Health Technology Assessment 2004; Vol. 8: No. 12

APPENDICES ONLY

Go to main text

Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review

A Pandor, J Eastham, C Beverley, J Chilcott and S Paisley

March 2004

Health Technology Assessment
NHS R&D HTA Programme

How to obtain copies of this and other HTA Programme reports.
An electronic version of this publication, in Adobe Acrobat format, is available for downloading free of charge for personal use from the HTA website (http://www.hta.ac.uk). A fully searchable CD-ROM is also available (see below).

Printed copies of HTA monographs cost £20 each (post and packing free in the UK) to both public **and** private sector purchasers from our Despatch Agents.

Non-UK purchasers will have to pay a small fee for post and packing. For European countries the cost is £2 per monograph and for the rest of the world £3 per monograph.

You can order HTA monographs from our Despatch Agents:

– fax (with **credit card** or **official purchase order**)
– post (with **credit card** or **official purchase order** or **cheque**)
– phone during office hours (**credit card** only).

Additionally the HTA website allows you **either** to pay securely by credit card **or** to print out your order and then post or fax it.

Contact details are as follows:

HTA Despatch
c/o Direct Mail Works Ltd
4 Oakwood Business Centre
Downley, HAVANT PO9 2NP, UK

Email: orders@hta.ac.uk
Tel: 02392 492 000
Fax: 02392 478 555
Fax from outside the UK: +44 2392 478 555

NHS libraries can subscribe free of charge. Public libraries can subscribe at a very reduced cost of £100 for each volume (normally comprising 30–40 titles). The commercial subscription rate is £300 per volume. Please see our website for details. Subscriptions can only be purchased for the current or forthcoming volume.

Payment methods

Paying by cheque
If you pay by cheque, the cheque must be in **pounds sterling**, made payable to *Direct Mail Works Ltd* and drawn on a bank with a UK address.

Paying by credit card
The following cards are accepted by phone, fax, post or via the website ordering pages: Delta, Eurocard, Mastercard, Solo, Switch and Visa. We advise against sending credit card details in a plain email.

Paying by official purchase order
You can post or fax these, but they must be from public bodies (i.e. NHS or universities) within the UK. We cannot at present accept purchase orders from commercial companies or from outside the UK.

How do I get a copy of *HTA on CD*?

Please use the form on the HTA website (www.hta.ac.uk/htacd.htm). Or contact Direct Mail Works (see contact details above) by email, post, fax or phone. *HTA on CD* is currently free of charge worldwide.

The website also provides information about the HTA Programme and lists the membership of the various committees.

Appendix 1

Electronic bibliographic databases searched

1. Biological Abstracts
2. CINAHL
3. Cochrane Controlled Trials Register (CCTR)
4. Cochrane Database of Systematic Reviews (CDSR)
5. Database of Abstracts of Reviews of Effectiveness (DARE)
6. EBM Reviews
7. EMBASE
8. Health Management Information Consortium (HMIC)
9. HTA Database
10. MEDLINE
11. PreMEDLINE
12. Science Citation Index

Appendix 2

Other sources consulted

1. Bandolier
2. Canadian Co-ordinating Centre for Health Technology Assessment (CCOHTA)
3. Current Controlled Trials (CCT)
4. Current Research in Britain (CRiB)
5. Department of Health
6. eGuidelines
7. INAHTA (International Network of Agencies for Health Technology Assessment) Clearinghouse
8. MRC (Medical Research Council) Funded Projects Database
9. National Guideline Clearinghouse (NGC)
10. National Research Register (NRR)
11. NCCHTA (National Coordinating Centre for Health Technology Assessment)
12. Research Findings Register (ReFeR)
13. ScHARR Library Catalogue
14. Scottish InterCollegiate Guideline Network (SIGN)
15. Trent Working Group on Acute Purchasing
16. Turning Research into Practice (TRIP) Database
17. Wessex DEC (Development and Evaluation Committee) Reports
18. West Midlands DES (Development and Evaluation Services) Reports

Appendix 3

Search strategies used in the major electronic bibliographic databases

Biological Abstracts

1985–2001
SilverPlatter WebSPIRS
Search undertaken November 2001

#1 (neonat* or newborn*) and screen*
#2 (inborn error*) and metabolism
#3 (mass or ms or tandem) and spect*
#4 #1 and #2 and #3

CDSR and CCTR

2001 (Issue 4)
The Cochrane Library, Update Software (CD-ROM version)
Search undertaken November 2001

#1 NEONATAL SCREENING*:ME
#2 (NEONAT* NEAR SCREEN*)
#3 (NEWBORN* NEAR SCREEN*)
#4 (#1 OR #2 OR #3)
#5 MASS-SCREENING*:ME
#6 INFANT-NEWBORN*:ME
#7 (#5 AND #6)
#8 (#4 OR #7)
#9 METABOLISM-INBORN-ERRORS*:ME
#10 ((INBORN NEAR ERROR*) NEAR METABOLISM)
#11 (#9 OR #10)
#12 (#8 AND #11)

CINAHL

1982–2001
Ovid Biomed
Search undertaken November 2001

1 exp health screening/
2 exp infant, newborn/
3 1 and 2
4 (neonat$ adj2 screen$).tw
5 (newborn$ adj2 screen$).tw
6 or/3-5
7 exp metabolism, inborn errors/
8 (inborn adj2 error$).tw
9 or/7-8
10 6 and 9
11 spectrum analysis/
12 (mass adj2 spect$).tw
13 (ms adj2 spect$).tw
14 (tandem adj2 mass).tw
15 or/11-14
16 10 and 15

CRD Databases (NHS DARE, EED, HTA)

CRD website – complete databases
Search undertaken November 2001

(neonat and screen) or (newborn and screen)/All fields AND (mass and spect) or (ms and spect) or (tandem and spect)/All fields

EMBASE

1980–2001
SilverPlatter WebSPIRS
Search undertaken November 2001

#1 'newborn-screening' / all subheadings
#2 neonat* near2 screen*
#3 newborn* near2 screen*
#4 'mass-screening' / all subheadings
#5 'newborn-' / all subheadings
#6 #4 and #5
#7 #1 or #2 or #3 or #6
#8 explode 'inborn-error-of-metabolism' / all subheadings
#9 inborn near2 error* near2 metabolism
#10 #8 or #9
#11 #7 and #10
#12 explode 'mass-spectrometry' / all subheadings
#13 mass near2 spect*
#14 ms near2 spect*
#15 tandem near2 mass
#16 #12 or #13 or #14 or #15
#17 #11 and #16

MEDLINE

1966–2001
Ovid Biomed
Search undertaken November 2001

1 neonatal screening/
2 (neonat$ adj2 screen$).tw
3 (newborn$ adj2 screen$).tw
4 mass screening/
5 exp infant, newborn/
6 4 and 5
7 or/1-3, 6
8 exp metabolism, inborn errors/
9 (inborn adj2 error$).tw
10 or/8-9
11 7 and 10
12 exp spectrum analysis, mass/
13 (mass adj2 spect$).tw
14 (ms adj2 spect$).tw
15 (tandem adj2 mass).tw
16 or/12-15
17 11 and 16

Science Citation Index

1981–2001
Web of Science
Search undertaken November 2001

Title=(neonat* or newborn*) and screen* and (inborn error*) and metabolism and (mass or ms or tandem) and spect*; DocType=All document types; Languages=All languages; Databases=SCI-EXPANDED; Timespan=All Years

Appendix 4

Search terms used in Ovid MEDLINE (1995–January 2002) for each of the individual inborn errors of metabolism

3-Methylcrotonyl-CoA carboxylase deficiency

1 (methylcrotonyl adj2 carboxylase adj2 deficien$).tw
2 methylcrotonylglycinuria.tw
3 methyl crotonyl glycinuria.tw
4 methylcrotonyl glycinuria.tw
5 mcc$ deficien$.tw
6 (methyl crotonyl adj2 carboxylase adj2 deficien$).tw
7 carbon-carbon ligases/df
8 or/1-7

3-Hydroxy-3-methylglutaryl-CoA lyase deficiency

1 3 hydroxy 3 methylglutaryl coa lyase deficien$.tw
2 (hmg adj2 lyase deficien$).tw
3 hydroxymethylglutaricaciduria.tw
4 (hydroxy adj2 methyl adj2 glutaric adj2 aciduria).tw
5 hydroxymethylglutaryl-coa synthase/df
6 3 hydroxy 3 methylglutaryl coenzyme lyase deficien$.tw
7 (hmg adj2 coenzyme adj2 deficien$).tw
8 (hmg adj2 coa adj2 deficien$).tw
9 oxo-acid-lyases/df
10 or/1-9

Arginase deficiency

1 argininaemia.tw
2 argininemia.tw
3 ((arginase or arg1) adj2 deficien$).tw
4 hyperargininemia.tw
5 hyperargininaemia.tw
6 hyperargininemia/
7 or/1-6

Argininosuccinic aciduria

1 argininosuccinic aciduria.tw
2 argininosuccinicaciduria.tw
3 (argininosuccinase or argininosuccinate or asl or asal) adj3 deficien$).tw
4 or/1-3

Carnitine translocase deficiency

1 exp carnitine acyltransferases/df
2 carnitine acylcarnitine translocase deficien$.tw
3 cac deficien$.tw
4 cact deficien$.tw
5 or/1-4

Citrullinaemia

1 citrullinaemia.tw
2 citrullinuria.tw
3 (argininosuccinate adj2 (synthase or synthetase) adj2 deficien$).tw
4 aas deficien$.tw
5 citrullinemia.tw
6 citrullinemia/
7 or/1-5

CPTI

1 carnitine o-palmitoyltransferase/df
2 carnitine palmitoyltransferase deficien$.tw
3 cpt deficien$.tw
4 or/1-3

CPTII

1 carnitine o-palmitoyltransferase/df
2 carnitine palmitoyltransferase deficien$.tw
3 cpt deficien$.tw
4 or/1-3

GAI

1 glutaryl coa.af
2 glutaricaciduria.af

3 gcdh.af
4 ga 1.af
5 glutaric aciduria.af
6 glutaric acidemia.af
7 glutaric acidaemia.af
8 or/1-7

GAII

1 glutaryl coa.af
2 glutaricaciduria.af
3 gcdh.af
4 ga 1.af
5 glutaric aciduria.af
6 glutaric acidemia.af
7 glutaric acidaemia.af
8 or/1-7

Homocystinuria

1 homocystinuria/
2 homocystinuria.tw
3 hypermethioninaemia.tw
4 hypermethioninemia.tw
5 ((cystathionine or cbs) adj2 deficien$).tw
6 or/1-5

Hyperornithinaemia

1 hyperornithinemia.tw
2 hyperornithinaemia.tw
3 (ornithine adj2 aminotransferase deficien$).tw
4 ((oat or okt) adj2 deficien$).tw
5 hoga.tw
6 or/1-5

Isovaleric acidaemia

1 isovaleric acidaemia.tw
2 isovaleric acidemia.tw
3 isovaleric aciduria.tw
4 ivd deficien$.tw
5 (isovaleric acid adj2 dehydrogenase deficien$).tw
6 (isovaleryl adj2 dehyrogenase deficien$).tw
7 isovalericacidemia.tw
8 isovalericacidaemia.tw
9 or/1-8

LCHAD

1 trifunctional protein deficien$.tw
2 exp 3-hydroxyacyl coa dehdrogenase/df
3 multienzyme complexes/df
4 (long chain adj4 dehydrogenase deficien$).tw
5 lchad.tw
6 hadh deficien$.tw
7 (hydroxyacyl adj3 dehydrogenase).tw
8 long chain.tw
9 7 and 8
10 hydroxydicarboxlicaciduria.tw
11 hydroxydicarboxlic aciduria.tw
12 or/1-6, 9, 10-11

Long-chain hydroxyacyl-CoA dehydrogenase deficiency

1 trifunctional protein deficien$.tw
2 exp 3-hydroxyacyl coa dehdrogenase/df
3 multienzyme complexes/df
4 (long chain adj4 dehydrogenase deficien$).tw
5 lchad.tw
6 hadh deficien$.tw
7 (hydroxyacyl adj3 dehydrogenase).tw
8 long chain.tw
9 7 and 8
10 hydroxydicarboxlicaciduria.tw
11 hydroxydicarboxlic aciduria.tw
12 or/1-6, 9, 10-11

Maple syrup urine disease

1 maple syrup urine disease/
2 maple syrup urine disease.tw
3 msud.tw
4 branched chain ketoaciduria.tw
5 keto acid decarboxylase deficien$.tw
6 or/1-5

MCAD

1 mcad.af
2 acadm.af
3 mcadh.af
4 medium chain acyl coa.af
5 or/1-4

Methylmalonic acidaemia

1 methylmalonic acidemia.tw
2 methylmalonic acidaemia.tw
3 methylmalonic aciduria.tw
4 methylmalonicaciduria.tw
5 mcm deficien$.tw
6 or/1-5

Ornithine carbamoyltransferase deficiency

1 ornithine carbamoyltransferase deficiency disease/
2 ((ornithine carbamoylase or ornithine carbamoyltransferase or OCT or ornithine transcarbamylase) adj2 deficien$).tw
3 or/1-2

Phenylketonuria

1 exp phenylketonurias/
2 phenylketonuria$.tw
3 or/1-2

Propionic acidaemia

1 propionic acidemia.tw
2 propionic acidaemia.tw
3 propionic aciduria.tw
4 propionyl coa carboxylase deficien$.tw
5 pa deficien$.tw
6 or/1-5

Tyrosinaemia type I

1 tyrosinemias/
2 tyrosinemi$.tw
3 tyrosinaemi$.tw
4 ((fumarylacetoacetase or fah) adj2 deficien$).tw
5 or/1-4

VLCAD

1 vlcad.tw
2 (very long chain adj3 dehydrogenase deficien$).tw
3 or/1-2

Appendix 5

Search filters used in Ovid MEDLINE for the individual inborn errors of metabolism

Epidemiology

1 epidemiology/
2 exp morbidity/
3 exp mortality/
4 exp survival analysis/
5 exp disease susceptibility/
6 disease progression/
7 natural history.tw
8 epidemiolog$.tw
9 or/1-8

Diagnosis

1 exp "sensitivity and specificity"/
2 di.xs
3 du.fs
4 specificity.tw
5 or/1-4

Treatment

1 randomized controlled trial.pt
2 dt.fs
3 tu.fs
4 random$.tw
5 or/1-4

Screening

1 exp mass screening/

Appendix 6

Reference list of excluded studies: tandem mass spectrometry

Abdenur JE, Chamoles NA, Guinle AE, Schenone AB, Fuertes AN. Diagnosis of isovaleric acidaemia by tandem mass spectrometry: false positive result due to pivaloylcarnitine in a newborn screening programme. *J Inherit Metab Dis* 1998;**21**:624–30.

Abdenur JE, Chamoles NA, Schenone AB, Guinle A, Fusta M, Gaggioli D. Supplemental newborn screening of aminoacids (AA) and acylcarnitines (AC) by electrospray tandem mass spectrometry (ESI-MS/MS): experience in Argentina. *J Inherit Metab Dis* 2000; **23** (Suppl 1): 13.

Albers S, Waisbren SE, Finkelson LA, Korson MS, Bailey IV, Levy HL. A model for medical evaluation of expanded screening with MS-MS. *J Inherit Metab Dis* 2000;**23** (Suppl. 1): 6.

Albers S, Marsden D, Quackenbush E, Stark AR, Levy HL, Irons M. Detection of neonatal carnitine palmitoyltransferase II deficiency by expanded newborn screening with tandem mass spectrometry. *Pediatrics* 2001;**107**:E103.

Bartlett K, Pourfarzam M. Tandem mass spectrometry – the potential. *J Inherit Metab Dis* 1999;**22**:568–71.

Biberoglu G, Hasanoglu A, Tumer L, Ezgu FS, Genc B. Screening inborn errors of metabolism from dried blood spotting using electrospray tandem mass spectrometry. *J Inherit Metab Dis* 2001;**24** (Suppl 1): 4.

Carpenter KH, Wilcken B. Neonatal diagnosis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and implications for newborn screening by tandem mass spectrometry. *J Inherit Metab Dis* 1999;**22**:840–1.

Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001;**50**(RR-3):1–36.

Chace DH, DiPerna JC, Mitchell BL, Sgroi B, Hofman LF, Naylor EW. Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. *Clin Chem* 2001;**47**:1166–82.

Charrow J, Goodman SI, McCabe ERG, Rinaldo P. Tandem mass spectrometry in newborn screening. *Genet Med* 2000;**2**:267–9.

Clayton PT, Doig M, Ghafari S, Meaney C, Taylor C, Leonard JV, *et al*. Screening for medium chain acyl-CoA dehydrogenase deficiency using electrospray ionisation tandem mass spectrometry. *Arch Dis Child* 1998; **79**:109–15.

Goyens P, Van Thi HV, Herremans N, De Meirleir L, Gerlo E, De Laet C, *et al*. Diagnosis of methylmalonic aciduria (MMA) by neonatal screening using tandem mass spectrometry. *J Inherit Metab Dis* 2001;**24** (Suppl 1):7.

Ito T, van Kuilenburg ABP, Bootsma A, Haasnoot AJ, van Cruchten A, Wada Y, *et al*. Rapid screening of high risk patients for disorders of purine and pyrimidine metabolism using HPLC–electrospray tandem mass spectrometry of liquid urine or urine soaked filter paper strips. *Clin Chem* 2000;**46**:445–52.

Jensen UG, Brandt NJ, Christensen E, Skovby F, Norgaard-Pedersen B, Simonsen H. Neonatal screening for galactosemia by quantitative analysis of hexose monophosphates using tandem mass spectrometry: a retrospective study. *Clin Chem* 2001;**47**:1364–72.

Johnson DW. A rapid screening procedure for the diagnosis of peroxisomal disorders: Quantification of very long-chain fatty acids, as dimethylaminoethyl esters, in plasma and blood spots, by electrospray tandem mass spectrometry. *J Inherit Metab Dis* 2000; **23**:475–86.

Kwon C, Farrell PM. The magnitude and challenge of false-positive newborn screening test results. *Arch Pediatr Adolesc Med* 2000;**154**:714–18.

Leonard JV, Dezateux C. Screening for inherited metabolic diseases in newborn infants using tandem mass spectrometry. *BMJ* 2002;**324**:4–5.

Liebl B, Fingerhut R, Roschinger W, Muntau A, Knerr I, Olgemoller B, *et al*. [Model project for updating neonatal screening in Bavaria: concept and initial results]. *Gesundheitswesen* 2000;**62**:189–95.

Liebl B, Nennstiel-Ratzel U, Fingerhut R, Olgemoller B, Roscher A. Audit of MS-MS based newborn screening model program in Bavaria. Interim report. *J Inherit Metab Dis* 2000; **23** (Suppl 1): 5.

Meyburg J, Schulze A, Kohlmuller D, Hoffmann GF, Linderkamp O, Mayatepek E. Differences in acylcarnitine profile between term and preterm infants. *J Inherit Metab Dis* 2000; **23** (Suppl 1), 12.

Mills KA, Mushtaq I, Johnson AW, Whitfield PD, Clayton PT. A method for the quantitation of conjugated bile acids in dried blood spots using electrospray ionization-mass spectrometry. *Pediatr Res* 1998;**43**:361–8.

Mushtaq I, Logan S, Morris M, Johnson AW, Wade AM, Kelly D, *et al*. Screening of newborn infants for cholestatic hepatobiliary disease with tandem mass spectrometry. *BMJ* 1999;**319**:471–7.

Naughten ER, Yap S, Mayne PD. Newborn screening for homocystinuria: Irish and world experience. *Eur J Pediatr* 1998;**157** (Suppl 2):S84–7.

Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol* 1999;**14** (Suppl 1):S4–8.

Poplawski NK, Ranieri E, Harrison JR, Fletcher JM. Multiple acyl-coenzyme A dehydrogenase deficiency: diagnosis by acyl-carnitine analysis of a 12-year-old newborn screening card. *J Pediatr* 1999;**134**:764–6.

Presto-Elgstoen KB, Zhao JY, Anacleto JF, Jellum E. Potential of capillary electrophoresis, tandem mass spectrometry and coupled capillary electrophoresis-tandem mass spectrometry as diagnostic tools. *J Chromat A* 2001;**914**:265–75.

Rashed MS, Rahbeeni Z, Ozand PT. Screening blood spots for argininosuccinase deficiency by electrospray tandem mass spectrometry. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):170–3.

Roe CR, Wiltse HE, Sweetman L, Alvarado LL. Death caused by perioperative fasting and sedation in a child with unrecognized very long chain acyl-coenzyme A dehydrogenase deficiency. *J Pediatr* 2000;**136**:397–9.

Sander J, Janzen N. Optimized laboratory technics for neonatal screening for phenylketonuria and other hyperphenylalaninemias. *Monatsschrift Kinderheilkunde* 1999;**147**:480–5.

Sander J, Janzen N, Sander S, Melchiors U, Steuerwald U. Neonatal screening for inborn errors of metabolism using tandem mass spectrometry. *Monatsschrift Kinderheilkunde* 2000;**148**:771–7.

Sansom C. Tandem mass spectrometry: the tool of choice for diagnosing inborn errors of metabolism. *Mol Med Today* 1999;**5**:95.

Schulze A, Kohlmueller D, Mayatepek E. Sensitivity of electrospray–tandem mass spectrometry using the phenylalanine/tyrosine-ratio for differential diagnosis of hyperphenylalaninemia in neonates. *Clin Chim Acta* 1999;**283**:15–20.

Shigematsu Y, Hata I, Kikawa Y, Mayumi M, Tanaka Y, Sudo M, *et al*. Modifications in electrospray tandem mass spectrometry for a neonatal-screening pilot study in Japan. *J Chromat B* 1999;**731**:97–103.

Shigematsu Y, Fujisawa K, Hata I, Mayumi M, Tanaka Y, Sudo M. Newborn screening by tandem mass spectrometry; results of pilot study and retrospective analysis in Japan. *J Inherit Metab Dis* 2000;**23** (Suppl 1):6.

Smith WE, Millington DS, Koeberl DD, Lesser PS. Glutaric acidemia, type I, missed by newborn screening in an infant with dystonia following promethazine administration. *Pediatrics* 2001;**107**:1184–7.

Sweetman L. Newborn screening by tandem mass spectrometry: gaining experience. *Clin Chem* 2001;**47**:1937–8.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Public Health Med* 1998;**20**:331–43.

Wilcken B, Wiley V, Keow GS, Carpenter K. Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry. *J Pediatr* 2001;**138**:581–4.

Wood JC, Magera MJ, Rinaldo P, Seashore MR, Strauss AW, Friedman A. Diagnosis of very long chain acyl-dehydrogenase deficiency from an infant's newborn screening card. *Pediatrics* 2001;**108**:E19.

Appendix 7

Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Hoffman *et al*., 2001[9] (Abstract)	*Screening* **Study type** NR **Period** NR **Country** USA (Wisconsin Newborn Screening Program)	*Screening* Tandem MS	*Screening* **Sample type** NR **Age at sampling** NR **Ethnicity** NR	*Screening* Acylcarnitine profile (confirmatory test method not reported)	*Screening* Acylcarnitines: cut-off for majority maintained at mean +4 SD; however, some acylcarnitines adjusted to mean ≥ 5 SD. In addition to acylcarnitine levels, used multiple concentration ratio-based criteria to the abnormal profiles for certain disorders	*Screening* **Total** Approx. 50,000 **True positives** 5 **False negatives** NR **False positives** 8 **True negatives** 49,987 **Sensitivity** Could not be calculated with certainty **Specificity** 99.984% **PPV** 38.462%	The authors screened approx. 50,000 specimens using tandem MS and identified 5 confirmed cases of inborn errors of metabolism (4 MCAD and 1 SCAD deficiency)
Lin *et al*., 2001[19]	*Screening* **Study type** NR **Period** NR **Country** Taiwan	*Screening* ESI/tandem MS	*Screening* **Sample type** Dried blood spots **Age at sampling** NR **Ethnicity** NR	*Screening* Amino acid profile PKU/HPA, Tyr, MSUD, Hcys, ASD, ALD Acylcarnitine profile PPA/MMA, MCD, IVA, GAI, GAII, MCAD, LCAD/ VLCAD, HMG	*Screening* The cut-off level for each compound or ratio was set as the mean +4SD (shown below) **Disorder** / Ref. conc. (μM) Mean (SD) PKU/HPA 49.9 (10.36) Tyr 85.5 (35.72) MSUD 144.5 (33.82) Hcys 24.2 (8.68) ASD 14.1 (9.15) ALD 14.1 (9.15) PPA/MMA 2.25 (1.22)	*Screening* **Total** 2100 **True positives** 2 **False negatives** NR **False positives** 27 **True negatives** 2071 **Sensitivity** Could not be calculated with certainty **Specificity** 98.713% **PPV** 6.897%	Based on the upper cut-off levels for each compound or ratio (mean +4 SD) 29 infants were suspected to have inherited metabolic disorders. Further evaluations showed that only 2 infants (1 identified as HPA and one with IVA) actually had inborn errors of metabolism (true incidence 0.09%, with false-positive rate of 1.29%)

continued

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
					SCAD/EMA 0.22 (0.121) MCD 0.138 (0.06) IVA 0.01 (0.007) HMG 0.13 (0.079) GAI 0.016 (0.025) GAII 0.146 (0.09) MCAD 0.185 (0.125) LCAD/VLCAD 0.028 (0.044) (Confirmation based on GC/MS analysis of urine)		The authors reported that the majority of false positives using tandem MS occurred in ALD and GAI categories. These were attributed to the use of only one indicator in screening these disorders. In addition, determination of the upper cut-off level of citrulline level for ALD screening is difficult; however, this can be accurately diagnosed by measuring elevations of ASA and its anhydrides in urine If the cut-off levels were lowered to mean +3 SD, 67 false positives would have been identified (specificity: 97%; PPV: 3%) The authors also compared the results from the tandem MS screening with those for traditional screening methods (only for PKU and Met) and found no false negatives in the 2100 studied samples using the current cut-off value

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Muenzer *et al.*, 2001[8] (Abstract)	*Screening* *Pilot study* **Study type** NR **Period** August 1997 to March 1999 **Country** USA (NeoGen Screening Inc., Pennsylvania)	*Screening* *Pilot study* Tandem MS	*Screening* *Pilot study* **Sample type** NR **Age at sampling** NR **Ethnicity** NR	*Screening* *Pilot study* Selected amino acid, organic acid and fatty acid oxidation disorders	*Screening* *Pilot study* NR	*Screening* *Pilot study* **Total** 194,384 *Screening* *Pilot study* **True positives** 31 **False negatives** NR **False positives** 228 **True negatives** 194,125 *Screening* *Pilot study* **Sensitivity** NA **Specificity** 99.883% **PPV** 11.969%	Metabolic disorders were confirmed for 31 infants. Disorders included MCAD deficiency (*n* = 14), long-chain fatty acid oxidation disorder (*n* = 1), HPA (*n* = 6), hypermethioninaemia (*n* = 1), citrullinaemia (*n* = 3), argininosuccinic aciduria (*n* = 1) and 5 organic acidurias

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV		Comments
	Screening programme **Study type** NR **Period** August 1999 to June 2000 **Country** USA (North Carolina Newborn Screening Program)	*Screening programme* Tandem MS	*Screening programme* **Sample type** NR **Age at sampling** NR **Ethnicity** NR	*Screening programme* Amino acids and acylcarnitines	*Screening programme* NR	*Screening programme* **Total** 131,776	*Screening programme* **True positives** 27 **False negatives** 3 **False positives** NR (authors reported false-positive rate as <0.85%) **True negatives** NR	*Screening programme* **Sensitivity** 90.000% **Specificity** NA **PPV** NA	Metabolic disorders were confirmed for 27 infants. Disorders included MCAD deficiency ($n = 10$), SCAD deficiency ($n = 1$); HPA ($n = 9$) and 7 organic acidurias Initial cut-offs resulted in false-positive detection rates of >1.9%, but revised cut-offs resulted in a <0.85% false-positive rate Since tandem MS screening began in North Carolina, disorders among 3 infants (1 with late-onset methylmalonic aciduria and 2 with GAI) were missed by tandem MS screening, but the disorders were diagnosed clinically before the age of 1 year for all 3 infants

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	 Sensitivity (%) Specificity (%) PPV	Comments
Rashed *et al.*, 1999[17]	*Screening* **Study type** Prospective cohort study **Period** June 1995 to June 1998 **Country** Saudi Arabia	*Screening* Automated ESI/tandem MS (using a CAMPA for automated processing and flagging of abnormal profiles)	*Screening* **Sample type** Dried blood spots (from newborn infants with minimum birth weight of 2 kg) **Age at sampling** Mean: 24.87 ± 16.4 hours **Ethnicity** NR	*Screening* Amino acids, acylcarnitines with argininosuccinic acid screen added in March 1996 [positive results: repeat analysis of second blood spot and analysis of urine for organic acids (in some cases) by GC/MS]	*Screening* Parameters used by algorithm were selected by comparing metabolic profiles from known cases of organic acidaemia and amino acid disorders with profiles from control samples Large set of data files (n = 1100) from newborn population was chosen for establishing control cut-off values. Sample criteria included newborn infants with minimum birth weight of 2.01 kg and sample analysis in <72 hours from time of collection Cut-off values were based on the percentile method with the 99.5 percentile as the upper cut-off limit and 0.5 percentile as the lower cut-off limit (used for some key metabolites)[25]	*Screening* **Total** 27,624	*Screening* **True positives** 20 **False negatives** 0 (due to early time of sample collection, authors cannot ascertain that no cases were missed) **False positives** 67 **True negatives** 27,537	*Screening* **Sensitivity** 100.000% **Specificity** 99.757% **PPV** 22.989%	The authors screened 27,624 blood spots and identified 20 cases of inherited metabolic disorders: PKU (HPA) (n = 3), MSUD (n = 2), ALD (n = 2), ASD (n = 1), non-ketotic hyperglycaemia (n = 1), glutaric CoA dehydrogenase deficiency (n = 2), MMA (n = 4), PPA (n = 1), IVA (n = 2), MCAD (n = 2). In addition, 26 cases were lost to follow-up. The frequency of amino acid and acylcarnitine disorders in this population was 1:1381 (20:27,624) No false-negative results were identified; however, due to the early times of sample collection (mean = 24.87 ± 16.4 hours), the authors could not establish whether any cases were missed Some false-positive results were eliminated on repeat analysis of a second blood spot using tandem MS and by GC/MS analysis of urine for organic acids in some cases

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Roscher *et al*., 2000[18] (Abstract)	*Screening* **Study type** Prospective, cohort study **Period** NR; however, 3-year study **Country** Germany (Bavaria Newborn Screening Programme)	*Screening* Tandem MS (using newly developed multi-analyte pattern recognition analysis)	*Screening* **Sample type** NR **Age at sampling** Day 3 (participation rate > 98%) **Ethnicity** NR	*Screening* 7 disorders routinely screened using tandem MS, disease range expanded under pilot criteria	*Screening* NR	*Screening* **Total** 166,000 (repeat samples using tandem MS was 0.3%, *n* = 498) **True positives** 49 **False negatives** 0 (so far) **False positives** 449 **True negatives** 165,502 **Sensitivity** 100.000% **Specificity** 99.729% **PPV** 9.839%	*Screening* The authors screened 166,000 newborns using tandem MS and identified 49 confirmed cases of inborn errors of metabolism (1:3390) that required treatment and/or counselling. The detection frequency of treatable fatty acid oxidation disorders was 1:9000. Besides 13 MCAD deficiencies, the first cases of LCHAD deficiency, carnitine uptake defect and CPTI were detected in prospective newborn screening. MCC deficiency (3 isolated cases and 2 asymptomatic mothers) appeared to be the most frequent organic acid disorder. Other defects included severe variants of VLCAD deficiency and MAD deficiency [2 newborns were identified in newborn screening, but died (day 3) before diagnosis]

