

Molecular Identification of the Most Prevalent Mutations of Glucose-6-Phosphate Dehydrogenase (G6PD) Gene in Deficient Patients in Khorasan Province of Iran

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) enzyme catalyses the first step in pentose phosphate pathway (conversion of glucose-6-phosphat to 6-phospho gluconat) which provides cells with pentoses and reduction power in the form of NADPH. In the present study we have analyzed the G6PD gene mutations in 76 patients with a history of favism in Khorasan province in Iran. DNA samples were analyzed for the presence of certain known G6PD mutations by the appropriate (PCR/RFLP analysis). The results showed that similar to Gilan, Golestan and Mazandaran provinces, Mediterranean mutation is the most common G6PD variant in this area (66%). 12% of samples had Chatham mutation and none of the samples had Cosenza mutation. In comparison with the three provinces of Caspian sea shore, more samples did not have three investigated variants (22%) which required further sequencing to search for other mutations.

Keywords: G6PD; Khorasan province; Chatham; Cosenza; Mediterranean; Favism

Introduction

The housekeeping enzyme glucose-6-phosphate dehydrogenase (G6PD) catalyzes the first step in pentose phosphate pathway (conversion of glucose-6-phosphat to 6-phosphoglucono lactone) with the concomitant reduction of NADP⁺. This pathway is an important source of NADPH that is essential for

maintaining adequate intracellular level of reduced forms of glutathione and other sulphhydryl groups. By preserving and regenerating reduced forms of glutathione as well as promoting the stability of catalase, NADPH plays a major role in the stability of cell to withstand oxidative stress. In red blood cells, since G6PD is the only source of NADPH, defense against oxidative damage is dependent on its activity [1-3]. The

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G6PD gene is located on the Xq28 region of X chromosome. It contains 13 exons and 12 introns and is 18.5 kb in length. The active enzyme is composed variably of two or four identical 515 amino acid subunits; each monomer has a molecular weight of 59 kDa. G6PD deficiency is the most common human enzymopathy and affects 400 million people world wide. Although the majority of people with this disease are asymptomatic, some of the clinical symptoms associated with deficiency are acute hemolytic anemia in association with infection or following the ingestion of some drugs or fava beans (favism), neonatal jaundice and in severe deficiency, chronic non-spherocytic hemolytic anemia (CNSHA) [4-6].

The aim of this study was to determine of mutations of G6PD gene in deficient patients in Khorasan province, Iran. In the first step, we screened Mediterranean variant because it is one of the most common G6PD variants. Then two other mutations, Chatham and Cosenza were investigated in this area. [7-11]

Material and Method

In total 76 blood samples were obtained from male and female patients with favism at Doctor Sheikh Hospital in Mashhad, Khorasan, Iran. These patients were recruited following acute anemia triggered by ingestion of fava beans. Suspected patients were diagnosed as G6PD-deficient using Dye Reduction Test [1,12]. Genomic DNA was extracted from peripheral blood leukocytes by salting out and DNA extraction kit. 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 enzyme. Digestion products were analyzed on an acrylamid gel [7,8,11]. All DNA samples were screened for the C→T mutation at nt563, which is characteristic of G6PD Mediterranean, using F-Med (5'...CCC CGA AGA GGA ATT CAA GGG GGT...3'), R-Med (5'...GAA GAG TAG CCC TCG AGG GTG ACT...3') primers and PCR amplification followed by digestion by MboII restriction endonuclease. Amplification was carried out for 35 cycles (each cycle consists of one minute at each of the following temperatures: 95°C, 60°C, 72°C) by using 0.5 unit of Taq DNA polymerase in a final volume of 25 µl. The G6PD-med mutation at the base position 563 creates one MboII site in exon 6 and 7 of the G6PD gene. The amplification product was digested with MboII for 4 h at 37°C and the digestion products were analyzed on 8% acrylamid gel [10,11]. Samples that were not G6PD-med were screened for Chatham mutation, using 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') primers. Amplification was carried out for 10 cycles: 95°C, 30 s and 70°C, 1 min and 20 cycles: 95°C, 65°C, 72°C each temperature 1 min) and digestion with BstxI. The remaining samples were examined for Cosenza mutation using F-cos (5'...GCA GCC AGT GGG ATC AGC AAG...3') and R-cos (5'...GGC AAG GAG GGT GGC CGT GG...3') primers (30 cycles: each cycle consists of 1 min for 95°C and 64°C temperature and 80 s for 72°C) and digestion with Eco81I on agarose gel [7-11].

