

## CASE STUDY

# A novel mutation in *SLC37A4* gene in a Sri Lankan boy with glycogen storage disease type Ib associated with very early onset neutropenia

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Glycogen storage disease type Ib (GSD Ib) [MIM 232220] is caused by mutations in the gene encoding microsomal glucose-6-phosphate translocase (G6PT) (Veiga-Da-Cunha et al, 1998). This solute carrier family 37, member 4 gene (*SLC37A4*) is located on chromosome 11q23.3 and encodes a 429 amino acid polypeptide (Veiga-Da-Cunha et al, 1998). This protein transports glucose-6-phosphate into the endoplasmic reticulum, where the enzyme glucose-6-phosphatase converts glucose-6-phosphate into glucose and inorganic phosphate. Mutations in *SLC37A4* result in accumulation of glucose-6-phosphate in cells. The *SLC37A4* gene has 9 exons and so far 83 mutations have been reported (Human Gene Mutation Database, 2011). Here, we report the identification of a novel mutation in the *SLC37A4* gene in a Sri Lankan child and the associated clinical phenotype.

This child was a three month old baby boy – the first child born to a consanguineous Sinhalese couple. The child had had pyrexia and umbilical sepsis few days after birth and recurrent, generalized pustular skin eruptions on and off since then. His abdomen was protruding. He had had persistent neutropenia, confirmed by haematological tests from the age of 3 weeks. Physical examination showed a grossly enlarged liver. He weighed 5.8Kg (Birth weight: 3.6Kg). The weight was within the 50th percentile.

Investigation findings were as follows: Fasting blood glucose: 66mg/dl (reference interval: 65-110mg/dl); plasma lactate: 89.8mg/dl (reference interval: 4.5-19.8mg/dl); serum uric acid: 8.54mg/dl (reference interval: 2.4-7.0mg/dl); serum triglycerides: 1236mg/dl (reference interval: 10 -200mg/dl); serum cholesterol: 242mg/dl (reference interval: 140-239 mg/dl); Alanine transaminase (ALT): 225.3U/l (reference interval: <40U/l); Aspartate transaminase (AST): 566.5U/l (reference interval: <40U/l),

Alkaline phosphatase: 795.5U/l (reference interval: 98-279U/l) and Gamma glutaryl transferase: 960.1U/l (reference interval: 7-50U/l).

The neutrophil count in the peripheral blood was frequently fluctuating between  $0.2 \times 10^3/\mu\text{l}$  and  $0.9 \times 10^3/\mu\text{l}$  (reference interval:  $1.5-9.0 \times 10^3/\mu\text{l}$ ) throughout this period. Bone marrow biopsy and peripheral blood picture were otherwise normal except for the low neutrophil counts. Based on the above clinical and laboratory findings, a clinical diagnosis of GSD type I was determined. First mutation analysis of the glucose-6-phosphatase (G6PC) gene was undertaken to confirm/exclude GSD Ia. No mutations were found in the *G6PC* gene. This was followed by screening for mutations in *SLC37A4* gene to confirm/exclude GSD Ib. Genetic testing in both instances was done after obtaining written informed consent from the parents. Genomic DNA was extracted from the child's peripheral venous blood. All the exons and intron-exon boundaries of the *G6PC* and *SLC37A4* genes were analyzed. Direct sequencing of PCR products was performed on both forward and reverse strands using the same primers for PCR. Cycle sequencing was performed with a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). The sequence was read following capillary electrophoresis on an ABI 3130 Genetic Analyzer (Applied Biosystems). The sequence was compared with the reference sequence (NG\_013331.1 RefSeqGene) in the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>). Detailed methods can be provided on request.

Sequence analysis showed that codon 50 of the *SLC37A4* gene was converted from GGG to GAG by the substitution of a Guanine nucleotide by an Adenine nucleotide (Figure 1).

This results in the substitution of Glycine (G) by Glutemic acid (E) in the amino acid sequence of the G6PT protein [p.G50E].

