

Molecular Genetics of Glucose-6-Phosphate Dehydrogenase Deficiency in Mexico

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ABSTRACT: Several studies carried out between 1965 and 1985 showed that G-6-PD deficiency in Mexico is heterogeneous at the biochemical level and that the G-6-PD A- phenotype is relatively common. We have now investigated the molecular basis of G-6-PD deficiency in Mexico. Up-to-date 60 chromosomes with G6PD mutations have been studied, 16 in previous studies and 44 in the present work. Molecular analysis of DNA from G-6-PD deficient Mexican mestizos and their relatives show that G-6-PD A- genotypes are relatively common but also that in Mexico G-6-PD deficiency is heterogeneous at the DNA level. Thus, five different genotypes have been observed: G-6-PD A-^{202A/376G} (41 chromosomes), G-6-PD A-^{376G/968C} (14 chromosomes), G-6-PD Seattle^{844C} (3 chromosomes), G-6-PD "Mexico City"^{680A} (1 chromosome) and G-6-PD Guadalajara^{1159T} (1 chromosome). The G-6-PD A-^{202A/376G}, G-6-PD A-^{376G/968C} and G-6-PD Seattle^{844C} mutations in Mexico are on the same *Pvu II*/*Pst I*/*1311*/*Nla III* haplotypes as found in individuals from Africa, Spain and the Canary Islands. Consequently, these mutations were probably imported to Mexico through African slaves and/or the Spanish immigrants during and after the colonization.

Keywords: G6PD, erythrocyte, population genetics

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is one of the most common hereditary disorders. The clinical manifestations include neonatal jaundice, chronic hemolytic anemia and drug or food induced hemolysis. G-6-PD shows great genetic heterogeneity; over 400 variants have been described on the basis of their biochemical characteristics. Some of these variants achieve polymorphic frequencies in certain populations (1-4).

Analysis of the DNA sequence of the coding portion has made it possible to identify mutations

in G-6-PD variants. At least 97 distinct hematologically important alleles involving mutations scattered through the G-6-PD gene have been identified (5). In addition, 6 polymorphic sites-- 2 in the G-6-PD coding region and 4 in introns-- have been described (6-10). These polymorphic sites have been helpful in assessing: i) the evolutionary origin of different mutations in the G-6-PD gene, ii) unique origin versus recurrent origin of different G-6-PD variants; and iii) the clonal origin of tissues and diseases (7, 9-15).

The frequency of G-6-PD deficiency in Mexico ranges from 0.39% to 4.09% with the highest prevalence rates occurring in Mestizos

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from both Pacific Coast (Guerrero State, 4.09%) and Gulf Coast (Tabasco State, 3.75%) and the lowest frequencies in several Indian groups (0.57%) as well as in mestizos from Northeastern Mexico (0.66%) and Northwestern Mexico (0.39%) (16-20). G-6-PD deficiency in Mexico is heterogeneous at the biochemical level. The most common variant -- defined by a fast electrophoretic mobility -- resembles G-6-PD A-, and deficient variants with normal and slow electrophoretic mobility have also been observed. Some variants identified and characterized according to the standardized methods recommended by the WHO (21) include G-6-PD Castilla, G-6-PD Tepic, G-6-PD Distrito Federal, G-6-PD Mexico, G-6-PD Chiapas, G-6-PD Guadalajara, G-6-PD Jalisco, G-6-PD Morelia, G-6-PD Canton and G-6-PD Trinacria. The last two variants were identified in individuals with Chinese and Italian ancestors respectively (22-29). DNA analysis of the first three variants has shown that all three variants are examples of G-6-PD A-^{202 A/376 G} (30).

We have previously demonstrated that the class I variant G-6-PD Guadalajara has a substitution at nt 1159 C→T (31). On the other hand, a preliminary study of a small group of seven G-6-PD deficient individuals showed the genotypes G-6-PD A-^{202 A/376 G}, G-6-PD A-^{376 G/968 C}, and G-6-PD Seattle^{844 C} (32).

We now present molecular analysis results of the G-6-PD gene in 74 individuals from 11 Mexican mestizo families with G-6-PD deficiency.

MATERIALS AND METHODS

Patients

Eleven unrelated G-6-PD deficient males and 63 relatives were studied. The index cases were ascertained through a screening program for inborn errors of red cell metabolism in newborns with jaundice and patients with hemolytic

anemia. Blood samples from the eleven families were obtained.