Results

After MboII digestion of PCR products (a 583bp fragment encompassing exons 6 and 7), the normal sample showed 4 fragments (24, 60, 120, 379 bp) on Acrylamid gel but in Mediterranean samples (C563T, ser 188 phe) two fragments of 276 bp and 103 bp were seen instead of the normal fragment of 379 bp. In total, 51 samples out of 76 showed this mutation (Fig. 1). The other samples were then examined for Chatham mutation (G1003A, Ala 335 Thr), after BstxI digestion of PCR products, samples that showed this mutation, produced 100 bp and 30 bp fragments instead of 130 bp, 9 samples out of 25 showed this mutation (Fig. 2). Finally, 16 remaining samples were analyzed for Cosenza mutation (G13C.ARG99Pro). Samples that had this mutation produced 316 and 233 bp fragments instead of 548 bp fragment. None of the samples have shown Cosenza mutation (Fig. 3).

Discussion

More than 130 different molecular abnormalities have been described for G6PD deficiency. The frequency of G6PD deficiency in the Middle East (Kuwait, Egypt, Iran, Syria, Lebanon and Jordan) varies widely, ranging from 1% for Egyptians to 11.55% for Iranians. Initially investigation of Mediterranean, Chatham and Cosenza, mutants was carried out in Northern provinces (Mazandaran, Gillan, Golestan) of Iran since fava beans are common in the diet of the mentioned provinces population. After research in these three provinces, Khorasan province has been assessed. Final results revealed that Mediterranean mutant is common in four provinces (Gillan = 86/4%, Golestan = 69%, Mazandaran = 66/2%, Khorasan = 66%). The prevalence of Mediterranean mutant declines gradually from Gillan province toward Khorasan province [7-11].

In Gillan and Mazandaran provinces the prevalence of Mediterranean and Chatham mutant are nearly the same.

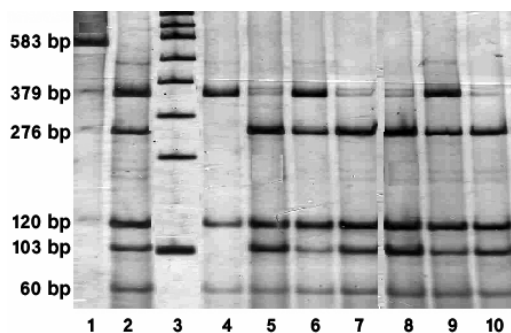


Figure 1. Restriction digestion analysis of PCR products related to G6PD Mediterranean mutation with MboII enzyme on acrylamid gel. Lane 1: PCR product; Lane 2: positive control; Lanes 5-10: G6PD Mediterranean mutation; Lane 3: molecular weight marker (100 bp Ladder); Lane 4: normal sample.

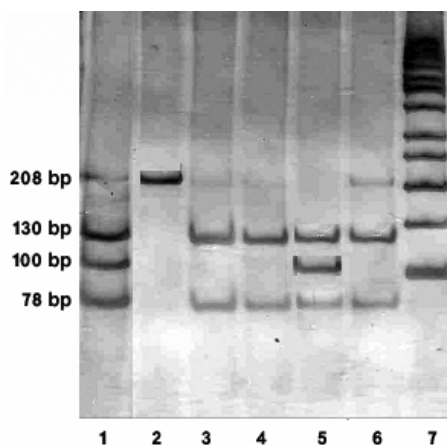


Figure 2. Restriction digestion analysis of PCR products related to G6PD Chatham mutation with BstXI enzyme on acrylamid gel. Lane 1: positive control; Lane 2: PCR product; Lanes 3, 4, 6: normal sample; Lane 5: G6PD Chatham mutation; Lane 7: molecular weight marker (50 bp Ladder).

