it has also seen the greatest improvement over time, due to the improved management of meconium ileus. In the past, the prognosis for infants with meconium ileus was very poor. Today, excluding those who do not survive the first 30 days of life, the long-term clinical course of these patients may be similar to those without it.[33]

As a result of these developments, infants born with CF today have a reasonable life expectancy. Thus, whereas in the 1960s median survival was under 15 years, by the 1970s it had increased to 10–25 years, and regression analysis predicts that median survival may have increased to over 40 years for children born in the 1990s.[70] These optimistic predictions, which are based on

statistical modelling techniques derived from mortality trends in young CF patients, may not be applicable to older patients given the accumulative long-term effect of the disease.

Prognostic indicators

There are several predictors of mortality, including: respiratory function, mode of presentation, nutritional status, gender, height, weight, blood gas levels, and chest condition after first course of treatment following diagnosis. These are interrelated so that, for example, malnutrition renders the patient susceptible to infection which leads to impaired lung function.

The presence of chronic *P. aeruginosa* or *B. cepacia* infection is a major determinant of prognosis. The life expectancy of patients with *B. cepacia* is about half that of uninfected patients (SC FitzSimmons; personal communication, 1998)*. Simply measuring the forced expiratory volume in 1 second (FEV_1) is by itself a very strong prognostic indicator. In one Canadian study among adults, an FEV_1 value under 30% of that expected for their age was associated with a 60% mortality rate over the next 2 years.[76] By comparison, those with an FEV_1 value over 50% had a mortality rate of under 5%.

Finally, in several studies a sex difference in survival has been observed, with males living longer than females.[68,70–73,76–78]

Residence and occupation are factors which have been shown to correlate with mortality. National statistics on mortality rates for CF in 1959–86 were analysed in relation to these factors.[78] The rates according to Regional Health Authorities varied by two- to three-fold. The highest mortality rate was seen in manual occupations; however, most death certificates gave the occupation of the parent or spouse. When the individual's own occupation was given the reverse was true, presumably reflecting a tendency for healthier patients with CF to take on manual jobs. The authors of this study raise the possibility that the observed social class effect is confounded by a tendency for those in manual occupations not to attend specialist centres. This is not seen in Yorkshire Regional CF Centre where the social class of attendees is similar to that of the general population in the region.[79]

Quality of life

A large survey was undertaken in 1990 to evaluate the social characteristics of UK adults with CF.[80] A questionnaire was sent to all 1052 members of the Association of CF Adults and 866 (82%) responded. A total of 285 (33%) were cohabiting compared with 61% in the general population. Educational achievement was not impaired: while a higher than average number left school without any qualifications, a greater than average proportion completed higher education. Most were in paid employment although the proportion was less than in the general population (54% compared with 69%).

A study has attempted to formally compare quality of life in those with and without CF.[81] A questionnaire was developed from the Nottingham Health Profile supplemented by six questions specifically relating to CF. Among 240 adults with CF, quality of life was, on average, comparable with that for minor non-acute medical conditions.

* Affiliations of authors of personal communications are given in the Acknowledgements (page 79).

Chapter 4
Treatment advances

The purpose of this chapter is to provide a brief outline of emerging and potential new treatments. Many established therapies for CF such as physiotherapy and antibiotic treatment have been introduced in the absence of well-designed multicentre randomised trials. Although it can be argued that proving effectiveness by such rigorous means is not always necessary, there is now mounting pressure to do so. Indeed, some of these treatments have now been formally validated by randomised controlled trials (RCTs). As the CF population continues to rise, so do the numbers reaching adulthood. This brings new challenges in terms of types of treatments and additional health costs. CF patients already undergo time-consuming daily treatment regimes and increasing this burden with new therapies in the absence of full evaluation may lead to non-adherence with established treatments. For these reasons, new treatments must be shown to contribute to the patient's quality of life by reducing morbidity and mortality.

The limited scope of the current review compared with the enormity of the literature does not permit a systematic review of every treatment. Such work is being carried out by other research groups in cooperation with the CF Cochrane Group. They are assessing the quality of each study by critically assessing the design, statistical analysis and outcomes measured. By mid-1997 two treatments had undergone systematic review and four treatment protocols had been registered. In addition, the NHS Health Technology Assessment programme is currently considering funding reviews of potential therapies.

Emerging therapies

Many of the new treatments emerging for CF are concerned with reducing the considerable morbidity and mortality associated with infection of the respiratory tract. Several approaches have been used, which aim to treat different stages of the disease by preventing and eradicating early infection and controlling chronic infection. These include correcting the ion transport system in lung epithelial cells and using post-infection anti-inflammatory drugs. However, most interest has been generated in the potential of gene therapy as a cure for respiratory symptoms.

Anti-inflammatory drugs

Recombinant human deoxyribonuclease

As described in chapter 3, infection and the resulting inflammatory response leads to high concentrations of neutrophil DNA which increase mucus viscosity. In addition to causing local tissue damage, this creates favourable conditions for further bacterial growth. In 1990, Shak and colleagues[82] produced recombinant human deoxyribonuclease (rhDNase) which was shown *in vitro* to reduce mucus viscosity. Clinical trials have shown that the use of rhDNase produces a significant increase in lung function within days as measured by both FEV_1 and forced vital capacity (FVC).[83–90] The results of two studies,[87–89] which were 6 months or more in duration, are shown in *Table 1*. In one of these,[87] a slight decrease in the frequency of exacerbations and number of hospitalisations was also observed. When treatment withdrawal was examined, the improvement in lung function was found not to be sustained.[89]

In the longest trial reported to date, treatment was studied for only 2 years and, consequently, the long-term effects of rhDNase on morbidity and mortality are not yet known. Based on results to date, both the US CF Foundation and the UK CF Trust have endorsed rhDNase treatment for patients with lower respiratory tract infections. Given the annual cost of the treatment, about £7500 per patient,[91] centres have agreed to monitor the progress of their patients and discontinue treatment when clinical benefit cannot be demonstrated.

Steroidal anti-inflammatory drugs

Steroidal anti-inflammatory drugs aim to reduce inflammation by blocking the production of cytokines which attract neutrophils into the lung tissue. They therefore act centrally to control the immune response. Although clinical trials report improved FEV_1 and FVC, together with reduced levels of inflammatory markers, using oral prednisolone,[92–94] serious side-effects such as glucose intolerance, cataract formation and growth retardation preclude its long-term use.[95,96] Inhaled corticosteroids, which are widely used to treat inflammatory responses, produce similar benefits to oral corticosteroids but with reduced side-effects.[97,98]

TABLE 1 Clinical trials of rhDNAse lasting 6 months or more: results from two studies

Study	Type	Age (years)	Number of doses[a] per day	Duration (months)	Number of patients	Improvements[b] (%)	
						FEV_1	FVC
UK[88,89]	Open label	16–55	1	18	52	8.0	1.2
			2	6	59	6.2	7.2
USA[87]	RCT	5 or more	1	6	322	5.8	3.8
			2	6	321	5.6	3.0

[a] A single dose is 2.5 mg
[b] Compared with baseline measurements in UK study and with placebo group in US study

Non-steroidal anti-inflammatory drugs

Ibuprofen is known to decrease inflammatory reactions by inhibiting neutrophil aggregation.[99] In a recent 4-year, double-blind RCT, daily treatment with ibuprofen was shown to reduce the rate of decline in lung function in younger, relatively mildly affected patients by 40% compared with a placebo group.[100] Although side-effects such as epistaxis and conjunctivitis are rare, long-term use of ibuprofen may be associated with nephrotoxicity.[101,102]

Amiloride

Amiloride is a diuretic agent which has been shown in clinical trials to reduce the viscosity of mucus in the lungs and to improve cough clearance without any known side-effects.[103–105] It is believed to work by blocking the sodium channel, thereby increasing the electrolyte content in the airway lumen which leads to rehydration of the airway secretions.[106] In addition, amiloride has specific bactericidal activity which may prove beneficial in treating or preventing lung infection. In trials to date, the greatest benefit has been seen in younger patients and those without a previous history of pneumonia. However, although one trial reported a slowing in the rate of FVC decline, FEV_1 was unaffected.[105] In addition, subsequent trials have failed to show a clinically significant benefit.

Chloride secretagoues

In the presence of amiloride, the triphosphate nucleotides ATP and UTP (uridine triphosphate) have been shown to increase chloride secretion across nasal epithelia.[107] There is potential for treatment using aerosolised UTP. The efficacy and safety of this method of treatment is currently under investigation.

Other pharmacological approaches currently being investigated in Phase 1 clinical trials include aminoglycosides, 8-cyclophenyl-1,3-dipropylxanthine (CPX) and phenyl butyrate. These methods are very precise, acting directly on the CFTR protein in a mutation-specific manner.

Gene replacement therapy

Gene replacement therapy involves the introduction of a normal copy of a gene into the cells which carry two defective copies, thereby restoring normal gene function. A number of potential methods for transferring genes into human cells have been studied; these included direct transfection, virus-mediated transduction and receptor-mediated transfer.

Viral systems

Some of the most studied methods are based on a virus-mediated system, such as retroviruses,[108] adenoviruses[109,110] and adeno-associated viruses.[111,112] Viral vectors are used to carry recombinant DNA, comprising viral and CFTR cDNA, to respiratory epithelia where they become incorporated into the host cells. In order to maximise the duration of expression so that the number of repeat doses can be reduced, the recombinant DNA should be integrated into the genome. Expression from episomal DNA will be short-lived because of the dilution effect during cell replication.

All of these methods have limitations and safety considerations. For example, direct infection of the respiratory tract with retroviruses is difficult to achieve as CFTR cDNA expression requires actively dividing cells; relatively few epithelial cells in the lungs are replicating ones. In addition, there is the risk of insertional mutagenesis. Although adenoviruses are able to achieve expression in the absence of replication,[113,114] their immunogenic properties make them unlikely candidates for gene therapy. The adeno-associated virus (AVV) is incorporated into the host genome and thus the

***TABLE 2** Clinical trials of liposome-mediated gene transfer: results from three studies*

Study	Method	Age of patients (years)	Number	Improvements	
				Potential difference	SPQ
London[115]	Aerosol	> 18	9	2 (22%)	N/A
Edinburgh[116]	Aerosol	> 16	8	0	0
Oxford[117]	Instillation	> 16	8	2 (25%)	5 (63%)
All			25	4 (16%)	5 (31%)

SPQ, Halide sensitive fluorophore 6-methoxy-N-(3-sulphopropyl)-quinolium
N/A, Not applicable

duration of expression may be longer. However, with recombinant AVV this does not occur as the vector exists episomally. Despite these problems, clinical trials using nasal mucosa are currently under way.

Non-viral systems

Liposomes are lipid particles which can be combined with CFTR DNA and fused with cell membranes. The advantages of this method over viral systems include safety, ease of transfer and a reduced risk of immune response which makes repeated administration more feasible.

The results of double-blind, randomised, placebo-controlled trials on the efficacy and safety of liposome-mediated gene delivery on nasal epithelium have recently been reported by three UK groups.[115–117] From *Table 2* it can be seen that there was restoration of chloride function in 16% (4:25) of patients for up to 15 days after treatment. Moreover, unlike the adenovirus trials, there was no evidence of increased inflammation between the active and placebo treatment groups. Although the studies demonstrated an effect on chloride function, there was no correction of sodium level to within the normal range. In five patients the presence of functional CFTR cDNA in the nasal epithelium was demonstrated by fluorescent techniques (SPQ). Several factors were suggested to have affected the results including interference from tissue sampling, the presence of upper respiratory tract infections, possible uneven gene transfer, liposome formulation and the length of tissue contact time during administration. Other difficulties encountered with this method relate to the inefficiency of gene transfer and poor level of expression. Even with these problems, however, a trial of liposome-mediated gene transfer using aerosol administration in the lungs has already been completed.

Summary of gene therapy trials

With all methods of gene therapy there are a number of fundamental questions which will need to be answered in coming years. These include the consequences of unregulated CFTR expression, the minimum amount of CFTR cDNA required for normal cell function and the long-term effects of repeated administration. In addition, many clinical trials to date have been based on nasal tissue. Trials on the lower respiratory tract present new challenges, not least of which is the requirement for a reliable and easily administered method of measuring the results.

Chapter 5

Genetics

CF is inherited as a simple Mendelian autosomal recessive condition. Non-carriers have two normal CFTR genes, while asymptomatic carriers (heterozygotes) possess one normal copy and one mutated copy of the gene. Those individuals affected by CF have mutations in both copies of the CFTR gene. The risk of having affected children is dependent on the carrier status of the parents. In couples where both partners are carriers, the risk is one in four for every child. If they have two pregnancies there is a one in two chance $(1-(3/4)^2)$ that at least one child will have CF; in three pregnancies, the risk is two in three $(1-(3/4)^3)$.

CFTR gene

Family studies performed in the 1940s provided the first evidence that CF was a genetic disorder.[118] Over 40 years later the CFTR gene responsible for the disease was cloned; situated on the long arm of chromosome 7 at q31, it spans over 250 kb and comprises 27 exons. The mRNA transcript is also very large, being about 5 kb in length.

CFTR protein

The protein coded for by the CFTR gene is manufactured in the nucleus and undergoes a complex series of processes before finally being sited on the cell membrane.[119] This includes a two-stage glycosylation process in the endoplasmic reticulum and golgi apparatus followed by folding. Comprising 1480 amino acids residues, it functions as a cyclic AMP-regulated chloride channel.[120–122]

CFTR protein consists of five domains: two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBDs) and a regulatory domain (RD). The latter three domains are situated in the cytoplasm while the MSDs, which form the main pore of the chloride channel; span the lipid bilayer (*Figure 1*).[123] Both the RD and the NBDs are involved in regulating channel activity. The first stage is phosphorylation of the RD by cyclic AMP-dependent protein kinase.[119,124–126] The NBDs then bind and hydrolyse ATP, thus providing the energy required to enable the channel to open and close.[127] Absence of phosphate from the RD renders the channel impermeable to chloride ions.

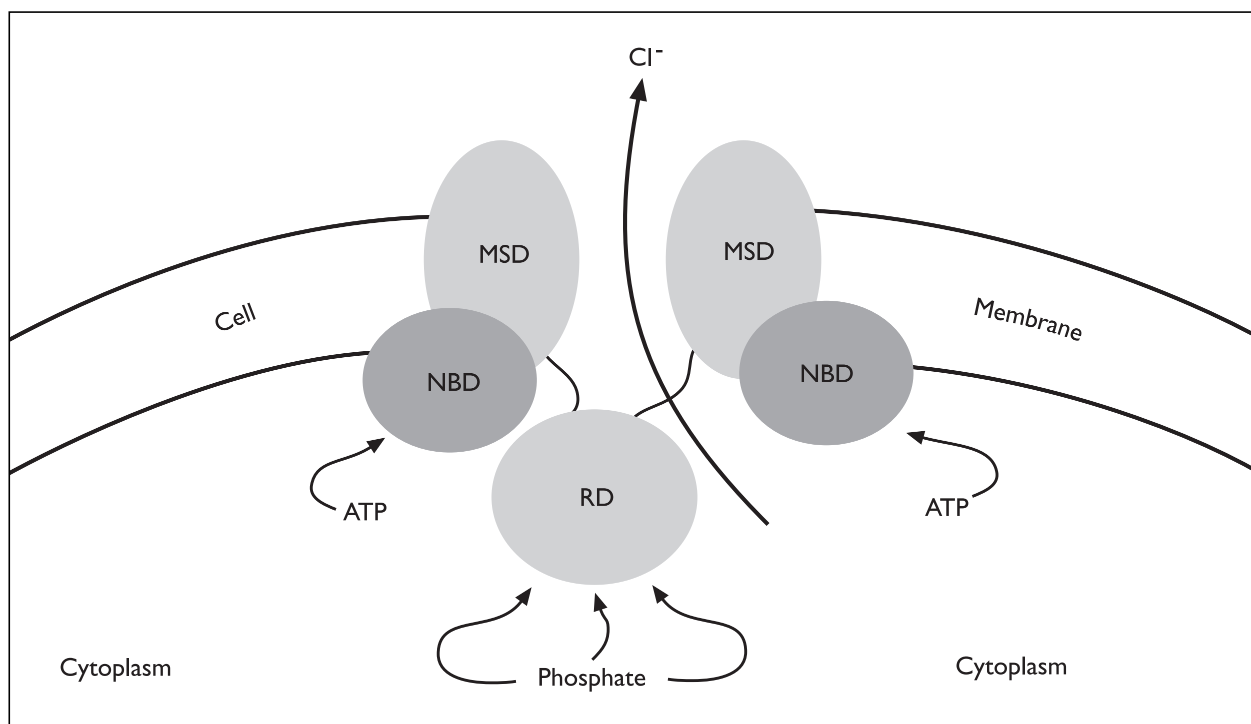

FIGURE 1 *CFTR protein (MSD, membrane-spanning domain; NBD, nucleotide-binding domain; RD, regulatory domain) (adapted from Welsh & Smith, 1993[123])*

***TABLE 3** CFTR mutations commonly referred to in this report*[137,138]

Mutation	Location	Type	Amino acid change
ΔF508	exon 10	deletion	loss of phenylalanine
G542X	exon 11	nonsense	glycine → stop
G551D	exon 11	missense	glycine → aspartic acid
621+1G→T	intron 4	splice	5' splice signal
N1303K	exon 21	missense	asparagine → lysine
W1282X	exon 20	nonsense	tryptophan → stop
R553X	exon 11	nonsense	arginine → stop
R117H	exon 4	missense	arginine → histidine
R347H	exon 7	missense	arginine → proline
A445E	exon 9	missense	alanine → glutamic acid
R334W	exon 7	missense	arginine → tryptophan
3849+10kbC→T	intron 19	splice	aberrant splicing
1717–1G→A	intron 10	splice	3' splice signal
D1270N	exon 20	missense	aspartic acid → asparagine

→, Changed to

The CFTR protein has been identified in the epithelia lining the airways,[128] sweat glands,[9] pancreas,[129] intestine, liver,[130] testes[131] and choriod plexus.[132,133]

Protein function

In addition to its primary role in chloride conductance, the CFTR protein is thought to have other physiological functions such as sodium absorption.[134] In the lungs, the sol phase and mucus hydration are maintained by both chloride secretion and sodium absorption. Normally the osmotic gradient created by the efflux of chloride ions enables water to flow on to the mucosal surface. In CF patients, the electrochemical balance is disrupted because of mutations in the CFTR gene, which cause reduced chloride secretion and increased sodium absorption.[106,135] As direct consequence the airway surface becomes dehydrated, leading to the progressive respiratory problems outlined in chapter 3.

CFTR mutations

Type of mutation

To date over 800 mutations in the CFTR gene have been identified, although not all have been found to be disease causing.[136] Few of them have been reported on more than 100 chromosomes and many are extremely rare, some reported only in a single case. In addition, at least 100 neutral polymorphic changes have been identified. A constantly updated database of all mutations can be found on the Internet.[136] All of the common mutations referred to by name in this report are listed in *Table 3*, together with their location, type and the specific effect on the amino acid sequence. In general, DNA mutations can be classified as: point mutations; small deletions or insertions (< 100 bp); large deletions or insertions (up to several thousand bp); gross rearrangements such as chromosome translocations; and allele expansions. They can be further classified according to their effect on the transcript: nonsense, missense, frameshift and splice.

The deletion of the three nucleotides encoding phenylalanine residue 508 (ΔF508) was the first CFTR mutation to be described[9] and is by far the most common mutation worldwide. About half of the CFTR mutations are missense, the bulk of the remainder being nonsense, frameshift and splice site mutations.[137] Large structural rearrangements and promoter mutations are very rare, and disruption of the gene by chromosomal translocation has never been observed. Intronic mutations which activate a cryptic splice site and lead to the incorporation of cryptic exons into the mRNA have also been reported.[139] They are described in more detail below. *De novo* mutations in CFTR have been reported but are surprisingly rare, given the large size of the gene.[140] Complex alleles, in which more than one mutation is present, have also been observed.[141,142]

Specific mutations vary in frequency among carriers in different ethnic groups. For example,

***TABLE 4** CFTR mutations: classification according to mechanisms causing protein dysfunction*[123,144]

Class	Mechanism	Mutation
I	Production	G542X
		ΔF508
		621+G→T
II	Processing	ΔF508
		N1303K
III	Regulation	G551D
IV	Conduction	R117H
		R334W
		R347H
V	Transcription	A445E
		3849+10kbC→T

in Northern European communities ΔF508 accounts for about three-quarters of all disease-causing mutations. However, less than one-third of Ashkenazi Jewish carriers of CF have the ΔF508 mutation, whereas half have the W1282X mutation (rarely found in non-Jewish carriers). Other populations are not as homogeneous so that each mutation may only account for a small percentage of heterozygotes.

Effect on CFTR protein

Mutations do not seem to be evenly distributed throughout the CFTR gene.[143] There is a tendency for them to cluster into hot spots which seem to correspond to the MSD and NBD of the protein, perhaps reflecting the functional importance of these domains.

There are five possible mechanisms by which mutations cause loss of CFTR channel function. They can disturb protein synthesis by altering production, processing, regulation, conduction and transcription. The standard classification is shown in *Table 4*, together with examples of how the common mutations are classified. Any specific mutation may cause more than one type of dysfunction; thus ΔF508 affects both processing[145] and regulation.[146]

Class I mutations which affect protein production result in little or no full-length protein. In this case the mutation may produce either a premature stop signal and no protein, an unstable mRNA with no detectable protein, or an unstable protein which may be degraded rapidly or be non-functional. Intracellular trafficking of the CFTR protein may be disrupted by Class II mutations. Here glycosylation fails to occur within the endoplasmic reticulum and, as a result, the product is either degraded or mislocalised.[147,148] Thus the protein is either missing or present in reduced quantities at the cell surface.

Other mutations in the CFTR gene may only affect the protein once it has become lodged in the membrane. Class III mutations, which occur in the NBDs, lead to decreased chloride channel activity by reducing either NBD activity or its affinity for ATP. Class IV mutations are relatively rare and occur in the MSDs of the CFTR gene. In addition to altering the conductance of the chloride channel, they may also reduce the length of time that it is open.[149] Class V mutations lead to low levels of mRNA either by producing an unstable protein or due to alternative splicing of pre-mRNA transcripts.

Nonsense and frameshift mutations are predicted to encode null alleles, as are many splice site mutations. However, some splice site mutations result in the production of a mixture of normal and mutant mRNA. Transcripts lacking certain exons are detected *in vivo* in normal individuals at levels of up to 92% of the total transcript. These alternatively spliced isoforms appear to have no biological role,[150] despite the resulting reduced level of synthesis of normal CFTR mRNA and protein.

Intronic variants

Within intron 8, the 3' splice site for exon 9 has a variable number of thymidine nucleotides in the polypyrimidine tract. Alleles with five, seven or nine thymidines have been observed, 7T being the most common allele in the general population.[151] The length of the poly-T tract can influence splicing of exon 9. In particular, the 5T allele is associated with higher levels of exon 9 skipping and the resultant production of a reduced quantity of functional transcript.[152] The ΔF508 mutation is exclusively associated with the 9T allele.[152,153] Although the three intronic variants are, of themselves, benign findings, in combination with a CF mutation they might result in a mild form of the disease. For example, when R117H is combined with 5T the level of CFTR mRNA is more than halved.[154]

Phenotype–genotype relationship

Kerem and colleagues[7] postulated that the severity of phenotype in CF may be related to presence of 'mild' or 'severe' mutations. It was also predicted that the severe mutation would only produce a severe phenotype if it was either present in its

homozygous state or in combination with another severe mutation.[155]

The possibility that the clinical course of CF might be predicted by genotyping has prompted numerous investigations. Many mutations have already been categorised according to clinical severity. In their homozygous state or in combination with each other, ΔF508, W1282X, G542X, N1303K have all been labelled severe mutations. However, R117H, R347H, R334H, A455E, R334W and 3849+10kbC→T, when found in their homozygous state, in combination with each other, or even together with a severe mutation, are thought to confer a milder phenotype. The genetic basis for this may be related to the mechanism by which they cause CFTR dysfunction (see *Table 4*).

