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CORRESPONDENCE

A Novel G6PD p. Gly 321 Val Mutation Causing Severe Hemolysis in an Indian Infant

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Dear Editor,

An 11-month-old baby boy belonging to the Brahmin community from Bhavnagar district of Gujarat, born in non consanguineous marriages was referred to us with a Hb-7.3 g/dL, reticulocyte count-20.0% and a history of jaundice, sepsis and recurrent blood transfusions. He was given double exchange blood transfusion for treating neonatal jaundice. His C-reactive protein level was high (46.8 mg/L), direct and indirect Coombs tests, osmotic fragility and heat stability tests were negative. The peripheral blood smear showed anisopoikilocytosis, polychromasia, microspherocytes, target cells, tear drop cells, few fragmented cells, adequate platelets with increased eosinophil (8%).

Hemoglobin analysis on HPLC did not show the presence of hemoglobinopathies (Hb A_2: 2.9%, Hb F: 1.7%). Flow cytometric analysis using eosin-5-maleimide dye suggested the absence of red cell membrane protein defect. Screening for enzymopathies showed the presence of G6PD deficiency (0.3 IU/g Hb, 3.3% of normal). Subsequently, family studies suggested that father was G6PD deficient (0.9 IU/g Hb, 9.9% of normal) while the mother (2.59 IU/g Hb, 28.4% of normal) and an older sister (2.8 IU/g Hb, 30.8% of normal) showed intermediate activity. Mutational analysis was carried out by Sanger sequencing using the Big Dye Terminator v3.1 Cycle Sequencing kit on a 3730xl DNA analyzer (Applied Biosystems). It showed the presence of a novel G6PD c. 962 G → T mutation in exon 9 which changes the amino acid from glycine to valine at position 321 in the proband and mother while the father and sister had G6PD Jammu (c. 871 G→A) mutation. Subsequently, we sequenced 50 healthy individuals from the same ethnic group. This mutation was not found in any of them, indicating that it was unlikely to be a polymorphism. The biochemical characteristics of the novel G6PD mutated enzyme showed increased Km for G6P, NADP and substrate analogue utilization with decreased thermostability and a bimodal pH curve (Fig. 1) along with normal electrophoretic mobility and classified as Class I variant.

Since this mutation has been reported for the first time and the individual has been originated from Bhavnagar district of Gujarat, hence it was named G6PD Bhavnagar. PolyPhen (Phenotyping polymorphism) software version 2.2 and Sorting Intolerant from tolerant (SIFT, version 6.2.1) software predicted the score of 1.00 and 0.01 respectively which suggests that the novel mutation may have a damaging effect on the protein structure as the mutated amino acid position falls in the evolutionary conserved region. Further, the stability of the p. Gly321Val protein was found to be decreased by using I-Mutant software. The computer based protein modeling on existing G6PD crystal structure (PDB: 1QKI) showed that the G6PD Bhavnagar mutation lies exactly at the dimer interface (Fig. 2a). Valine, a nonpolar amino acid forms additional ionic interaction with D324 (aspartic acid) and restrict the freedom of movement at residue 324 leading to rigidity and subsequent instability of the enzyme as compared to Glycine at position 321 (Fig. 2b, c). Thus, this variation makes the structure of the protein less flexible.

Glucose-6-phosphate dehydrogenase deficiency is the most common erythroenzymopathy affecting around 10%

