Identification of Two Novel Deletion Mutations in Glucose-6-Phosphate Dehydrogenase Gene Causing Hemolytic Anemia

By Akira Hirono, Hisaichi Fujii, and Shiro Miwa

Among over 50 distinct mutations causing glucose-6-phosphate dehydrogenase (G6PD) deficiency, only two deletion mutations have so far been reported. Using nonradioisotopic single-strand conformation polymorphism analysis, we found two additional deletion mutations in two Japanese G6PD-deficient patients with nonspherocytic hemolytic anemia. Case no. 1 had a 3-nucleotide deletion in exon 6 predicting a deletion of a serine at amino acid 188 or 189, which caused a class 1 variant G6PD Tsukui. Case no. 2 had a 3-nucleotide deletion in exon 5 predicting a deletion of a lysine at residue 95, which caused a class 2 variant G6PD Urayasu.

The 188th serine, which might be deleted in G6PD Tsukui, is located close to the putative G6P binding site. The 188th serine is also involved in the amino acid substitution in G6PD Mediterranean, but the kinetics of these two variants are totally different. The residue with an amino acid deletion in G6PD Urayasu was distant from the substrate binding sites and was located in a region with low sequence homology among species. The different properties of variants having mutations in exons 5 and 6 suggest that these two exons code distinct functional domains of the enzyme.

Existence of over 400 variants and an increasing number of identified mutations provide a great opportunity to elucidate the structure-function relationships of human G6PD.

Herein, we describe two novel deletion mutations causing nonspherocytic hemolytic anemia. Putative structure-function relationships of exons 5 and 6 are also discussed.

MATERIALS AND METHODS

Patients

Case no. 1. A case report of the patient with G6PD Tsukui and the enzymological properties of the variant enzyme have been published elsewhere. The propositus has a younger brother who also has G6PD deficiency. In the present study, a blood sample was obtained from him.

Case no. 2. The patient, S.K., is an 8-year-old Japanese boy. When he was 3-years-old, he had excreted dark urine after taking medicine for a cold. A panel of red blood cell enzyme assays showed that he was deficient in G6PD activity. After the episode, he has had no symptoms, and repeated laboratory data including hemoglobin concentration, reticulocyte count, and serum indirect-reacting bilirubin level showed no signs of chronic hemolysis. His mother and other family members had no history of anemia.

Polymerase chain reaction-based single-strand conformation polymorphism (PCR-SSCP) Analysis

Synthetic oligonucleotide primers used for the PCR and the direct sequencing were the same as those described previously. Blood samples were obtained from the patients and a normal male control by venipuncture following informed consent. Genomic DNA was isolated from leukocytes and purified by a standard method. PCR-SSCP analysis was performed by our modified method using a mini gel system and ethidium bromide staining.

Direct Sequencing

Direct sequencing of amplified exon sequences was performed by an automated DNA sequencer using a dye terminator protocol (model 373A; Applied Biosystems, Foster City, CA).

Biochemical Characterization of a Variant G6PD

Partial purification and biochemical characterization of a variant enzyme of case no. 2 were performed according to the standard methods recommended by the World Health Organization scientific group.
RESULTS

Molecular Analysis of Variant Gene

PCR-SSCP analysis showed that exon 6 in case no. 1 and exon 5 in case no. 2 showed clear mobility shifts on the gel (Fig 1). By subsequent direct sequencing analysis, we found a 3-bp deletion in each case (Fig 2). The deletion in case no. 1 could be in any serial 3 bases within nucleotides 561 to 567, predicting the deletion of a serine at amino acid 188 or 189. By the direct amplification of a sequence containing the deletion, we could easily confirm the mutation in the patient’s gene.

The deletion in case no. 2 was possibly in any serial 3 bases within nucleotide 281 to 285. The 3-bp deletion predicted a single amino acid deletion of the 95th lysine. Because the mutation created a new Sac I site, the existence of the base deletion was confirmed in the patient’s gene. By the restriction analysis also showed that the proband’s mother, who showed normal red blood cell G6PD activity, did not have the base deletion, suggesting that the mutation had arisen spontaneously (data not shown).

