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# Mutation analysis of glucose-6-phosphate dehydrogenase (G6PD) variants in Costa Rica

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**Summary.** Glucose-6-phosphate dehydrogenase (G6PD) deficiency has previously been reported among both the black and white populations of Costa Rica. All 28 G6PD A− samples were found to be of the common G6PD A$-^{376G/202A}$type. A previously described mutation associated with nonspherocytic hemolytic anemia, G6PD Puerto Limón, was found to be due to a G→A transition at nucleotide (nt) 1192, causing a glu→lys substitution. Mutations in this region of the G6PD molecule seem invariably to be associated with chronic hemolytic anemia. G6PD Santamaria had been described previously in two unrelated white subjects. We found that both did, indeed, have the same mutations. In this variant the A→G substitution at nt 376 that is characteristic of G6PD A was present, but an A→T mutation at nt 542, apparently superimposed on the ancient G6PD A mutation, resulted in an asp→val substitution. Thus, the gain of a negative charge at amino acid 126 was counterbalanced by the loss of a charge at amino acid 181, giving rise to a variant with the G6PD A mutation but with normal electrophoretic mobility.

## Introduction

Over 400 putative variants of glucose-6-phosphate dehydrogenase (G6PD) have been distinguished from one another by biochemical characterization in the last 30 years (Beutler 1990). Analysis of the DNA sequence of the coding portion of the G6PD gene has made possible precise classification of G6PD variants for the first time. Our studies and those of others have revealed that the number of different mutations is probably substantially less than had previously been believed on the basis of biochemical characterization; a recent review of 46 ostensibly distinct variants revealed only 23 different mutations (Beutler 1991).

The predominantly white population of Costa Rica, formerly a Spanish colony, is largely of Spanish origin. The black population, representing basically migrants from Jamaica, is considered to have originated in West Africa. Several different G6PD variants have been described among the populations of Costa Rica. These include G6PD Puerto Limón, a variant with severe deficiency causing hereditary nonspherocytic hemolytic anemia (Elizondo et al. 1982) and G6PD Santamaria (Saenz et al. 1984), in which hemolysis was observed only under stress. In addition, investigation of 1097 children of the San José province revealed a high incidence of G6PD deficiency among black boys, most of the deficient children having residual enzyme with fast mobility, so that it could be classified as G6PD A−. Interestingly, a few black boys with G6PD deficiency were found to have enzyme with normal electrophoretic mobility (Chaves et al. 1988). This is a very unusual finding: deficient samples from persons of African origin are almost always electrophoretically rapid, and are thus phenotypically classified as G6PD A−.

We have now analyzed deficient variants in Costa Rica at the DNA level, identifying the mutations both in enzyme-deficient black males and in the previously reported cases in whites.

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## Materials and methods

### *G6PD-deficient blood samples*

Blood for analysis was obtained from the patient originally described to have G6PD Puerto Limón and from the two patients originally described to have G6PD Santamaria. Blood samples from 289 black males from the province of Limón were screened for G6PD deficiency using the fluorescent spot test (Beutler 1966). We detected 29 deficient samples. All of the G6PD deficient samples were shipped from Costa Rica to La Jolla, Calif., where DNA was extracted using the Applied Biosystems DNA extractor.

### *DNA sequence analysis*

All DNA samples were screened for the A→G mutation at nucleotide (nt) 376 that is characteristic of G6PD A− and G6PD A+ and for the G→A mutation of nt 202 that is characteristic of the

most common form of G6PD A−, using polymerase chain reaction (PCR) amplification and digestion with the appropriate restriction endonuclease as previously described (Hirono and Beutler 1988; Beutler et al. 1991b). When both of these mutations were present the subject was classified as G6PD A$-^{376G/202A}$, the common G6PD A− genotype (Beutler 1989). When these mutations were not present, the entire coding region was sequenced using PCR-amplified DNA as previously described (Beutler et al. 1991a). Mutations that were encountered were confirmed by sequencing the reverse strand.

## Results

### *Samples from black males*

Of the 29 black male samples 28 were found to be G6PD A$-^{376G/202A}$. A repeat sample from the 29th patient was obtained and found to have normal G6PD activity and normal electrophoretic mobility of the enzyme.

### *G6PD Puerto Limón*

The mutation of G6PD Puerto Limón was found to be a G→A transition at nt 1192, causing a substitution of the amino acid lysine for the normal glutamate at amino acid 398.

### *G6PD Santamaria*

Both samples of G6PD Santamaria were, indeed, found to have the same mutation, confirming the impression from their biochemical properties that they were the same, although found in unrelated persons (Saenz et al. 1984). Two mutations were found. One of these was the A→G substitution at nt 376 characteristic of G6PD A+ and G6PD A−. However, the mutation at nt 202 characteristic of G6PD A− was not present. Instead an A→T transversion was identified at nt 542, causing a substitution of valine for the normal aspartic acid.

