Original article

Dihydropyrimidinase Deficiency, a Progressive Neurological Disorder?

C. W. M. Putman[1]
J. J. Rotteveel[1]
R. A. Wevers[2]
A. H. van Gennip[3]
J. A. J. M. Bakkeren[2]
R. A. De Abreu[2]

Departments of [1]Paediatric Neurology, [2]Clinical Chemistry of the Institutes of Neurology and Paediatrics, University Hospital Nijmegen, and [3]Department of Clinical Chemistry, Academic Medical Centre, Amsterdam, The Netherlands

Abstract

A case of a child presenting with congenital abnormalities at birth is reported. The early development remained severely retarded and acquired skills minimally. The head circumference centile decreased. Magnetic resonance imaging showed progressive neuronal atrophy and secondary delay in myelination. Dihydropyrimidine concentrations in body fluids were quantitated by NMR spectroscopy. Enzymatic assay in the liver biopsy revealed total deficiency of dihydropyrimidinase (DHP) (5,6-dihydropyrimidine amidohydrolase; EC 3.5.2.2). As such, the patient is the first with enzymatically proven DHP deficiency. Thus far dihydropyrimidinuria has been reported in three other patients with a variety of neurological abnormalities. A relation of the enzyme deficiency with the neurodegenerative clinical course in our patient is suggested.

Key words: Dihydropyrimidinuria – Dihydropyrimidinase deficiency – 5,6-dihydropyrimidine amidohydrolase – Neurodegenerative disease – NMR spectroscopy

Introduction

Dihydropyrimidinase (5,6-dihydropyrimidine amidohydrolase; EC 3.5.2.2) is the second enzyme involved in the breakdown of the pyrimidine bases uracil and thymine. It catalyses the degradation of dihydrouracil to ß-ureidopropionic acid and dihydrothymine to ß-ureidoisobutyric acid (Fig. 1). The first case of dihydropyrimidinuria in humans has been reported by *Duran* et al in an infant presenting with convulsions (4, 5). Otherwise, the child had a normal development at 19 months. *Henderson* et al subsequently described an infant with DHP deficiency and severe developmental delay (8). In Japan a case with dihydropyrimidinuria was detected within a metabolic screening program in a healthy girl, 6 months of age (9). The suspected enzymatic deficiency of dihydropyrimidinase in these cases has as yet not been confirmed. We present a new case with enzymatically proven DHP-deficiency with severe neurological symptoms. Quantification of the relevant metabolites in urine, plasma and CSF was done by H-NMR spectroscopy.

Case

Clinical presentation

The patient, a girl, was the first child of healthy consanguineous parents (first cousins) from Morocco. The family history did not reveal hereditary neurological disorders. Pregnancy and delivery were unremarkable. On investigation at birth dysmorphic features were noted: plagiocephaly, an anteriorly displaced anus, short perineum and open anovestibular fistula, clubfoot at the right and hip lateralization at the left side, hypoplasia of the end phalanges and nails of the third finger and toe. Furthermore facial dysmorphic features such as coarse face, cupped ears and a broad nasal bridge were noted. Bodyweight at birth was 3590 gram, length was 50 cm. Apgar scores were 7 and 8 at 0 and 5 min, respectively. She had feeding difficulties shortly after birth. Colostomy was carried out because of the anorectal abnormality. Between the age of 3 and 26 months she was hospitalized on several occasions for surgical interventions and for neurological and metabolic evaluation in view of her severe developmental delay and convulsions. No organomegaly was present. Cardiac examination was normal. Length and weight followed the 80th centile. The head circumference decreased slowly in 2 years from the 60th centile to the 30th centile. Mental development was absent. Visual fixation occurred sporadically after the age of one

