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# Diagnosis of dihydropyrimidinase deficiency in a Chinese boy with dihydropyrimidinuria

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Dihydropyrimidinase deficiency is an autosomal recessive inborn error of metabolism characterised by the presence of dihydropyrimidinuria. Its clinical presentation is variable and has also been reported in asymptomatic subjects. We report the first case of dihydropyrimidinase deficiency in Hong Kong, which is also the first reported in a Chinese subject. The patient was a 32-month-old boy who presented with language development delay. Biochemical analysis confirmed markedly increased urinary excretion of dihydrouracil and dihydrothymine, whilst DNA testing confirmed that the patient was compound heterozygous for two missense mutations, one known (p.R302Q) and the other was novel (p.N16K).

## Introduction

Dihydropyrimidinase (DHP [enzyme commission 3.5.2.2]), also known as 5,6-dihydropyrimidine amidohydrolase, is the second enzyme in the three-step catalytic pathway of pyrimidine uracil and thymine. It catalyses the reversible hydrolytic ring opening of 5,6-dihydrouracil and 5,6-dihydrothymine and converts them to N-carbamyl- $\beta$ -alanine and N-carbamyl- $\beta$ -aminoisobutyric acid, respectively. The enzyme is almost exclusively expressed in liver and kidney as a major 2.5-kb transcript and a minor 3.8-kb transcript.<sup>1</sup> The human DHP gene, *DPYS*, has been mapped to chromosome 8q22 by fluorescence in-situ hybridisation, and consists of 10 exons spanning more than 80 kb in the human genome.<sup>2</sup>

Dihydropyrimidinase deficiency (MIM 222748) is an autosomal recessive inborn error of pyrimidine metabolism characterised by the presence of dihydropyrimidinuria. The first case of DHP deficiency was first reported by Duran et al.<sup>3</sup> The patient was a Turkish male infant presenting with convulsion at 8 weeks after birth; at age 19 months the child appeared to be normal.<sup>3</sup> Up till now, this disease has been reported in over 20 patients with a variable clinical phenotype. Most DHP deficiency patients present with neurological disturbance, ranging from developmental delay,<sup>4</sup> mental retardation,<sup>4,5</sup> seizures,<sup>3,5</sup> hypotonia,<sup>5,6</sup> autism,<sup>5</sup> and a neurodegenerative clinical course.<sup>6</sup> Other presenting features included dysmorphic features,<sup>6,7</sup> growth retardation, failure to thrive, and a variety of gastro-intestinal problems.<sup>5,8</sup> On the other hand, DHP deficiency has also been reported in asymptomatic individuals.<sup>9-11</sup> In this case report, we describe the clinical, biochemical, and genetic findings of the first Chinese case of DHP deficiency.

### Key words

Alanine/urine; Amidohydrolases/deficiency; Liver/metabolism

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## Case report

Our patient was a 32-month-old Chinese boy born to healthy non-consanguineous parents. He was born at full term by elective caesarean section. His birth weight was 3.47 kg and his Apgar scores were 8 at both 1 and 5 minutes after birth. His postnatal course was unremarkable except for a transient period of neonatal jaundice beginning on day 3, and the peak bilirubin level of 254  $\mu$ mol/L, the level readily normalised after phototherapy. The patient was referred to Department of Paediatrics, Tseung Kwan O Hospital in January 2011 for delay in language development. At the age of 2 years, he still could not produce a single recognisable word. Motor development was appropriate for his age. Socially the child showed poor eye contact, but no restriction of other interests or ritualistic behaviour. On physical examination, his weight was at 75th centile, his height at the 97th centile, and his head circumference was in the 25th centile, all of which were normal and there were no dysmorphic features. Neurological examination yielded nil abnormal. Routine blood test showed that his complete blood picture, renal function test, blood gas, random glucose and ammonia levels were not abnormal. Liver function was unremarkable except for a mildly elevated alanine aminotransferase level of 78 U/L. Magnetic resonance imaging

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of the brain yielded no abnormality. Biochemical investigations for suspected inherited metabolic disease were performed. Urine organic acid analysis by gas chromatography–mass spectrometry showed markedly increased excretion of uracil, thymine, dihydrouracil, and dihydrothymine (Fig 1). Notably,  $\beta$ -alanine and  $\beta$ -aminoisobutyric acid were not detected in the urine amino acid analysis. Plasma amino acid analysis showed a normal pattern.

