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An Occipito-temporal Syndrome in Adolescents with Optimally Controlled Hyperphenylalaninaemia

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Summary: The study included 16 adolescents with optimally controlled hyperphenylalaninaemia (McKusick 26160), of whom six did not require treatment according to conventional criteria. All except the two patients with lowest median serum phenylalanine level throughout childhood (most values at 200–300 μmol/L) had white matter abnormalities detectable with magnetic resonance imaging. The lesions were particularly prominent in the watershed regions between the posterior and middle cerebral arteries. In most patients with moderate or severe hyperphenylalaninaemia frontal white matter lesions were present as well. Normal proton magnetic resonance spectra indicated that the lesions were stable. Occipital EEG abnormalities were frequent, and deficient performance on a pattern-recognition test was a characteristic neuropsychological finding. Serum phenylalanine levels at about 300 μmol/L or below throughout childhood and early adolescence may be required to avoid lesions. The present study demonstrates the limitations of even an optimally controlled dietary regimen in hyperphenylalaninaemia.

The advent of magnetic resonance imaging (MRI) has revealed abnormalities of white matter composition in the periventricular regions in patients with phenylketonuria and low intelligence or severe neurological symptoms (Thompson et al 1990; Bick et al 1991). The patients in these studies were characterized by late onset of dietary treatment, if any, and/or early discontinuation of treatment. In a recent brief report, MRI abnormalities were reported in early-treated patients as well (Thompson et al 1991). In the present study we report lesions and brain dysfunction in adolescents with optimally controlled hyperphenylalaninaemia, even in patients not thought to require treatment according to conventional criteria. By 'optimally controlled' hyperphenylalaninaemia we mean (1) achievement of therapeutic levels of serum phenylalanine before 4 weeks of age; (2) regular determination of serum phenylalanine levels (weekly in the first 2 years of life, later at least monthly); (3) median levels of

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serum phenylalanine at about 420 μmol/L or below up to 8 years of age. A gradual increase to around 900 μmol/L at 12–15 years of age has been accepted.

The term 'phenylketonuria' is avoided here. Patients on a diet low in phenylalanine do not have phenylketones in the urine. Furthermore, 'phenylketonuria' stigmatizes patients as less intelligent, in spite of normal intellectual development owing to modern dietary treatment.

SUBJECTS AND METHODS

Sixteen adolescents with hyperphenylalaninaemia in the age range 13.5–20 years (median 15.7) were studied. Clinical neurological and ophthalmological examinations were normal, and the academic or professional careers of these patients were unremarkable. Five had severe hyperphenylalaninaemia (phenylalanine tolerance <25 mg/kg at age 5 years), five had moderate hyperphenylalaninaemia (tolerance between 25 and 70 mg/kg at age 5 years), and six had mild hyperphenylalaninaemia (tolerance >70 mg/kg at age 5 years) (Güttler and Lou 1990). The latter group did not require dietary treatment according to conventional criteria (Güttler 1980) and corresponds to groups designated as persistent or benign HPA by other authors.

Phenylalanine concentrations were determined in serum using microadaptations of the fluorimetric assay system developed by McCaman and Robins (Güttler 1980). Phenylalanine tolerance, determined at the age of 5 years, was expressed in terms of the fractional amount of dietary phenylalanine (in mg per kg body weight per day) that the patients could ingest while serum phenylalanine levels remained below 600 μmol/L (Güttler 1980). For the purpose of detecting hyperphenylalaninaemia mutations, genomic DNA was isolated from leukocytes and regions of the phenylalanine hydroxylase gene containing mutations were amplified by the polymerase chain reaction with use of Taq-polymerase (Okano et al 1991). The amplified samples were immobilized on zeta-probe membranes with a dot–blot manifold, and the presence or absence of mutations was determined through allele-specific oligonucleotide hybridization analyses (Okano et al 1991).

Magnetic resonance imaging was performed at 1.5 T (Siemens Magnetom H-15 or SP 63/84). Sagittal T_1-weighted images were obtained using an inversion recovery sequence (TR = 2.45 s, TE = 28 ms, TI = 400 ms). Axial T_2-weighted images were obtained by a double spin-echo sequence (TR = 1.8 s, TE = 90 ms). The images were evaluated blindly together with the images of five normal siblings of the same range of age serving as controls. MR scans were classified as slight leukoencephalopathia (i.e. hyperintensity only around the occipital horns in T_2 images), prominent leukoencephalopathia (i.e. hyperintensity in T_2 images extending from the occipital horns into the frontoparietal white matter), and normal (no hyperintensity). In two patients, water-suppressed proton magnetic resonance spectroscopy was performed at 1.5 T (Siemens SP 63/84) using the head coil. The STEAM-sequence was used (Frahm et al 1989). Shimming was performed on the whole head as well as on the volume of interest (VOI). Line broadness of the water signal at half maximum was about 4 Hz. A $3 \times 3 \times 3\,cm^3$ volume of interest was selected posterior to the occipital horn, including the lesion but avoiding CSF. Spectra were recorded at echo times of 46, 135, and 270 ms, repetition time 1.5 s, and 256 acquisitions. The FID was multiplied

