

# A novel duplication at the putative DNA polymerase alpha arrest site and a founder mutation in Chinese in the *IVD* gene underlie isovaleric acidaemia

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Isovaleric acidaemia is a rare inherited organic acidaemia associated with a characteristic odour in affected patients. Fewer than 40 causative mutations have been reported to date. We report a case in a Hong Kong Chinese neonate who presented with respiratory distress and acute encephalopathy requiring aggressive resuscitation and treatment. Residual gross motor developmental delay was still observed at the age of 16 months. The child was subsequently found to harbour a known missense mutation (c.A1199G [p.Y371C]) and a novel 4-bp duplication (c.1148\_1151dupGCTA [p.Y355X]) in the *IVD* gene. We suggest that the former is a founder mutation in the Chinese population and propose an explanation for the duplication event. Strategies that may achieve early diagnosis and prompt treatment include raising awareness of this condition, implementation of a tandem mass spectrometry neonatal screening programme, and local acquisition of appropriate medications for these metabolic diseases.

## Introduction

Isovaleric acidaemia (IVA, MIM #243500) is an autosomal recessive inborn error of metabolism. It is caused by a defect in isovaleryl-CoA dehydrogenase, a mitochondrial matrix enzyme that catalyses the oxidation of isovaleryl-CoA to 3-methylcrotonyl-CoA in the leucine degradation pathway. Deficiency of this enzyme leads to an accumulation of isovaleryl-CoA and its toxic metabolites. Patients with IVA typically present during the neonatal period with acute encephalopathy, vomiting, dehydration, severe metabolic acidosis, and a disturbed mental status.<sup>1</sup> Estimated to occur less frequently than other organic acidaemias, the reported incidence of IVA ranges from 1 in 250 000 to 1 in 62 500 in non-Chinese populations,<sup>2,3</sup> and is 1 in 360 000 in Taiwanese.<sup>1</sup> One suspected case was reported in Hong Kong in 1997,<sup>4</sup> but there have been no confirmed cases and the local incidence is largely unknown. To date, fewer than 40 mutations causing this rare condition have been reported worldwide and most have been point mutations or single-nucleotide insertions/deletions.<sup>1,5,6</sup> Here we report a case of IVA in a Hong Kong Chinese neonate who was compound heterozygous for a novel 4-bp duplication together with a missense mutation known to be common in other Chinese populations. We also propose a possible cause of the duplication event. Furthermore, we conclude that the missense mutation is likely to be a founder mutation in the Chinese population and should be screened for in every case occurring in Hong Kong.

### Key words

Founder effect; Gene duplication; Hong Kong; Isovaleryl-CoA dehydrogenase

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## Case report

Our patient was the first child born to non-consanguineous ethnic Chinese parents. He was delivered uneventfully at 38 weeks' gestation with a birth weight of 3.35 kg, was put on mixed feeding, and discharged home on day 3. Retrospectively, it was noted that he had been described as drowsy and feeding poorly since birth. He presented to the hospital on day 8 when his parents observed that he had dyspnoea and he was intubated on admission for respiratory distress. His level of consciousness had deteriorated and a characteristic disagreeable 'sweaty feet' odour was noted. Investigations revealed a high anion gap (20 mmol/L) metabolic acidosis (pH 7.25), hyperammonaemia (214  $\mu$ mol/L, reference range [RR], 56-92  $\mu$ mol/L), and hypocalcaemia with an ionised calcium level of 0.75 mmol/L (RR, 1.12-1.30 mmol/L). Pancytopenia developed soon after, with his haemoglobin level dropping from 168 to 121 g/L, platelet count from  $101 \times 10^9$ /L to  $10 \times 10^9$ /L, and neutrophil count from  $1.1 \times 10^9$ /L to  $0.1 \times 10^9$ /L within 1 day. Urinary metabolite screening by gas chromatography-mass spectrometry detected significant hyperexcretion of isovalerylglycine, 3-hydroxyisovaleric acid, 4-hydroxyisovaleric acid, and methylsuccinic acid. Liquid chromatography-tandem mass spectrometry also detected extremely high C5-acylcarnitine levels (9.7  $\mu$ mol/L; RR, 0.04-0.22  $\mu$ mol/L) together with a severe reduction in free carnitine level (8  $\mu$ mol/L; 12-46

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## 異戊酸血症中IVD基因內的DNA多聚酶alpha停滯位上的新型重複序列及華人患者的創造者突變