In view of biochemical diagnosis of DHP deficiency, genetic testing for *DPYS* mutations was performed on the patient and his parents after obtaining parental informed consent. The nine coding exons and flanking intronic regions of the *DPYS* gene were amplified by polymerase chain reaction (PCR) on the subject's genomic DNA from peripheral blood specimens, and direct sequencing of amplification products performed in both the forward and reverse directions. The PCR-amplified fragment sequences were compared with NCBI Reference Sequences, NG\_008840.1 and NM\_001385.2. Two missense mutations were found in the proband's DNA, a novel c.48C>G transversion that changes asparagine to lysine at codon 15 in exon 1 (p.N16K), and a c.905G>A transversion that

## 二氫嘧啶尿症華籍男童確診二氫嘧啶酶缺乏症

二氫嘧啶酶缺乏症指新陳代謝出現常染色體隱性先天性障礙，其一特徵是出現二氫嘧啶尿症。它的臨床表現多變，過去也有出現於無徵狀患者的病例。本文報告香港首宗二氫嘧啶酶缺乏症病例，也是華人首宗病例報告。患者為32個月大的男童，因語言發展遲緩入院。生物化學分析證實二氫尿嘧啶和二氫胸腺嘧啶的泌尿排泄明顯增加。DNA測試證實患者的兩種基因複合雜合錯義突變：已知的p.R302Q和新型的p.N16K。

changes arginine to glutamine at codon 302 in exon 5 (p.R302Q) [Fig 2]. Each parent was heterozygous for one of these two mutations (N16K in the mother and R302Q in the father), indicating that the proband is a compound heterozygote.

## Discussion

Deficiency of DHP is a rare inborn error of the pyrimidine degradation pathway, with only around 20