with a Gaussian function with a half-width of 512 ms. Phase correction was made using the system software. No additional postprocessing or baseline correction was made.

Sixteen-channel EEG was recorded on a Dantec Concerto during rest, hyperventilation, and flash. Visual evoked potential (VEP) was performed using a TV checkerboard pattern-reversal stimulation with a check size of 1.2° and 2.4° changing position every 0.5 s. Potentials were recorded from the occipital region; 200 acquisitions were summarized.

The hyperphenylalaninaemia group was examined with a neuropsychological test battery designed to measure verbal and visuospatial learning and memory, and visuomotor functioning. Verbal learning and retention were measured with two tests: a paired-associate test comprising 15 cards with a nonsense syllable on the front and a consonant on the back, and a free-recall test with six lists containing 21 nouns each. Verbal short-term memory was measured with a digit span test, while visual perception and visual short-term memory were measured with a more difficult version of the Metric Test originally designed by Warrington and James (Lezak 1983). The first part of the test comprises 20 patterns (Figure 1); each pattern is shown for 2 s, and the subject is required to identify the pattern among four possible. In the second part, the stimulus picture is shown for 10 s before the response picture is presented upside-down. Visuomotor performance was measured with three well-known neuropsychological tests: Symbol Digit Modalities Test, Trail A, Trail B (Lezak 1983), and a computerized continuous visual reaction time test in which the subject is required to react to 100 visual stimuli appearing on a monitor at varying intervals from 2 to 6 s. Forty 8-graders (23 girls and 17 boys) from a school in the neighbourhood were tested with the same battery and used as control group (age range 15.3–16.5 years,

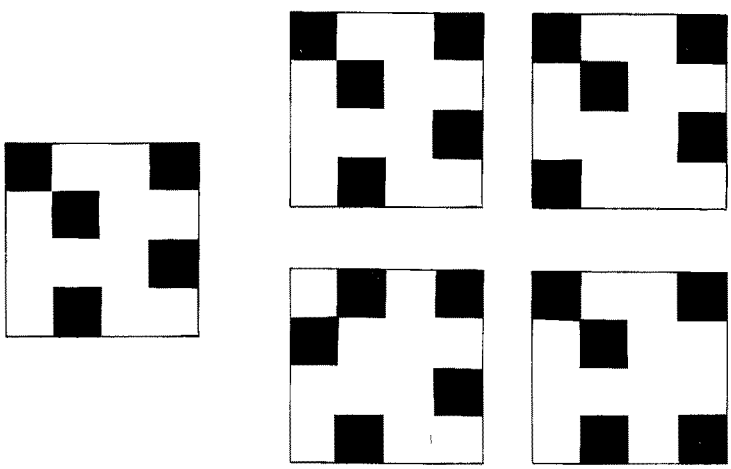

Figure 1 The Metric Figures Test comprises 20 stimulus figures like the above (left). Following a 2-s exposure of each figure, the subject must choose the identical figure from a set of four similar figures (right). On this test the performance of the combined hyperphenylalaninaemia group was significantly inferior to that of the controls

mean age 15.8 years). The mean IQ of the combined hyperphenylalaninaemia groups was 105 (range 82–133) at age 8 years.

RESULTS

A clear correlation was observed between mutation genotype and clinical phenotype. Patients with severe hyperphenylalaninaemia were either homozygous or heterozygous for the IVS-12 and/or the R408W mutations. Patients with moderate hyperphenylalaninaemia were heterozygous for the R261Q or the Y414C mutations. Patients with mild hyperphenylalaninaemia carried the Y414C mutation. Patient no. 3 with serum phenylalanine levels throughout childhood of 200–300 μmol/L and normal MR images is presumably a carrier of the IVS-12 mutation.

