Glucose-6-Phosphate Dehydrogenase Deficiency Does Not Result From Mutations in the Promoter Region of the G6PD Gene

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In this study, we investigated whether glucose-6-phosphate dehydrogenase (G6PD) promoter mutations are responsible for G6PD deficiency. We analysed the G6PD proximal promoter and the 5′ untranslated region (UTR) in 65 G6PD-deficient individuals, in which no mutations have been found in the G6PD gene coding sequences, using a nonradioactive polymerase chain reaction/single-strand conformation polymorphism (PCR/SSCP) analysis. We identified no sequence variations in the G6PD core promoter or in the 5′ UTR of these G6PD-deficient individuals, which indicates that G6PD deficiency is not associated with promoter mutations in the G6PD locus. J. Clin. Lab. Anal. 17:90–92, 2003. © 2003 Wiley-Liss, Inc.

Key words: G6PD deficiency; promoter; mutations; SSCP

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a very common X-linked inherited disorder. The vast majority of G6PD-deficient individuals are asymptomatic, but a variety of clinical manifestations, ranging from neonatal jaundice to mild or even severe haemolytic anaemia, have been reported in G6PD-deficient individuals (1).

Over 200 mutations in the housekeeping G6PD gene have been demonstrated to reduce or diminish G6PD production in red cells (2). In line with previous population-based studies, this remarkable genetic heterogeneity may reflect carrier adaptation as well as the fact that inherited deficiency of G6PD may confer protection against severe *P. falciparum* malaria (Ref. 3 and references therein).

G6PD deficiency is extremely prevalent and heterogeneous in Greece (4), as it is in other Mediterranean countries, especially because malaria is endemic. However, in a significant number of G6PD-deficient individuals, no molecular defect has been documented in the G6PD coding regions, which suggests that genetic defects outside G6PD coding sequences are responsible for the absence of G6PD in red cells. In this regard, regulatory sequences, such as the promoter or the untranslated regions (UTRs), can be considered to be attractive candidates.

In this communication, we report the results from a systematic study to determine whether G6PD deficiency is a result of G6PD promoter mutations.

MATERIALS AND METHODS

Case Selection and Biochemical Analysis

A total of 4,293 unrelated adult male individuals, recruited for the Hellenic Armed Forces from all over Greece, were included in this study. Informed consent was obtained from each participant. By means of fluorescent screening, 172 G6PD-deficient individuals were identified. These individuals were further analysed by an NADP-linked spectrophotometric assay to determine G6PD activity, as described in Ref. 5.

DNA Analysis

Genomic DNA (100–200 ng) was extracted from peripheral blood leukocytes and subjected to polymerase chain reaction (PCR) for the amplification of the entire G6PD coding sequence, as previously described (4). In addition, PCR amplification of the G6PD promoter and 5′UTR was performed, using 25 pmoles

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of primer G6p-FW (5′-AATTGGGGATGCAGAG-CAGC-3′) and primer G6p-RV (5′-GAAGTGTAC-GACCGTTTCCG-3′). The PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of 1 min denaturation at 94°C, 40 sec annealing at 58°C, and 40 sec elongation at 72°C. The 261-bp PCR products were then analysed, using single strand conformation polymorphism (SSCP) analysis, and electrophoresed in 10% native polyacrylamide gels, as described in Ref. 4. When necessary, the SSCP analysis was challenged by altering the SSCP electrophoretic conditions, including adjusting the temperature (from 4°C to 8°C, in increments of 1°C), the length of the gel run (20 or 40 cm), and the duration of the gel run (12 or 24 hr).

TABLE 1. Overall frequencies of the G6PD variants identified in the Hellenic population

G6PD variant	Chromosomes[a] (n)	Frequency (%)
Meditteranean	362	76.9
Hermoupolis	16	3.4
Cassano	11	2.3
Seattle	6	1.3
Ierapetra	3	<1
Serres	2	<1
Acrokorinthos	1	<1
Harilaou	1	<1
Mutation not within the G6PD locus	65	13.8
Total	471	100

[a]Ref. (4) and this study.

RESULTS AND DISCUSSION

All of the G6PD-deficient cases were asymptomatic, with G6PD activity ranging from 0.2% to 5% of normal. Mutation analysis using PCR/SSCP confirmed the genetic heterogeneity previously observed in the G6PD coding regions for the Hellenic population (4). The mutation frequencies are summarised in Table 1. However, no genetic alteration was identified in 23 G6PD-deficient cases, which indicates that the genetic defect responsible for the reduced G6PD levels may lie outside the coding region of the gene. The possibility that large deletions caused G6PD deficiency by removing part or the entire G6PD gene seems unlikely, because in this case no PCR products would have been generated with the primer sets used to amplify the coding region.

Next, we analysed the G6PD promoter and 5′ UTR to explore the possibility that G6PD deficiency is a result of transcriptional or post-transcriptional impairment of G6PD gene expression. The fragment of the promoter region amplified in our study encompassed the G6PD core promoter element (Fig. 1a), which was previously implicated in transcriptional regulation of the locus (6).

Fig. 1. **a:** Schematic representation of the G6PD promoter and 5′ UTR, and of the PCR fragment used to identify candidate promoter mutations in G6PD-deficient individuals. **b:** Representative PCR/SSCP analysis of the human G6PD promoter region, showing no alteration of the electrophoretic pattern. The samples in lanes 3 and 4 were sequenced to exclude the possibility that a sequence variation was present in the fragment analysed. SS, single-stranded; DS, double-stranded. **c:** DNA sequence of the human G6PD promoter and its 5′ UTR. The TATA box and GC motifs are depicted in black boxes, the CpG and CpNpG islands are in boldface, and the primer sequences used for this study are underlined. Capital C corresponds to the transcriptional start site.

In particular, the amplified region contains the TATA box and three GC motifs, previously shown to bind Sp1 and Sp3 transcription factors (Fig. 1c) (7). For those samples in which the presence of G6PD mutations in the coding region of the gene was excluded, PCR/SSCP analysis of the G6PD promoter and 5′ UTR failed to identify any unusual mobility shift of the fragment analysed in a total of 65 G6PD-deficient individuals (Ref. 4 and present study), indicating that no sequence alterations are located within that fragment (Fig. 1b). To confirm the absence of SSCP electrophoretic mobility shifts, all of the samples were examined in various electrophoretic conditions (i.e., different temperatures, and lengths and durations of the gel run (see Materials and Methods section)), which considerably increased the SSCP resolution of our screening approach. Furthermore, we performed DNA sequence analysis in 15 of the PCR/SSCP-analysed cases, with negative results. These data suggest that promoter or 5′ UTR mutations are probably not implicated in reducing G6PD levels. One cannot exclude the possibility that as-yet-unidentified sequence polymorphisms exist in this region; however, they would probably have no functional consequence in G6PD gene transcription.

An alternative hypothesis is that epigenetic, rather than genetic, changes influence G6PD gene transcription. Promoter CpG or CpNpG island methylation has been implicated in transcriptional silencing of various genes (reviewed in Ref. 8). Also, Toniolo et al. (9) have shown that G6PD expression is associated with the methylation status of promoter CpG islands. Therefore, it would be interesting to examine whether altered G6PD levels in G6PD-deficient individuals can be attributed to epigenetic changes in the G6PD promoter.

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