A Novel *de novo* Mutation in the *G6PD* Gene in a Korean Boy with Glucose-6-phosphate Dehydrogenase Deficiency: Case Report

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive hemolytic anemia caused by a mutation in the *G6PD* gene on Xq28. Herein, we describe a Korean boy with G6PD deficiency resulting from a novel mutation in *G6PD*. A 20-month-old boy with hemolytic anemia was referred for molecular diagnosis. He had no relevant family history. The G6PD activity was severely decreased at 0.2 U/g Hb (severe deficiency). Direct sequencing analyses on the *G6PD* gene revealed that he was hemizygous for a novel missense variant, c.1187C>G (p.Pro396Arg), in exon 10 of G6PD. Family study involving his parents revealed the *de novo* occurrence of the mutation. This is the first report of genetically confirmed G6PD deficiency in Korea.

Key words: glucose-6-phosphate dehydrogenase deficiency; *G6PD*; mutation; Korean.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect, affecting more than 400 million people worldwide [1]. G6PD is the first enzyme in the pentose phosphate pathway and provides an intracellular reducing source in the form of nicotinamide adenine dinucleotide phosphate (NADPH) [1]. Since G6PD is the only NADPH-producing enzyme in red blood cells, defense against oxidative damage is strongly dependent on its activity [2]. G6PD deficiency is an X-linked, hereditary genetic defect arising from mutations in the *G6PD* gene [1]. It is estimated that 7.5% of the global population carries one or two *G6PD* mutant alleles, with 2.9% being deficient for this enzyme [3]. In G6PD deficiency, the NADPH concentrations cannot be maintained. Oxidative damage then occurs, leading to an abnormal rupture of the cell wall with hemolytic anemia. Although affected individuals with G6PD deficiency are usually asymptomatic, this defect may cause neonatal jaundice as well as mild hemolytic anemia to chronic non-spherocytic hemolytic anemia triggered by infections, specific foods, or drugs [2]. Detecting G6PD-deficient individuals is important so that they may avoid trigger factors for hemolytic anemia and use a substitute medication when necessary.

It is documented that G6PD deficiency is highly prevalent in malaria-endemic countries, such as Africa, the Middle East, South Asia, and the Mediterranean region, conferring resistance against malaria [1]. To the best of our knowledge, no case of G6PD deficiency has been confirmed by molecular genetic analysis in Korea. Herein, we report the first genetically confirmed case of G6PD deficiency in Korea from a novel *de novo* mutation.

Case report

The patient was a 20-month-old boy with a clinical history of chronic hemolysis and several episodes of acute exacerbation. He is the first child born to a non-consanguineous marriage. There was no history of hematological disorders in his family. At birth, he suffered from

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severe neonatal jaundice and was treated with phototherapy. At the age of two months, he presented with anemia (hemoglobin level of 7.7 g/dL). Peripheral blood smear showed normochromic normocytic anemia, many nucleated RBCs, and polychromasia. Both direct and indirect antiglobulin tests were negative. After receiving transfusion, his condition improved; however, intermittent hemolytic crisis occurred concomitant with infection during follow-up. At the time of molecular genetic investigation, his hemoglobin level was 10.2 g/dL with a reticulocyte count of 15.6%. On physical examination, his height and weight were below the 10th percentile for age, possibly due to chronic anemia. There was no hepatosplenomegaly. The G6PD enzyme level in the red blood cells of the patient was significantly decreased at 0.2 U/g Hb (reference range of 7.9-16.3 U/g Hb), which was confirmed by repeated tests.

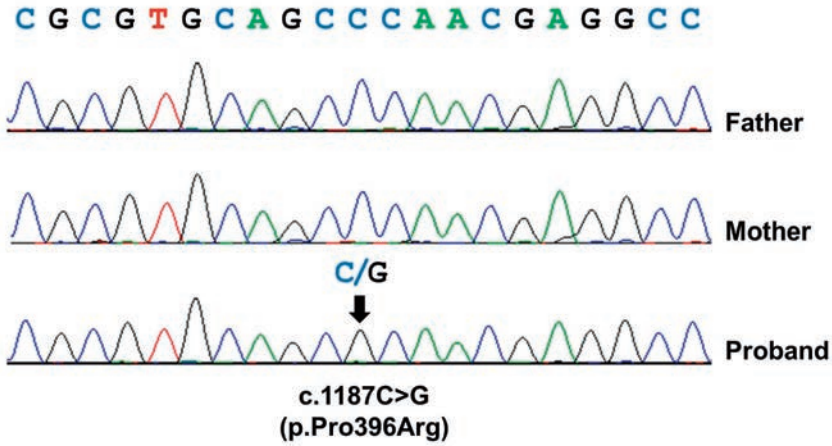

Figure 1. Sequencing analysis of the G6PD gene. *The patient was hemizygous for a novel missense mutation (c.1187C>G, p.Pro396Arg; arrow) of G6PD gene. Neither of the patients had the mutation.*