In the blind evaluation of MR images all five controls were found to be normal. This was also the case with two patients in the mild hyperphenylalaninaemia group with lowest median values of serum phenylalanine throughout childhood (mainly about 200–300 μmol/L). All other patients had varying degrees of white matter abnormalities. It is noteworthy that the median serum phenylalanine levels in all other patients were in the range of about 300–500 μmol/L for the first 8 years of life (Figure 2). The moderate and severe groups followed an identical trend to the time of examination at about 15 years of age, when they reached serum levels of 700–1000 μmol/L. In contrast, the mild group showed only a slight increase with age (Figure 2). This indicates a threshold for generation of MRI-detectable white matter abnormalities through the first 15 years of life of about 300 μmol/L.

The characteristic pattern found in all pathological images was regions of high signal intensity in the vicinity of the occipital horn (Figure 3), implying altered tissue composition with an excess of water (Zimmermann et al 1986). Examples of more extended lesions were almost exclusively seen in patients with moderate or severe hyperphenylalaninaemia (Table 1). Volumes of interest including altered white matter posterior to the occipital horn were selected for proton MR spectroscopy in two patients, with an example shown in Figure 3. The relative size of the peaks was identical to that seen in normal controls (Figure 4). Lactate could not be identified (Jung et al 1990).

EEG revealed cerebral dysfunction in occipital and occipito-temporal regions in the majority of cases in all three groups, and in several instances paroxysmal activity (Table 1). The background activity was normal in all cases. Visual evoked potentials showed normal latencies and amplitudes in all patients.

The performance of the hyperphenylalaninaemia group was significantly inferior to that of the controls on the first part (Figure 1) of the Metric Figures Test ($p < 0.01$). Regression analysis showed this difference to be significant also when test scores were corrected for mean performance on the total neuropsychological test battery. No significant differences were found for any of the other tests.

DISCUSSION

It is remarkable that white-matter abnormalities were seen in almost all patients. These patients had been under intensive clinical and dietary control throughout

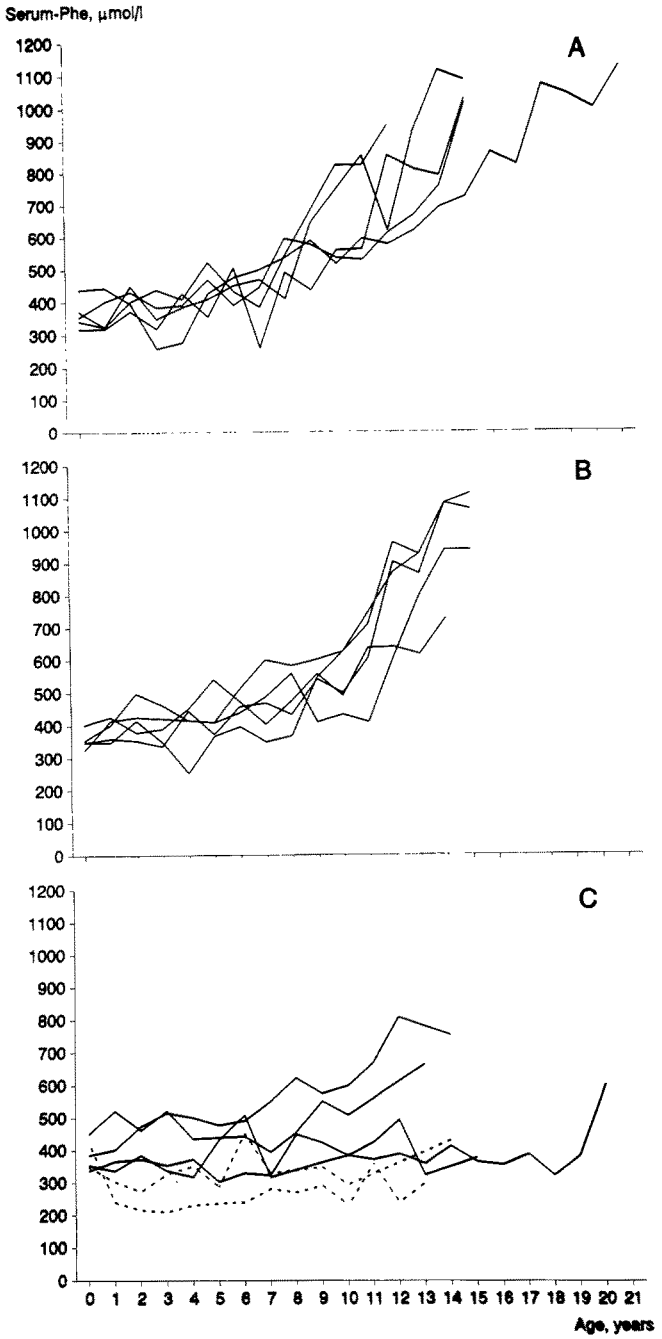

Figure 2 Yearly median serum values of phenylalanine (µmol/L) of (A) the five patients with severe hyperphenylalaninaemia, (B) the five patients with moderate hyperphenylalaninaemia, and (C) the six patients with mild, untreated hyperphenylalaninaemia, until the time of investigation in adolescence. Broken lines indicate the two patients showing normal MR scans

