Review of neonatal screening programme for phenylketonuria

Isabel Smith, Beverley Cook, Martin Beasley

Abstract

Objective—To review the neonatal screening programme during 1984-8.

Design—Analysis of data from screening laboratories and paediatricians.

Subjects—All live births in United Kingdom.

Main outcome measures—Structure of programme; number of infants tested and number with phenylketonuria; number of infants missed; ages at testing and treatment.

Results—The proportion of infants tested approached 100%. The incidence of phenylketonuria was 11.7/100 000 births (445 subjects): 273 had classic phenylketonuria and three had defects of cofactor metabolism. One child with phenylketonuria was known to have been missed compared with three in 1979-83 and six in 1974-8. Seven subjects had been missed over the 15 years due to negative test results. All seven had been tested with the bacterial inhibition assay, although only 53% of infants had been so tested; the difference between the expected and observed proportion was significant (Fisher's exact test, $p=0.017$). Eleven infants with classic phenylketonuria were not tested by 14 days of age and 23 (8%) did not start treatment until after 20 days, an improvement compared with 36 (15%) in 1979-83. There were, however, wide regional variations (0% to 27% treated after 20 days).

Conclusion—The screening programme achieves high coverage and effectiveness, although some children are still missed. A national practice for screening may help reduce regional variations.

Introduction

About one in 10 000 children born in the United Kingdom has phenylketonuria, defined here as blood phenylalanine concentrations persistently greater than 240 $\mu\text{mol/l}$.^{1,2} Most such infants are either homozygous or doubly heterozygous for one of the many mutations in the gene for phenylalanine hydroxylase³; a small proportion have mutations affecting the metabolism of the hydroxylase cofactor, tetrahydrobiopterin.^{1,4} The phenylketonuria register has reviewed the United Kingdom screening programme since 1964; methods of data collection have been described.^{1,2} We have reviewed the programme's performance during 1984-8

and compared it with the performance in 1974-8 and 1979-83.

Methods

We reviewed data from all laboratories testing newborn infants for phenylketonuria in the United Kingdom. The numbers of infants tested were compared with the numbers of live births, obtained from Office of Population Censuses and Surveys data. Regions have been identified by letters in the results section to maintain anonymity.

Results

In 1988, 26 laboratories measured phenylalanine for the screening programme, testing between 3000 and 100 000 infants a year. In Scotland, Northern Ireland, Wales, and eight English regions one laboratory received blood specimens from the whole region. One laboratory served two regions apart from one district, which sent most specimens to an adjoining region. Two regions were served by two laboratories (in one region soon to become one), one region was served by three laboratories, and one by seven, including the laboratory in a neighbouring region. Infants born in the United Kingdom (or abroad) to members of HM Forces were tested at the Royal Army Medical College.

Nine laboratories (covering 43% of infants tested) used a bacterial inhibition assay to measure phenylalanine, six used fluorimetry (23% of infants), and 11 used either thin layer or paper chromatography (34% of infants). Before screening for congenital hypothyroidism was introduced in 1982,^{5,6} 23 out of the then 37 laboratories used bacterial assay. A positive result, indicating the need for further investigation, was usually taken as phenylalanine concentration $>240 \mu\text{mol/l}$, but two laboratories used thresholds of 200 $\mu\text{mol/l}$, two 250 $\mu\text{mol/l}$, one 300 $\mu\text{mol/l}$, and one 480 $\mu\text{mol/l}$. All laboratories participated in a national external quality assurance scheme.⁷ Routine secondary testing for bipterin defects in infants with phenylketonuria began in 1978,^{1,8} and a supraregional assay service based on dried blood spots was introduced in 1985 for measuring dihydropteridine reductase and total bipterin concentrations.⁹

COVERAGE OF PROGRAMME

Estimates of the numbers of infants tested approached or exceeded 100% of live births (table I). The excess of infants tested over live births was accounted for by the varying degrees of difficulty which laboratories had in distinguishing repeat tests from tests in new infants. These results were similar to those recorded in 1979-83 and better than those recorded in 1974-8,² when a shortfall of 1-3% was recorded most years.