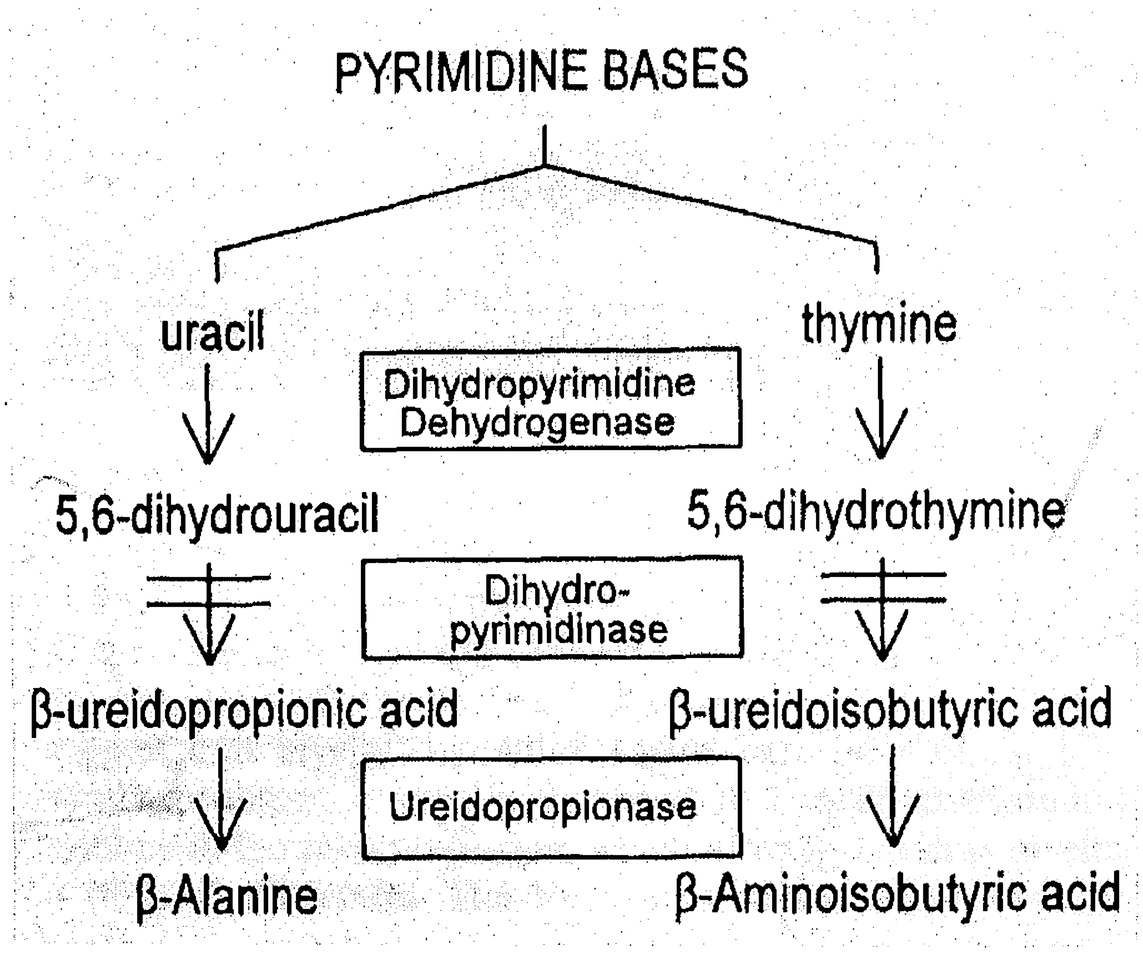

Fig. 1 Scheme of pyrimidine degradative pathways.