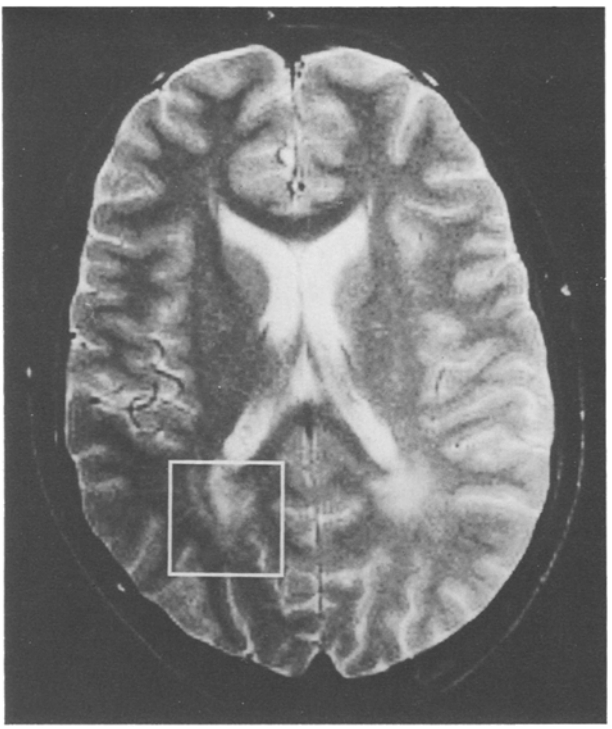

Figure 3 T_2 image of the brain of patient 12, showing the characteristic abnormality with increased signal intensity posterior to the occipital horn. The square indicates the $3 \times 3 \times 3\,cm^3$ volume of interest selected for proton spectroscopy

childhood from the first weeks of their lives. It appears that in order to avoid such lesions serum phenylalanine levels at or below 300 μmol/L should be maintained even through adolescence. This would in most cases necessitate a dietary regimen so rigorous that adverse psychological effect on family life could occur.

What, then, are the functional consequences of these lesions? Our study indicates that they are rather limited. Occipital EEG abnormalities, sometimes with paroxysmal activity, and conceivably an increased seizure susceptibility, are evident and have been previously reported (Pietz et al 1988). But normal visual acquity, visual fields and visual evoked potentials show that myelin function in the optic radiation is not grossly abnormal. More subtle dysfunctions, primarily of complex pattern perception, are detectable, however. This may have implications for professions with high demands on visual perceptual skills, but is not likely to influence daily activities.

The pathogenesis of the lesions is poorly understood. Increased signal intensity is seen in demyelinating diseases, for instance multiple sclerosis or Alexander disease. In these disorders the proton spectrum is, however, clearly abnormal, with a high choline peak indicating an excess of non-membrane-bound choline-containing substances (Grodd et al 1991). Alternatively, increased signal intensity may suggest defective myelin synthesis, as seen for instance in Pelizeus–Merzbacher disease. This

Table 1 MRI abnormalities in spin echo-images occipitally, frontally, and in inversion recovery (IR) images; presence indicated by '+'. EEG abnormalities also shown

Patient	T_2 *Occipital*	T_2 *Frontal*	*IR*	*EEG*
Mild				
1	+	+	+	3–5 Hz Sharp waves bioccipitally
2	+			Normal
3				Normal
4				2.5–4 Hz Spikes bioccipitally
5	+			3–5 Hz Sharp waves bioccipitally
6	+			2–4 Hz Bioccipitally
Moderate				
7	+			Normal
8	+	+	+	3–6 Hz Sharp waves bioccipitally
9	+	+	Not done	Normal
10	+	+	+	2–4 Hz Sharp waves bioccipitally
11	+	+		Normal
Severe				
12	+			2–4 Hz Sharp waves bioccipitally
13	+			3–4 Hz Biocippitally
14	+	+		2.5–5 Hz Tempero-occipitally
15	+	+	+	4–7 Hz Tempero-occipitally
16	+	+	+	Normal

disorder, however, is characterized by a very low choline peak (Krägeloh-Mann, personal communication). The present study is the first report on proton spectroscopy in hyperphenylalaninaemia. The normal proton spectrum indicates that the lesions are not associated with neuronal damage or ongoing myelin degradation, in good agreement with the assumed benign nature of the derangement in the present patient groups. In untreated severe hyperphenylalaninaemia progressive demyelination seems likely as judged by patho-anatomical studies (Malamud 1966). The location of the characteristic lesion in the present study is similar to that reported by other groups (Thompson et al 1990; Bick et al 1991) and may give a clue to the pathogenesis. It is situated at the watershed region between the two longest cerebral arteries, the middle and the posterior. By analogy, for instance, to hypoxic–ischaemic brain lesions (Lou 1989), watershed localization suggests a deficiency of supply through the

