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Molecular Analysis of the G6PD-Mediterranean Mutation in Kuwait

Key Words

Glucose-6-phosphate
dehydrogenase deficiency
G6PDMed
Kuwait

Abstract

Objective: Glucose-6-phosphate dehydrogenase (G6PD) deficiency has been reported to be quite common in Kuwait, though neither the underlying biochemical variants nor the mutations involved have ever been analyzed. The 563C→T mutation underlies one of the most common G6PD-deficient variants, G6PDMed. It is observed on two different haplotypes (Mediterranean and Asian), as judged by a 1311C→T polymorphism. The objective of this study was a population and haplotype analysis of the 563C→T mutation in Kuwait. **Methods:** DNA was extracted from the peripheral blood of 110 unrelated individuals (population group) and 20 family members of G6PDMed-positive probands. The population group comprises native Kuwaitis and Bedouins residing in Kuwait (63 males and 47 females). Detection of both 563C→T and 1311C→T was carried out by using the PCR/RFLP technique. **Results:** Seven individuals (4 hemizygotes, 2 heterozygotes and 1 homozygote) were identified in the population group, giving a G6PDMed frequency of 0.0509 (0.0635 for males and 0.0425 for females). Haplotype analysis of the G6PDMed chromosomes was informative for all cases except for 1 heterozygous female. Of the 7 unrelated G6PDMed chromosomes from Kuwait, 6 had the Mediterranean and 1 had the Asian haplotype.

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Introduction

The glucose-6-phosphate dehydrogenase (G6PD)-deficiency is one of the most common genetic defects in humans. Clinically, it may present with neonatal jaundice, acute hemolytic anemia induced by infection, drugs or ingestion of fava beans (a condition known as favism) or chronic hemolysis (nonspherocytic hemolytic anemia) [1]. The gene that codes for G6PD is located on the X chromosome in the region q28 and consists of 13 exons. The complete genomic sequence is known and this has enabled the molecular analysis of the G6PD variants. Based on biochemical characteristics, more than 400 variants of G6PD have been identified, of which less than a hundred have been characterized at the DNA level [1, 2]. Some of the G6PD variants are quite common, being polymorphic in many populations and even exceeding a frequency of 0.5 in some ethnic groups. Such a high frequency is thought to be due to a selective advantage of G6PD-deficient variants against malaria leading to their accumulation in areas endemic for the disease. Among such common variants is G6PDMed, which was originally identified in patients from Sardinia, and then from other Mediterranean and neighboring regions including the Middle East [3–12]. In addition, G6PDMed has been reported to be present even so far a field as in Pakistan, India, Singapore and Indonesia [13–16].

The molecular basis of G6PDMed is a C to T substitution at nucleotide 563 (563C→T) of exon 6 that replaces serine for phenylalanine at position 188 [3, 4]. G6PDMed seems to have originated independently in Europe and Asia. The evidence is based on the observation that a polymorphic silent mutation C→T at nucleotide 1311 of the G6PD gene (exon 11) is almost always present on G6PDMed chromosomes from the Mediterranean, and

rarely occurs on corresponding Asian chromosomes [5, 16, 17]. The G6PDMed mutation creates a restriction site for *Mbo*II, therefore allowing its detection by a simple PCR/RFLP test. It thus provides a convenient tool for population analysis of the mutation.

G6PDMed was reported as one of the main variants producing severe G6PD deficiency in the Arabian peninsula [10–12]. Kuwait, like other countries in the peninsula, is also known to have a high frequency of G6PD deficiency [18], though, neither the underlying biochemical variants nor the mutations involved have ever been studied. Using the PCR/RFLP technique, we have looked for the 563C→T mutation as well as the 1311C→T polymorphism in the Kuwaiti population and herein report the results of this study.

