LETTER TO THE EDITOR

Severe neonatal jaundice due to a de novo glucose-6-phosphate dehydrogenase deficient mutation

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency worldwide and has long been associated with reduced-efficiency erythrocytes [1]. Acute haemolysis induced by exposure to oxidative stress, related to infection, drugs or fava bean ingestion, is the most frequent clinical presentation; however, G6PD class 1 deficiency causes chronic haemolysis and could be a major contributor to neonatal hyperbilirubinemia [2]. To date, various different mutations in G6PD gene have been identified, most frequently on exon 10 and a minor cluster on exons 6, 7 and 8 [3]. Most of these mutations lead to amino acid replacements close to the dimer interface within the 'structural' NADP+-binding site, suggesting that the integrity of these regions is very important for the formation of stable dimers [4, 5]. The present letter reports a case of a newborn infant with severe class 1 G6PD deficiency associated with a de novo G6PD mutation.

The patient was a male, born at 39 weeks by Caesarean section (second pregnancy). The parents of Spanish origin were nonconsanguineous, and no family members had a history of anaemia or jaundice. A few hours after birth, he developed clinical respiratory distress accompanied by skin pallor, jaundice, hepatomegaly and splenomegaly. Blood tests showed a haemoglobin (Hb) level of 90 g/L, total bilirubin level of 11.2 mg/dL (normal 1–12 mg/dL) and direct bilirubin level of 4.4 mg/dL. A peripheral blood smear did not reveal spherocytes. Phototherapy and an exchange transfusion were needed at 15 h of life to treat severe hyperbilirubinemia (17.4 mg/dL). The patient was discharged after 21 days, with a probable diagnosis of ABO incompatibility (proband blood group A RhD+, mother: O RhD+, slightly positive direct antiglobulin test).

At 6 weeks of life, follow-up blood tests showed persistence of regenerative anaemia with Hb 72 g/L, reticulocyte count 328 9 103/lL, total bilirubin 0.5 mg/dL, lactate dehydrogenase 213 U/L (5–510), haptoglobin 58 mg/dL (50–200) and no spherocytes, and signs of oxidative stress or other pathological findings were observed in the blood smear. Analysis of enzyme activity showed reduced G6PD activity (145 mU/109 red blood cells; normal value: 221–570 mU/109 red blood cells). The direct antiglobulin test was negative, neurological status was normal and physical examination did not reveal splenomegaly. Subsequently, baseline Hb levels stabilized at 90 to 120 g/L and reticulocytes at 180 to 320 9 103/lL, while bilirubin levels normalized without transfusions, also a second assay of G6PD activity confirmed severe deficiency (30 mU/109 red blood cells). At 9 months old, the patient was admitted to hospital with haemolytic anaemia (Hb 55 g/L, reticulocytes 196 9 103/lL, lactate dehydrogenase 2250 U/L, total bilirubin 1.2 mg/dL) secondary to an infectious process and received a single-unit red blood cell transfusion.

Genetic studies were performed after informed consent. Genomic DNA was extracted from peripheral blood of the proband and mother, using standard methods. The G6PD gene was amplified by polymerase chain reaction from exon 2 to 13, amplified in genomic DNA of the proband and mother, and analysed by direct sequencing with the dideoxy chain termination reaction using an ABI PRISM 3130 Genetic Analyser and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequencing analysis of the G6PD gene on the proband revealed a previously described cytosine-to-thymine transition at position c.827 on exon 8, resulting in an amino acid change (proline to leucine) at the residue 276 (c.827C>T; p.Pro276Leu). This mutation was absent in genomic DNA from the proband's mother. The degree of mosaicism for the identified mutation was assessed by bidirectional sequencing in independent DNA samples from the proband obtained from peripheral blood containing EDTA buccal cells and hair. Sequencing confirmed the results previously obtained

with the peripheral blood DNA and did not reveal a double peak at the mutation site (Figure 1). Human leukocyte antigen – short tandem repeat markers were analysed in the proband and his mother using Luminex (Gen-Probe Transplant Diagnostics, Stamford, CT, USA) to confirm the mother-child relationship.