When making phenotype–genotype comparisons a number of factors need to be taken into consideration. Thus pulmonary function is confounded by age[156] and also possibly by gender, although this is uncertain.[66,68,72,78,157] However, controlling for age can be problematic. For example, including only young patients will limit the amount of information on mortality[158] and may also bias against patients with a milder phenotype who are not diagnosed until they are older. Conversely, an all-adult study group will selectively omit deceased patients for whom mutation analysis is not possible.[159] Also presenting results in broad age bands can make subtle differences less obvious.[160] Similarly, phenotype–genotype comparisons can be confounded by geographical variability in prognosis related to treatment differences. Although pooling results in multi-national or cross-sectional studies may overcome this, it may also obscure specific genetic and environmental factors that are present within an ethnic isolate.[160]

Pancreatic function

The most discriminatory clinical feature of phenotype–genotype studies is pancreatic function.[161] Corey and colleagues[162] first reported on the high degree of familial concordance in function between siblings and concluded that PI and PS represent different phenotypes. A number of studies have since observed a substantially lower frequency of PI in patients with mild mutations such as R117H, A455E, R334W or 3489+10kbC→T compared with those with severe mutations who are homozygous for ΔF508 or W1282X. The results from seven studies are summarised in *Table 5*: overall the proportions with PI are 32% and 98% in those with mild and severe mutations, respectively, a statistically significant difference ($p < 0.0001$). The association between pancreatic function and genotype is not absolute, however, as can be seen from the table. Even homozygous patients with severe mutations can be PS,[169] and PI patients can be have mild mutations on both chromosomes.[165,170]

Lung function

The comparison of CF patients having one mild mutation with those having two severe mutations have, in general, produced inconclusive results in

***TABLE 5** Pancreatic insufficiency in CF according to mutation type (mild or severe): results from seven studies*

Study	Genotype	Mild		Severe[a]	
		Number of patients	PI (%)	Number of patients	PI (%)
Canada I[161]	R117H/*	11	0	279	277 (99)
Cystic Fibrosis Genotype–Phenotype Consortium[163 b]	R117H/ΔF508	23	3 (13)	23	22 (96)
The Netherlands[164 b]	A455E/*	33	7 (21)	33	31 (94)
Spain I[165]	R334W/*	15	9 (60)	82	79 (98)
Spain II[166]	R334W/*	10	4 (33)	28	28 (100)
Israel[167]	3849+10kb C→T/*	15	5 (33)	57	57 (100)
Canada II[168 b]	A455E/*	22	13 (59)	22	22 (100)
All		**129**	**28 (32)**	**527**	**516 (98)**

[a] *Includes (ΔF508 and W1282X either in their homozygous or in combination with each other)*
[b] *Age matched*
* *Another mutation*

***TABLE 6** Lung function in CF according to mutation type (mild or severe): results from nine studies*

Study	Number of patients (mean age)		Statistical significance:[c] mild versus severe		
	Mild[a]	Severe[b]	*P. aeruginosa*	FEV_1	FVC
Denmark[159]	46 (17)	172 (14)	NS	NS	NS
USA[171]	24 (17)	27 (24)	*	*	NS
Israel[167]	15 (20)	57 (11)	*	NS	NS
Italy[158]	85 (9)	23 (9)	0.005	NS	NS
Cystic Fibrosis Genotype–Phenotype Consortium[163]	23 (23)	23 (23)	*	NS	*
Spain I[165]	15 (12)	82 (8)	*	NS	NS
The Netherlands[164]	33 (23)	33 (23)	0.02	0.002	0.04
Spain II[166]	12 (20)	28 (10)	0.0036	NS	NS
Canada[168]	22 (18)	22 (17)	NS	0.003	0.02

NS, Not statistically significant

** Not compared*

[a] *At least one mild mutation (series included R334W, A455E, 3489+1C→T) except for USA and Italy, where a single ΔF508 with an unknown mutation was taken to be mild*

[b] *Two severe mutations (series included ΔF508 and W1282X)*

[c] *For* P. aeruginosa *either the proportion infected or age at onset; for lung function either mean or proportion < 70% predicted (Denmark)*

terms of bacterial colonisation and lung function (see *Table 6*). The most clear-cut findings were observed in an age- and sex-matched study carried out in The Netherlands.[164] In addition, the number of patients colonised with *P. aerugnosa* was significantly greater in ΔF508 homozygotes than compound heterozygotes ($p < 0.02$).

As pancreatic insufficiency is associated with more severe lung disease,[169] a clearer correlation between genotype and respiratory phenotype might be expected for all the studies. One explanation is poor study design. Three studies did not match for age and thus the group with mild disease were older than the group with severe disease.[165–167] Although older CF patients would be expected to have poorer lung function, the fact that this was not observed may in itself suggest a relationship between genotype and respiratory phenotype. Furthermore, in two studies the presence of a mild mutation was not determined directly but inferred by the absence of homozygosity for ΔF508.[158,171]

Another explanation for the lack of clear correlation in all studies is the effect of extrinsic sources of variability. Among patients with the same genotype there are large variations in lung.[163,171–174] This may be due principally to the responsiveness of patients to different treatments, which will invariably mask some clinical features. Furthermore, the lungs, more than other tissues, are subject to highly variable environmental factors including nutrition, passive smoking and cross-infection. In addition, there may be biochemical factors other than those relating to chloride transport such as sodium absorption.

Liver disease

Although familial concordance for clinical liver disease has been observed,[175,176] genotype studies have failed to establish any correlation with specific mutations.[159,176,177]

Infertility

Although the majority of affected males are infertile, congenital bilateral absence of the vas deferens (CBAVD) may be the only or principal clinical feature in some cases. However, the relative frequency of these males compared with the more typical CF population is not known.

The frequency of CFTR mutations among males presenting with CBAVD alone have been reported in 12 studies; in most of these all exons were scanned. The results are summarised in *Table 7*: overall, 17% were found to carry two disease-causing mutations and 47% only had one mutation. Although a proportion of these patients can be shown to have two CFTR mutations, they are in the minority and earlier suggestions that

TABLE 7 *CFTR mutations in males with CBAVD: results from 12 studies*

Study	Number of males	Mutations tested	Mutations (%)		
			None	One	Two
England[178]	26	All exons	17 (65)	7 (27)	2 (8)
France I[179]	23	All exons	8 (35)	11 (48)	4 (17)
USA I[180]	49	All exons	9 (18)	31 (63)	9 (18)
USA II[181]	7	12	2 (29)	5 (71)	0 (0)
France II[142]	8	All exons	2 (25)	4 (50)	2 (25)
Israel[182]	36	17	18 (50)	13 (36)	5 (14)
Spain[183]	30	Exon 18	8 (27)	19 (63)	3 (10)
Canada[184]	25	6	16 (64)	5 (20)	4 (16)
Italy[185]	67	All exons	23 (24)	28 (42)	16 (24)
Europe/USA[139]	102	All exons	29 (28)	54 (52)	19 (19)
France III[186]	38	Exon 11	17 (45)	15 (39)	6 (16)
Scotland[187]	30	14	9 (30)	15 (50)	6 (20)
All	**441**		**158 (36)**	**207 (47)**	**76 (17)**

isolated CBAVD was a genital form of CF are unfounded.[188–190]

The distribution of CFTR mutations observed in CBAVD males with two mutations differs from that seen among CF patients in general. In *Table 8* the distribution between the 152 CFTR alleles of the patients considered in *Table 7* (76) is compared with that expected from a large international collaborative project. The most common mutations were ΔF508 and R117H, which were found in 36% and 22% of alleles, respectively, compared with 66% and 0.3% of CFTR chromosomes worldwide. Furthermore, there were no cases in which ΔF508, G551D, W1282X and N1303K were found in combination with each other or in their homozygous state. The most common genotype was R117H/ ΔF508 but this is not exclusive to isolated CBAVD and is also seen in patients with more typical, albeit milder, cases of CF.

TABLE 8 *Frequency distribution of CFTR mutations in CF patients and in CBAVD males with two mutations*

Mutation	CF patients[191]		CBAVD males[a]	
	Number of CF chromosomes	%	Number of CF chromosomes	%
ΔF508	28,946	66	55	36
G542X	1062	2.4	0	0
G551D	717	1.6	4	2.6
N1303K	589	1.3	1	0.7
W1282X	536	1	2	1.3
R553X	322	0.7	1	0.7
R117H	133	0.3	33	22
R347H	NI	–	8	5.3
D1270N	NI	–	5	3.3
Other			43	28
Total	32,305	73.5	152	100

NI, Not included in survey

[a] *Among the 76 cases from* Table 7

Phenotypes for CF carriers

Infertility

The CF carrier frequency in males with isolated CBAVD is more than ten times higher than that in the general population – 47% compared with 4% (*Table 7*). Recent evidence suggests that other aetiological factors, either extragenic or intragenic, may be involved in the development of CBAVD in male carriers.[182] For example, intronic variants at the 5T allele, which cause reduced levels of CFTR mRNA, have been found in male carriers with CBAVD at a significantly higher rate than in the general population.[139,192,193] The isolated nature of CBAVD could be explained by the increased sensitivity of the developing vas deferens to CFTR levels *in utero*, relative to other tissues.

Respiratory diseases

Despite the influence of environmental factors on the presence or absence of respiratory diseases, associations have been found in CF carriers. The contribution of a second rare undetected mutation or a variant polymorphism to these observations are not known.

In the USA, a three-fold lower risk of developing childhood asthma in carriers of the ΔF508 mutation compared with non-carriers has been reported,[194] although in another study performed in the UK the frequency of asthma amongst carriers was found to be similar to that in the general population.[195] In contrast to these findings, a more recent Danish study has reported a positive association between ΔF508 heterozygosity and asthma with an increased susceptibility amongst carriers to decreased pulmonary function.[196] The possibility that the ΔF508 mutation is in linkage disequilibrium with the gene that causes asthma may offer some explanation as to why different populations have produced conflicting results.[196]

CFTR mutations have been clearly linked to an increased risk of disseminated bronchiectasis.[197–201] For example, in a French study of 65 patients, eight were found to be carriers of ΔF508 and two were compound heterozygotes.[200] Associations have also been reported for chronic bronchitis in three studies[197,201,202] but not in a fourth.[203] In addition, an excess of carriers has been reported in those with Pseudomonas bronchitis and allergic bronchopulmonary aspergillosis[199] and also in those with chronic sinusitis.[204]

Digestive tract cancers

The above-average incidence of gastrointestinal cancers in CF patients (see chapter 3) has led to suggestions that CF carriers may also be at increased risk compared with non-carriers.[205] Although several studies are currently investigating this, no results are available at present. One group[206] is studying parents of CF patients, who are obligate carriers; however, the results may be confounded by any tumorogenic effects of the stress associated with caring for an affected individual.

Chapter 6

Prevalence and risk

Birth prevalence

Since not all those with two disease-causing mutations will present clinically, both a clinical and genetic definition of CF are possible. However, the proportion with subclinical disease is likely to be low and so both definitions should lead to similar prevalence figures.

Before the discovery of the CFTR gene, prevalence rates were determined either by epidemiological studies or neonatal screening programmes.[207] In predominantly Caucasian populations, most researchers found a prevalence between 1 in 1500 and 1 in 3500. Under-ascertainment will have influenced the epidemiological studies and under-diagnosis the neonatal screening studies. The most reliable rates are from total population registers which use multiple sources of ascertainment. There are seven such registers, in Canada[61] (1 in 3000), Czechoslovakia[208] (1 in 3300), Israel[209] (1 in 5200 in a Jewish population), The Netherlands[210] (1 in 3600), Sweden[211] (1 in 7700), the USA[212] (1 in 3200) and the UK (see below).

The ongoing UK register began in 1982 under the auspices of the British Paediatric Association.[71] Data are obtained by questionnaires sent to all consultant members of the Association, the British Thoracic Society and the British Association of Paediatric Surgeons. Other sources are the UK Association of CF Adults and any death certificate on which CF or any of its synonyms are mentioned. Any discrepancies between death certification and clinical cases are clarified using patient records. Data collection has been repeated in 1985, 1986, 1988, 1990, 1992 and 1995.[72,73]

Although the annual birth prevalence has been published for the years 1968–94,[73] late diagnoses mean that prevalence rates are only reliable until 1987. Over this period the total prevalence rate was 1 in 2400.

Risk calculation

Although CF is a simple Mendelian recessive condition, the carrier frequency cannot be derived from the Hardy-Weinberg principle. This is because of adult morbidity, male infertility and, especially in the past, high childhood mortality. As such, almost all CF births occur when both parents are carriers. Since there is a 25% chance that a child will inherit a defective gene from both parents and so have CF, the carrier frequency (F) can be inferred from the birth prevalence (P) by the relationship

$$P = \frac{1}{4} F^2$$

In the UK, where the birth prevalence is 1 in 2400, the carrier frequency can then be taken to be 1 in 24.

Risk given carrier status

The risk of having an affected child in couples where both parents are known to be carriers is one in four. When only one parent is a carrier and the other is untested the risk is 25% of the risk that the untested parent is a carrier. In the UK this is one in 96.

Because of the large number of disease-causing CF mutations, a negative result in any given genetic test does not exclude the possibility of being a CF carrier. In these circumstances, the risk of an affected pregnancy will depend on the specific mutations detected by the test and the estimated proportion of CF carriers who have those mutations. In *Table 9* it is shown how these risks may be calculated for a population with a 1 in 2400 birth prevalence and with a genetic test that detects 85% of carriers. The theoretical outcomes of a single pregnancy in one million unaffected couples are considered in the table. Of the 71,276 in whom only one parent is shown to be a carrier, 110 couples will have a CF pregnancy, a risk of 1 in 648. Although this is substantially lower than the one-in-four risk for carrier couples, it is high compared with the 1 in 15,000 risk for those couples in whom one parent is not found to be a carrier and the other is untested (55 + 10 from 35,638 + 927,469) or the 1 in 93,000 risk when neither parent is found to be a carrier (10 of 927,469). The probabilities of having an affected child, given the carrier status of the parents, from tests capable of detecting different proportions of mutations, are shown in *Table 10*. These risks do not take account of any previous unaffected pregnancies which will reduce the estimated risk in the current pregnancy, although not substantially.[213]

***TABLE 9** Hypothetical CF screening of 1,000,000 couples [affected pregnancies in parentheses]*

Parent carrier status[a]	Test results[b]				
	Both carriers	**Mother only**	**Father only**	**Neither carriers**	**Total**
Both carriers	1255	221	221	39	1736
	[314]	[55]	[55]	[10]	[434]
Mother only	0	35,417	0	6250	41,667
Father only	0	0	35,417	6250	41,667
Neither	0	0	0	914,930	914,930
Total	**1250**	**35,638**	**35,638**	**927,469**	**1,000,000**

[a] Population with 1 in 24 carrier frequency
[b] Assuming the test detects 85% of carriers

***TABLE 10** Risk of CF pregnancy according to parents' screening results and proportion of detectable mutations*

Mutations detectable (%)	Parents' screening results			
	+/–	**–/NT**	**+/– or –/NT**	**–/–**
50	1 in 190	1 in 4500	1 in 3100	1 in 8800
55	1 in 210	1 in 5000	1 in 3300	1 in 11,000
60	1 in 230	1 in 5600	1 in 3600	1 in 14,000
65	1 in 270	1 in 6400	1 in 4000	1 in 18,000
70	1 in 310	1 in 7500	1 in 4500	1 in 24,000
75	1 in 370	1 in 8900	1 in 5300	1 in 35,000
80	1 in 460	1 in 11,000	1 in 6400	1 in 54,000
85	1 in 620	1 in 15,000	1 in 8300	1 in 95,000
90	1 in 920	1 in 22,000	1 in 12,000	1 in 210,000

/, Result for each parent
+, Mutation detected
–, None detected
NT, Not tested

UK carrier detection

A good estimate of the frequency distribution of different CFTR mutations in the UK is available from a survey of all 22 clinical molecular genetics laboratories.[214] A total of 9807 affected chromosomes were studied for 56 different mutations. By far the most common mutation was ΔF508, which was present in 7387 (75%) followed by G551D (302, 3.1%), G542X (165, 1.6%), 621+1G→T (91, 0.9%), 1717-1G→A (56, 0.6%), N1303K (45, 0.5%), R117H (45, 0.5%), R553X (45, 0.5%), and 1898+1G→A (45, 0.5%). A test which includes the six most common mutations in the UK will therefore detect 82% of affected CFTR genes. The three commercial kits currently available (see chapter 8) detect 8, 12 and 31 mutations, accounting for 82%, 83% and 85% of carriers, respectively. Even if all 56 mutations found by the laboratories were included, the proportion detected would only be 86%.

In the survey of the 22 laboratories there was notable geographical variability in mutation frequency throughout the country. For example, R117H was more frequent in Scotland and Northern Ireland whereas G551D was more common in Scotland. However, a breakdown of the most common mutations according to region was not published and we have used other sources to quantify this.

The results of 16 surveys from different parts of the UK are shown in *Table 11*. It would

TABLE 11 *Common CFTR mutations according to UK region*

Region[a]	Number tested	Mutated chromosomes						
		ΔF508	G551D	G542X	621+1	1717–1	N1303K	All (%)
England [191]								
North-West	1245	1017	49	15	11	4	6	1102 (88)
North-West	365	288	11	5	3	2	4	313 (86)
North	232	190	5	2	2	4	0	203 (88)
Birmingham	1198	798	19	15	10	NT	NT	842 (70)
East Anglia	527	420	11	10	2	0	4	447 (85)
London	944	715	30	18	10	4	5	782 (83)
London	486	329	18	20	NT	NT	6	373 (77)
London	354	282	12	9	1	5	1	310 (88)
South	482	333	14	5	3	3	5	363 (75)
South	252	188	5	4	4	3	0	204 (81)
Scotland								
Edinburgh[191]	836	571	44	31	9	8	6	669 (80)
Grampian[215]	117	96	8	2	1	1	0	108 (92)
Wales[191]								
Cardiff	158	114	7	4	5	0	1	131 (83)
Cardiff	183	131	4	5	21	2	1	155 (85)
Northern Ireland								
Belfast[191]	456	258	17	9	8	9	1	302 (66)
Belfast[216]	412	280	21	9	9	NT	0	319 (78)

NT, Not tested

[a] *All assumed to be European Caucasians and some overlap may exist between studies*

appear that testing for the most common six mutations would detect a higher proportion in Scotland, Wales and the north of England. Using these studies, the proportion detected would be 85% in those regions and 79% elsewhere. Given that the commercial kits detect more than six mutations, for the rest of this report we have taken these proportions to be 86% and 80%, respectively.

Clinical over-diagnosis of CF will have influenced overall results from the UK laboratories and might account for some of the observed regional differences. For example, in Birmingham the proportion of CF chromosomes with the ΔF508 mutation was 67% but in bordering regions this proportion was found to be 72%, 76%, 79%, 80%, 81% and 82%. This could be the result of more clinicians in Birmingham incorrectly referring normal patients for CF testing compared with those in bordering regions.[214]

Ethnic minorities

Asians

The birth prevalence of CF in India and Pakistan is unknown but a study of pancreatic necropsy material from India suggests that the figure is very low. There have been two studies in the UK. A report from Birmingham relates to three children with CF born to parents of Asian origin in the city.[217] The authors wrote to all local paediatricians and, as a result, consider that these were the only such cases occurring in an estimated total of 30,000 births. A later and larger study in the West Midlands identified 13 cases from the records of the two regional CF clinics for children and adults.[218] From the relative proportion of Asian patients and births in the locality, the birth prevalence was estimated as 1 in 12,000. In a study in the USA, a much lower prevalence of 1 in 40,000 was found but the study is considered to be flawed because of substantial under-diagnosis.[219] In the absence

***TABLE 12** ΔF508 in Asian CF patients: results from five studies*

Study	Number of chromosomes	Number of consanguineous parents	ΔF508
USA[219]	26	2	11 (42%)
West Midlands, UK[218]	22	4	6 (27%)
Manchester, UK[191]	24	not known	7 (29%)
Leeds, UK[220]	18	not known	8 (44%)
Newcastle, UK[221]	2	1	0
All	92	7	32 (35%)

of further information, it can be assumed that the birth prevalence of CF in UK residents of Asian origin is only 1 in 12,000, about one-fifth of that in Caucasians. This implies a carrier frequency of 1 in 55.

There have been five studies of CFTR mutations in Asian patients, including four in the UK; the results are summarised in *Table 12*. Of the 92 affected chromosomes, the ΔF508 mutation was found in 32 (35%). Three of the studies, including 52 chromosomes, tested for further mutations.[219,221,222] No other mutation occurred frequently but there were cases with S549N, I19, V5201, 1161delC and 296+12T→C. In two studies the number of cases that were the result of consanguineous relationships was reported: 7 cases out of 25 (28%).[218,219]

Ashkenazi Jews

Ashkenazi Jews have a similar CF prevalence to other Europeans. The birth prevalence of CF in Israel has been estimated for 1981–87 using the records of all CF centres.[209] National statistics on ethnic origin were used to estimate the prevalence of CF separately for those whose families came from Europe (largely but not all Ashkenazi) and for those of Asian or African origin (Sephardi). There were 63 cases among those of European origin, a prevalence of 1 in 3300, and 33 in those of Asian or African origin, an overall prevalence of 1 in 9400; the lower prevalence appeared to be concentrated in those from Morocco, Iran and Iraq. In the UK, Jews are mainly of Ashkenazi origin, although there are some well-defined Sephardic centres of population. Thus a carrier frequency of 1 in 29 can be used for risk calculations.

Studies of chromosomes from CF patients in Israel show that, in those of Ashkenazi origin, ΔF508 accounts for less than one-third of mutations whereas almost half are associated with the W1282X mutation.[209,223] In a total of 261 CF chromosomes tested, these two mutations, together with G542X, detected 221 (85%) and 247 (95%), respectively, when 3849+10kbC→T, N1303K and 1717-1G→A are added. Similar proportions have been found in Jews of Ashkenazi origin outside Israel: in the USA, from 265 chromosomes, 115 (43%) had W1282X, 94 (35%) had ΔF508 and 17 (6%) had G542X.[191]

Others

Prevalence is extremely low in families of Afro-Caribbean origin and in those from the Far East.[207] A recent large national study of CF incidence in the USA included 37 Blacks and four Orientals among 868 patients, giving birth prevalence estimates of 1 in 15,000 and 1 in 31,000, respectively.[212] These figures imply gene frequencies of 1 in 61 and 1 in 88, respectively. Previous smaller local studies had reported rates of 1 in 17,000 for Blacks in Washington, DC,[224] and 1 in 90,000 for Orientals in Hawaii.[225] There are no published incidence figures for either ethnic group outside the USA. The prevalence in black Americans may be higher than for Afro-Caribbeans from other countries because of the high proportion with white ancestors. This may also have influenced the mutation frequency distribution. In four studies, a total of 118 chromosomes from US Blacks were tested: 44 (37%) had a ΔF508 mutation, two (1.7%) had 621+1G→T and two (1.7%) had G542X.[191,226]

Validity of risk calculation

CF carrier frequency

The derivation of carrier frequency from birth prevalence assumes random mating of carriers, equal numbers of male and female carriers, no pre- or post-zygotic selection for or against affected or carrier zygotes, no excess or deficit of intra-uterine lethality, and no correlation between family size and

carrier status. With CF, there is no reason to invoke non-random mating and an abnormal foetal loss rate is unlikely as the expected proportion of affected or carrier births is observed in carrier couples. There have been claims for altered sex ratios and increased fertility in carrier females but the evidence is inconsistent and putative effects too small to influence calculations in a specific period.[227–230]

Mutation detection

The estimated proportion of CF chromosomes with the different mutations is derived from studies of affected individuals. It is possible that the frequency distribution of any mutations is different in carriers. This would happen, for example, if some genotypes were more likely to result in a viable zygote than others. There is some evidence for this in Ashkenazi Jews. In 3892 chromosomes from healthy individuals undergoing CF screening in Israel and the USA, there were equal numbers with W1282X and ΔF508 mutations, whereas in patients with CF the ratio was almost two to one.[231] It is not known whether the phenomenon exists in other populations but the results of CF screening studies in predominantly non-Jewish populations do not appear to support this (see chapter 9).

Chapter 7

Screening and diagnosis

Aims of screening

The ultimate public health purpose of genetic screening is prevention. With CF there are two possibilities, namely, primary or secondary prevention aimed at reducing the birth prevalence of the disorder and tertiary prevention aimed at improving prognosis by appropriate management when the diagnosis is brought forward through screening. A second purpose is the provision of information for its own sake, which in general also appears to be of value.[232]

The two aims are not mutually exclusive so that, for example, the early diagnosis of one affected child in a family may lead to the avoidance of further affected children. Similarly, as a consequence of an affected pregnancy being detected and terminated, family studies may be initiated which bring forward the diagnosis of some affected relatives.

Reducing affected births

The ability to reduce the number of affected births is contingent upon the identification of carrier couples who are at high risk of an affected pregnancy. Once they have been identified, there are several preventative options, of which the first is prenatal diagnosis and selective abortion of affected pregnancies. Other options are to: avoid pregnancy; change partners; have artificial insemination using either donor sperm or egg; or pre-implantation diagnosis with selective implantation of an unaffected zygote.

Improving prognosis

The aim is to ensure that diagnosis of the disorder takes place before the onset of clinical symptoms. Although there is currently no cure for CF, there is evidence to suggest that early intervention may be associated with reduced morbidity at least in childhood. The question of whether or not a long-term morbidity and mortality benefit can be established is addressed below (see chapter 12).

Screening strategies

There are three possible broad strategies aimed at preventing affected births through carrier detection but the only screening strategy aimed at improving prognosis is the routine testing of neonates.