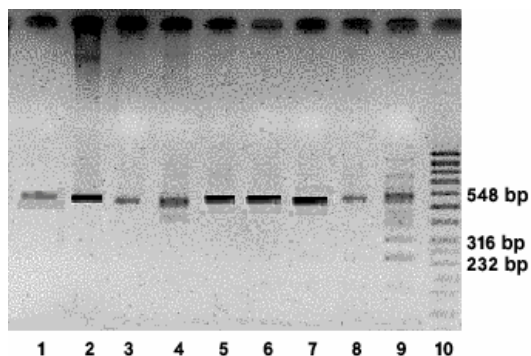


Figure 3. Restriction digestion analysis of PCR product related to G6PD Cosenza mutation with Eco8II enzyme on agarose gel. Lane 1: PCR product; Lanes 2-8: normal sample; Lane 9: positive control; Lane 10: molecular weight marker (50 bp Ladder).

In Khorasan province 16% of patients had Mediterranean mutation, 12% had Chatham mutation and Cosenza mutation was not detected. In contrary to the three Northern provinces in which most of the patients suffer from one of the three mentioned mutations, in Khorasan province 22% of patients did not show the three investigated mutations, therefore search for other mutations is required [7-11].

Khorasan province is the biggest province in Iran with about 313335 square Kilometers areas and 6013200 populations. Khorasan province is located in north east of Iran which neighbors to Turkmenistan from north and Afghanistan from east. The major ethnic groups in this region are Persian, Turkmen, Afghan, Turk and minorities of Kurd and Arab. Because this province is close to Afghanistan and Turkmenistan probably the percent of Unknown G6pd deficient variants can be expected to be similar to these countries. In Neighbour city which is located in this province Fava bean is planted immeasurably. In pre spring, severe hemolytic anemia has been detected in favism patients.

Mediterranean G6PD is a common G6PD variant around the Mediterranean Sea and Middle Eastern and fava bean cultivation is widespread especially throughout the Mediterranean region. [13,14,18,23].

In Kuwait and Saudi Arabia G6PD Mediterranean and A- genotypes were characterized as the most common variants among the G6PD deficient population, which suggest that gene flows from the Indian subcontinent, sub-Saharan Africa and other parts of the Mediterranean may have contributed to the observed G6PD mutation seen in these countries. [24,25]

G6PD Mediterranean is the commonest deficient variant in other Asian countries such as Turkey (77%), Iran (69%), India (60/4%) and Pakistan [12,15,22].

High prevalence of G6PD Mediterranean is also accounted for G6PD deficiency in Kurdish Jews. [19]

In African countries around the Mediterranean Sea such as Egypt and Libya, G6PD Mediterranean is the most common variant. The most common variants in Algeria are G6PD A- (46%) and G6PD Mediterranean (23%), both of which were associated with favism. G6PD Aures and G6PD Santamaria also are seen in this country. Thus, G6PD deficiency in Algeria is heterogeneous, suggesting that there has been significant gene flow, both from sub-Saharan Africa and from other Mediterranean parts [16,21].

In European countries such as Italy (70%), Sardinia, Spain and Greece which are near to the Mediterranean sea, G6PD Mediterranean is the most common variant [17,20,26].

The four most frequent polymorphic variants found in Spain are G6PD A-, G6PD Mediterranean, G6PD

Union and G6PD Seattle. The fact that G6PDA- is the most common variant within Spanish population and regarding the presence of G6PD Aures and G6PD Santamaria which are polymorphic in Algeria, it can be concluded that a significant gene flow from Africa to Europe occurred through Spain [27].

Molecular characterization revealed that in southeast Asia which is far from the Mediterranean sea, G6PD Mediterranean is less common and other variants are prevalent for example G6PD Mahodol was seen in Myanmar. G6PD Vanua Lava was found among Indonesians, G6PD Viangchan was observed in Laos and Thailand and G6PD Coimera was found in Malaysia [28-30].

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