# Phenylketonuria due to phenylalanine hydroxylase deficiency: an unfolding story

Medical Research Council Working Party on Phenylketonuria

**Efficient neonatal screening for phenylketonuria and the availability of complex diets for lifelong use have virtually eliminated severe mental handicap from the disease. Nevertheless, there remains a high risk of fetal damage in offspring of women with the disease, and the possibility that the diets themselves may be harmful cannot be excluded. Search for a preventive treatment for the disease has been greatly aided by advances in molecular genetics. For example, in mice modified liver cells have been implanted, which have not only corrected the phenylalanine defect but have remained healthy for the normal life span of the animal. Overall, however, prevention and treatment have not progressed as quickly as was hoped, and research and development must be pursued vigorously to take account of contemporary perceptions of the disorder.**

Phenylketonuria (persistent hyperphenylalaninaemia >240 μmol/l, relative tyrosine deficiency, and excretion of an excess of phenylketones) occurs in approximately one in 10 000 births in the United Kingdom.[1,2] Except for 1–2% of subjects with defective metabolism of tetrahydrobiopterin, these infants have a recessively inherited deficiency in the hepatic enzyme phenylalanine hydroxylase. The virtual disappearance of children with severe mental handicap from this cause is the result of highly efficient neonatal screening and early treatment with a diet low in phenylalanine. Such a diet is complex and depends on the use of manufactured substitutes for many natural foods (meat, fish, eggs, nuts, dairy products, bread, cakes) (see figure). This makes the diet difficult to sustain over long periods and management requires the services of a specialist metabolic team. As the first generation of well managed subjects reaches adulthood there is anxiety about their neurological progress, both in early childhood and later, and about the effects of maternal phenylketonuria on the next generation. Alongside the clinical concerns there has been significant progress in our understanding of the molecular genetics. This paper reviews the clinical implications of some recent work and considers future issues for health service research and development with special reference to the United Kingdom.

## Phenotypic and genetic variation

It has long been known that phenylalanine hydroxylase deficiency exhibits a wide and continuous range of clinical and biochemical severity, varying from a symptomless disorder with phenylalanine accumulation only just greater than in obligate heterozygotes to a severely handicapping condition with plasma phenylalanine concentrations over 20 times normal. The enzyme deficiency varies from absence of detectable activity to a residual activity of up to 25% or more.[3] Recent genetic studies have provided an explanation for this variation. Over 40 different mutations of the phenylalanine hydroxylase gene have been identified.[3] A high proportion of affected subjects are therefore compound heterozygotes rather than homozygotes, although particular mutations may occur with a frequency of over 60% in certain populations[4] and there may be close association with particular haplotypes,[5] indicating that founder effect has had an important influence on the distribution of mutations across nations.

The gene for phenylalanine hydroxylase, which is on chromosome 12, contains 13 exons and messenger RNA is not readily available. A screen of coding regions for mutations still presents a considerable challenge even with the much improved technology made available by the polymerase chain reaction, and it is possible that very many different mutations remain to be discovered. Association between genotype and phenotype has been observed and is supported by expression studies.[6] By using virus vectors phenylalanine hydroxylase genes carrying eight different mutations have each been inserted into a hepatoma cell line without natural hydroxylase activity. The enzyme activity of each modified cell line was assayed and the results used to calculate the expected enzyme activity resulting from the various combinations of mutations in vivo. In subjects carrying the various gene combinations estimated enzyme activity showed a remarkably good correlation with previously determined measures of biochemical severity (diagnostic phenylalanine concentrations, phenylalanine tolerance during treatment, and response to a standard protein load).

These advances in molecular genetics mean that by using a combination of haplotype and mutation analysis prenatal diagnosis is now possible in the great majority of couples who already have a child with phenylketonuria, although this has been undertaken infrequently because paediatricians and parents see early treated children as "healthy." Carrier testing is also practicable in close relatives but—except in communities with a high frequency of particular mutations[4]—carrier testing will not be practicable in the general population, including the unrelated spouses of known carriers, until it is possible cheaply to scan the hydroxylase gene for the many known mutations.

The therapeutic potential of these genetic advances has been indicated by recent gene transfer experiments by Woo in an inbred strain of mouse with phenylalanine hydroxylase deficiency (S L C Woo, paper delivered at annual meeting of European Metabolic Group (Milupa) Copenhagen, May 1991). Using virus vectors, Woo successfully inserted normal mouse genes for phenylalanine hydroxylase into cultured liver cells from the mutant mouse. These modified liver cells were then reimplanted by injection via the spleen. The injected cells remained healthy within the liver parenchyma and corrected the phenylalanine defect for the normal life span of the mouse (even though the cells made up only a small percentage of liver cells).