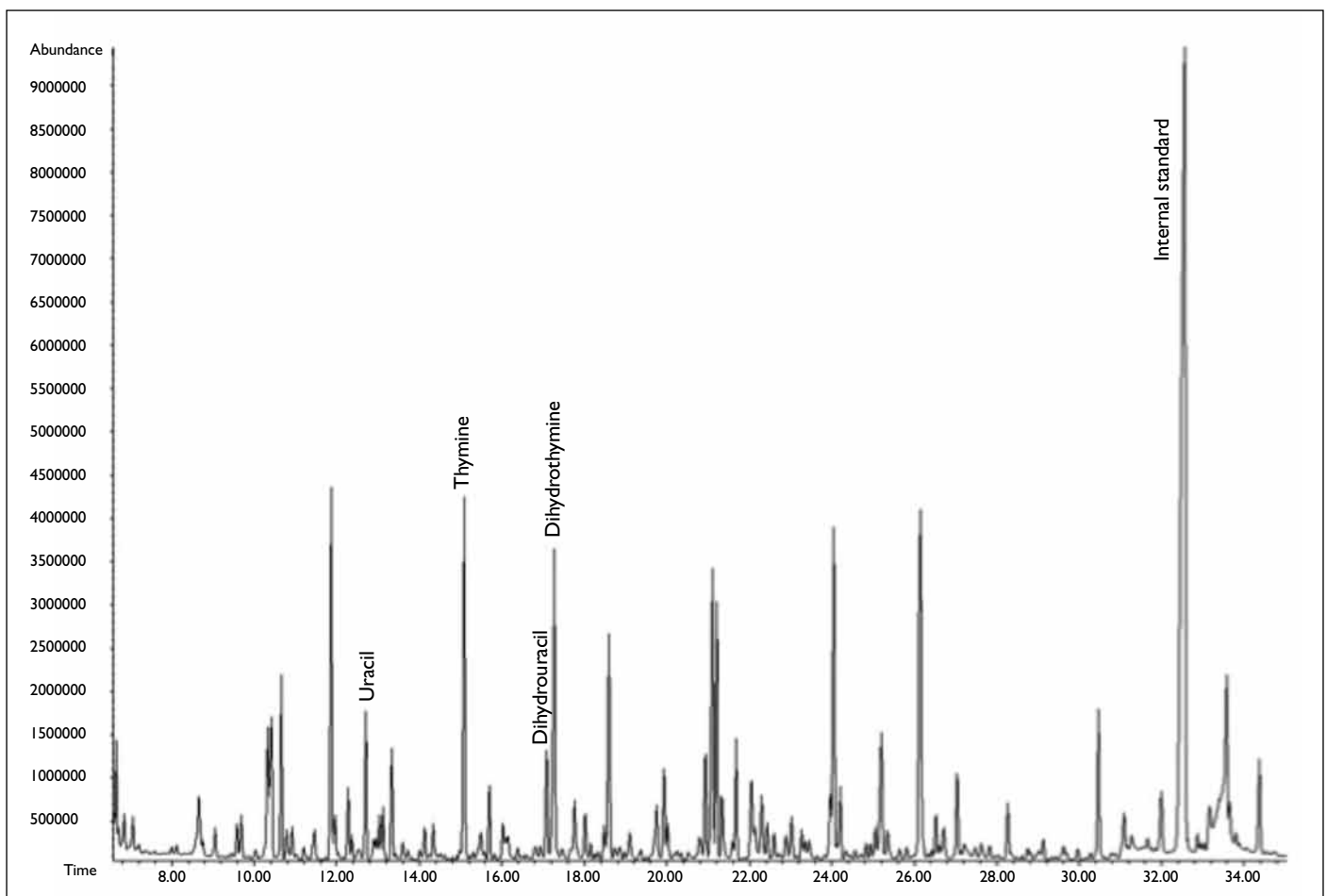


FIG 1. Chromatogram of urine organic acid analysis showing markedly elevated levels of uracil, thymine, dihydrouracil, and dihydrothymine in urine

patients having been reported in literature. In a study by Sumi et al,<sup>11</sup> after analysing urine samples from 21 200 healthy Japanese infants, two asymptomatic cases of dihydropyrimidinuria were encountered. The authors suggested that in Japan, the estimated prevalence of the deficiency was approximately 1/10 000.<sup>11</sup> The clinical phenotype of patients with DHP deficiency is highly variable. Notably, this disorder has also been reported in asymptomatic individuals identified by population screening for pyrimidine metabolism disorders<sup>9,10</sup> or family screening of relatives of DHP deficiency patients.<sup>5,11</sup> Furthermore, it has been demonstrated by family studies that siblings who shared the same *DPYS* mutations as the clinically affected index patient can be asymptomatic, suggesting that additional environmental factors may be involved in triggering the clinical phenotype. Also, a direct causal relationship between the genetic and biochemical changes in DHP deficiency and various clinical phenotypes, such as developmental delay as in our patient, cannot be definitively proven. This information too should be conveyed to the patient's parents during genetic counselling.

The pathophysiological mechanism of the various clinical features of DHP deficiency is not completely understood. The pyrimidine metabolic pathway is involved in the biosynthesis of both  $\beta$ -alanine and  $\beta$ -aminoisobutyric acid; the former was a structural homologue of  $\gamma$ -aminobutyric acid and glycine, and the latter a partial agonist of the glycine

receptor. Thus, pyrimidine metabolism is implicated in regulating neurotransmission in the central nervous system, and it has even been postulated that a decrease in  $\beta$ -alanine and  $\beta$ -aminoisobutyric acid may be responsible for the neurological manifestations of DHP deficiency.<sup>12,13</sup>

Our case was found to have compound heterozygote for mutations c.48C>G (p.N16K) and c.905G>A (p.R302Q). Analysis of the crystal structure of human DHP showed that the point mutation p.R302Q prevents oligomerisation of DHP subunits and formation of an enzyme homotetramer. Functional analysis has also confirmed that mutant DHP enzymes containing the p.R302Q have only 3.9% residual activity.<sup>5</sup> The other mutation c.48C>G (p.N16K) was novel and changed a highly evolutionarily conserved asparagine to lysine at codon 16. We have performed a *DPYS* gene study on 150 ethnically matched normal control subjects, in whom this mutation was not found. Also, computational analysis using PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), which predicts damaging effects of missense mutations, indicated that this novel mutation was 'probably damaging' with a score of 1.000 using the HumDiv dataset. All these findings suggest that the novel mutation c.48C>G (p.N16K) is damaging to the DHP enzyme function.

