Dihydropyrimidine Dehydrogenase Deficiency: Metabolic Disease or Biochemical Phenotype?

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Abstract Dihydropyrimidine dehydrogenase (DPD) deficiency is an autosomal recessive disorder of pyrimidine metabolism that impairs the first step of uracil and thymine degradation. The spectrum of clinical presentations in subjects with the full biochemical phenotype of DPD deficiency ranges from asymptomatic individuals to severely affected patients suffering from seizures, microcephaly, muscular hypotonia, developmental delay and eye abnormalities.

We report on a boy with intellectual disability, significant impairment of speech development, highly active epileptiform discharges on EEG, microcephaly and impaired gross-motor development. This clinical presentation triggered metabolic workup that demonstrated the biochemical phenotype of DPD deficiency, which was confirmed by enzymatic and molecular genetic studies. The patient proved to be homozygous for a novel c.2059-22T>G mutation which resulted in an in-frame insertion of 21 base pairs (c.2059-21_c.2059-1) of intron 16 of DPYD.

Family investigation showed that the asymptomatic father was also homozygous for the same mutation and enzymatic and biochemical findings were similar to his severely affected son. When the child deteriorated clinically, exome sequencing was initiated under the hypothesis that DPD deficiency did not explain the phenotype completely. A deletion of the maternal allele on chromosome 15q11.2-13-1 was identified allowing the diagnosis of Angelman syndrome (AS). This diagnosis explains the patient's clinical presentation sufficiently; the influence of DPD deficiency on the phenotype, however, remains uncertain.

Introduction

Dihydropyrimidine dehydrogenase (DPD) deficiency is a rare autosomal recessive disorder that was first described in the 1980s (van Gennip et al. 1981; Bakkeren et al. 1984). Dihydropyrimidine dehydrogenase catalyses the first and rate-limiting step of uracil and thymine degradation. Biochemical hallmark of enzyme deficiency is the excretion of uracil and thymine in urine, which is easily detected by basic metabolic work-up. DPD deficiency has been associated with severe, early-onset neurological symptoms such as seizures, microcephaly, muscular hypotonia, global developmental delay and eye abnormalities (e.g. microcornea, nystagmus and hypoplastic macula) (Yau et al. 2004; Van Kuilenburg et al. 1999; Braakhekke et al. 1987; Bakker et al. 1994). EEG-abnormalities encompass various types of epileptic discharges (Braakhekke et al. 1987). However, the biochemical phenotype has also been observed in asymptomatic individuals (Van Kuilenburg et al. 1999; Braakhekke et al. 1987; Van Gennip et al. 1994).

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Brain magnetic resonance imaging (MRI) in individuals with DPD deficiency showed normal brain morphology in some individuals; non-specific findings (e.g. cerebral atrophy or white matter T2-hyperintensity) have also been described (Enns et al. 2004).

Angelman syndrome (AS) has initially been described in 1965 in three unrelated children with “puppet-like” gait, microcephaly, dysmorphic facial features, paroxysms of laughter and severe mental disability (Angelman 1965). AS is caused by loss of function of the ubiquitin protein ligase E3A (UBE3A) gene on the maternally derived chromosome 15. In 75% of cases, an interstitial deletion of the maternal part of the chromosome is causative. Genomic imprinting defects, paternal uniparental disomy of chromosome 15 and intragenic mutations or deletions of UBE3A gene have also been reported as causes for AS (Buiting et al. 2016).

As in DPD deficiency, the MRI-features in AS are rather unspecific. Thinning of the corpus callosum, enlargement of the lateral ventricles and cerebral atrophy with frontal and temporal predominance have been described. The EEG changes in AS encompass three quite distinctive main patterns: rhythmic high amplitude 2–3 Hz activity predominantly over the frontal regions; rhythmic high amplitude 4–6 Hz activity or 4–5 Hz spike waves over the parieto-occipital regions after eye closure. These patterns may appear isolated or in various combinations and often evolve over time in affected individuals (Buiting et al. 2016; Boyd et al. 1988). The major characteristic in AS is, however, neither the MRI nor the EEG but development and behaviour, which can be characterised as severe intellectual disability, absence of speech, paroxysmal periods of laughter, sleeping disturbances including reversal of the day-night rhythm, and extreme hyperactivity which can result in uncontrolled movements due to ataxia.

Case Report

Our patient was the first male child born to healthy, consanguineous (second cousins) Turkish parents (Fig. 1). He was born at term by secondary caesarean section; auxologic parameters were normal and neonatal adaptation uneventful.

At 4 weeks, the child had been hospitalised with an acute infection, feeding difficulties and failure to thrive, and metabolic workup was initiated. Determination of uracil and thymine concentrations in urine using reversed-phase HPLC hyphenated with electrospray tandem mass spectrometry (van Lenthe et al. 2000) demonstrated excessive excretion of uracil and thymine, indicating DPD deficiency (Table 1).

