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Regular paper

Dihydropyrimidine dehydrogenase deficiency presenting with psychomotor retardation in the first Polish patient

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Dihydropyrimidine dehydrogenase (DPD) deficiency is a rare defect of the first step of the pyrimidine catabolic pathway. Patients with a complete enzyme deficiency may be clinically asymptomatic or suffer from neurological abnormalities of various severity. We report a case of an 8-year-old girl with psychomotor retardation and mild course of the disease. Analysis of urine showed strongly elevated levels of uracil and thymine, and no DPD activity could be detected in peripheral blood mononuclear cells. Sequence analysis of the DPD gene (*DPYD*) revealed that our patient was homozygous for the common splice-site mutation IVS14+1G >A, which suggest that the carrier status for this mutation may be not rare in the Polish population.

Keywords: dihydropyrimidine dehydrogenase deficiency, child, psychomotor retardation

INTRODUCTION

Dihydropyrimidine dehydrogenase (DPD; EC. 1.3.1.2) catalyzes the degradation of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively (Fig. 1). Liver cells exhibit high DPD activity but for diagnostic purposes peripheral blood mononuclear (PBM) cells and skin fibroblasts are used instead. Patients with a complete DPD deficiency present with high concentrations of uracil and thymine in urine, blood and cerebrospinal fluid (Bakkeren *et al*., 1984).

Congenital DPD deficiency was originally described in 1984 in a child with psychomotor retardation and seizures (Bakkeren *et al*., 1984). Until now, approximately 50 cases of the disease have been reported. Most of the patients suffered from seizures, mental retardation, microcephally, and muscle tone disorders whereas some individuals were asymptomatic (Van Kuilenburg *et al*., 1999).

Human DPD gene (*DPYD*) is present as a single copy gene on chromosome 1p22 and consists of 23 exons (Wei *et al*., 1998). The estimated number of individuals homozygous for the most common IVS14+1G>A mutation is 1.2 in 10000 (Van Kuilenburg *et al*., 2001). The G to A mutation in IVS14+1G>A leads to the skipping of exon 14 immediately upstream of the mutated splice donor site in the process of DPD pre-mRNA splicing. As a result, the mature DPD mRNA lacks a 165-nucleotide (nt) segment encoding the amino acids 581–635 (Wei *et al*., 1998; Van Kuilenburg *et al*., 2001).

No clear correlation between the genotype and phenotype could be established in 17 families presenting 22 patients with complete deficiency of DPD (Van Kuilenburg *et al*., 1999). In this group of patients, seven different mutations were identified, including two deletions, one splice site mutation, and four missense mutations.

DPD is also the main enzyme catalyzing the degradation of 5-fluorouracil which is used in oncologic chemotherapy. Patients with DPD deficiency are prone to develop severe 5-fluorouracil-associated toxicity, and its usage in such patients may even re-

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Abbreviations: CRC, colorectal cancer; FU, 5-fluorouracil.

sult in their death (Tuchman *et al*., 1985; Van Kuilenburg *et al*., 2001).

So, decreased DPD activity due to mutation heterozygosity usually is asymptomatic, but causes increased toxicity of 5-fluorouracil, while deficiency of DPD activity due to pathogenic mutations (in homozygous or compound heterozygous forms) results in a rare disease with an autosomal recessive genetic trait.

In this paper, the first case of a Polish patient with a DPD deficiency, presenting with mental retardation and speech disabilities, is reported.

MATERIALS AND METHODS

GC-MS urinary organic acids analysis was performed by the method described by (Chalmers & Lawson, 1975) with modification.

Analysis of pyrimidine bases and DPD activity. The concentrations of pyrimidine bases and their degradation products in urine and plasma were determined using reversed-phase HPLC combined with electrospray tandem mass spectrometry, and detection was performed by multiple-reaction monitoring. Stable-isotope-labeled reference compounds were used as internal standards (Van Lenthe *et al*., 2000).

The activity of DPD was determined in peripheral blood mononuclear (PBM) cells using radiolabeled thymine followed by separation of radiolabeled thymine from radiolabeled dihydrothymine using reversed-phase HPLC (Van Kuilenburg *et al*., 2000).