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Wiley, *et al.*, 1999[16] (includes additional information and results reported in two abstracts by Wilcken *et al.*, 2000[5] and Wilcken and Wiley, 2001[4])	*Screening* **Study type** Prospective cohort study **Period** Not clear; however, results from 1998 **Country** Australia [New South Wales Newborn Screening Programme: screens all babies born (approx. 95,000 per year) in New South Wales and Australian Capital Territory]	*Screening* Micromass Quattro II electrospray tandem MS (automated sampler and computer-assisted software for automated processing and flagging)	*Screening* **Sample type** Dried blood spots **Age at sampling** >48 hours (usually day 3) **Ethnicity** NR	*Screening* Amino acids and acylcarnitines (positive results: retested original sample in-house and as required using capillary electrophoresis, thin-layer chromatography or DNA mutational analysis. If marginally abnormal repeat analysis of dried blood spot, urine for a metabolic screen of organic acids and amino acids and plasma amino acids and/or acylcarnitines)	*Screening* Analytes / Cut-off for repeat sample (μmol/l) **Amino acids** Alanine 900 Citrulline 75 Glycine 1000 Leucine/isoleucine 500 Methionine 80 Phenylalanine 150 Tyrosine 500 **Acylcarnitines** Carnitine 5–125 Acetyl carnitine 8–160 Propionyl carnitine 9 Butyryl carnitine 1.6 Isovaleryl carnitine 1.4 3-Hydroxy isovaleryl carnitine 1.0 Hexanoylcarnitine 0.8 Octanoylcarnitine 1.0 Decanoylcarnitine 1.5 Decenoylcarnitine 0.8 Myristylcarnitine 1.5 Tetradecenoylcarnitine 1.5 Palmitoylcarnitine 8.5	*Screening* **Total** 196,000 (consecutive samples)	*Screening* **True positives** 46 **False negatives** 3 (known false-negative cases: tyrosinaemia type 1, non-ketotic hyperglycinaemia and cobalamin C defect) **False positives** 164 **True negatives** 195,790	*Screening* **Sensitivity** 93.878% **Specificity** 99.916% **PPV** 21.905%	The authors screened 196,000 blood spots and identified 46 cases of inherited metabolic disorders with a repeat sampling rate of 0.1% (210/196,000). The detection rate with tandem MS was approximately the expected rate, based on 20 years' previous experience in New South Wales or known mutation frequencies. The authors found 28 cases of PKU (21 expected), 2 biopterin defects, 6 MCAD deficiencies (6 expected), 2 with other defects of fatty acid oxidation [SCAD (n = 1), β-ketothiolase deficiency (n = 1)], 3 with organic acid defects [vitamin B_{12}-deficient babies of vegan mothers (n = 2), glutaric CoA dehydrogenase deficiency (n = 1)] and 5 with other amino acidopathies [MSUD (n = 1), tyrosinaemia type II (n = 1), HPA (n = 3)]. Overall, this

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
					[Cut-off values based on population data and retrospective samples with proven disorders. The database can provide individual cut-off levels (sample outside 99.5 percentile) as well as ratios of analytes]		yielded a frequency of approximately 1 in 4261 (46:196,000) The authors concluded that some disorders can be detected with confidence (PKU, many organic acidaemias, classic MSUD) and some always (MCAD deficiency, glutaric acidaemia) using tandem MS. Disorders that could not be reliably detected included mild MSUD, homocystinuria, tyrosinaemia type I and probably most fatty acid defects. Furthermore, this method coupled with the ability of the database to provide ratios of various analytes increased the sensitivity and specificity for the detection of inborn errors of metabolism

Appendix 7 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: amino acids and acylcarnitines*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Zytkovicz *et al.*, 2001[7]	*Screening* **Study type** Prospective cohort study **Period** Not clear, data from 1 February 1999 (reported 2-year summary) **Country** USA (New England Newborn Screening Program: specimens from Massachusetts, Maine, New Hampshire, Vermont, Rhode Island)	*Screening* Micromass Quattro LC triple quadrupole tandem MS (automated sampler and computer-assisted software for automated processing and flagging)	*Screening* **Sample type** Dried blood spots **Age at sampling** 1–3 days **Ethnicity** NR	*Screening*	*Screening*	See below	*Screening* The authors screened >160,000 blood spots using tandem MS and identified 22 newborns with amino acid disorders [PKU (*n* = 7), HPA (*n* = 11), MSUD (*n* = 1), hypermethioninaemia (*n* = 1), argininosuccinase lyase deficiency (*n* = 1) and argininaemia (*n* = 1)] and 20 infants with fatty and organic disorders [MCAD deficiency (*n* = 10; 4 homozygous for 985A → G mutation), SCAD deficiency (presumptive *n* = 5), PPA (*n* = 2), carnitine palmitoyltransferase II deficiency (*n* = 1), 3-methylcrotonyl-CoA carboxylase deficiency (*n* = 1), and VLCAD deficiency (presumptive *n* = 1)]

Marker and ratios	Flag limit	Total	Flagged	TN	FN	FP	TP	PPV (%)	Sensitivity (%)	Specificity (%)
Amino acid disorders										
Phe	139[a]	257,000	92	256,908	NR	74	18 (7 PKU, 11 HPA)	19.565	NA	99.971
Phe/Tyr	1.5	257,000	64	256,936	NR	46	18 (7 PKU, 11 HPA)	28.125	NA	99.982
Leu	373[a]	257,000	19	256,981	NR	18	1 MSUD	5.263	NA	99.993
Leu/Phe	5	257,000	8	256,992	NR	7	1 MSUD	12.500	NA	99.997
Met	67[a]	257,000	71	256,929	NR	70	1 HMet	1.408	NA	99.973
Met/Phe	1	257,000	32	256,968	NR	31	1 HMet	3.125	NA	99.988
Tyr	442[a]	164,000	42	163,958	NR	42	0	–	NA	99.974
Tyr/Phe	6	164,000	38	163,962	NR	38	0	–	NA	99.977
Orn	300[a]	164,000	10	163,990	NR	10	0	–	NA	99.994
Orn/Cit	10	164,000	5	163,995	NR	5	0	–	NA	99.997
Cit	100[a]	164,000	20	163,980	NR	19	1 ASL	5.000	NA	99.988
Cit/Arg	2	164,000	3	163,997	NR	2	1 ASL	33.333	NA	99.999
Arg	132[a]	164,000	6	163,994	NR	5	1 Arg	16.667	NA	99.997
Arg/Orn	1	164,000	3	163,997	NR	2	1 Arg	33.333	NA	99.999

[a] μmol/l.
[Phe]: marker for PKU and HPA; [Leu]: marker for MSUD; Met: marker for Hcys and HMet; [Tyr]: marker for Tyr; [Orn]: marker for HHH syndrome; [Cit]: marker for ASS and ASL deficiency; [Arg]: marker for Arg.
Note: retested original samples of positive results; confirmation of the disorders was according to standard metabolic procedures; in addition, MCAD deficiency blood spots with C8 concentrations > 0.5 μmol/l prompted analysis for 985A → G mutation using DNA analysis.

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments

Marker and ratios	Flag limit (μmol/l[a])	Total	Flagged	TN	FN	FP	TP	PPV (%)	Sensitivity (%)	Specificity (%)
Acylcarnitine disorders										
C3	8[a]	164,000	36	163,964	NR	34	2 PPA	5.556	NA	99.979
C3-2M-DC	0.8	164,000	6	163,994	NR	6	0	–	NA	99.996
C5	1.2	164,000	35	163,965	NR	35	0	–	NA	99.979
C5OH	0.8	164,000	24	163,976	NR	23	1 MCC	4.167	NA	99.986
C5:1	0.08	164,000	7	163,993	NR	7	0	–	NA	99.996
C5-3M-DC	0.12	164,000	21	163,979	NR	21	0	–	NA	99.987
C5-DC	0.21	164,000	32	163,968	NR	32	0	–	NA	99.980
C4	1.9	164,000	33	163,967	NR	28	5 SCAD[b]	15.152	NA	99.983
C8	0.5[a]	184,000	52	183,948	NR	42	10 MCAD	19.231	NA	99.977
C14:1	0.9	164,000	4	163,996	NR	3	1 VLCAD[b]	25.000	NA	99.998
C16	12[a]	164,000	2	163,998	NR	1	1 CPTII	50.000	NA	99.999
C16OH	0.1	164,000	5	163,995	NR	5	0	–	NA	99.997

[b] Presumptive cases.

[C3]: primary marker for PPA, MMA and MCD; [C5]: primary marker for IVA and 2-MBCD deficiency; [C5OH] or isomers: primary or secondary markers for BKT, HMG and MCC deficiency and MCD, additional markers include triglylcarnitine and 3-methylglutarylcarnitine; [C5-DC]: primary marker for GAI and secondary marker for GAII; [C4] and isomerisobutyrylcarnine: primary markers for SCAD and isobutyryl-CoA dehydrogenase deficiency; [C8, C6 and C10:1]: C8 primary marker for MCAD deficiency, with C6 and C10:1 good secondary markers; C10 and C8 are also good primary markers for GAII; [C14:1]: primary marker for VLCAD deficiency; [C16]: primary marker for CPTII and carnitine-acylcarnitine translocase deficiency; [C16OH]: primary marker for LCHAD deficiency.

Note: Retested original samples of positive results; confirmation of the disorders was according to standard metabolic procedures; in addition, MCAD deficiency blood spots with C8 concentrations > 0.5 μmol/l prompted analysis for 985A → G mutation using DNA analysis.

Approximately 0.3% of all newborns screened were flagged for either amino acid or acylcarnitine markers. The sensitivity of the tandem MS method could not be calculated with certainty due to a lack of information regarding false negatives; however, the cumulative specificity was 100%. The PPV for all amino acid and acylcarnitine disorders was 8% (22 of 260) and 9% (20 of 233), respectively. If flagged amino acids and there flagged ratios are used, the PPV increases to 14% (22 of 153)

ALD, arginosuccinase deficiency; Arg, arginaemia; ASD, arginosuccinic acid synthetase deficiency; ASL, arginosuccinate lyase; ASS, arginosuccinate synthetase; BKT, β-ketothiolase; CPTI, carnitine palmitoyltransferase type I; CPTII, carnitine palmitoyltransferase type II; EMA, ethylmalonic acidaemia; FN, false negative; FP, false positive; Hcys, homocystinuria; HMet, hypermethioninaemia; HMG, 3-hydroxy-3-methylglutaric aciduria; IVA, isovaleric aciduria; MAD, multiple acyl-coenzyme A dehydrogenase; 2-MBCD, 2-MBCD, 2-methylbutyryl-coenzyme dehydrogenase; MCC, 3-methylcrotonyl-coenzyme A carboxylase; MCD, multiple carboxylase deficiency; MMA, methylmalonic aciduria; PPA, propionic aciduria; PPV, positive predictive value; SCAD, short-chain acyl-coenzyme A dehydrogenase; TN, true negative; TP, true positive; Tyr, tyronsinaemia; VLCAD, very long-chain acyl-coenzyme A dehydrogenase.

Appendix 8

Effectiveness of neonatal screening using tandem mass spectrometry: medium-chain acyl-coenzyme A dehydrogenase deficiency only

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	 Sensitivity (%) Specificity (%) PPV	Comments
Andresen *et al.*, 2001[23]	*Screening*	*Screening*	*Screening*	*Screening*	*Screening*	*Screening*	*Screening*	*Screening*
	Study type Prospective cohort study **Period** 1 December 1992 to 31 January 2001 **Country** USA (Pennsylvania, Ohio, New Jersey, Illinois, Florida, North Carolina)	VG Quattro quadropole tandem MS with laboratory-based data system (Micromass)[22] (operated in static liquid secondary ionisation mode)	**Sample type** Dried blood spots **Age at sampling** < 72 hours **Ethnicity** NR	MCAD deficiency (verified in at least two separate analyses of blood spot and in most cases repeat blood-spot specimen) (samples having acylcarnitine profiles indicative of MCAD deficiency were also assayed for the 985A → G mutation using DNA analysis and 199T → C mutation using a mutation specific assay validated by DNA analysis)	Detection of 'diagnostic' acylcarnitine profiles, i.e. elevated C6, C8, C10 and C10:1 (mild profile: octanoylcarnitine concentration 0.5–2.0 μmol/l and octanoylcarnitine: decanoylcarnitine ratio 2–4; severe profile: octanoylcarnitine concentration > 2.0 μmol/l and octanoylcarnitine: decanoylcarnitine ratio of > 4)	**Total** 930,078 (includes 80,371 blood spots reported in study by Ziadeh *et al.*, 1995[67]) **True positives** 62 **False negatives** NR **False positives** 0 **True negatives** 930,016	**Sensitivity** Could not be calculated with certainty **Specificity** 100.000% **PPV** 100.000%	An MCAD deficiency frequency of 1:15,001 (62:930,078) was observed. From the 930,078 screened samples, the authors expected to find 36 to 38 985A → G homozygotes. The results showed that of the 62 acylcarnitine-positive blood spots 63% (39) were homozygous for the 985A → G mutation (1:23,848), indicating that tandem MS-based screening methods detect the expected number of 985A → G homozygous newborns; however, the frequency of the 985A → G mutant allele in newborns with a positive acylcarnitine profile is much lower than that observed in clinically affected patients (80%) Note that screening for MCAD deficiency was solely based on

continued

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
							diagnostic acylcarnitine profiles A new mutation, 199T → C, was identified and was present in a large proportion of the acylcarnitine-positive samples. Overexpression experiments showed that 199T → C was a mild folding mutation that exhibited decreased levels of enzyme activity only under stringent conditions. A carrier frequency of 1 in 500 in the general population makes the 199T → C mutation one of the three most prevalent mutations in the enzymes of fatty acid oxidation

Appendix 8 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: MAD deficiency only*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*)	Results True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	Results Sensitivity (%) Specificity (%) PPV	Comments
Carpenter *et al.*, 2001[20]	*Screening* **Study type** Prospective cohort study **Period** April 1998 to March 2001 **Country** Australia [New South Wales Newborn Screening Programme: screens all babies born (>99%) in New South Wales and Australian Capital Territory]	*Screening* Micromass Quattro II electrospray tandem MS	*Screening* **Sample type** Dried blood spots **Age at sampling** Median 3 days, and over 99% sampled before day 6 **Ethnicity** NR	*Screening* MCAD deficiency (samples having acylcarnitine profiles indicative of MCAD deficiency were also assayed for the 985A → G mutation, analysis of plasma, repeat blood-spot acylcarnitines and urinary organic acids and fibroblast fatty acid oxidation)	*Screening* Octanoylcarnitine concentration ≥ 1 μmol/l [threshold value based on a retrospective analysis of newborn screening samples from 13 patients born between January 1981 and June 1997 – dried blood-spot samples were obtained between 4 and 6 days (n = 12) and one at day 10 – later diagnosed clinically with MCAD deficiency (11 homozygous for the common MCAD mutations 985A → G, and 2 heterozygous), and prospective analysis of newborn screening samples from 24,000 newborns] Patients diagnosed with MCAD deficiency if one or more of the following criteria were met: homozygous for 985A → G mutation, raised hexanoylglycine and suberglycine in	*Screening* **Total** 275,653 (consecutive neonates undergoing routine newborn screening)	*Screening* **True positives** 12 (including 1 probable mild case) **False negatives** NR **False positives** 11 **True negatives** 275,630	*Screening* **Sensitivity** Could not be calculated with certainty **Specificity** 99.996% **PPV** 52.174%	*Screening* 11 babies were diagnosed with MCAD deficiency and 1 additional patient was considered to be a carrier at low risk of developing symptoms. Of the remaining 11 babies who screened positive but did not meet the diagnostic criteria for MCAD deficiency, four infants died in the neonatal period from a variety of causes before a second sample could be taken. Absence of MCAD deficiency was confirmed by enzyme analysis in cultured skin fibroblasts in one patient, and further information from clinicians and post-mortem findings eliminated MCAD deficiency from the other patients who died before analysis Authors reported 1:68,913 (4:275,653) newborns homozygous for the 985A → G

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
					urine, increased hexanoylcarnitine, octanoylcarnitine or decenoylcarnitine in plasma, etc.		mutation, which is considerably lower than the predicted birth prevalence from Australian figures (1:29,500, 95% CI 1:11,500 to 1:8700), but within the 95% CI Newborn screening using tandem MS can detect almost all patients with MCAD deficiency who would later have developed symptoms

Appendix 8 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: MAD deficiency only*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	Results Sensitivity (%) Specificity (%) PPV	Comments
Chace *et al*., 1997[22]	*Screening* **Study type** Prospective cohort study **Period** September 1992 to January 1997 **Area** USA (Neo Gen Screening, Pennsylvania, and North Carolina Newborn Screening Program)	*Screening* VG Quattro quadrupole tandem MS with laboratory-based data system (Micromass) (operated in static liquid secondary ionisation mode)	*Screening* **Sample type** Dried blood spots **Age at sampling** < 72 hours **Ethnicity** NR	*Screening* MCAD deficiency (verified by DNA mutation analysis)	*Screening* Octanoylcarnitine concentration ≥ 0.3 μmol/l [maximum levels of octanoyl-, hexanoyl- and decanoylcarnitines from 113 normal neonatal blood spots, aged < 72 hours, were below 0.3 μmol/l. Maximum levels of octanoyl-, hexanoyl- and decanoylcarnitines from 16 MCAD-deficient patients, aged < 72 hours, were well above 0.3 μmol/l; also above upper values of normal controls (>2.5 μmol/l), whereas maximum levels of octanoyl-hexanoyl-, decenoyl and decanoylcarnitines from 16 MCAD-deficient patients aged between 8 days and 11 years were above upper values of normal controls (>0.5 μmol/l) but to a lesser degree than the neonatal period ($p < 0.0001$, Mann–Whitney U-test)]	*Screening* **Total** 283,803 (Neo Gen Screening, *n* = 267,303; and North Carolina Newborn Screening Program, *n* = 16,500)	*Screening* **True positives** 16 **False negatives** 0 (known false-negative results) **False positives** 0 **True negatives** 283,787	*Screening* **Sensitivity** 100.000% **Specificity** 100.000% **PPV** 100.000%	The combined experience of prospective newborn screening in Pennsylvania and North Carolina showed an MCAD frequency of 1:17,706 (16:283,303). Nine of these 16 MCAD-deficient patients were homozygous for the 985A → G mutation and 7 were compound heterozygotes The authors also found that the diagnostic acylcarnitines (octanoyl-, hexanoyl-, decenoyl and decanoylcarnitines) were higher in the newborn period (<72 hours) than those observed in older patients (between 8 days and 11 years). The octanoylcarnitine was still > 0.3 μmol/l, but the diagnostic distinction was more difficult

Appendix 8 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: MAD deficiency only*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
Pourfarzam *et al.*, 2001[21]	*Screening* **Study type** Retrospective cohort study **Period** 1 January 1991 to 20 July 1993 **Country** UK (Northern region of the NHS)	*Screening* ESI tandem MS	*Screening* **Sample type** Dried blood spots **Age at sampling** NR **Ethnicity** NR	*Screening* MCAD deficiency	*Screening* Octanoylcarnitine concentration > 0.3 μmol/l with high octanoylcarnitine: hexanoylcarnitine ratio: > 4.0 (criteria based on 18 neonates with MCAD deficiency)	*Screening* **Total** 100,600 **True positives** 8 **False negatives** 0 **False positives** 0 **True negatives** 100,592 **Sensitivity** 100.000% **Specificity** 100.000% **PPV** 100.000%	*Screening* The authors analysed the concentrations of acylcarnitines in stored neonatal blood spots (up to 5 years) and reviewed patients with high octanoylcarnitine concentrations at the age of 7–9 years Of the 8 MCAD-deficient patients 7 were homozygous with detectable suberglycine, phenylpropionylglycine and hexanoylglycine in urine specimens (features not detected in patients with low octanoylcarnitine/ hexanoylcarnitine ratio) and 1 was heterozygous (confirmed by study of fibroblast fatty acid oxidation) for the 985A → G mutation. Of these, 1 patient died of gastroenteritis at the age of 17 months, before diagnosis, 4 others had life-threatening illnesses (1 had neonatal apnoea related to MCAD

Appendix 8 cont'd *Effectiveness of neonatal screening using tandem mass spectrometry: MAD deficiency only*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
							deficiency and 3 had severe or recurrent episodes of encephalopathy), 1 had mild symptoms of MCAD deficiency and 2 had no symptoms The incidence of MCAD deficiency in this population was 1:12,600, with the 985A → G transition accounting for 94% of mutant alleles. These findings were consistent with earlier studies of clinically detected cases in the UK The authors did not identify any false-negative results after examination of the regional registers for metabolic diseases and deaths. The specificity of screening was 100%; however, the sensitivity of the test was difficult to ascertain, because many occurrences of MCAD deficiency not diagnosed on clinical grounds

Appendix 9

Effectiveness of tandem mass spectrometry: laboratory-based studies

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	Results Sensitivity (%) Specificity (%) PPV	Comments
Chace *et al*., 1998[24]	**Study type** Retrospective, analytical study **Period** 1992 to 1994 **Country** USA (samples from California Newborn Screening Program)	Fluorometry vs isotope dilution liquid secondary ion tandem MS	**Sample type** Dried blood spots **Age at sampling** < 24 hours **Ethnicity** NR	PKU	*Fluorometry* Phenylalanine concentration ≥ 258 μmol/l (4.3 mg/dl) (metabolic specialist made final diagnoses of cases of classical PKU or variant PKU)	*Fluorometry* **Total** 203 (initially 208 specimens, but 5 serial samples from 1 infant with PKU)	*Fluorometry* **True positives** 19 (12 confirmed classical PKU; 7 confirmed variant PKU) **False negatives** NR, assumed zero **False positives** 91 **True negatives** 93	*Fluorometry* **Sensitivity** 100.000% **Specificity** 50.543% **PPV** 17.273%	Comparison of results obtained by fluorometry with those obtained by tandem MS reveals a strong correlation (Pearson correlation coefficient: 0.817) Phenylalanine measurement by tandem MS, with a cut-off of 180 μmol/l (3.0 mg/dl) or by fluorometry, with a cut-off of ≥ 258 μmol/l (4.3 mg/dl), detected all variant and classical cases of PKU. Tandem MS greatly reduced the number of false-positive results from 91 to 3 and is a more accurate method for measuring phenylalanine concentration

continued

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
					Tandem MS Phenylalanine concentration: ≥ 180 μmol/l (3.0 mg/dl) Primary HPA: phenylalanine/tyrosine molar ratio ≥ 2.5 Variant PKU: mean phenylalanine concentration of 430 μmol/l (7.1 mg/dl) Classical PKU: mean phenylalanine concentration of 1188 mmol/l (19.6 mg/dl) in second specimen	*Tandem MS* **Total** 203 (initially 208 specimens, but 5 serial samples from 1 infant with PKU) *Tandem MS* **True positives** 19 (12 confirmed classical PKU; 7 confirmed variant PKU) **False negatives** NR, assumed zero **False positives** 3 **True negatives** 181 *Tandem MS* **Sensitivity** 100.000% **Specificity** 98.370% **PPV** 86.364%	Simultaneous quantification of phenylalanine and tyrosine by tandem MS further reduced the number of false positives to 1 using a cut-off of phenylalanine/tyrosine molar ratio ≥ 2.5

Appendix 9 cont'd *Effectiveness of tandem mass spectrometry*

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*)	Results Sensitivity (%) Specificity (%) PPV	Comments
Rashed *et al*., 1997[25]	**Study type** Retrospective, analytical study **Period** NR **Country** Saudi Arabia	Automated ESI–tandem MS (using a CAMPA for automated processing and flagging of abnormal profiles)	**Sample type** Dried blood spots **Age at sampling** NR **Ethnicity** NR	Amino acids, acylcarnitines (PKU, Tyr, MSUD, Hcys, NKG, ASD, ALD, PYG/PIP, PRO, MCAD, SCAD/EMA, LCAD/VLCAD, GAII, HMG, BKT, PPA/MMA, MCD, IVA, GAI	*CAMPA* Parameters used by algorithm were selected by comparing metabolic profiles from known cases of organic acidaemia and amino acid disorders with profiles from control samples Large set of data files (n = 1100) from newborn population was chosen for establishing control cut-off values. Sample criteria included newborn infants with minimum birth weight of 2.01 kg and sample analysis in < 72 hours from time of collection Cut-off values were based on the percentile method with the 99.5 percentile as the upper cut-off limit and 0.5 percentile as the lower cut-off limit (used for some key metabolites)	*CAMPA* **Total** 559 (449 normal; 119 abnormal tandem MS data files)	*CAMPA* **True positives** 119 **False negatives** 0 **False positives** 91 **True negatives** 349	*CAMPA* **Sensitivity** 100% **Specificity** 79% **PPV** 57%	The sensitivity of CAMPA for flagging cases with known metabolic disorders was 100% and the weighted average cumulative specificity for the two tests was 83%. The difference between the sensitivity and specificity was <17% The authors reported that the variability in the acylcarnitine values was responsible for >85% of 'false flagging', [in particular, propionylcarnitine and butyryl (or isobutyryl) carnitine ratios just above the cut-off values were responsible for approximately 25% of the falsely flagged samples], <5% was due to borderline increase of one or more of the key amino acids [methionine, leucine (+isoleucine), alanine, glycine and phenylalanine] and the remaining 10% were a result of borderline low values for one or more of these amino acids

Authors, year	Study type Period Country	Screening test	Sample type Age at sampling Ethnicity	Target conditions	Threshold for disease identification	Results Total screened (*n*) True positives (*n*) False negatives (*n*) False positives (*n*) True negatives (*n*) Sensitivity (%) Specificity (%) PPV	Comments
							In most falsely flagged data files, a single parameter was the reason the flag was set up, whereas for most truly abnormal data files, multiple flags were set; thus, values obtained for concentrations or ratios were noticeably higher than the cut-off values
						CAMPA **Total** 1151 [1004 normal (new batch), 147 abnormal (including 119 abnormal) data files from neonatal screening samples] *CAMPA* **True positives** 147 **False negatives** 0 **False positives** 153 **True negatives** 851 *CAMPA* **Sensitivity** 100% **Specificity** 85% **PPV** 49%	The authors concluded that the CAMPA method demonstrated a high sensitivity (100%) in flagging abnormal profiles and a high cumulative specificity (83.1%). This method also gives a high throughput capacity (96-well microplate batch process), allowing analysis of hundreds of samples (500–1000 samples per instrument) per day

NKG, non-ketotic hyperglycinaemia; PYG/PIP, pyroglutamic/pipecolic acidaemia.

Appendix 10

Reference list of excluded studies: phenylketonuria

Ades AE, Walker J, Jones R, Smith I. Coverage of neonatal screening: failure of coverage or failure of information system. *Arch Dis Child* 2001;**84**:476–9.

Ades AE, Walker J, Jones R, Thompson S, Leonard JV, Smith I. Obstacles to timely neonatal screening in North Thames. *J Med Screen* 1998;**5**:183–6.

Allen KR, Degg TJ, Rushworth PA, Smith M, Henderson MJ. Measurement of phenylalanine and tyrosine in plasma by high-performance liquid chromatography using the inherent fluorescence of aromatic amino acids. *Ann Clin Biochem* 1999; **36** (Pt 2):207–11.

Applegarth DA, Toone JR, Lowry RB. Incidence of inborn errors of metabolism in British Columbia, 1969–1996. *Pediatrics* 2000;**105**:e10.

Azen C, Koch R, Friedman E, Wenz E, Fishler K. Summary of findings from the United States Collaborative Study of children treated for phenylketonuria. *Eur J Pediatr* 1996;**155** (Suppl 1):S29–32.

Baumeister AA, Baumeister AA. Dietary treatment of destructive behavior associated with hyperphenylalaninemia. *Clin Neuropharmacol* 1998; **21**:18–27.

Berenbaum SA. Neuropsychological follow-up in neonatal screening: issues, methods and findings. *Acta Paediatr* 1999;**88** (Suppl 432):83–7.

Blau N, Thony B, Spada M, Ponzone A. Tetrahydrobiopterin and inherited hyperphenylalaninemia. *Turk J Pediatr* 1996;**38**:19–35.

Boulat O, McLaren DG, Arriaga EA, Chen DD. Separation of free amino acids in human plasma by capillary electrophoresis with laser induced fluorescence: potential for emergency diagnosis of inborn errors of metabolism. *J Chromatogr B Biomed Sci Appl* 2001;**754**:217–28.

Bowman JE. To screen or not to screen: when should screening be offered? *Community Genet* 1998;**1**:145–7.

Burgard P, Bremer HJ, Buhrdel P, Clemens PC, Monch E, Przyrembel H, *et al*. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. *Eur J Pediatr* 1999;**158**:46–54.

Burlina AB, Bonafe L, Ferrari V, Suppiej A, Zacchello F, Burlina AP. Measurement of neurotransmitter metabolites in the cerebrospinal fluid of phenylketonuric patients under dietary treatment. *J Inherit Metab Dis* 2000;**23**:313–16.

Cabalska MB, Nowaczewska I, Sendecka E, Zorska K. Longitudinal study on early diagnosis and treatment of phenylketonuria in Poland. *Eur J Pediatr* 1996; **155** (Suppl 1):S53–5.

Chace DH, Sherwin JE, Hillman SL, Lorey F, Cunningham GC. Use of phenylalanine-to-tyrosine ratio determined by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. *Clin Chem* 1998;**44**:2405–9.

Ciske JB, Hoffman G, Hanson K, Annable KM, Wolff J, Litsheim T, *et al*. Newborn screening in Wisconsin: program overview and test addition. *Wisconsin Medical Journal* 2000;**99**:38–42.

Cleary MA, Walter JH, Wraith JE, White F, Tyler K, Jenkins JP. Magnetic resonance imaging in phenylketonuria: reversal of cerebral white matter change. *J Pediatr* 1995;**127**:251–5.

Coelho JC, Wajner M, Burin MG, Vargas CR, Giugliani R. Selective screening of 10,000 high-risk Brazilian patients for the detection of inborn errors of metabolism. *Eur J Pediatr* 1997;**156**:650–4.

Corsello G, Bosco P, Cali F, Greco D, Cammarata M, Ciaccio M, *et al*. Maternal phenylketonuria in two Sicilian families identified by maternal blood phenylalanine level screening and identification of a new phenylalanine hydroxylase gene mutation (P407L). *Eur J Pediatr* 1999;**158**:83–4.

da Silva LC, Carvalho TS, da Silva FB, Pires RF, Giugliani R, Pereira ML. Aspartame loading test in PKU heterozygous individuals bearing severe and moderate mutations. *Clin Genet* 2000;**58**:86–8.

de Freitas O, Izumi C, Lara MG, Greene LJ. New approaches to the treatment of phenylketonuria. *Nutr Rev* 1999;**57**:65–70.

Desviat LR, Perez B, Gamez A, Sanchez A, Garcia MJ, Martinez-Pardo M, *et al*. Genetic and phenotypic aspects of phenylalanine hydroxylase deficiency in Spain: molecular survey by regions. *Eur J Hum Genet* 1999; **7**:386–92.

Dezateux C. Evaluating newborn screening programmes based on dried blood spots: future challenges. *Br Med Bull* 1998;**54**:877–90.

Dhondt JL, Paux E, Farriaux JP. Need for a standardized procedure in the preparation of phenylalanine calibrators. *Early Hum Dev* 1996;**45**:277–85.

Dhondt JL, Loeber J, Elvers LH, Paux E. Preparation of the first European working standard for phenylalanine determination in dried blood spots. *J Med Screen* 1998; **5**:63–6.

Dotremont H, Francois B, Diels M, Gillis P. Nutritional value of essential amino acids in the treatment of adults with phenylketonuria. *J Inherit Metab Dis* 1995;**18**:127–30.

Eastman JW, Sherwin JE, Wong R, Liao CL, Currier RJ, Lorey F, *et al*. Use of the phenylalanine:tyrosine ratio to test newborns for phenylketonuria in a large public health screening programme. *J Med Screen* 2000;**7**:131–5.

Eiken HG, Knappskog PM, Boman H, Thune KS, Kaada G, Motzfeldt K, *et al*. Relative frequency, heterogeneity and geographic clustering of PKU mutations in Norway. *Eur J Hum Genet* 1996;**4**:205–13.

Eisensmith RC, Martinez DR, Kuzmin AI, Goltsov AA, Brown A, Singh R, *et al*. Molecular basis of phenylketonuria and a correlation between genotype and phenotype in a heterogeneous southeastern US population. *Pediatrics* 1996;**97**:512–16.

Enns GM, Martinez DR, Kuzmin AI, Koch R, Wakeem CK, Woo SL, *et al*. Molecular correlations in phenylketonuria: mutation patterns and corresponding biochemical and clinical phenotypes in a heterogeneous California population. *Pediatr Res* 1999;**46**:594–602.

Fan GX, Qing LX, Jun Y, Mei Z. Molecular studies and prenatal diagnosis of phenylketonuria in Chinese patients. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):63–5.

