Novel Point Mutation in Exon 12 of the Glucose-6-Phosphate Dehydrogenase Gene: G6PD Flores

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In Portugal there are a wide variety of G6PD deficiency associated mutations. In an individual from the island of Flores of the Azorean archipelago, we report a new mutation in the G6PD gene that gives rise to a "moderate rate of G6PD deficiency" (12.6% of the normal activity) according to WHO criteria. Direct sequencing revealed a C→A point mutation at position 1387 with the consequent substitution of an Argine by

Serine. We designated this new mutation as G6PD FLORES. The mutation is associated with haplotype I (– – + – –), using six intragenic RFLPs. This information may also be seen as contributing to the clarification of the genetic makeup of the Azorean population, founder mutations, and/or gene flow. *J. Clin. Lab. Anal.* 18:129–131, 2004. © 2004 Wiley-Liss, Inc.

Key words: haplotype; linkage disequilibrium; Azores; Portugal

INTRODUCTION

Red cell glucose-6-phosphate dehydrogenase deficiency (G6PD) is a sex-linked hereditary disorder, probably the most common human enzymopathy, affecting over 100 million individuals worldwide (1). There are multiple inherited molecular forms of this enzyme that are distinguished from the normal G6PD B and from each other by biochemical and molecular characteristics (2,3). Although over 400 G6PD variants based on biochemical studies have been reported, fewer than 127 mutations on the G6PD gene have been identified at the DNA level (4).

Among those with reduced enzymatic activity, the commonest are the G6PD A[–], which is virtually confined to the Negroid population, and the Mediterranean mutation found in Caucasoids and Asiatics (5).

In Portugal, the prevalence of G6PD deficiency in males is 0.51% ± 1.09 (6), and in the Portuguese Azorean archipelago, it is 0.23% ± 0.9 (7).

There are a wide variety of G6PD deficiency associated mutations in the Portuguese population (7). In this study we report a new mutation in the G6PD gene that gives rise to a mild enzymatic deficiency in an individual from the island of Flores of the Azorean archipelago.

MATERIALS AND METHODS

Population

Blood samples with EDTANa₂ as an anticoagulant were collected randomly from 863 Portuguese males (ages 18–21 years) living in the Azores.

Laboratory

All blood samples were screened by the NADP reduction fluorescent method (8). The G6PD activity in the screened deficient samples was determined as recommended by the World Health Organization (1). Samples from both a normal and a G6PD deficient control group were processed simultaneously.

Genomic DNA was extracted from peripheral blood leukocytes following the procedure described by Miller et al. (9).

Polymerase chain reaction (PCR) was carried out in a total volume of 50 µl using 300 ng of genomic

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DNA, 25 pmol of each oligonucleotide (5'-CTgCA-TACCTgTgggCTATg and 5'-gAggCATgAggTAgCTC-CAC), 0.2 mM of each dNTP, 0.24 U DNA Polymerase (AmpliTaQ, PE Biosystems), and 1 × reaction buffer (10 × PCR Buffer, PE Biosystems, Foster City, CA) to obtain a 250 bp fragment of DNA containing the exon 12 of the G6PD gene. After an initial denaturation temperature at 95°C for 5 min, a total of 35 cycles of amplification were performed (one cycle consists of 95°C for 30 sec, 59°C for 30 sec, and 72°C for 45 sec), in a DNA Thermal Cycle 9600 (Perkin-Elmer, Boston, MA).

The amplification products were subjected to single-strand conformation polymorphism (SSCP) analysis by using 95% formamide, 10mM Tris-HCl pH 8.0, 10 mM EDTA, 0.3% bromophenol blue, and 0.3% xylene cyanol and then by heating to 95°C for 3 min followed by rapid cooling at 4°C. Electrophoresis was performed in a nondenaturing 0.5 × MDE gel (FMC), at 40 W, at room temperature in a 0.6 × TBE buffer. The gel was stained by the AgNO₃ method (ethanol 10% for 15 min, nitric acid 1% for 4 min, deionized water for 1 min, AgNO₃ 0.012 M for 20 min, deionized water for 30 sec, sodium carbonate 0.28 M formaldehyde) and fixation with acetic acid 10% for 5 min and deionized water for 5 min for the visualization of the bands. Any mobility shift of single-stranded DNA fragment when compared with a normal control was indicative of an alteration. Fragments showing a mobility shift by SSCP analysis were sequenced using a direct PCR-sequencing procedure by dideoxy chain-termination method (10). Amplified DNA was purified by the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), and the sequencing reactions were performed using the AmpliCycle Sequencing Kit (Perkin-Elmer, Boston, MA) incorporating [α^{35} S] dCTP (Amersham, Freiburg, Germany). Any mutation encountered was confirmed by sequencing the opposite strand.

As described by Rodrigues et al. (7), six intragenic polymorphic sites were also analyzed: exon 5, nt 376 A → G, *Fok I*; intron 5, nt 611 C → G, *PvuII*; intron 8, nt 163 C → T, *BspHI*; exon 10, nt 116 G → A, *PstI*; exon 11, nt 1311 C → T, *BclI*; and intron 11, nt 93 T → C, *NlaIII*. All restriction digestions were performed according to the manufacturer's instructions (New England Biolabs, Beverly, MA). The polymorphisms were recorded as (+) if the restriction site was present and as (-) if it was absent.

RESULTS AND DISCUSSION

The G6PD deficient sample under study was characterized by its enzymatic activity in red blood cells and by analyzing PCR-amplified products from all exons of the G6PD gene by the SSCP method. Direct sequencing

Fig. 1. Sequencing gel of part of exon 12 in both a control and a patient sample. The arrow indicates the position of a G → T transition at base 1387, which replaces an arginine by a serine.

of exon 12 was performed because a shift was observed in the SSCP gel.

The individual presented a level of G6PD activity on RBC lysate of 0.69 ± 0.14 I.U./gHb (mean $\pm$ 1 SD; $n = 1$, in triplicate), 12.6% of the normal activity (5.48 ± 0.8 I.U./gHb), and was thus considered as having a "moderate rate of G6PD deficiency" according to WHO criteria (1).

Direct sequencing revealed a C → A point mutation at position 1387 (Fig. 1) with the consequent substitution of an Arginine by Serine. We designated this new mutation as G6PD FLORES. The mutation is associated with haplotype I (— + + — —).

Further research in the Azorean archipelago may further identify other individuals that carry the same mutation and if successful, may in turn suggest its origin or a founder effect.

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