New Glucose-6-Phosphate Dehydrogenase Mutations Associated With Chronic Anemia


We have identified the glucose-6-phosphate dehydrogenase mutations responsible for enzyme deficiency in nine individuals with chronic nonspherocytic hemolytic anemia. We found the variants Tokyo, Iowa, Shinshu, and Guadalajara in British subjects and Kobe in an Italian. In addition we have determined the variant Corum has the mutation 820 G → A and have found in British subjects the mis-sense mutations 224 T → C, 486 G → A and 833 C → T which have not been described before. Some, but not all, of the mutations involve amino acids located near putative substrate binding sites.

DEFICIENCY OF THE ENZYME glucose-6-phosphate dehydrogenase (G6PD) is associated with hemolytic anemia in two different situations. First, in areas in which malaria has been endemic, G6PD-deficient alleles have reached high frequencies (1% to 50%) and deficient individuals, though essentially asymptomatic in the steady state, have a risk of acute hemolytic attacks. Secondly, sporadic cases of G6PD deficiency occur at very low frequencies anywhere in the world, and they usually present with a more severe phenotype, namely chronic nonspherocytic hemolytic anemia. In these patients, the G6PD activity in most cells must be high enough to support normal development and metabolism, whereas in red blood cells, it is low enough to lead to chronic anemia; the most likely mechanism whereby this can happen is decreased stability of the enzyme. Presumably, the number of mutations that will be found in this category is limited to the number of amino acid changes that will produce an enzyme molecule with these properties.

The mutations responsible for about 60 G6PD variants have been determined and some interesting structure/function relationships have been noted. In this paper, we present the G6PD mutations responsible for enzyme deficiency in nine individuals with chronic nonspherocytic hemolytic anemia. Five of the mutations have been reported before, whereas four are new mutations.

MATERIALS AND METHODS

All patients were male. The diagnosis of chronic nonspherocytic hemolytic anemia (CNSHA) was based on the following criteria: (1) anemia, (2) elevated reticulocytes and bilirubin, (3) absence or rarity of spherocytes on morphology, and (4) normal osmotic fragility (not done in all cases). When the combination of criteria was satisfied and a quantitative G6PD assay yielded a result of normal or less, we regarded the most likely diagnosis as CNSHA caused by G6PD deficiency.

DNA samples were subjected to polymerase chain reaction (PCR) amplification of exons, SSCP analysis, DNA sequencing, and restriction enzyme analysis as described previously. Biochemical characterization of G6PD was performed as described previously.

RESULTS AND DISCUSSION

Mutations causing CNSHA. In all the patients included in this series the diagnosis of chronic nonspherocytic hemolytic anemia was documented by conventional hematologic criteria. In the absence of any other known cause, and in view of G6PD deficiency in a nonendemic area, this CNSHA was attributed to G6PD deficiency. This condition is usually associated with severely deficient red blood cell G6PD levels. It is not always possible in these cases to get a great deal of biochemical information from a small blood sample, but the PCR techniques used to determine the mutations can be applied to very small samples and are independent of the enzyme activity. If biochemical analysis of the variant enzymes is required, it can be obtained by making the recombinant variant protein in bacteria.

G6PD exons were amplified from subjects DNA samples and the PCR products examined for mutations by searching for mobility shifts using the single-strand conformation polymorphism (SSCP) procedure as previously described. Two of the mutations presented here, Shinshu and Guadalajara, did not show up as band shifts in SSCP and were determined by systematic sequencing of all exons. The results of the mutation analysis, together with some biochemical and hematologic data is shown in Table 1. It has been observed previously that many G6PD mutations causing severe anemia tend to be clustered in exon 10 and this fact, together with the raised Km NADP of some of the variant enzymes, led Hirono et al to suggest the NADP binding site may be located in this part of the molecule. Of the nine mutations that we present here, three of the five mutations that we find for the second time are indeed in exon 10, the exceptions being G6PD Kobe and G6PD Shinshu; the three new ones are not in this cluster but are more or less randomly distributed in the central portion of the molecule.

