CASE REPORT

Diagnosis of dihydropyrimidine dehydrogenase deficiency in a neonate with thymine-uraciluria

Dihydropyrimidine dehydrogenase deficiency is an inborn error of pyrimidine metabolism characterised by thymine-uraciluria, convulsive disorders and developmental delay in paediatric patients, and an increased risk of toxicity from 5-fluorouracil treatment. This report is of the first patient with dihydropyrimidine dehydrogenase deficiency diagnosed in Hong Kong. The patient was a 2-day-old male neonate of Pakistani origin who presented with convulsions. Diagnosis was made by gas chromatographic-mass spectrometric detection of thymine-uraciluria and by molecular detection of a G to A point mutation in a 5'-splicing site leading to skipping of exon 14 in the DPYD gene of chromosome location 1q22. The results showed that the patient and his mother were homozygous and the father heterozygous for the splice site mutation. The mother also had thymine-uraciluria but was clinically asymptomatic.

Introduction

Dihydropyrimidine dehydrogenase (DPD, enzyme commission 1.3.1.2) is the initial and rate-limiting enzyme in the metabolism of pyrimidine bases. This enzyme catalyses the reduction of thymine and uracil to β-aminoisobutyric acid and β-alanine. Dihydropyrimidine dehydrogenase may have important roles for neurodevelopment as this pathway is the only endogenous source of the neurotransmitter β-alanine.1 Dihydropyrimidine dehydrogenase deficiency is an autosomal recessive disorder that was first described in paediatric patients with thymine-uraciluria.2 Phenotypic variation is large, with convulsive disorders, microcephaly, motor retardation, and mental retardation the most frequent manifestations.3

Dihydropyrimidine dehydrogenase is also important in that it is responsible for the degradation of more than 80% of 5-fluorouracil (5-FU), a pyrimidine analogue.4 Studies have shown that DPD deficiency causes severe toxicity with standard therapeutic doses of 5-FU.4,5 Complete or partial deficiency is estimated to be present in 3% of adults with cancer. Even in complete deficiency states, affected patients may be asymptomatic prior to 5-FU treatment.5

In DPD deficiency, seven different mutations have been reported, including one splice site G to A point mutation, two deletions, and four missense mutations. Analysis has shown that the splice site mutation is the most common (52%).5 This mutation leads to skipping of exon 14, which is located immediately upstream of the mutated splice donor site during the splicing of DPD mRNA.

Key words:
Fluorouracil;
Infant;
Purine-pyrimidine metabolism, inborn errors;
Thymine-uraciluria

二氨嘧啶脱氨酶缺乏是一種先天性嘧啶新陳代謝缺陷，在小童患者中會呈現胸腺嘧啶一尿嘧啶，痙攣性癲癇和發育遲緩的特徵，並會增加5-氟尿嘧啶治療的毒性。本報告報導在香港診斷的首名二氨嘧啶脫氨酶缺乏患者。患者是一名巴基斯坦籍的兩天大男嬰，求診時呈現抽搐徵狀。由胸腺嘧啶一尿嘧啶的氣相質譜測定，以及在5'-節點中G到A點轉換導致損傷了染色體1q22位的DPYD基因中外頸子14。結果顯示患者對節點轉換，與其母親的是純合，而與其父親的是雜合。此外，診斷過程中發現患者的母親同樣有胸腺嘧啶一尿嘧啶，但並沒有出現臨床徵狀。

Introduc
As a consequence, a 165 bp fragment is lacking in the mature DPD mRNA, resulting in a deletion of 55 amino acid residues in the DPD protein. This case report is the first description of DPD deficiency occurring in Hong Kong.

**Case report**

A 2-day-old male neonate of Pakistani origin presented with hypotonia, seizures, and an in-sucking chest. The peripartum period was uneventful. The parents were cousins with a strong family history of consanguinity. Urinary metabolic screening for reducing substances, glucose, keto-acids, ketone bodies, and cystines were negative and the urine amino acid pattern was normal. Broad-spectrum metabolic profiling by gas chromatographic-mass spectrometric analysis detected a massive increase in thymine and uracil, however, with 5-hydroxymethyluracil, an abnormal metabolite, evident. At the age of 3 weeks, the same biochemical abnormalities were observed in this child. A tentative diagnosis of pyrimidine dehydrogenase deficiency was made.

Urine samples were collected for biochemical analysis from the patient’s 4-year-old sister and his parents. Both his sister and his mother had normal urine amino acid patterns but significant thymine-uraciluria. 5-Hydroxymethyluracil was detected. No abnormality was detected in the father’s urine.

Screening for the common G to A splice site mutation was performed on genomic DNA from peripheral blood specimens using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. The resulting fragments were separated in 2% agarose gel and visualised by ethidium bromide staining.

**Discussion**

The common G to A splice site mutation of the DPYD allele was detected in this Pakistani pedigree. Phenotypic variation was demonstrated in this family. Although the patient’s mother was homozygous for the splice site mutation, she was asymptomatic. The patient’s sibling had thymine-uraciluria, but appeared clinically unaffected. This large phenotypic variability has also been observed in other studies. It has been suggested that there may be a second gene linked to the DPYD allele which is required for full manifestation of DPD deficiency or that additional factors may be involved in determining clinical outcome. As DPD is required for the synthesis of β-alanine, the lack of sufficient dietary intake of this amino acid during an early developmental stage may contribute to the clinical abnormalities seen in patients who are DPD deficient.

The G to A splice site mutation has been found in a heterozygous state in 2.2% of Finnish and 2.7% of Taiwanese subjects. In addition, there appears to be some homogeneity for this mutation in northern Europe where most DPD deficiency patients are of Dutch origin (55%); in The Netherlands, screening for inborn errors of pyrimidine metabolism are included in the general screening programme. Another study has shown a heterozygous splice site mutation in 0.8% of Caucasians. The pedigree in this report is of Pakistani origin. Pakistani children who exhibit...
thymine-uraciluria and are homozygous for the splice site mutation have also been reported.\textsuperscript{3,6} This report highlights the importance of thorough metabolic investigation to establish a cause in children who present with convulsive disorders and developmental delay.

This exon 14 splice site mutation has also been suggested to be a common mechanism for DPD deficiency and severe 5-FU toxicity in patients with cancer.\textsuperscript{6,11} Even incomplete deficiency of DPD is sufficient to cause severe toxicity with 5-FU.\textsuperscript{6} It is therefore recommended that screening for DPD deficiency should be conducted before commencement of 5-FU therapy.\textsuperscript{6,11} While DPD deficiency is a rare inborn error of metabolism, the importance of DPD itself is increasingly recognised in the field of cancer therapy and pharmacogenomics.\textsuperscript{12} It has led to the development of DPD inhibitors, a new class of drugs that help to optimise 5-FU treatment.\textsuperscript{12,13} The potential role of pharmacogenomics lies in facilitating the development of new drugs, such as these, that increase the safety and efficacy of therapeutic drug use.\textsuperscript{14,15}

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References