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## Neuropsychological impairment in early treated subjects

Untreated phenylketonuria is, above all, a disorder of the central nervous system in which varying degrees of delayed development and other signs of serious brain disease, such as epilepsy, become apparent in early infancy.[2] The benefits of early treatment in ameliorating the clinical impact of the disease are well established. Nevertheless, early treated subjects as a group exhibit various detectable abnormalities (box 1). Children and adults have mean intelligence quotients (IQs) roughly half a standard deviation lower than those of unaffected siblings[7] and population norms.[8] The full clinical relevance of this reduction in IQ has been appreciated only recently[8] following the reports of a steady rise of mean IQ scores in the general populations of Western nations,[9] including the United Kingdom.[10]

Reviewed in the light of these population shifts, the findings in subjects with phenylketonuria suggest that a high proportion of early treated subjects—not just a poorly managed minority—exhibit some degree of intellectual impairment and that the major impact is in early childhood before there is any question of relaxing or stopping treatment. Interestingly, virtually all published studies from around the world have reported similar findings. There is also evidence of slower acquisition of language[11] and, in the school years, a higher frequency of learning difficulties[7 12] and behavioural disturbance—hyperactivity, anxiety, and poor concentration being prominent features.[13] Measurement of specific abilities in children and young adults have shown slowed response times, deficits in sustained attention, and impaired problem solving ability.[14-17]

Although these impairments must be of concern, we emphasise that most early treated children fall within the broad normal range of general ability and attend ordinary schools. However, abnormal neurological signs (notably unusually brisk tendon reflexes and tremor) are common in older subjects,[2] and overt neurological deterioration has been reported in a few early treated young adults.[18 19] Upper motor neurone disturbance rather than intellectual decline was a prominent feature, and magnetic resonance imaging disclosed abnormalities in the subcortical white matter.[19] Neurological deterioration in adulthood has also been recorded in some late and untreated subjects,[19 20] and in a few in whom a neuropathological report was available the findings suggested active and diffuse demyelination as well as hypomyelination and reduced brain size.[20]

Rather similar, though usually less severe, magnetic resonance imaging changes than those recorded in association with neurological deterioration have also been observed in symptomless subjects.[21 22] Of 26 clinic subjects aged 8 to 30 years studied in London,[21] none had unequivocally normal appearances on magnetic resonance imaging, indicating that white matter changes are very much commoner than overt neurological disease. The likelihood is that the changes on magnetic resonance imaging are a marker of abnormalities in myelin (such as vacuolation, expanded extracellular spaces, oedema, or demyelination). Recent reports suggest that oedema may be an important component of the changes.[22]

**Box 1**

**Neuropsychological impairments in early treated phenylketonuria***

*Children on strict diet*

Reduction in average intellectual ability (IQ > 2 SD below population mean in 8-10% of subjects compared with expected 2%)

Delayed acquisition of speech

Increased frequency of behavioural problems

Reduced educational progress

Slowed response times and impaired executive function on neuropsychological testing

*Older subjects on normal or near normal diet*

Increased frequency of electroencephalographic and visual evoked potential abnormalities

Changes in cortical white matter on magnetic resonance imaging

Abnormal neurological signs (unusually brisk tendon jerks and tremor); overt neurological deterioration in a few subjects

*Severity related to quality of control of blood phenylalanine concentrations.

## Relation between neurological problems and hyperphenylalaninaemia

The degree of intellectual impairment occurring in early treated preschool children is closely associated with the degree of hyperphenylalaninaemia to which the subjects have been exposed.[8 23] In a large British study intelligence decreased linearly and independently with increasing age at the start of treatment and poorer average control of blood phenylalanine concentrations.[8] Longer periods of unusually low phenylalanine concentrations were also independently associated with worse outcome. Relaxation or withdrawal of treatment before mid-childhood has been associated with a further decline in intellectual ability.[24-27] Other studies have shown worse school progress in children who stopped diet at 6 years of age compared with children who continued treatment.[28] Transient changes in neuropsychological function have also been shown to occur in response to short term changes in plasma phenylalanine concentrations.[29 30] In the magnetic resonance imaging studies[21] subjects who showed the more severe white matter changes had worse recent phenylalanine control and were more likely to have been on a normal diet without protein substitute for longer (A J Thompson, I Smith, D P Brenton, personal communication). Phenylalanine control in early childhood seemed to play little part.

