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Short Communication

Identification of novel point mutations in the dihydropyrimidine dehydrogenase gene

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Dihydropyrimidine dehydrogenase deficiency (McKusick 274270) is an autosomal recessive disorder leading to thymine-uraciluria. Dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2) catalyses the first and rate-limiting step in the catabolism of uracil, thymine and the analogue 5-fluorouracil. Patients do not exhibit a characteristic clinical phenotype, although in about half the cases with a complete or near-complete deficiency of the enzyme convulsive disorders are observed (Berger et al 1984; van Gennip et al 1989, 1994; Braakhekke et al 1987). In patients with a nearly complete enzyme defect, the initial diagnosis can be made on the presence of large amounts of both thymine and uracil in the patient's body fluids, whereas the diagnosis can be confirmed by measurement of the enzyme activity in either peripheral mononuclear cells or fibroblasts (Van Kuilenburg et al 1996).

The recent cloning of the dihydropyrimidine dehydrogenase cDNA now allows detection of the defect at the molecular level (Yokota et al 1994). We previously described a 165 base pair deletion in mRNA-derived cDNA, caused by exon skipping, in a patient with a complete deficiency of DPD (Meinsma et al 1995). Analysis of the flanking intron sequences revealed that exon skipping was due to a G → A point mutation in the invariant GT splice donor sequence in the intron downstream of the skipped exon (Vreken et al 1996). So far, no other mutations in the DPD gene have been described.

We now report a new frameshift mutation (ΔC1897) and two missense mutations (T85C and G2658A) leading to amino acid substitutions C29R and R886H.

METHODS

RNA and DNA were isolated from cultured fibroblasts by the guanidinium thiocyanate method (Chomczynski and Sacchi 1987) and standard procedures (Hoar 1987), respectively.

cDNA synthesis and RT-PCR reactions for amplifying DPD cDNA were carried out essentially as described previously (Meinsma et al 1995; Vreken et al 1996). Four over-

Figure 1 Pedigrees and DPD enzymatic activities of the families investigated in this study. The DPD enzymatic activity measured in fibroblasts is expressed as nmol product/h per mg protein below the symbols (control values 1.12 ± 0.7 nmol product/h per mg protein; mean $\pm$ 1SD, $n = 15$). Hatched symbols correspond to normal individuals, hatched symbols indicate individuals with intermediate DPD activity (carriers) and filled symbols indicate the index patients in each family. nd; not detectable, na; not investigated

lapping DPD cDNA fragments were amplified and used for direct sequence analysis with fluorescent labelled primers on an Applied Biosystems 377 automated DNA sequencer.

Amplification of the genomic region containing a DPD exon and its flanking sequences was carried out as described previously (Vreken et al 1996). PCR products were purified using a Qiaquickspin PCR purification kit and used for direct sequencing.

DPD activity in fibroblasts was measured as described previously (Van Kuilenburg et al 1996).

RESULTS AND DISCUSSION

Clinical and biochemical characterization: Patient A is the second child of consanguineous parents (first cousins). The first child died before her first birthday with severe neuromotor retardation and febrile convulsions. At the age of 9 months the patient showed febrile convulsions, severe neuromotor retardation and a spastic tetraplegia. Cerebral MRI revealed ventriculomegaly with white-matter hypodensity. In addition, microcephaly and growth retardation were noted. At the age of 6 years thymine-uraciluria was noted (data not shown) and dihydropyrimidine dehydrogenase deficiency was suspected. Analysis of DPD enzymatic activity in fibroblasts of the patient confirmed the diagnosis (Figure 1). Surprisingly, the father of the patient was also completely DPD deficient, while he had no known history of convulsions. The mother of the patient showed intermediate DPD activity consistent with her obligate carrier status.

Patient B showed severe growth retardation (length <3rd centile) and was submitted to the hospital at the age of 8 years for treatment of an upper airway infection. During her stay in the hospital a hypokalaemia (2.5 mmol/L) was noted and the diagnosis of Bartter syndrome suspected. Metabolic screening at that time revealed thymine-uraciluria (data not shown). Analysis of DPD enzymatic activity in fibroblasts of the patient confirmed

dihydropyrimidine dehydrogenase deficiency (Figure 1). The patient showed no convulsions or other neurological abnormalities. Unfortunately, no material from family members of this patient was available for study.