One carrier screening strategy is aimed at identifying carrier couples directly, either during pregnancy or when it is being planned. Another strategy takes an indirect approach, by aiming to identify carriers among all individuals of reproductive age in the general population. This will *ipso facto* lead to all carrier couples eventually being identified. The third strategy is to use systematic testing within the families of affected individuals ('cascade' screening). This will lead to the identification of both individual carriers and carrier couples.

Direct carrier couple screening

In the UK most women are offered antenatal screening for neural tube defects, Down's syndrome and ultrasound-detectable structural abnormalities. DNA testing for CF could be incorporated into these existing programmes. Two screening methods have been proposed to identify carrier couples during pregnancy.

- ***Sequential or stepwise*** Carrier testing is offered to the expectant mother, and only if she is found to be a carrier is a sample requested from the father. There are three possible outcomes: both parents are carriers, only the woman is a carrier, and if the woman is not a carrier, her partner is not tested. Under 0.2% would be classified as carrier couples with a one-in-four chance of an affected infant; less than 4% would be discordant, with only the mother identified as a carrier and having an intermediate risk; the rest would have a negligible risk (see *Table 10*). The risk in the intermediate group can be reduced by undertaking additional DNA analysis for more mutations on the partners of carriers but this will have only a marginal effect.

- ***Couple or paired*** Carrier testing is offered to couples and samples are obtained from both parents at the outset. However, the DNA testing is performed exactly as in sequential screening so that only a small percentage of the fathers' samples are actually tested. With this strategy, a carrier female is not informed of the result

until her partner's becomes available thus avoiding unnecessary anxiety. There are two ways of reporting the screening result in couple screening. These are either to disclose the carrier status of each partner as in sequential screening or to use a non-disclosure approach whereby a result is reported as 'positive' for carrier couples and otherwise as 'negative'. The risk in those with negative results will be greater than that for women in sequential screening who are not found to be carriers (see *Table 10*). Non-disclosure treats the couple, rather than the individual, as the screening unit and aims to avoid anxiety in the intermediate risk category; however, it does lead to increased cost because of having to re-test couples who have changed partners in subsequent pregnancies (see chapter 13).

The same methods could be used to test couples outside pregnancy. However, there are organisational difficulties with such pre-conceptional screening. It is not normal practice at present for couples to seek pre-conceptional advice although some women may do so if they see their general practitioners (GPs) at this time for rubella anti-body testing. Thus, it is unlikely that large numbers of women (or couples) would be persuaded to attend specifically for CF screening.

Population screening

In a properly constituted screening programme of this kind, a target population would be identified and systematically offered a DNA test. This target population would include school leavers and individuals of reproductive ages on GP lists.

An individual carrier has a one in 96 chance of CF in each pregnancy whereas those found not to be carriers have a much lower risk (see *Table 10*). Once an individual conceives the risks will change to that for couples, depending on the carrier status of the partner. The number of couples with intermediate risks will be double that for antenatal or pre-conceptional screening.

The problem with population screening is that the carrier status of a future reproductive partner will substantially modify the risk to either very high (one in four), intermediate or very low (see *Table 10*) and, until this is known, the information is of limited practical value. For some in the higher risk group there may be a long period before the risk is clarified, with attendant anxiety and the possibility of the information becoming mislaid or distorted in the interim.

Cascade screening

Following the clinical diagnosis of an affected individual, the genetic services normally offer counselling and DNA testing to family members. Close relatives are tested first and, depending on the results, more distant relatives might be contacted. This approach is well established in clinical genetics and some would not regard it as screening *per se*. With CF screening a distinction has been made between this practice and a more active type of cascade testing, whereby a systematic approach is taken to identify all affected families. Active cascade screening in its most complete form begins with a concerted attempt at case-finding.

The population is probably highly motivated and knowledgeable about the disease and, when a rare mutation is identified in the family, carriers of this as well as the more common ones can be sought. However, only a small fraction of individuals with CF will be in affected families.

Neonatal screening

In the UK, all newborn infants are subject to routine testing for phenylketonuria and hypothyroidism using a heel-prick blood sample absorbed on to a Guthrie card. Some hospitals routinely use spare blood spots to screen for CF (see chapter 11). Neonatal screening provides the opportunity to treat respiratory and gastrointestinal symptoms promptly, the ultimate aim being to minimise long-term damage and improve morbidity. In addition, early diagnosis offers parents more reproductive choice by informing them of their carrier status prior to subsequent pregnancies.

Selective screening

There are three situations in which it has been suggested that screening can be offered to selected groups.

Assisted reproduction

Couples seeking assisted reproduction because of male infertility may receive artificial insemination of donor sperm or more recently intracytoplasmic sperm injection (ICSI). In both situations, although for different reasons, CF screening is important.

With donor insemination there is the possibility that the donor is a CF carrier. As each sperm donor has the potential to father up to ten offspring, there is a high chance that at least one

of the offspring will be affected by CF. This means that if he is a carrier, the risk that at least one foetus will be affected by CF is one in ten ($1-[\frac{3}{4} \times \frac{1}{24} + \frac{23}{24}]^{10}$).

A common reason for performing ICSI is CBAVD, which accounts for approximately 6% of infertility caused by obstructive azoospermia and about 1–2% of all male infertility.[233] Because of the high frequency of CFTR mutations found in infertile males with CBAVD (see *Table 7*), at least one of the partners needs to be screened when ICSI is performed for this indication.[234] Couples should also be counselled on the risk of having offspring affected by a mild variant of CF. Given the high frequency and range of CFTR mutations found in such men, it may prove more cost-effective to screen the female for the most common mutations. If she tests negative, the risk of a child conceived by ICSI having CF or CBAVD would be about one in 1500.[235]

Prenatal diagnosis

Foetal DNA is available for CF testing when invasive prenatal diagnosis has been carried out, for reasons unrelated to CF. By testing this material or initially screening the parents before such testing, detection can be achieved without extra hazard to an unaffected foetus.

Ultrasound echogenic bowel

During routine second trimester ultrasound examination for foetal anomalies, echogenic bowel is observed in 0.6–1.4% of foetuses.[236–239] It has been suggested that this is an indication for parental CF screening. Although only a small proportion of CF infants have meconium ileus, it is possible that there are gastrointestinal abnormalities earlier in pregnancy that regress.

Measures of screening performance

Ultimately the decision whether or not to introduce any of the screening strategies for CF in the UK will depend on a variety of different factors. Nonetheless, the starting point of this decision-making process must be an assessment of the potential performance of the screening tests involved.[240,241]

The most important measures of the performance of a screening test quantify the ability to distinguish affected from unaffected individuals. The usual measures are the sensitivity or detection rate (proportion of affected individuals with positive results) and the false-positive rate (proportion of unaffected individuals with positive results). An alternative way of expressing the latter is the specificity, which is 100% minus the false-positive rate.

The purpose of screening is to identify a group at high risk for further action and to reassure the remainder that their risk is low. The predictive value of the test quantifies these risks. The positive predictive value is the probability that an individual with a positive result is indeed affected, and the negative predictive value is the chance of being unaffected given that the result is negative. These parameters are a function of prevalence of the disorder in the population being tested as well as of the sensitivity and specificity of the test itself.

To be effective any screening strategy needs to make an impact on one or more outcome measures. In addition to the sensitivity of the test, this will depend on the uptake rate of the screening test, the acceptability of the diagnostic procedure and other options offered to those with positive results, and the effect of these on the outcome being measured. The impact of screening can be assessed both in the population being targeted and overall.

Chapter 8

Screening technologies

Detecting CFTR mutations

As described in chapter 5, there are many different types of CFTR mutation. No one set of mutation-detection methods can cover all of them and it is likely that a small proportion of mutations will escape detection whatever method is chosen.

The detection method of choice will depend on whether testing is being undertaken for the purposes of screening or diagnosis. In the case of the former, cost considerations are likely to limit the mutations tested for either to ΔF508 alone or, particularly in a genetically heterogeneous population, a small number of mutations detected simultaneously ('multiplexing'). With diagnostic testing, either the target mutation is known, for example from the parental DNA, and testing will be focused, or a non-specific search of the CFTR gene is needed ('scanning').

The following is a brief outline of the methods for specific mutation testing and general mutation scanning. For a more detailed account see Taylor.[242]

Sample processing

All of the methods require an initial step whereby DNA is extracted from the sample. For detection of CFTR mutations, DNA can be extracted from peripheral white blood cells, mouthwashes, buccal scrapes, dried blood spots (Guthrie cards), amniotic fluid, chorionic villus and cells from urine samples. Even with small volume samples (for example under 1 ml of blood) or less purified starting material, the extracted DNA can be amplified before further processing. The most common technique is the polymerase chain reaction (PCR), which uses the enzyme DNA polymerase to process and copy a specified sequence.

Although DNA is fairly stable, it can be degraded by nucleases released from ruptured cells or by hydrolysis in the presence of divalent cations (e.g. magnesium). For this reason, blood samples are best collected using ethylenediaminetetraacetic acid (EDTA) or acid citrate dextrose (ACD) anticoagulants and transported without freezing. In such samples, good quality DNA can be extracted for up to 1 week after collection provided that prolonged exposure to temperatures above 25 °C is avoided. Guthrie cards are an ideal storage medium; the DNA is dried quickly after collection and remains stable, potentially for years.

Long-term storage of extracted DNA is undertaken for research purposes. When frozen it can be stored indefinitely, although repeated freezing–thawing is not recommended. The spare blood spots remaining after neonatal phenylketonuria and hypothyroidism screening are a valuable national research resource; however, it has recently been noted that they are not being stored according to recommended guidelines.[243]

Detection methods

Testing for specific mutations can be carried out using a variety of methods depending on the mutation concerned. This will involve some or all of the following steps:

- digestion of the DNA with a restriction enzyme
- separation of products by gel electophoresis
- blotting
- detection of the product with a radioactive, fluorescent or chemiluminescent probe.

In some cases a mutation gives rise to or destroys a restriction endonuclease site in the DNA fragment. The first detection assay to be described was for such a mutation in sickle cell disease, which causes loss of a *Dde*I endonuclease site.[244] The assay involved restriction enzymes, gel electrophoresis, Southern blotting and the use of a radio-labelled gene probe which produced results in about a week. Today a non-radioactive PCR-based assay is used and the results are produced within 1 day. It should be noted, however, that conventional PCR-based assays do not work well when large DNA fragments including allele expansions are to be detected.

Other methods that have been used to detect specific mutations include the dot–blot. Here the sequence is tested for its ability to hybridise with an oligonucleotide that matches either the wild-type or the mutant sequence. Dot–blot assays have been conducted with either the oligonucleotide or the gene sequence ('reverse' dot–blot) as the labelled probe. Reverse dot–blots enable a single sample to be used to probe for several mutations at once.[245]

An extreme version of the reverse dot–blot assay is the GeneChip® (licensed by Affymetrix Inc., USA). Here, a photolithographic technique using removable masks, similar to those used in semiconductor chip fabrication, enables a densely packed array of oligonucleotides to be synthesised on a small glass or silicon support. This technology has been applied to mutation searching in the CFTR gene.[246] Two types of light-generated DNA probe arrays have been used by researchers to test for a variety of mutations in the gene. One array, made up of 428 probes, is designed to scan through the length of CFTR exon 11 and identify differences from the wild-type reference sequence. The second type of array contains 1480 probes chosen to detect known deletions, insertions, or base-substitution mutations. The validity of the probe arrays was established by hybridising them with fluorescently labelled control oligonucleotide targets. Characterised mutant CFTR genomic DNA samples were then used to further test probe array hybridisation specificity. Ten unknown patient samples were genotyped using a tile CFTR probe array assay. The genotype assignments were identical to those obtained by PCR product restriction fragment analysis.

Another method of mutation detection is the amplification refractory mutation system (ARMS™) which can be multiplexed to detect several mutations in one tube.[247] Generally the products are analysed by gel electrophoresis, which limits the throughput, although high capacity gels have been described which use the microtitre format, MADGE (Microtitre Array Diagonal Gel Electrophoresis). The Taqman assay (Perkin-Elmer Ltd) can detect specific mutations in a closed system using a fluorescence quenching effect and is commercially available, though not configured as a CF test. A similar approach which can multiplex several mutations in a single, closed tube system called ‘molecular beacons’ has also been described.[248] Solid phase minisequencing, in which the base at a specific position is determined by primer extension, has been well proven as a high throughput mutation testing method and has now been adapted to an array (‘chip’) format, although it is not yet commercially available.[249]

Another approach is the oligonucleotide ligation assay (OLA). This was originally proposed as a way of improving the specificity of DNA probe assays.[250] The principle behind it is based on the ability of DNA ligase to join adjacent pieces of DNA. If two probes were located immediately adjacent to one another then they could be joined by DNA ligase to form a larger probe, which would be easily differentiated by size from the two starting probes. Ligase will only seal the nick created by adjacent probes if there is a perfect match around the nick site, ligation of mismatched bases being strongly disfavoured.[251] Detection of point mutations is usually achieved by setting up the OLA reaction with two competing allelic probes which are ligated to a common probe only when they are perfectly complementary to the target DNA. Use of a thermostable ligase enables repeat cycles of annealing, ligation and dissociation to be conducted, amplifying the signal in a linear fashion. By combining a PCR pre-ligation assay with oligonucleotide ligation, a 60-allele multiplex OLA assay is possible.[252]

When scanning for an unidentified mutation in a patient, it is usual to start by examining the most frequently mutated exons. Apart from direct sequencing, methods for non-specific mutation scanning merely detect a mutation, which then needs to be characterised, usually by sequencing the DNA. If the population manifests a high number of neutral polymorphisms (variation in the sequence with no effect on the phenotype) such scanning methods are not efficient as they will generate considerable additional sequencing. These methods have usually been used in a research as opposed to a diagnostic setting. As research tools they have only recently begun to be adapted to high throughput settings.

Current commercial techniques

At present there are three commercially available kits for CFTR testing. They are designed to work with different types of samples but may not all perform to specification on DNA extracted from blood spots.

First, a reverse dot–blot assay manufactured marketed as INNO–LiPA® (Innogenetics, Belgium) detects eight mutations (ΔF508, N1303K, W1282X, R553X, G542X, 1717-1G→T, G551D and ΔI507). A membrane containing oligonucleotide probes designed to detect DNA labelled by the user is supplied, together with reagents for the non-radioactive dot–blot assay. The whole test takes a day to perform. A six mutation (ΔF508, G542X, G551D, R553X, W1282X and N1303K) reverse dot–blot assay (Roche Molecular Systems Inc., USA) was used for many of the pilot studies of genetic screening for CF in the USA but it is not commercially available.

Next there is an ARMS assay kit available. The CF4 kit (initially manufactured by Cellmark Diagnostics, Zeneca Ltd, UK) can detect four mutations (ΔF508,

G551D, G542X, and 621+1G→T) and was used in many pilot studies of genetic screening. It is now marketed together with the CF12 kit under the Elucigene™ brand name (Zeneca Diagnostics). The CF12 kit detects 12 mutations (ΔF508, G542X, G551D, N1303K, W1282X, 1717-1G→A, R553X, 621+1G→T, R117H, R1162X, 3849+10kbC→T, R334W). The user is supplied with ARMS reaction premixes and Taq polymerase. After amplification of the DNA, products are resolved by agarose gel electrophoresis and the amplification products seen enable the test genotype to be inferred.

Lastly there is a PCR/OLA kit (Perkin-Elmer Ltd, UK). This uses a 15-plex PCR followed by a 60-plex OLA to detect 31 mutations in one tube. Sequence code separation together with a dedicated, expensive 4-colour fluorescent DNA sequencer (ABI PRISM® Sequencer or ABI PRISM® 310 Gene Analyser) and custom software is needed to detect mutations. The system is designed to detect: ΔF508, F508C, ΔI507, Q493X, V520F, 1717-1G→A, G542X, G551D, R553X, R560T, S549R, 3849+10kbC→T, 3849+4A→G, R1162X, 3659delC, W1282X, 3905insT, N1303K, G85E, 621+1G→T, R117H, Y122X, 711+1G→T, 1078delT, R347P, R347H, R334W, A455E, 1898+1G→A, 2183AA→G, and 2789+5G→A. The mutations include all those detected by the INNO-LiPA and Zeneca kits.

In some localities which have a particularly high frequency of ΔF508 it may be appropriate to test for this mutation alone. Although this is technically feasible, there are no commercial kits for this purpose. A conventional PCR and fragment size analysis, ARMS or OLA assay format would each provide a low-cost route for such an assay.

High throughput

Testing for CFTR mutations has so far been conducted on a fairly low throughput scale (under 20 samples per day), even in the population-based pilot screening studies.

Although much effort has been devoted to optimising various mutation detection methods, DNA extraction is time-consuming and may become rate limiting in high-throughput settings. Automated extraction protocols are available but none have as yet been adapted for buccal samples or Guthrie cards. Extraction from such samples is an extremely simple matter, which would be amenable to batch processing.

Mutation testing assays have generally been more adaptable to low-cost, higher-throughput methods, although with improvements in some mutation-scanning techniques and the prospect of GeneChip diagnostics this may not be the case for much longer. However, the cost and reliability of GeneChip analysis is not yet known.

A possible means of increasing throughput and thus save on costs is to pool groups of, say, eight samples and only test them individually if the pool contains a CF mutation. However, this approach would require careful assessment in parallel with individual testing before it could be used and this has not, as yet, been undertaken.

Biochemical markers

Meconium albumin and lactase

The albumin content of meconium in neonates with CF is increased compared with unaffected neonates. This is thought to be caused by the inability of foetuses with CF to digest amniotic fluid in combination with the impairment of pancreatic function.[253] The test is performed either by qualitative dip stick (the 'BM meconium' test)[254] or by various immunoassay methods.[255,256] The discriminatory power of meconium testing is poor with high false-positive and false-negative rates.[257,258] Testing for lactase as well as albumin leads to a slight improvement in test performance.[259] Most of the screening programmes originally based on meconium testing have changed to IRT testing, alone or in combination with DNA testing.

IRT

The serum level of IRT in neonates with CF is, on average, raised compared with unaffected neonates; this is thought to be due to back leakage from blocked pancreatic exocrine ducts.[260] Measurement generally involves the use of dried blood spots from heel-prick samples using radioimmunoassay. Among the factors associated with false-positive IRT results are ethnicity, CF carrier status and perinatal health. False-negative results are related to the age of the infant and the presence of meconium ileus.

In one American study the false-positive rate for African-Americans was about three times that of whites.[261] To our knowledge, a similar effect in other ethnic groups has not been reported. The relationship between carrier status and IRT level is discussed in chapter 11. Infants with pulmonary diseases, such as respiratory distress syndrome, transient neonatal tachypnea and meconium aspiration, and those with low Apgar scores at 1 and 5 minutes caused by perinatal asphyxia are

more than twice as likely to have raised IRT levels than apparently normal neonates.[261]

In CF, serum IRT levels decline with increasing age at a greater rate than in unaffected neonates.[260,262] The proportion of CF cases with meconium ileus detected by IRT testing is much lower than those without meconium ileus (see chapter 11). Both surgical intervention and the use of different reagents for the IRT test have been shown to reduce levels.[263–266]

Sweat test

The presence of CF in an individual can be confirmed by performing a sweat test, otherwise known as the quantitative pilocarpine iontophoresis test (or QPIT). This involves stimulation by pilocarpine iontophoresis, collection of at least 100 mg of sweat, and measurement of the chloride concentration with or without sodium. For diagnostic purposes, sweat chloride is more discriminatory than sodium.[267] Concentrations greater than 60 mmol/l in combination with typical clinical features are regarded as diagnostic. Repeat sweat testing is required for borderline results (between 40 and 60 mmol/l) and for cases in which clinical presentation conflicts with chloride levels. In those cases in which sweat testing repeatedly gives ambiguous results, measurements of nasal potential difference may aid diagnosis.[268] Also extensive genotyping or pancreatic stimulation testing may be indicated.

The interpretation of electrolyte concentrations also needs to take account of the age of the individual being tested. For example, 10% of unaffected adolescents have levels exceeding 60 mmol/l[269] and a higher cut-off point of 70 mmol/l needs to be used. False-positive results have been associated with the presence of Klinefelter's syndrome, untreated hypothyroidism, eczema and a number of other conditions.[270] Also, carriers have levels intermediate between those with CF and non-carriers. In one study, the mean sweat chloride concentration was 15 mmol/l for 128 ΔF508 carriers compared with 99 mmol/l in 115 CF patients and 11 mmol/l in 184 unaffected neonates.[269]

In those with CF, the sweat test result may also be related to the CFTR mutations involved. In one study, three CF neonates with a ΔF508/R117H genotype had equivocal sweat test results.[271] Among 14 patients with recurrent pulmonary disease, with a 3849+10kbC→T mutation in either its homozygous or heterozygous state, seven (50%) had negative or equivocal sweat tests.[174] Another study investigated 23 patients with CF-like lung disease but normal sweat tests.[272] Thirteen patients were found to have the 3849+10kbC→T mutation (two homozygous, nine in combination with ΔF508 and two with W1282X), and all had nasal bio-electric properties similar to CF. In a third report, two compound 3849+10kbC→T/ΔF508 heterozygotes were reported with negative sweat tests and mild symptoms.[273]

Amniotic fluid enzymes

In the 1980s the method of choice for prenatal diagnosis for CF was the determination of markers in the amniotic fluid, particularly reduced levels of the microvillar enzymes γ-glutamyl transpeptidase and aminopeptidase M,[274] and isoforms of alkaine phosphatase.[275] Prospective studies demonstrated that high detection rates could be achieved at the cost of false-positive results, which would be acceptable in populations at high risk.[276,277]

Although this approach has been superseded by genetic diagnosis, there are circumstances where biochemical marker levels may be informative. For example, when only one parent is a proven carrier the risk of an affected pregnancy is not great but prenatal diagnosis may still be requested. If the foetus is shown to have inherited the known mutation, the risk will double and biochemical assay may provide some reassurance or indicate that gene scanning is necessary.

Neonatal testing protocols

A number of protocols have been devised using meconium or IRT alone, in combination with each other or together with DNA testing. The most widely used combinations are summarised in *Table 13*. There is no universally agreed policy regarding strategy, cut-off levels and timing of the test. As a consequence, screening performance varies greatly between programmes.

Single stage

Most neonatal screening programmes using IRT perform the test about 1 week after birth. IRT has low discriminatory power in the first day or two of life, since transient hypertrypsinaemia in some unaffected neonates is indistinguishable from that seen in neonates with CF. IRT levels in unaffected neonates generally decline much faster than in those with CF, leading to increasing discriminatory power.[278] The chosen cut-off for IRT is particular to a laboratory, as it is empirically derived from the distribution of levels in the local population.

TABLE 13 *Neonatal testing protocols*

Protocol	Stages	Description
Meconium	1	Meconium lactase or albumin test only.
IRT	1	IRT test only.
IRT + meconium	2	IRT test initially; meconium test if IRT level raised.
IRT + IRT	2	IRT test initially; second sample tested for IRT if first raised.
IRT + DNA	2	IRT test initially; first sample tested for CFTR mutations if IRT level raised.
IRT + meconium + IRT	3	IRT test initially; meconium test if IRT level raised and, if positive, refer for sweat testing; IRT test on second sample if meconium test negative and initial IRT level very high.
IRT + DNA + IRT	3	IRT test initially; first sample tested for CFTR mutations if IRT level raised; IRT test on second sample in neonates with a single mutation.

Multi-stage testing

The discriminatory power of a single IRT determination at 1 week is not great and a two-stage protocol is now widely adopted in order to reduce the number of false-positives and to improve the positive predictive value. With an IRT + IRT protocol a repeat test is performed on a second blood sample, taken at about 4 weeks of age, in neonates with raised initial IRT. The cut-off level may differ between the two IRT determinations. Centres may use a lower cut-off level for the first IRT than would be adopted for a single-stage IRT protocol to reduce the chance of generating extra false-negatives.

With an IRT + DNA combination, DNA testing for one or more common CFTR mutations is carried out using the initial sample if the IRT level is raised. This avoids the need for a second sample as with the IRT + IRT protocol. In addition, it obviates the need for a sweat test in many neonates; those with two CFTR mutations are diagnosed directly and those with no mutations need not be approached further. Both the second IRT test and the sweat test are naturally anxiety-provoking, particularly for those who are eventually shown not to have the disorder (see chapter 13).

The three-stage IRT + DNA + IRT protocol was devised to reduce the number of false-positive sweat tests generated by the IRT + DNA protocol.[279] Neonates with one proven CFTR mutation are recalled for a second IRT at about 4 weeks of age, just as for the IRT + IRT protocol. Although this will prolong the period of uncertainty, it will avoid the greater anxiety associated with the sweat test. With the IRT + meconomium + IRT protocol, both meconium and blood-spot samples are provided for each neonate. IRT is measured in all cases and meconium tested if the IRT level is raised. A second IRT is only performed in those with extremely raised initial IRT and negative meconium levels.