異戊酸血症是一種罕有的酸血症，患者一般會發出腳臭氣味。到目前為止只發現少於40個致病基因變異。本文報告一名香港華籍嬰兒出現呼吸窘迫症狀及急性腦部病變，需要積極急救與治療。他於16個月大的時候仍有殘餘運動功能發展遲緩的情況，最後發現嬰兒的IVD基因內有已知的錯義突變 (c.A1199G [p.Y371C]) 及新型的4-bp重複序列 (c.1148\_1151dupGCTA [p.Y355X])。我們認為這種錯義突變正是華人患者的創造者突變。本文並為該重複序列提出闡釋。要早期診斷並及時治療，必須提升對此病的認識，為新生兒提供串聯質譜檢測的篩檢計劃，並且隨時預備合適的藥物醫治這種新陳代謝疾病。

μmol/L) and other acylcarnitines in his plasma. A diagnosis of IVA was made. Computed tomography of his brain revealed small cerebellar haematoma, up to 1.9 cm in size. Levocarnitine was started on day 3 and parenteral nutrition supplying 0.5 g/kg/day of protein, 3 g/kg/day of lipids and 10% dextrose was given. The child was also given intravenous arginine and oral sodium benzoate, thiamine, riboflavin, and biotin. Double-volume exchange transfusions were performed 4 times within 24 hours aiming to eliminate the toxic isovaleric acid metabolites in his blood. The child responded gradually with both a clinical and haematological improvement. The specific treatment for IVA, L-glycine, was not available locally and thus had to be ordered urgently from Melbourne, Australia. It was given orally from day 17, and the parenteral nutrition was replaced by an oral milk formula with low leucine content. Clinical improvement was observed and he was extubated on day 19. The odour quickly subsided and was absent at the time of his discharge on day 32. At 10 weeks he was clinically well and active, with social smiling yet hypotonic with a severe head lag. Gross motor developmental delay was still noted when he was followed up at the age of 16 months. At the age of 16 months, a developmental assessment showed that he had normal cognitive function with a delay in gross motor aspects.

### Mutation analysis

Informed consent was obtained from the parents for mutation analysis. Genomic DNA was extracted, using a QIAamp blood kit (Qiagen, Hilden, Germany), from peripheral whole blood taken from the patient and his parents. For the patient, all coding exons with the flanking intronic regions of the IVD gene were amplified (primer sequences, polymerase chain reaction [PCR] mixture components and cycling conditions available upon request). Both strands were sequenced using the amplification primers

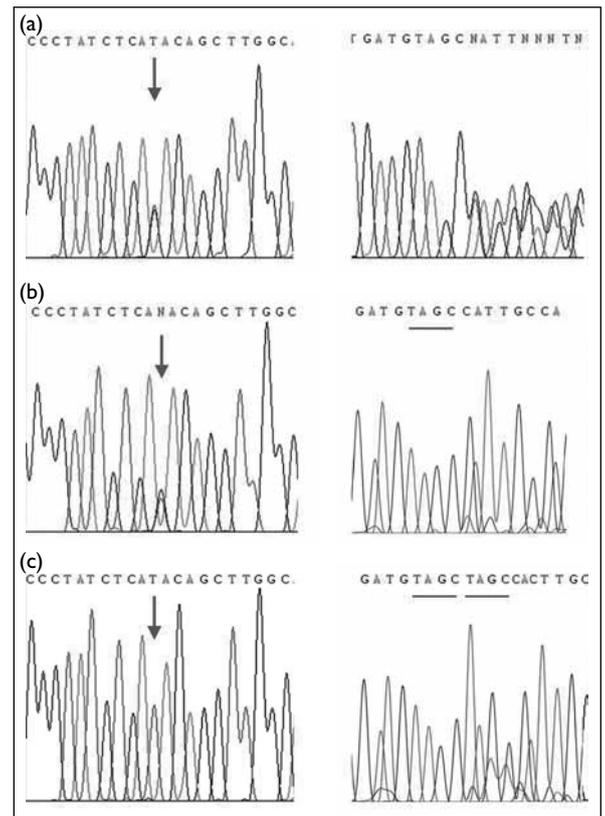


FIG. Segregation of the two mutations was demonstrated by sequencing with different primers

The duplication and missense mutation were detected on different alleles. Reverse complementary sequences are shown. (a) Sequenced with reverse amplification primer: Both mutations c.A1199G (left) and c.1148\_1151dupGCTA (right) appeared to be heterozygous in the patient. (b) Sequenced with primer 5'-GGTCCCAGCCCCTATCTCAC-3' (a primer annealing to c.A1199G). The allele without duplication is shown. (c) Sequenced with primer 5'-GGTCCCAGCCCCTATCTCAT-3' (a primer annealing to wild-type c.A1199). c.1148\_1151dupGCTA is shown

and BigDyeDeoxy terminator cycle sequencing reagents (Applied Biosystems, Foster City [CA], US). The sequencing reaction products were purified by Centri-Sep spin columns (Princeton Separations, Adelphia [NJ], US) and detected on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Compound heterozygosity was demonstrated by sequencing the PCR product of exon 12 with primers specific to wild-type and mutated missense sequences (5'-GGTCCCAGCCCCTATCTCAT-3' and 5'-GGTCCCAGCCCCTATCTCAC-3' respectively) annealing to the missense mutation site (Fig).