It is generally accepted that some of the small nucleotide deletions occur between short nucleotide repeats by mispairing during DNA duplication. Because each of our present cases also had such direct repeats in the region with the deletion (Fig 2), we concluded that the deleted nucleotides in case no. 1 were either serial 3 bases CTC, TCC, or CCT in nucleotide 561 to 565, and those in case no. 2 should be AGA in nucleotide 281 to 283.

Biochemical Characterization

Table 1 shows the biochemical properties of the variant enzyme in case no. 2. Because it showed severe enzyme deficiency but it did not cause chronic hemolytic anemia, the variant was considered to be classified as class 2. Except for the decreased activity and the fast electrophoretic mobility, the variant had almost normal properties. Because the properties of this variant are considered to be unique, we designated it G6PD Urayasu.

DISCUSSION

A single amino acid deletion of G6PD Urayasu is located in the region coded by exon 5 of G6PD gene. The mutation of G6PD Urayasu is unique because it is the only known deletion mutation that does not cause chronic hemolytic anemia. The normal thermostability may explain the relatively mild clinical expression of the variant. G6PD A,13 G6PD Quing Yuan,14 and G6PD Ilesha15 are known to have their single disease-causing amino acid substitutions in this region. The fact that all of these three variants show normal or mildly deceased enzyme activities, normal kinetic properties, and unremarkable clinical manifestations suggest that this region does not closely relate to the important function of the enzyme. The consistently low homology in sequences from various species in this region16 may be supportive.

An amino acid deletion of G6PD Tsukui is located in the region coded by exon 6 of the G6PD gene. This region might be functionally important because it includes a highly conserved amino acid stretch (Arg-198 to Glu-206) that is considered to be involved in the substrate G6P binding. Seven biochemically characterized variants have so far been reported to be caused by mutations around the putative G6P binding site (Table 2).6,15,17,20

G6PD Tsukui and G6PD Mediterranean15 are of special interest because the deletion (G6PD Tsukui) or the substitution by a phenylalanine (G6PD Mediterranean) of the same Ser-188 causes variants with totally different properties (Ta-
It is noteworthy that other variants having mutations in the G6P-binding domain also have kinetic properties similar to those of either G6PD Tsukui or G6PD Mediterranean and can be separated into two types from the kinetic point of view. The "Mediterranean type" includes G6PD Santamaria,15 G6PD Mediterranean,15 G6PD Coimbra,18 and G6PD Sibari,19 which are characterized by decreased Michaelis constant (Km) for both G6P and NADP* and low substrate specificity, and the "Tsukui type" includes G6PD Shinshu,20 G6PD Minnesota20 and G6PD Tsukui, which are characterized by their decreased affinity and the increased stringency for the substrate binding. Because the latter variants cause chronic hemolytic anemia and the former do not, the low substrate affinity of the latter variants might be one of the factors responsible for the more severe clinical expression of those variants.21 Existence of two types of variants suggests that the amino acid changes of each type of variants cause a contrary effect to the binding site. Future x-ray crystallographic analysis particularly on Ser-188 should help elucidate the precise mechanism of the structural alteration and its relationship to the kinetic abnormality of these variants.

ACKNOWLEDGMENT

We are indebted to Drs Tadashi Miyamori (Municipal Ida Hospital, Kawasaki, Japan) and Hirokuni Yoshida (Juntendo Urayasu Hospital, Juntendo University, Urayasu, Japan) for providing the blood samples of G6PD deficiency patients. The biochemical characterization of G6PD Urayasu was performed by Drs Kenzaburo Tani, Takayuki Morisaki, Hitoshi Kanno, Hiromi Ogura, Hisashi Tsutsumi, and Keisuke Takahashi in the Department of Pathological Pharmacology, Institute of Medical Science, Tokyo University (Tokyo, Japan).