Materials and Methods

Peripheral blood was collected from 110 unrelated individuals (63 males, 47 females) as well as from 20 family members of probands with G6PDMed. DNA was extracted using the 'salting-out' technique [19]. The population group comprises native Kuwaitis and Bedouins residing in Kuwait. Primers for PCR amplification of the fragment that includes the site for the 563C→T mutation were chosen based on the G6PD gene sequence deposited in EMBL (accession number X55448 Z29527) using the computer program 'Oligo 5.0' (National Biosciences, Inc.). The sequences of these primers are as follows:

Sense: 5'-TGATCCTCACTCCCCGAAGA-3'
Antisense: 5'-GCTTGGCCCCACCTCAGCAC-3'

The primers used for the amplification of the fragment encompassing the 1311C→T mutation have been previously reported [5]. The antisense primer has a mismatch, which provides a *Bcl*I site only in the presence of the mutation.

The PCR mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatine, 50 μM of each dNTP, 0.4 μM of the primers, 0.75 units of Taq polymerase (Perkin Elmer) and 200–1,000 ng of genomic DNA in a total volume of 25 μl.

Thermocycling was performed in the Perkin Elmer 9600 system using the following conditions: initial

Fig. 1. The partial map of G6PD gene with the sites of 563C→T mutation (**A**) and 1311C→T polymorphism (**B**). The positions of PCR primers are shown by horizontal arrows. ↓ = The normal site; * = the site created by mutation.

denaturation at 94°C for 3 min followed by 30 cycles of 45 s at 94°C, 45 s at 55°C, 45 s at 72°C with a final extension at 72°C for 7 min.

For the RFLP analysis, the PCR products were digested with either the restriction enzyme *Mbo*II for 3 h at 37°C or *Bcl*I for 3 h at 50°C and then subjected to agarose gel electrophoresis. The gel was prepared at a concentration of 1.5 or 2.25% (w/v) agarose in 1 × Tris-borate-EDTA (TBE) buffer with ethidium bromide at a final concentration of 0.5 µg/ml. Electrophoresis was carried out in 1 × TBE buffer at 100 V for 20 min, after which the gel was photographed under UV light.

Results

The expected sizes of the PCR products as well as the *Mbo*II and *Bcl*I restriction fragments corresponding to the 563C→T and 1311C→T mutations, respectively, in the G6PD gene are shown in figure 1. In the case of the 563C→T mutation (fig. 1A), the 230-bp PCR product is cut by *Mbo*II into fragments of 104, 98 and 28 bp. As to the wild-type sequence, a pattern that includes 202-

and 28-bp fragments is observed. For the silent 1311C→T mutation (fig. 1B), the corresponding patterns after *Bcl*I digestion are as previously reported: 203 bp for the wild-type sequence and 180 and 23 bp for the mutant. Representative results showing these patterns in individuals with and without mutations are shown in figures 2 and 3.

Of the 110 individuals analyzed, 7 were found to have the 563C→T mutation: 4 hemizygous males, 2 heterozygous and 1 homozygous females. Thus, the G6PDMed mutation was present in 7.2% of randomly selected Kuwaiti individuals while severely deficient variants (hemizygous or homozygous for the mutation) in 4.5%. Investigation of 20 relatives of G6PDMed probands revealed 5 hemizygous males and 1 homozygous female.

The frequency of the 563C→T mutation in the Kuwaiti population was estimated using only the data for 110 randomly collected samples (table 1). Of the 157 X chromosomes analyzed, 8 had the 563C→T mutation, giving a frequency of 0.0635 for males and

Fig. 2. The detection of 563C→T mutation by electrophoresis in 1.5% agarose gel. Lanes 1–4, 6 and 8 = normal; lane 5 = heterozygote for 563C→T; lane 7 = hemizygote for 563C→T.

Fig. 3. 1311C→T polymorphism analysis of Kuwaiti G6PDMed chromosomes (electrophoresis in 2.25% agarose gel). Lane M = DNA ladder (100, 200, 800, 1,200 and 2,000 bp); lane 1 = undigested PCR fragment; lanes 2, 4, and 5 = heterozygotes for 1311C→T; lane 3 = homozygote for 1311C→T; lane 6 = hemizygote for 1311C→T; lane 7 = wild-type (control sample negative for G6PDMed).

Table 1. Frequency of G6PDMed (563C→T) mutation in Kuwaiti population

	Number of			G6PDMed gene frequency
	individuals	X chromosomes	G6PDMed chromosomes	
Males	63	63	4	0.0635
Females	47	94	4	0.0425
Total	110	157	8	0.0509

0.0425 for females. The overall frequency for this allele in the Kuwaiti populations is 0.0509.