No evidence for a threshold of phenylalanine effects on intelligence or behaviour emerged from the analysis of United Kingdom data.[8 13 27] Subjects with milder phenylketonuria did consistently better than those with a more severe disorder, but this advantage disappeared when allowance was made for their better phenylalanine control. The view that a "threshold of effect" exists for the effects of phenylketonuria on brain development is based largely on the apparently normal intelligence of subjects with the mildest forms of the disorder (so called "benign" hyperphenylalaninaemia), whose plasma phenylalanine concentrations are < 1000 μmol/l (normal 50-120 μmol/l) and who do not usually receive treatment. However, some IQ data for this group of subjects are available,[31] and when these are re-examined in the light of revised population IQ norms[9] a similar reduction in mean IQ is observed to that seen in early treated subjects. An association between the degree of phenylalanine accumulation and mean IQ is also apparent—more severe form, mean IQ 98; milder form, mean IQ 103 (expected population mean IQ approximately 110).

Despite the weight of evidence linking the severity of impairment with the degree of hyperphenylalaninaemia there remain some difficulties in interpreting the data. For example, a recent German study,[32] which aimed at controlling blood phenylalanine concentrations between 60 and 300 μmol/l, produced IQ results no better than those in British subjects, in whom the

aim was more relaxed control (180-600 μmol/l), though the blood phenylalanine value was on average 100 μmol/l lower in the German subjects. We do not know whether peaks (and troughs) in phenylalanine control are more (or less) harmful than persistent but moderate hyperphenylalaninaemia. Nor is it certain that the reported associations between long term outcome and exposure to phenylalanine accumulation are entirely "cause and effect." The possibility that some neurological damage may occur before delivery and be more pronounced in infants with classic than atypical disease (and therefore be associated with rather than caused by worse phenylalanine control) cannot be completely discounted. Nor can we discount the possibility that some factor in the diet itself has harmful effects.

Nevertheless, it seems highly probable that the persisting biochemical abnormalities (phenylalanine excess or some closely related change) at least make a substantial contribution to the neurological impairment. Taken together these data link neurological outcome in early treated subjects closely to the quality of phenylalanine control throughout life. This conclusion has important implications for the management of affected subjects, and guidelines on treatment need to be revised in the light of these recent findings.

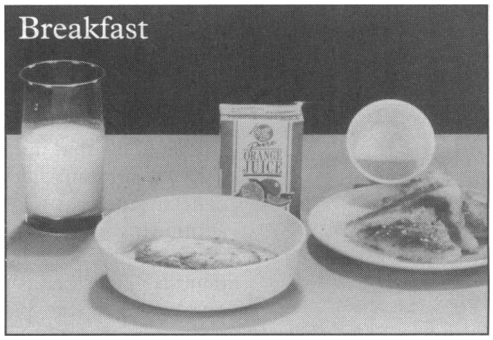
## Maternal phenylketonuria

There is a high risk of fetal damage in the offspring of women with phenylketonuria. In classic phenylketonuria microcephaly, mental handicap, and impaired fetal growth are likely to occur in over 80% of subjects, accompanied by malformations in the heart or other organs in at least 20%.[33] Fortunately dietary intervention from before conception seems to have a favourable influence on outcome, which seems to be closely associated with the degree of hyperphenylalaninaemia very early in gestation.[34,35] Congenital anomalies are uncommon in the offspring of women with phenylalanine concentrations below 900 μmol/l at the time of conception.[33-36] However, head size, birth weight,[34,35] and intelligence[36] have been shown to be inversely and linearly associated with maternal phenylalanine concentrations, again without any obvious threshold in effect. The data suggest that optimal outcome for the fetus can be achieved only when maternal phenylalanine concentrations are close to normal from early gestation.

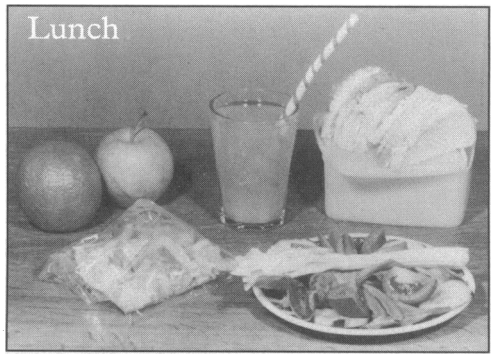
In an ongoing study (A Stewart, D Brenton, personal communication) of 15 infants conceived under dietary control (mean phenylalanine values in first trimester 213-496 μmol/l—twice to four times normal) Griffiths developmental quotients at one year were within the normal range and head circumferences were at or above the 10th centile. However, strict neurological assessment showed that even in these infants minor neurological impairments were detectable, which may indicate a risk of cognitive deficits later. The findings suggest that in the management of pregnant women phenylalanine control needs to be, if anything, even stricter than in children with phenylketonuria.