## Discussion

G6PD Puerto Limón is a severely deficient G6PD variant associated with hereditary nonspherocytic hemolytic anemia. It is thus categorized as a class 1 variant in the usual classification (Yoshida et al. 1971). While it was once believed that such variants would prove to be very heterogeneous, our recent studies have indicated that many such variants that were believed to be distinct are, in reality, identical to each other on DNA sequence analysis (Beutler 1991). However, the mutation we identified in G6PD Puerto Limón is one that has not been encountered previously. Its position at nt 1192 places it in a domain of the G6PD molecule that is involved in the binding of NADP (Hirono et al. 1989). Mutations in this region seem invariably to be associated with hereditary nonspherocytic hemolytic anemia (Beutler 1991). The mutation of G6PD Puerto Limón is located between the mutations of G6PD Anaheim and G6PD Nashville, identical variants with a mutation at nt 1178, and G6PD Riverside, with a mutation at nt 1228. Both of these variants are associated with nonspherocytic hemolytic anemia. The substitution of a positively charged lysine at amino acid 398 for the negatively charged glutamic acid normally present accounts for the reported very rapid electrophoretic mobility of the enzyme (Elizondo et al. 1982).

The other variants we have investigated in Costa Rica were not associated with chronic hemolytic anemia; one of the patients with G6PD Santamaria had some evidence of hemolysis but the other was perfectly normal. These variants therefore represent examples of the extensive polymorphism involving this enzyme that has apparently evolved as a mechanism of protection against Falciparum malaria (Luzzatto et al. 1986). G6PD deficiency among people of African origin is phenotypically almost always G6PD A−. Sequence analysis has shown, however, that G6PD A− is actually heterogeneous. At nt 376 an A→G substitution, which occurs also in G6PD A, is always present. This mutation, causing the substitution of asparagine by aspartic acid is responsible for the rapid electrophoretic mobility of G6PD A and G6PD A−. The deficiency is due to the second mutation, which most commonly is a G→A substitution at nt 202. Occasionally, however, the second mutation is different: a T→C may be present at nt 968 or a G→T at nt 680 (Hirono and Beutler 1988; Beutler et al. 1989). The forms of G6PD A- characterized by two of these second mutations, those at nt 202 and at nt 968, have also been found among Spaniards, in whom the mutation had previously been thought to be a unique variant, G6PD Betica (Beutler et al. 1989).

The fact that virtually all African deficiency mutations of G6PD arose in the context of G6PD A has led us to suggest that G6PD A might have been the predominant mutation in Africa at one time (Beutler et al. 1989). Alternatively, it has been suggested that deficiency mutations might be better tolerated in the context of the nt 376 mutation (Yoshida 1989). The existence among persons of African descent of G6PD mutations in which the residual enzyme manifested normal electrophoretic mobility (Chaves et al. 1988), as had been found in Costa Rica, was therefore of special interest.

Unfortunately the original subjects in which such a mutation had been observed were no longer available for study, and none of the 29 black males we studied fell into this category. However, although found in white subjects, G6PD Santamaria may provide an explanation for the existence of African deficient variants with normal electrophoretic mobility, in that we already recognize that G6PD deficiency in Spain reflects G6PD deficiency in Africa. This variant, which has normal electrophoretic mobility, was found to have the G6PD A mutation at nt 376. However, the aspartic acid that was gained at amino acid 126 was lost by the mutation at nt 542, which causes a change from aspartic acid to valine at amino acid 181. The net change in amino acid composition is a loss of an asparagine and the gain of a valine with no change in charge. The double-banded pattern originally reported in the subjects with this enzyme remains unexplained, but could be due to aggregation of the mutant enzyme molecules as is believed to occur also

in G6PD Tel-Hashomer (Kirkman et al. 1969). While G6PD Santamaria cannot be considered to be an example of G6PD A− phenotypically, it does represent a fourth example of a deficiency mutation arising in the context of the nt 376 A→G transition. The fact that the mutant was originally described in two unrelated males (Saenz et al. 1984) could be the result of remote, unknown kinship, but could also indicate that this is yet another polymorphic form of G6PD deficiency. Consistent with the latter interpretation is the fact that no hematologic abnormalities were regularly present in the absence of stress. It remains to be seen whether this mutant will also be found in persons of African ancestry as have been other Spanish G6PD deficient variants, but it could explain the occasional finding of G6PD deficient variants with normal electrophoretic mobility among Africans.

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