Assay of G-6-PD Activity and G-6-PD Electrophoresis

Quantitative spectrophotometric assay for enzyme activity (33) and G-6-PD electrophoresis (34) were carried out on the red cells of the 74 individuals. All index cases showed a variant with fast electrophoretic mobility like G-6-PD A-. Additionally, among the relatives 9 G-6-PD deficient males and 24 heterozygous females (electrophoretic phenotype B A-) were identified. The mean G-6-PD activity in the deficient hemizygotes and in the heterozygotes was 12% and 59% respectively versus the mean activity (13.78±2.19 IU/g Hb) in the relatives with normal electrophoretic mobility. Thus, the candidate mutations were those involved in the three known G-6-PD A- genotypes which have been observed in both African and non-African populations G-6-PD A-^{202 A/376 G}, G-6-PD A-^{376 G/680 T} and G-6-PD A-^{376 G/968 C}.

Identification of G-6-PD Mutations and Polymorphic Sites

Genomic DNA was extracted from the leukocytes of the whole blood by means of standard methods (35). The relevant exons were amplified by PCR and then analysed with the restriction enzymes *Nla III*, *Fok I*, *Bst NI* and *Nci I* for the detection of substitutions at nucleotides 202, 376, 680 and 968 respectively (36). In the G-6-PD deficient individuals as well as in a previously described Mexican family with the variant G-6-PD Seattle^{844 C} (32) the four polymorphic sites at intron 5 (*Pvu II*), nt 1116 (*Pst I*), nt 1311 (*I311*) and intron 11 (*Nla III*) previously reported (6, 7, 8, 10) were examined using appropriate endonucleases (6,7,8,10). The site was recorded as positive (+) if it was cleaved and negative (-) if it was not.

RESULTS

G-6-PD Mutations and Haplotypes

The mutations responsible for G-6-PD deficiency are shown in Table 1. Fifteen out of 20 male individuals with the biochemical phenotype of G-6-PD A- showed the mutations A-^{202A/376G} whereas in the other five subjects the mutations A-^{376G/968C} were documented. The G-6-PD activity in the males with the first and the second genotypes were 1.854±0.39 and 1.352±0.37 IU/g Hb respectively (mean ± SE).

This difference is not statistically significant.

Eighteen out of 24 heterozygous females showed the genotype Gd^B/Gd A-^{202A/376G} and the other 6 the genotype Gd^B/Gd A-^{376G/968C}. Molecular analysis showed that three women initially classified by biochemical phenotype as homozygous normal were actually heterozygous. Thus, of 44 chromosomes analyzed 33 showed the mutations A-^{202A/376G} and 11 the mutations A-^{376G/968C}. Results of haplotype analysis (*Pvu II* / *Pst I* / *1311* / *Nla III*) of these mutations as well as of the G-6-PD Seattle^{844C} mutation are shown in Table 2.

Table 1. Mutations Responsible for G-6-PD Deficiency

Mutation	No. chromosomes
A- ^{202A/376G}	15 ^a (18) ^b
A- ^{376G/968C}	5 ^a (6) ^b
Total	20(24)

^a Chromosomes from deficient hemizygous subjects

^b Chromosomes from heterozygous subjects

Table 2. Association of G-6-PD A- and G-6-PD Seattle with different haplotypes

Variant	n	Haplotype ^c			
		<i>Pvu II</i>	<i>Pst I</i>	1311	<i>Nla III</i>
G-6-PD A- ^{202A/376G}	15 ^a (18) ^b	+	+	-	+
G-6-PD A- ^{376G/968C}	5 (6)	-	+	-	-
G-6-PD Seattle ^{844C}	2 (1)	-	+	-	-

^a Chromosomes from deficient hemizygotes

^b Chromosomes from heterozygotes

^c Site is recorded as + if it is cleaved or if the 1311 C→T mutation is present

Table 3. G-6-PD Mutations Identified In Mexican Mestizo Subjects

Variant	n ()*	Haplotype				Reference
		Pvu II	Pst I	1311	Nla III	
G-6-PD A ^{-202A/376G}	5 (5)	+	+			30
G-6-PD Guadalajara ^{1159T}	1 (1)					31
G-6-PD "Mexico City" ^{680A}	1 (1)					31
G-6-PD A ^{-202A/376G}	3 (3)					32
G-6-PD A ^{-376G/968C}	3 (2)					32
G-6-PD Seattle ^{844C}	3 (1)	-	+	-	-	32, present work
G-6-PD A ^{-202A/376G}	33 (9)	+	+	-	+	Present work
G-6-PD A ^{-376G/968C}	11 (2)	-	+	-	-	Present work
Total	60 (24)					

*No. total of chromosomes analyzed with mutations (No. chromosomes from unrelated G-6-PD deficient index cases).

