RAPID COMMUNICATION

G6PD Nara: A New Class 1 Glucose-6-Phosphate Dehydrogenase Variant With an Eight Amino Acid Deletion

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In the course of molecular studies on Japanese glucose-6-phosphate dehydrogenase (G6PD) variants using single-strand conformation polymorphisms (SSCP) analysis, we found an unusual class 1 G6PD variant that had nucleotide deletion in exon 9. The patient showed chronic nonspherocytic hemolytic anemia associated with frequent episodes of severe hemolytic attack. The hemolysate exhibited no measurable activity. Although the partially purified enzyme had detectable activity, we could not perform kinetic studies because of its extreme instability. Nucleotide sequencing showed a unique 24 bp deletion at nucleotide 953-976 that predicts an eight amino acid deletion of TKGYLDPP at residue 319-326. While this is one of the most drastic structural alterations found in G6PD variants, the region with the amino acid deletion was distant from both the G6P and NADP+ binding sites and was located in a domain with low sequence homology among species. The comparatively low functional importance of the deleted region may have saved the patient from lethal tissue dysfunction.

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GLUCOSE-6-PHOSPHATE dehydrogenase (G6PD) is a housekeeping enzyme that is essential for every cell to be viable. G6PD deficiency is a common genetic disorder that may cause hereditary nonspherocytic hemolytic anemia in a range of clinical severities. Although the same X-linked enzyme is expressed in every tissue, the functional defects are limited to erythrocytes in almost all cases of G6PD deficiency. This suggests that it would be lethal if the enzyme defect is so severe as to cause dysfunction of tissues other than erythrocytes. Recent molecular studies have elucidated that most of the variant enzymes are produced by one or two missense mutations in the structural gene.1,2

The only two exceptions are G6PD Sunderland,3 which is associated with a 3-bp deletion, and G6PD Vancouver,4 with three missense mutations. We have been performing a survey on the molecular abnormalities of Japanese G6PD variants using single-strand conformation polymorphisms (SSCP) analysis.1 In the course of this survey, we encountered an unusual class 1 G6PD variant that had a nucleotide deletion in exon 9 of the G6PD gene. We report here a molecular analysis of this unique Japanese G6PD variant.

MATERIALS AND METHODS

Case report. The patient, T.T., is a 6-year-old Japanese boy. He had severe neonatal hyperbilirubinemia and hemolytic anemia at birth and required blood transfusions. When he was 2 months old, he had the first episode of acute hemolytic attack associated with bacterial infection of a finger and was admitted to the Nara Prefectural Hospital. During the ensuing 6 years, he had 21 episodes of acute intravascular hemolysis, most of which were associated with viral or bacterial infections. During each episode, the patient was admitted and received blood transfusions. At his fourth admission, when he was 8 months old, a diagnosis of G6PD deficiency was established. The last episode of acute intravascular hemolysis occurred at the age of 6 associated with influenza A virus infection. At the time of this admission, he was pale and icteric. The liver was palpable 4.5 cm and the spleen was palpable 7.5 cm beneath the respective costal margins. No lymph node swelling was observed. Pertinent laboratory data included the following: packed cell volume, 21.0%; red blood cell count, 1.51 × 1012/mm3; hemoglobin concentration, 5.5 g/100 mL; reticulocyte count, 99.6%; serum total bilirubin, 8.3 mg/100 mL, of which 7.7 mg/100 mL was indirect reacting. The chronic nonspherocytic hemolytic anemia with hemoglobin concentration of around 8 g/100 mL and marked reticulocytosis had persisted in symptom-free periods. The blood for the present study was taken at the symptom-free period just before the last acute hemolytic episode. The patient showed no signs of chronic granulomatous disease, cataract, or other disorders involved in tissues other than erythrocytes. His mother and other family members had no history of anemia.

Materials. Restriction endonucleases and other modifying enzymes were purchased from Takara Shuzo (Kyoto, Japan). For the polymerase chain reaction (PCR),6 Thermus aquaticus (Taq) DNA polymerase (Cetus, Norwalk, CT) or Thermus flavus DNA polymerase (Tub DNA polymerase; Amersham International, Amersham, UK) was used. All other reagents were of analytical grade.

PCR-single-strand conformation polymorphisms (SSCP) analysis. Fresh blood samples were obtained from subjects with G6PD deficiency and a normal control by venipuncture following informed consent. Genomic DNA was isolated from leukocytes. Each exon sequence was amplified by PCR using the oligonucleotide primers described previously2 and purified by 8% polyacrylamide gel electrophoresis. The PCR-SSCP analysis was performed according to a standard protocol with modifications of using a compact gel system (8 × 8 × 0.1 cm) and ethidium bromide staining. When the mobility shift of DNA bands was detected, the corresponding amplified DNA segment was cloned into plasmid pBluescript (Stratagene, La Jolla, CA) and sequenced by using a DNA sequencer (Applied Biosystems, Foster City, CA, 373A).

Analysis of genomic DNA. The 163-bp segment including the nucleotide 953-976 was amplified from genomic DNA from a propositus, his mother, and a normal control using a pair of primers.

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G6PD VARIANT WITH AMINO ACID DELETION

Predicting an amino acid deletion of TKGYLDDP at residue 319-326.