During 1984-8, 445 infants were born with persistent phenylketonuria, an incidence of 11.7/100 000 live

Medical Research Council/
Department of Health
Phenylketonuria Register,
Department of Child
Health, (Institute of Child
Health, London)
WC1N 1EH

Isabel Smith, FRCP,
honorary senior lecturer
Beverley Cook, BSC, research
assistant
Martin Beasley, BSC,
computer programmer

Correspondence to:
Dr Smith.

BMJ 1991;303:333-5

TABLE I—Differences in numbers of infants reported as tested for phenylketonuria and number of live births, 1984-8

Region	Difference (% not screened)				
	1984	1985	1986	1987	1988
England	-3262 (0.5)	1250*	2306*	1612*	521*
Wales	-763 (2.1)	1051*	120 (0.3)	-712 (1.9)	156*
Scotland	737*	-833 (1.2)	375*	963*	763*
Northern Ireland	-98 (0.3)	-40 (0.1)	-150 (0.5)	95*	-262 (0.9)

*Estimates of infants tested exceeded live births owing to inclusion of repeat tests.

births (table II). The infants were classified into three groups according to diagnostic phenylalanine concentrations—classic >1200 $\mu\text{mol/l}$, possibly atypical 900–1200 $\mu\text{mol/l}$, or atypical <900 $\mu\text{mol/l}$. Most infants had classic phenylketonuria. As in previous years the incidence of all forms of phenylketonuria in Northern Ireland was consistently double that in the rest of the country.

INFANTS MISSED BY THE SCREENING PROGRAMME

Only one infant with phenylketonuria was known to have been missed during 1984–8 compared with three in 1979–83 and six in 1974–8. Table III gives details of all the cases missed over 15 years; since a previous report the number of known missed cases during 1974–8 had risen from five to six.² Seven children were missed because of a negative test result, all of whom had been tested with the bacterial assay, although only 633 of the 1195 children with phenylketonuria had been tested by this method. The difference between the actual and expected proportions was significant (Fisher's exact test, $p=0.017$).

Three of the infants with negative test results, including the child missed during 1984–8, had mild accumulation of phenylalanine at diagnosis (400–720 $\mu\text{mol/l}$), suggesting that phenylalanine concentrations in the neonatal period may have been close to normal. This was confirmed in one infant, the most recent, whose original Guthrie card gave a phenylalanine result of <240 $\mu\text{mol/l}$ when retested by thin layer chromatography 14 months later.

TABLE II—Number of infants born with classic, possibly atypical, and atypical phenylketonuria 1984–8

Region	No of births	No with phenylketonuria			All forms	Incidence/ 100 000 births
		Classic (phenylalanine >1200 $\mu\text{mol/l}$)	Possibly atypical (phenylalanine 900–1200 $\mu\text{mol/l}$)	Atypical (phenylalanine <900 $\mu\text{mol/l}$)		
England	3 141 176	210	59	77	346	10.2
Wales	186 310	13	2	2	17	9.9
Scotland	330 047	32	12	8	52	12.7
Northern Ireland	139 112	18	4	8	30	24.9
All United Kingdom	3 796 645	273	77	95	445	11.0
Incidence/100 000 births		7.2	2.0	2.5	11.7	

TABLE III—Details of cases of hyperphenylalaninaemia known to have been missed in screening programme 1974–88

Year of birth	No (%) cases missed	How missed	Mode of diagnosis
1974–8	6 (1.6)	4 Negative test result	2 Developmental delay 1 Tested when sibling detected on screening 1 Tested at adoption
		1 Specimen lost	Developmental delay
		1 No specimen	Tested at 5 months
1979–83	3 (0.8)	2 Negative test result	1 Developmental delay 1 Diarrhoea, failure to thrive, neurological abnormality*
		1 Specimen lost	Detected when sibling screened
1984–8	1 (0.2)	1 Negative test result	Developmental delay*

*Infant had cofactor defect.