## Mechanisms of damage

In considering which strategies might be useful in trying to improve outcome in phenylketonuria it would be helpful to understand the mechanisms involved in the damage. Despite the difference in degree there are obvious similarities in the character of the neurological abnormalities in early and late treated children, suggesting common mechanisms.[2] However, the identity of the critical biochemical events leading to cerebral damage, and to damage to the fetus in pregnancy, remains uncertain.




*Breakfast for 6 year old on low phenylalanine diet (8 g natural protein (400 mg phenylalanine) per day.) Whole wheat cereal provides 2 g natural protein (100 mg phenylalanine). Sugar is phenylalanine free. Orange juice and jam provide another 50 mg phenylalanine. Bread and milk substitutes and amino acid supplement (10 g as paste with mineral and vitamins added) are virtually phenylalanine free and are prescribable for phenylketonuria*




*Lunch for 6 year old on low phenylalanine diet (8 g natural protein (400 mg phenylalanine) per day*). Crisps provide 1 g natural protein (50 mg phenylalanine). Salad vegetables, fruit, and spread in sandwiches provide another 50 mg phenylalanine. Bread substitute and amino acid supplement (10 g as orange flavoured drink containing minerals and vitamins) are virtually protein free and prescribable for phenylketonuria*

**8 g Natural protein per day would be too high an intake for young children with most severe types of phenylalanine hydroxylase deficiency, for whom 300 mg per day or less would be needed.*

The ketoacid derivatives of phenylalanine, although potentially harmful, seem unlikely to reach high enough concentrations in the brain to account for neurological damage.[37] Owing to the competitive nature of amino acid transport across the blood-brain barrier and across the placenta, the brain in patients with phenylketonuria and the fetus in women with phenylketonuria are exposed to both high phenylalanine concentrations and low concentrations of the other large neutral amino acids, especially tyrosine.[38] One direct consequence of the amino acid changes is the well recognised reduction in dopamine and serotonin turnover.[2] The scale of the amine abnormality is proportional to the degree of hyperphenylalaninaemia and probably depends on the combined effects of competitive inhibition of tyrosine and tryptophan hydroxylases (by phenylalanine) and a deficiency of the amino acid substrates for these enzymes.

Transient deterioration in neuropsychological function has been shown convincingly during short periods of experimental hyperphenylalaninaemia, and it has been argued that the changes may be due to neurotransmitter deficiency.[29,30] However, this remains speculation and in any case seems an unlikely explanation for microcephaly, hypomyelination, and myelin loss. Inhibition by phenylalanine of certain key reactions in

brain protein synthesis[39] or in the synthesis of myelin sulphatides,[40] or both, seems a more probable cause of myelin damage. Whether common mechanisms underlie damage to the brain and to the fetus is not known.

The above findings suggest there are some additional therapeutic strategies which may be of value in limiting the effects of phenylalanine. Animal[39] and human[38 41] studies show that uptake of phenylalanine by the brain can be substantially inhibited by increased plasma concentrations of other amino acids induced by administration of large doses of amino acids such as tyrosine, tryptophan, or the branched chain compounds. The same is likely to be true of phenylalanine uptake across the placenta. Therefore, the quantity, composition, and diurnal distribution of the amino acid supplements used in treatment may influence the effects of raised plasma phenylalanine concentrations, especially if these are not too far above the normal range. Judicious choice of quantity and timing of amino acid intake could therefore be of benefit. Pharmacological doses of tyrosine, tryptophan, or branched chain compounds may also have a therapeutic role when phenylalanine concentrations are higher.[41] An alternative approach to reducing phenylalanine accumulation is oral treatment with the enzyme phenylalanine ammonia lyase.[42] The aim is to improve dietary tolerance to phenylalanine by inducing breakdown of phenylalanine in the gut before absorption. Both these approaches require further investigation in controlled trials.

## Future research and development

A low phenylalanine diet cannot fully substitute for the fine tuning of phenylalanine turnover normally exerted by hepatic phenylalanine hydroxylase. The dietary control of plasma phenylalanine concentrations requires rigorous restriction of natural protein intake, often to less than 6 g per day. Frequent biochemical monitoring and regular ingestion of unpalatable substitutes for protein, minerals, vitamins, and energy are also needed. It is particularly difficult to maintain smooth phenylalanine control in subjects with severe enzyme deficiency, in whom even a minor feverish illness or fall in energy intake may lead to a rise in phenylalanine concentrations. Aiming at "normal" concentrations runs the risk of inducing phenylalanine deficiency, which several lines of evidence suggest is harmful to both nutrition and brain development.