The deletion mutation of G6PD Nara is one of the most marked sequence alterations found in mutant G6PD genes. The unstable nature of G6PD Nara and the resulting severe clinical expression of persisting hemolytic anemia with frequent episodes of acute intravascular hemolysis seem to be compatible with the marked structural alteration. Among 50 mutations found in variant G6PD genes, four missense mutations have been identified in exon 9.10-13 A class 3 variant with G6PD A- phenotype is of particular interest because one of its missense mutations is located at nucleotide 968 that is deleted in G6PD Nara.11 Except for the reduced enzyme activity, they show no notable kinetic abnormalities and none of them causes chronic hemolytic anemia. The region with the deletion in G6PD Nara is distant from both the putative G6P and NADP+ domains,14,15 and the homology in amino acid sequences from various species is consistently low in this region.2 These findings suggest that the region with deletion in G6PD Nara is not likely to be associated with the essential function of the enzyme. Recently, Maeda et al4 reported that three missense mutations caused G6PD Vancouver, a “null” variant with neutrophil dysfunction as well as chronic hemolytic anemia.16 It is somewhat surprising that the functional abnormalities and the clinical manifestations of G6PD Vancouver are much more severe than those of G6PD Nara, despite the much larger number of involved amino acids in the latter. This discrepancy might be attributed to a difference in functional importance of the mutated domain.

**RESULTS**

In the course of molecular studies on Japanese G6PD variants using PCR-SSCP analysis,3 we found that exon 9 of a class 1 variant gene was shorter than that of normal G6PD. Subsequent sequencing analysis showed a 24-bp deletion in exon 9 of the mutant gene. This deletion does not alter the reading frame and should produce a mutant protein eight amino acids shorter than the normal (Fig 1). By the direct PCR amplification of a fragment containing the nucleotide 953-976 from genomic DNA, we could confirm the deletion mutation in the variant gene (Fig 2). The mother was found to be heterozygous for the mutant allele. In addition to the deletion, a nucleotide substitution of G to A at nucleotide 17 of intron 11 was found in the mutant gene.

Routine enzyme assay showed no measurable G6PD activity in the patient’s hemolysate. Partial purification and characterization of the variant enzyme were tried by the standard method.8 The variant enzyme was extremely thermolabile and it showed complete loss of activity after 10 minutes of incubation at 46°C. The instability was marked also at 25°C, and because of the rapid loss of activity during the assay procedure, kinetic studies could not be performed on this variant. High concentrations of NADP+ up to 2 mmol/L did not protect the enzyme from rapid inactivation.

**DISCUSSION**

In this study, we found a 24-bp deletion in a class 1 variant G6PD gene. This is the second case of G6PD deficiency caused by nucleotide deletion, but the number of involved amino acids is much greater than that of the first case.3 Because its unusual molecular abnormality and the extreme instability seemed to be quite unique, we designated this variant G6PD Nara. The existence of two direct tetranucleotide (CCAC) repeats in the region with the nucleotide deletion strongly suggests that the deletion occurred between the tetranucleotides by mispairing in DNA duplication (Fig 1).9 Therefore, although the deletion could be in any serial 24 bases within nucleotide 953-980, we concluded that the deleted nucleotides should be nucleotide 953-976 predicting an amino acid deletion of TKGYLDDP at residue 319-326.

The deletion mutation of G6PD Nara is one of the most marked sequence alterations found in mutant G6PD genes. The unstable nature of G6PD Nara and the resulting severe clinical expression of persisting hemolytic anemia with frequent episodes of acute intravascular hemolysis seem to be compatible with the marked structural alteration. Among 50 mutations found in variant G6PD genes, four missense mutations have been identified in exon 9.10-13 A class 3 variant with G6PD A- phenotype is of particular interest because one of its missense mutations is located at nucleotide 968 that is deleted in G6PD Nara.11 Except for the reduced enzyme activity, they show no notable kinetic abnormalities and none of them causes chronic hemolytic anemia. The region with the deletion in G6PD Nara is distant from both the putative G6P and NADP+ domains,14,15 and the homology in amino acid sequences from various species is consistently low in this region.2 These findings suggest that the region with deletion in G6PD Nara is not likely to be associated with the essential function of the enzyme. Recently, Maeda et al4 reported that three missense mutations caused G6PD Vancouver, a “null” variant with neutrophil dysfunction as well as chronic hemolytic anemia.16 It is somewhat surprising that the functional abnormalities and the clinical manifestations of G6PD Vancouver are much more severe than those of G6PD Nara, despite the much larger number of involved amino acids in the latter. This discrepancy might be attributed to a difference in functional importance of the mutated domain.

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Although nucleotide deletions or nonsense mutations are common molecular abnormalities that may cause a variety of genetic disorders, they are quite rare in G6PD deficiency cases. The extremely low frequency of amino acid deletions as a cause of G6PD deficiency might imply that severe tissue dysfunction usually associated with such drastic structural aberration is presumably lethal unless the involved region is functionally insignificant. The comparatively low functional importance of the deleted region in G6PD Nara may have saved the patient from lethal tissue dysfunction.

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REFERENCES

17. McKusick VA: Mendelian Inheritance in Man. Baltimore, MD, The Johns Hopkins University,
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