Fan GX, Jun Y, Rui-guan C. Neonatal screening of phenylketonuria and congenital hypothyroidism in China. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):17–19.

Green A. Neonatal screening: current trends and quality control in the United Kingdom. *Rinsho Byori* 1998; **46**:211–16.

Greeves LG, Patterson CC, Carson DJ, Thom R, Wolfenden MC, Zschocke J, *et al*. Effect of genotype on changes in intelligence quotient after dietary relaxation in phenylketonuria and hyperphenylalaninaemia. *Arch Dis Child* 2000;**82**(3):216–21.

Griffiths P, Tarrini M, Robinson P. Executive function and psychosocial adjustment in children with early treated phenylketonuria: correlation with historical and concurrent phenylalanine levels. *J Intellect Disabil Res* 1997;**41** (Pt 4):317–23.

Griffiths P, Ward N, Harvie A, Cockburn F. Neuropsychological outcome of experimental manipulation of phenylalanine intake in treated phenylketonuria. *J Inherit Metab Dis* 1998;**21**:29–38.

Griffiths P, Smith C, Harvie A. Transitory hyperphenylalaninaemia in children with continuously treated phenylketonuria. *Am J Ment Retard* 1997; **102**:27–36.

Gu XF, Chen RG. Current status of neonatal screening in China. *J Med Screen* 1999;**6**:186–7.

Guldberg P, Levy HL, Hanley WB, Koch R, Matalon R, Rouse BM, *et al*. Phenylalanine hydroxylase gene mutations in the United States: report from the Maternal PKU Collaborative Study. *Am J Hum Genet* 1996;**59**:84–94.

Guldberg P, Zschocke J, Dagbjartsson A, Henriksen KF, Guttler F. A molecular survey of phenylketonuria in Iceland: identification of a founding mutation and evidence of predominant Norse settlement. *Eur J Hum Genet* 1997;**5**:376–81.

Guldberg P, Rey F, Zschocke J, Romano V, Francois B, Michiels L, *et al*. A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. *Am J Hum Genet* 1998;**63**:71–9.

Guthrie R. The introduction of newborn screening for phenylketonuria. A personal history. *Eur J Pediatr* 1996;**155** (Suppl 1):S4–5.

Hanley WB, Platt LD, Bachman RP, Buist N, Geraghty MT, Isaacs J, *et al*. Undiagnosed maternal phenylketonuria: the need for prenatal selective screening or case finding. *Am J Obstet Gynecol* 1999; **180**:986–94.

Hellekson KL, National Institutes of Health. NIH consensus statement on phenylketonuria. *Am Fam Phys* 2001;**63**:1430–2.

Hitzeroth HW, Niehaus CE, Brill DC. Phenylketonuria in South Africa. A report on the status quo. *S Afr Med J* 1995;**85**:33–6.

Hutchesson AC, Hall SK, Preece MA, Green A. Screening for tyrosinaemia type I. *Arch Dis Child Fetal Neonatal Ed* 1996;**74**:F191–4.

Huttenlocher PR. The neuropathology of phenylketonuria: human and animal studies. *Eur J Pediatr* 2000;**159** (Suppl 2):S102–6.

Infante JP, Huszagh VA. Impaired arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid synthesis by phenylalanine metabolites as etiological factors in the neuropathology of phenylketonuria. *Mol Genet Metab* 2001;**72**:185–98.

Kalsner LR, Rohr FJ, Strauss KA, Korson MS, Levy HL. Tyrosine supplementation in phenylketonuria: diurnal blood tyrosine levels and presumptive brain influx of tyrosine and other large neutral amino acids. *J Pediatr* 2001;**139**:421–7.

Kimura T, Ikeda H, Akaba K, Guldberg P, Guttler F, Maki K, *et al*. Mutation analysis of phenylketonuria in Yamagata prefecture, Japan. *Pediatr Int* 2001;**43**:1–3.

Koch R, Moseley K, Ning J, Romstad A, Guldberg P, Guttler F. Long-term beneficial effects of the phenylalanine-restricted diet in late-diagnosed individuals with phenylketonuria. *Mol Genet Metab* 1999;**67**:148–55.

Leandro P, Rivera I, Ribeiro V, de Almeida IT, da Silveira C, Lechner MC. Mutation analysis of phenylketonuria in south and central Portugal: prevalence of V388M mutation. *Hum Mutat* 1995;**6**:192–4.

Leuzzi V, Fois D, Carducci C, Antonozzi I, Trasimeni G. Neuropsychological and neuroradiological (MRI) variations during phenylalanine load: protective effect of valine, leucine, and isoleucine supplementation. *J Child Neurol* 1997;**12**:338–40.

Levy HL, Lobbregt D, Platt LD, Benacerraf BR. Fetal ultrasonography in maternal PKU. *Prenat Diagn* 1996;**16**:599–604.

Levy HL, Lobbregt D, Barnes PD, Poussaint TY. Maternal phenylketonuria: magnetic resonance imaging of the brain in offspring. *J Pediatr* 1996;**128**:770–5.

Levy PA, Miller JB, Shapira E. The advantage of phenylalanine to tyrosine ratio for the early detection of phenylketonuria. *Clin Chim Acta* 1998;**270**:177–81.

Lillevali H, Ounap K, Metspalu A. Phenylalanine hydroxylase gene mutation R408W is present on 84% of Estonian phenylketonuria chromosomes. *Eur J Hum Genet* 1996;**4**:296–300.

Lin WD, Wu JY, Lai CC, Tsai FJ, Tsai CH, Lin SP, *et al*. A pilot study of neonatal screening by electrospray ionization tandem mass spectrometry in Taiwan. *Acta Paediatr Taiwan* 2001;**42**:224–30.

Lines D, Magarey A, Raymond J, Robertson E. Tyrosine supplementation in phenylketonuria. *J Paediatr Child Health* 1997;**33**:177.

Lugovska R, Vevere P, Andrusaite R, Kornejeva A. Newborn screening for PKU and congenital hypothyroidism in Latvia. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):52–3.

Merrick J, Schwarz G, Sela BA. Incidence of phenylketonuria in Israel. *Isr Med Assoc J* 2000;**2**:801–2.

Mowat DR, Hayden MC, Thompson SM, Wilcken B. Maternal phenylketonuria: a continuing problem. *Med J Aust* 1999;**170**:592–5.

Muenzer J, Frazier DM, McCandless SE. Incidence and follow-up evaluation of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001;**50** (RR-3): 28–9.

Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol* 1999;**14** (Suppl 1):S4–8.

O'Donnell KA, Tighe O, O'Neill C, Naughten E, Mayne PD, McCarthy TV, *et al*. Rapid detection of the R408W and I65T mutations in phenylketonuria by glycosylase mediated polymorphism detection. *Hum Mutat* 2001;**17**:432.

Okano Y, Isshiki G. Newborn mass screening and molecular genetics of phenylketonuria in east Asia. *Southeast Asian J Trop Med Public Health* 1995; **26** (Suppl 1):123–9.

Ounap K, Lillevali H, Klaassen T, Metspalu A, Sitska M. The incidence and characterization of phenylketonuric patients in Estonia. *J Inherit Metab Dis* 1996;**19**:381–2.

Ounap K, Lillevali H, Metspalu A, Lipping-Sitska M. Development of the phenylketonuria screening programme in Estonia. *J Med Screen* 1998;**5**:22–3.

Ozalp I, Coskun T, Tokatli A, Kalkanoglu HS, Dursun A, Tokol S, *et al*. Newborn PKU screening in Turkey: at present and organization for future. *Turk J Pediatr* 2001;**43**:97–101.

Perez B, Desviat LR, De Lucca M, Schmidt B, Loghin-Grosso N, Giugliani R, *et al*. Mutation analysis of phenylketonuria in south Brazil. *Hum Mutat* 1996; **8**:262–4.

Pietz J, Landwehr R, Kutscha A, Schmidt H, de Sonneville L, Trefz FK. Effect of high-dose tyrosine supplementation on brain function in adults with phenylketonuria. *J Pediatr* 1995;**127**:936–43.

Ponzone A, Spada M, Ferrero GB, Ponzone R, Ferraris S. Newborn feeding and screening for phenylketonuria. *Acta Paediatr* 1999;**88**:347–8.

Prince AP, McMurray MP, Buist NR. Treatment products and approaches for phenylketonuria: improved palatability and flexibility demonstrate safety, efficacy and acceptance in US clinical trials. *J Inherit Metab Dis* 1997;**20**:486–98.

Randell EW, Lehotay DC. An automated enzymatic method on the Roche COBAS MIRA S for monitoring phenylalanine in dried blood spots of patients with phenylketonuria. *Clin Biochem* 1996;**29**:133–8.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol* 1999;**23**:183–93.

Ratrisawadi V, Horpaopan S, Chotigeat U, Sangtawesin V, Kanjanapattanakul W, Ningsanond V, *et al*. Neonatal screening program in Rajavithi Hospital, Thailand. *Southeast Asian J Trop Med Public Health* 1999; **30** (Suppl 2):28–32.

Reilly AA, Bellisario R, Pass KA. Multivariate discrimination for phenylketonuria (PKU) and non-PKU hyperphenylalaninemia after analysis of newborns' dried blood-spot specimens for six amino acids by ion-exchange chromatography. *Clin Chem* 1998;**44**:317–26.

Rivero A, Allue JA, Grijalba A, Palacios M, Merlo SG. Comparison of two different methods for measurement of phenylalanine in dried blood spots. *Clin Chem Lab Med* 2000;**38**:773–6.

Rohr FJ, Lobbregt D, Levy HL. Tyrosine supplementation in the treatment of maternal phenylketonuria. *Am J Clin Nutr* 1998;**67**:473–6.

Rouse B, Azen C, Koch R, Matalon R, Hanley W, de la Cruz F, *et al*. Maternal Phenylketonuria Collaborative Study (MPKUCS) offspring: facial anomalies, malformations, and early neurological sequelae. *Am J Med Genet* 1997;**69**:89–95.

Rouse B, Matalon R, Koch R, Azen C, Levy H, Hanley W, *et al*. Maternal phenylketonuria syndrome: congenital heart defects, microcephaly, and developmental outcomes. *J Pediatr* 2000;**136**:57–61.

Schulze A, Kohlmueller D, Mayatepek E. Sensitivity of electrospray–tandem mass spectrometry using the phenylalanine/tyrosine-ratio for differential diagnosis of hyperphenylalaninemia in neonates. *Clin Chim Acta* 1999;**283**:15–20.

Scriver CC. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants, by Robert Guthrie and Ada Susi, *Pediatrics*, 1963;**32**:318–343. *Pediatrics* 1998;**102**:236–7.

Scriver CR, Byck S, Prevost L, Hoang L. The phenylalanine hydroxylase locus: a marker for the history of phenylketonuria and human genetic diversity. PAH Mutation Analysis Consortium. *Ciba Foundation Symposium* 1996;**197**:73–90.

Sinai LN, Kim SC, Casey R, Pinto-Martin JA. Phenylketonuria screening: effect of early newborn discharge. *Pediatrics* 1995;**96**:605–8.

Smith ML, Hanley WB, Clarke JT, Klim P, Schoonheyt W, Austin V *et al*. Randomised controlled trial of tyrosine supplementation on neuropsychological performance in phenylketonuria. *Arch Dis Child* 1998; **78**:116–21.

Start K. Treating phenylketonuria by a phenylalanine-free diet. *Professional Care of Mother and Child* 1998; **8**:109–10.

Streetly A. The national newborn screening programme: an audit of phenylketonuria and congenital hypothyroidism screening in England and Wales. London: Department of Public Health Medicine, UMDS Guy's and St Thomas's Medical Schools; 1998.

Streetly A, Grant C, Pollitt RJ, Addison GM. Survey of scope of neonatal screening in the United Kingdom. *BMJ* 1995;**311**:726.

Sueoka H, Moshinetsky A, Nagao M, Chiba S. Mutation screening of phenylketonuria in the Far East of Russia. *J Hum Genet* 1999;**44**:368–71.

Sueoka H, Nagao M, Chiba S. Rapid mutation screening of phenylketonuria by polymerase chain reaction-linked restriction enzyme assay and direct sequence of the phenylalanine hydroxylase gene: clinical application in northern Japan and northern China. *Genet Test* 2000;**4**:249–56.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Public Health Med* 1998;**20**:331–43.

Trefz FK, Aulela-Scholz C, Blau N. Successful treatment of phenylketonuria with tetrahydrobiopterin. *Euro J Pediatr* 2001;**160**:315.

van Spronsen FJ, Smit PG, Koch R. Phenylketonuria: tyrosine beyond the phenylalanine-restricted diet. *J Inherit Metab Dis* 2001;**24**:1–4.

Verma IC. Burden of genetic disorders in India. *Indian J Pediatr* 2000;**67**:893–8.

Wang ST, Pizzolato S, Demshar HP. Receiver operating characteristic plots to evaluate Guthrie, Wallac, and Isolab phenylalanine kit performance for newborn phenylketonuria screening. *Clin Chem* 1997;**43**:1838–42.

Wasant P, Naylor EW, Liammongkolkul S. Detection of inherited metabolic disorders via tandem mass spectrometry in Thai infants. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):154–9.

Wasant P, Svasti J, Srisomsap C, Liammongkolkul S, Naylor EW, Matsumoto I. Inherited metabolic disorders in Thailand – Siriraj experience. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):124–37.

Wasant P, Liammongkolkul S, Srisawat C. Neonatal screening for congenital hypothyroidism and phenylketonuria at Siriraj Hospital, Mahidol University, Bangkok, Thailand – a pilot study. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):33–7.

Wilcken B, Wiley V. Tandem mass spectrometry in the New South Wales newborn screening program. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001;**50** (N RR-3): 33.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999; **88** (Suppl 432):48–51.

Wyatt D. PKU treatment in 1950. *Am J Nurs* 2001; **101**:13.

Yanling Y, Qiang G, Zhixiang Z, Chunlan M, Lide W, Xiru W. A clinical investigation of 228 patients with phenylketonuria in mainland China. *Southeast Asian J Trop Med Public Health* 1999; **30** (Suppl 2):58–60.

Yannicelli S, Ryan A. Improvements in behaviour and physical manifestations in previously untreated adults with phenylketonuria using a phenylalanine-restricted diet: a national survey. *J Inherit Metab Dis* 1995; **18**:131–4.

Zschocke J, Graham CA. A fluorescent multiplex ARMS method for rapid mutation analysis. *Mol Cell Probes* 1995;**9**:447–51.

Zschocke J, Graham CA, Carson DJ, Nevin NC. Phenylketonuria mutation analysis in Northern Ireland: a rapid stepwise approach. *Am J Hum Genet* 1995; **57**:1311–17.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001; **47**:1945–55.

Appendix 11

UK studies of birth incidence: phenylketonuria

Authors, year	Study design Duration of study Country Total screened	Patient type Age at sampling (years) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments
Hutchesson *et al.*, 1998[30]	**Study design** Retrospective cohort study **Duration of study** 22 April 1981 to 21 April 1991 **Country** Neonatal Screening Service Programme, West Midlands, England **Total screened** 707,720	**Patient type** All neonatal infants born in the West Midlands NHS region (covering counties of West Midlands, Hereford, Worcester, Shropshire, Staffordshire and Warwickshire). Data derived from West Midlands Neonatal Screening Programme, regional register for patients with inborn errors of metabolism and population frequencies from national census **Age at sampling** 6–10 days of age **Gender (M/F)** NR	**Outcomes** Frequencies of inborn errors of metabolism (major disorders of amino acid, organic acid, carbohydrate metabolism and storage disorders) in different ethnic groups and underlying gene frequencies **Diagnostic test** The Neonatal Screening Service programme for Birmingham used heparinised plasma and tested for PKU by amino acid chromatography (as opposed to dried blood spots and the Guthrie microbiological assay for phenylalanine, used for the rest of the region) **Threshold for disease identification** NR	**Births and frequencies of inborn errors of metabolism affecting the major disorders of amino acid, organic acid, carbohydrate metabolism and storage disorders in the West Midlands between 1981 and 1991** Autosomal recessive inborn errors |	No. of births[a] (%)	No. of diagnoses (%)	Cumulative Incidence (95% CI) | |---|---|---|---| | Total	707,720	263	1:2691 (1:2475, 1:3037) | | North-west European	605,331 (85.5)	160 (60.8)	1:3783 (1:3240, 1:4445) | | Pakistani	28,903 (4.1)	91 (34.6)	1:318 (1:259, 1:394) | | Indian	31,062 (4.4)	5 (1.9)	1:6212 (1:2662, 1:19,133) | | Afro-Caribbean	16,887 (2.4)	1 (0.4)	1:16,887 (1:3031, 1:667,470) | | Other ethnic groups and mixed race	25,537 (3.6)	6 (2.3)	1:4256 (1:1955, 1:11,598) | [a] Number of neonates from whom a sample was collected for PKU screening at 6–10 days of age. The overall incidence of recorded inborn errors of metabolism was ten times higher among Pakistanis than in white children (1:318 vs 1:3760). In contrast, only 5 Indian children (incidence 1:6212) and 1 Afro-Caribbean child were identified (incidence 1:16,887) with autosomal recessive inborn errors	Authors reported that they were unable to identify those who died before collection of neonatal sample, children with inborn errors of metabolism may have died without recognition of the underlying diagnosis, particularly with tyrosinaemia type I and MCAD deficiency, which can present with neonatal death, and some may have failed as yet to present clinically, particularly MCAD deficiency, which is frequently underdiagnosed

continued

Authors, year	Study design Duration of study Country Total screened	Patient type Age at sampling (years) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments
		Ethnicity North-west European, Pakistani, Indian, Afro-Caribbean, other ethnic groups and mixed race	**Confirmation of disease** Confirmation of inborn errors of metabolism, for children born in the region during the assessment period, was derived from laboratory records	**Selected comparative diseases of the most common autosomal recessive inborn errors of metabolism in the West Midlands region between 1981 and 1991**	The authors concluded that the results illustrate the interplay between gene frequency and parental consanguinity in determining disease frequencies in different populations, and indicate predictable disease frequencies in the absence of consanguineous marriage

	Cases (families)	North-west European (95% CI))	Pakistani (95% CI)
Disease frequency			
PKU	52 (50)	1:12,611 (1:9512, 1:17,104)	1:14,452 (1:4001, 1: 119,337)
Tyr	13 (10)	1:302,655 (1:83,786, 1: 2.5×10^6)	1:2628 (1:1468, 1:5263)[a]
MCAD deficiency	9 (9)	1:67,259 (1:35,430, 1:147,089)	0 (<1:9350)
Gene frequency			
PKU	52 (50)	1:112 (1:98, 1:131)	1:713 (1:210, 1: 5750)[b]
Tyr	13 (10)	1:550 (1:289, 1:1581)	1:144 (1:87, 1:271)[c]
MCAD deficiency	9 (9)	1:259 (1:188, 1:384)	0 (<1:395)[c]

[a] $p < 0.001$ vs frequency in north-west Europeans; [b] $p < 0.01$ vs frequency in north-west Europeans; [c] $p < 0.05$ vs frequency in north-west Europeans.

The incidence of PKU was similar in Pakistani and white children (1:12,611 vs 1:14,452); however, the gene frequency was significantly lower (1:112 vs 1:713, $p < 0.01$).
Two cases of PKU were identified in mixed race children (1 mixed Jordanian/European and 1 of Afro-Caribbean/Arabic origin). No cases of PKU were observed among Indians
The incidence of tyrosinaemia type 1 was significantly higher in the Pakistani population (1:2628 vs 1:302,655; $p < 0.001$) than in the north-west European subjects. Similar findings were observed with gene frequency

Authors, year	Study design Duration of study Country Total screened	Patient type Age at sampling (years) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments
				(1:144 vs 1:550, respectively, $p < 0.05$). This is because of the effect of consanguinity, which increases both anticipated disease and gene frequency rates The gene frequency for MCAD deficiency was significantly lower in the Pakistani population than in north-west European children. This illustrates that the diagnosis for MCAD deficiency is rare outside those of north-west European ethnicity	

M: male; F: female.

Appendix 12

Effectiveness of treatments for phenylketonuria: dietary interventions

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments
Poustie and Rutherford 2002[31] (Cochrane systematic review, updated 13 Nov 2001)	**Search dates** 1966 to 25 January 2001 **Databases searched** MEDLINE, EMBASE, handsearching journals and abstracts of conferences, reference lists, Cystic Fibrosis and Genetic Disorders trials register and manufacturers of very low or phenylalanine-free protein supplements **Inclusion/exclusion criteria** Randomised or pseudo-randomised trials comparing phenylalanine restricted diet to either relaxation or termination of dietary restrictions in patients with PKU (any age)	**Type of studies** RCTs or pseudo-randomised studies **Type of participants** Individuals of any age with PKU and other forms of phenylalanine hydroxylase deficiency diagnosed by the Guthrie test or other recognised, validated screening method in which dietary intervention was initiated early in life **Sample no.** 251 (see results) **Age** See results **Gender (M/F)** See results **Ethnicity** See results **Type of interventions** Phenylalanine-restricted diet with phenylalanine-free or very low phenylalanine amino acid supplement, started early in life and either continued,	**Statistical techniques** Pooled estimate of treatment effect for each outcome across studies and calculated weighted mean difference **Tests of heterogeneity** Yes, using standard χ^2 test or ANOVA **Outcome measures** Blood phenylalanine and tyrosine concentrations, weight gain/body mass index/ Z scores/centiles/ other indices of nutritional status or growth, neuropsychological performance, intelligence, energy and	**Patient and study characteristics of included studies** Study: Clarke, 1987[110]; Griffiths, 1998[111,112]; Holtzman, 1975[113]; US/PKU collaborative Sample no.: 9; 16; 10; 216 Gender (M/F): 3/6; 10/6; 6/4; NR Mean age: 14.4 years; 12.6 years; NR; NR Ethnicity: NR; NR; NR; NR Duration: 10 weeks; 6 months; 2 years; 12 years **Results** **Comparison**: PKU patients started on diet at diagnosis: diet continuation vs discontinuation or relaxation later in life *Outcome: Blood phenylalanine level (μmol/l)* 0–3 months[a]: 29; 30; $p = 0.00$; –672.203 (–813.799 to –530.608) 3–6 months[b]: 5; 5; $p = 0.00$; –871.200 (–1261.542 to –480.858) 6–12 months[b]: 5; 5; $p = 0.00$; –913.500 (–1370.426 to –456.574) After 1 year[c]: 42; 48; $p = 0.00$; –751.540 (–883.412 to –619.667) *Outcome: Weight* 0–3 months[b]: 44; 44; $p = 0.15$; 0.200 (–0.072 to 0.472) 3–6 months[b]: 44; 44; $p = 0.30$; 0.200 (–0.156 to 0.556) 6–12 months[b]: 44; 44; $p = 0.70$; 0.100 (–0.381 to 0.581)	Majority of studies involved only small number of subjects. Several included studies failed to provide details on allocation concealment and method of randomisation sequence. Only 2 of the 4 included studies used intention-to-treat analysis More than 30 different assessments of neuropsychological performance were evaluated in the 4 included studies. Of these different assessments, only 3 were used in more than 1 study; however, final data from these assessments were not available. In addition, the following outcome measures were not measured in any of the studies: blood tyrosine concentration, eating behaviour, quality of life and mortality

continued

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments
	Assessment of validity and quality Yes, two reviewers independently selected trials and assessed methodological quality which included method of randomisation, generation of randomisation sequence, blinding level and patients loss to follow-up or excluded from the study	discontinued or relaxed at any point during life of patient	nutrient intake, eating behaviour, quality of life and death	See table below	As would be expected, blood phenylalanine concentrations (assessed in all the trials) were significantly lower in subjects with PKU following a phenylalanine-restricted diet than in those on a less restricted or relaxed diet. IQ was significantly higher in subjects who continued on the phenylalanine-restricted diet than in those who terminated the diet; however, these findings are based on only 1 study Based on the results of the this review, the authors reported that no firm conclusions could be made about the effectiveness of specific dietary interventions in PKU owing to a lack of good-quality RCTs

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: IQ				
>12 months[b]	53	62	$p = 0.03$	–5.000 (–9.595 to –0.405)
Outcome: Calorie intake (kcal/kg)				
0–3 months[b]	44	44	$p = 0.80$	1.000 (–9.064 to 11.064]
3–6 months[b]	44	44	$p = 0.40$	3.000 (–4.116 to 10.116)
6–12 months[b]	44	44	$p = 0.07$	6.000 (–0.507 to 12.507)
Outcome: Protein intake (g/kg)				
0–3 months[b]	44	44	$p = 0.00$	0.000 (–0.398 to 0.398)
3–6 months[b]	44	44	$p = 0.40$	0.100 (–0.131 to 0.331)
6–12 months[b]	44	44	$p = 0.30$	0.100 (–0.109 to 0.309)

Test for heterogeneity: [a] $p < 0.05$, [b] NA (data from 1 study only), [c] $p > 0.05$.

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments

Comparison: PKU patients started on strict diet since diagnosis: diet continuation vs discontinuation or relaxation later in life

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: Blood phenylalanine level (μmol/l)				
0-3 months[a]	20	21	$p = 0.00$	–698.667 (–869.444 to –527.889)
3–6 months[b]	5	5	$p = 0.00$	–871.200 (–1261.542 to –480.858)
6–12 months[b]	5	5	$p = 0.00$	–913.500 (–1370.426 to –456.574)
> 12 months[c]	42	48	$p = 0.00$	–751.632 (–883.505 to –619.759)

Test for heterogeneity: [a] $p < 0.05$, [b] NA (data from 1 study only), [c] $p > 0.05$.

Comparison: PKU patients started on strict diet since diagnosis, later relaxed diet: diet reestablishment vs continuation

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: Blood phenylalanine level (μmol/l)				
0–3 months[b]	9	9	$p = 0.00$	–614.000 (–867.271 to –360.729)
3–6 months[b]	0	0	NA	NA
6–12 months[b]	0	0	NA	NA
>12 months[b]	0	0	NA	NA

Test for heterogeneity: [b] NA (data from 1 study only).

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments

Comparison: PKU patients at diagnosis: low phenylalanine diet vs moderate phenylalanine diet

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: Blood phenylalanine level (μmol/l)				
0–3 months[b]	66	66	$p = 0.00$	–127.100 (–185.045 to –69.155)
3–6 months[b]	0	0	NA	NA
6–12 months[b]	66	66	$p = 0.00$	–157.300 (–217.179 to –97.421)
At 2 years[b]	66	66	$p = 0.02$	–84.700 (–158.018 to –11.382)
At 3 years[b]	63	65	$p = 0.20$	–48.400 (–125.394 to 28.594)
At 4 years[b]	64	63	$p = 0.11$	–78.700 (–174.492 to 17.092)
At 5 years[b]	62	65	$p = 0.11$	–72.600 (–162.352 to 17.152]
At 6 years[b]	0	0	NA	NA
Outcome: Weight				
0–3 months[b]	44	44	$p = 0.15$	0.200 (–0.072 to 0.472)
3–6 months[b]	44	44	$p = 0.30$	0.200 (–0.156 to 0.556)
6–12 months[b]	44	44	$p = 0.70$	0.100 (–0.381 to 0.581)

Appendix 12 cont'd *Effectiveness of treatments for phenylketonuria*

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: IQ				
At 4 years[b]	58	53	$p = 0.30$	–3.000 (–8.768 to 2.768)
At 6 years[b]	66	66	$p = 0.50$	–2.000 (–7.408 to 3.408)
Outcome: Calorie intake (kcal/kg)				
0–3 months[b]	44	44	$p = 0.80$	1.000 (–9.064 to 11.064)
3–6 months[b]	44	44	$p = 0.40$	3.000 (–4.116 to 10.116)
6–12 months[b]	44	44	$p = 0.07$	6.000 (–0.507 to 12.507)
Outcome: Protein intake (g/kg)				
0–3 months[b]	44	44	$p = 0.00$	0.000 (–0.398 to 0.398)
3–6 months[b]	44	44	$p = 0.40$	0.100 (–0.131 to 0.331)
6–12 months[b]	44	44	$p = 0.30$	0.100 (–0.109 to 0.309)

Test for heterogeneity: [b] NA (data from 1 study only).