Genetic diagnosis

Carrier couples identified through antenatal screening or any of the other screening strategies described in chapter 7 will have the option of invasive prenatal diagnosis. As carrier couples have an extremely high risk of having an affected child, the preferred invasive method for obtaining foetal DNA is by chorionic villus sampling (CVS). Amniocentesis and sampling of foetal blood directly from the umbilical cord are also possible options. Whichever method is used, care must be taken to avoid contamination with maternal DNA.

Pre-implantation genetic diagnosis is another option for those wishing to avoid termination of pregnancy. This uses *in vitro* fertilisation techniques to allow the selection and transfer of healthy embryos to the uterus. Thus carrier couples can embark on the pregnancy with the reassurance that it is free from the condition. The technique involves the extraction of single cells (blastomeres) from early embryos followed by PCR of the specific DNA sequence. While homozygous normal and affected embryos are relatively straightforward to diagnose, heterozygosity is complicated by the phenomenon of allelic dropout; this occurs when one of the two heterozygous alleles fails to amplify, leading to misdiagnosis of carriers as either homozygous normal or unaffected. The rate of allelic dropout can be as high as 33% but is reduced to less than 5% with fluorescent PCR[280] or dual amplification for a polymorphism closely linked to ΔF508.[281] In addition to CF carriers, compound heterozygotes carrying a mutation other than

ΔF508 may also be misdiagnosed due to allelic dropout.[282] Research is currently being conducted into the possibility of testing for four mutations in single cells.

Infants at high risk of CF identified by neonatal screening, family history or clinical presentation will, in general, be referred for diagnostic sweat testing. Although DNA testing could be used, the number of CFTR mutations that occur make it impractical. However, for clinically suspected cases with a normal or borderline sweat test result, further genetic studies will be required.

Chapter 9

Assessment of antenatal screening

Theoretical screening performance

With a simple Mendelian condition such as CF, it is a simple matter to predict the proportion of test results that will be classified as positive, the false-positive rate, the detection rate and the positive predictive value. With antenatal screening for CF, the proportion of partners needing a test and the risk in discordant couples are also important performance indicators that can be readily calculated. The predicted values for the principle performance indicators are given in *Table 14*. Five sets of figures are given for screening: UK regions with high mutation detection, regions with lower detection, and Asians, Ashkenazi Jews, and Blacks. In all five, the positive predictive value is one in four.

Practical experience

There have been 11 published studies reporting the results of antenatal screening pilot projects. Five were undertaken in the UK, of which only the Edinburgh study is on-going; the remainder were in Germany, Denmark and the USA. The methods used are presented in *Table 15* and the results summarised in *Table 16*.

Aberdeen, UK

Women attending a maternity hospital in the city were randomised into either couple or sequential screening arms.[283] For administrative ease this was performed on a weekly basis in which 5 consecutive weeks of sequential testing were followed by 1 week of couple testing. Mouthwash samples were tested for four mutations, using the Cellmark Diagnostics kit, which account for 92% of known CF carriers in the Grampian region.[215] No practical difficulties were encountered. In total, screening was offered in 2002 pregnancies and the uptake rates for the two strategies were comparable. In the sequential arm, 91% of women accepted the offer as did 98% of the partners of carriers, and in the couple screening arm, uptake was 89%. These rates were similar to that experienced locally with antenatal screening for Down's syndrome and neural tube defects. In the course of the study, two carrier couples were detected and both accepted prenatal diagnosis. Neither of the foetuses was affected by CF.

Edinburgh, UK

This is by far the largest and most sustained antenatal screening project. The investigators have reported on every practical, technical and psychosocial aspect of antenatal CF screening.[284–289] Samples were tested for six mutations, four using the Cellmark Diagnostics kit and two (R553X and ΔI105) using an in-house assay. The mutations covered by the tests accounted for 85% of those in the local population.[290] Initially a blood sample was used for the women and a mouthwash for the partners but this was soon changed to a mouthwash for both.

For the first 2 years of the study (October 1990 to December 1992), only one obstetric unit in the city

***TABLE 14** Antenatal screening: theoretical performance in UK*[a]

Situation[b]	Mother carrier	CF risk discordant couples	Carrier couples	Detection rate	False-positive rate (per 1000)
Scotland, Wales and Northern England	3.6%	1 in 660	1.3 in 1000	74%	1.0
Elsewhere in UK	3.3%	1 in 460	1.1 in 1000	64%	0.8
Asians	0.6%	1 in 340	< 0.1 in 1000	12%	< 0.1
Ashkenazi Jews	3.3%	1 in 2200	1.1 in 1000	90%	0.8
Blacks	0.7%	1 in 410	< 0.1 in 1000	17%	< 0.1

[a] Assuming that a multi-mutation commercial assay is used

[b] Based on the following carrier frequencies and proportion of mutations detected: Scotland, Wales and northern England, 1:24, 86%; elsewhere in the UK, 1:24, 80%; Asians, 1:55, 35%; Ashkenazi Jews, 1:29, 95%; Blacks, 1:61, 41%

TABLE 15 *Antenatal screening: methods used in 11 studies*

Study	Setting	Strategy	Medium	Mutations[a]
UK				
Aberdeen[283]	Hospital	Sequential and couple	Mouthwash	CF4
Edinburgh[284,285]	Hospital	Sequential and couple	Blood and mouthwash	CF4, R553X, ΔI105
Leeds[291]	Hospital and GP	Sequential	Blood	ΔF508
Manchester[292]	GP	Mixed	Mouthwash	CF4, W1282X
Oxford[293]	Hospital	Couple	Buccal smear	CF4, R553X, W1383X, R1283M
Elsewhere				
Copenhagen, Denmark[294]	Hospital	Sequential	Blood	CF4, N1303K, W1282X
East Berlin, Germany[295]	Hospital	Sequential	Blood	ΔF508 (plus R553X, G551D for partners)
Los Angeles, USA[296]	Family practice	Sequential	Buccal smear	CF6
Maine, USA[297]	Family practice	Couple	Buccal smear	CF4, R553X, W1383X, R1283M
Rochester, USA[298]	Hospital	Sequential	Blood	CF6, plus 10 others
San José USA[299]	Hospital	Sequential	Blood	CF6, R117H, 621+1G→T, I507, 1717-1G→A, R560T, S459N

[a] *CF4 = ΔF508, G542X, G551D, 621+1G→T; CF6 = ΔF508, G542X, G551D, W1282X, R553X, N1303K*

was involved and a sequential screening strategy was adopted. Subsequently, the project was extended to include a second maternity unit and couple screening was offered at both centres. In January 1994 the formal study ended and antenatal CF screening became integrated into routine antenatal care. The transfer of the programme from a research to a routine NHS setting did not result in a marked reduction in uptake.[286]

By May 1995 a total of 22,601 individuals or couples had been offered screening and 17,544 (78%) had accepted. Uptake was 83% for sequential screening and 99% of partners of carriers agreed to be tested. The uptake fell to 76% after couple screening began but this could be attributable to the inclusion of a second obstetric unit in a different part of the city.

The latest results available are until March 1997, by which time 25,026 had been screened (DJH Brock; personal communication, 1997). A total of 941 carriers (1 in 27) and 36 carrier couples (1 in 700) have been identified. Prenatal diagnosis has been performed on 44 occasions for 33 couples. This includes 27 couples having one diagnostic procedure, five having two and one each having three and four. As a result, 13 CF pregnancies have been detected prenatally and terminated.

Leeds, UK

From the beginning of 1993, for a 21-month period, CF testing was offered to pregnant women attending for antenatal care at two hospital obstetric units in Leeds and Hull and at eight general practices in the greater Leeds area.[291] A sequential testing approach was adopted; however, couple screening was provided on request by attending partners. Testing was for ΔF508 only, as this accounts for 80–90% of CF carriers in Yorkshire.[300] Of the 6071 women offered screening, 3773 (62%) accepted. There was a difference in uptake between the hospitals sites (78% in Hull, 60% in Leeds) and in the general practices (70%). DNA testing identified 130 carriers (3.4% of test) and three carrier couples. Only one couple had prenatal diagnosis and the foetus was found to be a carrier; in the second case, CVS was attempted but failed, and in the third testing was refused.

TABLE 16 *Antenatal screening: results from 11 studies*

Study	Number of women	Screening accepted	Partners of carriers tested	Invasive prenatal diagnosis in carrier couples	Termination of CF pregnancy
UK					
Aberdeen[283]	1641	1487 (91%)	47/48 (98%)	2/2	0/0
	361	321 (89%)	N/A		
Edinburgh[285] [b]	6030 16,571	4978 (83%) 12,566 (76%)	189/190 (99%) N/A	33/36	13/13
Leeds[291]	6071	3773 (62%)	127/130 (98%)	1/3	0/0
Manchester[301]	623	529 (85%)	10/10 (100%)	1/1	0/0
Oxford[293]	810	543 (67%)	N/A	0/0	–
Elsewhere					
Copenhagen, Denmark[294]	3054	2443 (80%)	not known (94%)	0/0	–
East Berlin, Germany[295]	638	637 (99%)	20/20 (100%)	1/1	1/1
Los Angeles, USA[296]	4739	3192 (67%)	47/55 (85%)	1/1	1/1
Maine, USA[297]	not known	1682 (not known)	N/A	1/1	1/1
Rochester, USA[298]	3646	3334 (59%)	96/109 (88%)	4/5	0/0
San José USA[299]	6617	5161 (78%)	116/142 (86%)	7/7	1[a]/2
Total	52,801	38,964 (74%)	651/704 (92%)	51/57 (89%)	17/18 (94%)

N/A, Not applicable as couple screening

[a] *Concordant twins homozygous for ΔF508*

[b] *DJH Brock; personal communication, 1997*

Manchester, UK

Between 1991 and 1994, eight general practices in the Manchester area with a list size of 42,000 were recruited into the study.[292,301–303] Patients were screened for the four mutations in the Cellmark Diagnostics kit, accounting for 85% of CF carriers in the locality and, in addition, W1282X was also included for patients of Ashkenazi Jewish descent. Screening was offered at the first antenatal booking visit to 623 individuals and 529 (85%) accepted the offer. They were then randomised to a disclosure couple and a sequential screening arm so that the practicalities and psychological aspects of the two strategies could be compared. One carrier couple was identified and prenatal diagnosis revealed a normal foetus.

Oxford, UK

A non-disclosure couple-screening strategy was adopted for women attending one antenatal clinic in the city's only maternity unit.[293,304] A buccal smear sample was used: initially three mutations were determined (Δ508, G551D, R553X) by in-house assay but thereafter this was supplemented by the Cellmark Diagnostics kit, with its range extended for the purposes of this study to include two further mutations (W1282X, R1283M), taking the total to seven. Of the 810 individuals offered screening, 543 (67%) accepted; fathers were present in the antenatal clinic in 294 cases and all but 32 of the remainder provided a postal sample. A total of 18 carriers and no carrier couples were detected.

Copenhagen, Denmark

Over the 2-year period from June 1990 to June 1992, CF screening was offered to 3054 women attending an obstetric unit in the city for routine antenatal care.[294,305] A sequential screening strategy was adopted based on blood sampling. Women were tested for ΔF508 and partners of carriers were

tested for six mutations, four using the Cellmark Diagnostics kit and two by in-house assay (N1303K, W1282X). The prevalence of CF is low in Denmark, with one estimate of 1 in 4700,[75] but ΔF508 accounts for 88% of CFTR mutations and the six mutations together will detect a further 2%.[306] The screening offer was accepted by 2443 women (80%) and when a carrier was found, 94% of their partners were tested but no carrier couples were identified.

East Berlin, Germany

Between 1990 and 1993, women attending two antenatal clinics were offered CF screening using a sequential screening strategy.[295] Initially whole blood samples and later dried blood spots from the women were tested for ΔF508. Partners of carriers were tested for two additional mutations (R553X, G551D). Uptake was very high with only one of the 638 offered the test declining. In all, 18 women were identified as carriers including two false-positives caused by a technical error. All partners accepted screening and one carrier couple was identified. The pregnancy was terminated following diagnosis of a foetus homozygous for ΔF508.

Los Angeles, USA

CF screening was offered to women attending for antenatal care in two settings: an academic medical centre and a large health maintenance organisation.[296] A sequential screening strategy was employed. Buccal smear samples were tested, using the Roche reverse dot–blot assay, for six mutations (ΔF508, G542X, G551D, R553X, W1282X, N1303K) which are appropriate for the ethnic diversity of the local population. Of the 4739 women offered screening, 3688 (78%) agreed to attend a CF instruction session after which 3192 (67%) were tested. A total of 55 women were found to be carriers (one in 58); 47 of their partners (85%) were tested and one carrier couple was identified. Following prenatal diagnosis, a ΔF508 homozygous foetus was found and the pregnancy was terminated.

Maine, USA

Primary prenatal care practitioners in the state were recruited to the programme. A couple-screening strategy was used based on buccal smears and, after DNA extraction in a local laboratory, samples were sent to the UK and tested for the same mutations as the Oxford programme.[297,307] Of the 74 doctors approached, 69 agreed to participate in the programme but they were not asked to keep a record of those offered screening so that information on uptake is not available. Over a 16-month period, 1682 couples were screened, including 83 (5%) in whom a repeat sample was needed because of technical failure. One carrier couple was identified; prenatal diagnosis revealed an affected foetus homozygous for ΔF508 and the couple opted for termination of pregnancy.

Rochester, USA

All 124 prenatal care providers (111 obstetricians, 13 family physicians) with delivery rights at five hospitals in the city were recruited to the programme.[298] A total of 68 agreed to participate and 37, all of them obstetricians, eventually submitted patients. A sequential strategy was adopted using a blood sample to test for six mutations in the Roche assay in the early part of the study; ten further mutations (R117H, R334W, R347P, A455E, ΔI507, 1717-1G→A, S549N, R560T, 621+1G→T, 3849+10kbC→T) were added later. There was no charge for the test.

Of 5646 pregnant women in the participating practices, 3334 (59%) were screened and 109 were found to be carriers (one in 52). The partners of 96 (88%) carriers were tested and five were found to be carriers. Four of the carrier couples had prenatal diagnosis and one neonatal diagnosis; none of them had CF.

San José, USA

This programme was based on members of a large health maintenance organisation. Over 10 months from December 1991 to September 1992, pregnant women attending a prenatal care education session were offered CF screening.[299] A sequential screening strategy was used and a blood sample from each woman was sent to one of two laboratories. One laboratory tested for the six most common mutations (ΔF508, G542X, G551D, R553X, W1282X, N1303K) and the other tested for an additional six (R117H, 621+1G→T, ΔI507, 1717-1G→A, R560T, S549N). Initially the allocation was at random to each laboratory but, after a time, all went to the latter. All partners of carriers were tested for 12 mutations. Those of African or Oriental ancestry were excluded because of low CF prevalence.

Of the 6617 women offered screening, 5161 (78%) agreed to participate. There was a strong correlation between social class and screening uptake. A total of 142 carriers were identified as well as one woman who was a compound heterozygote (ΔF508/R117H). Seven of the carriers miscarried before the partner could be tested and, of the remainder, 116 (86%) were tested and seven carrier couples were found. All accepted prenatal diagnosis but three miscarried before this could be performed (one had prenatal diagnosis in a sub-

sequent pregnancy) and, although in one case, homozygous ΔF508 concordant twins were diagnosed the parents decided to continue the pregnancy. The two children (aged 2 and 5 years) of one carrier couple identified by screening were examined and found to be compound heterozygotes for G551D/R117H.

Summary of pilot studies

Summaries of the screening performance indicators are presented in *Table 16* for each study, together with an overall figure for all studies combined.

Uptake

Taking all the studies together the uptake rate was 74%, which reduced to 70% when account is taken of carriers in sequential screening whose partners refused testing. There was little overall difference in uptake between the sequential and couple screening approaches, although the combined results are confounded by other between-centre differences. Within-centre comparison is possible for Aberdeen and Edinburgh but the latter included different hospitals in the sequential and couple screening phases of the study. The Aberdeen results suggest that if the uptake is lower for couple screening, it is not by a large amount. Even if couple screening has a lower uptake, this will be compensated for by less than complete uptake in partners in sequential screening.

Confounding could also affect the comparison of overall uptake rates for the different settings. The uptake rate was variable between studies and within the same study between sites (for example, 60% and 78% for two hospitals and 70% for the GPs in Leeds). The reasons for this variability are not known.

Prenatal diagnosis and termination of pregnancy

The overall uptake rate of prenatal diagnosis in carrier couples was 89%. This is similar to that reported in Copenhagen, Denmark, for families with an affected child embarking on subsequent pregnancies.[294] Once an affected foetus had been detected, termination of pregnancy was carried out in all but one case.

Confirmation of UK prevalence and prediction

The combined results from UK centres also confirm that the assumptions about the relationship between gene frequency and prevalence are correct (see chapter 6). The observed carrier frequency for women screened sequentially in Aberdeen, Edinburgh, Leeds and Manchester together was 1 in 28, which is exactly the expected rate if the frequency is 1 in 24 and the tests detect 86% of mutations.

The false-positive and detection rates were also in line with the theoretical predictions presented above. The overall false-positive rate was 0.1%. Comprehensive information on detected and missed cases is only available from the large Edinburgh study (DJH Brock; personal communication, 1997). Of the 25 CF pregnancies in the study population, six were not screened, two of which were detected and terminated because of a family history. Of the 19 screened pregnancies, 15 (80%) CF pregnancies were detected although only 13 (68%) were terminated. So, in Edinburgh, screening and traditional genetic services together reduced the birth prevalence by 60%.

Organisational matters

The 11 pilot studies also demonstrated the feasibility of incorporating CF screening into routine antenatal care. None of them reported any practical difficulties. In some studies undertaking couple screening, a large number of men were attending antenatal clinics with their partners. It also appeared to be straightforward to obtain samples from couples when a woman had attended by herself. Psychological aspects, patient and health professional preferences, knowledge and cost implications arising from the studies are summarised in chapter 13.

Chapter 10

Assessment of other genetic screening

Pre-conceptional screening

Family planning clinics

A study of routine CF screening was carried out in four NHS family planning clinics in south-west Hertfordshire, UK.[308] Attendees of both sexes were asked for a mouthwash sample which was tested for three common mutations (ΔF508, G551D, R553X) using an in-house assay. A total of 431 individuals were offered testing and 374 (87%) accepted, including 14 of the 18 men (78%).

Pre-nuptial

Since 1985, a charitable organisation, Dor Yesharim, has provided genetic screening for young orthodox Ashkenazi Jews. The programme was established to screen for Tay–Sachs disease but has been extended to include CF and Canavan's disease. The ultimate objective is to prevent the marriage of two carriers, so a non-disclosure couple strategy is adopted. Blood samples were, until recently, tested for five CF mutations (ΔF508, W1282X, G542X, N1303K, 3849+10kbC→T) which are common among Ashkenazi Jews and are now tested for 12 mutations, using the Zeneca kit. The test results have been reported on 6076 screened individuals aged under 18 years, 232 of whom were found to be CF carriers.[309] As the programme is not population-based there is no information on uptake and the number of carrier couples was not reported.

Pre-implantation genetic diagnosis

There are over 60 units carrying out this procedure for a range of conditions; the units include four centres licensed to do so in the UK: in London at the Hammersmith Hospital, University College Hospital and St Thomas's Hospital; and at the Leeds Teaching Hospitals. The accumulated world-wide experience in the diagnosis of CF has been published up to June 1995.[282] At that stage, 46 unaffected embryos had been transferred from 52 cycles, leading to 15 pregnancies after *in vitro* fertilisation, with seven unaffected births and one CF birth (due to misdiagnosis). By the end of 1996 a total of 54 couples had been treated (Joyce Harper; personal communication, 1997). There were 95 cycles yielding 82 embryos suitable for biopsy. Sixteen clinical pregnancies were achieved leading to nine unaffected and one CF birth: two patients miscarried and four continued at the time of reporting.

Population screening: general practice

There have been four studies of general population CF screening in general practice, three in the UK and one in the USA. The studies considered two broadly different approaches: namely, to invite patients individually for testing or to offer screening opportunistically to those attending the surgery for unrelated reasons. The results are summarised in *Table 17*.

Baltimore, USA

Four of the sites in a city-wide health maintenance organisation were used for the study.[310] All those

TABLE 17 *General practice screening: results from four studies comparing two approaches*

Study	Approach offered	Number offered	Number accepted (%)	Number of carriers detected
Baltimore[310]	letter	2713	101 (3.7)	8
	opportunistic	608	143 (24)	
North London[311]	letter	3951	234 (5.9)	28
	opportunistic	1208	556 (46)	
South Wales[312]	letter	1182	231 (20)	26
	opportunistic	359	238 (66)	
South-west Hertfordshire[308]	letter	852	87 (10)	29
	opportunistic	513	340 (66)	
All	letter	8698	653 (7.9)	91
	opportunistic	2688	1277 (48)	

aged 18–44 years who were registered with the practice were approached. At two sites a letter of invitation was sent to all 2713 individuals in the age range (and to couples where only the woman was) inviting them to attend a seminar on CF screening. At the other two sites, 608 eligible individuals who attended for a scheduled visit over a 7-month period were opportunistically offered screening without having to return for a further visit. A mouthwash sample was used and tested for six mutations (ΔF508, G542X, G551D, R553X, W1282X, N1303K). No charge was made for the test.

In the letter invitation group, 471 (17%) returned a questionnaire expressing an interest in screening, 109 (4.0%) attended an education session and 101 (3.7%) were screened. In the attendee approach group, 235 (39%) expressed an interest in being screened and 143 (24%) were actually tested. Eight carriers were detected in 226 samples, the remaining 18 being technical failures.

North London, UK

Over a 15-month period patients aged between 18 and 45 years who were registered with an inner city general practice were offered CF carrier testing.[311] A mouthwash sample was tested for four mutations (ΔF508, G542X, G551D, 621+1G→T) and, additionally, for W1282X if they were Ashkenazi Jews. A total of 3951 were invited by letter to make a screening appointment and 1208 were invited opportunistically when they attended the practice for unrelated reasons. For each group three different approaches were used.

In the letter group, 502 were selected at random to receive a letter only and 498 a letter plus a leaflet; the remaining 2953, who received a letter only, were those who had not attended the surgery by the end of the study. Overall only 234 (5.9%) accepted the offers: 12%, 9.5% and 4.3% for the three approaches, respectively.

The opportunistic group were either approached with a leaflet offering immediate testing (471) or a face-to-face invitation with immediate testing (649), or an appointment to return for testing (88). A total of 556 (46%) were screened: 17%, 70% and 25% for each of the three approaches, respectively.

Of the 957 individuals who were screened, 28 were carriers (1 in 34). Of these, 15 had partners, of whom 11 were tested (73%) but none was found to be a carrier. Cascade testing occurred in 14 first-degree relatives of carriers and five carriers were found.

South Wales, UK

Over a 21-month period in 1991–93, two general practices participated in a study of screening among those aged 16–45 years.[312] Testing was by mouthwash, using the Cellmark Diagnostics kit, for four mutations which accounted for 84% of carriers in Wales.[313] In one practice, 1541 individuals were offered screening either by letter with a specified appointment time (739), opportunistically (359) or, at the end of the study, by letter with no specific appointment time (443). In the letter invitation group, 231 individuals (20%) accepted, 22% with appointment times and 15% without; in the opportunistic group, 238 (66%) accepted the offer. In the second practice, 135 couples were offered screening by letter with a specified appointment; only two (1.5%) accepted.

In total, 481 individuals were tested, including 12 who invited themselves, leading to the identification of 26 carriers and two carrier couples (the number of partners tested was not specified). After the end of the formal study, the number tested increased to 604 of whom 33 were carriers (1 in 18); cascade screening of 58 relatives led to the identification of 18 carriers.

South-west Hertfordshire, UK

CF screening was provided for men and women aged 16–44 years at three general practices.[308] The technical details are the same as the parallel study in family planning clinics (see above). At one of the practices, 852 patients were sent a letter together with a leaflet inviting them to make an appointment at the surgery for screening. At the other two practices, 513 patients who were waiting to be seen at the surgery were approached to be screened. In the letter invitation group, only 87 were screened, an uptake rate of 10% which was unrelated to age and sex. In the opportunistic group, 340 (66%) were screened. Among the 884 non-Jewish, white individuals tested at either the general practices or family planning clinics, 29 carriers were found (1 in 30).

Summary of GP studies

All four studies had a low uptake when a letter was used to invite patients for screening and, taken together, the overall rate was 7.9%. In the same studies, opportunistic screening achieved an overall uptake rate of 48% and, in the UK practices, it was 55%. None of the studies used the opportunistic approach for long enough to examine the proportion of those on GPs' lists who would eventually have been screened. This will depend on attendance rates, which vary with age, gender and parity. For example, in both the

studies offering opportunistic screening which reported the gender of attendees (Baltimore and south-west Hertfordshire), there were almost three times as many women as men. In order for the eventual uptake rate to approach that observed among attendees, sustained organisation and staff motivation would be required.