TABLE IV—Characteristics and outcome of infants born with phenylketonuria due to defective cofactor metabolism, 1974–88

Case No	Year of birth	Result of routine test	Enzyme deficiency	Age at diagnosis (months)	Treatment	Outcome
1	1980	Not done	Dihydropteridine reductase	14	Yes	Death aged 33 months
2	1981	Positive	Dihydropteridine reductase	3	Yes	Mild handicap ^a
3	1981	Not done	Synthesis defect	7	Yes	Death aged 7 months
4	1982	Not done	Dihydropteridine reductase	48	Partial	Moderate handicap ^b
5*	1983	Not done	Synthesis defect	4	No	Death aged 4 months ^a
6	1985	Positive	Dihydropteridine reductase	4	Yes	Normal
7*	1986	Not done	Dihydropteridine reductase	14	Yes	Moderate handicap
8†	1987	Positive	Synthesis defect	7	Yes	Moderate handicap

*Child missed on screening.

†Diagnostic investigation delayed due to family problems and apparently normal development.

CHILDREN WITH DEFECTIVE COFACTOR METABOLISM

Three children known to have cofactor defects were identified during 1984–8 compared with five during 1979–83 and none during 1974–8 (table IV). During 1979–88 the prevalence of cofactor defects was 1% in infants with phenylketonuria. Details of three children have been published.^{1,8,10} Another child with a fatal neurological illness was also reported to have a possible (but unproved) cofactor abnormality,¹¹ but a healthy sibling with phenylketonuria was born subsequently, making it unlikely that the proband had a cofactor defect.

Two of the 10 children missed in the neonatal screening programme had defects of cofactor metabolism, including the infant missed between 1984 and 1988. Of the six who were detected by screening, only three had been routinely tested to rule out a cofactor defect. The others presented because of symptoms (delayed development, feeding problems, hypotonia, hypokinesia, myoclonic epilepsy). Three died: two rather suddenly after phenylketonuria was diagnosed and one after a more prolonged period of neurological deterioration.

AGE AT DIAGNOSIS AND START OF TREATMENT

Table V shows the numbers of infants with different forms of phenylketonuria detected by screening, those treated, and the proportion treated before 21 days of age. In 1984–8, 251 (92%) infants with classic and 67 (91%) with possibly atypical disease had started treatment by 20 days of age. Only 15 (58%) infants with atypical disease had started treatment by 20 days; these infants had serial monitoring of blood phenylalanine concentrations before treatment was decided on. In all forms of the disorder the proportions receiving treatment by 20 days had increased compared with previous years.^{2,5}

REGIONAL VARIATIONS

Table IV summarises the regional differences with respect to the timing of the initial screening test and the start of treatment. Only infants with classic phenylketonuria born on the British mainland and not of a service family have been included in this comparison so that differences in regional policy with respect to atypical forms of phenylketonuria and problems of geography would not influence the results.

The median age at first test was 6–7 days in most regions, but in three regions where health visitors rather than midwives collected the blood (regions F, G, and L) median ages at first test were 10–11 days. Eleven of the infants with classic phenylketonuria (2%) were first tested after 14 days of age, five of whom were from region L. Median age at the start of treatment ranged from 9.5 to 17 days with intervals between screening test and treatment ranging from three to 10.5 days. Twenty three infants started treatment after 20 days, with regional proportions varying from none in six regions to over a quarter in region L. Late collection of blood (after 14 days) was the main cause of delay in seven infants, an unusually long interval (more than seven days) between initial test and treatment in nine and a combination of both factors in seven.