Received August 19, 1996; revised, accepted December 30, 1996

Neuropediatrics 28 (1997) 106–110

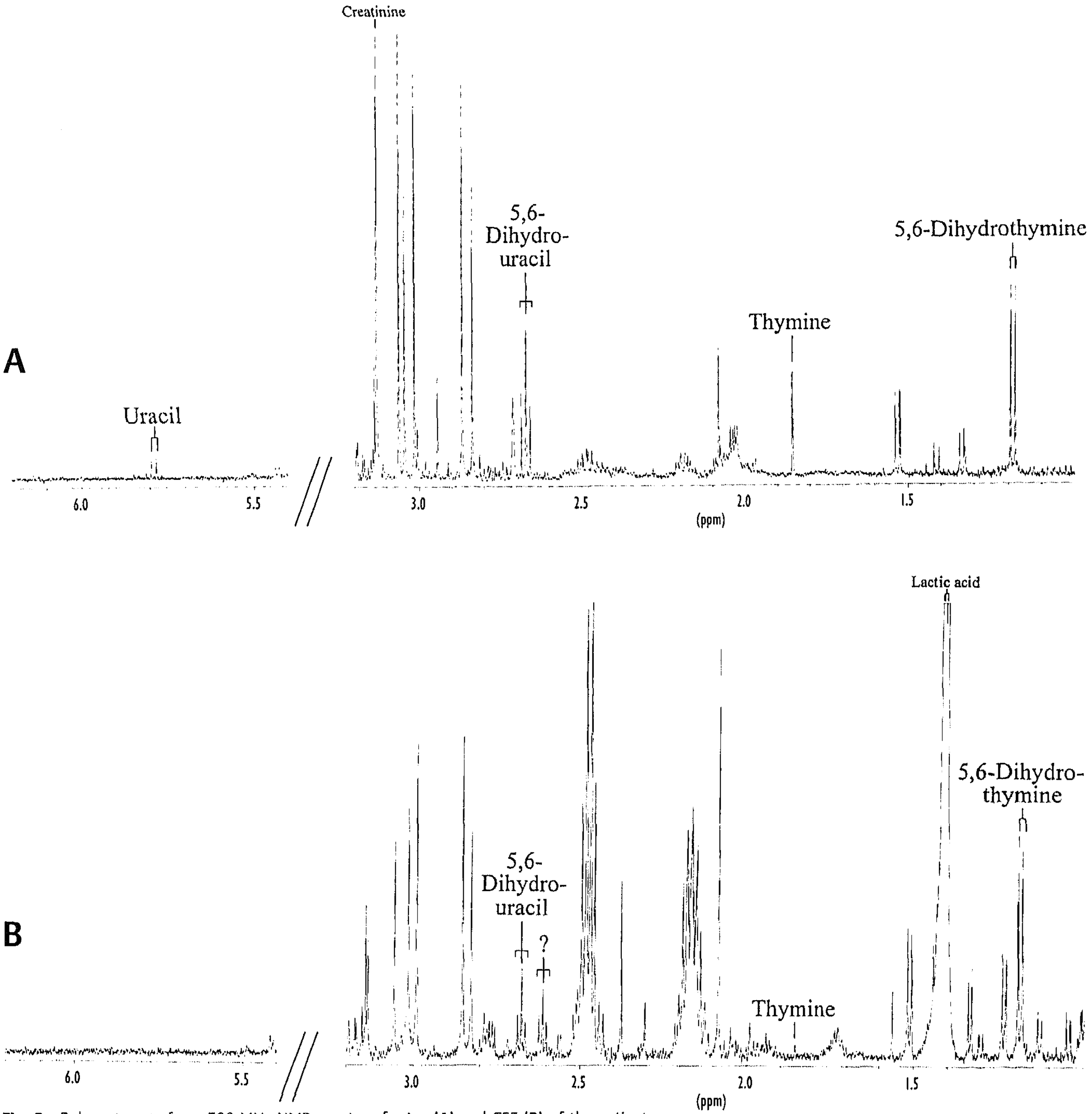

Fig. 2 Relevant parts from 500 MHz NMR spectra of urine **(A)** and CSF **(B)** of the patient.

year. Auditory reactions were absent except for reflex myoclonic reactions after one year. Myoclonic seizures were noted since the age of 3 months. Pupillary hippus was often the only, but persisting epileptic phenomenon. Repeated fundoscopic examination revealed disc pallor suggestive for hypomyelination. Bulbar reflexes were absent or decreased interfering with efficient feeding. The motor performance was predominantly choreatic. Gradually pyramidal signs appeared. The tone was always decreased to severe floppiness resulting in frog position and head-lag.

Additional examinations

Laboratory

Routine biochemical and haematological investigations were normal, except for a persisting and unexplained increase of alkaline phosphatase (700 U/l to 1700 U/l; normal value: <120 U/l), but normal gammaGT, ALAT and ASAT. Chromosomes were 46 XX in blood and fibroblasts. Spinal fluid examination was within normal limits, including amino acids and brain-specific proteins (S-100, myelin basic protein and neuron specific enolase). Urine analysis for mucopolysaccharides, oligosaccharides, monosaccharides and polyols, and neuraminic acid was normal. Analysis of the lysosomal enzymes in the leukocytes

Table 1 Quantification of relevant metabolites in various body fluids based on H-NMR spectroscopy.