Population screening: other

School

There have been two studies of CF screening among schoolchildren. The first involved 15–17 year-old students from four schools in Montreal, Canada.[314,315] When CF screening was introduced, routine screening for Tay–Sachs disease and β-thalassaemia had been well-established at the schools for some years. Parental consent was requested, a blood sample was obtained and, in the first phase, tested for ΔF508 only; this was later extended to include four other mutations (G551D, G542X, W1282X, S549N). A total of 1399 students were offered the test but only 574 (41%) accepted the offer. The uptake rate was considerably lower than the 70% seen in the same schools for Tay–Sachs disease and β-thalassaemia. Nine CF carriers were found, all of whom had the ΔF508 mutation.

A study of community-wide screening in two towns with a high CF prevalence in New South Wales, Australia (see below), targeted, among other groups, the two main schools. Students aged over 16 years were offered CF screening for ΔF508 only.[316] An overall uptake rate is not available but separate rates of 42% and 70% were reported for each school. Among the 186 students tested, eight carriers were identified, including two carriers in the nine students with a family history of CF.

Workplace

Both male and female employees of Guy's Hospital, London, working in the Division of Molecular and Medical Genetics, were invited for screening through a poster advertising campaign in the hospital.[317] A mouthwash sample was used and testing was for four mutations (ΔF508, G551D, G542X, 621+1G→T). Of the 110 members of staff in the division (most of whom were of reproductive age), 23 (21%) were screened and no carriers were identified. Although the employees' knowledge of CF was not formally measured, it is likely that they were well informed, so the low uptake cannot be fully explained by poor awareness.

Community

For 12 months a screening campaign was conducted in two county towns in New South Wales, Australia.[316] These were chosen because of a high CF prevalence rate in the locality and the consequently increased community awareness. With a combined population of 14,940, the residents included an extended family with eight CF patients, and three other families with one adult and two affected children. The campaign was based principally on advertising by radio, television and newspaper interviews, posters in doctors' surgeries and community talks. In the two main schools, relevant information was incorporated into the syllabus, leaflets were distributed to parents and testing was provided for those aged over 16 years. Informal talks were also given in several workplaces. Testing was for ΔF508 only, initially using a hair sample and, later, a mouthwash.

A total of 610 individuals were tested, comprising 7.9% of residents aged 16–55 years. Those screened included 42 (6.9%) with a family history of CF, a disproportionate number, and 63 (10%) through cascade screening. Of the 47 ΔF508 carriers detected, 28 were directly as a result of general population screening, giving a frequency of 1 in 20; the remainder were from cascade screening. For 21 of the 28 carriers, cascade screening was instigated and, of the nine couples who were of reproductive age, eight also had their partners tested.

Cascade screening

Genetic testing in relatives of CF probands is widespread but is generally passive, in that the initiative to request screening is left with families. A study in Ontario, Canada, has examined the extent to which relatives are actually screened when such an approach is taken.[318] A consecutive series of 118 CF patients from 115 families were included and, following genetic counselling, the availability of free testing for family members was raised with adults with CF or the parents of children with CF. Subsequent examination of pedigrees revealed that screening had been carried out in 54 (38%) of 143 siblings, 76 (9.1%) of 835 grandparents, aunts and uncles, and 22 (3.3%) of third-degree relatives.

In three of the general population screening studies above (the general practices in North London and South Wales, and the community campaign) it would appear that a more systematic or 'active' approach was taken. From the total of 82 carriers detected by screening, 135 relatives were tested leading to the detection of 42 further

carriers. However, the studies do not give any details of the number of relatives approached and the degree of relatedness.

There have been three studies which provide sufficient details for active cascade screening to be assessed. One study relates to a single family and in two studies such screening was attempted in a large series that included all affected families in the locality. In addition, a model has been constructed to predict the proportion of carriers in the general population who could be identified by cascade screening.

New South Wales, Australia

In this study, active cascade screening in a single family is described in detail.[319] There were five generations of the family, including 230 living descendants of a couple who are assumed to be carriers of the ΔF508 mutation. The cascade began when a woman having four siblings who had died of CF in childhood approached the genetic services. The woman, then in her forties, wanted to know the CF risk in her grandchildren. This involved testing her parents, who were obligate carriers; both were found to have the ΔF508 mutation. The cascade then moved on to their siblings before progressing to the younger generations.

Of the 230 living family members, screening was not performed on 30 because either they had CF (8), or they were too young (18), or they did not have children (4). Also there was no need to test 101 relatives because testing had revealed that their parent was not a carrier. Of the remaining 99, screening was accepted by 49 and 24 carriers were identified.

Manchester, UK

In 1993, paediatricians and physicians throughout the North Western Regional Health Authority were asked to provide the regional genetics service with the names of CF patients.[320] A total of 537 living patients (excluding affected siblings) were identified, many of whom were already known to the service. The series was supplemented with 70 deceased patients. The programme involved approaching each index family, constructing a family pedigree and offering CF screening to relatives. The families themselves contacted relatives with the offer of testing. Children were not screened, except to exclude CF in a sibling, and those of childbearing age, especially couples, were specifically targeted. The discovery of a carrier led to an extension of the cascade to their close relatives. Mouthwash samples were tested using the Cellmark Diagnostics four-mutation assay, together with testing for W1282X in Ashkenazi Jews, and for rarer mutations which were known to occur in a family.

The results have been published for the first year of the project when, out of 141 index families approached, 129 (91%) agreed to contact relatives. Of 959 relatives contacted, 943 (98%) agreed to be tested. In total, 1122 relatives were tested including a small number of cascades started through the discovery of carriers without a family history. Of these, 427 (38%) were found to be carriers. Also, 441 partners of carriers were tested and 23 were carriers. Prenatal diagnosis was offered in nine pregnancies to eight carrier couples: one couple refused testing, in one pregnancy CF was detected but termination was declined, and three affected pregnancies were terminated.

North Carolina, USA

A register of probands was compiled from the records of a large regional CF centre.[321] The centre contacted each patient or their parents by letter. This was followed by a telephone call to obtain a family pedigree and the contact address of all first-, second- and third-degree relatives. Screening was by mouthwash using the Roche six mutation assay; no charge was made for the test.

Of the 427 proband families found, 107 were excluded; 80 lived outside the study area and 27 were either in another research project, had an unclear CF diagnosis or did not have one of the mutations being screened for. Of the remaining 320, 68 (21%) could not be reached by telephone, 61 (19%) refused to participate, 33 (10%) did not provide contact information on relatives, and 49 (15%) had no eligible relatives in the study area.

This left just 109 probands (34%) who identified 1648 relatives, of whom only 699 satisfied the study criteria: aged over 18 years, not currently pregnant, not previously tested, contactable or residing in the area. All were invited by letter to consider CF screening, with a follow-up telephone call to confirm participation. The researchers were unable to make telephone contact with 151 or, if contacted, they were unable to decide about screening, and a further 34 were found to be ineligible when contacted. Of the remaining 514, the offer of screening was accepted by 299 (58%). The location of the test, at home or in the clinic, was subject to a randomised trial. Uptake was significantly higher with the home-based approach (67% compared with 44%,

$p < 0.01$). Uptake was also higher in first-degree relatives than in more distant ones: 85% compared with 56%. In total, 120 carriers were detected; 63 of the 92 with partners (68%) accepted screening and five carrier couples were identified.

Model prediction

Holloway and Brock[322] constructed a model to estimate the number of relatives that would need to be tested if cascade screening extended to second cousins, and to estimate what proportion of carriers in the general population would be detected. The principal assumptions were a carrier frequency of 1 in 25, a mutation detection rate of 85%, and that no new mutations occurred in the family. The prior probability of being a carrier was computed for all the common categories of unaffected relatives up to second cousins in the same and previous generation as the proband. Census information on completed family size in Scotland was used to estimate the average number of siblings (1.8) and offspring (2.5).

In any family, the greater the number of older relatives it is possible to test, the fewer need to be tested in younger generations as carriers can be excluded from whole branches of the family. Two cascade strategies were considered: in the first, all of the grandparents' generation are assumed to be available for testing and the cascade began with them; in the second, none are available but all the parent's generation can be tested so the cascade starts with them.

The model predicts that when the cascade begins in the grandparents' generation there are 54 unaffected relatives involved, of whom 28 would be tested and 14 carriers found, ten in the same generation as the proband or the parents. When the cascade begins in the parents' generation there are 78 relatives, of whom 35 would be tested and 11 carriers detected. The model also predicts that under 15% of carriers in the population would be detectable using a cascade screening strategy that stopped at second cousins. Extending the cascade further is unrealistic and, if undertaken, would rapidly reduce the yield of carriers detected per test.

Selective screening

Assisted reproduction

In 1995 sperm from 22 prospective and current donors in Leeds were tested for ΔF508.[280] Despite the local policy at the time of excluding potential carriers on the basis of family history of genetic disease, two were found to be carriers.

In the USA, surveys carried out after 1995 found that only 25% of semen donors[323] and 22% of oocyte donors[324] were routinely screened for CF. In the UK, although the British Andrology Society endorses testing sperm donors for autosomal recessive disorders, including Tay–Sachs disease and sickle-cell anaemia, screening for CF is not mandatory.[325] The Human Fertilisation and Embryology Authority are currently reviewing this situation with a view to recommending that it be carried out routinely (R Deech; personal communication, 1997). Meanwhile, for the purposes of this review, we wrote to all 99 *in-vitro* fertilisation and donor insemination units in the UK to ascertain their policy on the matter. Of the 76 units that responded, 38 are involved in sperm donation. In four units, taking a family history was the only method of excluding CF carriers among potential donors. Genetic screening with one or more CFTR mutation was routinely used to exclude carriers in 32 units; in four units it was the only method used and in 29 a combination of family history and DNA testing was used. The remaining two did not indicate their policy. Our survey asked for samples of information material on screening given to potential donors. Only six units provided information leaflets and only three of them reported that they discussed the implications of carrier testing.

Prenatal diagnosis

There have been three reports of routine CF screening when invasive prenatal diagnosis is being carried out for unrelated reasons.

In a study in the USA, screening was offered to 1617 couples using a foetal sample and testing for ΔF508.[326] Only 562 couples (35%) accepted, with a higher uptake rate in those having CVS (44%) rather than amniocentesis (19%). The cost of screening was borne by the patients, which might have influenced uptake. The latest published results show that in the 3237 foetuses tested so far, 104 carriers and no ΔF508 homozygotes have been found.[327]

In a study in Copenhagen, Denmark, sequential CF screening was offered to women having prenatal diagnosis, mainly because of advanced maternal age. The study design was the same as that for those attending for routine antenatal care described in chapter 9.[294] The uptake rate was high: of 3545 offered screening, 3474 (98%) accepted. Three carrier couples were found and the CVS

samples already obtained for the primary indication were tested. One homozygous CF foetus was detected and the couple opted to terminate the pregnancy.

The third study was in Milan, Italy.[328] Screening was offered to couples having first or early second trimester CVS. The foetal sample was tested for ΔF508 and, if it was heterozygous, general mutation scanning was carried out in the parent without this mutation before confirming the result on foetal tissue. The acceptance rate was extremely high (98%) and testing was carried out for 802 foetuses including 44 twins. There were 12 carriers and one affected foetus with a ΔF508/M348 genotype was detected; the pregnancy was terminated.

In one series of 116 amniocenteses performed because of echogenic bowel, foetal DNA was tested for ΔF508; one homozygote was detected and the pregnancy terminated.[329] A second study found seven cases in 145 patients with echogenic bowel among 7400 having routine scans.[239] The high overall CF prevalence suggests that this study is biased but within the abnormal ultrasound group a trend towards higher CF risk with increasing echogenicity was seen, so the association may be real.

Chapter 11

Practical experience of neonatal screening

There are reports in the literature relating the detailed experience of 20 neonatal screening programmes for CF including six in the UK. The results are summarised in *Tables 18* and *19*. In addition, four studies have retrospectively analysed samples collected in prospective neonatal screening programmes.

UK programmes

All neonates in the UK are screened for phenylketonuria and hypothyroidism. Six programmes have additionally provided routine screening for CF: East Anglia, Leeds, Northamptonshire, Northern Ireland, Trent, and Wales and West Midlands.

TABLE 18 *Neonatal screening: results of 20 prospective programmes in the UK and elsewhere*

Study	Protocol	Number	First positive screened	Sweat test	False-positive
UK					
East Anglia[330]	IRT + IRT	211,344	1150	99	20
Leeds[331]	Mixed	81,778	not known	not known	not known
Northants[a]	IRT + IRT	104,000	510	53	23
Northern Ireland[332]	IRT + IRT	108,424	5120	136	102
Trent[333]	IRT + IRT	311,857	1849	114	26
	IRT + DNA + IRT	125,973	726	21	2
Wales & West Midlands[334]	IRT + IRT	227,183	944	95	33
Elsewhere					
Brittany, France[335]	IRT + DNA	32,300	379	not known	not known
Collaborative study, France[336]	IRT + IRT	513,440	5948	not known	not known
Colorado, USA[337]	IRT + IRT	461,364	884	128	74
New South Wales[338]	IRT + IRT	1,015,000	7362	577	335
	IRT + DNA	189,000	1968	111	102
New Zealand[339]	IRT + IRT	210,751	1399	101	29
Normandy, France[340]	IRT	79,800	253	253	234
North-east Italy[341,342]	Mixed	773,206	not known	not known	not known
	IRT + meconium + IRT	157,992	1320	215	173
North-east Netherlands[343]	meconium	94,043	not known	not known	not known
Queensland[344]	IRT + IRT	180,000	not known	not known	not known
South Australia[345]	IRT + DNA	108,871	1220	89	82
Victoria[346]	IRT + DNA	130,708	1142	97	82
Vienna, Austria[347]	IRT	19,992	119	119	108
West Pennsylvania, USA[348]	IRT + IRT	105,734	827	201	181
Wisconsin, USA[349,350 b]	IRT	220,865	369	369	323
	IRT + DNA	104,308	2056	123	113

[a] *SJ Evans; personal communication, 1998*
[b] *PM Farrell; personal communication, 1997*

***TABLE 19** Neonatal screening: incidence of CF in 20 programmes*

Study	Meconium ileus[a]	CF: number detected	CF: number missed	CF: detection rate
UK				
East Anglia[330]	18	79	1	99%
Leeds[331]	5	25	1	96%
Northants[b]	not known	30	4	88%
Northern Ireland[332]	12	34	14	71%
Trent[333]	23	88	6	94%
	6	44	3	94%
Wales & West Midlands[334]	6	62	10	86%
Worldwide				
Brittany, France[335]	not known	11	2	85%
Collaborative study, France[336]	15	100	7	93%
Colorado, USA[337]	12	54	7	89%
New South Wales[338]	80	242	30	89%
	9	53	0	100%
New Zealand[339]	not known	72	6	92%
Normandy, France[340]	3	19	1	95%
North-east Italy[341,342]	not known	144	37	80%
	3	42	0	100%
North-east Netherlands[343]	4	19	5	79%
Queensland[344]	not known	not known	not known	–
South Australia[345]	7	33	0	100%
Victoria[346]	9	38	3	93%
Vienna, Austria[347]	not known	11	1	92%
West Pennsylvania, USA[348]	not known	20	0	100%
Wisconsin, USA[349,350 c]	11	46	4	92%
	6	15	0	100%

[a] Wherever possible cases of meconium ileus have been excluded from all other columns
[b] SJ Evans; personal communication, 1998
[c] PM Farrell; personal communication, 1997

The latter programme was suspended in 1989 and restarted in 1997 for Wales only. In 1991, when only four centres were screening, it was estimated that 16% of UK infants were screened for CF.[351] Currently the proportion may be closer to 25%.

East Anglia

Neonatal screening using IRT was first introduced to East Anglia in 1980 and, since 1982, all infants born in the region have been included. All samples are tested in Peterborough using an IRT + IRT protocol. By 1989, 211,344 neonates had been screened and 1150 required a second IRT; 99 screened positive, of whom 79 were diagnosed with CF.[330] There were a further 18 cases of meconium ileus and only one reported false-negative result at a maximum follow-up of 10 years. When results for a further year are included, the total screened was 238,990 and the number of cases 107, giving a prevalence of 1 in 2200.[352]

Leeds, West Yorkshire

Neonatal screening for CF was first introduced into one of the two maternity units in the city in 1975. Initially a meconium-only protocol was used but, in 1990, this was changed to IRT + meconium. Up to December 1994, 81,778 infants had been screened.[331] There were a total of 37 cases of

CF detected; 25 individuals were found through routine screening (including four neonates who were tested because of family history), seven were false-negatives and five presented with meconium ileus. In December 1995, the screening method was replaced by a three-stage IRT + DNA + IRT protocol and the service was extended to the second maternity unit. Since then about 14,000 neonates have been screened and six cases of CF have been identified.

Northamptonshire

Screening began in 1982 and covers all births in the county; testing is carried out in Northampton. So far about 104,000 neonates have been screened using an IRT + IRT protocol (SJ Evans; personal communication, 1998). A raised first IRT level was found in 510 neonates and 53 were referred for sweat testing after the second IRT determination. CF has been confirmed in 30 neonates as a result of screening, although an unspecified number had meconium ileus and would have been detected regardless of screening. So far, there have been four false-negative results.

Northern Ireland

Neonatal screening for CF commenced in 1983 using an IRT + IRT protocol.[332] All births in the province are included and the samples are tested in Belfast. In the first 4 years, 108,424 neonates were screened, of whom 5120 (5%) required a second sample. Sweat testing was performed on 136 (0.1%) and the diagnosis was confirmed in 34 neonates. In addition, 12 affected neonates presented with meconium ileus and, although they were detected, only two had raised IRT levels. By 1988, after a maximum follow-up period of 5 years, 14 further cases had presented with false-negative screening results. The positive predictive value was 25%, excluding those with meconium ileus. The median age at diagnosis before screening was 9 months, which was reduced to 4 weeks following the introduction of screening.

Since 1987 a further 228,500 neonates have been screened, of whom 87 were found to have CF, 22 had meconium ileus and there were four false-negatives (H Leslie; personal communication, 1997). Taking both periods together, there were 173 cases of CF, a prevalence of 1 in 1900.

Trent

Neonatal screening services for Derbyshire, Leicestershire, Lincolnshire, Nottinghamshire and South Yorkshire are provided by a laboratory in Sheffield. Routine screening of neonates for CF began in 1989 using an IRT + IRT protocol. In 1994, this was replaced by an IRT + DNA + IRT protocol, in which the only mutation tested for was ΔF508. The totals screened by the two protocols are 311,857 and 125,973, respectively.[333] The number with raised IRT results in the initial analysis was 2575 (0.6%) and the effect of the DNA step was to reduce the number of repeat samples required for the second IRT test from 0.6% to 0.05%. The need for a sweat test was halved with 114 (0.04%) referrals on the two-stage protocol and 21 (0.02%) when the third stage was introduced. This increased the positive predictive value of the screening process from 77% to 96% when both DNA-detected homozygotes and those requiring the sweat test from persistently raised IRT levels are regarded as positive screening results. The detection rate was comparable for both protocols, with only one false-negative in the three-stage protocol that might have otherwise been detected by the two-stage protocol. This case involved a compound heterozygote with R553X and an unidentified mutation, and so the neonate did not go on to have a second IRT. The main disadvantage to the three-stage protocol was the inadvertent identification of 36 carriers of ΔF508, many of whom had negative sweat tests. For these infants, the GP was notified with a recommendation for referral to a genetics centre. In total, 170 neonates with CF were identified with a prevalence of 1 in 2600.

A more detailed analysis of 91 anonymised ΔF508 carriers has recently been carried out.[353] These infants were screened negative, being hypertrypsinaemic on the first IRT sample but not on the second. Sequencing of the CFTR gene has revealed a further 20 compound heterozygotes, nine of which also carry the R117H mild mutation. Although these would have been reported as screened negative, six infants, all with mild mutations, have since been diagnosed following clinical presentation.

Wales and West Midlands

From 1985 to 1989, all infants in these two health regions received neonatal screening for CF.[334,354] This was part of the RCT of screening described in chapter 12. A two-stage IRT + IRT protocol was employed and samples were tested in two regional laboratories. Over the 5-year period, a total of 227,183 neonates were screened, of whom 944 had raised IRT levels; 95 required a sweat test which led to the identification of 62 infants with CF. In addition, there were six cases of meconium ileus; all were screened and had positive results. By 1991, ten false-negative cases had surfaced. The incidence of CF, including meconium ileus, was 1:2900. In 1997, routine neonatal screening for CF

was reintroduced in Wales using an IRT + DNA protocol, with funding from the Welsh Office for 2–3 years.

Other programmes

There have been 14 reports of screening programmes from Australia, Austria, France, Denmark, Italy, The Netherlands, New Zealand and the USA.

Brittany, France

Since 1988 all neonates in Brittany have been screened for CF. Initially an IRT + IRT protocol was used but this was replaced in 1993 by an IRT + DNA protocol.[335] The DNA test scans the entire coding regions of exons 7, 10 and 11 in the CFTR gene, which have been shown retrospectively to identify, in Brittany, 98% of disease-causing mutations.[355] Over an 18-month period, from January 1993 to June 1994, 32,300 neonates were screened using this protocol with 379 (1.2%) requiring DNA analysis. A total of 11 neonates had two mutations but, at the end of the study period, CF had only been confirmed by sweat testing in ten. The additional case, a compound heterozygote with a novel mutation, had a borderline sweat test and it remains to be seen whether it is a pathogenic or normal polymorphism. There were 40 carriers, although more extensive DNA testing revealed two more compound heterozygotes with rare mutations and negative sweat tests. No other false-negatives have been reported.

Collaborative study, France

Eleven laboratories collaborated over a 2-year period in a study carried out by the French Association for Neonatal Screening.[336] Samples were tested with an IRT + IRT protocol. A total of 513,440 neonates were screened, 100 CF cases were detected, with seven false-negative results. There were an additional 15 neonates with meconium ileus; all were tested and only 13 had positive screening results. The study was not continued because the false-positive rate was regarded by the investigators as being too high. The number with positive initial IRT tests (5948; 1.2%) was reported but the number needing a sweat test was not.

Colorado, USA

Since 1982 all infants born in the state have been screened using an IRT + IRT protocol. By September 1987 461,364 had been screened, 884 (0.2%) required a repeat test but this was only carried out for 693.[337] Sweat tests were required in 128 (0.03%), leading to the detection of 54 cases of CF. There were seven false-negatives and, in addition, 12 neonates presented with meconium ileus.

New South Wales, Australia

All infants born in the state have been screened since 1981 and this was extended to include the Capitol Territory in 1986.[338] Until 1992, the protocol used was IRT + IRT after which it was IRT + DNA, for ΔF508 only. In the initial period, 1,015,000 infants were screened and 7362 (0.7%) required a repeat test. Following this, 577 required a sweat test and 242 infants with CF were detected. At follow-up, 30 false-negative cases had emerged. There were also 80 cases of meconium ileus.

Since the change in protocol 189,000 infants have been tested and DNA analysis performed on 1968 (1.0%). Excluding nine infants with meconium ileus, there were 44 CF homozygotes detected by the test, and 111 neonates with a single ΔF508 mutation had a sweat test, which revealed that nine of them were affected. Six of the nine neonates with meconium ileus who had screening tests had positive results. There were no false-negative results apart from the three cases of meconium ileus.

New Zealand

Neonatal screening for CF was first introduced as a pilot study in 1981.[339] Over a 4-year period, 210,751 infants were screened using an IRT + IRT protocol, with 1399 infants (0.7%) requiring a second IRT test. The sweat test was performed on 101 (0.05%) and 72 infants with CF were found, including an unspecified number with meconium ileus. A further six cases with false-negative results have emerged on follow-up.

Normandy, France

A pilot study was carried out between February 1980 and May 1982 using a one-stage IRT protocol.[340] All births in the regions of Basse-Normandy and Haute-Normandy were included and samples were tested in Caen. Some 79,800 neonates were tested, 253 (0.3%) had raised IRT levels, 19 individuals with CF were detected and there was one false-negative. Three infants with meconium ileus were also tested and all had IRT values below the cut-off point.

North-east Italy

Since 1973, neonatal screening for CF has been available for births in three regions: Veneto, Trentino-Alto Adige and Friuli-Venezia Giulia. Until April 1981, a one-stage meconium protocol was used and, thereafter, a three-stage IRT + meconium + IRT protocol. All tests were carried

out in a single laboratory in Verona. Over the period 1973 to August 1988, 773,206 neonates were screened out of a total population of 1,148,345.[341] In total, 144 neonates of CF were diagnosed through screening and there were 37 with false-negative results. No information has been published on the proportion with false-positive screening results, or on the results separated according to the protocol used. However, results are available for the later period, September 1988 – August 1991, when only the IRT + meconium + IRT protocol was used.[342] At that time, 157,992 infants had been tested and repeat samples were requested in 405 (0.3%). Altogether 215 (0.1%) had a sweat test and 42 infants with CF were diagnosed. There have been no reports of false-negatives apart from all three individuals with meconium ileus who were tested.