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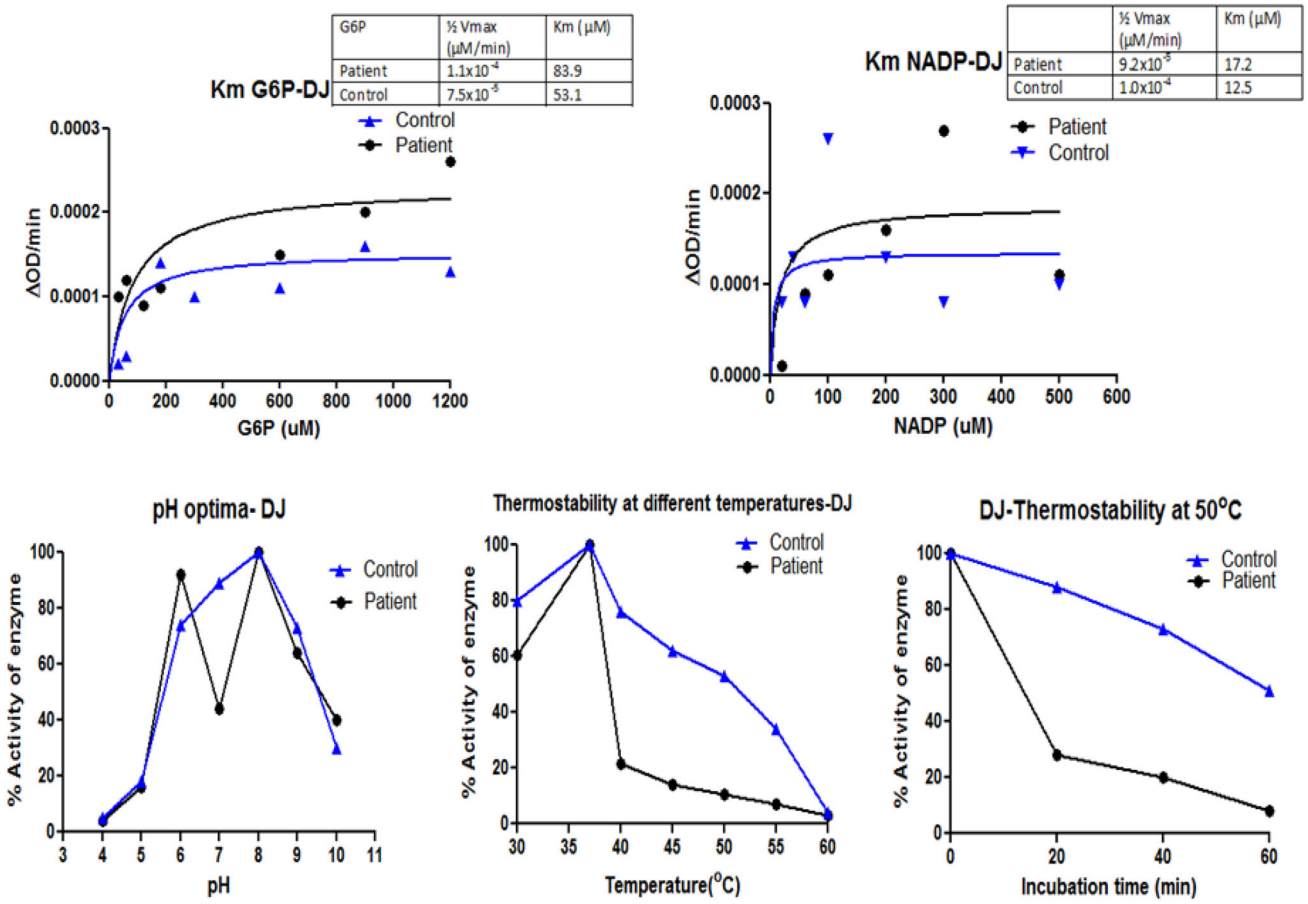

Fig. 1 Kinetic characteristics of the novel variant G6PD (962G→T)

of the world population and characterised by wide clinical and biochemical heterogeneity. Mostly G6PD deficient individuals are asymptomatic, until and unless exposed to oxidative stress (drugs, infection etc.) which can induce hemolysis [1]. The most severe phenotypic expression of G6PD deficiency is chronic nonspherocytic hemolytic anemia (CNSHA).

G6PD Bhavnagar mutation at c. 962 G→T is an unreported electrophoreticaly normal variant in exon 9 with severe chronic non spherocytic hemolytic anemia. There are 14 biochemically characterised G6PD variants reported from Indian population. Of these, only G6PD Jamnagar showed normal electrophoretic mobility with 23.5% enzyme activity of normal, with low Km for G6P, biphasic pH optima, much decreased thermostability and normal rate of substrate analogue and was classified as a Class III variant. Although G6PD Bhavnagar showed 3.3% enzyme activity of the normal however, its substrate affinity was found to be low as compared to G6PD Jamnagar which makes it a unique variant.

G6PD Oklahama, a Class I electrophoreticaly normal variant has similar biochemical properties with G6PD Bhavnagar except for the utilization of the substrate analogue. Other variants that have similar biochemical properties with G6PD Bhavnagar but differ in electrophoretic mobility are G6PD Riverside (Class I), G6PD Adana (Class II) and G6PD Kyoto (Class I). G6PD Frankfurt, a Class III electrophoretically normal variant shared similar features with G6PD Bhavnagar except for normal values of Km NADP [2].

A total of 14 mutations have been reported in exon 9 and five of them are associated with CNSHA [3]. Many mutations encoding class I variants directly affect the subunit interface of the G6PD dimer [4] and has the most deleterious effects on the structure and stability of protein. In the present case, the 3D structure of the novel mutation p.Gly321Val suggests that the variant is located in a region of the G6PD dimer interface crucial for enzymatic activity and probably the changes in the local interactions of the protein chain are responsible for structure distortion which further causes destabilization of the enzyme and hence severity.