Mutation analysis of *DPYD*. DNA was isolated from leukocytes using the Wizard Genomic DNA Purification Kit. PCR amplification of all 23 coding exons and flanking intronic regions was carried out by using intronic primer sets, as described before (Van Kuilenburg *et al*., 2000). Sequence analysis of genomic fragments amplified by PCR was carried out on an Applied Biosystems model 3100 automated DNA sequencer using the dye-terminator method.

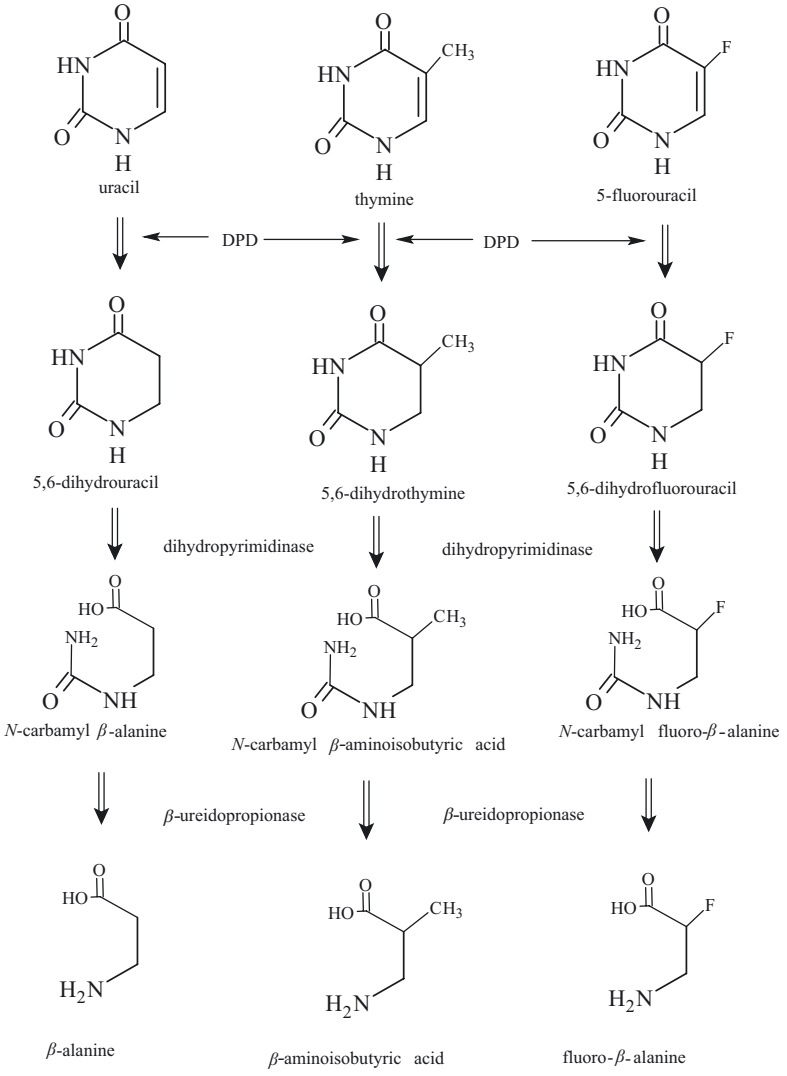

Figure 1. Catabolic pathway of pyrimidines.

RESULTS

Case Report

The girl was born after a healthy non-complicated pregnancy and spontaneous labour, her brith mass was 3800 g, and after clinical examination her clinical condition was rated at 8 points on the Apgar scale. The family history is unsignificant, parents are young and unrelated, and her brothers (6 and 2 year-old) are healthy.

The patient's early history revealed psychomotor retardation; she sat when 9 months old, walked at the age of 16 months and spoke when 2 years old, and her speech was retarded from the very beginning. At the age of 2 years, at a clinical neurologic examination she was moderately hypertonic, showed signs of visual-motor incoordination, small manual disabilities, and mental retardation of moderate severity. At present at the age of 8 years, she is suffering from mild mental retardation without neurological symptoms. MRI scan of the head did not show any