North-east Netherlands

A neonatal screening programme for CF was instigated in 1973 using a one-stage meconium protocol.[343,356,357] All doctors in the provinces of Groningen, Friesland, Drenthe and Overijssel could request a test. The screening programme was halted in July 1979. By that time 94,043 neonates had been screened but 116,955 had not. Nineteen neonates with CF were detected by screening, there were five false-negative results and four infants with meconium ileus.

Queensland, Australia

Neonatal CF screening began in 1982. Samples from all infants in Queensland are tested in Brisbane with an IRT + IRT protocol modified in recent years so that a specific human monoclonal antibody test is used rather than the traditional assay. By 1986, 180,000 neonates had been screened, 0.8% needed a second IRT test and 0.04% were referred for diagnostic testing.[344] Insufficient details have been published from this study to determine the detection and false-positive rates.

South Australia

In December 1989, CF was added to the three other disorders (phenylketonuria, hypothyroidism and galactosaemia) routinely tested for in the South Australian neonatal screening programme based in Adelaide.[345,358,359] An IRT + DNA protocol was used with five mutations (ΔF508, ΔI506, G551D, G542X, R553X) which account for 80% of CFTR mutations found in the region. By 1993, 108,871 infants had been tested, of whom 1220 (1.1%) had raised IRT levels. DNA testing revealed 26 infants with CF and 89 neonates with only one identifiable mutation. Of those referred for diagnostic sweat testing, a further seven were confirmed. The positive predictive value of the screening programme overall was 29%; for those undergoing the sweat test it was only 8%.

Victoria, Australia

All neonates in the state are screened for phenylketonuria and congenital hypothyroidism and testing is carried out in a single laboratory in Melbourne.[346] In 1989, this programme was extended to include CF, with a pilot phase based on a two-stage IRT + IRT protocol which was replaced by an IRT + DNA protocol in July 1990. This uses ΔF508 only, which accounts for over 70% of CFTR mutations found in the region.

Over the period 1991–92, 130,708 neonates were screened using the IRT + DNA protocol; 1142 (0.9%) had a raised IRT levels, 23 were homozygotes, and 97 had a sweat test because they had one copy of ΔF508. This led to the diagnosis of 15 infants with CF. There were three false-negatives, two with raised IRT but not ΔF508 and one normal IRT. Nine infants had meconium ileus, of whom only two had positive screening tests.

Vienna, Austria

Since January 1988, a one-stage IRT protocol has been used in the six obstetric units within the city.[347] Up until April 1991, 19,992 infants had been screened, 119 (0.6%) had raised IRT levels and 11 infants with CF were found among the 88 who attended for a sweat test. An unspecified number of infants with meconium ileus were excluded and there has been one CF case with a false-negative result.

Western Pennsylvania, USA

From April 1987 to August 1991 neonates were screened for CF using an IRT + IRT protocol.[348] Over the 4-year period, 105,734 newborns were screened, 827 (0.8%) had initially raised IRT levels and 201 were positive after the second test. Twenty neonates with CF were confirmed on sweat testing and no false-negatives were reported.

Wisconsin, USA

Neonates throughout the state are routinely tested for six conditions (phenylketonuria, congenital hypothyroidism, galactosaemia, biotindiase deficiency, congenital adrenal hyperplasia and haemoglobinopathies). Since 1985, a randomised trial of neonatal CF screening has been operating in which all neonates are screened but only half are disclosed (see chapter 12).

For the first 6 years of the study, 220,865 neonates in the disclosed arm of the trial were screened using a one-stage IRT protocol.[262,349,360] During this period, 369 (0.2%) were referred for sweat testing of whom 46 neonates were confirmed as having CF. There were four false-negatives and 11 infants with meconium ileus, of whom five had positive screening tests. From July 1991, an IRT + DNA protocol was used based on ΔF508 only.[350] By the middle of 1994, a further 104,308 infants from the disclosure arm of the trial had been screened; 2056 (2.0%) had a raised IRT level, ten homozygotes were found and 123 (0.1%) with one mutation were referred for sweat testing (PM Farrell; personal communication, 1998). In total, 21 CF patients were detected by screening and there was one false-negative: six of the 22 had meconium ileus including the false-negative. Screening performance was improved following the introduction of a IRT + DNA protocol, increasing sensitivity to 93% and the positive predictive value to 19%.

Retrospective studies

Copenhagen, Denmark

Samples from 1081 neonates were tested anonymously using an IRT + DNA protocol with analysis for ΔF508 only.[361] In Denmark, 88% of disease-causing CFTR mutations are due to ΔF508.[306] Twelve (1.0%) neonates had a raised IRT level and the DNA test revealed that one of them was homozygous-affected and another was a carrier.

North-east Italy

Samples from the ongoing screening programme using a IRT + meconium + IRT protocol taken between January 1993 and December 1994 were tested for DNA mutations.[362] All samples with raised initial IRT results were tested for ΔF508, R1162X and N1303K. In Italy these three mutations account for only 61% of CFTR mutations.[363] Given this, a retrospective study was undertaken to evaluate a combined IRT + DNA + meconium + IRT protocol. Over the study period, 95,553 neonates were screened and 32 neonates with CF were detected using the existing protocol. DNA analysis revealed two further individuals who would have been detected with the extended protocol but the number requiring a sweat test would have increased from 59 to 89. Had the second IRT test been used in the full protocol, the detection rate would have been unchanged but only 78 sweat tests would have been needed. Following the pilot study, the combined protocol without the second IRT test was adopted for routine screening using a reverse dot–blot assay for 14 mutations which account for 85% of Italian CFTR mutations.[363]

Trent, UK

Samples from the IRT + IRT phase of the study were retrospectively tested for ΔF508.[333] A total of 1124 unaffected neonates had a raised initial IRT and normal second IRT result. There were 59 ΔF508 heterozygotes.

Vienna, Austria

Samples from 22 neonates with raised IRT levels in the one-stage IRT protocol were tested for ΔF508.[347] There was one homozygote and two heterozygotes, one of whom was affected by CF.

Western Pennsylvania, USA

For a 2-year period, samples from the IRT + IRT based programme were tested for DNA mutations.[348] All samples with initially raised IRT levels were tested, together with those for which other tests had been inconclusive. The principal mutation was ΔF508 but G551D and R553X were also determined if there was only one mutation. The results are not reported in a way which allows detailed examination but the authors conclude that had an IRT + DNA protocol been used instead of IRT + IRT, the false-positive rate would have decreased.

Summary of screening performance

The studies detailed above represent a wide variation in screening protocol, cut-off level, duration of follow-up and prevalence. While this may formally preclude pooling the results, this has been done in order to provide a guide to expected performance. To do this some *ad hoc* decisions on classification have had to be made and operational definitions adopted. Thus, cases of meconium ileus have been excluded from the calculation of the main performance indicators. This is because CF will be detected quickly following such a presentation so that screening is of little potential value. Also the screening results are not always available for meconium ileus cases in the published reports. In contrast, those cases in which clinical symptoms or information on family history emerged after the initial screening test have not been excluded. In some centres the protocol was not followed strictly such that, for example, a DNA analysis or sweat test may have been performed without waiting for a repeat IRT test. The screening result has been classified as positive in these cases.

***TABLE 20** Neonatal screening: summary performance indicators according to protocol*

Protocol	Rate	Numbers
First positive rate		
One-stage	2.3 per 1000	741:320,657
IRT + IRT	8.0 per 1000	25,993:3,269,097
IRT + DNA	12.0 per 1000	6765:565,187
Three-stage	7.2 per 1000	2046:283,965
False-positive rate		
One-stage	2.1 per 1000	665:320,575
IRT + IRT	0.3 per 1000	823:2,754,898
IRT + DNA	0.7 per 1000	384:532,745
Three-stage	0.6 per 1000	175:283,876
Positive predictive value		
One-stage	10%	76:741
IRT + IRT	45%	681:1504
IRT + DNA	25%	139:559
Three-stage	27%	86:322
Detection rate		
One-stage	90%	95:106
IRT + IRT	90%	781:866
IRT + DNA	97%	150:155
Three-stage	97%	86:89

Not all the studies reported complete information on the numbers of neonates involved at each stage in the screening process and the CF status. To calculate summary performance figures only those with the detailed data relevant to the performance indicator involved have been included. A summary is given in *Table 20*.

False-positive rate

In the centres undertaking screening with multi-stage protocols, there were a total of 34,804 positives on the initial IRT test, a rate of 8.4 per 1000. This was a much higher rate than the 2.3 per 1000 in the three studies with one-stage IRT protocols. Following the additional stages there was a decline in the positive rate: for those using an IRT + IRT protocol the decline was 92%, for IRT + DNA 93%, and for three-stage protocols 88%.

Taking all the studies which reported the relevant information together, there were 3,892,094 unaffected neonates tested, 2047 neonates required a sweat test and the false-positive rate was 0.5 per 1000. The false-positive rate for those using one-stage protocols was 2.1 per 1000, five times greater than the 0.4 per 1000 for multi-stage protocols.

In the DNA-based protocols, there were 6386 neonates with positive IRT tests excluding those shown to have CF by DNA or sweat tests. A total of 487 individuals were found to be carriers (1 in 14). Using these results and the retrospective studies, it can be seen from *Table 21* that this is an excess. On the basis of either the observed prevalence in the screened neonates or the expected prevalence from local population studies, the expected carrier frequency is 1 in 33 whereas the observed rate is 1 in 16. It is also noteworthy that in the retrospective study from Copenhagen, Denmark, the median IRT level in carriers was 26 μg/l compared with 20 μg/l in non-carriers and 380 μg/l in individuals with CF.[361] One explanation for the phenomenon is neonatal transitory hypertrypsinaemia in CF carriers; however, it is also possible that among the carriers there are some with additional undetected CFTR mutations.

Positive predictive value

A total of 3019 neonates were either referred for a sweat test or had a DNA diagnosis, including 982 with CF. The overall positive predictive value for the screening process was therefore 33% but this was critically dependant on the protocol. In one-stage protocols, because of the high false-positive rate the positive predictive value was 10% whereas for multi-stage protocols it was 40%. Again, as the false-positive rate was even lower for the IRT + IRT protocol so the positive predictive value was higher at 45% than for the IRT + DNA protocol at 25%. Considering only those referred for a sweat test as positive, the positive predictive value of the IRT + DNA protocol was reduced to 8%. The addition of the second IRT to this protocol in Trent improved this to 48% without noticeably increasing the number of false-negatives.

Detection rate

In total there were 1423 known cases of CF among the screened neonates. Of these all but 142 were detected as a result of screening, a detection rate of 90%. The detection rate in studies based on single-stage protocols was 90%, for IRT + IRT it was 90%, for IRT + DNA 97%, and for three-stage protocols 97%. Firm conclusions on the effect of repeat testing on detection cannot be readily made because of other differences between studies.

These detection rates are necessarily overestimates caused by the under-ascertainment and under-diagnosis of false-negatives, which may emerge with longer follow-up. None of the studies investigated those with negative screening results for an extended period. Most only reported false-negatives that had emerged during the course of

***TABLE 21** CF carriers in neonates with raised IRT levels found from neonatal screening studies*

Study	Type	Number tested	Number of carriers	Expected[a]	
				Number	Frequency
Trent[333]	Prospective	682	38	21	1 in 33
	Retrospective	1124	59	35	1 in 32
Brittany, France[335]	Prospective	366	38	10	1 in 38
New South Wales[338]	Prospective	1915	102	60	1 in 33
Normandy, France[364]	Prospective	130	9	3	1 in 42
North-east Italy[365]	Retrospective	656	45	15	1 in 45
Pennsylvania, USA[348]	Prospective	462	21	12	1 in 37
South Australia[345]	Prospective	971	65	33	1 in 30
Wisconsin, USA[349]	Prospective	155	18	3	1 in 44
All		**6461**	**395**	**193**	

[a] *From local population: based on the frequency of ΔF508 only, except for South Australia and North-east Italy*

the screening study. This was usually under 5 years, so the average follow-up period was under 3 years. Moreover, the reports do not generally indicate whether systematic and intensive surveillance methods were used to find false-negatives.

One way of assessing the extent of overestimation in the detection rate is to compare the overall CF prevalence in the study with that expected from known prevalence rates. Many reports specify an expected prevalence for the locality but for most this is probably unreliable. Indeed, in some regions the best available prevalence estimates are from the screening programmes themselves. However, in the UK the national CF survey[73] provides a good independent prevalence estimate of 1 in 2400. Taking all six UK programmes there were 471 infants with CF, including 70 with meconium ileus (15%) in 1,170,559 neonates screened, making an observed prevalence of 1 in 2500. If all the difference between the observed and expected rates is due to under-ascertainment of false-negatives, the detection rate for the UK centres would be 86%.

In all the studies combined, there were 229 infants with meconium ileus. Screening tests were said to have been performed on 70 and 28 had positive results, a detection rate of 40%.

Chapter 12

Efficacy of neonatal screening

Rationale

Apart from the 10–20% with meconium ileus, neonatal screening will bring forward the age at diagnosis in up to 90% of cases. The average age at diagnosis in the absence of screening is less than 12 months. Although this 'lead time' is not great, it does allow earlier intervention. The treatment provided at this very early stage is not only believed to be effective in itself but is claimed to confer long-term benefits that would be missed by delayed treatment. Thus it is argued that although CF *per se* leads to the secretory problems in various systems, provided the pathological processes caused by infection and malnutrition are kept under control, end-stage disease can be avoided.[366] This amounts to the concept of a 'point of no return', separating the stable CF disease of the young uninfected infant with CF from the stage of progressive lung function decline which follows the onset of chronic infection and inflammation. Hence, early intervention might lead to improved long-term morbidity and mortality since, it should be noted, even very young infants frequently have infection and inflammation by the time a clinical diagnosis of CF is made.[10]

The concept of early treatment may have an intuitive appeal for clinicians but that is not sufficient reason in itself to recommend screening. There is a need to establish direct, or at least indirect, evidence of efficacy. In several studies there have been attempts to provide such evidence by comparing clinical progress in screened and non-screened individuals. However, although this appears to be relatively straightforward there are several inherent biases which can lead to misleading and inconclusive results.

Potential biases

Test results and disease severity

It is possible that those missed by CF screening represent a subgroup with a different level of disease severity from CF in general. If so, comparison of outcome in screen-detected with missed cases would be biased. Insofar as screening has a detection rate far less than 100%, the comparison of screened with unscreened cases will also be biased, albeit to a lesser extent. This type of bias certainly occurs for those with meconium ileus since this relatively severe presentation is associated with normal IRT levels. However, CF neonates with meconium ileus are detected early in any case and our assessment should be limited to other types of case. Insofar as the IRT test measures a consequence of CFTR dysfunction, it is conceivable that those missed by the test will have a less severe course. Similarly, the DNA-based protocols use common mutations which are known to be related to a more severe phenotype. Therefore both testing modalities bias against screening.

Mode of presentation and presence of symptoms

Screening is actively directed at asymptomatic individuals. Thus it is necessarily the case that individuals with CF detected by screening will have fewer symptoms than those presenting clinically. Such a difference between screen-detected and clinically presented CF is not a proof of efficacy but rather a measure of potential lead time.

Another biased comparison arising from symptoms is the duration of hospitalisation in infancy. Some of the time spent in hospital will be while symptoms are being investigated with a view to arriving at a diagnosis of CF. Since this is not needed for those detected by screening, comparison with a control group will be biased. Avoiding a lengthy period of evaluation prior to diagnosis is an undoubted advantage of screening but the consequent reduced hospitalisation should not be seen as proof of better prognosis.

Age at presentation and disease severity

In the absence of screening, the age at diagnosis will depend on the presence and severity of clinical symptoms, and the standard of medical care. This has a large effect as is seen by comparing the mean age of diagnosis in CF patients with the severe and mild genotypes (see *Table 22*). Thus any control series with a restricted age distribution can be biased in a way which favours screening. Whenever possible the series should include late presenting cases.

Time and place differences

Comparison of screened and control cases of CF from different periods is subject to bias caused by

TABLE 22 Age of CF diagnosis according to mutation type (mild or severe): results from six studies

Study	Mild mutation[a]			Severe mutation[b]		
	Number	Mean age (years)		Number	Mean age (years)	
		Current	At diagnosis		Current	At diagnosis
Spain I[165]	15	12	8	82	8	2.2
Israel[167]	15	21	13	57	11	0.8
Cystic Fibrosis Genotype–Phenotype Consortium[163]	23	24	10	23	23	2.5
The Netherlands[367]	33	23	15	33	22	3.1
Spain II[166]	12	20	15	28	10	2.4
Canada[168]	22	18	11	22	17	1.0
All	120	20	12	245	13	1.9

[a] Patients with at least one mild mutation: R117H, 3849+10kbC→T, A455E, R334W
[b] Patients with two severe mutations: ΔF508 or W1282X

health improvements in the population generally, including well-documented advances in treatment and survival, and even a healthier life style.

Geographical comparisons can be biased for related reasons. The environmental factors which interact with the disease to alter outcome do not apply equally to all geographical localities. Also, improvements in treatment are not readily available in all locations at the same time. In particular, much of the improved prognosis seen in recent years has been attributed to the large specialist CF centres.[74,75,368,369] Such centres are not available to all CF patients and this will introduce bias. Moreover, there is a tendency to refer the more severe cases to specialist centres. Finally, organisational differences between CF centres may influence health outcomes. Clinics which allow patients of all ages to mix freely, compared with those which segregate older patients from newly diagnosed ones, have a significantly lower prevalence of Pseudomonas infections.[370]

Exclusions and omissions

Removing cases from any analysis always raises the possibility of bias and should be avoided. The following are some of the situations in which exclusions might bias studies of neonatal CF screening.

- Loss of patients to follow-up can bias the mortality rates, as the vital status of fitter and, hence, more mobile patients are more likely to be unknown to medical carers than patients with poor prognosis. This can be overcome by using the correct statistical technique of actuarial analysis whereby patients lost to follow-up only contribute to the survival curve up to the date when they were last known to be alive. The opposite bias occurs when series of adults are studied. They are necessarily survivors and will include a disproportionate number who have avoided chronic infection and/or may have milder genotypes.
- To avoid 'lead-time' bias, survival curves should start from the time of birth rather than age at diagnosis. Even if screening is ineffective, the average time of survival from the time of diagnosis will be greater for screened patients, the difference being the average lead time.
- CF patients presenting with meconium ileus are often screened, particularly in programmes screening for other neonatal conditions, unless they undergo surgery before the test can be done. Although in recent times the prognosis for such patients is similar to those without meconium ileus, this was not always so. There is no good reason to include patients with meconium ileus as they are all readily diagnosed. To be certain of avoiding bias, studies which exclude patients with meconium ileus from screening should also exclude them from the control series.

Minimising bias

The only fully unbiased study design would be an RCT of screening with a long-term follow-up and

mortality as the principal end-point. Such studies are impractical and virtually impossible to organise and, because of the timescale involved, interim decisions on whether or not to introduce screening will have to be made on the basis of evidence from potentially biased studies. Although all biases cannot be overcome, minimising them wherever possible will give more credence to the results.

Even randomised trials are not entirely without bias in practice. First, there is the question of what constitutes long-term follow-up. A study in The Netherlands found that 17% of patients attending a CF centre had been diagnosed after age 16 years.[367] Similarly, in a study in the USA, 14% of CF adults were reported as having been diagnosed after age 18 years.[171] A second problem is that the study may be analysed in a way that introduces bias. The data analysis should be performed on an 'intention-to-treat' basis so that those randomised to be screened are included in the screened arm of the trial even if they were not in fact screened. Similarly, anyone in the control arm who was actually screened should remain a control.

Outcome measures

Mortality can be assessed in terms of actuarial survival and age-standardised rates. With the current relatively high survival rate in CF, differences in these measures may not emerge for many years.

Lung condition is a strong predictor of mortality and could be used as a shorter-term surrogate end-point. This can be quantified by the extent of chronic lung infection and by measures of lung function. Both FEV_1 and FVC results, expressed as a percentage of the predicted levels for age and height, can be reliably measured after age 5 years. In younger children, maximum flow at functional residual capacity, thoracic gas volume and airways resistance are used. The extent of pathological lung damage can also be determined using numerical chest radiograph scoring systems, such as the Chrispin–Norman, the Brasfield and the Northern. These record the presence or absence of several radiographic features and, of the three systems, the Northern score correlates more consistently with respiratory function tests.[371] There are also blood indices of chest condition such as the IgG level.

There are two widely used measures of overall well-being, the Shwachman–Kulczycki and US National Institutes of Health (NIH) scores. Both are correlated to the long-term course of the disease but are subject to inter-observer variability and prone to bias through subjective interpretation.[372] Both tests are more reliable in older children and adults than in those aged under 5 years. In addition, care is needed when comparing individuals of different ages. Improvements in treatment since the Shwachman–Kulczycki and NIH scoring systems were devised may have reduced their prognostic value to some extent.

Apart from clinical measures of well-being, a few studies have attempted to construct quality-of-life measures for CF (see the 1997 review by Abbott and colleagues).[373] The aim is to encompass both physical and psychosocial functioning from the patient's perspective. However, so far all but one of the published studies have been based on too few patients to evaluate their validity and none have been used in the assessment of screening.

Other measures relating to the disease process itself include the extent of malnutrition, the frequency and extent of exacerbations, and associated hospitalisations. Particularly during childhood, body weight and height indicate the extent of malnutrition, as do serum vitamin A and E levels. In children the rate of growth is also an important indicator of lung function but it is confounded by dietary intake and pancreatic function.

All the outcomes discussed above relate to potential benefits of screening. Nonetheless, it is possible for the outcome to be worse in some patients as a result of screening. The possibility that the clinical diagnosis of CF might be delayed in those with false-negative screening results has been raised.[374]

RCTs

There have been two RCTs of neonatal CF screening.[334,375] The specific screening protocols for these are described in chapter 11.

Wisconsin, USA

In this study all neonates were screened but the disclosure of the test result was subject to randomised trial.[375] Equal numbers were allocated according to hospital number into an early diagnosis and a control arm. Those in the early diagnosis arm had immediate disclosure of results. In the control arm, disclosure took place when there was a positive family history, clinical symptoms or at 4 years of age. The screening protocol changed over the duration of the study (see chapter 11).

A total of 650,341 neonates were randomised and the first analysis of the results has been published.[376] There were 74 patients in the disclosed arm, 15 with meconium ileus, 54 detected through screening and a further five who were diagnosed before 4 years of age. In the control arm, 18 infants with meconium ileus and 40 others were diagnosed before the study was unblinded, and a further nine were disclosed at 4 years of age. The published analysis excluded those with meconium ileus and was restricted to a subset of patients, 56 in the disclosure arm and 40 controls, for whom parental agreement was obtained for follow-up.

There were many differences between the CF patients in the two arms of the study. Some were entirely expected and do not relate to efficacy. Thus, at the time of diagnosis, the length or height and weight of those in the disclosure arm were greater, after appropriate adjustment for age, than in the control arm. The growth and nutritional parts of the Shwachman–Kulczyzki scores showed similar effects.

Some differences between the study arms may be chance occurrences but could also be due to bias in the way that the subset for follow-up was selected. There were statistically significant differences in mutation type (a lower frequency of ΔF508 in controls; $p < 0.001$) and pancreatic function (more PS in the controls; $p < 0.04$).

The 96 cases were followed-up for up to 10 years and routine 3-monthly assessments were made to determine nutritional status and clinical severity. Taking the whole 10-year period, those in the disclosure arm had more favourable weights and heights. For example, children in the control arm were three times more likely to suffer from severe malnutrition, defined either as weight or height below the normal tenth percentile. However, it is not valid to include measurements carried out before the age of 4 years as they are dominated by the biased state at the time of symptomatic diagnosis (see above). The weight and height curves for the two arms progressively converge over time and it is unclear whether or not any apparent advantage after the age of 4 years is statistically significant. Such an analysis is not included in the published report.

Over a 9-year period, 123 patients were assessed at 6-monthly intervals for airway colonisation with *P. aeruginosa*. Prevalence and incidence rate of infection were similar in both cases and controls[376] but this may be due to patient management policy.[377] Unlike in the UK, it is not local policy to use prophylactic antibiotics, so that the opportunity to prevent chronic lung infection will have been lost for some screen-detected cases. Also, in the early years of the study chronically infected and newly diagnosed cases were not segregated, thus aiding the spread of infection.

Wales and West Midlands, UK

Between January 1985 and December 1989, neonates in these two Regions were randomised according to the week of birth into a screening and control arm. Over the study period, infants born on every second week were screened. A total of 474,142 births were randomised and at the end of the study period there were 144 patients with CF, 65 identified by screening, 13 screening false-negatives and 66 unscreened infants presenting with clinical symptoms. The excess of cases in the screening arm might be due in part to chance but in the expectation that relatively more late-presenting cases will eventually surface in the control arm.