Currently, no specific treatment is available for DHP deficiency. However, identification of this disorder is crucial, because patients with inborn

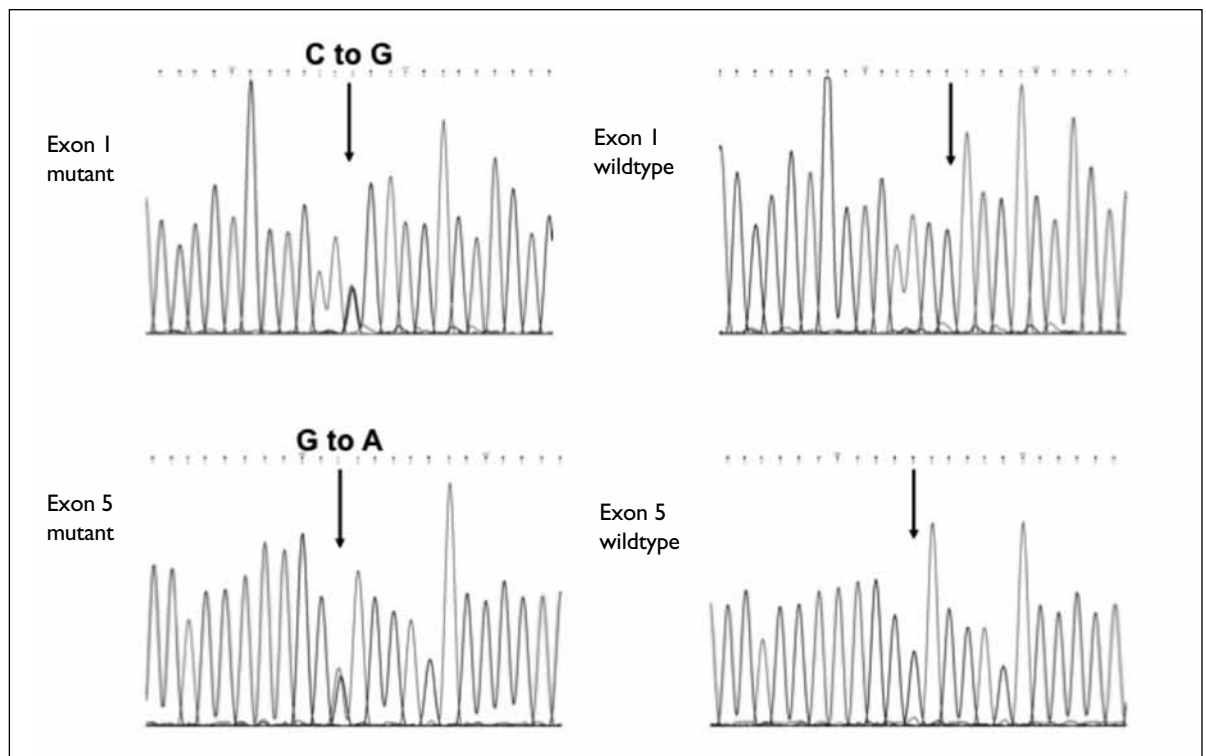


FIG 2. Direct sequencing chromatograms of *DPYS* sequence alterations in exon 1 and exon 5

error of pyrimidine metabolism cannot metabolise fluoropyrimidine chemotherapeutic agents, such as 5-fluorouracil (5-FU) and its prodrug capecitabine. It has been reported that partial-to-complete deficiency of dihydropyrimidine dehydrogenase (the first enzyme in the three-step pyrimidine catalytic pathway) accounted for up to 43% of patients with 5-FU-related toxicity.<sup>14</sup> The contribution of this deficiency in 5-FU toxicity has also been increasingly

recognised. In a case report, severe 5-FU toxicity was attributed to a partial deficiency of DHP due to heterozygous missense mutation in *DPYS* gene, which illustrates the importance of identifying not only DHP deficiency patients, but also carriers.<sup>15</sup> Dihydropyrimidinase deficiency should be regarded as a model of how knowledge in pharmacogenomics translates to improving clinical outcomes by reducing serious adverse drug reactions.

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