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Griffiths *et al.*, 1995[35]	**Study design** Cross-sectional mixed design with matched pairs, independent samples and non-repeated measures **Randomisation method** Not applicable; however, tests were randomised and first to last sequence counterbalanced **Duration of study** Period of study NR **Setting/location** NR, Scotland, UK	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** Mean neonatal blood phenylalanine level before treatment for T1 (1977 ± 500 μmol/l) vs. T2 (1912 ± 1077 μmol/l), p = NS; initiation of treatment for T1 vs T2, p = NS; mean phenylalanine level when tested for T1 (348 ± 167 μmol/l) vs T2 (1014 ± 216 μmol/l), $p < 0.001$. Average ages of matched younger and older controls did not differ significantly from those of PKU	**Type of interventions** Phenylalanine-restricted diet vs diet termination **Dosage/ outcomes** Outcome measures included a battery of neuro-psychological tests **Patient types** PKU patients (from West of Scotland register) consisted of younger children who were still on diet and older adolescents and adults who discontinued dietary treatment at median age of 10.17 years (range	**Median age** T1: 7.53 years (range 5.58–9.75 years) T2: 20.59 years (range 13.58–28.42 years) T3: 7.59 years (range 5.66–9.75 years) T4: 20.54 years (range 13.58–27.92 years) **Gender (M/F)** T1: 7/3 T2: 7/3 T3: NR T4: NR **Ethnicity** NR	Mean ± SD scores for neuropsychological test Tests: Young T1 / Young T3 / Old T2 / Old T4 Reaction time (s): 3.31 ± 0.89 / 2.95 ± 0.52 / 2.37 ± 0.77 / 2.20 ± 0.47 Peg transfer (s): 88.50 ± 19.56 / 72.70 ± 12.80 / 51.50 ± 5.42 / 46.90 ± 4.75 Matching figures: 4.90 ± 1.52 / 4.80 ± 1.55 / 7.20 ± 1.40 / 8.80 ± 1.23 Letter cancellation: 3.33 ± 0.77 / 3.51 ± 1.46 / 7.36 ± 3.52 / 9.76 ± 5.06 Verbal fluency: 18.80 ± 8.95 / 19.00 ± 9.63 / 33.80 ± 9.43 / 38.90 ± 8.63 Design fluency: 11.90 ± 2.56 / 12.20 ± 2.35 / 18.20 ± 3.43 / 24.50 ± 3.41 Rey verbal learning: 42.40 ± 10.09 / 43.00 ± 7.65 / 46.70 ± 8.08 / 55.10 ± 7.40 Rey labyrinth: 10.50 ±4.74 / 13.10 ±4.98 / 14.50 ±3.95 / 17.60 ±1.08 Data are performance scores expressed as total correct items, except for Reaction time and peg transfer tasks, which are scored in seconds (the lower the value the better the performance).	Authors concluded that the results from the neuropsychological test failed to provide compelling evidence that prolonged exposure to unrestricted phenylalanine during adolescence and early adulthood is harmful to cognitive and motor functioning and do not necessarily support a diet-for-life policy Delaying dietary termination until 10 years of age is sufficient to prevent a substantial reduction in cognitive and motor ability; however, there

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
		samples (T1 vs T3, p = NS; T2 vs T4, p = NS) **Intention-to-treat analysis** NR	9.50–11.42 years). All diagnosed with classical PKU and treated within 3 months of birth, with treatment starting at median age of 15 days (range 12–20 days) for younger group and 31 days for (range 8–75 days) for older group (p = NS). All controls free from major developmental disorders **Patient numbers** Patients with PKU: T1: 10 (young, on diet) T2: 10 (old, off diet) Matched non-clinical control on basis of age, gender and approximate socio-economic status: T3: 10 (young) T4: 10 (old) **Loss to follow-up** NR	*F*-ratios from two-factor ANOVA Tests: Reaction time (s); Peg transfer (s); Matching figures; Letter cancellation; Verbal fluency; Design fluency; Rey verbal learning; Rey labyrinth Factor: Age/diet *F* 15.20, 63.35, 48.47, 26.50, 36.23, 97.73, 9.59, 11.28; *P* 0.001, 0.001, 0.001, 0.001, 0.001, 0.001, 0.01, 0.01 Factor: Diagnosis *F* 1.50, 7.22, 2.75, 1.79, 0.84, 12.31, 2.89, 5.07; *P* NS, 0.05, NS, NS, NS, 0.01, NS, 0.05 Interaction *F* 0.19, 2.28, 3.53, 1.05, 0.71, 10.17, 2.17, 0.04; *P* NS, NS, NS, NS, NS, 0.01, NS, NS Age/diet factor refers to younger, on-diet PKU children and controls versus older, off-diet PKU adolescents/adults and controls. Diagnosis factor refers to all PKU subjects (i.e. both on and off diet) vs all controls. Owing to developmental factors favouring older patients, a highly significant age effect on each measure was observed. Findings for the diagnosis factor illustrated that subjects with PKU had significantly poorer ability than controls on Peg transfer, Design fluency and Rey labyrinth test, irrespective of age or treatment status. Design fluency was the only test to show significant interaction between diagnosis and treatment status, the older, off-diet PKU group doing differentially worse than the younger on-diet group (Scheffé, $p < 0.001$)	were indications that termination of dietary control at the age of 10 years appeared to be associated with deficits in frontal executive functioning. Furthermore, inferences to wider PKU populations should be made with caution owing to small sample sizes and limited functions test

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Robinson *et al.*, 2000[36]	**Study design** Prospective cohort study with historical controls **Randomisation method** NA **Duration of study** Period of study NR **Setting/location** Willink Biochemical Genetics Unit, Manchester, England, UK	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** Mean haemoglobin values and mean cell volumes in each subgroup were not significantly different from normal **Intention to treat analysis** NR	**Type of interventions** Effect of different degrees of dietary interventions on vitamin B_{12} and folate levels **Dosage/outcomes** Vitamin B_{12} and folate status **Patient types** Adolescents and adults with classical PKU **Patient numbers** T1: strict diet (strict low-phenylalanine diet with amino acid, mineral and vitamin supplements, $n = 22$) T2: relaxed diet (total protein intake of approx. 1 g/kg per day with approx 50% from natural protein and 50% from amino acid,	**Median age** (range 11–38 years) T1: 24 years T2: 21 years T3: 22 years T4:NR **Gender (M/F)** NR **Ethnicity** NR	**Blood phenylalanine, blood vitamin B_{12} and erythrocyte folate values in patients with and without classical PKU** Intervention T1 / T2 / T3 / T4 Mean blood phenylalanine levels (μmol/l) 500 / 1100 / 1220 / NR $n = 22$ / $n = 30$ / $n = 31$ / NR Mean vitamin B_{12} values (ng/l) 468.7 ± 199.7 / 332.8 ± 128 / 275.3 ± 95 / 411.9 ± 148.75 $p = 0.077$ / $p = 0.0034$ / $p = 0.0001$ $n = 22$ / $n = 30$ / $n = 31$ / $n = 1676$ Mean erythrocyte folate values (μg/l) 476 ± 258 / 471 ± 190.5 / 350 ± 166.1 / 201 ± 92.8 $p < 0.0001$ / $p < 0.0001$ / $p < 0.0001$ $n = 11$ / $n = 21$ / $n = 30$ / $n = 1502$ *p*: probability value for *t*-test of difference in means between PKU and normal population (statistically significant, $p < 0.05$). Vitamin B_{12} levels were significantly lower in the PKU groups on relaxed or unrestricted diets than in the normal population. Folate levels were significantly elevated in all PKU groups; however, some patient samples were insufficient for the assay of erythrocyte folate	Authors concluded that adolescents and adult patients with PKU who have stopped or relaxed their diet might be at risk from vitamin B_{12} deficiency. These authors recommend continued medical and dietetic supervision and having the vitamin B_{12} status monitored

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			mineral and vitamin supplements, $n = 30$) T3: unrestricted diet (no formal protein restriction and not taking amino acid supplements, $n = 31$) T4: normal population (data from the Dietary and Nutritional Survey of British Adults published in 1990, $n = 1676$) **Loss to follow-up** NR			

ANOVA: analysis of variance; NS: not significant

Appendix 13

Effectiveness of treatments for phenylketonuria: tyrosine supplementation

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments
Poustie and Rutherford 2002[32] (Cochrane systematic review, updated 29 Aug 2001)	**Search dates** 1966 to 25 June 2001 **Databases searched** MEDLINE, EMBASE, handsearching journals and abstracts of conferences, reference lists, Cystic Fibrosis and Genetic Disorders trials register and manufacturers of dietary products used in the treatment of PKU **Inclusion/exclusion criteria** Randomised or pseudo-randomised trials comparing the use of tyrosine supplementation versus placebo in patients with PKU in addition to, or instead of, a phenylalanine-restricted diet. Patients treated for maternal PKU were excluded	**Type of studies** RCTs or pseudo-randomised studies **Type of participants** Individuals of any age with PKU and other forms of phenylalanine hydroxylase deficiency diagnosed by the Guthrie test or other recognised, validated screening method in which dietary intervention was initiated early in life **Sample no.** 56 (see Results) **Age** See Results **Gender (M/F)** See Results **Ethnicity** See Results **Type of interventions** Enteral supplementation of tyrosine (in patients with PKU, who have been treated with a low-phenylalanine diet from diagnosis and who	**Statistical techniques** Pooled estimate of treatment effect for each outcome across studies and calculated weighted mean difference **Tests of heterogeneity** Yes, using standard χ^2 test or ANOVA **Outcome measures** Blood phenylalanine and tyrosine concentrations, weight gain and any other indices of nutritional status or growth, neuropsychological performance, intelligence, quality of life and death	**Patient and study characteristics of included studies** Mazzocco, 1992; Pietz, 1995; Smith, 1998 Sample no.: 9; 24; 23 Gender (M/F): 4/5; 11/13; 11/12 Mean age (year): 10.25; 20.08; 11.3 Age range (year): 6.5–13.25; 16–25; 6–28 Ethnicity: NR; NR; NR Duration: NR; 24 weeks; 12 to 16 weeks Tyrosine intervention (supplement dosage): 2500 mg/day; 100 mg/kg/day; 100 mg/kg/day **Comparison**: All patients with PKU *Outcome: Blood phenylalanine concentration (μmol/l)* 0–3 months[c]: Treatment (*n*) 51; Control (*n*) 51; Overall effect $p = 0.60$; Weighted mean difference (fixed) (95% CI) 24.478 (–72.790 to 121.747) 3–6 months: NA; NA; NA; NA 6–12 months: NA; NA; NA; NA *Outcome: Blood tyrosine concentration (μmol/l)* 0–3 months[a]: 51; 51; $p = 0.00$; 22.996 (12.895 to 33.097) 3–6 months: NA; NA; NA; NA 6–12 months: NA; NA; NA; NA *Outcome: Neuropsychological performance* 0–3 months[c]: 42; 42; $p = 0.50$; –9.363 (–34.859 to 16.133) 3–6 months: NA; NA; NA; NA 6–12 months: NA; NA; NA; NA Test for heterogeneity: [a] $p < 0.05$, [c] $p > 0.05$.	Authors reported that the 3 included studies were double blind and placebo controlled with adequate allocation concealment. In all 3 studies, only small numbers of subjects were involved and the duration of the treatment and control arms was brief. Two studies failed to provide details of the method of randomisation sequence and only 1 of the 3 included studies used intention-to-treat analysis IQ could not be assessed because the results of all the patients were combined and compared with a non-PKU group. In addition, the following outcome measures were not measured in any of the studies:

continued

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments
	Assessment of validity and quality Yes, two reviewers independently selected trials and assessed methodological quality which included method of randomisation, generation of randomisation sequence and patients loss to follow-up or excluded from the study	continued or discontinued their diet later in life) compared with no tyrosine supplementation or placebo		**Comparison**: Patients with PKU continued on diet since diagnosis Period | Treatment (*n*) | Control (*n*) | Overall effect | Weighted mean difference (fixed) (95% CI) *Outcome: Blood phenylalanine concentration (μmol/l)* 0–3 months[c] | 30 | 30 | $p = 0.70$ | 19.250 (–98.040 to 136.540) 3–6 months | NA | NA | NA | NA 6–12 months | NA | NA | NA | NA *Outcome: Blood tyrosine concentration (μmol/l)* 0–3 months[c] | 30 | 30 | $p = 0.01$ | 13.674 (3.042 to 24.306) 3–6 months | NA | NA | NA | NA 6–12 months | NA | NA | NA | NA *Outcome: Neuropsychological performance* 0–3 months[b] | 21 | 21 | $p = 0.90$ | 4.500 (–73.957 to 82.957) 3–6 months | NA | NA | NA | NA 6–12 months | NA | NA | NA | NA Test for heterogeneity: [b] NA (data from 1 study only), [c] $p > 0.5$. **Comparison**: Patients with PKU on diet from diagnosis who no longer follow the diet Period | Treatment (*n*) | Control (*n*) | Overall effect | Weighted mean difference (fixed) (95% CI) *Outcome: Blood phenylalanine concentration (μmol/l)* 0–3 months[b] | 21 | 21 | $p = 0.70$ | 36.000 (–138.064 to 210.064) 3–6 months | NA | NA | NA | NA 6–12 months | NA | NA | NA | NA *Outcome: Blood tyrosine concentration (μmol/l)* 0–3 months[b] | 21 | 21 | $p = 0.00$ | 109.000 (76.618 to 141.382) 3–6 months | NA | NA | NA | NA 6–12 months | NA | NA | NA | NA	weight gain, other measures of nutritional status, quality of life and death As would be expected, the blood tyrosine concentrations were significantly higher in subjects receiving tyrosine supplements than those in the placebo group. No other significant differences were found in any of the other outcomes Based on the data currently available, the authors concluded that there was not enough evidence to show the effect of tyrosine supplementation to the diet of people with PKU

Authors, year	Search dates Databases searched Inclusion/exclusion criteria Assessment of validity and quality	Type of studies Type of participants Sample no. Age Gender (M/F) Ethnicity Type of interventions	Statistical techniques Tests of heterogeneity Outcome measures	Results (odds ratios, relative risks, confidence intervals, etc.)	Comments

Period	Treatment (*n*)	Control (*n*)	Overall effect	Weighted mean difference (fixed) (95% CI)
Outcome: Neuropsychological performance				
0–3 months[b]	21	21	$p = 0.40$	–11.000 (–37.959 to 15.959)
3–6 months	NA	NA	NA	NA
6–12 months	NA	NA	NA	NA

Test for heterogeneity: [b] NA (data from 1 study only), [c] $p > 0.05$.

Appendix 14

Effectiveness of treatments for phenylketonuria: fatty acid supplementation

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Agostoni *et al.*, 1995[33]	**Study design** Single-blind, RCT **Randomisation method** Time-balanced randomisation table **Duration of study** 6 months **Setting/location** NR, Italy	**Inclusion/exclusion criteria** NR **Power calculation** NR **Baseline comparability** Yes, no differences between PKU groups for bleeding times, lipid values and fatty acid levels in plasma total lipids before supplementation **Intention-to-treat analysis** NA	**Type of interventions** T1: fish oil containing LCPUFA of the n-3 series T2: blackcurrant seed oil containing compounds of both n-6 and n-3 series immediately beyond limiting step for LCPUFA synthesis T3: normal free diet (control) **Dosage/outcomes** One commercially available capsule in a triglyceride form (500 mg) per 4 kg body weight per day for 6 months. Subjects received 5–8 capsules (2.5–4 g oil) per day on basis of individual body weight. Outcomes included plasma lipid and fatty acid status	**Age (years)** Range 5–10 **Gender (M/F)** Included both genders, but ratios NR **Ethnicity** NR	Significant decrease from baseline observed for triglycerides in T1 group (data shown below); in contrast, no significant changes from baseline were found for total cholesterol, HDL cholesterol and LDL cholesterol in the T2 group Significant increases in total polyunsaturated fatty acids observed in fatty acid composition of plasma total lipids in both PKU groups. Significant increases from baseline for n-3 LCPUFA in T1 group (data shown below). These values were also significantly higher than T3 group. Only plasma dihomo-γ-linolenic acid (20:3n-6) significantly increased in T2 group No other significant changes were observed from baseline for the following fatty acids: 18:2n-6; 20:4n-6; 22:4n-6 and 22:5n-6. In addition, bleeding time values in the T1 and T2 group did not differ from baseline values and were not significantly different compared with T3 (data not shown, but provided in original paper)	No adverse reactions reported during study period In children with PKU, fish oil supplementation significantly decreased triglycerides and significantly increased *n*-3 LCPUFA. In contrast, the blackcurrant oil displayed no significant changes in the lipid profile from baseline, except for n-6 linolenic acid Authors concluded that a more complete source of LCPUFA of both the n-6

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			Patient types Treated children with PKU monitored for clinical symptoms and nutritional follow-up **Patient numbers** T1: 10 (with PKU) T2: 11 (with PKU) T3: 12 (healthy children) **Loss to follow-up** T1: 0/10 T2: 0/11 T3: 0/12		**Plasma lipid (mmol/l) and fatty acid composition (weight %) of plasma total lipids before and after supplementation** Lipid or fatty acid: T1 Baseline; T1 >6 months; T2 Baseline; T2 >6 months; T3 Control *Plasma lipid* Triglycerides: 1.17 ± 0.14; 0.68 ± 0.16[ab]; 123 ± 0.45; 1.09 ± 0.47; 1.03 ± 0.25 *Fatty acid* Total monounsaturated: 32.3 ± 5.4; 28.3 ± 3.9[a]; 32.5 ± 5.8; 30.8 ± 4.9; 28.1 ± 4.0 20:3n-6: 1.60 ± 0.48; 1.21 ± 0.32[ab]; 1.48 ± 0.36; 2.09 ± 0.36[ab]; 1.69 ± 0.45 20:5n-3: 0.24 ± 0.05; 1.96 ± 0.79[ab]; 0.26 ± 0.06; 0.27 ± 0.06[b]; 0.61 ± 0.27 22:5n-3: 0.42 ± 0.18; 1.20 ± 0.15[ab]; 0.38 ± 0.11; 0.32 ± 0.09; 0.41 ± 0.13 22:6n-3: 0.65 ± 0.10; 2.94 ± 0.88[ab]; 0.66 ± 0.12; 0.73 ± 0.08[b]; 1.78 ± 0.52 Total polyunsaturated: 39.1 ± 4.6; 41.9 ± 3.6[a]; 38.3 ± 6.0; 41.3 ± 5.7; 39.9 ± 3.6 [a] significantly different from baseline, $p < 0.05$; [b] significantly different from control, $p < 0.05$.	and n-3 series should be supplied and investigated further for dietary supplementation of PKU patients

Appendix 14 cont'd *Effectiveness of treatments for phenylketonuria: fatty acid supplementation*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Agostoni *et al.*, 2001[34]	**Study design** Double-blind, randomised, placebo-controlled trial **Randomisation method** NR **Duration of study** 12 months **Setting/location** NR, Italy	**Inclusion/exclusion criteria** NR **Power calculation** Yes, require 9 per group at 90% power **Baseline comparability** Yes, no significant differences between groups for basal blood phenylalanine and mean blood phenylalanine during supplementation **Intention-to-treat analysis** No	**Type of interventions** T1: long-chain PUFA T2: placebo (olive oil) T3: reference dietary and biochemical data from healthy subjects **Dosage/outcomes** Gelatine capsules (500 mg oil per capsule) supplying either 26% fatty acid as LCPUFA (including 4.6% γ-linolenic acid, 7.4% arachidonic acid, 5.5% eicosapentaenoic acid and 8% docosahexaenoic acid) or placebo (olive oil). Daily dosage of supplement provided 0.3–0.5% of individual daily	**Mean age (years)** T1: 10 ± 7 T2: 10 ± 5 T3: NR **Gender (M/F)** T1: 5/5 T2: 6/4 T3: NR **Ethnicity** NR	At baseline, HPA children (T1 and T2) had significantly lower levels of saturated fatty acid, docosahexaenoic acid (22:6n-3), non-significantly lower eicosapentaenoic acid (20:5n-3), and significantly higher levels of monounsaturated fatty acid, 20C polyunsaturated derivative (20:3n-9) and a trend towards higher linolenic acid levels than the reference (T3) group (similar observations were observed in erythrocyte lipids; data not shown) At the end of the 12-month period, supplemented HPA (T1) children with long-chain PUFA showed an increase by around 100% in the baseline docosahexaenoic acid levels in plasma phospholipids and were significantly different from the unsupplemented group (T2). Similar findings in erythrocytes, but percentage changes in docosahexaenoic acid were not significantly higher than those in the unsupplemented group. Arachidonic acid levels were quite similar between groups (T1, T2 and T3) in plasma phospholipids and erythrocyte lipids. Therefore, supplementation resulted in higher n-3 PUFA levels without modifications in the n-6 series Blood lipid levels (total cholesterol, HDL cholesterol and LDL cholesterol) did not significantly change at the end of treatment (data not shown)	The authors concluded that a balanced supplementation with LCPUFA in treated HPA children may improve the docosahexaenoic acid status without adversely affecting the arachidonic acid status

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			energy requirements as LCPUFA or placebo equivalent **Patient types** Children treated from first month of life were detected by newborn screening and diagnosed as having type I HPA according to predefined protocols **Patient numbers** T1: 12 T2: 12 T3: 18 **Loss to follow-up** T1: 2/12 T2: 2/12 T3: NR		LCPUFA (weight %) in plasma phospholipids (mean ± SD) Baseline: T3 (Reference) / T2 / T1; End of treatment: T2 / T1 Saturated: 49.2 ± 2.5 / 44.9 ± 3.0[a] / 43.9 ± 1.9[ab] / 45.8 ± 2.9 / 44.5 ± 2.0 Mono-saturated: 12.9 ± 1.1 / 18.1 ± 3.9[a] / 18.3 ± 3.8[ab] / 16.3 ± 3.3 / 16.8 ± 2.9 18:2n-6: 21.3 ± 2.5 / 20.1 ± 2.8 / 20.8 ± 4.1 / 21.4 ± 2.1 / 21.7 ± 3.6 20:4n-6: 9.0 ± 1.4 / 8.6 ± 1.3 / 9.5 ± 1.7 / 8.3 ± 2.0 / 9.0 ± 1.7 n-6 series: 34.0 ± 2.4 / 33.9 ± 3.5 / 34.9 ± 3.8 / 35.2 ± 2.4 / 34.5 ± 2.3 18:3n-3: 0.10 ± 0.03 / 0.17 ± 0.09[a] / 0.13 ± 0.03 / 0.17 ± 0.11 / 0.15 ± 0.05 20:5n-3: 0.32 ± 0.10 / 0.29 ± 0.13 / 0.26 ± 0.16 / 0.21 ± 0.13 / 0.32 ± 0.20 22:6n-3: 2.6 ± 0.5 / 1.8 ± 0.5[a] / 1.6 ± 0.3[ab] / 1.6 ± 0.4 / 3.1 ± 1.6[b] n-3 series: 3.6 ± 0.5 / 2.8 ± 0.7[a] / 2.5 ± 0.4[ab] / 2.5 ± 0.6 / 4.1 ± 1.8[b] Poly--unsaturated: 37.8 ± 2.3 / 37.0 ± 3.1 / 37.8 ± 3.6 / 37.9 ± 2.6 / 38.7 ± 2.2 20:3 n-9: 0.09 ± 0.05 / 0.24 ± 0.10[a] / 0.34 ± 0.17[ab] / 0.14 ± 0.06 / 0.12 ± 0.08 [a] Significantly different from reference, $p < 0.05$; [b] significant difference at baseline or end of treatment between T1 and T2, $p < 0.002$.	

Appendix 14 cont'd *Effectiveness of treatments for phenylketonuria: fatty acid supplementation*

Appendix 15

Effectiveness of treatments for phenylketonuria: treatment during pregnancy

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Waisbren *et al.*, 2000[37]	**Study design** Longitudinal, prospective cohort study (Maternal PKU Collaborative Study) **Randomisation method** NA **Duration of study** Ongoing, started in 1984 **Setting/location** 78 metabolic clinics and obstetric offices, Canada, Germany and the USA	**Inclusion/exclusion criteria** Offspring (who reached their 4th birthday) of all pregnant women with PKU or MHP were included **Power calculation** NR **Baseline comparability** NR **Intention-to-treat analysis** NR	**Type of interventions** Women with PKU offered low-phenylalanine diet before or during pregnancy with aim of maintaining metabolic control (plasma phenylalanine ≤ 605 μmol/l). Women with MHP had plasma phenylalanine <605 μmol/l on normal diet and were not treated **Dosage/outcomes** Outcomes measures include children's scores on cognitive and behavioural assessments compared by maternal metabolic status at 0–10 weeks, 10–20 weeks and after 20 weeks of gestation	**Age (year)** Not reported, however outcome assessed at 4 years of age: **Gender (M/F)** Not reported **Ethnicity** Not reported **Other** Statistical power calculated at more than 80% for each subgroup comparison with non HPA group if differences of 10 points found on standardised tests, but not large enough to detect	**Maternal characteristics** The majority of women with PKU attained metabolic control after 10 gestational weeks. Maternal IQ was lower in these women and their assigned plasma phenylalanine level was higher **Maternal characteristics of children receiving preschool evaluation** Study group / No. of cases / Maternal full-scale IQ[a] / Maternal assigned plasma phenylalanine level (mg/dl) T1 / 17 / 91 ± 11 / 22.5 ± 5.6 T2 / 26 / 83 ± 10 / 23.0 ± 7.3 T3 / 47 / 83 ± 11 / 24.3 ± 7.1 T4 / 59 / 80 ± 10 / 26.4 ± 6.7 T5 / 33 / 95 ± 13 / 6.3 ± 1.7 T6 / 71 / 101 ± 13 / NR [a] Number of subjects 229. Data are shown as mean ±SD. **Cognitive effects in offspring** Scores on the McCarthy GCI decreased as weeks to metabolic control increased. For example, offspring of women with PKU who had metabolic control before pregnancy had a mean ±SD score of 99 ± 13, in contrast to a score of 71 ± 19 for maternal metabolic control achieved after > 20 weeks. A linear relationship between number of weeks of gestation until maternal metabolic control and the McCarthy GCI score suggested a dose–response association ($r = -0.58$; $p < 0.001$).	No details on rates of follow-up, treatment compliance or adherence to study protocol were reported Authors concluded that delayed development in offspring of women with PKU is associated with lack of maternal metabolic control before or early in pregnancy. Treatment at any time during pregnancy may reduce severity of delay

continued

Author, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			Patient types 253 children of women with PKU (n = 149), with untreated MHP (n = 33) or without known metabolic problems (comparison group, n = 71) were followed up to 4 years of age **Patient numbers** 253 (149 children of women with PKU categorised into 4 treatment groups relating to timing of maternal metabolic control): T1: before pregnancy (n = 17) T2: > 0 up to 10 weeks (n = 26) T3: > 10 up to 20 weeks (n = 47) T4: > 20 weeks or never in control (n = 59)	differences of 5 or fewer points in untreated MHP prior to pregnancy groups	The percentage of children attaining scores 1 (≤ 86) and 2 SD (≤ 72) below the mean increased as metabolic control decreased. For example, 47% of offspring whose mothers did not have metabolic control by 20 weeks gestation had a GCI score 2 SD below the norm, in contrast to 6% for those having metabolic control before pregnancy **McCarthy scales of children's abilities** Study group / No. of cases / GCI score / % of subjects with GCI score ≤ 86 / ≤ 72 T1 / 17 / 99 ± 13 / 24 / 6 T2 / 26 / 89 ± 17 / 42 / 12 T3 / 47 / 84 ± 18 / 51 / 32 T4 / 59 / 71 ± 19 / 78 / 47 T5 / 33 / 99 ± 14 / 21 / 0 T6 / 71 / 107 ± 20 / 13 / 6 Data are shown as mean ±SD. **Behavioural effects in offspring** Overall 30% of children born to mothers with PKU had social and behavioural problems according to the total behaviour problems index of the Achenbach Child Behaviour Checklist. Few children were rated as having somatic problems, anxiety or delinquency **Results from 2 year vs 4 year assessments** Scores obtained when the children were 4 years old, using the McCarthy scales, were significantly lower than the scores on the infant development test (Bayley scales) at 2 years of age (Bayley scales, mean ± SD: mental index 96 ± 23 vs	

Appendix 15 cont'd *Effectiveness of treatments for phenylketonuria: treatment during pregnancy*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			T5: untreated MHP (n = 33) T6: non-HPA control (n = 71) **Loss to follow-up** NR		McCarthy scales: GCI, 85 ± 21; $p < 0.01$; and Bayley scales: motor index 98 ± 19 vs McCarthy scales: motor scale, 91 ± 17, $p = 0.002$). For children in the non-HPA comparison group, the differences were not significant	

MHP: mild hyperphenysalaninaemia; GCI: General Cognitive Index.

Appendix 16

Reference list of excluded studies: tyrosinaemia type I

Anonymous. Evaluating newborn screening program data systems – Georgia, 1998. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1101–4.

Blau N, Beck M, Matern D. Tetrahydrobiopterin induced neonatal tyrosinaemia. *Eur J Pediatr* 1996;**155**:832.

Boulat O, McLaren DG, Arriaga EA, Chen DD. Separation of free amino acids in human plasma by capillary electrophoresis with laser induced fluorescence: potential for emergency diagnosis of inborn errors of metabolism. *J Chromatogr B Biomed Sci Appl* 2001;**754**:217–28.

Depetris-Boldini C, Galetto R, Videla MP, de Kremer DR. Increased excretion of coproporphyrin I in a patient with hereditary tyrosinaemia type I: relevant changes with NTBC treatment. *J Inherit Metab Dis* 1999; **22**:227–30.

Hitzeroth HW, Niehaus CE, Brill DC. Phenylketonuria in South Africa. A report on the status quo. *S Afr Med J* 1995;**85**:33–6.

Holme E, Lindstedt S. Tyrosinaemia type I and NTBC (2-(2-nitro-4-trifluoromethyl benzoyl)-1,3-cyclohexanedione). *J Inherit Metab Dis* 1998;**21**:507–17.

Neto EC, Schulte J, Anele E, Becker D, Rubim R, Lewis E, *et al*. Persistent tyrosinemia detected by thin-layer chromatography. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):151.

Pronicka E, Rowinska E, Bentkowski Z, Zawadzki J, Holme E, Lindstedt S. Treatment of two children with hereditary tyrosinaemia type I and long-standing renal disease with a 4-hydroxyphenylpyruvate dioxygenase inhibitor (NTBC). *J Inherit Metab Dis* 1996;**19**:234–8.

Ros J, Vilaseca MA, Lambruschini N, Mas A, Lindstedt S, Holme E. NTBC as palliative treatment in chronic tyrosinaemia type I. *J Inherit Metab Dis* 1999;**22**:665–6.

Schulze A, Frommhold D, Hoffmann GF, Mayatepek E. Spectrophotometric microassay for delta-aminolevulinate dehydratase in dried-blood spots as confirmation for hereditary tyrosinemia type I. *Clin Chem* 2001;**47**:1424–9.

Subramanyam SB. Saudi aminoacidemias: a six-year study. *Indian J Pediatr* 1996;**63**:641–4.

van Spronsen FJ, Smit GP, Wijburg FA, Thomasse Y, Visser G, Heymans HS. Tyrosinaemia type I: Considerations of treatment strategy and experiences with risk assessment, diet and transplantation. *J Inherit Metab Dis* 1995;**18**:111–14.

Verma IC. Burden of genetic disorders in India. *Indian J Pediatr* 2000;**67**:893–8.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999;**432** (Suppl 88):48–51.

Wilson CJ, Van Wyk KG, Leonard JV, Clayton PT. Phenylalanine supplementation improves the phenylalanine profile in tyrosinaemia. *J Inherit Metab Dis* 2000;**23**:677–83.

Appendix 17

Effectiveness of treatments for tyrosinaemia type I: orthotopic liver transplantation

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Mohan *et al.*, 1999[40]	**Study design** Retrospective analysis of clinical and biochemical data **Randomisation method** NA **Duration of study** Between 1989 and 1997 Setting/location Liver Unit, Birmingham Children's Hospital, England, UK	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Liver transplantation **Dosage/outcomes** Outcomes included hepatic dysplasia, hepatocellular carcinoma, renal function, tubular function and quality of life of post-transplant **Patient types** Patients with TT1. Diagnosis in each case was established by increased plasma tyrosine concentration and detection of increased urinary SA and was confirmed by fumarylacetoacetase deficiency in skin fibroblasts. All patients were managed with dietary therapy,	**Median age** 64 months at OLT (range 5–127 months) **Gender (M/F)** 5:3 **Ethnicity** NR **Other** Median weight 24 kg (range 6–25 kg)	Clinical features at diagnosis of TT1 patients between 1989 and 1997 (n = 17) Clinical features — No. Hepatomegaly — 11 Coagulopathy — 11 Failure to thrive — 11 Developmental delay — 9 Rickets (renal) — 8 Hypoglycaemia — 6 Cardiomyopathy — 6 Neurological crises — 2 Age at presentation < 2 months — 8[a] 2–6 months — 3 > 6 months — 6 [a] Four were detected by neonatal screening. All patients had biochemical and/or radiological evidence of liver dysfunction and raised α–fetoprotein levels (range 56–119,000 kU/l). **Treatment** Before introduction of NTBC, 7 patients presented with TT1 between 1989 and 1992. Of these 7 patients, 6 underwent OLT. Liver transplantation was contraindicated in 1 patient Between 1992 and 1997 10 patients were diagnosed with TT1, who started NTBC treatment [mean starting dose 0.6 mg/kg (range 0.55–1 mg/kg) and mean current dose 0.74 mg/kg (range 0.5–2.5 mg/kg)] in addition to dietary therapy. Two of these 10 patients went on to have an OLT. The indications were non-response to NTBC in 1 child and	The authors concluded that liver transplantation was an effective treatment for TT1, resulting in clinical and biochemical improvements and good quality of life. With the development of NTBC therapy, the indications for OLT in TT1 are non-response to NTBC, risk of malignancy and poor quality of life related to dietary restriction and frequency of blood sampling

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			± NTBC and/or OLT and followed up clinically and biochemically **Patient numbers** 8 (initially, 7 patients presented with TT1 between 1989 and 1992 before introduction of NTBC and 10 diagnosed with TT1 who started on NTBC treatment in addition to dietary therapy between 1992 and 1997. Eight of these 17 patients went on to have OLT) **Loss to follow-up** 2/8 (1 from primary non-function and 1 from chronic rejection)		development of hepatic dysplasia associated with poor quality of life in the second patient. In total, 8 patients were transplanted [median age at OLT 64 months (range 5–127 months), with a median weight of 24 kg (range 6–25 kg); M/F ratio 5/3)]. Of the 8 children transplanted, 4 received a whole liver graft; 3 received reduction hepatectomy and 1 had a split liver **Outcomes following transplantation** **Biochemical assessment** Plasma tyrosine and raised α-fetoprotein levels returned to normal in all cases. Urinary SA was reduced but persisted in small amounts (median 7.7 μmol/mmol creatinine) **Renal function** Before transplantation the glomerular filtration rate (using height:creatinine ratio) was normal in 5 out of 6 survivors. 3/6 survivors developed renal dysfunction, with a fall in the glomerular filtration rate (using height:creatinine ratio). This stabilised following a reduction of cyclosporin A or a change to mycophenolate mofetil, suggesting that the main cause of renal dysfunction was nephrotoxicity due to cyclosporin A. Hence the authors recommended regular monitoring of renal function post-OLT and a reduction in immunosuppressive therapy **Clinical outcome** Hypertrophic cardiomyopathy was resolved within 1 year in patients in which it occurred ($n = 3$). Hypoglycaemia not responding to diet therapy or NTBC was present in	

Appendix 17 cont'd *Effectiveness of treatments for tyrosinaemia type 1*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
					1 patient before transplantation and was resolved following transplantation. **Complications and quality of life** There were 2 deaths, 1 from primary non-function and 1 from chronic rejection. Late complications in survivors (n = 6) included post-transplant lymphoproliferative disease of the iris, which resolved and renal dysfunction in 3 of the 6 survivors (fall in glomerular filtration rate). This was attributed to cyclosporin toxicity and a change in the immunosuppressive regimen. The 1-year actuarial survival rate was 88%, while the 5-year actuarial rate was 73%. The median follow-up post-OLT was 6.7 years (range 1–7 years) The quality of life after OLT in survivors was good. All survivors were in mainstream schools and had a reduction in hospital visits (median 2/year), venepuncture (median 5 blood samples/year), medication (median 2/day) and dietary freedom, in contrast to patients receiving NTBC therapy who had more hospital visits (median 4/year), frequent venepuncture (median 35 blood samples/year), extra medication (median 5/day) and a restricted diet	

TT1: tyrosinaemia type I; SA: succinylacetone.