Discussion

Our review suggests that the neonatal screening programme is generally working according to the Department of Health's recommendation (that infants are tested between the 6th and 14th days after birth). Laboratories are also recommended to report negative as well as positive results and district staff to check against the district birth register to confirm that a result is received on every infant.^{2,7} The success of the

TABLE V—Number of infants detected by screening who received treatment and who started a low phenylalanine diet aged 20 days or less according to severity of phenylketonuria

	Classic			Possibly atypical			Atypical		
	No of infants	No treated	No (%) treated aged ≤20 days	No of infants	No treated	No (%) treated aged ≤20 days	No of infants	No treated	No (%) treated aged ≤20 days
1974-8	266	266	210 (79)	54	51	23 (45)	51	11	4 (36)
1979-83	240	240	204 (85)	60	60	51 (85)	71	19	6 (32)
1984-8	273	273	250 (92)	77	74	67 (91)	95	26	15 (58)

TABLE VI—Regional comparisons in treated infants with classic phenylketonuria born 1984-8

Region	No of infants	Method of test	Age at first test (days)	Age at start of treatment (days)		
				Median	Range	No (%) aged >20
A	32	Bacterial	6.5	14.5	7-28	4 (12)
B	18	Bacterial	7	13	7-28	2 (11)
C	13	Thin layer chromatography	7	13	9-34	3 (23)
D	9	Fluorimetry	6	13	7-16	
E	8	Fluorimetry*	8	14	6-29	1 (13)
F	25	Paper chromatography	11	15	11-22	1 (4)
G	18	Thin layer chromatography	10	13	6-17	
H	18	Bacterial*	6	13	7-55	1 (6)
I	22	Fluorimetry	6	9.5	7-17	
J	9	Thin layer chromatography	7	13	9-21	1 (11)
K	18	Bacterial	6.5	12.5	8-17	
L	17	Fluorimetry*	11	17	8-47	5 (27)
M	5	Paper chromatography	7	12	10-19	
N	19	Thin layer chromatography	7	12	7-47	3 (16)
O	8	Bacterial	6.5	16.5	6-18	
P	16	Bacterial	7	14	7-21	1 (6)
Q	14	Bacterial	6.5	14.5	7-24	1 (7)

*Method of largest laboratory in region when more than one.

screening programme depends on the professionalism and diligence of midwives and health visitors.

In a recent survey in an inner city district of London, in which an attempt was made to match laboratory screening reports to the district register of births,¹² coverage was estimated to be only 93.5%. Lack of a suitable record system to fully assess coverage is likely to have contributed to this low figure. Between 1984 and 1988 we found no infants who had been missed because they had not been tested, suggesting that coverage is much higher over the country as a whole. Nevertheless, we were unable to obtain absolute figures for coverage because repeat tests could not always be identified. However, assuming that the rate of repeat tests was around 1% (a higher proportion than occurred in most laboratories) and that at least half of these repeat tests had been identified, the data suggest that by 1984-8 coverage approached, but probably did not reach, 100% of infants surviving the first week.

The frequency of false negative test results also seems to have fallen to a low level. Results during 1974-88 suggest that the bacterial assay is significantly more likely than chemical methods to lead to false negative results. This conclusion is strengthened by the results of a survey, in 1969-73, which recorded false negative results in three children tested by the bacterial method.² However, another case has been reported as missed in 1990 after routine testing using fluorimetry.

The genetic heterogeneity of infants with phenylalanine hydroxylase deficiency³ is well illustrated by our survey. Although infants with defective cofactor metabolism account for only around 1% of affected subjects, it is simple to arrange routine testing for such defects,⁹ and this should be carried out in all infants with phenylketonuria. Another four infants with cofactor defects were born in 1989 and 1990, and two in 1991. In all the defect was diagnosed by routine testing

after a positive neonatal screening result. No false negative results on cofactor testing have been identified.

Although most infants in our survey had been tested by 14 days of age and had started treatment by 20 days, there was considerable regional variation. After other factors are allowed for (for example, severity of the disorder and quality of phenylalanine control) intelligence quotient (IQ) falls linearly by 1 point for each week's delay in starting treatment.¹³ Hence every effort should be made to organise screening so that, within the limits imposed by the need to make a secure diagnosis, treatment starts as soon as possible.