For enzymatic testing, peripheral blood mononuclear (PBM) cells were isolated and the activity of DPD was

Fig. 1 Family tree over four generations

Table 1 DPD activity and pyrimidine bases in affected family members

	Tissue	DPD activity (nmol/mg/h)	Uracil (μmol/mmol creatinine)	Thymine (μmol/mmol creatinine)
Son	PBM cells	0.2		
	Fibroblasts	< 0.02		
	Urine		562	288
Father	PBM cells	0.1		
	Fibroblasts	<0.02		
	Urine		141	42
Controls	PBM cells ^a	9.9 ± 2.8		
	Fibroblasts ^b	1.1 ± 1.2		
	Urine ^c		11.8 ± 9.1	0.5 ± 0.6

^a Data from van Kuilenburg et al. (2002)

^b Data from van Kuilenburg et al. (2000b)

^c Data from van Kuilenburg et al. (2004b)

determined in a reaction mixture containing 35 mM potassium phosphate (pH 7.4), 2.5 mM MgCl₂, 1 mM dithiothreitol, 250 μM NADPH and 25 μM [4-¹⁴C]-thymine. Separation of radiolabeled thymine from radiolabeled dihydrothymine was performed by reversed-phase HPLC with online detection of the radioactivity, as described before (van Kuilenburg et al. 2000b). The patient showed an almost complete deficiency of DPD activity (Table 1).

DNA was isolated from blood using standard techniques. PCR amplification of all 23 coding exons and flanking intronic regions of DPYD was carried out using intronic primer sets, essentially as described before (van Kuilenburg et al. 2000a). Sequence analysis of genomic fragments

From the age of 12 months, the paediatrician noticed developmental impairment and generalised muscular hypotonia. At 19 months, the child presented with failure to thrive and microcephaly (body weight and head circumference <3rd centile). He was able to sit unsupported but had not yet started to walk. His movements were poorly coordinated. Speech development, play behaviour and social skills corresponded to a developmental age of approximately 10 months. MRI of the brain revealed unspecific symmetric T2-hyperintense lesions in the occipital white matter region. EEG registered 4–5 Hz

During the further course, the clinical phenotype of AS evolved more and more. At 3 years, the parents reported paroxysm of laughter, he showed hyperactivity and ataxia, he was microcephalic and the characteristic EEG pattern with rhythmic frontal high amplitude 2–3 Hz activity and rhythmic high amplitude 4–6 Hz activity in addition to the previously described 4–5 Hz spike waves over the parieto-occipital regions was recorded.

In the patient presented here, overall developmental delay triggered basic metabolic work-up, which resulted in the identification of DPD deficiency. Enzymatic and molecular genetic studies were consistent with the biochemical phenotype. However, as the healthy father had the identical genetic and biochemical phenotype and further studies in the index patient showed signs unusual for DPD deficiency, additional studies were performed that finally demonstrated AS. Characteristic signs of AS such as microcephaly, delayed development and characteristic EEG features were encountered in the patient (Buiting et al. 2016).

This broad phenotypic spectrum indicates a variable penetrance of DPD deficiency. Additional factors such as other metabolites in pyrimidine catabolism, and oxidative stress or febrile infections as triggers may be involved in the development of the clinical phenotype and may even lead to sudden, eventually devastating neurologic disease in

formerly asymptomatic individuals (Fiumara et al. 2003; Van Kuilenburg et al. 2006). The mechanism behind this, however, has not been elucidated yet. Furthermore, it has been suggested that mutations in other, yet unknown genes and epigenetic influences may be linked to mutations in *DPYD* and thus involved in the development of the clinical phenotype (Van Kuilenburg et al. 1999).

Epilepsy is a feature often encountered in DPD deficiency. Therefore, it is an interesting observation that in DPD patients, plasma beta-aminoisobutyric acid (β -AIB), a downstream metabolite in thymine catabolism, is significantly decreased (van Kuilenburg et al. 2004a). The molecular structure of β -AIB is very similar to gamma-aminobutyric acid and glycine, two of the most important inhibitors of neuronal synaptic transmission. It has therefore been hypothesised that β -AIB may also be involved in inhibitory synaptic regulation. Following this line of thought, low β -AIB may contribute to the imbalance between excitatory and inhibitory agents and thus play a role in seizure aetiology in DPD. Since plasma concentrations of β -AIB levels are very low in DPD patients this parameter may unfortunately not be useful to differentiate between mild and severely affected individuals (Van Kuilenburg et al. 2004a).

We conclude that more investigations are necessary to elucidate possible pathogenic mechanisms behind the variable phenotype of DPD. We recommend thorough diagnostic workup of patients with the DPD biochemical phenotype beyond DPD in case the clinical course is suggestive of another disease or not consistent with clinical observations in DPD deficiency.

Compliance with Ethical Guidelines

All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Conflict of Interest

M. Fleger, J. Willomitzer, R. Meinsma, M. Alders, J. Meijer, R. C. M. Hennekam, A. B. P. van Kuilenburg and M. Huemer declare that they have no relevant conflict of interest.

Details of the Contributions of Individual Authors

M. Fleger, M. Huemer and A. B. P. van Kuilenburg designed the research and drafted the manuscript. R. Meinsma, M. Alders, J. Meijer and R. C. M. Hennekam performed the experiments and critically revised the manuscript. J. Willomitzer provided clinical data and revised the manuscript.

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