Appendix 18

Reference list of excluded studies: homocystinuria due to cystathionine β-synthase deficiency

Accinni R, Bartesaghi S, De Leo G, Cursano CF, Achilli G, Loaldi A, *et al*. Screening of homocysteine from newborn blood spots by high-performance liquid chromatography with coulometric array detection. *J Chromat* 2000;**896**:183–9.

Andersson HC, Shapira E. Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). *J Pediatr* 1998;**132**:121–4.

Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet Med* 1999;**1**:146–50.

Anonymous. Evaluating newborn screening program data systems – Georgia, 1998. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1101–4.

Cruysberg JR, Boers GH, Trijbels JM, Deutman AF. Delay in diagnosis of homocystinuria: retrospective study of consecutive patients. *BMJ* 1996;**313**:1037–40.

Dawson PA, Cox AJ, Emmerson BT, Dudman NP, Kraus JP, Gordon RB. Characterisation of five missense mutations in the cystathionine beta-synthase gene from three patients with B6-nonresponsive homocystinuria. *Eur J Hum Genet* 1997;**5**:15–21.

Dudman NP, Guo XW, Gordon RB, Dawson PA, Wilcken DE. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. *J Nutr* 1996;**126** (4 Suppl): 1295S–300S.

Gat-Yablonski G, Mandel H, Fowler B, Taleb O, Sela BA. Homocystinuria in the Arab population of Israel: identification of two novel mutations using DGGE analysis. *Hum Mutat* 2000;**16**:372.

Gaustadnes M, Kluijtmans LA, Jensen OK, Rasmussen K, Heil SG, Kraus JP, *et al*. Detection of a novel deletion in the cystathionine beta-synthase (CBS) gene using an improved genomic DNA based method. *FEBS Lett* 1998;**431**:175–9.

Guttormsen AB, Ueland PM, Nesthus I, Nygard O, Schneede J, Vollset SE, *et al*. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (≥ 40 micromol/liter). The Hordaland Homocysteine Study. *J Clin Invest* 1996;**98**:2174–83.

Harrison DA, Mullaney PB, Mesfer SA, Awad AH, Dhindsa H. Management of ophthalmic complications of homocystinuria. *Ophthalmology* 1998;**105**:1886–90.

Kaur M, Kabra M, Das GP, Suri M, Verma IC. Clinical and biochemical studies in homocystinuria. *Indian Pediatr* 1995;**32**:1067–75.

Kim CE, Gallagher PM, Guttormsen AB, Refsum H, Ueland PM, Ose L, *et al*. Functional modeling of vitamin responsiveness in yeast: a common pyridoxine-responsive cystathionine beta-synthase mutation in homocystinuria. *Hum Mol Genet* 1997;**6**:2213–21.

Kuhara T, Ohse M, Ohdoi C, Ishida S. Differential diagnosis of homocystinuria by urease treatment, isotope dilution and gas chromatography–mass spectrometry. *J Chromatogr B Biomed Sci Appl* 2000; **742**:59–70.

Moat SJ, Bonham JR, Tanner MS, Allen JC, Powers HJ. Recommended approaches for the laboratory measurement of homocysteine in the diagnosis and monitoring of patients with hyperhomocysteinaemia. *Ann Clin Biochem* 1999; **36**(Pt 3):372–9.

Naughten ER, Yap S, Mayne PD. Newborn screening for homocystinuria: Irish and world experience. *Eur J Pediatr* 1998;**157** (Suppl 2):S84–7.

Peterschmitt MJ, Simmons JR, Levy HL. Reduction of false negative results in screening of newborns for homocystinuria. *N Engl J Med* 1999;**341**:1572–6.

Ray M, Kumar L, Prasad R. Homocystinuria with early thromboembolic episodes and rapid response to high dose pyridoxine. *Indian Pediatr* 1997;**34**:67–9.

Reilly AA, Bellisario R, Pass KA. Multivariate discrimination for phenylketonuria (PKU) and non-PKU hyperphenylalaninemia after analysis of newborns' dried blood-spot specimens for six amino acids by ion-exchange chromatography. *Clin Chem* 1998;**44**:317–26.

Sakura N, Ono H, Nomura S, Ueda H, Fujita N. Betaine dose and treatment intervals in therapy for homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency. *J Inherit Metab Dis* 1998;**21**:84–5.

Sebastio G, Sperandeo MP, Panico M, de Franchis R, Kraus JP, Andria G. The molecular basis of homocystinuria due to cystathionine beta-synthase deficiency in Italian families, and report of four novel mutations. *Am J Hum Genet* 1995;**56**:1324–33.

Smith KL, Bradley L, Levy HL, Korson MS. Inadequate laboratory technique for amino acid analysis resulting in missed diagnoses of homocystinuria. *Clin Chem* 1998;**44**:897–8.

Snyderman SE, Sansaricq C. Newborn screening for homocystinuria. *Early Hum Dev* 1997;**48**:203–7.

Subramanyam SB. Saudi aminoacidemias: a six-year study. *Indian J Pediatr* 1996;**63**:641–4.

Taylor RH, Burke J, O'Keefe M, Beighi B, Naughton E. Ophthalmic abnormalities in homocystinuria: the value of screening. *Eye* 1998;**12**(Pt 3a):427–30.

Verma IC. Burden of genetic disorders in India. *Indian J Pediatr* 2000;**67**(12):893–8.

Walter JH, Wraith JE, White FJ, Bridge C, Till J. Strategies for the treatment of cystathionine beta-synthase deficiency: the experience of the Willink Biochemical Genetics Unit over the past 30 years. *Eur J Pediatr* 1998;**157** (Suppl 2):S71–6.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 19

Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency): dietary supplementation

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Franken *et al.*, 1996[46]	**Study design** Before and after intervention **Randomisation method** NA **Duration of study** 6 weeks of treatment **Setting/location** NR; however, University Hospital Nijmegen, The Netherlands	**Inclusion/exclusion criteria** Criteria for inclusion included: regularly visiting hospital, good compliance to homocysteine-lowering treatments, prolonged elevated fasting homocysteine levels in blood despite homocysteine-lowering treatment for at least 2 years, and at least 14 years of age **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Thiamin administration **Dosage/outcomes** 2 or 3 daily doses of 25 mg thiamin orally (mean ± SD mg thiamin dosage for the subjects was 0.9 ± 0.25 mg thiamin supplementation/kg body weight; range 0.48–1.43) **Patient types** All subjects were homozygotes for homocystinuria due to cystathionine synthase deficiency. None of the patients was on a regimen of methionine restriction, except for 1 (subject 9). In addition to conventional homocysteine-	**Mean age** 24.4 ± 6.2 years (range 14–34 years) **Gender (M/F)** NR **Ethnicity** NR **Other** **Mean weight** 72.3 ± 10.6 kg (range 52.5–88.0 kg) **Mean duration of homocysteine lowering treatment** 9.0 ± 2.4 years (range 5–17 years)	**Fasting blood concentrations of total homocysteine, methionine, thiamin (vitamin B_1) and transamine metabolites in cystathionine synthase-deficient patients before and after 6 weeks of thiamin treatment** Subject | Homocysteine (μmol/l) Before | Homocysteine (μmol/l) After | Methionine (μmol/l) Before | Methionine (μmol/l) After | Therapy 1 | 143 | 48 | 98 | 30 | 750 B_6, B_{12} 2 | 46 | 49 | 43 | 42 | 400 B_6, FA, B_{12} 3 | 81 | 82 | 291 | 123 | 750 B_6, FA, betaine 4 | 82 | 79 | 94 | 54 | 750 B_6, FA, betaine 5 | 58 | 56 | 161 | 58 | 750 B_6, FA, betaine 6 | 113 | 101 | 222 | 93 | 500 B_6, FA, betaine 7 | 82 | 120 | 139 | 188 | 500 B_6, FA, betaine 8 | 85 | 103 | 57 | 72 | 750 B_6, FA, B_{12}, betaine 9 | 128 | 109 | 643 | 490 | 250 B_6, FA, betaine MPR Mean ± SD | 91 ± 25 | 83 ± 22 | 194 ± 127 | 128 ± 94 Normal | 5–18 | | 16–47	The authors concluded that thiamin (vitamin B_1) supplementation could not be used as an additional homocysteine-lowering treatment to vitamin B_6, vitamin B_{12}, FA and betaine in most homozygotes for homocystinuria

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			lowering therapy, 6 patients received 25 mg of thiamin hydrochloride for 6 weeks three times daily and three patients twice daily (subjects 2, 6 and 7). These three patents received only in total 50 mg thiamin hydrochloride, conforming with their conventional homocysteine-lowering treatment, which was also twice daily **Patient numbers** 9 **Loss to follow-up** NR		See below	

Subject	Thiamine (μmol/l)		Transamination (μmol/l)		Therapy
	Before	After	Before	After	
1	98	170	0.3	0.3	750 B_6, B_{12}
2	96	130	0.3	0.3	400 B_6, FA, B_{12}
3	110	190	0.6	0.3	750 B_6, FA, betaine
4	120	210	BD	BD	750 B_6, FA, betaine
5	100	210	0.3	0.3	750 B_6, FA, betaine
6	NP	NP	0.4	0.2	500 B_6, FA, betaine
7	NP	210	BD	BD	500 B_6, FA, betaine
8	110	NP	0.3	0.3	750 B_6, FA, B_{12}, betaine
9	230	330	1.0	0.7	250 B_6, FA, betaine MPR
Mean ± SD	123 ± 30	207 ± 38			
Normal	47–142		BD to 1.2		

B_6; vitamin B_6; FA was given in a daily dose of 5 mg; B_{12}, vitamin B_{12} was given in a 2 monthly dose of 1 mg i.m.; betaine was given in a daily dose of 6 g.

The vitamin B_1 plasma concentration in the homozygotes for homocystinuria increased from 123 ± 30 nmol (n = 7) before thiamin treatment to 207 ± 38 (n = 7) after 6 weeks of treatment. The fasting blood methionine concentration decreased significantly from 194 ± 127 μmol/l to 128 ± 94 μmol/l after thiamin treatment (n = 9; $p < 0.06$)

The mean fasting plasma homocysteine concentrations did not differ significantly before and after thiamin treatment (91 ± 25 to 83 ±22 μmol/l, respectively) as did serum transamination metabolites in all patients

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Wilcken and Wilcken, 1997[47]	**Study design** Retrospective analysis of clinical and biochemical data **Randomisation method** NR **Duration of study** Not clear however, duration of treatment: T1: mean 16.6 years T2: mean 11 years **Setting/location** NR; however, New South Wales (NSW), Australia	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Effect of pyridoxine (vitamin B_6), FA and hydroxocobalamin treatment **Dosage/outcomes** All patients received pyridoxine 100–200 mg/day and FA 5 mg/day, and most had intermittent hydroxocobalamin by injection according to serum B_{12} status, measured twice yearly. Pyridoxine-non-responsive patients all received in addition 6–9 g/day oral trimethylglycine (betaine) given in two divided doses. Diet was not closely monitored, but general advice was given to reduce intake of foods with a high methionine content. During last 4 years, vitamin B_{12} therapy (1 mg by intramuscular injection every 1–3 months) has been given to pyridoxine-non-responsive patients irrespective of their serum B_{12} levels Outcomes included vascular disease	**Mean age** 30 years (range 9–66 years) **Gender (M/F)** NR **Ethnicity** NR	**Pyridoxine-responsive group (T1)** The 17 patients who were pyridoxine responsive all maintained plasma total free homocyst(e)ine levels <20 μmol/l over an average treatment period of 16.6 years. In this group, there were 2 vascular deaths, 1 fatal pulmonary embolus and 1 myocardial infarction, whereas without treatment 21 deaths (data derived from untreated patients with CβS deficiency, which was reviewed by Mudd *et al.*, 1985[114]) would have been expected, $\chi^2 = 14.22$, $p = 0.0001$, RR 0.09 (95% CI 0.02 to 0.38) **Non-responsive to pyridoxine group (T2)** The 15 patients who were non-responsive to pyridoxine additionally received 6–9 g of betaine daily, which resulted in a substantial decline (mean 74 ± 14%) in plasma total free homocyst(e)ine levels, persisting during an average (postbetaine) treatment period of 11 years (mean ± SD levels: 33 ± 17 μmol/l; $n = 15$). In this group, there were no events during 258 patient-years of treatment ($p < 0.005$ vs expected untreated). 19 subjects had a total of 19 major and 15 minor operations requiring anaesthetic and there were 3 successful pregnancies, 1 in a patient receiving betaine. There were no thromboembolic complications **Other information** Authors reported that during 539 patient-years of treatment in all 32 patients, there were	Authors concluded that effective treatment of CβS deficiency (both pyridoxine responsive and non-responsive) markedly reduces the increased cardiovascular risk associated with homocystinuria and that the additional effect of betaine therapy in pyridoxine-non-responsive patients is substantial and produces sustained reduction of elevated plasma homocyst(e)ine, thereby contributing to reducing cardiovascular risk

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			Patient types Patients with homocystinuria due to CβS deficiency diagnosed in the state of NSW, Australia. Diagnosis on basis of characteristic clinical features plus elevated levels of plasma methionine and free homocyst(e)ine with low free cyst(e)ine. All patients had increased urinary homocysteine Patients were categorised as being either pyridoxine responsive [plasma homocyst(e)ine <20 μmol/l] or pyridoxine-non-responsive **Patient numbers** T1: pyridoxine responsive (n = 17) during 281 patient-years of treatment T2: pyridoxine non-responsive (n = 15) during 258 years of treatment **Loss to follow-up** 10 patients died at 2–30 years of age. Of these, 8 were definite vascular deaths, 1 was presumed vascular death and the other was due to an accident, unrelated to homocystinuria		2 vascular deaths, whereas, if untreated, 21 events would have been expected. Therefore, the treatment markedly reduced the RR to 0.09 (95% CI 0.02 to 0.38, χ^2 = 14.22, p = 0.0001)	

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Yap and Naughten 1998[48]	**Study design** Retrospective analysis of clinical and biochemical data **Randomisation method** NA **Duration of study** 1971 to 1996 **Setting/location** National Centre for Inherited Metabolic Disorders, Dublin, Ireland	**Inclusion/exclusion criteria** Methionine and free homocysteine levels during initial period of stabilisation, pyridoxine challenge and subsequent periods of proven illness were excluded from the calculations for lifetime medians and ranges **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of intervention** Effect of pyridoxine or restriction of dietary methionine **Dosage/outcomes** All patients received oral pyridoxine 50 mg 3 times daily. In pyridoxine-non-responsive patients, dietary management was begun by restricting dietary methionine and using a methionine-free, cystine-supplemented synthetic mixture. Two-thirds of total protein intake was derived from synthetic methionine-free, cystine-supplemented mixture and remaining third from natural methionine-containing food. Patients with deficient plasma B_{12}	**Mean age** range 2.5 to 23.4 years **Gender (M/F)** 12:13 **Ethnicity** NR	**Patient demographics** Over a 25-year period up to 1996, 25 cases of homocystinuria were diagnosed, 21 of whom were identified on screening. All were pyridoxine non-responsive and 5 cases were breast-fed. Four other cases were detected clinically, 3 of whom were breast-fed and 1 was pyridoxine responsive **Complications among patients with homocystinuria** Detected by screening Total no. / Without complications (group 1)[a] / With complications (group 2)[b] / Missed on screening (group 3)[c] Total detected: 25 / 18[d] / 3 / 4 Ectopia lentis: 6 / 0 / 2 / 4 Osteoporosis (radiological): 2 / 0 / 1 / 1 Mental disability: 4 / 0 / 2 / 2 Thromboembolism: 0 / 0 / 0 / 0 [a] All dietary treated cases. [b] All subjects non-compliant with prescribed diet (indicated by diet history and poor biochemical control). [c] Presented with complication after 2 years of age. [d] One died at 8 years of age in a drowning accident. **Clinical and biochemical findings** Treatments were begun before 6 weeks of age for patients in groups 1 and 2, whereas those in group 3 started upon presentation and diagnosis. The mean period of follow-up for groups 1 and 2 was 14.7 years (range 2.5–23.4 years). For patients in group 3 the mean period of follow-up was 14.7 years (range 11.7–18.8 years)	Despite the small number of patients and varying lengths of follow-up, the authors concluded that homocystinuria due to CβS deficiency was a potentially treatable disorder. Newborn screening, early treatment, good dietary compliance and the maintenance of a lifetime median plasma free homocysteine < 11 μmol/l appear to protect, at least up to 23.4 years, against the overt recognised complications of untreated homocystinuria

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			and folate were given supplements **Patient types** All patients with homocystinuria due to CβS deficiency detected in Ireland between 1971 and 1996, either by the national newborn screening programme (n = 21 with blood methionine concentrations >100 μmol/l) or by clinical presentation. All patients were started on treatment upon diagnosis. 24 of the 25 were patients were non-responsive to pyridoxine. Diagnosis was based on clinical presentation and elevated levels of blood methionine and free homocysteine with		Of the 25 patients with 365.7 patient-years of treatment, no homocystinuria-related complications were found in 18 dietary-treated cases. In these 18 subjects (group 1), 3 developed increasing myopia and all had higher lifetime median free homocysteine levels (range 18–48 μmol/l) compared with the remaining 15 patients who had lifetime median free homocysteine levels < 11 μmol/l Among the 3-screened non-dietary compliant cases (group 2), 2 presented with ectopia lentis, 1 had osteoporosis and 2 had mental disabilities. In this group, poor dietary compliance (reflected by diet history and higher plasma free homocysteine levels) for between 2 and 8 years significantly increased the risk of developing complications. In group 3, where 4 cases were missed by screening, 3 presented with ectopia lentis after the age of 2 years All 25 patients had lifetime median methionine levels ranging from 47 to 134 μmol/l and no patients developed any thromboembolic events (age range 2.5–23.4 years). Four patients had mental disability, whereas the remaining 21 patients achieved age-appropriate education standards	

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			low cystine. Upon discharge from hospital, patients initially attended an outpatient clinic fortnightly and thereafter at 4–6 weekly intervals. They were reviewed at least 4 times per year Outcomes measures included growth parameters, biochemical control of methionine, free homocysteine and cystine, annual ophthalmological examination, clinical cardiovascular, IQ and dietary assessments **Patient numbers** 25 **Loss to follow-up** 1/25 (1 patient died due to a drowning accident)			

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Yap *et al.*, 2001[49]	**Study design** Observational study with concurrent controls **Randomisation method** NA **Duration of study** Not clear; however, all patients with homocystinuria due to CβS deficiency detected in Ireland from 1971 either by the national newborn screening programme or by clinical presentation[48] **Setting/location** National Centre for Inherited Metabolic Disorders, The Children's Hospital, Ireland	**Inclusion/exclusion criteria** 2 patients detected through newborn screening were excluded from this study: 1 died from drowning, and psychometric data from 1 patient (postgraduate psychology student) may not be valid due to her knowledge, training and refusal to be assessed **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Evaluation of intellectual abilities of early treated individuals with CβS deficiency using standardised age-appropriate psychometric tests for full-scale IQ, verbal IQ and performance IQ **Dosage/outcomes** All patients diagnosed with CβS deficiency were started on oral pyridoxine while on a normal diet to ascertain their clinical responsiveness to pyridoxine. After confirmation of pyridoxine non-responsiveness (indicated by a persistently high or rising plasma methionine and free homocystine),	**Mean age** 16.5 years (range 4.4–24.9 years) at assessment **Gender (M/F)** NR **Ethnicity** NR	**Characteristics of patients with CβS deficiency** Group *n* Mean (range) age (years): At assessment; Start of treatment; Lifetime free homocystine median (μmol/l) 1 13 14.4 (4.4–24.9) 0.05 (0.02–0.1) 13 (4.0–11) 2 6 19.9 (13.8–25.5) 0.07 (0.02–0.12) 27 (11–49) 3[a] 2 18.9/18.8 2.4/2.9 4.5/8.5 4[a] 2 22.4/11.7 – 5 10 19.5 (7.8–32.9) – [a] Data presented for each of the 2 patients in this group. Group 1: newborn screened (compliant); group 2: newborn screened (poorly compliant); group 3: late detected; group 4: untreated; group 5: unaffected siblings (controls). **Intellectual abilities of early treated individuals with CβS deficiency** There were no statistically significant differences between the newborn-screened compliant group and the unaffected control group in the psychometric parameters assessed, except for a higher full-scale IQ in the patient group ($p = 0.0397$) The newborn-screened, good compliance group ($n = 13$) with a total of 187.7 patient-years of treatment had a mean full-scale IQ of 105.8 (range 84–120), verbal IQ of 110.3 (range 88–117) and performance IQ of 98.6 (range 78–118). These patients did not have any other recognised homocystinuria-related complications and had lifetime free homocystine medians < 11 μmol/l. In contrast, the control group ($n = 10$) had a mean full-scale IQ of 102 (range	The authors concluded that despite the relatively small numbers, the data suggest that early treatment with good biochemical control (lifetime free plasma homocystine median < 11 μmol/l) appeared to prevent mental retardation in pyridoxine-non-responsive, CβS deficient patients

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			subjects started on dietary management of methionine restriction and a methionine-free, cystine-supplemented synthetic amino acid mixture Patients deficient in plasma B_{12} and folate were given supplements. Betaine was used as an adjunct to treatment only in those patients (late adolescent/young adults) who became poorly compliant to treatment (since 1996). In the late detected pyridoxine-non-responsive patients, betaine was started with cofactor (pyridoxine, B_{12} and folate) supplementation Outcome measures included		76–116), verbal IQ of 107 (range 81–123) and performance IQ of 96.6 (range 76–115) Patients in the newborn-screened, poorly compliant group (n = 6) had a mean full-scale IQ of 80.8 years (range 40–103), verbal IQ of 87.3 (range 46–113) and performance IQ of 75.2 (range 46–87). Correspondingly, the lifetime free homocystine medians were inversely related to full-scale IQ. The patients in this group received a total of 118.9 patient-years of treatment The 2 late-detected patients had full-scale IQ of 80 and 102, verbal IQ of 75 and 107, performance IQ of 92 and 94, and lifetime free homocystine medians of 4.5 and 8.5 μmol/l. The 2 untreated patients had full-scale IQ of 52 and 53, verbal IQ of 58 and 61, and performance IQ of 52 and 52, before starting treatment Group n Mean (range): Full-scale IQ / Verbal IQ / Performance IQ 1 (13): 105.8 (84–120) / 110.3 (88–117) / 98.6 (78–118) 2 (6): 80.8 (40–103) / 87.3 (46–113) / 75.2 (46–87) 3[a] (2): 80/102 / 75/107 / 92/94 4[a] (2): 52/53 / 58/61 / 52/52 5 (10): 102 (76–116) / 107 (81–123) / 96.6 (76–115) [a] Data presented for each of the 2 patients in this group. Group 1: newborn screened (compliant); group 2: newborn screened (poorly compliant); group 3: late detected; group 4: untreated; group 5: unaffected siblings (controls).	

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			biochemical monitoring, control and IQ tests **Patient types** Pyridoxine-non-responsive patients with CβS deficiency from 18 families attending the National Centre for Inherited Metabolic Disorders, Ireland. Patients were divided into three groups depending on the time of starting treatment. Newborn-screened patients were further divided into two groups depending on their compliance as defined by their biochemical control. Criterion for good compliance was defined as a treatment lifetime plasma free homocystine median < 11 μmol/l			

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			19 patients were detected through newborn screening. Of these, 13 were compliant to treatment (group 1) with no complications, and the remaining 6 developed complications due to poor compliance (group 2). Two were detected late (group 3) and 2 were untreated (group 4). All newborn-screened patients had started on a methionine-restricted, cystine-supplemented diet within 6 weeks of birth, whereas 2 late-detected patients began treatment at 2.4 and 2.9 years. The control group (group 5) consisted of 10 unaffected siblings. The mode of diagnosis, age at			

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			which treatment started, treatment regimen and lifetime biochemical control, including educational achievements for 22 of the 23 patients, were included in a previous article[48] included in this review **Patient numbers** 23 **Loss to follow-up** NR			

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Yap *et al.*, 2001[50]	**Study design** Multicentre observational study with historical controls **Randomisation method** NR **Duration of study** Not clear; however, authors reported that newborn screening for homocystinuria in Manchester started in 1969 and Dublin in 1971. The study period was until the end of 1998 **Setting/location** Five centres in Ireland (Dublin), Australia (Sydney), The Netherlands	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of intervention** Effect of homocysteine-lowering therapy in reducing vascular events **Dosage/outcomes** Three main treatment regimens were used by all centres with minor modifications (see treatment regimen table). Initially, therapeutic doses of pyridoxine (B_6) were given in combination with folate. If response to B_6 was inadequate (pyridoxine-responsive patients were those whose total free plasma homocysteine was reduced to < 20 μmol/l with pyridoxine treatment), dietary	**Mean age** See results **Gender (M/F)** NR **Ethnicity** NR	**Characteristics of patients with CβS deficiency treated in Sydney, Nijmegen, Dublin, Manchester and London** Overall data Total no. of Cβ-S patients[a] 170 Deaths before treatment 7 Deaths during treatment[b] 7 Vascular events before/off treatment 33 Total no. followed up with treatment[c] 158 B_6 responders 70 B_6 non-responders 88 Mean period of treatment (years) B_6 responders 17.8 B_6 non-responders 17.9 Mean age (range) at start of treatment (years) 11 (0–57) Current (1998) mean age (years) 29.4 Range of ages (*n*) 4.5–70 < 10 years old 5 (3%) < 30 years old 57 (36%) > 50 years old 10 (6.3%) Overall data are reported here; however, data from each centre are also available in the original publication. [a] Sydney, $n = 40$; Nijmegen, $n = 30$; Dublin, $n = 28$; Manchester, $n = 31$; London, $n = 40$. [b] Of the 7 deaths that occurred during treatment, each of the Australian and Irish groups have 1 death unrelated to homocystinuria. The remaining 5 deaths were vascular deaths (pulmonary embolism, $n = 3$; myocardial infarction, $n = 1$; sagittal sinus thrombosis, $n = 1$). [c] Sydney, $n = 32$; Nijmegen, $n = 28$; Dublin, $n = 27$; Manchester, $n = 30$; London, $n = 41$.	The authors concluded that treatment regimens designed to lower plasma homocysteine significantly reduce cardiovascular risk in CβS deficient patients despite imperfect biochemical control; however, the authors of this study also acknowledged that it was not clear whether this results entirely from lowering the extremely high pretreatment levels of homocysteine or from some other aspect of the treatment

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
	(Nijmegen) and the UK (Manchester, London)		methionine restriction was attempted. However, B_6-non-responsive pyridoxine was continued to be given to many of the patients due to reports of its beneficial effects. The third therapeutic option was the use of betaine, mainly in patients non-responsive to B_6 All vascular events were diagnosed by appropriate contemporary diagnostic methods used by the respective teaching hospitals at the time of the events. The total number of expected vascular events, if the study population had		**Treatment regimens in each respective centre treating patients with CβS deficiency** **Centre** **Sydney** / **Nijmegen** Treatment regimens used Dietary methionine restriction (mg/day): General advice / 600 Pyridoxine (mg/day) Adult: 100–200 / 750 Child: / 200–500 Folate (mg/day): 5 / 5 Vitamin B_{12} (intramuscular or oral): Routine to all / If deficient Betaine (g/day): 6–9 / 6 Frequency of biochemical monitoring/year: 1–4 / 1–2 Criteria for B_6 responsiveness (μmol/l): tfHcy <20 tHcy <50 / tfHcy <20 or **Dublin** / **Manchester** Treatment regimens used Dietary methionine restriction (mg/day): 200–625 / 160–900 Pyridoxine (mg/day) Adult: 100–800 / 50–500 Child: 150 (neonate) / 150 Folate (mg/day): 5 / 5 Vitamin B_{12} (intramuscular or oral): If deficient Betaine (g/day): 3–6 / 4.5–15 Frequency of biochemical monitoring/year: ≥ 8–10 / 1–4 Criteria for B_6 responsiveness (μmol/l): Hcy-Hcy <5 <10 / Hcy-Hcy	

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			remained untreated was derived from the data of Mudd *et al.*, 1985[110] Data were analysed for each individual centre (data not presented) with final calculation from the pooled data (data presented) **Patient types** Patients with CβS deficiency who had been treated chronically (B_6 responders, $n = 70$; B_6 non-responders, $n = 88$) **Patient numbers** 158 (initially there were 170 patients, but only 158 were followed up with treatment)		**Centre** **London** Treatment regimens used Dietary methionine restriction (mg/day) 400–1375 Pyridoxine (mg/day) Adult 20–500 Child Folate (mg/day) 5–10 Vitamin B_{12} (intramuscular or oral) 50 μg orally Betaine (g/day) 2–6 Frequency of biochemical monitoring/year 2–4 Criteria for B_6 responsiveness (μmol/l) Hcy-Hcy <10 B_6 responsiveness is determined by serial homocysteine assessments in relation to B_6 administration, and the patient is classified as responsive when the homocysteine levels meet the criteria set by each respective centre.	

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			Loss to follow-up NR		**Overall data of patient years of treatment, predicted and actual number of vascular events and biochemical control of patients with CβS deficiency**	

	Overall data
Total patient-years of treatment	2821.6
B_6 responders	1243.8
B_6 non-responders	1577.8
Actual vascular events while on treatment (*n*)	17
Type of events (*n*)	(Pulmonary embolism, $n = 3$; myocardial infarction, $n = 2$; abdominal aortic aneurysm, $n = 2$; transient ischaemic attack, $n = 1$; sagittal sinus thrombosis, $n = 1$; deep vein thrombosis, $n = 5$; cerebrovascular accident, $n = 3$)
Predicted vascular events with untreated[a]	112
RR	0.09
95% CI	0.036 to 0.228)
p	< 0.0001

Overall data are reported here; however, data from each centre are also available in the original publication

[a] Derived from the data of Mudd *et al.*[110]

Appendix 19 cont'd *Effectiveness of treatments for homocystinuria (cystathionine β-synthase deficiency)*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
					Without treatment, 112 vascular events would have been expected (1 vascular event per 25 years was expected in 2821.6 patient-years of treatment). Instead, only 17 vascular events occurred in 12 patients who were undergoing treatment (RR, 0.09, 95% CI 0.036 to 0.228, $p < 0.0001$). Of the 17 vascular events, 12 occurred in eight B_6 responders at a mean age of 51.6 years (range 25–67 years) and 5 vascular events occurred in 4 B_6 non-responders at a younger mean age of 20.6 years (range 18–24 years). There were 5 vascular deaths during the treatment period, 2 in the B_6 responders (pulmonary embolism, myocardial infarction) and three among B_6 non-responders (pulmonary embolism, sagittal sinus thrombosis). In the total of 825 patient-years of betaine treatment (the longest period of betaine treatment was 17 years) there were no reports of significant side-effects. Plasma homocysteine levels were markedly reduced from pretreatment levels, but were several times higher than the mean for the respective normal population of each centre, despite the aim of achieving normal levels	

FA: folic acid; MPR: methionine-poor regimen; NP: not performed; BD: below detection limit; tf Hcy: total free homocysteine; Hcy-Hcy: free homocysteine (the disulphide).

Appendix 20

Reference list of excluded studies: maple syrup urine disease

Anonymous. Evaluating newborn screening program data systems – Georgia, 1998. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1101–4.

Boulat O, McLaren DG, Arriaga EA, Chen DD. Separation of free amino acids in human plasma by capillary electrophoresis with laser induced fluorescence: potential for emergency diagnosis of inborn errors of metabolism. *J Chromatogr B Biomed Sci Appl* 2001;**754**:217–28.

Burgos C, Civallero GE, de Kremer RD, Gerez de Burgos NM, Blanco A. Enzymatic method for branched chain alpha-ketoacid determination: application to rapid analysis of urine and plasma samples from maple syrup urine disease patients. *Acta Physiol Pharmacol The Latinoam* 1999;**49**:109–17.

Chace DH, Hillman SL, Millington DS, Kahler SG, Roe CR, Naylor EW. Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. *Clin Chem* 1995;**41**:62–8.

Chuang JL, Chuang DT. Diagnosis and mutational analysis of maple syrup urine disease using cell cultures. *Methods Enzymol* 2000;**324**:413–23.