The recommended timing for screening in the United Kingdom is later than that in most other countries. Though this has the advantage of achieving better discrimination between normal infants and those with phenylketonuria,¹⁴ a reduction in the age of testing to 5-8 days seems to have few disadvantages (except the temporary inconvenience which any change brings) and ought to be considered.

Finally, we would suggest that district health authorities, with only 3-4000 births a year, are not in the best position to decide policy for neonatal screening, though they have an essential role in seeing that the service is implemented. Small laboratories testing fewer than 20 000 infants a year are unlikely to give the best "cost per test" or to maintain adequate experience of screening events. To ensure that the best features of the present programme are fostered and that national neonatal screening policy is appropriately updated, we suggest that a supraregional, multidisciplinary working group should be established by the Department of Health.

We thank all the screening laboratory staff and paediatricians who provided the data for this review. We also thank Ms Sarah Tillotson for statistical advice, Dr Rodney Pollitt for advice on the manuscript, and the Medical Research Council and Department of Health for financial support.

- Smith I. The hyperphenylalaninaemias. In: Lloyd JK, Scriver CR, eds. *Genetic and metabolic disease. Butterworth's international medical reviews. Paediatrics* 5. London: Butterworth, 1985:166-209.
- Medical Research Council Steering Committee for the MRC/DHSS Phenylketonuria Register. Routine neonatal screening for phenylketonuria in the United Kingdom, 1964-78. *BMJ* 1981;282:1680-4.
- Okano Y, Eissensmith RC, Guttler F, Lichter-Konecki U, Konecki DS, Trefz FK, et al. Molecular basis of the phenotypic heterogeneity in phenylketonuria. *N Engl J Med* 1991;324:1232-8.
- Smith I. Disorders of tetrahydrobiopterin metabolism. In: Fernandez J, Saudubray J-M, eds. *Inborn metabolic diseases*. Berlin: de Gruyter, 1990:183-97.
- Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales, and Northern Ireland, 1982-84. *BMJ* 1988;296:1355-8.
- Department of Health. *Screening for early detection of congenital hypothyroidism*. London: HMSO, 1981. (HM(81)20.)
- Whitehead TP, Woodford FP. External quality assessment of clinical laboratories in the United Kingdom. *J Clin Pathol* 1981;34:947-57.
- Smith I, Leeming RJ, Cavannagh N, Hyland K. Neurological aspects of biopterin metabolism. *Arch Dis Child* 1986;61:130-7.
- Leeming RJ, Barford PA, Blair JA, Smith I. Blood spots on Guthrie cards can be used for inherited tetrahydrobiopterin deficiency screening in hyperphenylalaninaemic infants. *Arch Dis Child* 1984;59:58-61.
- Greeves LG, Leeming RJ, Hyland K, Dempsey SI, Carson DJ. Neuroblastoma in a patient with dihydropteridine reductase deficiency. *Eur J Pediatr* 1990;149:713-5.
- Westwood AS, Barr DG. Phenylketonuria with a progressive neurological disorder not responsive to tetrahydrobiopterin. *Eur J Pediatr* 1982;71:859-61.
- Hunter R, Roderick P, Ruta D, Stuz-Steiger T. "Heal to heal"—screening of newborn in Riverside health district by Guthrie testing (MSc report). London: London School of Hygiene, 1988.
- Smith I, Beasley MG, Aides AE. Intelligence and quality of dietary treatment in phenylketonuria (PKU). *Arch Dis Child* 1990;65:472-8.
- McCabe ERB, Mosher GA, McCabe L, Allen RJ, Berman JL. Newborn screening for phenylketonuria: predictive validity as a function of age. *Pediatrics* 1983;72:390-8.

(Accepted 30 May 1991)