RAPID COMMUNICATION

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

By Akira Hirono, Hisaichi Fuji, Masayuki Shimaa, and Shiro Miwa

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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G6PD VARIANT WITH AMINO ACID DELETION

Normal
...GluAlaThrLysGlyTyrLeuAspAspProThrVal...
G6PD Nara
...GAGCCCAAGGTTACCTGGAGGCCACGGT...

Fig 1. A 24-nucleotide deletion in G6PD Nara gene. The deletion occurred between two direct tetranucleotide repeats CCAC (underlined).

5'-GTCAAGGTGGTTGAAATGCATCT and 3'-GGTGAAACC-GTGGCACGCAGGAGA. The amplified DNA fragments were separated by 8% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining.

RESULTS

In the course of molecular studies on Japanese G6PD variants using PCR-SSCP analysis, 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. 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. 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). 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. 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. 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, and the homology in amino acid sequences from various species is consistently low in this region. 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 al reported that three missense mutations caused G6PD Vancouver, a “null” variant with neutrophil dysfunction as well as chronic hemolytic anemia. 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.

Fig 2. PCR amplification of a region containing the deletion. The propositus (P) showed a 139-bp amplified fragment which is 24-bp shorter than that of the normal control (C). The heterozygous mother (M) showed both normal and deleted fragments with heteroduplex bands.
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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