Edelmann L, Wasserstein MP, Kornreich R, Sansaricq C, Snyderman SE, Diaz GA. Maple syrup urine disease: identification and carrier-frequency determination of a novel founder mutation in the Ashkenazi Jewish population. *Am J Hum Genet* 2001;**69**:863–8.

Johnson AW, Mills K, Clayton PT. The use of automated electrospray ionization tandem MS for the diagnosis of inborn errors of metabolism from dried blood spots. *Biochem Soc Trans* 1996;**24**:932–8.

Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol* 1999;**14** (Suppl 1):S4–8.

Nellis MM, Danner DJ. Gene preference in maple syrup urine disease. *Am J Hum Genet* 2001;**68**:232–7.

Nyhan WL, Rice-Kelts M, Klein J, Barshop BA. Treatment of the acute crisis in maple syrup urine disease. *Arch Pediatr Adolesc Med* 1998;**152**:593–8.

Pang CP, Law LK, Mak YT, Shek CC, Cheung KL, Mak TW, *et al*. Biochemical investigation of young hospitalized Chinese children: results over a 7-year period. *Am J Med Genet* 1997;**72**:417–21.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol* 1999;**23**:183–93.

Reilly AA, Bellisario R, Pass KA. Multivariate discrimination for phenylketonuria (PKU) and non-PKU hyperphenylalaninemia after analysis of newborns' dried blood-spot specimens for six amino acids by ion-exchange chromatography. *Clin Chem* 1998;**44**:317–26.

Schadewaldt P, Bodner-Leidecker A, Hammen HW, Wendel U. Significance of L-alloisoleucine in plasma for diagnosis of maple syrup urine disease. *Clin Chem* 1999;**45**:1734–40.

Subramanyam SB. Saudi aminoacidemias: a six-year study. *Indian J Pediatr* 1996;**63**:641–4.

Verma IC. Burden of genetic disorders in India. *Indian J Pediatr* 2000;**67**:893–8.

Wasant P, Naylor EW, Liammongkolkul S. Detection of inherited metabolic disorders via tandem mass spectrometry in Thai infants. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):154–9.

Wasant P, Svasti J, Srisomsap C, Liammongkolkul S, Naylor EW, Matsumoto I. Inherited metabolic disorders in Thailand – Siriraj experience. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):124–37.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999;**88** (Suppl 432):48–51.

Yahya NA, Ismail Z, Embong KH, Mohamad SA. High performance liquid chromatography (HPLC) method for confirming thin layer chromatography (TLC) findings in inborn errors of metabolism children in Malaysia. *Southeast Asian J Trop Med Public Health* 1995; **26** (Suppl 1):130–3.

Zakiah I, Ashikin YN, Aisiah SM, Ismail HI. Inborn errors of metabolic diseases in Malaysia: a preliminary report of maple syrup urine diseases for 1993. *Southeast Asian J Trop Med Public Health* 1995;**26** (Suppl 1):134–6.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 21

Reference list of excluded studies: urea cycle disorders

Carpenter KH, Potter M, Hammond JW, Wilcken B. Benign persistent orotic aciduria and the possibility of misdiagnosis of ornithine carbamoyltransferase deficiency. *J Inherit Metab Dis* 1997;**20**:354–8.

Genet S, Cranston T, Middleton-Price HR. Mutation detection in 65 families with a possible diagnosis of ornithine carbamoyltransferase deficiency including 14 novel mutations. *J Inherit Metab Dis* 2000;**23**:669–76.

Kasahara M, Kiuchi T, Uryuhara K, Ogura Y, Takakura K, Egawa H, *et al*. Treatment of ornithine transcarbamylase deficiency in girls by auxiliary liver transplantation: conceptual changes in a living-donor program. *J Pediatr Surg* 1998;**33**:1753–6.

Kuhara T, Shinka T, Inoue Y, Ohse M, Zhen-wei X, Yoshida I, *et al*. Pilot study of gas chromatographic-mass spectrometric screening of newborn urine for inborn errors of metabolism after treatment with urease. *J Chromatogr B Biomed Sci Appl* 1999;**731**:141–7.

Leao M. Valproate as a cause of hyperammonemia in heterozygotes with ornithine-transcarbamylase deficiency. *Neurology* 1995;**45**:593–4.

Linnebank M, Homberger A, Rapp B, Winter C, Marquardt T, Harms E, *et al*. Two novel mutations (E86A, R113W) in argininosuccinate lyase deficiency and evidence for highly variable splicing of the human argininosuccinate lyase gene. *J Inherit Metab Dis* 2000;**23**:308–12.

Maestri NE, Clissold D, Brusilow SW. Neonatal onset ornithine transcarbamylase deficiency: a retrospective analysis. *J Pediatr* 1999;**134**:268–72.

Muenzer J, Frazier DM, McCandless SE. Incidence and follow-up evaluation of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. In: Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001; **50**(RR-3):28–9.

Oppliger Leibundgut EO, Wermuth B, Colombo JP, Liechti-Gallati S. Ornithine transcarbamylase deficiency: characterization of gene mutations and polymorphisms. *Hum Mutat* 1996;**8**:333–9.

Oppliger LE, Wermuth B, Colombo JP, Liechti-Gallati S. Identification of four novel splice site mutations in the ornithine transcarbamylase gene. *Hum Genet* 1996; **97**:209–13.

Peltola K, Heinonen OJ, Nanto-Salonen K, Pulkki K, Simell O. Oral lysine feeding in gyrate atrophy with hyperornithinaemia – a pilot study. *J Inherit Metab Dis* 2000;**23**:305–7.

Potter M, Hammond JW, Sim KG, Green AK, Wilcken B. Ornithine carbamoyltransferase deficiency: improved sensitivity of testing for protein tolerance in the diagnosis of heterozygotes. *J Inherit Metab Dis* 2001;**24**:5–14.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol* 1999;**23**:183–93.

Rashed MS, Rahbeeni Z, Ozand PT. Screening blood spots for argininosuccinase deficiency by electrospray tandem mass spectrometry. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):170–73.

Riudor E, Arranz JA, Rodes M, Rubio V, Sentis M, Burlina AB. Influence of dose and age on the response of the allopurinol test for ornithine carbamoyltransferase deficiency in control infants. *J Inherit Metab Dis* 2000;**23**:662–8.

Tokatli A, Coskun T, Ozalp I. Citrullinemia. Clinical experience with 23 cases. *Turk J Pediatr* 1998;**40**:185–93.

Tuchman M, Morizono H, Rajagopal BS, Plante RJ, Allewell NM. Identification of 'private' mutations in patients with ornithine transcarbamylase deficiency. *J Inherit Metab Dis* 1997;**20**:525–7.

Uchino T, Endo F, Matsuda I. Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. *J Inherit Metab Dis* 1998;**21** (Suppl 1):151–9.

Valik D, Miner PT, Jones JD. First U.S. case of adenylosuccinate lyase deficiency with severe hypotonia. *Pediatr Neurol* 1997;**16**:252–5.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 22

Effectiveness of treatments for ornithine transcarbamylase deficiency: dietary and pharmacological therapy

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Burlina *et al.*, 2001[56]	**Study design** Retrospective analysis of clinical and biochemical data from three different European Paediatric Centres **Randomisation method** NA **Duration of study** Median 26 months (range 17 to 42 months) **Setting/location** NR; however, first author from Italy	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of intervention** Effect of sodium phenylbutyrate **Dosage/outcomes** Sodium phenylbutyrate was given in 3–4 divided doses (median 352 mg/kg per day, range 125–484) at 8.9 and 4.9 years of age (median) in males and females, respectively Outcome measures included clinical and biological evaluations, including growth parameters and nutritional status **Patient types** Patients with ornithine transcarbamylase deficiency, aged 6 days to 14 years	**Mean age** Range 2–16 years at starting sodium phenylbutyrate treatment **Gender (M/F)** 4/5 **Ethnicity** NR	Overall, sodium phenylbutyrate was well tolerated, no adverse effects were detected during the treatment period and there were no hyperammonaemic episodes requiring hospitalisation Authors reported that in this retrospective study, it was not possible to obtain accurate data because patients were referred from different centres having distinct nutritional protocols and the treatment regimen was not sufficient for the increase in protein intake allowed. Despite these limitations, the authors observed that total protein intake increased from 0.84 g/kg per day (range 0.43–1.63) before starting treatment with phenylbutyrate to 0.95 g/kg per day (range 0.66–1.46) after 18 months of treatment. Total protein intake correlated significantly with increases in the dose of phenylbutyrate ($p < 0.001$), suggesting that an increase in the phenylbutyrate dose allowed an increase of 1 g in protein intake. In contrast to the decline in plasma ammonia levels, plasma glutamine levels did not show any significant variation during the treatment period Plasma concentrations of essential amino acids, including alanine, asparagines, leucine and isoleucine, remained normal. In addition, other routine haematological and biochemical measurements (liver and renal function tests) remained normal during the treatment period, suggesting a lack of toxicity effect No cognitive evaluation was performed during the treatment, but the authors presumed that the metabolic stability may have prevented neurological deterioration over the period of treatment	Authors concluded that treatment with sodium phenylbutyrate was safe and facilitated the clinical management of patients with ornithine transcarbamylase deficiency and enhanced their quality of life by achieving a better metabolic control despite a higher intake of natural protein; however, these authors encouraged further prospective research to define the optimal dosage of sodium phenylbutyrate and establish the requirements of a protein diet at different ages

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			at diagnosis. Diagnosis based on liver enzyme assay and or DNA mutation analysis. All patients were treated for at least 8 months (median 17 months; range 8–28 months) with a low protein diet (median 0.84 g/kg per day), arginine (5 patients) or citrulline (4 patients) supplementation and oral sodium benzoate (median 248 mg/kg per day, range 106–275). Sodium benzoate was replaced when phenylbutyrate became available **Patient numbers** 9 **Loss to follow-up** NR			

Appendix 23

Effectiveness of treatments for citrullinaemia: dietary and pharmacological therapy

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Maestri *et al.*, 1995[57]	**Study design** Analysis of clinical and biochemical data from 18 medical institutions throughout the USA and Canada **Randomisation method** NA **Duration of study** Not clear; however, 15-year period of study **Setting/location** Department of Pediatrics, Johns Hopkins University, USA	**Inclusion/exclusion criteria** NA **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Long-term therapeutic protocols: dietary management (limitation of dietary nitrogen) and oral administration of arginine freebase and sodium benzoate (and/or) sodium phenyl acetate or sodium phenylbutyrate **Dosage/outcomes** Treatment protocols were modified during the 15 year period of study due to availability of new drugs Protocol I included administration of sodium benzoate and arginine (subjects born before 1984 maintained on this protocol). Protocol II included sodium phenyl acetate	**Mean age** Not reported **Gender (M/F)** Not reported **Ethnicity** White (n = 15), Hispanic (n = 2), Black (n = 1), unknown or mixed racial background (n = 5) and not reported (n = 1)	**Long-term therapeutic protocols for study patients** Protocol I (1980)[a] / Protocol II† (1984)[a] / Protocol III‡ (1987)[a] Diet (/kg per day) Natural protein (g): 0.5–0.7 / 0.5–0.7 / 1.25–2.0 Essential amino acids: 0.5–0.7 / 0.5–0.7 / 0 Calories (kcal): As required / As required / As required Medications (mg/kg per day) Arginine freebase: 500–700 / 500–700 / 400–700 Sodium benzoate: 250 / 250 Sodium phenylacetate † or sodium phenylbutyrate ‡: 250 / 450–600 [a] Introduction of treatment protocol. **Start of therapy and survival** In 24 patients with neonatal onset of citrullinaemia born before 1990, who had been treated since birth with various therapeutic protocols designed to limit dietary nitrogen and to provide vehicles other than urea to excrete waste nitrogen, the cumulative survival rate was 87.5% at 5 years and approximately 72% at 10 years of age Overall, 15 patients survived (3 died during treatment protocol IIa, 4 died on protocol III regimen and 2 withdrew from therapy) during treatment with high doses of sodium phenylbutyrate **Developmental progress** Among the 15 surviving patients, 11 were classified as severely to profoundly mentally retarded (IQ < 55). The remaining 4 patients had IQ measurements in the low borderline to mentally retarded range (IQ 50–70)	The number of patients treated in each protocol regimen is not explicitly clear Authors concluded that these drugs, sodium benzoate, sodium phenyl acetate and sodium phenylbutyrate, were safe and that the current protocol (protocol IIIb) improved survival rates. However, survival was accompanied by mental retardation, growth retardation, risk of hyperammonaemic episodes and the need for lifetime adherence to strict medication and dietary management Note: participation in this study was voluntary and transfer to a newer protocol was neither strictly

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			(protocol IIa), first used in 1984, or sodium phenylbutyrate (protocol IIb), first used in 1985. Protocol III included high doses of sodium phenylacetate (protocol IIIa) or phenylbutyrate (protocol IIIb) and excluded sodium benzoate; patients were transferred to this protocol as it became available in 1987 Outcomes included metabolic, clinical and development data and an assessment of patient compliance with protocol **Patient types** Infants born between 1 January 1979 and 1 September 1989, in whom citrullinaemia was diagnosed		**Hyperammonaemic episodes** All patients had intercurrent hyperammonaemic episodes, the frequency of which decreased with the implementation of the current protocol. There was wide variation in the number and frequency of episodes in individual patients, but different treatment histories make strict comparisons among protocols difficult. On average, the 15 surviving patients had 1 episode per year (range 2–30 episodes) during 5.4–15.5 years of treatment **Anthropometric and other measurements** Overall, patients were growth retarded, had height-for-weight *Z* scores within 2 SD of the mean, and laboratory studies of plasma amino acids and of haematopoietic, renal and hepatic function were within normal limits, with the exception of slightly elevated serum aminotransferase values	enforced nor strongly recommended until evidence accumulated that a newer therapy was effective, and different treatment histories make strict comparisons among protocols difficult

Appendix 23 cont'd *Effectiveness of treatments for citrullinaemia*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			within the first month of life. Diagnosis based on elevated plasma ammonium levels (ranging from 266 to 2000 μmol/l), increased plasma citrulline levels (>1000 μmol/l) and no detectable plasma arginosuccinate. Patients were referred to the Department of Pediatrics, Johns Hopkins University School of Medicine, USA, for enrolment in ongoing clinical studies of sodium benzoate, sodium phenylacetate and sodium phenylbutyrate **Patient numbers** 24 **Loss to follow-up** 9 (7 died and 2 withdrew from therapy)			

Appendix 24

Reference list of excluded studies: methylmalonic, propionic and isovaleric acidaemias

Abdenur JE, Chamoles NA, Guinle AE, Schenone AB, Fuertes AN. Diagnosis of isovaleric acidaemia by tandem mass spectrometry: false positive result due to pivaloylcarnitine in a newborn screening programme. *J Inherit Metab Dis* 1998;**21**:624–30.

Andersson HC, Shapira E. Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). *J Pediatr* 1998;**132**:121–4.

Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. *Genet Med* 1999;**1**:146–50.

Berger I, Shaag A, Anikster Y, Baumgartner ER, Bar-Meir M, Joseph A, *et al*. Mutation analysis of the MCM gene in Israeli patients with mut(0) disease. *Mol Genet Metab* 2001;**73**:107–10.

Bonafe L, Troxler H, Kuster T, Heizmann CW, Chamoles NA, Burlina AB, *et al*. Evaluation of urinary acylglycines by electrospray tandem mass spectrometry in mitochondrial energy metabolism defects and organic acidurias. *Mol Genet Metab* 2000;**69**:302–11.

Bonham Carter SM, Watson DG, Midgley JM, Logan RW. Synthesis and characterisation of acyl glycines. Their measurement in single blood spots by gas chromatography–mass spectrometry to diagnose inborn errors of metabolism. *J Chromatogr B Biomed Appl* 1996; **677**:29–35.

Chace DH, DiPerna JC, Mitchell BL, Sgroi B, Hofman LF, Naylor EW. Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. *Clin Chem* 2001; **47**:1166–82.

Chemelli AP, Schocke M, Sperl W, Trieb T, Aichner F, Felber S. Magnetic resonance spectroscopy (MRS) in five patients with treated propionic acidemia. *J Magn Reson Imaging* 2000;**11**:596–600.

Fries MH, Rinaldo P, Schmidt-Sommerfeld E, Jurecki E, Packman S. Isovaleric acidemia: response to a leucine load after three weeks of supplementation with glycine, L-carnitine, and combined glycine-carnitine therapy. *J Pediatr* 1996;**129**:449–52.

Itoh T, Ito T, Ohba S, Sugiyama N, Mizuguchi K, Yamaguchi S, *et al*. Effect of carnitine administration on glycine metabolism in patients with isovaleric acidemia: significance of acetylcarnitine determination to estimate the proper carnitine dose. *Tohoku J Exp Med* 1996; **179**:101–9.

Jellum E, Dollekamp H, Blessum C. Capillary electrophoresis for clinical problem solving: analysis of urinary diagnostic metabolites and serum proteins. *J Chromatogr B Biomed Sci Appl* 1996;**683**:55–65.

Kuhara T, Shinka T, Inoue Y, Ohse M, Zhen-wei X, Yoshida I, *et al*. Pilot study of gas chromatographic–mass spectrometric screening of newborn urine for inborn errors of metabolism after treatment with urease. *J Chromatogra B Biomed Sci Appl* **731**:141–7.

Lin WD, Wu JY, Lai CC, Tsai FJ, Tsai CH, Lin SP, *et al*. A pilot study of neonatal screening by electrospray ionization tandem mass spectrometry in Taiwan. *Acta Paediatr Taiwan* 2001;**42**:224–30.

Marwah A, Ramji S. Propionic acidemia in the newborn. *Indian Pediatr* 1997;**34**:639–41.

Matern D, Seydewitz HH, Lehnert W, Niederhoff H, Leititis JU, Brandis M. Primary treatment of propionic acidemia complicated by acute thiamine deficiency. *J Pediatr* 1996;**129**:758–60.

Muenzer J, Frazier DM, McCandless SE. Incidence and follow-up evaluation of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001; **50**(No. RR-3),2001:28–9.

Nyhan WL, Bay C, Beyer EW, Mazi M. Neurologic nonmetabolic presentation of propionic acidemia. *Arch Neurol* 1999;**56**:1143–7.

Pang CP, Law LK, Mak YT, Shek CC, Cheung KL, Mak TW, *et al*. Biochemical investigation of young hospitalized Chinese children: results over a 7-year period. *Am J Med Genet* 1997;**72**:417–21.

Presto-Elgstoen KB, Zhao JY, Anacleto JF, Jellum E. Potential of capillary electrophoresis, tandem mass spectrometry and coupled capillary electrophoresis–tandem mass spectrometry as diagnostic tools. *J Chromatogra A* 2001;**914**:265–75.

Rashed MS, Ozand PT, Bucknall MP, Little D. Diagnosis of inborn errors of metabolism from blood spots by acylcarnitines and amino acids profiling using automated electrospray tandem mass spectrometry. *Pediatr Res* 1995;**38**:324–31.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol* 1999;**23**:183–93.

Ravn K, Chloupkova M, Christensen E, Brandt NJ, Simonsen H, Kraus JP, *et al*. High incidence of propionic acidemia in greenland is due to a prevalent mutation, 1540insCCC, in the gene for the beta-subunit of propionyl CoA carboxylase. *Am J Hum Genet* 2000; **67**:203–6.

Rosenblatt DS, Aspler AL, Shevell MI, Pletcher BA, Fenton WA, Seashore MR. Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). *J Inherit Metab Dis* 1997; **20**:528–38.

Rossi A, Cerone R, Biancheri R, Gatti R, Schiaffino MC, Fonda C, *et al*. Early-onset combined methylmalonic aciduria and homocystinuria: neuroradiologic findings. *AJNR Am J Neuroradiol* 2001;**22**:554–63.

Saudubray JM, Touati G, Delonlay P, Jouvet P, Schlenzig J, Narcy C, *et al*. Liver transplantation in propionic acidaemia. *Eur J Pediatr* 1999;**158** (Suppl 2):S65–9.

Shigematsu Y, Hata I, Kikawa Y, Mayumi M, Tanaka Y, Sudo M, *et al*. Modifications in electrospray tandem mass spectrometry for a neonatal-screening pilot study in Japan. *J Chromatogr B* 1999;**731**:97–103.

Sniderman LC, Lambert M, Giguere R, Auray-Blais C, Lemieux B, Laframboise R, *et al*. Outcome of individuals with low–moderate methylmalonic aciduria detected through a neonatal screening program. *J Pediatr* 1999;**134**:675–80.

Tokatli A, Coskun T, Ozalp I. Isovaleric acidemia. Clinical presentation of 6 cases. *Turk J Pediatr* 1998; **40**:111–19.

van der Westhuizen BM, Terblanche SE, Minnie LJ. Benzoylserine and benzoylthreonine in propionic acidaemia treated with sodium benzoate. *S Afr Med J* 1999;**89**:1273–4.

van't Hoff W, McKiernan PJ, Surtees RA, Leonard JV. Liver transplantation for methylmalonic acidaemia. *Eur J Pediatr* 1999;**158** (Suppl 2):S70–4.

Vockley J, Rogan PK, Anderson BD, Willard J, Seelan RS, Smith DI, *et al*. Exon skipping in IVD RNA processing in isovaleric acidemia caused by point mutations in the coding region of the IVD gene. *Am J Hum Genet* 2000;**66**:356–7.

Wasant P, Matsumoto I, Liammongkolkul S. Detection of inborn errors of metabolism in Thai infants via gas chromatography and mass spectrometry. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):160–5.

Wasant P, Naylor EW, Liammongkolkul S. Detection of inherited metabolic disorders via tandem mass spectrometry in Thai infants. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):154–9.

Wasant P, Svasti J, Srisomsap C, Liammongkolkul S, Naylor EW, Matsumoto I. Inherited metabolic disorders in Thailand – Siriraj experience. *Asian J Trop Med Public Health* 1999;**30** (Suppl 2):124–37.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 25

Effectiveness of treatments for methylmalonic acidaemia: cobalamin-responsive versus cobalamin-non-responsive patients

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Nicolaides *et al.*, 1998[58]	**Study design** Cross-sectional study **Randomisation method** NA **Duration of study** NR **Setting/location** Great Ormond Street Hospital for Children, London, UK	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Cobalamin-responsive and non-responsive and, early (presented in first month of life) and late onset (presented after first month) groups **Dosage/outcomes** Cobalamin responsive: low-protein diet and/or intramuscular injections with cyanocobalamin or hydroxycobalamin (1 mg daily for 5 days, $n = 6$) Non-responsive ($n = 29$): low-protein diet (some were treated with carnitine, $n = 13$) **Patient types** Patients with methylmalonic	**Mean age** Not reported; however, all living patients under 16 years of age **Gender (M/F)** Cobalamin responsive: Not reported Non responsive: 12/17 **Ethnicity** NR	**Summary of the neurological outcome of patients with methylmalonic acidaemia** Cobalamin responsive / Cobalamin non-responsive: Early onset / Cobalamin non-responsive: Late onset **Illness severity** Hyperammonaemia at presentation: 1/6 / 9/20 / 1/9 Severity score, median (≥ 95% CI[a]): 1 (1–2) / 6 (5–7) / 4 (1–6) **Neurological outcome** Delayed early development[b]: 2/6 / 14/18 / 3/9 Full-scale IQ, median (≥ 95% CI): 100 (77–102) / 75 (65–84) / 101 (83–125) Abnormal neurological signs: 2/6 / 7/18 / 6/9 Abnormal neuroimaging: 1/3 / 9/13 / 2/6 [a] ≥ 95% confidence interval for the median. [b] Younger than 2 years. There were significant differences between cobalamin-responsive and non-responsive groups in the severity, survival and incidence of neurological sequelae **Illness severity and survival** There was a significant difference between cobalamin-responsive and non-responsive patients in the severity of the illness. In general, the cobalamin-non-responsive group had a greater median difference severity score of 3.6 (95% CI 2.0	Authors concluded that the overall outcome of patients with methylmalonic acidaemia, particularly the early-onset group, remains unchanged, disappointing and unsatisfactory. Cobalamin-responsive patients had a better long-term outcome than non-cobalamin-responsive patients. All cobalamin-non-responsive patients were at risk of developing a progressive neurological disease, particularly the early-onset group. In view of the poor prognosis and to prevent further illness, alternative treatments need to be considered for the early-onset patients, such as liver transplantation; however, this

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			acidaemia who were seen and treated at the Great Ormond Street Hospital for Children, London, UK, between 1970 and 1996. Diagnosis based on increases in urinary methylmalonate and methylcitrate, raised methylmalonate in blood with normal plasma vitamin B_{12} levels and no detectable plasma homocystine In most patients diagnosis confirmed by enzyme studies on cultured skin fibroblasts. After diagnosis all patients treated with low-protein diet and intramuscular injections of cyanocobalamin or hydroxycobalamin		to 5.2, $p < 0.001$) and more encephalopathic episodes (median difference 1.4, 95% CI 2.4 to 6.3, $p < 0.001$) All 6 cobalamin-responsive patients had mild disease and its severity was not dependent on age at presentation, their neurological complications were less severe and they were all alive. In contrast, the cobalamin-non-responsive group consisted of 19 early- (13 females) and 9 late-onset patients (4 females). The early-onset group had more severe disease presentation with episodes of severe acidosis, hyperammonaemia (up to 1800 μM), and 6 collapsed requiring intensive care and ventilatory support. 14 patients in the early-onset cobalamin-non-responsive group had died (median survival 6.4 years, 95% CI 3.6 to 9.1 years), whereas all of the patients in the late-onset group were alive. Six patients in the early-onset cobalamin-non-responsive group died in the first year of life and 8 died between 15 months and 7 years of age Most late-onset patients presented in first year with either an episode of metabolic decompensation and subsequent development delay, or feeding difficulties with failure to thrive. Anorexia and feeding difficulties were seen in 25 of the 27 of cobalamin-non-responsive patients There were no significant differences between the groups with respect to protein intake, which varied between 1.0 and 1.8 g/kg per day. One-third of cobalamin-non-responsive patients had poor growth with height and weight below the third centile	procedure is not without risk

Appendix 25 cont'd *Effectiveness of treatments for methylmalonic acidaemia*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			(1 mg daily for 5 days). Response assessed by urinary methylmalonate measurements, and in responsive patients cobalamin injections were continued **Patient numbers** 35 [patients divided into cobalamin-responsive, $n = 6$, and non-responsive, $n = 29$, and early (presented in first month of life) and late-onset (presented after first month) groups] **Loss to follow-up** Not clear; however, 14 patients in the early-onset, cobalamin-non-responsive group died		**Neurological outcome** There were no significant differences between the early- and late-onset groups in abnormal neurological signs (7 of 18 and 6 of 9, respectively, $p = 0.13$), although early-onset patients had significantly reduced full-scale IQ (median difference 26, $p = 0.03$) and poor neurological and cognitive outcome. In both groups abnormal neurological signs continued to increase with age. In all patients, new neurological symptoms and signs developed following episodes of acute metabolic decompensation	

Appendix 26

Effectiveness of treatments for propionic acidaemia: dietary protein restriction in early-onset versus late-onset patients

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
van der Meer *et al*., 1996[59]	**Study design** Retrospective analysis of clinical data **Randomisation method** NA **Duration of study** NR **Setting/location** Hospital Necker Enfants Malades, Paris, France	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of interventions** Dietary management with low protein intake, with or without supplemental protein mixtures **Dosage/outcomes** Basis of therapy involved dietary management with low protein intake (natural) with or without supplemental protein mixtures In the 1970s dietary treatments for propionic acidaemia were based on restriction of natural protein. After 1980, treatment principles changed. Home tube feeding became routine daily treatment, with the addition of	**Mean age** See Results **Gender (M/F)** NR **Ethnicity** Early-onset patients: 5 of the 12 were non-European immigrants Late-onset patients: all originated from France	**Clinical and treatment data of patients with propionic acidaemia** Early onset / Late onset No. of patients: 12 / 5 Age at diagnosis: 9.3 days (3–19) / 16.3 months (3.5–36) Total time in hospital: 4.1 months (2–12) / 2.9 months (1–7) No. deceased: 5 / 2 Age at death: 3, 3.6, 5, 6 months and 9.5 years / 2.8 and 4 years Present age: 5.6 years (1–9.3) / 11.4 years (4–23) Patients treated with Tube feeding: 9 / 1 Carnitine: 11 / 3 Metronidazole: 6 / 2 Five (42%) of the early-onset and 2 (40%) of the late-onset patients died. The deceased early-onset patients had a median survival time of 0.4 years, whereas the late-onset patients died at the ages of 2.8 years and 4 years. At the time of the study, the median age of the living early-onset patients was 5.2 years (1–9.3 years), whereas the late-onset patients were 4, 7 and 23 years of age All patients were treated with natural protein restriction with the addition of carnitine (100 mg/kg per day) and later with metronidazole (20 mg/kg per day). The natural protein intake per day remained fairly stable during the first 3–4 years of life in the early-onset patients. After the 6th year of life, total protein intake remained fairly constant and seldom reached values > 13 g/day. The supplemental protein intake was higher in early-onset patients, and showed a steady but strong increase and levelled off from the age of 6 years	Authors concluded that the prognosis for patients with propionic acidaemia appeared to be satisfactory in terms of survival, neurological and mental development; however, new developments in medical techniques such as liver transplantation or somatic gene therapy might improve the quality of life for these patients in the future

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			carnitine (100 mg/kg per day) and later metronidazole (20 mg/kg per day). Changes in regimen were kept under strict surveillance and noted Outcome measures included disability, neuromotor, mental, psychological, visceral, sensory, social and nutritional outcomes **Patient types** Patients with propionic acidaemia (diagnosed since 1970) with early- (n = 12) or late-onset type (n = 5). In most patients, diagnosis confirmed with enzymic assay of propionyl CoA carboxylase. Biotin response was assessed in all		The natural protein intake of late-onset patients was higher and rose more rapidly to an almost normal protein intake after 3–4 years of life. The differences between the mean natural protein intake of early- and late-onset patients were significant in all age groups ($p < 0.0001$, t-test) Late-onset patients suffered more frequently from minor to intermediate neurometor, mental and psychological disabilities than the early-onset patients; this may be due to the delay in diagnosis. The authors also observed less frequent metabolic decompensations and hospitalisations with the introduction of nasogastric tube feeding Many patients showed a failure to thrive, particularly for height. This was attributed to the strong protein restriction during the first years of life	

Appendix 26 cont'd *Effectiveness of treatments for propionic acidaemia*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			patients, but none responded either clinically or biochemically. 12 patients presented within 3 weeks (mean 9.3 days, range 3–19 days) after birth (early-onset group), whereas 5 patients were diagnosed later (mean 16.3 months, range 3.5 months to 3 years of age) in life (late-onset group) **Patient numbers** 17 **Loss to follow-up** Not clear; however, 7 patients died (5 in the early-onset and 2 in the late-onset group)			

Appendix 27

Reference list of excluded studies: other defects of branched-chain acyl-coenzyme A metabolism

Bonafe L, Troxler H, Kuster T, Heizmann CW, Chamoles NA, Burlina AB, *et al*. Evaluation of urinary acylglycines by electrospray tandem mass spectrometry in mitochondrial energy metabolism defects and organic acidurias. *Mol Genet Metab* 2000;**69**:302–11.

Gallardo ME, Desviat LR, Rodriguez JM, Esparza-Gordillo J, Perez-Cerda C, Perez B, *et al*. The molecular basis of 3-methylcrotonylglycinuria, a disorder of leucine catabolism. *Am J Hum Genet* 2001;**68**:334–46.

Holzinger A, Roschinger W, Lagler F, Mayerhofer PU, Lichtner P, Kattenfeld T, *et al*. Cloning of the human MCCA and MCCB genes and mutations therein reveal the molecular cause of 3-methylcrotonyl-CoA: carboxylase deficiency. *Hum Mol Genet* 2001; **10**:1299–306.

Mitchell GA, Ozand PT, Robert MF, Ashmarina L, Roberts J, Gibson KM, *et al*. HMG CoA lyase deficiency: identification of five causal point mutations in codons 41 and 42, including a frequent Saudi Arabian mutation, R41Q. *Am J Hum Genet* 1998;**62**:295–300.

Muro S, Rodriguez-Pombo P, Perez B, Perez-Cerda C, Desviat LR, Sperl W, *et al*. Identification of novel mutations in the PCCB gene in European propionic acidemia patients. Mutation in brief no. 253. Online. *Hum Mutat* 1999;**14**:89–90.