Table 1. IVS14+1G>A mutation incidence in chosen countries on references

Country	Group examined	IVS14+1G>A allele frequency	References
France	102 French Caucasian patients with cancer, and 93 patients with 5-FU-related toxicity	1.96% and 2.2%	Mange *et al*., 2005
	80 French colorectal cancer CRC patients with toxicity following 5-FU	0%	Mange *et al*., 2007
Netherlands	1357 Dutch Caucasian patients with healthy individuals	0.91%	Van Kuilenburg *et al*., 2001
Germany	851 German Caucasian control and cancer individuals	0.94%	Raida *et al*., 2001
Portugal	73 Portuguese population with CRC	2.7%	Salgueiro *et al*., 2004
South Korea	Healthy Korean and 21 CRC patients with toxicity to 5-FU	0%	Cho *et al*., 2007
Egypt	247 Egyptian healthy subjects	0.02%	Hamdy *et al*., 2002
Taiwan	300 healthy Taiwanese subject	0%	Hsiao *et al*., 2004
Japan	150 healthy Japanese	0.7%	Ogura *et al*., 2005
USA	Healthy African-American n=149 and Caucasian n=109 volunteers	8.0% and 2.8%	Mattison *et al*., 2006
Turkey	218 individuals 56 patients with CRC and 162 healthy individuals	0.6%	Uzunkoy *et al*., 2007

abnormalities. An organic acid profile in the urine was established, using GC/MS, and it showed abnormal secretion of uracil and thymine which suggested DPD deficiency.

Biochemical and genetic analysis

Analysis of a urine sample of the patient showed strongly elevated levels of uracil — 611 μmol/mmol creatinine (ref. value 1–14 μmol/mmol creatinine) and thymine — 99 μmol/mmol creatinine (ref. value <1 μmol/mmol creatinine). Highly elevated concentrations of uracil (16.6 μM; controls <0.4 μM; n=40) and thymine (18.5 μM; controls <0.1 μM; n=40) were detected in plasma as well. The DPD activity in PBM cells was undetectable (<0.014 nmol/mg per h; controls: 9.9±2.8 nmol/mg per h). Analysis of *DPYD* for the presence of mutations showed that the patient was homozygous for the IVS14+1G>A mutation.

DISCUSSION

The most frequent manifestation of DPD deficiency are neurological symptoms such as seizures, psychomotor retardation, microcephaly, hypotonia and autistic features. Dysmorphy and ocular symptoms are sometimes observed. Asymptomatic cases have also been reported (Bakkeren *et al*., 1984; Diasio *et al*., 1988).

In our patient with a complete DPD deficiency a mild course of history involving mental retardation was observed from infancy till present. Additionally, increased muscle tone and disorders of visual-motor coordination were found. Enns *et al*. (2004) showed that children with DPD deficiency might present with white matter abnormalities. In our patient, an MRI scan of the brain revealed no abnormalities.

It is estimated that 1–3% of the Caucasian race might be carriers for a mutation in the gene encoding DPD (Van Kuilenburg *et al*., 2001). As for now it is not clear why in some individuals with a complete DPD deficiency no symptoms are present.

Recently, from among eight patients with a DPD deficiency, none have presented with seizures, which is considered to be the most frequent clinical manifestation. However, all those patients showed motor-developmental retardation, and the majority — mental retardation (Van Kuilenburg *et al*., 2002). To date, no clear genotype-phenotype correlations have been established (Van Kuilenburg *et al*., 1999).

DPD is also an essential enzyme in the metabolism of 5-fluorouracil, which is used in the chemotherapy of colon, breast, kidney, and ovarian cancer, and neoplasms of the central nervous system. In case of a DPD deficiency, 5-fluorouracil can not be degraded any more and will be converted into toxic 5-fluoronucleotides. Thus, patients with a DPD deficiency are at risk of developing severe 5-fluorouracil-associated toxicity (Van Kuilenburg *et al*., 2000; Ciccolini *et al*., 2006). The most prevalent mutation in patients suffering from DPD deficiency is the IVS14+IG>A mutation. The incidence of this mutation in different populations is shown in Table 1. There are no such data concerning the Polish population.

The detection of the IVS14+1G>A mutation in Poland, clearly indicates that also in this country there is a necessity of broadening diagnostic investigations in order to detect DPD deficiency, especially in the case of patients diagnosed with cancer, who might be eligible for the treatment with 5-fluorouracil-containing chemotherapy.

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