After written informed consent was given, the molecular genetic test was performed by direct sequencing of the *G6PD* gene to confirm G6PD deficiency. Genomic DNA was extracted from peripheral blood leukocytes. PCR and direct sequencing were performed by amplifying all coding exons and their flanking intronic regions of *G6PD* using primer pairs developed by the authors (available upon request) on the ABI Prism *3730xl* Genetic Analyzer (Applied Biosystems, Foster City, California, USA) using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The sequence chromatograms obtained were compared with the reference sequence of *G6PD*, NM_001042351.1. Identified variants were described following the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/). The patient was found to be hemizygous for a missense variant of *G6PD* (c.1187C>G; p.Pro396Arg) in exon 10. According to a review of the literature and mutation database, the variant has not been described previously. The p.Pro396Arg variant was absent in the single nucleotide polymorphism (SNP) database (dbSNP, http://www.ncbi.nlm.nih.gov/snp/) and Exome Sequencing Project database (http://evs.gs.washington.edu/EVS/). Bioinformatic analyses revealed that the affected residue, Pro396, is perfectly conserved from zebrafish to human (data not shown) and predicted Pro396Arg to be deleterious by Sorting Intolerant From Tolerant (SIFT, http://sift.bii.a-star.edu.sg/index.html) and Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2. Population screening revealed that the variant was not detected on screening of 100 control chromosomes. Thus, we concluded that the variant was the causative mutation in the patient. Family study was performed by involving the parents of the proband; neither of his parents carried the mutation **(Figure 1)**. The p.Pro396Arg is therefore a *de novo* mutation.

Discussion

In this report, we described a Korean boy who suffered from hemolytic anemia caused by a novel *de novo* mutation in the *G6PD* gene. The transversion mutation in exon 10 replaced the Pro396 residue with Arginine (c.1187C>G; p.Pro396Arg). The *G6PD* gene is located on the chromosome band Xq28 and consists of 13 exons spanning nearly 20 kb. To date, more than 180 different variants have been reported to be associated with G6PD deficiency [4]. Many variants of *G6PD* (mostly missense mutations) are spread throughout the entire coding region of the gene [4]. A wide range of enzyme activity levels and associated clinical symptoms have been described [4]. Several Korean cases of G6PD deficiency have been reported but lack molecular confirmation [5,6].

As for the genotype-phenotype correlations, the World Health Organization (WHO) has categorized *G6PD* mutations into five classes based on the

residual enzymatic activity and clinical manifestations [3], with Class I encompassing the severely deficient cases with chronic non-spherocytic hemolytic anemia, as in our patient. Of note, the mutations that cause the severe phenotype (Class I) are most frequent in exon 10 (amino acids 351-429, close to the dimer interface region) [4]. The interface subunit affects important interactions including hydrophobic and charge-to-charge or salt bridges [1]. Therefore, mutations located in this region have a significantly deleterious effect on the enzyme activity by reducing its stability, leading to a severe phenotype [1]. Pro396 in the region, mutated in our patient, is a completely conserved residue across species. A couple of mutations affecting the Pro396 residue but with a different amino acid changes, p.Pro396Ala and p.Pro396Leu, have been previously described in two families of Indian and Italian ethnic origins [7,8]. All affected individuals of the families had chronic non-spherocytic hemolytic anemia with very low (<1% of the normal value) enzyme activity, as in our patient.

The genotype analyses targeting Pro396 in the parents demonstrated that the mutation occurred *de novo* in the patient. A review of the literature showed a limited number of reports of *de novo* occurrence of G6PD deficiency [9-11]. A family study including parents is important to deliver accurate information in genetic counseling.

In conclusion, we identified a novel missense mutation (c.1187C>G; p.Pro396Arg) of the *G6PD* gene that causes a severe phenotype of disease. To the best of our knowledge, this is the first genetically confirmed case of G6PD deficiency in the Korean population.

References

1. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet 2008;371:64-74.
2. Mason PJ, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. Blood Rev 2007;21:267-283.
3. Glucose-6-phosphate dehydrogenase deficiency. WHO Working Group. Bull World Health Organ 1989;67:601-611.
4. Minucci A, Moradkhani K, Hwang MJ, Zuppi C, Giardina B, Capoluongo E. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: review of the "old" and update of the new mutations. Blood Cells Mol Dis 2012;48:154-165.
5. Jekarl DW, Oh EJ, Park YJ, Han K, Lee SW, Park CW. [Acute hemolysis and renal failure caused by hepatitis A infection with underlying glucose-6-phosphate dehydrogenase deficiency]. Korean J Lab Med 2007;27:188-191.
6. Kim MK, Yang CH, Kang SH, Lyu CJ, Kim KY. Glucose-6-phosphate dehydrogenase deficiency--report of 4 cases. J Korean Med Sci 1992;7:71-75.
7. Edison ES, Melinkeri SR, Chandy M. A novel missense mutation in glucose-6-phosphate dehydrogenase gene causing chronic nonspherocytic hemolytic anemia in an Indian family. Ann Hematol 2006;85:879-880.
8. Filosa S, Cai W, Galanello R, Cao A, de Mattia D, Schettini F, Martini G. A novel single-base mutation in the glucose 6-phosphate dehydrogenase gene is associated with chronic non-spherocytic haemolytic anaemia. Hum Genet 1994;94:560-562.
9. van Wijk R, Huizinga EG, Prins I, Kors A, Rijksen G, Bierings M, van Solinge WW. Distinct phenotypic expression of two de novo missense mutations affecting the dimer interface of glucose-6-phosphate dehydrogenase. Blood Cells Mol Dis 2004;32:112-117.
10. Costa E, Cabeda JM, Vieira E, Pinto R, Pereira SA, Ferraz L, Santos R, Barbot J. Glucose-6-phosphate dehydrogenase aveiro: a de novo mutation associated with chronic nonspherocytic hemolytic anemia. Blood 2000;95:1499-1501.
11. Minucci A, Concolino P, Vendittelli F, Giardina B, Zuppi C, Capoluongo E. Glucose-6-phosphate dehydrogenase Buenos Aires: a novel de novo missense mutation associated with severe enzyme deficiency. Clin Biochem 2008;41:742-745.