GSD type I or von Gierke disease includes a clinically, biochemically, and genetically heterogeneous group of autosomal recessive disorders. The basic defects reside in the impairment of the terminal steps of glycogenolysis and gluconeogenesis, at different levels. Mutations of the *G6PC* gene are responsible for the most frequent form of GSD type I, the subtype Ia. In GSD Ia, the white blood cell count is generally within reference ranges because leukocyte function is unaffected by the defect. In contrast, chronic neutropenia due to impaired function of neutrophils, is found in GSD Ib (Visser et al, 2000). The clinical manifestations of GSD type I include growth retardation, hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, and hyperlipidemia. In addition, patients with GSD Ib commonly have infectious complications which are caused by neutropenia and dysfunction of neutrophils. The diagnosis of GSD type I is based on its clinical presentation; *i.e.*, abnormal plasma concentrations of glucose, lactate, uric acid, triglycerides, lipids; liver biopsy to measure enzyme activity; and molecular genetic testing (Visser et al, 2000).

This child had all the clinical and biochemical parameters suggestive of GSD Ia, except persistent neutropenia. Individuals with GSD Ia who are homozygous for the p.G188R mutation in the *G6PC* gene have been reported with GSD Ib like phenotype with neutropaenia (Weston et al, 2000). This child was tested for mutations in the *G6PC* gene to exclude this possibility. In untreated patients with GSD Ib, neutropenia usually manifests after about 1 year of age (Weston et al, 2000). In this child however, neutrophils were significantly reduced, with recurrent skin sepsis almost from birth.

In summary, we identified a missense mutation in the *SLC37A4* gene [p.G50E] in a Sri Lankan child with clinical and laboratory findings suggestive of the diagnosis of GSD

Ib. This is a new mutation which has not been described before in other children with GSD Ib and it appears that it is associated with very early onset neutropaenia.

## ACKNOWLEDGEMENTS

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## COMPETING INTERESTS

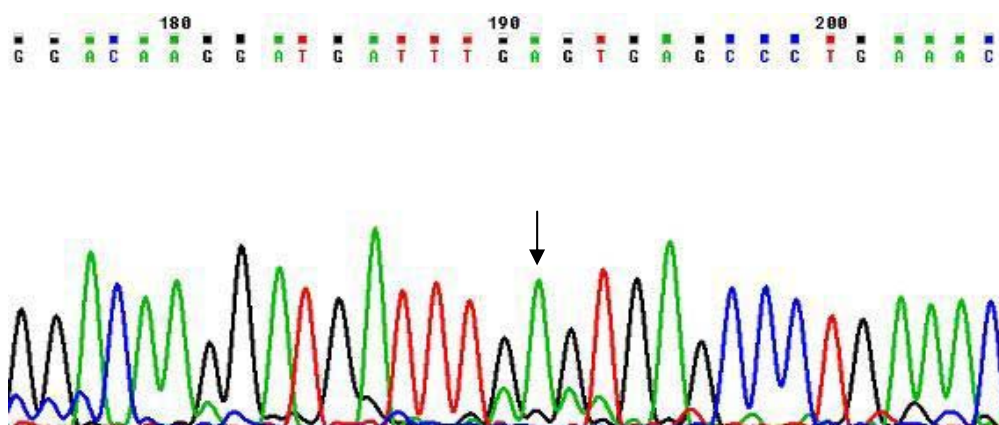
None declared.

## LIST OF ABBREVIATIONS

GSD - Glycogen Storage Disease  
G6PC - Glucose 6-phosphatase  
G6PT - Glucose-6-Phosphate Translocase  
SLC37A4 - Solute Carrier Family 37, Member 4 Gene

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**Figure 1.** Electropherogram of the sense strand nucleotide sequence of the *SLC37A4* gene. The arrow indicates Guanine → Adenine substitution at codon 50 in exon 1, resulting in the p.G50E missense mutation.