Material	Uracil	Thymine	Dihydrouracil	Dihydrothymine
Urine	144	230	760	490
Plasma	nd	nd	<30	49
CSF	nd	10	46	79

Urine concentrations in μmol/mmol creatinine. Plasma and spinal fluid concentrations in μmol/L. nd = not detectable. In plasma and CSF the pyrimidines and dihydropyrimidines are normally not detectable with NMR spectroscopy (detection limits for uracil and dihydrouracil <30 μmol/L, for thymine and dihydrothymine <10 μmol/L). In urine normal levels for these metabolites are below 10 μmol/mmol creatinine.

did not reveal deficiencies. Amino acids in urine and serum were normal. Metabolic screening of the urine by gas chromatography and mass spectrometry (1, 6) showed increased amounts of uracil, dihydrouracil, thymine and dihydrothymine. High-pressure liquid chromatography confirmed the elevation of pyrimidines. Dihydropyrimidines could not be detected by this method because of the low UV absorption range of these compounds.

Quantitation of dihydropyrimidinuria

By in vitro nuclear magnetic resonance (NMR) analysis of body fluids (12, 13) the increased pyrimidines and dihydropyrimidines were quantitated (Fig. 2, Table 1). The concentration of uracil and thymine was comparably low in plasma and in CSF. The concentration of the dihydropyrimidines was higher in CSF than in plasma. Furthermore an unidentified metabolite (triplet resonance 2.61 ppm; J coupling 6.6 Hz) was observed in the CSF, but not in plasma or urine. This metabolite has not been observed in any of the 60 other CSF samples, screened until now, of patients suspected to have an inborn error of metabolism. N-Carbamoyl-ß-alanine can be recognized by triplet resonances at 2.55 and 3.36 ppm. It was however not detectable in any of the body fluids, leading to the hypothesis that this patient must have dihydropyrimidinase deficiency and not ureidopropionase deficiency. The dihydropyrimidinase enzyme assay performed in the biopsy of the liver showed a complete deficiency, i.e. activity was below the detection level of 0.3 nmol/mg protein hr^{-1} (normal range 20–74 nmol/mg protein hr^{-1}, n = 8) (report on the assay is in preparation).

Morphology

Electron microscopic examination of the lymphocytes did not show inclusions. Biopsies of the liver and the quadriceps muscle showed normal morphology.

Neurophysiological examinations

The electroretinogram showed activity and the visual evoked potentials were present, consistent with some retinal activity and central visual signal propagation. The initial brainstem auditory evoked potentials were poorly reproductive. Repeat examinations showed an interpeak I–V delay consistent with a delayed brainstem conduction. The middle latency auditory evoked potentials could not be elicited. The cortical auditory evoked responses showed a retarded maturation. Repeated electroencephalograms showed an increasingly disturbed and slowing background activity and irritative activity, at last consistent with slow wave spiking (*Lennox Gastaut* features). EMG nerve conduction and muscular insertion studies were non-revealing.

Imaging

Neither ultrasound of the kidneys, liver and pelvis nor X-ray of the spine and skeleton showed abnormalities. Magnetic resonance imaging (MRI) of the brain was performed at the ages of 6, 18 and 27 months. The corpus callosum was thin, the widened subarachnoidal frontoparietal and temporal space was consistent with progressive cortical atrophy. The myelination was retarded. Brainstem, basal ganglia and cerebellum were normal (Fig. 3).

Discussion

An increased excretion of uracil and thymine, and in particular of dihydrouracil and dihydrothymine, was found in the urine of the patient. N-carbamoyl-ß-alanine was not elevated. This indicated that the defect should be located at the level of dihydropyrimidinase, thus DHP deficiency (Fig. 1). This could be verified by the enzymatic assay in the liver biopsy. No activity could be detected. As such, the patient was the first case with enzymatically proven DHP deficiency. The elevated amounts of uracil and thymine can be explained by the reversibility of the first step of pyrimidine degradation, which is catalysed by dihydropyrimidine dehydrogenase.

