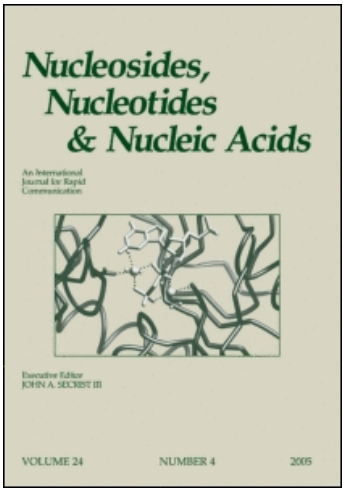

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ß-ALANINE AND ß-AMINOISOBUTYRIC ACID LEVELS IN TWO SIBLINGS WITH DIHYDROPYRIMIDINASE DEFICIENCY

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□ *Dihydropyrimidinase (DHP) deficiency is an inborn error of the pyrimidine degradation pathway, affecting the hydrolytic ring opening of the dihydropyrimidines. In two siblings with a complete DHP deficiency and a variable clinical presentation, a normal concentration of ß-alanine and strongly decreased levels of ß-aminoisobutyric acid were observed in plasma, urine and CSF. No major differences were observed for the concentrations of the ß-amino acids in plasma and urine between the symptomatic and asymptomatic sibling. Thus, the relevance of the shortage of ß-aminoisobutyric acid for the onset of a clinical phenotype in patients with DHP deficiency remains to be established.*

Keywords Dihydropyrimidinase; pyrimidines; *DPYS*; ß-alanine; ß-aminoisobutyric acid

INTRODUCTION

In humans, the pyrimidine bases uracil and thymine are degraded via a three step pathway to ß-alanine and ß-aminoisobutyric acid, respectively. Dihydropyrimidine dehydrogenase (DPD, EC 1.3.1.2) is the initial and rate-limiting enzyme, catalysing the reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively. The second step consists of a hydrolytic ring opening of the dihydropyrimidines which is catalysed by dihydropyrimidinase (DHP, EC 3.5.2.2). Finally, the resulting N-carbamyl-ß-aminoisobutyric acid and N-carbamyl-ß-alanine are converted in the third step to ß-aminoisobutyric acid and ß-alanine, ammonia and CO_2 by ß-ureidopropionase (ß-UP, EC 3.5.1.6).

To date, only 11 patients have been described with a complete DHP deficiency (MIM 222748). The clinical phenotype of these patients was highly variable, ranging from asymptomatic to seizures, mental retardation,

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growth retardation and dysmorphic features.[1–10] The pathophysiological mechanism is still unknown. In these patients with a DHP deficiency, a large accumulation of dihydrouracil and dihydrothymine was detected in urine, blood and cerebrospinal fluid and no activity of DHP could be detected in liver tissue.[5,11]

The pyrimidine degradation pathway plays an important role in the biosynthesis of ß-alanine and ß-aminoisobutyric acid.[12] ß-Alanine is a structural analogue of both γ-aminobutyric acid and glycine, which are the major inhibitory neurotransmitters in the central nervous system. Furthermore, ß-aminoisobutyric acid has been shown to be a partial agonist of the glycine receptor.[13] Thus, the altered homeostasis of these ß-amino acids, as observed in patients with a DPD deficiency, might underlie some of the clinical abnormalities encountered in patients with a DHP deficiency. In this study, we investigated the levels of the ß-amino acids in body fluids of two siblings with a DHP deficiency with variable clinical presentation.[10]

MATERIALS AND METHODS

The concentrations of ß-alanine and ß-aminoisobutyric acid in plasma, urine and cerebrospinal fluid (CSF) were determined with a dual-column reversed-phase HPLC procedure, combined with fluorescence detection of the orthophthaldialdehyde derivatives, as described before.[12]

RESULTS

The two siblings presented with strongly elevated levels of 5,6-dihydrouracil and 5,6-dihydrothymine in their body fluids.[10] Analysis of the DHP gene showed that both siblings were compound heterozygous for the missense mutations 1078T>C (W360R) in exon 6 and 1235G>T (R412M) in exon 7.[10] One of the siblings (patient 1) had a severe delay in speech development and white matter abnormalities on the cerebral MRI scan. Patient 2 was free of symptoms, and did not undergo an MRI scan.[10] To investigate whether the impaired degradation of the dihydropyrimidines would result in altered levels of ß-alanine and ß-aminoisobutyric acid, we measured the levels of these ß-amino acids in the body fluids of controls and the two siblings with DHP deficiency (Table 1). In both siblings, a low normal concentration of ß-alanine was observed in plasma which was 42% (patient 1) and 63% (patient 2) of the mean concentration of ß-alanine as observed in controls. In contrast, the levels of ß-aminoisobutyric acid in plasma were 12-fold and 5-fold lower in patient 1 and 2, respectively, when compared to controls (Table 1). A normal concentration of ß-alanine was observed in urine from both siblings whereas the concentration of ß-aminoisobutyric acid was approximately 7-fold lower