### Results

Two different mutations in exon 12, c.A1199G (p.Y371C) and c.1148\_1151dupGCTA (p.Y355X) were found in this patient. The former is a known disease-causing mutation but the latter has not been

reported before. No other unreported variation was detected in any *IVD* exon. The patient's father was found to be heterozygous for the missense mutation, and his mother was found to be heterozygous for the duplication.

By sequencing the PCR product of exon 12 with sequencing primers specific to wild-type and mutated missense sequences respectively, the two alleles were separated. It was shown that c.A1199G and c.1148\_1151dupGCTA were on different alleles in this patient (Fig).

## Discussion

While IVA is rare, early recognition and institution of treatment to remove toxic isovaleric acid is crucial to the outcome. We are presenting this case to alert clinicians to this disease. Our patient demonstrated classical IVA features: pancytopenia, hypocalcaemia, acidosis, and hyperammonaemia. The characteristic 'sweaty feet' odour should enable this condition to be easily recognised clinically. The advent of this case has made L-glycine available in Hong Kong, so new patients should benefit from this specific treatment, which combines with isovaleric acid to form isovalerylglycine, thus providing an alternate pathway for excreting the toxic metabolites.

Patients with IVA presenting with encephalopathy are at risk of adverse neurological outcomes due to the neurotoxicity of the metabolites.<sup>7</sup> An established neonatal screening programme could have detected this case before encephalopathy set in. Early institution of dietary and medical treatments could also have prevented his encephalopathy. This is supported by the experience of the tandem mass spectrometry neonatal screening programme in Taiwan.<sup>8</sup>

The missense mutation c.A1199G in *IVD* is a known disease-causing mutation and was a common finding in a previous study in Taiwan.<sup>1</sup> Its detection in our patient also suggests that it is a founder mutation in ethnic Han Chinese that is likely to be detected in other Chinese patients. On the other hand, c.1148\_1151dupGCTA, which produces a premature termination codon at the site of duplication in a strictly conserved region, has not been reported before. It is predicted that the resulting protein truncation will cause partial loss of the fatty-acyl binding pocket of *IVD* and therefore direct disruption of the catalytic function of the enzyme.<sup>9</sup>

Cooper and Krawczak<sup>10</sup> pointed out that an insertional mutation involving a sequence of less than 10-bp in a gene-coding region is non-random and highly dependent upon the local DNA sequence context. Although insertions occur much less

frequently than deletions, in principle they actually arise through similar mechanisms. Krawczak and Cooper<sup>11</sup> described a consensus sequence closely resembling DNA polymerase alpha arrest sites. They proposed that the arrest of DNA synthesis at the replication fork underlies both deletional and insertional mutagenesis,<sup>10,11</sup> possibly by increasing the probability of replication slippage errors or secondary structure formation. Many different insertions and deletions have been found to be associated with this consensus sequence TGRKRM. In our case, it was noted that TGGCTA (or TGTAGC on the reverse strand) at the site of duplication closely resembled the consensus sequence. It was thus likely that the duplication event occurred at this site when DNA synthesis was arrested in exon 12 in *IVD*.

In our case, both mutations were located in exon 12 of *IVD* (Fig). Compound heterozygosity is often confirmed by analysing parental samples. When two heterozygous mutations are located in close proximity, eg in the same exon, as in our patient, segregation of the two mutations on separate chromosomes can also be demonstrated by using wild-type/mutation-specific sequencing primers annealing to the site of one of the mutations. In cases of compound heterozygosity, sequencing with the former primer (ie 5'-GGTCCCAGCCCCTATCTCAT-3') shows the other mutation (ie c.1148\_1151dupGCTA), and sequencing with the latter primer (ie 5'-GGTCCCAGCCCCTATCTCAC-3') shows the absence of the other mutation. This approach is much simpler, more economical and time-saving than the traditional microsatellite analysis, in which multiple additional primers and amplification steps are required.

In summary, we report a genetically confirmed case of IVA in a Hong Kong Chinese neonate who presented with respiratory distress and acute encephalopathy, with cerebellar haemorrhages evident on imaging. Mutation analysis served to confirm the diagnosis and enable DNA-based prenatal diagnosis in future pregnancies. It also provided new insights concerning the mutational spectrum in *IVD*. In Chinese patients, the proposed founder mutation, c.A1199G, should be screened for first, enabling simpler mutation analysis. Allele segregation was demonstrated by using wild-type/mutation-specific sequencing primers, a simple and economical method compared with the traditional microsatellite analysis. The long-term neurological sequelae suffered by this patient may have been prevented by increased awareness, a tandem mass spectrometry-based neonatal screening programme, and local availability of the target medication for this rare metabolic disease.

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