Muroi J, Yorifuji T, Uematsu A, Shigematsu Y, Onigata K, Maruyama H, *et al*. Molecular and clinical analysis of Japanese patients with 3-hydroxy-3-methylglutaryl CoA lyase (HL) deficiency. *Hum Genet* 2000;**107**:320–6.

Pearson MA, Aleck KA, Heidenreich RA. Benign clinical presentation of 3-methylcrotonylglycinuria. *J Inherit Metab Dis* 1995;**18**:640–1.

Shotelersuk V, Srivuthana S, Wacharasindhu S, Dhamcharee V, Jaruratanasirikul S, Pangkanon S, *et al*. Establishing gas chromatography-mass spectrometry to diagnose organic acidemias in Thailand. *Southeast Asian J Trop Med Public Health* 2000;**31**:566–70.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 28

Reference list of excluded studies: medium-chain acyl-coenzyme A dehydrogenase deficiency

Abdenur JE, Chamoles NA, Specola N, Schenone AB, Jorge L, Guinle A, *et al*. MCAD deficiency. Acylcarnitines (AC) by tandem mass spectrometry (MS–MS) are useful to monitor dietary treatment. *Ad Exp Med Biol* 1999;**466**:353–63.

Andresen BS, Bross P, Jensen TG, Knudsen I, Winter V, Kolvraa S, *et al*. Molecular diagnosis and characterization of medium-chain acyl-CoA dehydrogenase deficiency. *Scand J Clin Lab Invest* 1995; **220** (Suppl):9–25.

Andresen BS, Dobrowolski SF, O'Reilly L, Muenzer J, McCandless SE, Frazier DM *et al*. Medium-chain acyl-CoA dehydrogenase (MCAD) mutations identified by MS/MS-based prospective screening of newborns differ from those observed in patients with clinical symptoms: identification and characterization of a new, prevalent mutation that results in mild MCAD deficiency. *Am J Hum Genet* 2001;**68**:1408–18.

Bonafe L, Troxler H, Kuster T, Heizmann CW, Chamoles NA, Burlina AB, *et al*. Evaluation of urinary acylglycines by electrospray tandem mass spectrometry in mitochondrial energy metabolism defects and organic acidurias. *Mol Genet Metab* 2000;**69**:302–11.

Bonham Carter SM, Watson DG, Midgley JM, Logan RW. Synthesis and characterisation of acyl glycines. Their measurement in single blood spots by gas chromatography-mass spectrometry to diagnose inborn errors of metabolism. *J Chromatogra B Biomed Sci Appl* 1996;**677**:29–35.

Brivet M, Slama A, Saudubray JM, Legrand A, Lemonnier A. Rapid diagnosis of long chain and medium chain fatty acid oxidation disorders using lymphocytes. *Ann Clin Biochem* 1995;**32**:154–9.

Carpenter K, Wiley V, Sim KG, Heath D, Wilcken B. Evaluation of newborn screening for medium chain acyl-CoA dehydrogenase deficiency in 275 000 babies. *Arch Dis Child Fetal Neonatal Ed* 2001;**85**:F105–9.

Chace DH, Hillman SL, Vanhove JLK, Naylor EW. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. *Clin Chem* 1997;**43**:2106–13.

Charrow J, Goodman SI, McCabe ERG, Rinaldo P. Tandem mass spectrometry in newborn screening. *Genet Med* 2000;**2**:267–9.

Clayton PT, Doig M, Ghafari S, Meaney C, Taylor C, Leonard JV, *et al*. Screening for medium chain acyl-CoA dehydrogenase deficiency using electrospray ionisation tandem mass spectrometry. *Arch Dis Child* 1998; **79**:109–15.

Costa CG, Struys EA, Bootsma A, Ten Brink HJ, Dorland L, Tavares dA I, *et al*. Quantitative analysis of plasma acylcarnitines using gas chromatography chemical ionization mass fragmentography. *J Lipid Res* 1997;**38**:173–82.

Costa CG, Dorland L, Holwerda U, de Almeida IT, Poll-The BT, Jakobs C, *et al*. Simultaneous analysis of plasma free fatty acids and their 3-hydroxy analogs in fatty acid beta-oxidation disorders. *Clin Chem* 1998; **44**:463–71.

Costa CG, Guerand WS, Struys EA, Holwerda U, Ten Brink HJ, Tavares dA I, *et al*. Quantitative analysis of urinary acylglycines for the diagnosis of beta-oxidation defects using GC-NCI-MS. *J Pharm Biomed Anal* 2000;**21**:1215–24.

de Vries HG, Niezen-Koning K, Kliphuis JW, Smit GP, Scheffer H, ten Kate LP. Prevalence of carriers of the most common medium-chain acyl-CoA dehydrogenase (MCAD) deficiency mutation (G985A) in The Netherlands. *Hum Genet* 1996;**98**:1–2.

Flath B, Rolinski B, Roscher AA. Simple high-performance liquid chromatographic method for the detection of phenylpropionylglycine in urine as a diagnostic tool in inherited medium-chain acyl-coenzyme A dehydrogenase deficiency. *J Chromatogr B Biomed Sci Appl* 1997;**694**:227–32.

Grosse S. Screening for medium chain acyl-CoA dehydrogenase deficiency is being evaluated. *BMJ* 2001;**322**:1062.

Hoffman G, Litsheim T, Laessig R. Implementation of tandem mass spectrometry in Wisconsin's newborn screening program. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001; **50** (RR-3),26–7.

Ioulianos A, Wells D, Harper JC, Delhanty JD. A successful strategy for preimplantation diagnosis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. *Prenat Diagn* 2000;**20**:593–8.

Johansson A, Guthenberg C, Ahlman H, Von Dobeln U, Hagenfeldt L. Prevalence of the 985A → G mutation in the medium-chain acyl-CoA dehydrogenase (MCAD) gene in Sweden. *Scand J Clin Lab Invest* 1999;**59**:289–91.

Kozak L, Hrabincova E, Rudolfoa J, Vrabelova S, Freibelger T. Screening of the most common medium-chain acyl CoA dehydrogenase (MCAD) deficiency mutation (K329E) in the Czech newborn population. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):49–50.

Lecoq I, Mallet E, Bonte JB, Travert G. The A985 to G mutation of the medium-chain acyl-CoA dehydrogenase gene and sudden infant death syndrome in Normandy. *Acta Paediatr* 1996;**85**:145–7.

Matsubara Y, Fujii K, Rinaldo P, Narisawa K. A fluorogenic allele-specific amplification method for DNA-based screening for inherited metabolic disorders. *Acta Paediatr* 1999;**88** (Suppl 432):65–8.

Muenzer J, Frazier DM, McCandless SE. Incidence and follow-up evaluation of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001; **50** (RR-3):28–9.

Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol* 1999;**14** (Suppl 1):S4–8.

Njolstad PR, Skjeldal OH, Agsteribbe E, Huckriede A, Wannag E, Sovik O, *et al*. Medium chain acyl-CoA dehydrogenase deficiency and fatal valproate toxicity. *Pediatr Neurol* 1997;**16**:160–2.

Pang CP, Law LK, Mak YT, Shek CC, Cheung KL, Mak TW, *et al*. Biochemical investigation of young hospitalized Chinese children: results over a 7-year period. *Am J Med Genet* 1997;**72**:417–21.

Patel JS, Leonard JV. Ketonuria and medium-chain acyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1995;**18**:98–9.

Rashed MS, Ozand PT, Bucknall MP, Little D. Diagnosis of inborn errors of metabolism from blood spots by acylcarnitines and amino acids profiling using automated electrospray tandem mass spectrometry. *Pediatr Res* 1995;**38**:324–31.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Semin Perinatol* 1999;**23**:183–93.

Romppanen EL. Oligonucleotide ligation assay: applications to molecular diagnosis of inherited disorders. *Scand J Clin Lab Invest* 2001;**61**:123–9.

Romppanen EL, Mononen T, Mononen I. Molecular diagnosis of medium-chain acyl-CoA dehydrogenase deficiency by oligonucleotide ligation assay. *Clin Chem* 1998;**44**:68–71.

Roscher A, Liebl B, Fingerhut R, Olgemoller B. Prospective study of MS-MS newborn screening in Bavaria, Germany. Interim results. *J Inherit Metab Dis* 2000;**23** (Suppl. 1):4.

Sander S, Janzen N, Janetzky B, Scholl S, Steuerwald U, Schafer J, *et al*. Neonatal screening for medium chain acyl-CoA deficiency: high incidence in Lower Saxony (northern Germany). *Eur J Pediatr* 2001;**160**:318–9.

Saudubray JM, Martin D, de Lonlay P, Touati G, Poggi-Travert F, Bonnet D, *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999;**22**:488–502.

Seddon HR, Gray G, Pollitt RJ, Iitia A, Green A. Population screening for the common G985 mutation causing medium-chain acyl-CoA dehydrogenase deficiency with Eu-labeled oligonucleotides and the DELFIA system. *Clin Chem* 1997;**43**:436–42.

Szalai C, Czinner A, Revai K. The frequency of medium-chain acyl-CoA dehydrogenase G985 mutation in the Hungarian population. *Eur J Pediatr* 1996;**155**:256.

Tanaka K, Gregersen N, Ribes A, Kim J, Kolvraa S, Winter V, *et al*. A survey of the newborn populations in Belgium, Germany, Poland, Czech Republic, Hungary, Bulgaria, Spain, Turkey, and Japan for the G985 variant allele with haplotype analysis at the medium chain Acyl-CoA dehydrogenase gene locus: clinical and evolutionary consideration. *Pediatr Res* 1997;**41**:201–9.

Tanner S, Sharrard M, Cleary M, Walter J, Wraith E, Lee P, *et al*. Screening for medium chain acyl-CoA dehydrogenase deficiency has still not been evaluated. *BMJ* 2001;**322**:112.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Public Health Med* 1998;**20**:331–43.

Thorpe C, Kim JJ. Structure and mechanism of action of the acyl-CoA dehydrogenases. *FASEB J* 1995; **9**:718–25.

Wang SS, Fernhoff PM, Hannon WH, Khoury MJ. Medium chain acyl-CoA dehydrogenase deficiency human genome epidemiology review. *Genet Med* 1999; **1**:332–9.

Wasant P, Matsumoto I, Liammongkolkul S. Detection of inborn errors of metabolism in Thai infants via gas chromatography and mass spectrometry. *Southeast Asian J Trop Med Pub Health* 1999;**30** (Suppl 2):160–5.

Wilcken B, Wiley V. Tandem mass spectrometry in the New South Wales newborn screening program. In Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001;**50** (RR-3):33.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999;**88** (Suppl 432):48–51.

Wilson CJ, Champion MP, Collins JE, Clayton PT, Leonard JV. Outcome of medium chain acyl-CoA dehydrogenase deficiency after diagnosis. *Arch Dis Child* 1999;**80**:459–62.

Ziadeh R, Hoffman EP, Finegold DN, Hoop RC, Brackett JC, Strauss AW, *et al*. Medium chain acyl-CoA dehydrogenase deficiency in Pennsylvania: neonatal screening shows high incidence and unexpected mutation frequencies. *Pediatr Res* 1995;**37**:675–8.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 29

UK studies of birth incidence: medium-chain acyl-coenzyme A dehydrogenase deficiency

Authors, year	Study design Duration of study Country Total screened	Patient type Age at sampling (year) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments
Pollitt and Leonard, 1998[65]	**Study design** Prospective surveillance study **Duration of study** March 1994 to March 1996 **Country** British Paediatric Surveillance Unit, UK **Total screened** NR	**Patient type** NR **Age at sampling** NR **Gender (M/F)** NR **Ethnicity** NR	**Outcomes** Diagnosis and outcome of MCAD deficiency in the UK **Diagnostic test** NR **Threshold for disease identification** NR **Confirmation of disease** NR; however, British Paediatric Surveillance Unit sent out orange cards on a monthly basis to all consultant paediatricians in the UK and the Republic of Ireland, accompanied by short case definitions of the disorders under surveillance (average return rate 94.4% in 1995). All notifications followed up by questionnaire requesting patient details, presentation, family history and diagnostic criteria. Supplemented by information from UK laboratories that were likely to have diagnosed and confirmed MCAD deficiency	103 initial notifications were received through the Surveillance Unit. Follow-up led to the identification of 55 patients. Many of the discrepancies between the number of returns and the number of patients were due to multiple reporting, illustrating the tendency for MCAD deficiency patients to be referred to specialist centres either before or after diagnosis. The laboratories identified an additional 7 cases **Incidence** Of the 62 affected individuals with MCAD deficiency, 57 were from England, giving an incidence of 4.5 cases per 100,000 births. Similar figures were found in Scotland, with 4.0 cases per 100,000 births (5 patients with MCAD deficiency were from Scotland). No reports were received from Wales, Northern Ireland or the Irish Republic **Diagnosis and outcome** In the 46 cases who presented with acute illness, 39 were diagnosed after a single episode, all but 4 within 30 days. Six patients were diagnosed after a second acute episode, between 39 and 369 days after the initial illness, and 1 patient was diagnosed after a third acute episode at the age of 12 years 11% had more than one episode before diagnosis and more than 50% of patients who presented with an acute episode were diagnosed within 30 days. 10 of the 62 cases died during the initial attack. Three of the 36 patients surviving one or more attacks showed signs of neurological damage in the immediate postrecovery period and 3 others had developmental delay or learning difficulties. However, a longer follow-up period would be necessary to reveal the milder neurodevelopmental deficits Diagnosed patients (n = 62) came from 54 families. These families contained another 6 confirmed cases of MCAD deficiency diagnosed before the study, and 5 other children had died in infancy, all of whom probably had MCAD deficiency	Authors reported the following limitations of the study: (1) study based on diagnosed cases only, estimating minimum incidence but giving no firm data on the proportion of cases who remain undiagnosed or asymptomatic; (2) short follow-up probably led to an underestimate of long-term sequelae; (3) some data were incomplete and there may be systematic underreporting of symptoms in the newborn period

continued

Author, year	Study design Duration of study Country Total screened	Patient type Age at sampling (year) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments

Clinical presentation

All, but 2 of the clinically affected cases presented with typical symptoms of MCAD deficiency. The age at the first episode ranged from 2 days to 4.39 years, with a median at 1.1 years. Neonatal episodes were significantly underreported because there was no systematic review of neonatal records

Reasons for diagnostic investigation for MCAD deficiency

Indication	Patient numbers
Previous family history	
Siblings known to have MCAD deficiency	6[a]
Sibling suspected to have died of MCAD deficiency	1
Siblings of newly diagnosed case	6
Presentation with an acute illness	
Survived	36[b]
Diagnosed post-mortem	10[c]
Others[d]	3
Total	62

[a] Including 1 prenatal diagnosis.
[b] One with a previously diagnosed sibling, but wrongly believed to be unaffected.
[c] One neonatal death with a previously diagnosed sibling.
[d] One case identified at 2 years of age; 1 reclassified from a diagnosis of glutaric aciduria type 2 made some years previously; 1 investigated because of an apparently unrelated congenital abnormality.

Outcome of MCAD-deficient patients at the time of study

Outcome	Patient numbers (%)
Asymptomatic	15 (24)
Full recovery from attack	30 (48)

Authors concluded that the frequency of MCAD deficiency in Scotland and England is high enough to justify the inclusion of the UK neonatal screening programme. Mortality from MCAD deficiency remains unchanged and morbidity is still considerable. Although a few are diagnosed in the neonatal period, most patients have their first detected attack after the age of 3 months and would benefit from early diagnosis. Although there is some degree of underdiagnosis in the UK, the authors found that a greater number of MCAD-deficient patients were being identified and that there is a geographical variation in MCAD deficiency in the British Isles

Author, year	Study design Duration of study Country Total screened	Patient type Age at sampling (year) Gender (M/F) Ethnicity	Outcomes Diagnostic test Threshold for disease identification Confirmation of disease	Results (cumulative incidence, prevalence, morbidity/mortality data, etc.)	Comments
				Neurological impairment/developmental delay 6 (10) Death 10 (16) No information 1 (2) Total 62 (100) **Other** DNA analysis for the common G985A → G mutation had been performed in 45 families. In 36, the affected children were homozygous for G985A → G mutation, whereas 9 were heterozygous. Thus, 90% of mutant alleles were G985 and the gender ratio in confirmed cases was 1.0	

Data extraction/evidence tables of other UK studies have been included and reported in other sections: Hutchesson *et al*. (1998)[30], see Appendix 11; Pourfarzam *et al*. (2001)[21], see Appendix 8.

Appendix 30

Reference list of excluded studies: defects of long-chain fatty acid catabolism

al Aqeel AI, Rashed MS, Wanders RJ. Carnitine-acylcarnitine translocase deficiency is a treatable disease. *J Inheri Metab Dis* 1999;**22**:271–5.

Andresen BS, Olpin S, Poorthuis BJ, Scholte HR, Vianey-Saban C, Wanders R, *et al*. Clear correlation of genotype with disease phenotype in very-long-chain acyl-CoA dehydrogenase deficiency. *Am J Human Genet* 1999;**64**:479–94.

Brown-Harrison MC, Nada MA, Sprecher H, Vianey-Saban C, Farquhar J Jr, Gilladoga AC, *et al*. Very long chain acyl-CoA dehydrogenase deficiency: successful treatment of acute cardiomyopathy. *Biochem Mol Med* 1996;**58**:59–65.

Carpenter KH, Wilcken B. Neonatal diagnosis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency and implications for newborn screening by tandem mass spectrometry. *J Inherit Metab Dis* 1999;**22**:840–1.

Chace DH, DiPerna JC, Mitchell BL, Sgroi B, Hofman LF, Naylor EW. Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. *Clin Chem* 2001;**47**:1166–82.

Costa CG, de Almeida IT, Jakobs C, Duran M, Poll-The BT. Medium-chain triglyceride loading has no diagnostic power in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1996; **19**:376–7.

Costa CG, Struys EA, Bootsma A, Ten Brink HJ, Dorland L, Tavares dAI, *et al*. Quantitative analysis of plasma acylcarnitines using gas chromatography chemical ionization mass fragmentography. *J Lipid Res* 1997;**38**:173–82.

den Boer ME, IJlst L, Wijburg FA, Oostheim W, van Werkhoven MA, van Pampus MG, *et al*. Heterozygosity for the common LCHAD mutation (1528g>C) is not a major cause of HELLP syndrome and the prevalence of the mutation in the Dutch population is low. *Pediatr Res* 2000;**48**:151–4.

Divry P, Vianey-Saban C, Mathieu M. Determination of total fatty acids in plasma: cis-5-tetradecenoic acid (C14:1 omega-9) in the diagnosis of long-chain fatty acid oxidation defects. *J Inherit Metab Dis* 1999; **22**:286–8.

Gillingham M, Van Calcar S, Ney D, Wolff J, Harding C. Dietary management of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD). A case report and survey. *J Inherit Metab Dis* 1999;**22**:123–31.

He G, Yang BZ, Roe DS, Teramoto R, Aleck K, Grebe TA, *et al*. Identification of two novel mutations in the hypoglycemic phenotype of very long chain acyl-CoA dehydrogenase deficiency. *Biochem Biophys Res Commun* 1999;**264**:483–7.

Ijlst L, Uskikubo S, Kamijo T, Hashimoto T, Ruiter JP, de Klerk JB, *et al*. Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: high frequency of the G1528C mutation with no apparent correlation with the clinical phenotype. *J Inherit Metab Dis* 1995;**18**:241–4.

Matern D, Strauss AW, Hillman SL, Mayatepek E, Millington DS, Trefz FK. Diagnosis of mitochondrial trifunctional protein deficiency in a blood spot from the newborn screening card by tandem mass spectrometry and DNA analysis. *Pediatr Res* 1999;**46**:45–9.

Mathur A, Sims HF, Gopalakrishnan D, Gibson B, Rinaldo P, Vockley J, *et al*. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. *Circulation* 1999;**99**:1337–43.

Muenzer J, Frazier DM, McCandless SE. Incidence and follow-up evaluation of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. In: Centers for Disease Control and Prevention. Using tandem mass spectrometry for metabolic disease screening among newborns: a report of a work group. *MMWR Morb Mortal Wkly Rep* 2001; **50**(RR-3):28–9.

Pande SV. Carnitine-acylcarnitine translocase deficiency. *Am J Med Sci* 1999;**318**:22–7.

Pollitt RJ. Disorders of mitochondrial long-chain fatty acid oxidation. *J Inherit Metab Dis* 1995;**18**:473–90.

Saudubray JM, Martin D, de Lonlay P, Touati G, Poggi-Travert F, Bonnet D, *et al*. Recognition and management of fatty acid oxidation defects: a series of 107 patients. *J Inherit Metab Dis* 1999;**22**:488–502.

Souri M, Aoyama T, Orii K, Yamaguchi S, Hashimoto T. Mutation analysis of very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency: identification and characterization of mutant VLCAD cDNAs from four patients. *Am J Hum Genet* 1996;**58**:97–106.

Spiekerkotter U, Schwahn B, Korall H, Trefz FK, Andresen BS, Wendel U. Very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency: monitoring of treatment by carnitine/acylcarnitine analysis in blood spots. *Acta Paediatr* 2000;**89**:492–5.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Public Health Med* 1998;**20**:331–43.

Tyni T, Palotie A, Viinikka L, Valanne L, Salo MK, Von Dobeln U, *et al*. Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with the G1528C mutation: clinical presentation of thirteen patients. *J Pediatr* 1997; **130**:67–6.

Van Hove JL, Kahler SG, Feezor MD, Ramakrishna JP, Hart P, Treem WR, *et al*. Acylcarnitines in plasma and blood spots of patients with long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *J Inherit Metab Dis* 2000;**23**:571–82.

Vianey-Saban C, Divry P, Brivet M, Nada M, Zabot MT, Mathieu M, *et al*. Mitochondrial very-long-chain acyl-coenzyme A dehydrogenase deficiency: clinical characteristics and diagnostic considerations in 30 patients. *Clin Chim Acta* 1998;**269**:43–62.

Vreken P, van Lint AE, Bootsma AH, Overmars H, Wanders RJ, van Gennip AH. Rapid diagnosis of organic acidemias and fatty-acid oxidation defects by quantitative electrospray tandem-MS acyl-carnitine analysis in plasma. *Ad Exp Biol* 1999;**466**:327–37.

Wasant P, Naylor EW, Liammongkolkul S. Detection of inherited metabolic disorders via tandem mass spectrometry in Thai infants. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):154–9.

Wasant P, Matsumoto I, Liammongkolkul S. Detection of inborn errors of metabolism in Thai infants via gas chromatography and mass spectrometry. *Southeast Asian J Trop Med Public Health* 1999;**30** (Suppl 2):160–5.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999; **88** (Suppl 432):48–51.

Wood JC, Magera MJ, Rinaldo P, Seashore MR, Strauss AW, Friedman A. Diagnosis of very long chain acyl-dehydrogenase deficiency from an infant's newborn screening card. *Pediatrics* 2001;**108**:E19.

Zytkovicz TH, Fitzgerald EF, Marsden D, Larson CA, Shih VE, Johnson DM, *et al*. Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program. *Clin Chem* 2001;**47**:1945–55.

Appendix 31

Reference list of excluded studies: glutaryl-coenzyme A dehydrogenase deficiency

Awaad Y, Shamato H, Chugani H. Hemidystonia improved by baclofen and PET scan findings in a patient with glutaric aciduria type I. *J Child Neurol* 1996;**11**:167–9.

Baric I, Zschocke J, Christensen E, Duran M, Goodman SI, Leonard JV, *et al*. Diagnosis and management of glutaric aciduria type I. *J Inherit Metab Dis* 1998; **21**:326–40.

Baric I, Wagner L, Feyh P, Liesert M, Buckel W, Hoffmann GF. Sensitivity and specificity of free and total glutaric acid and 3-hydroxyglutaric acid measurements by stable-isotope dilution assays for the diagnosis of glutaric aciduria type I. *J Inherit Metab Dis* 1999;**22**:867–81.

Chace DH, DiPerna JC, Mitchell BL, Sgroi B, Hofman LF, Naylor EW. Electrospray tandem mass spectrometry for analysis of acylcarnitines in dried postmortem blood specimens collected at autopsy from infants with unexplained cause of death. *Clin Chem* 2001; **47**:1166–82.

Drigo P, Piovan S, Battistella PA, Della PA, Burlina AB. Macrocephaly, subarachnoid fluid collection, and glutaric aciduria type I. *J Child Neurol* 1996; **11**:414–7.

Forstner R, Hoffmann GF, Gassner I, Heideman P, de Klerk JB, Lawrenz-Wolf B, *et al*. Glutaric aciduria type I: ultrasonographic demonstration of early signs. *Pediatr Radiol* 1999;**29**:138–43.

Hauser SE, Peters H. Glutaric aciduria type 1: an underdiagnosed cause of encephalopathy and dystonia-dyskinesia syndrome in children. *J Paediatr Child Health* 1998;**34**:302–4.

Hoffmann GF, Zschocke J. Glutaric aciduria type I: from clinical, biochemical and molecular diversity to successful therapy. *J Inherit Metab Dis* 1999;**22**: 381–91.

Hoffmann GF, Bohles HJ, Burlina A, Duran M, Herwig J, Lehnert W, *et al*. Early signs and course of disease of glutaryl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1995;**18**:173–6.

Hoffmann GF, Athanassopoulos S, Burlina AB, Duran M, de Klerk JB, Lehnert W, *et al*. Clinical course, early diagnosis, treatment, and prevention of disease in glutaryl-CoA dehydrogenase deficiency. *Neuropediatrics* 1996;**27**:115–23.

Kolker S, Ramaekers VT, Zschocke J, Hoffmann GF. Acute encephalopathy despite early therapy in a patient with homozygosity for E365K in the glutaryl-coenzyme A dehydrogenase gene. *J Pediatr* 2001;**138**:277–9.

Pang CP, Law LK, Mak YT, Shek CC, Cheung KL, Mak TW, *et al*. Biochemical investigation of young hospitalized Chinese children: results over a 7-year period. *Am J Med Genet* 1997;**72**:417–21.

Pfluger T, Weil S, Muntau A, Willemsen UF, Hahn K. Glutaric aciduria type I: a serious pitfall if diagnosed too late. *Eur Radiol* 1997;**7**:1264–6.

Rashed MS, Rahbeeni Z, Ozand PT. Application of electrospray tandem mass spectrometry to neonatal screening. *Sem Perinatol* 1999;**23**:183–93.

Smith WE, Millington DS, Koeberl DD, Lesser PS. Glutaric acidemia, type I, missed by newborn screening in an infant with dystonia following promethazine administration. *Pediatrics* 2001;**107**:1184–7.

Superti-Furga A, Hoffmann GF. Glutaric aciduria type 1 (glutaryl-CoA-dehydrogenase deficiency): advances and unanswered questions. Report from an international meeting. *Eur J Pediatr* 1997;**156**:821–8.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Pub Health Med* 1998;**20**:331–43.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatr* 1999;**88** (Suppl 432):48–51.

Zschocke J, Quak E, Guldberg P, Hoffmann GF. Mutation analysis in glutaric aciduria type I. *J Med Genet* 2000;**37**:177–81.

Appendix 32

Effectiveness of treatments for glutaryl-coenzyme A dehydrogenase deficiency: dietary management

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Bjugstad *et al.*, 2000[71]	**Study design** Retrospective analysis of archival data from published research **Randomisation method** NA **Duration of study** NR **Setting/location** NR; however, authors from Denver, USA	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NA **Intention-to-treat analysis** NA	**Type of intervention** Dietary **Dosage/outcomes** Most patients were started on a protein (lysine/tryptophan)-restricted diet supplemented with carnitine and/or riboflavin after the onset of symptoms. Of the 103 patients, 15.5% had no treatment and 84.5% were treated. Treatment consisted of diet only (5.8%), carnitine or riboflavin supplement (32.0%) and carnitine and riboflavin supplement (46.6%) Outcome measures included age at symptom onset, motor deficits,	**Mean age** NR **Gender (M/F)** NR; however, no gender differences were found in patients with GAI **Ethnicity** NR	More than 50% of patients had an onset of symptoms before 8 months of age, and nearly all children in whom symptoms developed had the symptoms within the first 3 years of life. Only 8.7% of patients were reported as being asymptomatic, approximately 20% eventually improved to lead a normal life with normal or no residual consequences of GAI, and 21.6% of patients died A forward stepwise, multiple linear regression analysis showed that the age at symptom onset contributed significantly to the variability in motor deficits and overall clinical outcome. Patients with symptom onset between 6 and 9 months had a much higher probability of a poor outcome, disease progression and death. In patients with gradually progressive symptoms (no precipitating illness), later onset of symptoms predicted better clinical outcome ($r = 51$, $p > 0.0004$). For patients who had a precipitating illness such as respiratory infection, vomiting or diarrhoea, no correlation was seen between the age at symptom onset and clinical outcome ($r = 0.003$, $p = 0.98$) Postsymptomatic treatment had no significant effect on the severity of motor deficits or the overall clinical outcome. Authors reported that there was a lack of data to analyse the statistical benefit of treatment when it was given before symptoms occurred. Only 6 patients started treatment before any motor symptoms were present, and all 6 have had a relatively normal development	Authors concluded that the age of onset can significantly predict the severity of motor deficits and overall clinical outcome; therefore, it is important to identify patients with GAI as early as possible. Dietary treatment given after the onset of GAI symptoms was not associated with a better prognosis

continued

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			cortical atrophy, basal ganglia atrophy, enlargement of cerebrospinal fluid spaces, changes in white matter, treatment (postsymptomatic treatment: only 6 patients with enough biographical information were found to have been treated for GAI before the onset of symptoms; no statistical analysis could be done to evaluate the benefits of presymptomatic treatment) and clinical outcome (changes in motor behaviour) **Patient types** Articles (n = 42) presenting 115 individuals with GAI were analysed. No			

Appendix 32 cont'd *Effectiveness of treatments for glutaryl-coenzyme A dehydrogenase deficiency*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			patients were double counted, i.e. when one patient was a subject in 2 articles, the data were combined **Patient numbers** 103 (initially 115 patients, but treatment data were available for only 103 subjects) **Loss to follow-up** NR			

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
Monavari and Naughten, 2000[69]	**Study design** Retrospective analysis of clinical data **Randomisation method** NA **Duration of study** NR **Setting/location** National Centre for Inherited Metabolic Disorders, The Children's Hospital, Dublin, Ireland	**Inclusion/exclusion criteria** NR **Power calculation** NA **Baseline comparability** NR **Intention-to-treat analysis** NA	**Type of intervention** Dietary **Dosage/outcomes** Management of disorder based on dietary management. Basic diet consisted of a synthetic protein drink (deficient in the amino acids lysine, hydroxylysine and tryptophan), natural protein restriction (ordinary food), but sufficient for growth, sufficient energy for growth, oral or intravenous supplementation with L-carnitine (100–200 mg/kg per day) and to avoid catabolic state. Total protein intake ranged from 1.5 to 3 g/kg body weight per day (range of natural protein,	**Mean age** NR **Gender (M/F)** NR **Ethnicity** NR	**Symptomatic patients** The age of presentation in 6 symptomatic patients ranged from 3 to 9 months, but the diagnosis was delayed (6–24 months) due to delay in organic acid results (1–16 months) or lack of awareness of the condition. All of the 6 late-diagnosed symptomatic patients suffered from dyskinetic cerebral palsy and 5 patients died **Patients detected as result of family screening** Six patients were diagnosed as a result of family screening, which was carried out because older siblings had been diagnosed previously. Four of the 6 patients were developing normally, 1 died and 1 had mild mental disability. The 5 remaining patients were aged between 3 and 9 years, and were diagnosed with the disorder between 1 and 6 weeks. In these presymptomatic patients, supplementation with L-carnitine and intensive dietary treatment with aggressive emergency management resulted in a favourable outcome. Overall, the data showed that early diagnosis and aggressive treatment lead to favourable outcomes with the prevention of major neurological sequelae	Authors concluded that dietary interventions were not effective in reversing the neurological damage in symptomatic patients; however, these interventions offer some hope to presymptomatic patients in that early intensive management can alter the natural history of GAI. The threat of disability or death remains, and emergency management needs to be initiated with illness at all ages

Appendix 32 cont'd *Effectiveness of treatments for glutaryl-coenzyme A dehydrogenase deficiency*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			0.5–2 g/kg per day; synthetic protein, 0.5–2 g/kg per day; tryptophan, 5–21 mg/kg per day). Patients also received riboflavin; however, all were non-responsive During periods of acute illness or subtle metabolic decompensation, patients reduced natural protein (even stopped it for a short period of 24–48 hours, then reintroduced it gradually as tolerated clinically and biochemically), continued synthetic protein, and increased energy intake by 20–50% by using oral and intravenous glucose and lipids; and L-carnitine intake			

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			was doubled to 200 mg/kg per day **Patient types** Patients were diagnosed with GAI at the Children's Hospital, Ireland. Six patients were diagnosed as a result of family screening and 6 were diagnosed late after symptomatic presentation. Diagnoses were based on abnormal organic acids in urine by capillary GC/MS and clinical suspicion. The disorder was confirmed by enzyme assay of glutaryl-CoA dehydrogenase activity in cultured skin fibroblasts			

Appendix 32 cont'd *Effectiveness of treatments for glutaryl-coenzyme A dehydrogenase deficiency*

Authors, year	Study design Randomisation method Duration of study Setting/location	Inclusion/exclusion criteria Power calculation Baseline comparability Intention-to-treat analysis	Type of interventions Dosage/ outcomes Patients Type Numbers Loss to follow-up	Age (years) Gender (M/F) Ethnicity	Results	Comments
			Patient numbers 12 **Loss to follow-up** 6 (5 in symptomatic group and 1 in the group detected by screening)			

Appendix 33

Reference list of excluded studies: multiple acyl-coenzyme A dehydrogenase deficiency

Pang CP, Law LK, Mak YT, Shek CC, Cheung KL, Mak TW, *et al*. Biochemical investigation of young hospitalized Chinese children: results over a 7-year period. *Am J Med Genet* 1997;**72**:417–21.