TABLE 1 Levels of ß-amino acids in DHP patients and controls

	Patient 1	Patient 2	Controls[a]
Plasma			
ß-alanine (μM)	1.6	2.4	3.8 ± 2.9 (n = 52)
ß-aminoisobutyric acid (μM)	0.2	0.5	2.3 ± 1.9 (n = 52)
Urine (μM/mM creatinine)			
ß-alanine	1.0	1.1	2.4 ± 3.0 (n = 44)
ß-aminoisobutyric acid	2.2	2.3	14.8 ± 11.7 (n = 44)
CSF			
ß-alanine (μM)	0.037	n.a	0.024 ± 0.013 (n = 32)
ß-aminoisobutyric acid (μM)	<0.01	n.a	0.015 ± 0.014 (n = 35)

[a]Data taken from van Kuilenburg et al.[12]
n.a. = not available.

than the mean concentration of ß-aminoisobutyric acid in controls. In CSF, a high-normal ß-alanine concentration was observed whereas the levels of ß-aminoisobutyric acid were undetectable (Table 1).

DISCUSSION

DHP deficiency is an autosomal recessive disease characterised by dihydrothymine-dihydrouraciluria. The DHP gene (*DPYS*) has been mapped to chromosome 8q22 and consists of 10 exons spanning >80 kB of genomic DNA.[9] The cDNA coding for human DHP contains an open reading frame of 1560 nucleotides, corresponding to a protein of 519 amino acids with a calculated molecular weight of 56629 Da.[9] To date, only 11 individuals have been described with a complete DHP deficiency[2–10] and various mutations have been identified in *DPYS* (Figure 1). Five of these 11 patients have been identified during a screening programme for inborn errors of pyrimidine degradation in Japan and these three children and two adults were healthy at the time of diagnosis.[6–9] In contrast, the clinical spectrum of the other 6 patients included seizures, mental retardation,

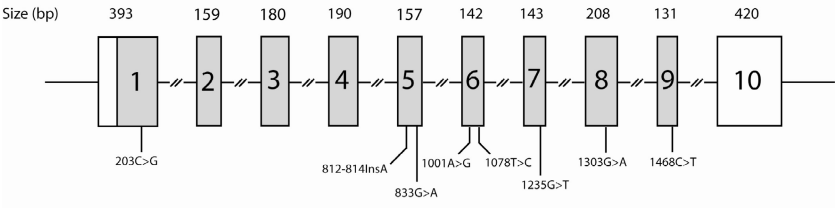

FIGURE 1 Genomic organisation of the DHP gene. The DHP gene consists of 10 exons encoding an open reading frame of 1560 bp (depicted in gray). The different mutations identified in patients with a DHP deficiency are indicated. The numbers correspond to the cDNA position.

growth retardation, delay in speech development, white matter abnormalities and dysmorphic features.[1,5,10]

To date, the pathological mechanism underlying the various clinical abnormalities is still unknown. The pyrimidine degradation pathway plays an important role in the synthesis of ß-alanine and ß-aminoisobutyric acid and it has been shown that in patients with DPD deficiency, the levels of ß-aminoisobutyric acid were strongly decreased or even undetectable in body fluid.[12] The concentrations of the ß-amino acids in our two patients with DHP deficiency were comparable to those observed for patients with DPD deficiency or ß-ureidopropionase deficiency.[12,14] However, no major differences were observed between the plasma levels of ß-alanine and ß-aminoisobutyric acid between the symptomatic and asymptomatic sibling. Although an altered homeostasis of the ß-amino acids might underlie some of the clinical abnormalities encountered in the DHP patients, the finding that some individuals with a complete DHP deficiency did not present with any clinical abnormalities suggest that additional factors may be involved determining the clinical outcome. A DHP deficiency is, therefore, probably a necessary, but not a sole, prerequisite for the onset of a clinical phenotype. It remains to be assessed whether supplementation of ß-aminoisobutyric acid results in clinical amelioration of the patient.

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