REFERENCES

2. Vuliamy T, Beutler E, Luzzatto L: Variants of glucose-6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. Hum Genet 78:369, 1988

Table 1. Biochemical Characteristics of G6PD Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Km G6P (μmol/L)</th>
<th>Km NADP+ (μmol/L)</th>
<th>Ki NADPH (μmol/L)</th>
<th>2-Deoxy G6P (% of G6P)</th>
<th>Gal-6P (% of G6P)</th>
<th>Deamino NADP+ (% of NADP+)</th>
<th>pH Optimum</th>
<th>Heat Stability</th>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shinshu</td>
<td>1.5</td>
<td>89</td>
<td>100</td>
<td>4.0</td>
<td>12.8</td>
<td>1.2</td>
<td>1.5</td>
<td>Normal</td>
<td>Labile</td>
</tr>
<tr>
<td>Santamaria</td>
<td>7.5</td>
<td>109</td>
<td>40</td>
<td>4.0</td>
<td>4.4</td>
<td>3.7</td>
<td>7.5</td>
<td>Normal</td>
<td>Stable</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>3.5</td>
<td>100</td>
<td>23</td>
<td>1.4</td>
<td>ND</td>
<td>25</td>
<td>24</td>
<td>Normal</td>
<td>Stable</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>31-71</td>
<td>2.6-6.6</td>
<td>4.9-12.9</td>
<td>1.8-6.4</td>
<td>&lt;4</td>
<td>51-69</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not determined; Ki NADPH, inhibition constant for NADP, reduced form; Gal-6P, galactose-6-phosphate.

Table 2. Biochemically Characterized G6PD Variants Caused by Mutations Around the Putative G6P Binding Site

<table>
<thead>
<tr>
<th>Variant</th>
<th>Exon</th>
<th>Amino Acid Number</th>
<th>Amino Acid Change</th>
<th>Affinity for Substrates</th>
<th>Specificity for Substrates</th>
<th>Class</th>
<th>Clinical Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shinshu</td>
<td>6</td>
<td>178</td>
<td>Asp → Gly</td>
<td>Low</td>
<td>High</td>
<td>1</td>
<td>HNSHA</td>
</tr>
<tr>
<td>Santamaria</td>
<td>6</td>
<td>181*</td>
<td>Asp → Val</td>
<td>High</td>
<td>Low</td>
<td>2</td>
<td>AHA</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>6</td>
<td>188</td>
<td>Ser → Phe</td>
<td>High</td>
<td>Low</td>
<td>2</td>
<td>AHA</td>
</tr>
<tr>
<td>Tsukui</td>
<td>6</td>
<td>189</td>
<td>Ser → del</td>
<td>Low-normal</td>
<td>High</td>
<td>1</td>
<td>AHA</td>
</tr>
<tr>
<td>Coimbra</td>
<td>6</td>
<td>198</td>
<td>Arg → Cys</td>
<td>High</td>
<td>Low</td>
<td>2</td>
<td>AHA</td>
</tr>
<tr>
<td>Sibari</td>
<td>6</td>
<td>212</td>
<td>Met → Val</td>
<td>High</td>
<td>Low</td>
<td>3</td>
<td>AHA</td>
</tr>
<tr>
<td>Minnesota</td>
<td>5</td>
<td>213</td>
<td>Val → Leu</td>
<td>Low-normal</td>
<td>High</td>
<td>1</td>
<td>HNSHA</td>
</tr>
</tbody>
</table>

Abbreviations: HNSHA, hereditary nonspherocytic hemolytic anemia; AHA, acute hemolytic anemia.

* With an additional amino acid substitution 126 Asn → Asp.


Identification of two novel deletion mutations in glucose-6-phosphate dehydrogenase gene causing hemolytic anemia

A Hirono, H Fujii and S Miwa