Multiple occurrence of mutations. Five of the mutations reported in this series have been described before in different parts of the world. The mutations previously found in Shinshu and Tokyo, Japan; Iowa; and Guadalajara, Mexico, have now turned up in Britain, and we report here the mutation previously found in Kobe, Japan, was found in an Italian subject. This is most likely to be caused by a second occur-

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<table>
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<tr>
<th>Variant Name (proposed or previous)</th>
<th>Ethnic Origin</th>
<th>Age*</th>
<th>Clinical manifestation (in addition to CNSHA)</th>
<th>Hematology</th>
<th>G6PD Activity in RBC IU/L</th>
<th>Biochemistry</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hb g/dL</td>
<td>Reticulocytes %</td>
<td>2d G6P dNADP</td>
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<tr>
<td>Swansea</td>
<td>Welsh</td>
<td>29</td>
<td>-</td>
<td>11.9</td>
<td>13.9</td>
<td>ND 15</td>
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<tr>
<td>Plymouth</td>
<td>English</td>
<td>45</td>
<td>Cardiomyopathy</td>
<td>13-14</td>
<td>12.5</td>
<td>14.0 72.0</td>
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<tr>
<td>Tokyo</td>
<td>Scottish</td>
<td>11</td>
<td>-</td>
<td>8-9</td>
<td>15</td>
<td>NA NA NA NA</td>
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<tr>
<td>Wexham</td>
<td>English</td>
<td>—</td>
<td>NNJ; episodes of severe anemia</td>
<td>12</td>
<td>5.6</td>
<td>9.5 16.7</td>
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<tr>
<td>Corum</td>
<td>Turkish</td>
<td>10</td>
<td>Presented with severe anemia</td>
<td>10-12</td>
<td>4</td>
<td>14.8 45.3</td>
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<tr>
<td>Iowa</td>
<td>English</td>
<td>51</td>
<td>Needed splenectomy at age 13</td>
<td>12</td>
<td>5.2</td>
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<tr>
<td>Kobe</td>
<td>Italian</td>
<td>12</td>
<td>NNJ</td>
<td>11</td>
<td>5.2</td>
<td>NA NA NA NA</td>
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<tr>
<td>Shinshu</td>
<td>English</td>
<td>54</td>
<td>Daughter aborted male fetus at 31 wks</td>
<td>12-14</td>
<td>9-17</td>
<td>NA NA NA Normal</td>
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<tr>
<td>Guadalajara</td>
<td>Irish</td>
<td>25</td>
<td>NNJ</td>
<td>12-14</td>
<td>9.3</td>
<td>15.0 23.0</td>
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</tbody>
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Abbreviations: NNJ, neo-natal jaundice; RBC, red blood cell; Hb, hemoglobin; ND, none detected; NA, not available.

* Age at time of mutation determination.
† % activity with G6P/NADP.
rence of the same mutation. The alternative explanation, that each pair of patients with the same mutation have a common ancestor seems less likely because of the evolutionary distance that would have to be postulated and the severely disadvantageous nature of the mutated allele.

In the case of the Guadalajara (387 Arg → Cys) variant, we have definite evidence that the mutation in the British subject is new. The base change responsible for this mutation in the Guadalajara and Belfast* mutations documented so far, only 3, including the Tokyo and Guadalajara mutations, occur by CpG → TpG changes. The other seven mutations reported here do not involve CpG dinucleotides may also influence the likely multiple incidence of the same mutations. However Table 2 shows that of the eight cases of multiple occurrence of CNSHA causing mutations documented so far, only 3, including the Tokyo and Guadalajara mutations, occur by CpG → TpG changes.

The fact that we are seeing several mutations for the second time from only 30 CNSHA mutations described implies that mutations capable of causing CNSHA are restricted to a rather small number of amino acid changes that produce an enzyme with low red blood cell activity, yet substantial activity in other tissues. The higher mutation rate at CpG dinucleotides may also influence the likely multiple incidence of the same mutations. However Table 2 shows that of the eight cases of multiple occurrence of CNSHA causing mutations documented so far, only 3, including the Tokyo and Guadalajara mutations, occur by CpG → TpG changes. The other seven mutations reported here do not involve CpG → TpG changes.

Evolutionary conservation. The G6PD amino acid sequence is now known for 10 organisms including rat, flies, yeasts, and bacteria.11.12 Of the 9 amino acids that are mutated in the cases presented here, 9 are conserved in Drosophila melanogaster, 7 in Saccharomyces cerevisiae, and only 1 in Escherichia coli. The figures for percentage conservation between human and these other G6PDs are 61, 47, and 38, respectively. It would seem from this that amino acids whose mutations cause severe deficiency are well conserved, but not invariant. Presumably mutations in some of the invariant amino acids (of which there are 54) would not be compatible with normal embryogenesis. When this analysis was extended to all known class I mutations,5 93% were found to affect residues conserved in D melanogaster, which was very significantly higher than would be expected by chance ($P < .001$).

**ACKNOWLEDGMENT**

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**REFERENCES**


2. Vulliamy TJ, Beutler E., Luzzatto L: Variants of glucose-6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. Hum Mutat 2:159, 1993

3. Hirono A, Kuhl W, Gelbart T, Forman L, Fairbanks VF,
Beutler E: Identification of the binding domain for NADP of human glucose-6-phosphate dehydrogenase by sequence analysis of mutants. Proc Natl Acad Sci USA 86:10015, 1989


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