MOLECULAR IDENTIFICATION OF THE MOST PREVALENT MUTATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) GENE IN DEFICIENT PATIENTS IN GILAN PROVINCE


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Abstract

Glucose-6-Phosphate Dehydrogenase (G6PD) is a cytosolic enzyme which its main function is to produce NADPH in the red blood cells by controlling the step from Glucose-6-Phosphate to 6-Phosphogluconate in the pentose phosphate pathway. G6PD deficiency is the most common X-chromosome linked hereditary enzymopathy in the world, that result in reduced enzyme activity and more than 125 different mutations causing it have been identified. In the present study we analyzed peripheral blood samples of 103 unrelated patients with G6PD deficiency in Gilan Province in the north of Iran. DNA samples from these subjects were analyzed for the presence of certain known G6PD mutations by the appropriate restriction enzyme digestion of fragments, amplified by PCR. In this way, it revealed that Mediterranean mutation (C563T, Ser 188 Phe) is predominant in this area and 89 samples out of 103 (86.4%) have this mutation, indicating a higher prevalence of this mutation, in Gilan in Comparison to Mazandaran (66.2%). Among other patients, 10 samples (9.71%) have Chatham mutation (G1003A, Ala 335 Thr), but none of the samples was found to have Cosenza mutation (G1367, Arg 459 Pro).

Keywords: Favism; Glucose-6-phosphate dehydrogenase; Gilan; Chatham; Cosenza; Mediterranean

Introduction

Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is the most common human genetic enzymopathy [1], affecting more than 200 million people world wide [2-6,21,22]. It is a cytoplasmic
enzyme that affects the production of the NADPH (Nicotine- Adenosine Dinucleotide Phosphate) coenzyme by controlling the first step in the pentose phosphate pathway [5,6,8-11]. In red blood cells, since G6PD is the only source of NADPH, defense against oxidative damage is dependent on its activity [7,9]. Inherited G6PD deficiency is associated with neonatal jaundice, chronic nonspherocytic hemolytic anemia, favism and food or drug induced acute hemolytic anemia [1-3,10,12]. Although the majority of people with this deficiency are asymptomatic, they may show acute hemolytic anemia in association with infections or following the ingestion of some drugs or fava beans [11,13,14]. The G6PD gene is located in the Xq28 region of X chromosome [1,5-7,9,11,15]. It contains 13 exons and 12 introns and is 18.5 kb in length [16], encodes a protein of 515 amino acids [16]. It is highly polymorphic and more than 400 different variants have been identified on the basis of their biochemical properties [3-5,9,12,17].

As fava beans are a common food in northern provinces of Iran, G6PD deficiency is diagnosed more frequently in this area. The aim of the present study was to look for the prevalence of G6PD gene in deficient patients in Gilan Province, Iran. Due to the fact that one of the most common G6PD variants is a Mediterranean variant (C563T, Ser 188 Phe), we screened it in Gilan at first [18]. Also we investigated two other mutations, Chatham (G1003A, Ala 335 Thr) and Cosenza (G1367C, Arg 459 Pro) in this area, because they previously have been showed in Mazandaran province, another state of Iran [18,19,21].

Materials and Methods

During the field work, 103 blood samples were obtained from unrelated male patients with favism (2-6 years), randomly selected at the 17 Shahrivar Hospital in Gilan, Iran. The red blood cell enzyme activity was assayed with the Beutler’s fluorescent spot test [7], to diagnose G6PD deficiency. Genomic DNA was extracted from peripheral blood leukocytes using salting out method [20]. The DNA region from the G6PD gene encompassing each point mutation was selectively amplified by PCR using specific oligonucleotide primers, followed by digestion with restriction enzymes. Digestion products were analyzed on a 12% acrylamide gel. A Biotech apparatus and Cinagen Taq DNA polymerase were used. Oligonucleotides:

F-Med (5'-...CCC CGA AGA GGA ATT CAA GGG GGT...-3'), R- Med (5'-...GAA GAG TAG CCC TCG AGG GTG ACT...-3'), F-Chat (5'-...CAA GGA GCC CAT TCT CTC CCT T...-3'), R-Chat (5'-...TTC TCC ACA TAG AGG ACG ACG GCT GCC AAA GT...-3'), F-Cos (5'-...GCA GCC AGT GCC ATC AGC AAG...-3') and R-Cos (5'-...GGG AAG GAG GGT GGT GCC GTG GG...-3'), as well as restriction enzymes MboII, BstXI and Bsu36I were purchased from TIB MolBiol Synthes labor and Fermentase company respectively [18,19].

All samples were tested for the Mediterranean mutations using F-Med and R-Med primers (35 cycles: one cycle consists of one minute at each of following temperatures 95°, 58°, and 72°C) and digestion with MboII. Samples that were not G6PD-Med, then were screened for Chatham mutations, using F-Chat and R-Chat primers (10 cycles 95°, 30 s and 70°, 1 min and 10 cycles 95°, 65°, 72°C each temperature 1 min), and digestion with BstXI. Remaining samples were examined for Cosenza mutation using F-Cos and R-Cos primers (30 cycles: one cycle consists of 1 min for 95° and 64°C temperature and 80 s for 72°C) and digestion with Bsu36I.

Results

All of 103 patients, obtained in this study, were diagnosed as G6PD deficient. DNA samples were analyzed for Mediterranean mutation (C563T, Ser 188 Phe). After MboII digestion of PCR production (a 583 bp fragment encompassing exons 6 and 7), the normal samples showed 4 fragments (24, 60, 120, 379 bp) on acrylamide gel [18]. But in Mediterranean samples two fragments of 276 bp and 103 bp were seen instead of the normal fragment of 379 bp. In total, 89 samples out of 103 (86.4%), showed this mutation that create the other recognition site for MboII enzyme (Fig. 1).

Other samples were then examined for two other mutations, Chatham (G1003A, Ala 335 Thr) and Cosenza (G1367C, Arg 459 Pro). The presence of BstXI and Bsu36I sites in the amplified fragments of exons 9 and 11-13 established the mutation of the Chatham and Cosenza, respectively. Ten samples out of 14, showed 100 bp and 30 bp fragments instead of 130 bp in RFLP investigation, indicating 9.71% Chatham mutation in our samples (Fig. 2).

Finally, remaining four samples were analyzed for Cosenza mutation and none of the samples showed this mutation (316 bp and 232 bp fragments instead of 548 bp fragment) (Fig. 3).

Discussion

Hundred and three favism patient samples in Gilan state – a north state in Iran – have been analyzed for the most prevalent mutations of G6PD gene; Mediterranean mutation (C563T, Ser 188 Phe), Chatham (G1003A, Ala
Our results showed that, 86.4% (89 out of 103) of patients had Mediterranean mutation, 9.71% (10 out of 103) of patients had Chatham mutation and 4% (4 out of 103) cases didn’t have these mutations and surprisingly, none of samples were found to be Cosenza mutation. Therefore, it requires sequencing the studied samples to search other mutations.

These results comparing with Mazandaran – another province in North of Iran – shows Mediterranean mutation is dominant variant in these areas [18,19]. It’s interesting that the Chatham mutation in Gilan has no high frequency such that observed in Mazandaran (27%) [18,19]. Comparing these issues with Middle Eastern and Italy, these alleles in Gilan are similar to Italian population (80-84% Mediterranean mutation in Italy), then confirming similarity of the mutation spectrum of Iranian population to Italian rather than Middle Eastern [23]. Mazandaran (27%) and surprisingly, none of the samples were found to be G6PD Cosenza. Therefore, it is required to sequence the studied samples to find other possible mutations. It seems that, frequency of G6PD variants is different in northern and other regions of Iran. It is likely that, Cosenza mutation is found in another area in Iran.
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References