DISCUSSION

Epidemiologic and biochemical studies had previously shown that G-6-PD deficiency in Mexico is heterogeneous. G-6-PD A⁻ seems to be the commonest variant (16-20, 22-29). Molecular analysis of DNA from G-6-PD deficient Mexican mestizos corroborates this observation. To date 60 chromosomes containing mutations have been studied -- 16 in previous studies (30,31,32) and 44 in the present work -- and five different genotypes have been observed (Table 3).

G-6-PD A⁻. In this and previous studies (30, 32) of Mexican G6PD deficient subjects 21 out of 24 (87 %) unrelated G-6-PD deficient index cases had a G-6-PD A⁻ variant: 17 individuals (80 %) had the A^{-202A/376G} mutations and 4 subjects (20%) had the A^{-376G/968C} mutations (Table 3). The origin of G-6-PD A^{-202A/376G} appears to be African (9,11). This variant achieves polymorphic frequencies in Africa, Spain, Italy, Canary Islands, Cuba, Costa Rica and Mexico and is always associated with both *Pvu II* and *Pst I* sites regardless of ethnic origin. The variant G-6-PD A^{-376G/968C} is common in Africa, Spain, Cuba, Canary Islands and Mexico (9,11,30,32,36,37,39-42). The G-6-PD A^{-202A/376G} and G-6-PD A^{-376G/968C} mutations in Mexico are

on the same *Pvu II* / *Pst I* / 1311 / *Nla III* haplotypes (Table 3) as found in individuals from Canary Islands (37). It is very probable that the A^{-202A/376G} and A^{-376G/968C} mutations were imported to Mexico through African slaves and/or the Spanish immigrants during and after the colonization. It has been estimated that in 1640 about 150,000 African slaves had already been brought to Mexico (43). On the other hand, about 450,000 peninsular Spanish people emigrated to America between 1506 and 1800. During the 19th and 20th centuries the number of Spanish immigrants was about 1.5 and 3 million respectively. The region that contributed the largest number of immigrants during the first half of 19th century was the Canary Islands (44,45).

G-6-PD Seattle^{844C}. This variant is common in Italy, Spain, Sardinia, Algeria and Canary Islands (37,41, 46-49). It has also been observed in a Brazilian white male with German ancestry (50). The origin of this variant is not known (3). The G-6-PD Seattle^{844C} mutation in Mexico is on the same haplotype (Table 3) as found in individuals from Ireland, Italy, Algeria and Canary Islands (37,38). Probably this mutation was imported to Mexico through the Spanish immigrants.

G-6-PD "Mexico City"^{680A}. This variant is characterized by a G→A transition at nt 680, the same nucleotide which is altered from G to T in one of the types of G-6-PD A-. In G-6-PD "Mexico City" the arginine at amino acid 227 is changed to glycine, while in one form of G-6-PD A- it has become a leucine. G-6-PD "Mexico City" has only been observed in Mexico (31).

G-6-PD Guadalajara^{1159T}. This variant was first observed in Mexico (27,31). It has also been found in Japan (51,52) and Ireland (53); in the Irish patient evidence was obtained suggesting a *de novo* mutation and one of the Japanese patients showed a one base deletion in intron 5. Furthermore, the mutation 1159 C→T has been observed associated with mutation nt 376 A→G in the variant "Mt Sinai"^{376G/1159T} (5). All these data strongly suggest a recurrent origin for the nt 1159 C→T mutation.

G-6-PD deficiency in Spain is also heterogeneous at both biochemical and DNA levels. The most common genotypes in descending order are: i) those corresponding to the G-6-PD A- variant; ii) G-6-PD Mediterranean^{563T}; and iii) G-6-PD Seattle^{844C} (46, 54-57). Other G-6-PD variants observed in Spain include G-6-PD Aures^{143C}, G-6-PD Union^{1360T}, G-6-PD Murcia^{209G}, G-6-PD Clinic^{1215A}, G-6-PD Tomah^{1153C}, G-6-PD Andalus^{1361A} and G-6-PD Santamaria^{376G/542T} (41,57,58). The last variant has also been observed in Canary Islands (37), Algeria (38) and in white subjects from Costa Rica (39).

Our results clearly show that some G-6-PD A- genotypes are relatively common and that G-6-PD deficiency is heterogeneous at the DNA level in Mexico. However, it will be necessary to study more G-6-PD deficient individuals from both general and selected populations in order to determine whether G-6-PD deficiency in Mexico is a reflection of G-6-PD deficiency in Spain and whether other genotypes contribute to the molecular heterogeneity. For instance, the G-6-PD deficient variants with normal

electrophoretic mobility previously observed by others (20) and by us in Mexican mestizos could correspond to the G-6-PD Mediterranean^{563T}, G-6-PD Aures^{143C}, G-6-PD Santamaria^{376G/542T} or to other variants.

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