Whether the dysmorphic as well as the clinical degenerative symptoms are attributable to dihydropyrimidinase deficiency, remains to be established. The syndromal abnormalities in our patient could not be classified according to a specific syndrome. *Coffin Siris* syndrome has been postulated, but the criteria were insufficient.

Three other cases with DHP-deficiency have been reported to date (Table 2). The first case, a girl presented by *Duran* et al (4, 5) manifested with convulsions and disturbed consciousness at

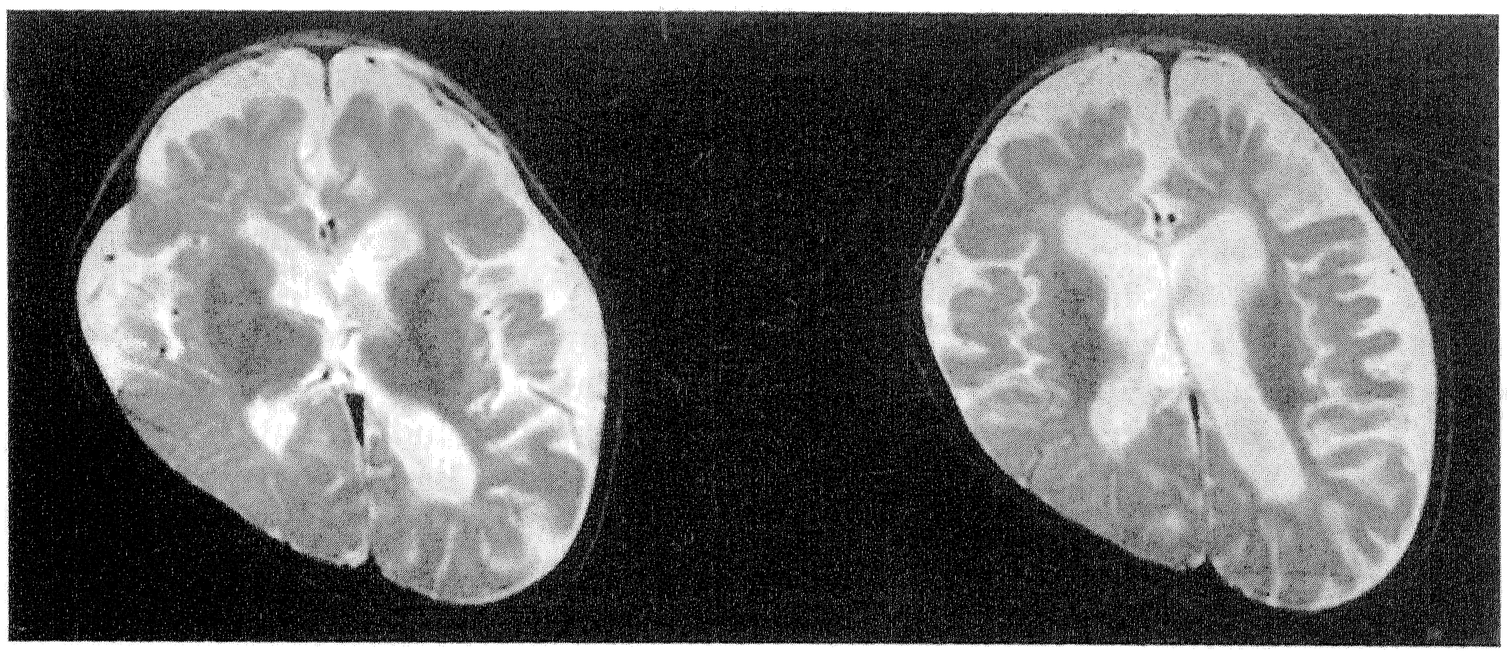

Fig. 3 MRI of the patient showing diffuse neuronal degeneration with secondary delay of myelination.

Table 2 Clinical presentation and degree of dihydropyrimidinuria.