The results of a follow-up study have been reported.[334] Siblings of patients with CF were excluded as were those presenting with meconium ileus, leaving 102 cases for analysis. However, it is not possible to draw any meaningful conclusions from this as the trial was not analysed on an intention-to-treat basis. Rather 58 screen-detected patients were compared with 44 patients who were either missed by screening (9) or detected following clinical presentation in the control arm (35). The results of this biased analysis are included in the next section on case–control studies. It should also be borne in mind that over the period of this trial, screen-detected patients were treated by general paediatricians with sub-optimal antibiotic regimes.

Case–control studies

There have been six case–control studies reported in the literature. The details of the screening protocol for each study are given in chapter 11. All the studies compared patients from a screening centre with controls who were detected clinically. Generally, all the cases are screen-detected but some include false-negatives. Also, in some of the studies the controls include patients missed by screening; however, more commonly they are clinically presenting cases arising before the advent of screening ('historical controls') or concurrent but from a locality where screening was not being performed ('geographical controls'). The details are summarised in *Table 23*.

***TABLE 23** Outcomes in screen-detected patients (cases) and clinically diagnosed patients (controls) from six studies*

Study	Length of follow-up (years)	Number of cases	Number of controls	Better in cases	Statistical significance (p value)
East Anglia[378]	1	7	5	Weight	not known
				Antibiotics	not known
				X-ray	< 0.01
North-east Italy[341]	10	144	75	*P. aeruginosa*	not known
				Shwachman	< 0.001
				Chrispin–Norman	< 0.001
				Height	< 0.001
				Weight	< 0.001
				Survival	< 0.000001
North-east Netherlands I[379]	11	23	27	Survival	0.05
North-east Netherlands II[379]	9	15	16	Height	NS
				Weight	NS
				Chrispin–Norman	< 0.01
				IgG	< 0.05
				Vitamin A	< 0.01
North-east Netherlands III[357]	12	13	24	Survival	< 0.05
				FEV_1	not known
				IgG	< 0.05
				P. aeruginosa	< 0.05
New South Wales I[380]	2	34	48	Hospitalisation	< 0.001
New South Wales II[381]	10	60	59	Shwachman	< 0.05
				FEV_1	< 0.05
				FVC	< 0.05
Queensland[344]	2	28	23	Antibiotics	< 0.025
				Weight	NS
Wales & West Midlands[334]	4	58	48	Hospitalisation	< 0.01
				Height	NS

NS, Not statistically significant

East Anglia, UK

A small series of seven individuals diagnosed by screening were compared with five controls diagnosed clinically before age 6 months.[378] The weight of screen-detected patients was greater for their age than that of controls throughout the first year of life. They also required fewer antibiotic treatments as a result of chest exacerbations and had better chest X-ray scores.

North-east Italy

This is the largest case–control study with a total of 144 screen-detected patients, 37 false-negatives and 75 unscreened controls.[341] The average age at diagnosis was 4 months for cases and 14 months for controls. At follow-up, six screen-detected patients (3%) and 14 controls (19%) had died. As the screen-detected individuals had been followed-up for a much shorter time than the controls, the cumulative mortality rate is biased but actuarial survival comparison showed that there was a long-term mortality difference.

Clinical evaluations were performed at 6-monthly intervals. At the end of the first hospital admission, the screen-detected individuals had more favourable indices than controls in the Shwachman–Kulkzycki score (81 compared with 70), Chrispin–Norman score (4.1 and 9.1), and weight standardised for sex and age (z scores of –1.4 and –1.8). Longer-term clinical analysis for up to 10 years has also been published. The screen-detected and false-negative patients are compared separately with controls showing a persistent difference between both the screened groups and the controls in Chrispin–Norman scores and in weight. Information on colonisation by *Pseudomonas* has been published for screen-detected patients only and the frequency is much lower compared with the unscreened group during the first 5 years of life.

North-east Netherlands

This study analysed medium-term survival and clinical outcome in 23 screened infants. The first analysis performed was a comparison with 27 unscreened controls born during the same period.[379] The latter group were not screened because they had been born in hospitals that had not joined the screening programme. Actuarial methods were used to compare survival up to 11 years of age, excluding patients and controls with meconium ileus (four and two, respectively). The survival curve was more favourable in the screened infants ($p = 0.05$); however, there was geographical bias since more of them had been treated in a specialised CF centre (47% compared with 25% of the controls).

Comparison of clinical outcome was made for 16 screened infants (four with meconium ileus) and 20 controls (two with meconium ileus) with up to 9 years follow-up. The screened patients spent more time in hospital than controls in the first 2 years of life.[382] At the beginning of the study period, the children were aged 5 years, on average, although the controls were about 6 months older and this may have distorted comparisons. At this time, the only statistically significant difference between the groups was in the Chrispin–Norman clinical score; Shwachman–Kulkzycki scores were higher in screened infants but not significantly so. At the end of the study period, when both groups were aged 8 years on average and 15 screened children and 16 controls were still alive, both scores showed significant differences. There were also reduced IgG ($p < 0.05$) and increased vitamin A levels ($p < 0.01$) in the screened children. Some of the differences at this stage may be the result of bias, since only survivors are being compared.

A second analysis of this case–control series was not performed on an intention-to-treat basis, rather screened false-negatives were excluded from the screened positives and considered together with the controls.[357] In addition, a further control group was included comprising 26 infants born after screening had stopped and followed-up for up to 7 years. Improvements in treatment over time would inevitably bias the new controls towards better prognosis but there is also evidence of ascertainment bias (a much lower frequency in those born during the period than in the other groups) which would select a more severe phenotype.

Lung function tests showed that the annual rate of decline was less for the screened group than controls. The rate of decline in FEV_1 was 1% per year after adjustment for age compared with 3% for the contemporary unscreened group and 2% for the later group. Trends in the levels of immunological markers were also compared and IgG levels were shown to increase with age in both control groups but not in the screened group. The screened and contemporary control groups were also compared in relation to the extent of chronic lung colonisation. The cumulative prevalence of *P. aeruginosa* was 15% (2:13) in the screened group and 52% (12:23) in controls ($p < 0.05$) but the prevalence of *S. aureus* was similar for both groups.

New South Wales, Australia

Patients in this study have been followed-up to 10 years of age. Preliminary analysis based on the first 2 years was concerned with the effect of screening on hospitalisation. In this there were 40 CF infants who had been screened for the disorder (cases) including six with meconium ileus; the controls were 56 infants (eight with meconium ileus) born before the screening programme began.[380] The following causes of admission were included: chest infection, intravenous antibiotics, malabsorption, diarrhoea, failure to thrive and intestinal obstruction (excluding meconium ileus).

The average duration of hospitalisation in patients with meconium ileus was high in both groups (16 and 12 days, respectively). Among those without meconium ileus, the control group had statistically significant longer average duration (4 and 27 days; $p < 0.01$) and more long stays (67% over 7 days compared with 21%; $p < 0.001$).

A newer analysis is now available for those with 10 years follow-up.[381] A total of 49 screened patients and 36 controls were compared for height, weight, lung function and clinical score. After adjustment for pancreatic insufficiency, cross-sectional data analysis showed that all outcome measures were better in screened individuals than in controls except for chest X-ray scores which were similar. The improvements in FEV_1, FVC and Shwachman–Kulczycki scores reached statistical significance with average differences of 9.4%, 8.4% and 5.3 points, respectively. Screened individuals were, on average, 1.7 kg heavier and 2.7 cm taller than controls but these differences were not statistically significant.

The authors considered changes in treatment over the study period that may have biased a comparison with historical controls. There were two changes, namely the introduction of a policy of unrestricted dietary management and more effective pancreatic enzyme supplementation. Dietary changes were introduced gradually, beginning shortly after screening started and the change in enzyme

regimen took place 1–2 years into the study. Consequently, had a concurrent control group been used it is likely that their prognosis would have been better than the historical controls. However, the authors argue that the change to a full fat diet will have disadvantaged the screen-detected patients. This arises because screening will have brought forward their date of diagnosis to before the change in policy and so they received a restricted diet. Had they remained unscreened the diet would have been *ipso facto* unrestricted. Similarly, improvement pancreatic enzyme therapy was introduced after most indiviiduals had been diagnosed through screening. Given that most of the historical control series had also been diagnosed by this time there is unlikely to have been a bias.

Queensland, Australia

A series of 28 infants without meconium ileus detected by screening in the first 4 years of the neonatal CF screening programme were compared with 23 unscreened infants born in the period immediately preceding this.[344] The comparison related to the first 2 years of life. Patient records, parental interviews and questionnaires were used to obtain information on general health. The controls were diagnosed on average 6 months after the cases: 21 (91%) were symptomatic at diagnosis compared with three (14%) of screen-detected individuals ($p < 0.001$); 39% required more than ten medical consultation to make the diagnosis. By age 2 years, all infants had been treated for chest infections but twice as many controls had three or more episodes (14 (61%) compared with nine (32%)). There was no difference between the groups in the number of hospital admissions, despite the fact that hospitalisation in the course of making the diagnosis does not appear to have been excluded. The weight of the controls was consistently lighter on average than that of the screen-detected infants at each 6-month interval throughout the follow-up period.

Wales and West Midlands, UK

As expected, the 44 controls were more symptomatic than the 58 screen-detected patients at the time of diagnosis which occurred, on average, at 51 weeks and 9 weeks, respectively.[334]

Multiple symptoms were seen in 75% of controls compared with 29% of screen-detected patients and none had minimal or no symptoms compared with 38%. There were three early deaths in the controls (two at 11 weeks and one at 22 months) and none in the screen-detected group. Before diagnosis more than twice as many controls required hospitalisation. Excluding admissions for diagnosis and initiation of treatment, in the first year of life there were an average of 1.3 admissions in the screen-detected group among cases and 3.2 in controls. The length of stay in hospital was, on average, 19 and 27 days, respectively ($p < 0.01$). There was no statistically significant difference between the groups in terms of height, weight, clinical score or blood indices at any age to a maximum follow-up of 4 years.

Other evidence on early treatment

Sibling pairs

This small study, carried out some time ago, is statistically powerful because it is a paired comparison. In 16 sibships, two children with CF were compared using several prognostic indicators. The younger sibling had a more favourable prognosis as measured by X-ray score, general score, residual lung volume and FEV_1.[383] Since the age of diagnosis is lower for the younger sibling, because doctors will have been alerted by the family history, this can be interpreted as proof of benefit. The improved prognosis may arise from early diagnosis and treatment. However, it can also be seen as a particularly well-matched type of controlled trial, in which genotype differences are eliminated. The study, nevertheless, has all the biases of historically controlled trials and, in addition, the younger child will have benefited from the parents previous experience in caring and rapid reaction to symptoms.

Survival according to age at diagnosis

The survival curves for CF cases in the Canadian Patient Data Registry have been analysed in relation to a number of known predictors of prognosis.[61] A proportional hazard model was used and this revealed an unusual pattern in relation to the age of diagnosis. Taking diagnosis under 6 months as the baseline, there was a statistically significant increased hazard for diagnoses in the second half of the first year of life. Thereafter the hazard declined until there was a statistically significant lower hazard for those diagnosed after age 10 years. One interpretation is that there are two competing effects: the beneficial consequence of early diagnosis and attenuation caused by late diagnosis of patients with milder forms of CF.

Randomised trial of early treatment

An alternative way of assessing the benefits of neonatal screening is to carry out a randomised trial in which prophylactic treatment is compared with symptomatic among screen-detected infants.

TABLE 24 Summary of clinical outcomes according to whether the study reported an improved or worse outcome in screened individuals

Hospital admissions[a]	Growth[b]	Lung status[c]	General health score	Survival
Improved				
Case–control*	RCT*	Case–control*	Case–control*	Case–control*
Case–control*	Case–control*	Case–control*	Case–control*	Case–control*
Treatment*	Case–control*	Case–control*	Siblings*	
	Case–control*	Case–control*		
	Case–control*	Case–control*		
	Case–control*	Case–control*		
	Case–control*	Treatment*		
	Siblings	Siblings		
Worse				
Case–control		RCT		
Case–control*				

[a] *Either number of hospitalisations or duration of admissions*
[b] *Either weight or height*
[c] *Either FEV_1, FVC, bacterial colonisation from culture, chest X-ray, frequency of lung infections or frequency of antibiotic treatment*
* *Statistically significant*

This mimics the situation in a trial of screening some extent except that the control group are known to have CF so they are likely to be treated earlier than otherwise. Thus the trial results are conservative.

Such a trial has been carried out for the anti-bacterial agent, flucloxacillin.[384,385] The 38 subjects, infants detected as part of the East Anglia screening programme, were randomised into a group of 18 to receive continuous prophylactic treatment and 20 controls who were to receive treatment only when they became symptomatic. Follow-up was for 2 years. Although the number included was small, statistically significant benefits were seen.

Outcome measures included pulmonary function, lung colonisation (*P. aeruginosa, S. aureus, H. influenza, S. pneumonia*), length and frequency of hospital admissions and clinical score. Pulmonary abnormalities were observed as early as 16 weeks in a proportion of infants from both groups. In the first year of life there was no significant difference between study arms in terms of respiratory function. There was a reduction on average in lung colonisation among those in the prophylactic arm. For one species, *S. aureus*, this reached statistical significance: affected specimens were found in three children during 6 infant months (17%) compared with 12 children during 32 infant months (60%). During the second year of life there was also a significant decrease in the number of hospital admissions (five compared with 12) and average duration of stay (2.2 and 6.4 days) in the prophylactic compared with the episodic treatment arm.

As often happens when only small numbers of patients are studied, the two groups were not entirely comparable. There was a chance allocation of fewer meconium ileus cases to the prophylactic arm (11% compared with 30%). However, this may not be important as within-group analysis showed that the hospital admission rates were similar in those with and without meconium ileus, as expected with modern treatment.

Summary

The balance of evidence for and against a beneficial effect from neonatal screening is summarised in *Table 24*. There is no completely unbiased evidence of a mortality benefit. Although two case–control studies found improved survival in screen-detected cases the results are subject to bias due to the use of historical and geographical controls. The Wisconsin RCT will eventually be provide an unbiased assessment of mortality but it will be some years before follow-up is long enough for a reliable survival comparison to be made.

Similarly there is evidence, albeit biased, that screening leads to a general improvement in

medium- to long-term morbidity as evidenced by the two clinical scoring systems used in both case–control studies and the study of sibling pairs. So far there is no information on clinical scores from the Wisconsin study.

Specific evidence of changes in the early natural history of the disorder resulting from screening is available in terms of lung status and growth. Nearly all the studies reported some improvement in indicators of lung involvement, such as infection rate and respiratory function. Although the opposite was found in the Wisconsin trial, this may be explained by a sub-optimal patient management policy, particularly in the early years of the study. Also the randomised trial of early compared with delayed anti-bacterial therapy shows that screening followed by early treatment could **in principle** improve lung status. Again, some improvement in growth, whether as increased height or weight, was reported in most studies. Unlike lung function, the Wisconsin trial did find that neonates randomised to the screening arm were relatively larger and heavier up to age 10 years, although the comparison is biased up to age 4 years.

Some but not all studies found that fewer or less prolonged hospital admissions were required in screen-detected cases compared with controls or in those having early treatment. However, only the Wales and West Midlands case–control study excluded hospitalisations for diagnostic work-up. This finding is thus a biased measure of treatment efficacy, although reduced hospitalisation prior to diagnosis is of itself a useful outcome of neonatal CF screening.

Taking all the studies together and considering the natural history of CF, we conclude that the balance of evidence favours neonatal screening. Although unbiased information on long-term prognosis is lacking there is a large body of information showing a short- to medium-term advantage. Although much of the evidence is potentially biased, it is consistent with unbiased results from the large randomised trial of screening and the trial of early treatment.

Chapter 13

Human and financial costs of screening

Hazards of prenatal diagnosis

Amniocentesis

The principal hazard of amniocentesis is miscarriage but the excess risk associated with the procedure is difficult to quantify precisely. Some 3–4% of mid-trimester pregnancies will miscarry without amniocentesis and, in a particular case of foetal loss following the procedure, it is only rarely possible to directly attribute the adverse outcome to the procedure. Cases of amnionitis or chronic amniotic fluid leakage would be attributable but these are relatively rare consequences. Studies of women having amniocentesis and matched controls are biased. When amniocentesis was new it was more available to women of higher social class with a lower miscarriage rate, so early studies were biased towards the safety of the procedure. Later, when the main indications were advanced age and abnormal biochemistry or ultrasound, factors associated with increased risk of miscarriage, the bias went the other way.

There has been only one randomised trial of amniocentesis.[386] The foetal loss rate in more than 2000 women randomised to have the procedure was 0.8% higher than in the control group. Although this is necessarily limited to the skills and experience of a single obstetric unit, the results provide the only unbiased estimate of hazard. Thus the excess miscarriage rate is usually quoted as between 0.5% and 1%.

CVS

The relative safety and efficacy of CVS at 9–12 weeks compared with second trimester amniocentesis has been studied in three major randomised trials, namely the Canadian, Danish and Medical Research Council European trials.[387–392] A systematic review of these studies has shown that transcervical CVS has a significantly higher foetal loss rate compared with amniocentesis; however, when performed transabdominally the rate is comparable.[393] The possibility of another important consequence of the procedure has also been raised, namely the causation of limb reduction defects in the foetus. An international registry of CVS organised by WHO has been monitoring the procedure so that any iatrogenic effects will not go unnoticed. The latest reported results based on 138,000 infants found no excess of limb reduction defects compared with data on the background prevalence of these conditions.[394]

Foetal blood sampling

The sampling of blood from the umbilical cord would appear to be more hazardous than either amniocentesis or CVS. However, there is no evidence that it results in more foetal losses than the other procedures. There have been no randomised trials but a meta-analysis has been performed on six series, each with more than 100 cases.[395] Patients with foetal pathological conditions were excluded because a compromised foetus is often the indication for carrying out this procedure. The miscarriage rate in the remainder was only 1.4% which is reassuringly low. There are other complications but they are not major.

Psychosocial aspects of CF screening

Outcome measures

Various outcomes have been measured that can be readily categorised into behavioural, cognitive and emotional. For example, the uptake rates for screening, prenatal diagnosis and termination of pregnancy are behavioural measures of acceptability. Similarly, completed family size among CF carriers is a measure of reproductive decision-making. Level of knowledge, understanding of information and ability to recall genetic risks comprise cognition, and emotion generally includes anxiety and stigmatisation.

Genetic screening: acceptance

The most common consistently elicited reasons for accepting or declining antenatal CF screening are given in *Table 25*. Those accepting did so mainly for reassurance about their own carrier status and to avoid the birth of an affected child. The overwhelming reason given for declining screening during pregnancy was unacceptability of pregnancy termination.

The low acceptance rates observed in population screening have been attributed to a number of practical and psychosocial reasons. For example, in some studies patients were required to make a

TABLE 25 *Inclusion in antenatal screening pilot programmes: most common reasons given for accepting and declining*[288, 291, 298, 299,301]

	Accepting	Declining
1	Reassurance of own carrier status	Abortion unacceptable
2	Prepare in case baby had CF	CF not common
3	Reassurance of baby's health	CF not serious
4	Avoid the birth of CF child	Low risk
5	To have all the tests during pregnancy	Partner would not want to be tested

TABLE 26 *Negative screening result: wrong interpretation in ten studies*

Study	Type	Time since test	Wrong meaning
'Not CF carrier'			
London[398]	Population	0 weeks	26 (6%)
		12 weeks	77 (17%)
New South Wales[316]	Population	12 weeks	147 (44%)
Cardiff[312]	Population	12 weeks	46 (25%)
Aberdeen[283]	Antenatal	~24 weeks	183 (19%)
Edinburgh[399 a]	Antenatal	3–4 years	164 (56%)
Copenhagen, Denmark[400]	Antenatal	1–2 years	131 (94%)
Montreal, Canada[314]	School	not known	not known (5%)
'No risk of CF baby'			
Aberdeen[283]	Antenatal	~24 weeks	179 (19%)
Manchester[301]	Antenatal	4 weeks	82 (29%)
Rochester, USA[298]	Antenatal	not known	64 (56%)
Leuven, Belgium[401]	Cascade	0–6 years	40 (58%)
Copenhagen, Denmark[400]	Antenatal	1–3 years	64 (46%)

[a] *Screen-negatives not contacted with results*

special visit to the centre for testing and this may have been a major disincentive. In one study, fear of stigma, perception of low risk and test sensitivity were all found to be predictors of uptake.[310] These factors suggest that, in this setting, the purpose of screening may be viewed purely as the identification of carriers, thus leading many to perceive the test as irrelevant and unimportant.[310]

Genetic screening: knowledge and understanding

The objective of providing clear and accurate information prior to testing is to increase knowledge, thus aiding informed decision making. With respect to genetic screening, however, pre-test information is complex as it necessarily includes facts on inheritance, laboratory procedures and risk calculations. This, and the fact that the general population has limited basic genetics awareness,[295,396] means that truly informed consent for CF screening is difficult to obtain.

The effectiveness of information giving has been measured in antenatal CF studies by comparing knowledge before and after testing. This has included clinical and genetic aspects of CF as well the ability to recall risk information in the short and long term. The use of leaflets and videos appears to improve general knowledge of CF and carrier testing;[299] however, understanding of the genetics of CF may be only superficial.[398]

Both knowledge and understanding are influenced by different antenatal testing strategies such as couple and stepwise screening. The latter necessarily involves disclosure of individual results while couple screening may also be non-disclosed. Although non-disclosure of individual results may be advantageous in terms of reduced counselling time and anxiety, there is evidence that it is less effective than stepwise screening at communicating information. For example, Miedzybrodzka and colleagues[283] found that 21% (53/253) of women who were given 'negative' couple screening results

were unaware that repeat testing would be required should they change partners. By comparison, only 6% (2/34) of carrier females screened by the step-wise approach misunderstood.

In an attempt to avoid giving false reassurance following negative results, many carrier screening studies used post-test as well as pre-test explanatory information regarding residual risks. The effectiveness of this has been measured by assessing the ability of those screened to recall risk information at different time intervals after receiving their results (*Table 26*). The general finding was that many women with negative results erroneously believe that they are definitely not carriers and that there was no risk of having an affected child. Also, as the time interval since the test increases, the tendency for both carriers and non-carriers to misunderstand the meaning of screening results increases.[398,399] This has the greatest implications for screening at school which relies on accurate recall of carrier status many years after being screened (see chapter 7).

Although the ability to recall risk information is of interest, it is of limited value as a measure of understanding. While external factors such as those discussed above can be influential, poor recall of the meaning of negative results is more likely to reflect the way in which people deal with risk information rather than poor understanding.[402–404] For example, the residual risk of a negative screening test may be regarded as insignificant and thus categorised as no risk. Hence, even with the most efficient screening programme, a number of individuals will appear to misconstrue risk information.

Genetic screening: anxiety and stigmatisation

A small percentage of those screened will experience anxiety as a direct result of screening. In one general population study, anxiety levels were found to be raised immediately after receipt of test results in carriers compared with non-carriers; however, after 3 months anxiety levels returned to normal.[311,398] With regard to antenatal testing, women identified as carriers in step-wise screening also experience increased anxiety. Again, this is only transient, with the anxiety level returning to normal following a negative result for their partner.[283,284] Even this short period of anxiety can be avoided by performing non-disclosure couple screening. Given that non-disclosure couple screening is less effective in terms of knowledge and understanding, couple sampling and disclosure of individual results may be a more suitable compromise.[405] Although it has been suggested that this may be problematic for discordant couples in whom only one partner is found to be a carrier,[289] recent studies suggest that the residual risk is well tolerated[295] and acceptable, insofar as prenatal diagnosis is not requested[298] and future reproductive planning is unaltered.[401]

With regard to carrier couples identified during antenatal screening, little is known about the long-term psychosocial effects. Although it is known that the uptake of prenatal diagnosis and termination of affected pregnancy are both relatively high at the time of initial screening, information on future pregnancies and reproductive behaviour of these couples is limited. Only the Edinburgh screening programme has follow-up information on subsequent pregnancies. Of 34 carrier couples identified by screening, prenatal diagnosis was performed once in 27 couples, twice in five couples, three times in one couple and four times in another (DJH Brock; personal communication, 1997).

CF carrier screening may also lead to stigmatisation of carriers, in terms of personal relationships and health or life insurance. Of 35 carriers identified through one screening programme, none reported feeling stigmatised in their personal relationships; they were actually able to discuss their results with friends and family.[283,284,400] With insurance, however, the situation is more complicated. In the USA, where health insurance is mostly private, there have been reports of CF carriers being discriminated against on these grounds.[406,407] This stems from a general misunderstanding amongst many American insurance companies that carriers for recessive disorders are pre-disposed to the condition.[408] The current position within the UK is equally unclear. Although insurance companies do not require applicants to undergo genetic testing, those who have already been screened are requested to disclose their results. At present the information is not acted upon but there is concern for the future as to how insurance companies will interpret these.

Neonatal screening: acceptance

The amount and quality of pre-test information given to parents is probably limited. The assumption is that most parents would want their baby to be tested. As with other screening situations, however, informed consent is a prerequisite for neonatal testing, particularly since the participant in this case is unable to provide informed consent themselves.