Haplotype analysis of the G6PDMed chromosomes using 1311C→T polymorphism was informative for all cases except for 1 female. This female was double heterozygote for both 563C→T and 1311C→T mutations; however, the lack of DNA samples from her family members did not allow the phase determination. In the other double heterozygous female the haplotyping was possible using DNA samples from siblings. All G6PDMed hemizygous males and 1 of 2 homozygous females revealed only the 1311C→T variant. However, 1 female homozygous for G6PDMed was a heterozygote for 1311C→T polymorphism. Thus, of the 7 unrelated G6PDMed chromosomes from Kuwait, 6 had the Mediterranean and 1 had the Asian haplotype.

Discussion

Kuwait has been reported to be an area with a high frequency of G6PD deficiency [18]. However, information on the underlying G6PD variants is lacking. Taking into consideration that the G6PDMed variant is common in neighboring populations, it was of interest to investigate whether this variant is also present in Kuwait. The direct detection

of the 563C→T mutation at the DNA level provides a major advantage over biochemical techniques especially for the identification of females heterozygous for G6PDMed. Using this approach, we have confirmed the presence of the 563C→T mutation in Kuwait and found it to be polymorphic in the population with an overall frequency of 0.0509. The observed difference between males and females (0.0635 and 0.0425, respectively) is statistically insignificant. However, it should be pointed out that there are at least two reasons why allelic frequencies can be unequal in males and females: nonrandom mating and sex-linked difference in selective forces. Both are relevant to the G6PD system (a high rate of consanguineous marriages in the Arabs and selective advantages of heterozygotes against malaria).

Many of the native Kuwaitis are descendants of the settlers from the eastern and central Arabian tribes (Bani Khalid and Utub) [20, 21]. In this respect, it is interesting to compare our data with the G6PDMed frequency reported for Saudi Arabia from where these tribes originate. However, there is some limitation when making such a comparison because the numerous population studies on G6PD deficiency carried out in Saudi Arabia were based on the use of biochemical techniques which can miss female heterozygotes, and therefore only the gene frequency for males can be considered reliable. The gene frequency for G6PDMed reported in Saudi Arabian males varies from 0.0282 to 0.2610 among the different provinces [10]. The frequency in Kuwait being 0.0635 in males is comparable to that reported for the central province of Saudi Arabia. This is in accord with the fact that many of the early settlers in Kuwait migrated from this region. There is no known history of malaria in Kuwait and the central province of Saudi Arabia was also described as having no measurable malaria

endemicity. However, the ancient Arabic tribes could have migrated to this area from the neighboring regions endemic for malaria.

The finding of the 563C→T mutation with the simultaneous presence of the silent polymorphism, 1311C→T, in 6 of the 7 Kuwaiti chromosomes is in agreement with the data available for the other populations of the Arabian Peninsula. Up to now 9 G6PDMed chromosomes from Saudi Arabia and 18 from Oman (all with the Mediterranean haplotype) have been reported [5, 11]. It is now widely accepted that G6PDMed spread in the Mediterranean and the Middle East with the expansion of the Greek civilization [1, 2]. Unexpectedly the Asian haplotype has also been detected in one of the Kuwaiti G6PDMed chromosomes. In fact, it is the first such chromosome reported from the Arabian Peninsula. The proband with this chromosome is aware that her ancestors migrated to Kuwait from Iran. However, the Iranian G6PDMed chromosomes are also known to have the Mediterranean haplotype [5, 17]. Interestingly, the G6PDMed chromosomes of Pushtoons and Afghans from northwest Pakistan are mainly of the Asian haplotype [13]. Thus, it is possible that the G6PDMed chromosome with the Asian haplotypes could be introduced to Kuwait from this region.

Our study represents the first report of the molecular analysis of G6PD deficiency in the Kuwaiti population. It is very likely that G6PDMed is not the only mutation responsible for G6PD deficiency in Kuwait. Hence, population studies using molecular tests for the detection of other common G6PD mutations such as 202G→A, and 376A→G that underly the common A- variant are required. Furthermore, the molecular analysis of G6PD variants among patients with clinical manifestations of G6PD deficiency is warranted.

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