Genomic sequence analysis: Since we previously identified a splice donor site mutation in two unrelated Dutch patients, we tested the patients and available family members for this mutation. A 333bp genomic DNA fragment encompassing a DPD exon and its flanking intron sequences was amplified and directly sequenced. The previously reported splice donor site mutation (Vreken et al 1996) was not detected in either patient or relatives. Patient A was shown to be heterozygous for a single base pair deletion at position C1897, whereas his father was homozygous for the Δ C1897 mutation. This mutation leads to a frameshift and a premature stop codon shortly thereafter. The mother of patient A and patient B showed no abnormalities in this genomic DPD region (data not shown).

cDNA sequence analysis: Total RNA isolated from fibroblasts of the patients and their relatives was subjected to RT-PCR in order to amplify four overlapping regions encompassing the complete DPD cDNA. No deviations in size compared to control samples could be detected. However, the father of patient A, homozygous for the Δ C1897 mutation at the DNA level, showed only very weak bands, suggesting that the mutant transcript exhibits reduced stability or reduced expression. Reduced mRNA stability of transcripts containing premature stop codons is a well known phenomenon and therefore the most likely explanation for this observation (Cheng and Maquat 1993; Ross 1995). Sequence analysis of the amplified fragments revealed that the father of patient A was, as expected, homozygous for the Δ C1897 mutation at the cDNA level. Patient A, however, did not show heterozygosity for the mutation at the cDNA level, suggesting that there is a clear difference in steady-state concentration of the Δ C1897 and the maternally inherited allele. Similar results were obtained from the brother of the father of patient A who is also a Δ C1897 heterozygote at the DNA level; his heterozygosity for the Δ C1897 mutation could not be detected clearly in the cDNA analysis. Since the patient lacks residual DPD enzymatic activity, it is suspected that there is another mutation present on the maternally inherited allele. So far, however, we were unable to detect this mutation in either patient A or his mother.

Patient B showed homozygosity for two novel missense mutations T85C and G2758A, leading to two amino acid substitutions C29R and R886H. Since it was not clear which of these mutations leads to the DPD-deficient phenotype, we introduced these mutations separately into a wild-type DPD cDNA and subcloned them into a pSE420 expression vector (details will be published elsewhere). Analysis of the mutant constructs revealed that the DPD enzymatic activity in mutant DPD-C29R was undetectable, whereas mutant DPD-R886H showed about 40% of the normal activity. These results suggest that the C29R mutation alone is sufficient to explain the DPD-deficient phenotype in this patient. The R886H mutation leads, at least in our expression system, to a significant amount of DPD activity and therefore cannot explain a DPD-deficient phenotype, suggesting that this mutation is, most likely, a (rare) polymorphism.

REFERENCES

- Berger R, Stoker-de Vries SA, Wadman SK, et al (1984) Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism. *Clin Chim Acta* **141**: 227–234.
- Braakhekke JP, Renier WO, Gabreëls FJM, De Abreu RA, Bakkeren JAJM, Sengers RCA (1987) Dihydropyrimidine dehydrogenase deficiency: neurological aspects. *J Neurol Science* **78**: 71–77.
- Cheng J, Maquat LE (1993) Nonsense codons can reduce the abundance of nuclear mRNA without affecting the abundance of pre-mRNA or the half-life of cytoplasmic mRNA. *Mol Cell Biol* **10**: 2269–2284.
- Chomczynski P, Sacchi N (1987) Single step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Anal Biochem* **162**: 156–159.
- Hoar DI (1987) Molecular diagnosis: new horizons in medicine. *Can Fam Physician* **33**: 401–404.
- Meinsma R, Fernandez-Salguero P, van Kuilenburg ABP, van Gennip AH, Gonzalez FJ (1995) Human polymorphism in drug metabolism: mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. *DNA Cell Biol* **14**: 1–6.
- Ross J (1995) mRNA stability in mammalian cells [Review]. *Microbiol Rev* **59**: 423–450.
- Van Gennip AH, Abeling NGGM, Elzinga-Zoetekouw L, Scholten LG, van Cruchten A, Bakker HD (1989) Comparative study of thymine and uracil metabolism in healthy persons and in a patient with dihydropyrimidine dehydrogenase deficiency. *Adv Exp Med Biol* **253A**: 111–118.
- Van Gennip AH, Abeling NGGM, Stroomer AEM, van Lenthe H, Bakker HD (1994) Clinical and biochemical findings in six patients with pyrimidine degradation defects. *J Inher Metab Dis* **17**: 130–132.
- Van Kuilenburg ABP, van Lenthe H, van Gennip AH (1996) Identification of a NADH-dependent activity of dihydropyrimidine dehydrogenase in man. *Anticancer Res* **16**: 389–394.
- Vreken P, van Kuilenburg ABP, Meinsma R, et al (1996) A point mutation in an invariant splice donor site leads to exon skipping in two unrelated Dutch patients with dihydropyrimidine dehydrogenase deficiency. *J Inher Metab Dis* **19**: 640–641.
- Yokota H, Fernandez-Salguero P, Guruya H, et al (1994) cDNA cloning and chromosome mapping of human dihydropyrimidine dehydrogenase, an enzyme associated with 5-fluorouracil toxicity and congenital thymine uraciluria. *J Biol Chem* **269**: 23192–23196.