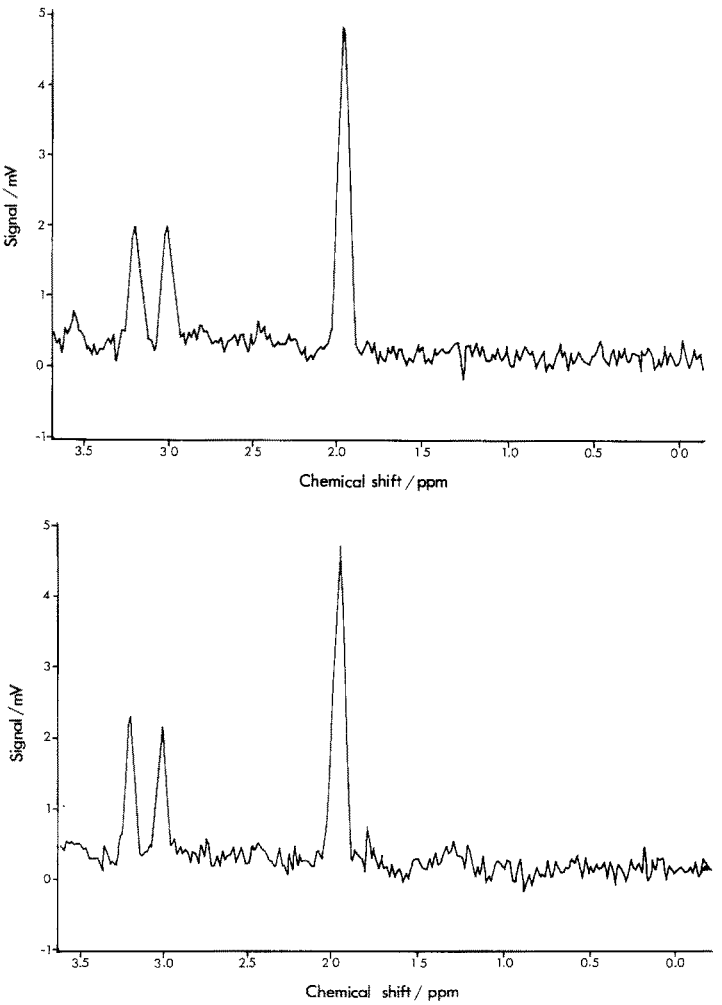

Figure 4 (a) Proton spectroscopy (TR = 1.5 s, TE = 270 ms, 256 acquisitions) of the volume of interest in Figure 3. Choline (3.2 ppm), phosphocreatine/creatine (3.0 ppm), and *N*-acetylaspartate peaks (2.0 ppm) are identified. The spectrum is essentially identical to a control spectrum (b).

arterial system, in this case for instance of essential amino acids. Decreased serum concentrations of essential amino acids is a well-known feature of hyperphenylalaninaemia (Gerdes et al 1991), and the transport across the blood–brain barrier is impaired, at least for other aromatic amino acids such as tryptophan and tyrosine that compete with phenylalanine for the same carrier (Gerdes et al 1991). A lack of essential amino acids is likely to affect membrane formation, possibly leading to increased water content of the white matter. These features are prominent in *post mortem* studies as well (Prensky et al 1968).

The fact that extended white-matter lesions were seen in the majority of patients in the moderate and severe hyperphenylalaninaemia groups and, with one exception, not in the mild group, support the concept of a role of excessive serum phenylalanine levels in pathogenesis, and indicate that the rather abrupt rise in serum levels in the former groups after the age of 8–10 years is critical for the extension of the lesions.

Our results suggest that there are limitations to the effect of dietary treatment in hyperphenylalaninaemia. This kind of therapy is seen to have shortcomings when evaluated with modern techniques; and although the consequences for the individual may be limited, they are detectable. The recent quest for genetic therapy in hyperphenylalaninaemia should also be seen in this light (Woo 1991 and personal communication).

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