Patient references	Sex	Age	Presentation and course	Dihydropyrimidinuria	
				Dihydrouracil	Dihydrothymine
4, 5	M	8 wk	Seizures Normal development Lost for follow-up	HPLC*: 790	450 µmol/L
9	F	11 mnth	Screening program, normal infant No follow-up	HPLC*: 626	451 µmol/mmol creat.
8	M	6 wk	Febrile seizures at 6 weeks Intractable seizure disorder Gross microcephaly Severe mental retardation Spastic quadriplegia, choreatic signs Lost for follow-up	HPLC*: 100–500	100–500 µmol/mmol creat.
Case	F	birth	Dysmorphic features (see above) Intractable seizure disorder Severe developmental delay Feeding problems Decrease centile head circumference Pyramidal/choreatic signs and symptoms EEG, EPs, MRI: progressive disease Follow-up: birth-onward	H-NMR**: 760	490 µmol/mmol creat.

* HPLC, high pressure liquid chromatography
** H-NMR, NMR spectroscopy (Table I)

the age of 8 weeks. She showed subsequently a normal development up to the age of 19 months. The second case reported by *Henderson* et al (8) however, presented with febrile convulsions at 6 weeks and subsequent frequent generalized seizures. On examination at 2½ years of age, gross microcephaly and severe developmental retardation were noted. Signs of spastic quadriplegia and jerky choreiform movements were observed. The third case (9) was discovered in a screening program for inborn errors of pyrimidine metabolism. The case showed no neurological symptoms at the time of diagnosis at the age of 11 months. Our case is remarkably similar to the one described by *Henderson,* but our patient showed more clearly clinical, neurophysiological and MRI features of a progressive neurodegenerative disease. Clinically there were lack of mental development, decreasing head circumference, a progressive seizure disorder and choreatic-pyramidal features. The evoked potential studies were concurrent with a retarded central conduction and maturation, and the EEG investigations pointed to deterioration, both with respect to the general background activity as to the nature of the irritative paroxysmal activity. The repeated MRIs were consistent with primary neuronal degeneration with secondary retarded myelination.

The differing severity of the neurological symptoms suggests that phenotypic expression might be broad, as can be seen in the other pyrimidine disorder, i.e. dihydropyrimidine dehydrogenase deficiency (2, 7). Furthermore, in our patient we are dealing with a complete deficiency of the enzyme, while in the other cases the deficiency has not yet been established on the enzymatic level. A relation of the degree of enzyme deficiency and the clinical presentation is currently not available.

The pathophysiological mechanism involved in the occurrence of neurological signs and symptoms in patients with pyrimidine disorders is unclear. In our patient the concentration of the two dihydropyrimidines was significantly higher in the CSF than in blood. This can be explained by active transport of dihydropyrimidines from blood to CSF over the blood-brain barrier. However, no transporters for these compounds are known. It is more likely that within the brain active biosynthesis of ß-alanine and possibly also of ß-aminoisobutyric acid occurs from uracil and thymine, respectively. *DeFeudis* and *Martin Del Rio* have emphasized the importance of ß-alanine as a putative neurotransmitter (3). From animal studies it appears that ß-alanine acts, together with glycine and GABA, as inhibitory amino acid mainly in the spinal cord, but also in cerebral cortical membranes (11). If ß-alanine plays a pathophysiological role, it should exert besides inhibitory also neuromodulatory functions in the infant brain, in view of the cerebral development in our patient. The clinical symptomatology in our patient is probably too complex to be explained only by decreased ß-alanine inhibition.

Furthermore, it has been described that uridine has anticonvulsant effects in animals with experimental seizures (10). This might indicate that pyrimidine compounds play a role in the regulation of central nervous system activity. Moreover, in cancer treatment, disturbances of pyrimidine metabolites by antimetabolites are thought to be responsible for neurotoxicity (14).

Our finding of higher dihydropyrimidine concentrations in the CSF than in plasma may be the beginning of an explanation for involvement of the CNS in DHP-deficiency. It remains unknown whether the low concentration of ß-alanine or the high concentration of the dihydropyrimidines is the most important factor for the clinical symptomatology. The as yet unknown metabolite observed only in the CSF of our patient may be of interest in this respect. It has not been confirmed in another case if this metabolite relates to DHP-deficiency.