Neonatal screening: knowledge and understanding

There is very little information on parents' knowledge of neonatal screening. In one study, parents whose babies had false-positive results were questioned about CF screening 6 weeks after the birth. Despite being given a explanatory leaflet prior to testing, only 25% of parents reported that they were aware that their child had been screened for CF.[409]

In the Trent screening programme, which incorporates DNA testing, the policy is to inform the GP when a carrier is identified and to suggest referral of the parents to the genetic counselling service. Follow-up of these cases revealed that of 45 carrier children, 25 referrals had been made, eight parents had had informal counselling from their GP and eight had not been informed of the results by the GP.[410] It is not known why some GPs decided against contacting parents or, indeed, what was discussed during the informal GP sessions. An alternative approach has been adopted in South Australia. In addition to offering genetic counselling and testing, all parents of carrier infants are given a written report and information leaflet. On follow-up, 61% of parents (34 couples, nine mothers and one father) of 63 carrier neonates identified by the screening programme, had undergone DNA testing.[345]

Neonatal screening: anxiety and false-positives

Both repeat sampling because of a raised IRT and sweat testing generate parental anxiety. Even following a negative sweat test, a small number of parents harbour residual anxiety about the infant's health. The Wisconsin study found that, despite intensive consultation, 5% of parents whose infants had a false-positive IRT result still believed their children might have CF when questioned a year later.[409] In more general neonatal screening programmes, 36% of parents of infants with false-positive screening tests had concerns about their child's health 2 weeks after receiving a normal sweat test result; in half of these the concern was said to be great.[411] This type of reaction may be more pronounced in neonatal screening programmes which incorporate DNA testing. For parents of infants identified as having one CFTR mutation, there may be continuing anxiety either relating to the actual screening experience itself or about the presence of an undetected mutation. Indeed, it has been shown that 1 year after screening, parents of neonates with false-positive results were more defensive and emotionally suppressed compared with age-matched controls.[412]

The burden of responsibility which requires parents to retain genetic information and accurately explain the implications of carrier status to their child at an appropriate age must also be considered.

Neonatal screening: anxiety and true-positives

The diagnosis of CF in a child is invariably very distressing for the parents. An early and relatively quick diagnosis through neonatal screening may well be less anxiety-provoking than that following clinical presentation. For many, screening removes the long period of anguish and uncertainty while non-specific symptoms are being investigated. In addition, parental confidence in doctors, which is of the utmost importance in the management of any chronic illness, may be jeopardised by a long delay in diagnosis. One adverse consequence of screening and early asymptomatic diagnosis is that it may lead a period of increased anxiety for parents as they wait for the first signs of illness.

With regard to the parent–child relationship, it is debatable whether diagnosis following neonatal screening is more or less detrimental than clinical diagnosis. One school of thought is that the delay from the onset of symptoms to diagnosis may help parents adjust to the reality of the child's condition.[413] Alternatively, diagnostic delay may lead to parental over-protectiveness and, as already mentioned, disillusionment with the medical profession. This issue has been addressed by two studies. In one study, the results were biased by parental self-selection and age differences.[413] For example, those who decided not to participate in the study may have done so because of over-protectiveness. Bias from age differences could be caused by the possible effect of the child's age on the parent–child relationship. In the second study, from the Wisconsin trial, parental stress in those diagnosed early through screening was compared with that in those presenting clinically. Although the differences did not reach statistical significance, 45% (9/20) of parents from the early diagnosis group had stress scores warranting psychiatric referral compared with only 5.9% (2/33) in the control group.[412]

Neonatal screening: reproductive decision making

Carrier couples with an affected child identified through neonatal screening are discussed below. Little is known about the reproductive intentions of those whose child had a false-positive screening result or was found to be a carrier. In one study,

parents of 104 infants were interviewed shortly after receiving a normal sweat test result.[409] Of these, 69% stated that they had not changed their reproductive plans, 8% had and 22% were uncertain.

Implications for the family

Carrier testing

As with all life-threatening conditions, the psychological burdens of CF on the affected individual and family are great. Added to this is the burden of genetic information which some might feel should be shared amongst relatives. While some families may encourage open discussion, the existing taboo and emotional barriers around genetic conditions means that many will find this difficult.[414] Thus, although close relatives may be aware of the clinical aspects of CF, poor communication may lead them to develop preconceived erroneous ideas about genetics which prevents them from seeking carrier testing. Siblings, for example, may instinctively believe that they are definitely carriers or perceive that birth order or other family myths control their carrier status. Some believe that CF is more common than it actually is and that being a carrier has implications for health.[415] Further psychological barriers to carrier testing include feelings of guilt, anxiety, self-stigmatisation and resentment.[416] Alternatively, the practical aspect of where to obtain testing may act as a barrier. On a wider scale, the perception that CF is restricted to the nuclear family may hinder the passage of genetic information to second degree relatives and beyond. Thus, when cascade screening is reliant solely on relatives informing each other, it becomes of limited value.[318] By educating and providing testing, genetic counselling seeks to overcome some of these difficulties and to improve the process of genetic information sharing.[417]

Views on screening and prenatal diagnosis

In general, parents and siblings support CF screening. One study asked 268 family members about the preferred screening method.[418] The popular preference was pre-conceptional screening (43%), closely followed by neonatal (49%); however, antenatal screening was not given as an option.

Before prenatal diagnosis became available for CF, approximately 63% of parents with an affected first-born child abstained from having more children.[397] It was predicted that this proportion would decrease with the introduction of prenatal diagnosis, particularly since surveys of parents yielded uptake rates based on reproductive intentions ranging from 44% to 84%.[397,419–421] Indeed, the most recent figures from a neonatal screening programme in the USA suggest that only 30% of such couples now avoid future pregnancy.[422] However, this decline is not entirely due to the availability of prenatal diagnosis. Only 11–29% actually opt for this during subsequent pregnancies.[397,421–423] One possible reason may relate to improvements in health care, as the perceived burden of CF in early childhood decreases.[421]

Surveys of affected individuals and close family members suggest that only about half find termination of an affected pregnancy acceptable (see *Table 27*). In these families, where the parents have opted for prenatal diagnosis and CF has been confirmed, termination rates vary considerably, from 42% to 100%.[276,277,426,427] However, there have been instances in which termination of pregnancy has occurred without prenatal diagnosis having been performed, because of the parents' fear of having another affected child.[421] It is not known how common an occurrence this is.

TABLE 27 *Termination of CF pregnancy: acceptability in affected families*

Study	Relationship to CF patient	Acceptable (number)
Colorado, USA[419]	Parent	39% (16)
Cardiff[420]	Parent	52% (15)
North-west Thames[418]	1st, 2nd or 3rd degree	58% (138)
London[424]	Parent	44% (70)
Leeds[425]	Patient themselves	23% (10)
Leeds[425]	Parent	84% (66)
San Francisco, USA[416]	Sibling or partner	21% (18)
New England, USA[423]	Parent	20% (45)
All		53% (373)

Even though few parents appear to opt for prenatal diagnosis in future pregnancies, one neonatal screening programme has reported a reduction in birth prevalence. Over a 10-year period there was an annual decrease in the number of cases in East Anglia amounting to 7–8% on average.[428] The reasons for this decline are unclear but could be attributable to both under-ascertainment of cases and the effects of genetic counselling on reproductive options.

Financial costs

Measures of cost

The cost-effectiveness of a screening programme is usually expressed as the average cost of detecting one affected individual. This can be readily estimated from the separate unit costs for each component of the screening process. For example, with antenatal genetic screening there is information giving, DNA testing, genetic counselling and prenatal diagnosis (some would also include the cost of therapeutic abortion). The average cost is computed from the estimated detection and false-positive rates, prevalence and uptake rates. Sensitivity analysis can then be used to vary one or more of the component costs and determine what aspect of the programme is most price-sensitive.

A more complex approach is to carry out a cost–benefit analysis in which the benefits are also measured and valued. In the example of antenatal screening, the most common method is to estimate the costs averted by detecting and terminating affected foetuses; these would include treatment and loss of output. Although useful, this method does not take into account the additional less quantifiable benefits and disbenefits of screening such as reassurance, information, and anxiety.

Two other approaches have also been used to address some of these issues by posing hypothetical questions to volunteers regarding their potential decision making. The first is willingness-to-pay which measures the value or benefit of a healthcare intervention such as screening. With this approach, individuals are simply asked the maximum amount they would be willing to pay for a particular service. The second approach is the standard gamble technique which measures preferences under conditions of uncertainty.[429] Here the subject is informed about the intervention and then taken through the decision-making process, during which they are asked which option they would take given a range of outcome values. In the context of antenatal screening, such outcomes would be the information obtained from screening regarding carrier status as well as the outcome of prenatal diagnostic testing. The average outcome value which elicits indifference in the decision whether or not to accept screening is then determined.

Antenatal screening

Cost-effectiveness in the UK has been estimated under a variety of conditions.[430] In this analysis, the four components of the screening process were costed both from the literature and from the Leeds pilot study; the results were summed according to the specific screening strategy adopted (sequential or couple), mutations tested for (ΔF508 alone or multiple), proportion of carriers detected (70–95%) and uptake (55–95%). Baseline assumptions were made about: the proportion with missing information on carrier status from previous pregnancies (20%), the proportion changing partners between pregnancies (20%), and the uptake of prenatal diagnosis (100%). Sensitivity analysis was performed by varying these assumptions.

Under the baseline assumptions, the cost of sequential screening ranged from £40,000 to £90,000 per affected pregnancy detected. Non-disclosure couple screening was more expensive, ranging from £46,000 to £104,000. From the sensitivity analysis, a 10% change in the assumed proportion with missing information from a previous pregnancy altered the cost by £4000. A 10% change in the proportion with new partners had a similar effect but only for non-disclosure couple screening, and the cost changed directly in proportion to the uptake of prenatal diagnosis. In some parts of the UK, the additional cost of non-disclosure couple screening may not be as great, as only a small proportion change partners between pregnancies.[431]

Counselling is an important component of screening but unless an appropriate level is adopted the cost will be insupportable.[430] Thus, in the analysis two levels were used: for all those who would potentially be screened a low-cost option (basic information in a leaflet with midwife or GP reinforcement), and for carrier couples expensive genetic counselling (by a nurse specialist). Others may consider that with sequential screening the expensive option is also needed for carrier women whose partners have yet to be tested.[286,297]

This analysis has been updated using information from the present review. In addition, more recent estimates for the unit cost of the DNA test have been used (the previous sensitivity analysis showed

that this was the single most important component of overall cost). The gene frequency and mutation detection rates are those given in *Table 14*, the uptake rate for screening is the overall value of 75% seen in UK pilot studies, and the uptake rate for prenatal diagnosis is assumed to be 89% for all the pilot studies combined. For laboratories able to commit to purchase 5000 tests per annum, Zeneca Diagnostics Ltd would be able to provide their Elucigene kits at £12 per test including royalties and licences (R Ferrie; personal communication, 1998). INNO-LiPA kits would be slightly more expensive: Murex Biotech Ltd could supply them at £15 per test excluding royalties but with a free automatic processor (B Coull; personal communication, 1998). The reagents needed for DNA extraction from 5000 samples would cost about £10,000, with 1.25 whole time equivalent technicians, at £12,000 a year, would be needed to carry out the tests. On-costs at 25% of salaries, plus a further 25% for administration, brings the total to about £89,000 or £18 per sample. *Table 28* shows that the cost per affected pregnancy detected for disclosure couple screening would range from £46,000 in Scotland, Wales and the north of England to £53,000 elsewhere in the UK. The cost is similar for Ashkenazi Jews but considerably higher than average for Asians and Blacks. Given the low cost per sample tested using the commercial multimutation kits, there would be no advantage in testing for ΔF508 alone using in-house reagents. One UK study was carried out in an area of high ΔF508 frequency and tested for this mutation alone.[291] The estimated cost per test was £16 (in 1995); the inclusion of licence fees would increase this but they would be offset by reduced cost with higher throughput testing.

A previous UK study estimated the cost per affected birth detected by antenatal screening to be £143,000.[432] Four non-UK studies obtained the following estimates: $450,000–860,000, depending on the screening strategy, in the USA, or £284,000–542,000 converted, using purchasing power parity in 1995;[433] $326,000 or £205,000, in Israel;[434] $1,658,000 or £1,043,000, in the USA;[435] and £177,000–213,000 in The Netherlands.[436] There are four main reasons for these estimates being much higher than ours. First, the cost of the DNA test in three studies was greater: $125 (£79), $72 (£45) and $100 (£62).[433–435] Second, one study assumed that only 30% of affected pregnancies detected would be terminated.[435] Third, two studies included the indirect costs of travelling to have the test and work loss.[434,436] Last, three of the studies considered screening for just one pregnancy,[432,433,435] which effectively doubled the cost.

On the basis of our estimates, the cost of screening for CF in the UK is higher than the cost for other established antenatal screening services although not markedly so. For example, maternal serum screening for Down's syndrome costs about £30,000 per affected pregnancy detected.[437–439]

A simple cost–benefit analysis shows that our estimated cost of screening is much less than the averted treatment costs. In a UK adult CF centre during the financial year 1989–90, the average cost of care was reported to be £8200 per patient.[440] In 1996, the average annual treatment cost for children in the UK was £10,567, ranging from £5310 in the 0–5 years age group to £12,945 in those aged over 15 years.[441] Four studies of lifetime treatment costs, including during childhood, from other countries are summarised in *Table 29*. A study in The Netherlands in 1991 estimated that the average cost of CF treatment in that year was £10,900; 42% for hospital care, 20% for home care and 37% for medication.[442] Costs were under £10,000 per year until age 15 years, when they began to rise steadily reaching a peak of £37,000 in the pre-terminal phase. Taking survival into account, based on the Dutch CF Register, the authors estimated a total of £410,000 over the lifetime of an average patient. Because the

TABLE 28 *Cost (£000s) per affected pregnancy detected according to the screening strategy and situation*

Situation	Sequential	Couple	
		Disclosure	None
Scotland, Wales & northern England	46.2	46.1	53.3
Elsewhere in UK	53.1	52.9	61.3
Asians	1400	1400	1600
Ashkenazi Jews	54.9	54.7	63.4
Blacks	1200	1200	1400

TABLE 29 Estimated lifetime costs of treating CF from four studies

Study	Year of costing	Average survival (years)	Treatment (000s)		
			Cross-sectional*	Lifetime	
				Crude	Discounted (rate)
The Netherlands[442]	1991	27	£10.9	£409	£164 (5%)
Israel[434]	1993	25	–	–	US$297 (5%)
USA[435]	1993	29	–	–	US$243 (5%)
USA[443]	1997	30	US$49	–	US$800 (3%)

* In the year of costing

financial benefits of preventing a CF case are cost-savings in the future, it can be argued that the costs should be discounted in order to determine their present value. When the Dutch study costs are discounted at a rate of 5% per annum, the estimated lifetime costs of treatment were reduced to £164,000. In a recent study in the USA, in 1997, much higher average treatment costs of $49,000 were found; this amounts to a total of $800,000 over a 30-year lifespan after discounting.[443] The two other studies[434,435] found intermediate discounted life-time costs. Thus all the estimates for the average lifetime treatment costs are less than our estimate for the average cost of detecting a case of CF by antenatal screening.

Four other cost–benefit analyses have been carried out for antenatal CF screening. Of these, three concluded that benefits exceed costs, provided that the carrier frequency is sufficiently high,[434,444] or that the cost of the DNA test is under $130 (£82).[445] The fourth study suggests that, under most assumptions, there would be no net savings.[435]

Non-economic benefits have been measured in two further studies. Willingness-to-pay analysis in Aberdeen suggests that this is about £18–22,[446,447] which is comparable to the £20 actual cost per woman offered screening in our analysis. This is much less than the £98 currently being charged to private patients by two UK laboratories. The laboratories both carry out a 'mail-order' service so it is not possible to derive population-based information on rate of uptake at this price.

A standard gamble technique was used to measure benefit in a small study, also in Aberdeen.[448] The authors determined what values for outcomes such as the foetal loss rate due to invasive prenatal diagnosis would be tolerated for the avoidance of CF. The results showed that 69% would prefer antenatal screening under reasonable assumptions about outcomes.

Estimated cost of neonatal screening

There have been four cost-effectiveness studies, three from the USA and one in the UK. The component costs are simpler to evaluate than with antenatal screening as they are largely limited to the cost of the laboratory determinations. It is reasonable to assume that the blood spot sample is already available and paid for by the existing neonatal screening tests. Although few include a sweat test, the cost is high and it could be argued that only sweat tests done on those with false-positive screening tests should be included, since the affected neonates would have been tested eventually anyway.

In the UK as part of a comprehensive evaluation of costs for existing and proposed neonatal screening methods, three of the laboratories currently screening for CF submitted cost estimates.[449] The total cost of screening 100,000 neonates for CF, including the sweat test and other investigations to confirm CF, was estimated to be £151,000 for the programme using an IRT + DNA + IRT protocol. For the two programmes using IRT + IRT, the costs were £154,000 and £218,000, respectively. This analysis yields a cost of £4400 per neonate detected or £6400 per case, excluding those with a family history or meconium ileus that would have been detected early without screening. The three American studies derived remarkably similar cost estimates of $6200,[450] $7000 for IRT only or IRT + DNA protocol,[451] and $10,000 for IRT or $11,000 for IRT + DNA.[439]

Cost–benefit analysis is more difficult to assess. Some financial gain must accrue from obviating the need for multiple investigations and hospitalisations as part of the differential clinical diagnosis

of CF during childhood. A small improvement in long-term lung condition attributable to early intervention in the first few months of life would translate into a large financial benefit over a lifetime. However, such an improvement remains to be established and it would need to be set against the additional cost of prophylaxis resulting from increased longevity. If screening does improve prognosis, the greater gain will be in terms of quality of life, which is particularly difficult to quantify.

These studies assume that neonatal screening is being carried out in the absence of genetic screening. If routine antenatal screening were to be introduced, the birth prevalence would be reduced and, consequently, the cost per affected neonate detected through routine neonatal screening would be much higher. Assuming 75% uptake of antenatal screening, 74% detection rate (Scotland, Wales and northern England), and 89% uptake of prenatal diagnosis, the prevalence would decrease by 49%. With a 64% detection rate (for other parts of the UK), the decrease would be 43%. So the cost of neonatal screening would rise to £9000–10,000 per neonate detected or £13,000–15,000 per case, excluding those with a family history or meconium ileus.

Another possibility is to restrict neonatal screening to infants where:

(a) antenatal screening was refused
(b) a carrier couple was detected but prenatal diagnosis was refused
(c) only the mother was found to be a carrier.

The birth prevalence in those offered neonatal screening would be higher than in the general population and so the cost per neonate detected would be reduced. Under the above assumptions, the birth prevalence would be 1 in 770 for Scotland, Wales and northern England and 1 in 810 elsewhere. So the cost of neonatal screening would reduce to £1400–1500 per neonate detected. If a family history of CF did not alter the acceptability of antenatal screening and the test results, the cost of neonatal screening would be £2100–2200 per neonate detected, after excluding cases with a family history or meconium ileus. In practice, both screening uptake and carrier frequency are likely to be higher in those with a family history; hence, excluding those cases will increase the cost somewhat.

Ethics

Screening tests differ from other tests performed in normal medical practice in that they are carried out proactively rather than in response to symptoms or concerns raised by patients. Although the efficacy of normal medical tests may not be quantifiable, they can be justified by the patient's need. This is not the case for screening tests; it is only ethically justifiable to offer screening if the full consequences can be predicted.

Genetic screening

Additional questions are raised by genetic screening. The Nuffield Council for Bioethics[452] have produced a report on the ethical issues that arise for the individual and for society as result of genetic screening. The NHS Central R&D Committee[453] endorsed the Nuffield report and raised its own concerns. They expressed the view that genetics differs from other biomedical areas in that it involves not only the individual being tested but other family members.

The Department of Trade and Industry,[454] in response to public concern and a report of the House of Commons Select Committee on Science and Technology, advised the Government to establish an Advisory Committee on Genetic Testing. The Committee has so far only published one report,[455] in which a code of practice and guidelines are suggested for genetic testing services supplied direct to the public. For CF testing, the code of practice relates to quality assurance for equipment, reagents and staff, and a voluntary registration scheme has been introduced.

Neonatal screening

There are three main ethical concerns with respect to neonatal screening. First, given the technology available and the feasibility demonstrated by antenatal screening, is it justifiable to wait until couples have already given birth before offering a diagnosis? Second, sweat testing is unable to discriminate between milder and more severe phenotypes. Given that it may be years before CF becomes evident in some cases, how does this affect their care and what is the effect on the family. Third, carrier identification in neonatal screening breaks confidentiality.

Chapter 14

Conclusions and recommendations for further research

This report brings together a large body of literature on CF. The natural history, genetics and prevalence of the disorder are summarised and various meta-analyses have been undertaken on published studies relating to screening. These form a sound basis for health planners to judge whether or not CF screening is worth considering and which screening approach to take. In the light of the review, we have come to a number of conclusions, including recommendations for future research. In doing so we have also taken account of other opinion leaders, including a statement on the subject recently published by the US NIH.[443] This statement by a consensus development panel recommended pre-conceptual and antenatal CF screening for couples without a family history of the disorder but did not support neonatal screening.

Genetic screening

It is clear that although prognosis has improved in recent decades, CF remains a severe condition. New treatment techniques, including those based on gene therapy, may eventually improve matters further but this cannot be guaranteed. Genetic screening has the potential to reduce the burden of disease. From what is known about the gene frequency of different CFTR mutations, it is possible to predict the discriminatory power of genetic screening. Of the various possible strategies, only antenatal screening has been shown to be practical. There is now a considerable body of experience in the practical delivery of antenatal screening services for CF. This has demonstrated that antenatal screening is acceptable to pregnant women and their partners, with minimal psychological burden. The cost of screening is not substantially greater than for other antenatal screening programmes and is far outweighed by the savings in averted treatment costs. Given this evidence we therefore conclude that:

- antenatal screening should be offered routinely to women and their partners in all maternity units.

No recommendations are made about the specific screening protocol to be used; this will depend on the population being screened and the preference of the local health professionals involved. Sequential screening is more cost-effective than couple screening but causes more anxiety; disclosure couple screening is a reasonable compromise. Screening Asians and Blacks is 20 times more expensive than other groups but localities with a small ethnic minority may find it more practical to test routinely.

Although antenatal screening is the most practical genetic screening approach, pre-conceptional testing would provide more reproductive options. Therefore, we conclude that:

- screening should be made available in the family planning clinic or GP setting for couples without a family history who request the test because a pregnancy is planned.

There are two situations in which assisted reproduction could increase the risk of a CF pregnancy. To avoid this we conclude that:

- screening should be made available in assisted reproduction units for those having ICSI and for sperm donors.

The cost-effectiveness calculations assume that DNA testing is carried out in high throughput laboratories with bulk purchasing of reagents. We therefore conclude that:

- in order to minimise cost and concentrate expertise, laboratories with an annual throughput of at least 5000 CF tests carry out screening tests.

Neonatal screening

There has been considerable experience worldwide with neonatal CF screening. This has provided reliable information on performance of different testing protocols. A two- or three-stage protocol based on IRT and DNA tests will yield

a high detection rate, a very low false-positive rate and favourable positive predictive value at extremely low cost.

However, the central question as to whether early diagnosis through screening improves long-term prognosis remains unanswered. There is no **direct** unbiased evidence that any long-term benefits will derive from screening. The two randomised trials of screening may eventually yield the required evidence but this is likely to take over a decade. Although direct evidence is lacking, there is a large body of **indirect** evidence, of which some but not all is biased, that is consistent with a long-term benefit. Moreover, there are short-term advantages, such as avoidance of prolonged hospitalisation for differential diagnosis that are certain to accrue. In view of the low false-positive rate and the low cost of the test we conclude that:

- each purchasing health authority could consider providing neonatal CF screening, either in combination with antenatal screening or alone.

Some health authorities may consider that, given the lack of proven long-term benefit, the cost is too high, even when used in combination with antenatal screening. Others may judge that although the apparent short-term benefits are not sufficient justification for neonatal screening, they should nonetheless introduce the service in the expectation of some as yet unproven long-term advantages.

Recommendations for further research

This review has revealed a number of gaps in the knowledge base. Hence the following recommendations are made for further research and development in the area of neonatal screening.

- The Wales and West Midlands trial is reanalysed on an intention-to-treat basis.
- Research is undertaken to determine the psychological and medical consequences for CF carriers and their families who have been found by DNA analysis of blood spots with high IRT levels.
- In order to investigate the efficacy of specific early treatments, more RCTs of screen-detected CF patients should be undertaken.

There is widespread concern that screening is often undertaken without due consideration of the facilities required to fully inform patient choice. We therefore recommend:

- the development and evaluation of innovative methods of providing information on genetic screening for CF, such as a free video, a national help-line and an Internet site
- an audit procedure to ensure that parents give informed consent to neonatal screening for CF and other disorders.

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This report was identified as a priority by the Population Screening Panel.

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