The c.827C>T mutation being observed in the proband and absent in his mother suggests that this is a spontaneously occurring new genetic alteration. The absence of mosaicism in the proband DNA samples suggests that this "de novo" event could have occurred in the very early stages of embryogenesis or in the mother's germ cell lines. Pro276 is a strictly conserved residue located in the loop between helices i2 and j, located in the a + b subdomain of G6PD, within the structural NADP+ binding region (Figure 2). This residue is exposed at the dimer interface, in a region where several variants associated with class 1 G6PD deficiency are clustered [6]. The loop where Pro276 is located stacks against a long loop in the adjacent monomer that connects strands K and L from the central antiparallel b-sheet, which harbours the structural NADP+ binding site (Figure 2). In particular, Pro276 establishes van der Waals contacts with the side chain of Ile380. The presence of a conserved proline at this position is likely to be required to fix the conformation of this loop in a position that favours conserved polar interactions with the adjacent monomer (e.g. Lys275 interaction with the main chain of Ala377 and Gly378). This mutation, p.Pro276Leu, will presumably interfere with G6PD dimer stability, as insertion of a larger leucine residue at this position would cause steric hindrance forcing the loops on adjacent monomers to move apart. This same G6PD mutation c.827C>T (p.Pro276Leu), denominated variant Hamburg, was previously described in a Caucasian neonate with severe neonatal cholestasis and chronic nonspherocytic haemolytic anemia [7]. In addition, a variant called G6PD Sugao c.826C>T (p.Pro276Ser) was previously described affecting the same amino acid in a G6PD-deficient patient with severe jaundice and anaemia at birth, requiring transfusions [6]. In both cases, the mothers of the proband were heterozygous for that specific mutation. Neonatal indirect hyperbilirubinemia is an emergency requiring prompt therapeutic attention and can be due to rare sporadic G6PD mutations. For this reason, clinicians should be aware of this aetiology.

References

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Legends

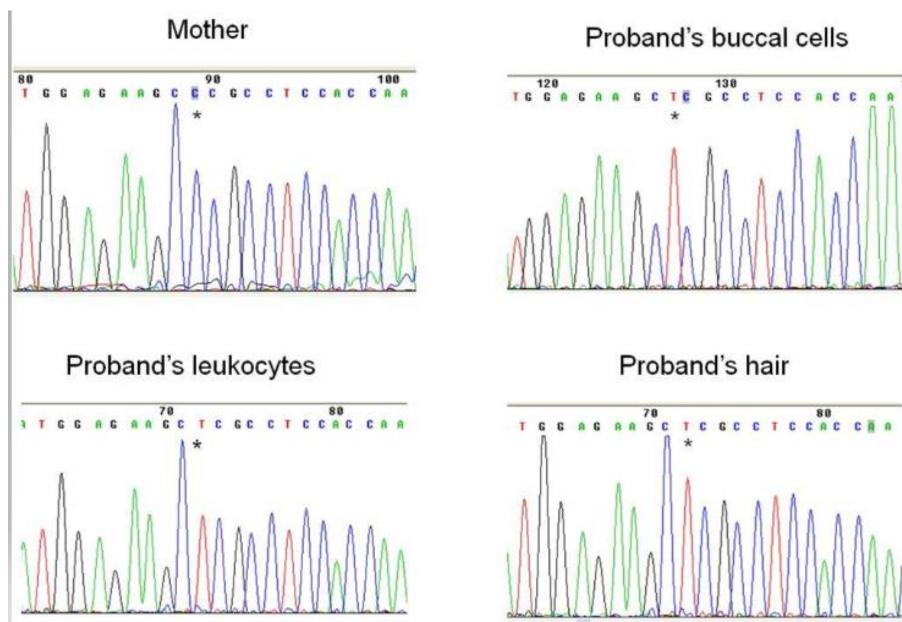

Figure 1. Sanger sequencing of genomic DNA obtained from probands leukocytes, buccal cells and hair and from mother's leukocytes. Note the nucleotide substitution at 827 position marked by a black star (wild type Citosine by Timine). Sequence did not reveal mosaicism in the proband.

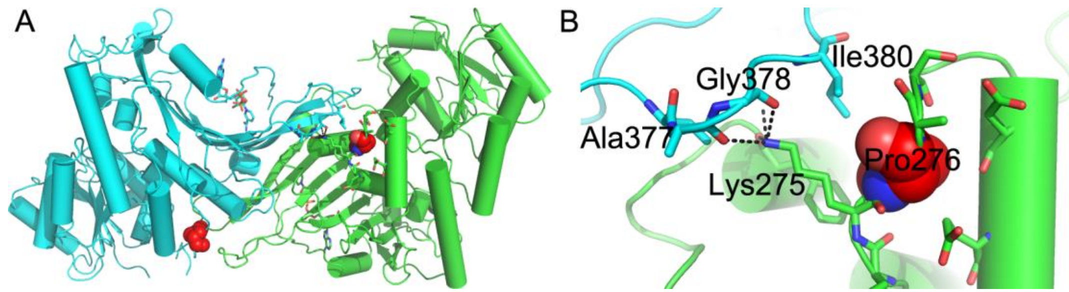

Figure 2. Structure of the human G6PD dimer. (a) Overall cartoon representation of G6PD dimer (G6PD subunits are coloured in green and blue). The mutated residue Pro276, is located at the intermonomer interface and is represented as red spheres). The structural NADP+ is shown as stick models. (b) Close view of the mutation site, showing the interacting residues in adjacent subunits. Hydroden bonds are represented as black dashed lines. The figure was prepared with PyMOL (The PyMOL Molecular Graphics System, Version 1.7.4 Schrodinger, LLC.) using the co-ordinates of the human G6PD Canton mutant 1QKI [5].