These arguments support the hypothesis that inborn errors of pyrimidine metabolism are associated with central nervous system dysfunction and disease. Further enzymatic and genetic studies are needed to explain the variability of the clinical expression of dihydropyrimidinase deficiency.

Acknowledgments

The authors thank *U. Engelke* for his invaluable assistance in measuring the NMR-spectra of our patient.

References

1 *Bakkeren, J. A. J. M., R. A. De Abreu, R. C. A. Sengers, F. J. M. Gabreëls, J. M. Maas, W. O. Renier:* Elevated urine, blood and cerebrospinal fluid levels of uracil and thymine in a child with dihydrothymine dehydrogenase deficiency. Clin. Chim. Acta 140 (1984) 247–256

2 *De Abreu, R. A.:* Dihydropyrimidine dehydrogenase deficiency: biochemical and genetic basis. In: *Gresser U.* (Ed.) Molecular Genetics, Biochemistry and Clinical Aspects of Inherited Disorders of Purine and Pyrimidine Metabolism. Berlin, Springer-Verlag (1993) 176–179

3 *De Feudis, F. V., R. Martin Del Rio:* Is ß-alanine an inhibitory neurotransmitter? Gen. Pharmacol. 8 (1977) 177–180

4 *Duran, M., P. Rovers, P. K. De Bree, C. H. Schreuder, H. Beukenhorst, L. Dorland, R. Berger:* Dihydropyrimidinuria. Lancet 336 (1990) 817–818

5 *Duran, M., P. Rovers, P. K. De Bree, C. H. Schreuder, H. Beukenhorst, L. Dorland, R. Berger:* Dihydropyrimidinuria: a new inborn error of pyrimidine metabolism. J. Inherit. Metab. Dis. 14 (1991) 367–370

6 *van Gennip, A. H., S. Busch, L. Elzinga, A. E. M. Stroomer, A. van Cruchten, E. G. Scholten, N. G. G. M. Abeling:* Application of simple chromatographic methods for the diagnosis of defects in pyrimidine degradation. Clin. Chem. 39 (1993) 380–385

7 *van Gennip, A. H., N. G. G. M. Abeling, A. E. M. Stroomer, H. van Lenthe, H. D. Bakker:* Clinical and biochemical findings in six patients with pyrimidine degradation defects. J. Inherit. Metab. Dis. 17 (1994) 130–132

8 *Henderson, M. J., K. Ward, H. A. Simmons, J. A. Duley, P. M. Davies:* Dihydropyrimidinase deficiency presenting in infancy with severe developmental delay. J. Inherit. Metab. Dis. 16 (1993) 574–576

9 *Ohba, S., K. Kidouchi, S. Sumi, M. Imaeda, N. Takeda, H. Yoshizumi* et al: Dihydropyrimidinuria: the first case in Japan. Purine and Pyrimidine Metabolism in Man VIII. Adv. Exp. Med. Biol. 370 (1994) 383–386

10 *Roberts, C. A.:* Anticonvulsant effects of uridine: comparative analysis of metrazol and penicillin induced foci. Brain Res. 55 (1973) 291–308

11 *Saransaari, P., S. S. Oja:* Characterization of sodium-independent beta-alanine binding to cerebral cortical membranes from 7-day-old and adult mice. Int. J. Dev. Neurosci. 12 (1994) 491–497

12 *Wevers, R. A., U. Engelke, A. Heerschap:* High-resolution H-NMR spectroscopy of blood plasma for metabolic studies. Clin. Chem. 40 (1994) 1245–1250

13 *Wevers, R. A., U. Engelke, U. Wendel, J. G. N. de Jong, F. J. M. Gabreëls, A. Heerschap:* Standardized method for high-resolution H-NMR of cerebrospinal fluid. Clin. Chem. 41 (1995) 744–751

14 *Wiley, R. G., R. J. Gralla, E. S. Casper, N. Kemeny:* Neurotoxicity of the pyrimidine synthesis inhibitor N-phospho-acetyl-L-aspartate. Ann. Neurol. 12 (1982) 175–183

Dr. J. J. Rotteveel

Department of Child Neurology
University Hospital Nijmegen
PO Box 9101
6500 HB Nijmegen
The Netherlands