In conclusion, the cause of CNSHA in the present case was mainly due to a class I novel G6PD deficient variant which leads to enzyme instability. Hemolytic anemia due to hemoglobinopathies, erythroenzymopathies and

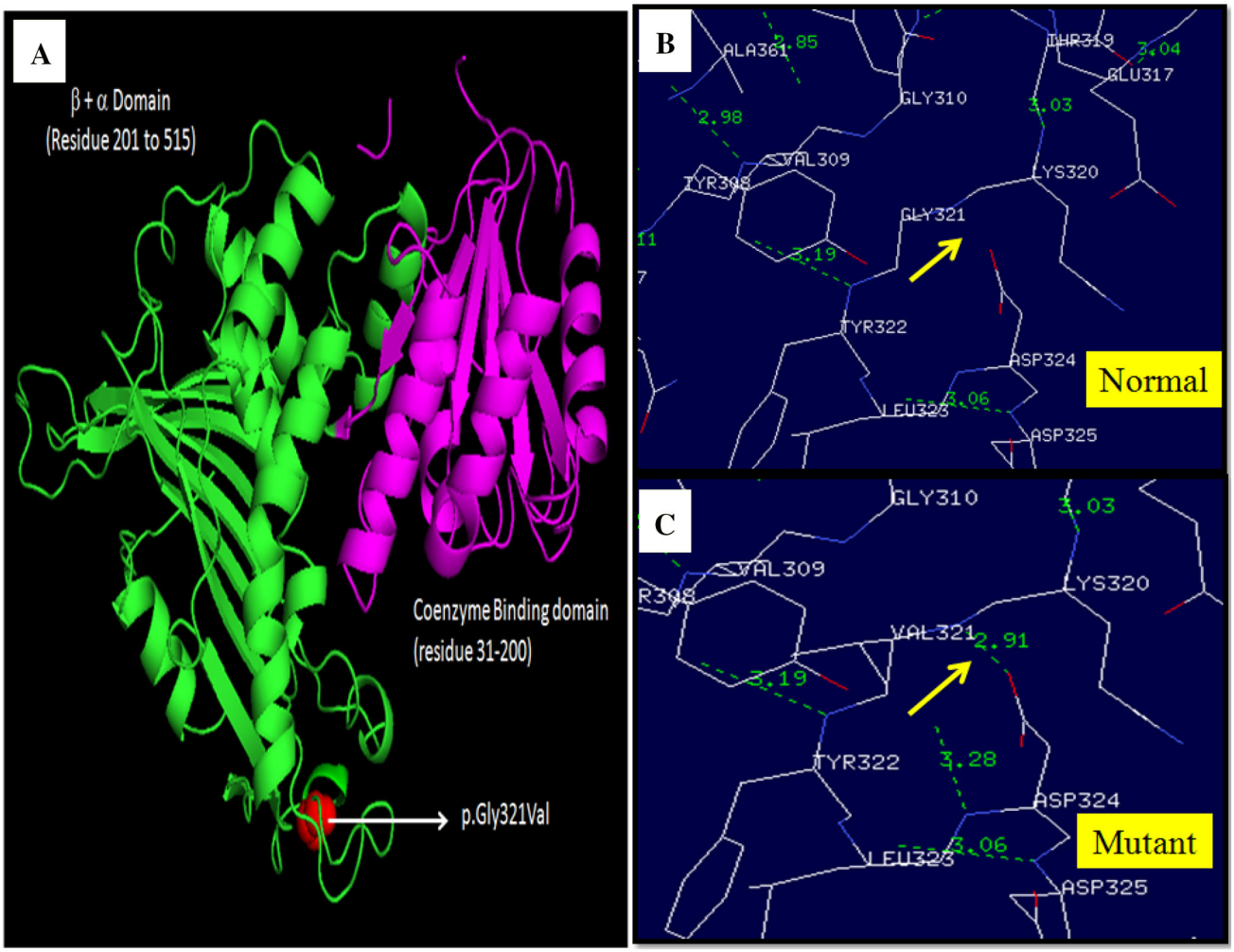

Fig. 2 X-ray crystallographic structure of human erythrocyte G6PD monomer generated from the atomic coordinates of the protein data bank entry 1QKI (PDB). The location of the mutation is shown in **a**. Representation of normal (**b**) and mutant (**c**) G6PD protein structural figure generated from the atomic coordination of Protein data bank entry 1QKI using the Swiss-PDB viewer (http://expasy.org/spdbv/)

membrenopathies are not uncommon in India and hence a systematic approach for screening should be undertaken. Molecular characterization leads to new insights in understanding the genotype–phenotype correlation and its probable protein conformation may help us to offer pre-natal diagnosis.

Compliance with Ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all the participants included in the study.

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