**Box 2**

**Specialist services for phenylketonuria**

*Metabolic team for screening, diagnosis, counselling, and long term management**

- Biochemical services
- Physician (paediatrician and adult physician)
- Dietitian
- Nurse specialist

*Neurological assessment*

- Neurology
- Neuropsychology
- Electrophysiology
- Magnetic resonance imaging

*Genetic counselling, carrier testing, prenatal diagnosis*

- Clinical geneticist
- Access to molecular genetics service

*Obstetrics*

- Preconception assessment and contraception advice
- Procedures for prenatal diagnosis
- Fetal assessment—size, congenital anomalies

*Best within specialist service for inherited metabolic disease.

**Box 3**

**Role of family doctor and community services**

- Screening process—communication with parents, blood collection, records (tests done, results received), audit of timing, coverage
- Ensure access to specialist services
- Special needs of affected children (where necessary, blood taking, telephone, housing, nursery placement, school, etc)
- Prescribing of special food products
- Promotion of long term dietary treatment and strict diet before conception
- Family planning advice

It is clear that better therapeutic strategies will be needed if we are substantially to improve outcome in subjects with phenylketonuria. Given the difficulties in implementing treatment, the human and financial costs, and the concerns about neurological progress and fetal outcome, phenylketonuria is a potential candidate for gene therapy if this proves "safe." Although the philosophical climate in the United Kingdom is right for such a development and the epidemiological background is well established, much more laboratory work is needed on the molecular genetics, enzyme function, gene transfer to human liver cells, and relevant liver cell biology if patients in the United Kingdom are to benefit from any advances which may occur.

Even if molecular genetics ultimately provides a better form of treatment there is still need to evaluate and, where possible, improve our present dietary management (box 2). Our working party has drawn up a set of revised guidelines on monitoring dietary management.[43] In addition, more information needs to be collected on the neurological status of early treated children from infancy onwards, and programmes of neuropsychological and neurophysiological assessment and brain imaging need to be developed to provide more precise measures of progress in individual subjects. This would enable improved therapeutic strategies to be properly evaluated. It is important that the national epidemiological project (the phenylketonuria register) should continue to monitor the screening programme and follow up adolescents and young adults, as well as women of fertile age and their offspring, so that long term outcome can be documented. A similar recommendation has been made concerning the American collaborative study on phenylketonuria.[44]

## Health service provision for phenylketonuria

The services needed for screening, diagnosis, and long term management of phenylketonuria are highly specialised (box 2). Because of the low incidence of the disorder such services can never be comprehensively and efficiently provided at district level. A regional or supraregional service within a wider service for inherited metabolic disease is likely to provide the most effective approach. Nevertheless, local services play an essential part in the screening programme[1] and in the day to day care of affected subjects (box 3). Many different disciplines need to be aware of the particular needs of such patients and the implications of new findings. In particular, it is important that the reasons for continuing a difficult diet (which includes expensive phenylalanine free protein substitute and other special food products, biochemical monitoring, and experienced dietetic advice) are clearly understood in terms of the neurological risks to the patient. Even more

important is the need for women of fertile age with phenylketonuria to be appropriately counselled and supported in order to ensure that their children are conceived under the best possible phenylalanine control. This requires the use of effective contraception until the woman can maintain a strict diet under home conditions (demonstrated by biochemical monitoring at least twice weekly).

## Conclusions

Outcome in early treated subjects with phenylketonuria is not as good as was thought just a few years ago and is much more closely associated with the quality of blood phenylalanine control at all ages than previously recognised. As in diabetes even the very best treatment fails to achieve perfect metabolic control. Maternal phenylketonuria is an important problem since the advantages of screening in one generation could be lost in the next unless preventive treatment can be implemented in a high proportion of affected women. Continuing research and development is needed for phenylketonuria, and services for children and adults need to be updated to take account of our new perceptions of this disorder.

*Members of the working party were:* Professor F Cockburn (chairman), Mrs B E Barwell, Dr D P Brenton, Dr Jean Chapple, Mrs Brenda Clark, Professor G Curzon, Dr D C Davidson, Dr A F Heeley, Ms Sheena C Laing, Dr I A F Lister-Cheese, Professor I McDonald, Dr Susan Malcolm, Dr R J Pollitt, Dr D Quinn, Dr G Rylance, Dr Isabel Smith (compiler of report), Dr Ann Stewart, Dr R Surtees, Dr A J Thompson, Dr Linda Tyfield, Professor M J Whittle.

Professor C R Scriver served as visiting commentator and Dr Jane Frew as secretary to the committee.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