Thomason MJ, Lord J, Bain MD, Chalmers RA, Littlejohns P, Addison GM, *et al*. A systematic review of evidence for the appropriateness of neonatal screening programmes for inborn errors of metabolism. *J Pub Health Med* 1998;**20**:331–43.

Wasant P, Matsumoto I, Liammongkolkul S. Detection of inborn errors of metabolism in Thai infants via gas chromatography and mass spectrometry. *Southeast Asian J Trop Med Pub Health* 1999;**30** Suppl 2:160–5.

Wiley V, Carpenter K, Wilcken B. Newborn screening with tandem mass spectrometry: 12 months' experience in NSW Australia. *Acta Paediatrica* 1999; **88** (Suppl 432):48–51.

Appendix 34

Base-case treatment strategies for inborn metabolic disorders

Condition	Cost of early treatment	Cost of treatment for additional lives saved
Tyrosinaemia type I	0.4 years of NTBC and total elemental diet	2 years (minus 0.4 years for early Tx) of NTBC and total elemental diet followed by liver transplant and 28 years of subsequent treatment
Homocystinuria (pyridoxine responsive)	5 years of low-protein diet plus supplements	60 years (minus 5 years for early Tx) of low-protein diet plus supplements
Homocystinuria (pyridoxine non-responsive)	2.5 years of total elemental diet	8 years (minus 5 years for early Tx) of low-protein diet plus supplements, followed by 32 years on general dietetic advice/co-factor treatment
MSUD	0.1 years of total elemental diet	40 years (minus 0. I years for early Tx) of total elemental diet
Urea cycle disorders (moderate)	0.5 years of low-protein diet plus supplements	40 years (minus 0.5 years for early Tx) of low-protein diet plus supplements
Urea cycle disorders (severe)	0.1 years of low-protein diet plus supplements	10 years (minus 0. I years for early Tx) of low-protein diet plus supplements
Methylmalonic acidaemia (neonatal)	0.1 years of low-protein diet plus supplements	30 years (minus I year for early Tx) of low-protein diet plus supplements
Propionic acidaemia (neonatal)	0.1 years of low-protein diet plus supplements	40 years (minus I year for early Tx) of low-protein diet plus supplements
Isovaleric acidaemia	0.5 years of general dietetic advice/co-factor treatment	50 years (minus 0.5 years for early Tx) of general dietetic advice/co-factor treatment
Branched-chain acyl-CoA metabolism	0.2 years of no diet/emergency regimen	5 years (minus 0.2 years for early Tx) of no diet/emergency regimen
MCAD deficiency	I year of no diet/emergency regimen	5 years (minus I year for early Tx) of no diet/emergency regimen
Long-chain defects	0.5 years of general dietetic advice/co-factor treatment	5 years (minus 0.5 years for early Tx) of general dietetic advice/co-factor treatment followed by 45 years of general dietetic advice/co-factor treatment at a reduced cost of £250
GAI	I year of general dietetic advice/co-factor treatment	Cost of treatment of additional lives saved: 50 years (minus I year for early Tx) of general dietetic advice/co-factor treatment
GAII	0.3 years of general dietetic advice/co-factor treatment	50 years (minus 0.3 years for early Tx) of general dietetic advice/co-factor treatment

Source: Pollitt *et al*. (1997).[1]
Tx, treatment.

Appendix 35

Base-case assumptions for current cost of each treatment category

	Base values for model	Cost (£): 2001	
	Treatment categories	**0–1 year**	**1–2 years**
1	Total elemental diet	2883	5481
2	Low-protein diet + supplements	1536	2287
3	No diet/emergency regimen	74	74
4	General dietetic advice/co-factor treatment (e.g. riboflavin, pyridoxine, carmitine, biotin)	529	529
(5)	Tyrosinaemia: NTBC treatment and diet; liver transplantation by 2 years	£13,454 in year 1, then £16,052 in year 2 until transplant (£52,750); then £5286 per year following transplant	

Source: Pollitt *et al*. (1997).[1]
Figures are based on 1996 costs up-rated for inflation using Health Service Cost Index.

Appendix 36

Base-case assumptions for treatment categories and average period between presymptomatic and symptomatic diagnosis

Condition	Treatment category	Additional treatment years
Amino acid disorders		
PKU	–	–
Tyrosinaemia type I	(5)	0.40
Homocystinuria (pyridoxine responsive)	4	5.00
Homocystinuria (pyridoxine non-responsive)	1	2.50
MSUD	1	0.10
Acylcarnitines		
Methylmalonic acidaemia	2	0.10
Propionic acidaemia	2	0.10
Isovaleric acidaemia	4	0.50
Branched-chain acyl-CoA metabolism	3	0.30
MCAD deficiency	3	1.00
Defect of long-chain fatty acid	4	0.50
GAI	4	1.00
GAII	4	1.00
Urea cycle disorders		
Urea cycle disorders (moderate)	2	0.50
Urea cycle disorders (severe)	2	0.10

Source: Pollitt *et al.* (1997).[1]
Additional treatment period is the period between symptomatic and asymptomatic diagnosis. Where disorders only require treatment in the short term, the treatment period is assumed to be 5 years.

Appendix 37

Cost-effectiveness searches

HEED

CD-ROM version
Search undertaken November 2001

Search terms

- neonat* AND screen* AND inborn error* AND spect*

Fields searched

- Abstract
- All data
- Article title
- Book title
- Keywords
- Technology assessed

NHS EED

CRD website – complete database
Search undertaken November 2001

(neonat and screen) or (newborn and screen)/All fields AND (mass and spect) or (ms and spect) or (tandem and spect)/All fields

Appendix 38

Economic evaluations and quality-of-life search filters used in Ovid MEDLINE

Economic evaluations

1 economics/
2 exp "costs and cost analysis"/
3 economic value of life/
4 exp economics, hospital/
5 exp economics, medical/
6 economics, nursing/
7 economics, pharmaceutical/
8 exp models, economic/
9 exp "fees and charges"/
10 exp budgets/
11 ec.fs
12 (cost or costs or costed or costly or costing$).tw
13 (economic$ or pharmacoeconomic$ or price$ or pricing).tw
14 or/1-13

Quality of life

1 exp quality of life/
2 quality of life.tw
3 life quality.tw
4 hql.tw
5 (sf 36 or sf36 or sf thirtysix or sf thirty six or short form 36 or short form thirty six or short form thirtysix or shortform 36).tw
6 qol.tw
7 (euroqol or eq5d or eq 5d).tw
8 qaly$.tw
9 quality adjusted life year$.tw
10 hye$.tw
11 health$ year$ equivalent$.tw
12 health utilit$.tw
13 hui.tw
14 quality of wellbeing$.tw
15 quality of well being.tw
16 qwb.tw
17 (qald$ or qale$ or qtime$).tw

Appendix 39

Model parameters and distribution assumptions for phenylketonuria screening

Parameters: PKU neonatal screening programme using existing technology and tandem MS

Variable	Base value	Model distribution	Source
Incidence of PKU	9 per 100,000	Poisson distribution	Literature review and Lord *et al.*, 1999[90]
False-positive rate (existing technology)	0.050%	Triangular: (0.0%, 0.05%, 1.7%)	Pollitt *et al.*, 1997[1]
False-negative rate (existing technology)	0.020%	Fixed: insufficient data to calculate a range	Pollitt *et al.*, 1997[1]
False-positive rate (tandem MS)	0.029%	Triangular: (0.022%, 0.029%, 0.035%) Lower and upper bounds based on 95% CIs for PKU diagnostic study	Zytkovicz *et al.*, 2001[7]
False-negative rate (tandem MS)	0.020%	Fixed. Assume same false-negative rates for both technologies	Literature review
Sample collection costs	Assumed identical for both technologies. These costs excluded from the comparison		
Laboratory cost per sample (existing technology)	£0.92 per sample	Normal: N(0.92, 0.12) Probabalistic estimate based on a simulation of the distribution of costs per sample by 3 types of existing technology used: Guthrie, fluorometry and chromatography. Weighted distribution of costs based on median values reported in Pollit *et al.*, 1997[1]	Pollitt *et al.*, 1997[1] Prices updated to 2001 for inflation using Health Services Cost Index See Table 11
Laboratory cost per sample (tandem MS)	£1.48 per sample	Normal: N(1.48, 0.1). Other probabilistic estimates based on different workload volumes for a single tandem MS system.	See Appendix 46 and Table 12
Repeat sampling rate (for inadequate sampling)	This refers to repeat sampling required due to poor-quality samples, etc., and is not due to additional sampling related to false positives and confirmation protocols. Assumed identical (1%) for both technologies		
Cost of obtaining a repeat specimen	£20.15	Log-normal distribution. Costs per home visit; based on two-third and one-third split between visits made by midwives and health visitors for repeat specimens; plus £1.15 for consumables	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit[93]
Confirmation cost per positive case	£60	Log-normal distribution. Includes estimate of laboratory test costs, referral and advice	Current laboratory prices for tests performed
Treatment costs	With false negatives assumed equal, treatment costs should be the same; these costs excluded		
Future healthcare and social care costs	With false negatives assumed equal, future health and social care costs should be the same; excluded		
Mortality/morbidity	With false negatives assumed equal, health outcomes should be the same; excluded		

Appendix 40

Model parameters and distribution assumptions for extension of phenylketonuria screening to include medium-chain acyl-coenzyme A dehydrogenase deficiency

Parameters: PKU neonatal screening using tandem MS with and without additional screening for MCAD deficiency

Variable	Base value	Model distribution	Source
Incidence of MCAD deficiency	8 per 100,000	Poisson distribution	Pollitt *et al.*, 1997[1]
False-positive rate for MCAD deficiency screening	0.023%	Triangular: (0.0159%, 0.0230%, 0.0297%) Lower and upper bounds are 95% CIs for MCAD deficiency	Literature reviewed
False negative rate (MCAD deficiency screening)	0.0001%	Normal: N(0.0001%, 0.00001%)	Assumption
Mean age of symptomatic presentation/ diagnosis	1	Fixed	Pollitt *et al.*, 1997[1]
Proportion of cases who remain asymptomatic	0.30	Uniform: U(0.25, 0.35)	Pollitt *et al.*, 1997[1]
Incremental cost of tandem MS for PKU	£54,900	Normal: N(£54,900, £15,899) for range 50,000–60,000 Other estimates based on different workload volumes	Derived from phase i model See results in Table 13.
Additional laboratory staffing costs for condition	£3500	Normal: N(3500, 350). Estimated cost for additional staff time (0.1 WTE, clinical scientist) for reporting and advice, etc. Fixed for volume ranges evaluated in model	Pollitt R: personal communication
Laboratory consumables per sample for MCAD deficiency	£0.10 per sample	Normal: N(0.10, 0.01)	Incremental consumables cost, per sample, for MCAD deficiency screen
Additional specimen collection costs for MCAD deficiency screening	£0.30 per sample	Log-normal distribution	Based on estimated additional time and materials (consultation with midwife)
Repeat sampling rate (for inadequate sampling)	This refers to repeat sampling required due to poor-quality samples, etc., and is not due to additional sampling related to false positives and confirmation protocols. These costs were included in the phase i model and are not included again here		
Cost of obtaining a repeat sample	£20.15	Log-normal distribution. Costs per home visit; based on two-third and one-third split between visits made by midwives and health visitors for repeat specimens, plus £1.15 for consumables	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit)[93]
Confirmation cost per positive case of MCAD deficiency	£130 (screened) £150 (unscreened)	Log-normal distributions	Pollitt *et al.*, 1997[1]; personal communication and current laboratory prices for tests
Cost of a paediatric referral	£64	Log-normal distribution	Unit Costs of Health and Social Care: 2001) Personal Social Services Research Unit)[93]
Acute 'presentation' episode cost	£1043	Log-normal: mean £1043 (range: £119–6712)	NHS Reference Costs, 2001[94]
Treatment cost: presymptomatic diagnosis Treatment cost: symptomatic diagnosis	£330 £256	Uniform: (330, 1320) Uniform: (256, 1024)	For base values, see Appendices 34–36

continued

Parameters: PKU neonatal screening using tandem MS with and without additional screening for MCAD Deficiency

Variable	Base value	Model distribution	Source
Proportion of screened cases who develop significant disability/impairments	0.00	Assumed zero. Evidence indicates no appreciable cognitive impairments or neurological damage due to effectiveness of early treatment; see Wilson *et al.*, (1999)[63] and Clayton *et al.*, (1998)[28]	
Proportion of symptomatic cases who develop significant disability/impairments	0.12	Uniform: U(0.10, 0.15). Note that these proportions only apply to symptomatic cases (i.e. incident cases minus the expected numbers who would remain asymptomatic in the absence of screening)	Iafolla *et al.*, 1994[60]; Pollitt *et al.*, 1997[1]; Tanner *et al.*, 2001[92]
Future healthcare and social care costs for moderate to severe disabilities and impairments	£88,000	Uniform: (£88,000, £290,000)	Based on Beecham *et al.*, 2001,[95] and Lord *et al.*, 1999[90]. See text
Life expectancy (with significant disability)	55 years	Triangular: (35, 55, 65)	Literature reviewed. See text
Life expectancy (for asymptomatic and those without significant disability)	75 years	Uniform: (75, 80). Figures taken from GAD Life Tables for 2001; 75 for males and 80 for females	Assumed normal life expectancy for those detected and treated before significant impairments develop, or for those who remain asymptomatic
Mortality proportion in screened cases	0.00	Assumed zero. Evidence indicates no subsequent deaths of diagnosed cases in recent years based on effectiveness of early treatment; see Andresen *et al.* (2001)[23], Carpenter *et al.* (2001)[20], Wilson *et al.* (1999)[63] and Clayton *et al* (1998)[28]	
Mortality proportion in unscreened cases	0.20	Uniform: U(0.15, 0.25)	Pollitt *et al.*, 1997[1] and literature reviewed
Discount rates	All future costs discounted at 6%; benefits (life-years gained) at 1.5%		

GAD: Government Actuaries Department.

Appendix 41

Model parameters and distribution assumptions for glutaric aciduria type I

Parameters: GAI

Variable	Base value	Model distribution	Source
Incidence of GAI	2 per 100,000	Poisson distribution	Pollitt *et al.*, 1997[1]
False-positive rate	0.023%	Triangular: (0.0159%, 0.023%, 0.0297%) Lower and upper bounds based on 95% CIs	Literature reviewed
False-negative rate	0.000%	Fixed	Zytkovicz *et al.*, 2001[7]
Mean age of symptomatic presentation/diagnosis	1	Fixed	Pollitt *et al.*, 1997[1]
Proportion of cases who remain asymptomatic	0.20	Uniform: U(0.15, 0.25)	Pollitt *et al.*, 1997[1]
Incremental cost of using tandem MS for PKU and MCAD deficiency screening	–£23,060	Triangular: (–142,498, –23060, 62,350). Based on 5th, 50th and 95th percentiles	Derived from PKU + MCAD deficiency model
Additional laboratory staffing costs	0	Assumed that for small number of additional cases detected, 'advice and referral' input from laboratory staff can be accommodated within additional staffing provided for MCAD deficiency (see Appendix 40)	
Additional laboratory costs per sample	£0.10 per sample	Normal: N(0.10, 0.01)	Incremental consumables cost, per sample, for GA1 screen
Repeat sampling rate (for inadequate sampling)	This refers to repeat sampling required due to poor-quality samples, etc., and is not due to additional sampling related to false positives and confirmation protocols. These costs were included in the phase i model and are not included again here		
Cost of obtaining a repeat sample	£20.15	Log-normal distribution. Costs per home visit; based on two-third and one-third split between visits made by midwives and health visitors for repeat specimens, plus £1.15 for consumables	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit)[93]
Confirmation cost per positive case (*nb*. advice and referral included elsewhere)	£130 (screened) £150 (unscreened)	Log-normal distributions.	Personal communication and laboratory prices for tests performed
Cost of a paediatric referral	£64	Log-normal distribution	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit)[93]
Acute 'presentation' episode cost	£1043	Log-normal: mean £1043 (range £119–6712)	NHS Reference Costs, 2001[94]
Treatment cost: presymptomatic diagnosis	£8831	Uniform: U(8831, 17,662), and U(8302, 16,604)	
Treatment cost: symptomatic diagnosis	£8302	Represents future treatment costs based on defined strategy for GAI. Discounted at 6%	
Proportion of screened cases (who survive) with significant disability/impairments	0.20	Normal: N(0.20, 0.01)	Monavari and Naughten, 2000[69]
Proportion of symptomatic unscreened cases (who survive) with significant disability/ impairments	0.90	Uniform: U(0.90, 1.0)	Hoffman and Zschocke, 1999[68]; Monavari and Naughten, 2000[69]

continued

Parameters: GAI			
Variable	**Base value**	**Model distribution**	**Source**
Future healthcare and social care costs	£83,000	Uniform: U(£88,000, £290,000)	Based on Beecham *et al.*, 2001[95] and Lord *et al.*, 1999.[90] See MCAD deficiency model.
Life expectancy (those with significant disability)	55 years	Triangular: (35, 55, 65)	Assumption same as for MCAD deficiency model
Life expectancy (survivors without significant disability)	65 years	Normal: N(65, 3.25)	Assume near normal life expectancy
Mortality proportion in screened cases	0.16	Normal: N(0.16, 0.08)	Monavari and Naughten, 2000[69]
Mortality proportion in symptomatic unscreened cases	0.83	Uniform: U(0.60, 0.83)	Pollitt *et al.*, 1997[1], Monavari and Naughten, 2000[69]
Discount rates	All future costs discounted at 6%; benefits (life-years gained) at 1.5%		

Appendix 42

Model parameters and distribution assumptions for homocystinuria (cystathionine β-synthase deficiency)

Parameters: homocystinuria

Variable	Base value	Model distribution	Source
Incidence of homocystinuria	1.5 per 100,000	Poisson distribution	Pollitt *et al.*, 1997[1]
False-positive rate	0.0272%	Triangular: (0.0209%, 0.0272%, 0.0336%) Lower and upper bounds based on 95% CIs	Zytkovicz *et al.*, 2001[7]
False-negative rate	0.000%	Fixed	Assumption
Mean age of symptomatic presentation/ diagnosis	3.75	Poisson	Pollitt *et al.*, 1997[1]
Proportion of cases who remain asymptomatic	0.0		Pollitt *et al.*, 1997[1]
Incremental cost of using tandem MS for PKU and MCADD screening	–£23,060	Triangular: (–142,498, –23,060, 62,350). Based on 5th, 50th and 95th percentiles	Derived from PKU + MCAD deficiency model
Additional laboratory staffing costs	0	Assumed that for small number of additional cases detected, 'advice and referral' input from laboratory staff can be accommodated within additional staffing provided for MCAD deficiency (see Appendix 40)	
Additional laboratory costs per sample	£0.10 per sample	Normal: N(0.10, 0.01)	Incremental consumables cost, per sample, for homocystinuria screen
Repeat sampling rate (for inadequate sampling)	This refers to repeat sampling required due to poor-quality samples, etc., and is not due to additional sampling related to false positives and confirmation protocols. These costs were included in the phase i model and are not included again here		
Cost of obtaining a repeat sample	£20.15	Log-normal distribution. Costs per home visit; based on two-third and one-third split between visits made by midwives and health visitors for repeat specimens; plus £1.15 for consumables	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit)[93]
Confirmation cost per positive case (NB. advice and referral included elsewhere)	£160 (screened) £160 (unscreened)	Log-normal distributions	Personal communication and laboratory prices for tests performed
Cost of a paediatric referral	£64	Log-normal distribution	Unit Costs of Health and Social Care: 2001 (Personal Social Services Research Unit)[93]
Acute 'presentation' episode cost	£1043	Log-normal: mean £1043 (range £119–6712)	NHS Reference Costs, 2001[94]
Treatment cost: presymptomatic diagnosis	£30507	Uniform: U(30,507, 61,014); U(20,531, 41,062)	
Treatment cost: symptomatic diagnosis	£20531	Represents future treatment costs based on defined strategy for homocystinuria. Discounted at 6%	
Proportion of screened cases (who survive) with significant disability/impairments	0.16	Normal: N(0.16, 0.16)	Yap and Naughten, 1998[48]
Proportion of symptomatic unscreened cases (who survive) with significant disability/ impairments	0.60	Normal: U(0.60, 1.0)	Estimate from Mudd, Levy and Kraus, in Scriver *et al.*, 2001[45]

continued

Parameters: homocystinuria			
Variable	**Base value**	**Model distribution**	**Source**
Future healthcare and social care costs	£83,000	Uniform: U(£88,000, £290,000)	Based on Beecham *et al*, 2001,[95] and Lord *et al.*, 1999[90]. See MCAD deficiency model
Life expectancy (those with significant disability)	55 years	Triangular: (35, 55, 65)	Assumption same as for MCAD deficiency model
Life expectancy (survivors without significant disability)	65 years	Normal: N(65, 3.25)	Assume near normal life expectancy
Mortality proportion in screened cases	0.0		Assumption
Mortality proportion in symptomatic unscreened cases	0.225	Normal: U(0.225, 0.0225)	Wilcken and Wilcken, 1997[47]
Mean age at death for mortality events in symptomatic unscreened cases	13 years	Normal: N(13, 2.6)	Wilcken and Wilcken, 1997[47]
Discount rates	All future costs discounted at 6%; benefits (life-years gained) at 1.5%		

Appendix 43

Base-case assumptions for incidence and proportion of cases affected for other inborn metabolic conditions

Condition	Incidence rate (per 100,000)	Proportion of cases affected
Amino acid disorders		
Tyrosinaemia type I	1	100%
MSUD	0.5	> 99%
Acylcarnitines		
Methylmalonic acidaemia	1.5	100%
Propionic acidaemia	0.8	100%
Isovaleric acidaemia	0.7	100%
Branched-chain acyl-CoA metabolism	1.0	80%
Defects of long-chain fatty acids	3	> 95%
GAII	2	> 95%
Urea cycle disorders		
Urea cycle disorders	2.5	> 95%

Source: Pollitt *et al.* (1997).[1]

Appendix 44

Base-case assumptions for mortality avoided and life expectancy for other inborn metabolic conditions

Condition	Mortality rate (%) in symptomatic cases	Life expectancy of presymptomatic and symptomatic survivors		
		Minimum	Most probable	Maximum
Amino acid disorders				
Tyrosinaemia type I	15	20	30	40
MSUD	20	30	40	50
Acylcarnitines				
Methylmalonic acidaemia	25	20	30	40
Propionic acidaemia	25	30	40	50
Isovaleric acidaemia	20	40	50	60
Branched-chain acyl-CoA metabolism	25	40	50	60
Defects of long-chain fatty acids	50	40	50	60
GAII	20	40	50	60
Urea cycle disorders				
Urea cycle disorders	35	10	25	40

Source: Pollitt *et al.* (1997).[1]

Appendix 45

Model construction and formulae

TABLE 18 *Model for PKU screening using tandem MS*

A	B	C	E	D
Inputs	**Option T_0**	**Existing PKU technologies**	**Option T_1**	**Tandem MS**
Parameters	Base values		Base values	
Cohort	100,000		100,000	= B3
Frequency	9.00		9.00	= B4
False-positive rate	0.050%		0.107%	
False-negative rate	0.00%		0.00%	
No. false positives	50	= B5*B3	17	= E5*E3
No. false negatives	0.000	= B6*B3	0.000	= E6*E3
No. detected presymptomatically	9.00	= B4 – B8	9.00	= E4 – E8
Repeat sampling for technical failure	1.00%		1.00%	= B10
Cost per new specimen	20.15		20.15	= B11
Cost per resample	21.07	= B11 + B15	21.68	=E11 + E15
Number of resamples required	1,050.00	= (B3*B10) + B7	1,017.00	= (E3*E10) + E7
Resampling total cost	22,123.50	= B13*B12	22,048.56	= E13*E12
Laboratory costs per screening per sample	0.920	= sample values from probabilistic distribution	1.53	= sample values from probabilistic distribution
Laboratory costs for cohort	114,123.50	= (B15*B3) + B14	175,048.56	= (E15*E3) + E14
Confirmation cost per initial positive case (average)	0.00	[Included in sample cost estimates]	60.00	['Advice and referral' for MS/MS option included in sample cost estimates]
Confirmation costs for cohort	0.00	[Included in sample cost estimates]	1,560.00	= (E7 + E9)*E17
Total cost (discounted)	£114,123.50	= B18 + B16	£176,608.56	= E18 + E16
Incremental cost	**£62,485**	**= E19 – B19**		

TABLE 19 *Model for (PKU+) MCAD screening using tandem MS*

A	B	C	E	D
Inputs	**Option T_0**	**Screening**	**Option T_1**	**No screening**
Cohort	100,000		100,000	= B3
Frequency	8		8	= B4
False-positive rate	0.0230%		0	
False-negative rate	0.0000%		0	
No. false positives	23	= B5*B3	0	
No. false negatives	0	= B6*B3	0	
Age at presymptomatic screen (years)	0		0	= B9
Detected presymptomatically	8	= B4*(1 – B6)	0	
Average age at symptomatic presentation (years)	1		1	= B11
Proportion that remain asymptomatic	30.00%		30.00%	= B12
Number detected symptomatically	0	= (B4 – B10)*(1 – B12)	5.60	= (E4 – E10)*(1 – E12)
Additional screening cost using MS/MS for PKU	57,363	= mean and distribution obtained from PKU model	0	
Additional laboratory costs for disorder screening	0.1		0	
Additional cost for an initial blood sample	0.3		0	
Additional laboratory and sample collection costs	40,000	= (B16*B3) + (B15*B3)	0	
Confirmation cost per initial positive case (average)	130		150	
Additional 'advice and referral' for all positive cases	3,500		0	
Confirmation costs for cohort	9,090	= ((B7 + B10)*B18) + (B10*1.5**B18) + B19	1,260	= (((E7 + E13)*E18)*1.5) + E19
Cost of acute episode (average) for 'symptomatic presentation'	1,043		1,043.00	= B21
No. of acute episodes	1.00		1.00	= B22
Acute care 'symptomatic presentation' costs	0	= B22*B21*B13	5,840.8	= E22*E21*E13
Costs of a paediatric referral for positive cases	64		64	= B24
Total costs of paediatric referral	512	= B24*B10	358.4	= E24*E13
Treatment costs per case (discounted)	330		256	
Treatment costs (discounted)	2,640	= B26*(B10 – B36)	1,146.88	= E26*(E13 – E35)
Proportion of symptomatic cases with significant disability	12.00%		12.00%	= B28
Life expectancy of those with disabilities	55		55	= B29
Number of cases with significant disabilities	0	= B28*(B13 – B35)	0.54	= E28*(E13 – E35)
Life-years of those with disabilities (discounted)	0	= B30*(–PV(1.5%, B29,1,0))	20.04	= E30*(–PV(1.5%, E29, 1,0))
Future health and social care costs of disability per case (discounted)	88,000		88,000	= B32
Future health and social care costs of disability (discounted)	0	= B32*B30	47,308.8	= B32*E30

continued

TABLE 19 *Model for (PKU+) MCAD screening using tandem MS (cont'd)*

A	B	C	E	D
Inputs	**Option T_0**	**Screeing**	**Option T_1**	**No screening**
Mortality rate of symptomatic cases	20.00%		20.00%	= B34
Neonatal/early infant mortality (events)	0	= B13*B34	1.12	= E34*E13
Life expectancy of survivors without disabilities	60.00		60.00	= B36
Discounted value of life expectancy gain (@ 1.5%)	39.38	= –PV(1.5%, B36, 1,,0)	39.38	= B37
Life-years of survivors without disabilities (discounted)	315.04	= B37*(B4 – 30 – B35)	249.77	= E37*(E4 – E30 – E35)
Total cost (discounted)	109,605.00	= B14 + B17 + B20 + B23 + B25 + B27 + B33	55,914.88	= E14 + E17 + E20 + E23 + E25 + E27 + E33
Total life-years gained (discounted)	315.04	= B38 + B31	269.80	= E38 + E31
Incremental cost	**53,690**	**= B39 – E39**		
Incremental benefit	**45**	**= B40 – E40**		

Appendix 46

Estimated costs for a single tandem mass spectrometry screening service

Using tandem MS in a laboratory for neonatal screening

Labour inputs	**Grade**	**WTE**	**Annual cost**
Preparation and loading of microtitre plates, etc.	MLSO grade I	1.00	£17,538
Reading and reporting results obtained	Clinical scientist	0.15	£ 5,175
			£22,713
Advice and referral	Clinical scientist	0.2	£6,900
Salary cost values	**Salary**	**Plus on-cost (@15%)**	
MLSO[a] grade I	£15,250	£17,548	
Clinical scientist	£30,000	£34,500	

[a] In the UK, a Medical Laboratory Scientific Officer (MLSO) is a biomedical scientist working in heathcare laboratories performing tests on human samples.

Appendix 47

Estimated current costs of tandem mass spectrometry

	Distribution			
	Base case	**Minimum**	**Most probable**	**Maximum**
Initial capital outlay	**£175,000**	**£165,000**	**£175,000**	**£180,000**
Asset life (years)	**7**	**5**	**7**	**10**
Rate of interest or discount (%)	**6**	**6**	Fixed	
Scrap value at end of asset life (% capital outlay)	**0**		Fixed	
Annual equivalent cost	**£31,349**			
Other annual expenses:				
Facility overheads (for accommodation of tandem MS system)	£2,000		Normal	
Maintenance (per annum)	£14,000		Normal	
Internal standards (per annum)	£2,000		Normal	
Basic solvents (per sample)	£0.03		Uniform (0.03, 0.05)	
Microtitre plates (each)	£1.00		Normal	
Expected annual number of samples analysed for typical neonatal laboratory	50,000		Varied to reflected scale	
Capital and related consumables costs per sample	**£1.03**			
Labour				
Labour cost for preparation, analysis and reporting	£22,700		Normal	
Labour costs for 'advice and referral'	£7,000		Normal	
Total cost per sample	**£1.61**	Probabilistic estimate (10,000 iterations)		

Source: Information on instrument and consumable costs obtained from two suppliers of tandem MS technology in the UK. Labour cost estimates in consultation with the Neonatal Screening Laboratory, Sheffield Children's Hospital (see Appendix 45)

Feedback

The HTA Programme and the authors would like to know your views about this report.

The Correspondence Page on the HTA website (http://www.ncchta.org) is a convenient way to publish your comments. If you prefer, you can send your comments to the address below, telling us whether you would like us to transfer them to the website.

We look forward to hearing from you.

The National Coordinating Centre for Health Technology Assessment,
Mailpoint 728, Boldrewood,
University of Southampton,
Southampton, SO16 7PX, UK.
Fax: +44 (0) 23 8059 5639 Email: hta@soton.ac.uk
http://www.ncchta.org

ISSN 1366-5278