SPREAD OF THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANT (G6PD-MEDITERRANEAN) IN ONE OF THE COASTAL PROVINCES OF CASPIAN SEA IN IRAN

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Abstract

In order to explore the nature of glucose-6-phosphate dehydrogenase (G6PD) deficiency in one of the coastal provinces of the Caspian Sea (Mazandaran) in Iran, we have analysed the G6PD gene in 74 unrelated G6PD-deficient males (2-6 year children) with a history of Favism, by using PCR and subsequent digestion by appropriate restriction enzymes, looking for the presence of certain known mutations. The results showed that 49 of 74 cases (66.21%) had the G6PD Mediterranean genotype and there were not other known mutations (such as G6PD Aures, G6PD A, and G6PD A$^-$) in rest of the samples. This is the first report on the molecular analysis of G6PD mutations in north of Iran and we have revealed the frequency and distribution of the most common G6PD variant (G6PD-Mediterranean) in this area.

Introduction

Glucose-6-Phosphate Dehydrogenase (G6PD, EC 1.1.1.49) deficiency is the most common human enzymopathy which has affected more than 400 million people worldwide [1,2]. The G6PD gene, mapped to chromosome Xq28, consists of 13 exons and encodes a protein of 515 amino acids [3,4]. More than 122 different mutations in the G6PD gene have been found to be the primary defect of about 177 variants of red cell G6PD deficiency [5]. Although the majority of people with this disease are asymptomatic, they may develop acute hemolytic anemia in association with infections following the ingestion of certain drugs or fava beans (Favism). In a few sporadic cases, G6PD deficiency is the cause of chronic nonspherocytic hemolytic anemia [6,7]. Most of the G6PD deficiency patients are African, Middle Eastern, and Southeast Asian ancestry. One of the most common G6PD variants is a Mediterranean one, it has been observed in several countries such as: Saudi Arabia [8,9], Bahrain [10,11], Oman, Iraq, Jordan, Lebanon and one case of Iran in 1990 [12], Turkey [13], Pakistan [14], Egypt [15], Greece [16,17],

Keywords: G6PD deficiency, Favism, Mutation, Mediterranean

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Italy [18], Spain [19]. The frequency of this variant of G6PD is 0.70 among Kurdish jews, probably the highest incidence of G6PD deficiency in any population [20]. There is one report in 1984 by Ohkura *et al.* that indicated 8.6 percent for incidence G6PD deficiency in Mazandaranian and Guilanian in Iran [21]. The aim of this study is the determination of the molecular basis of G6PD deficiency in the coastal provinces of the Caspian Sea in the north of Iran. There are three provinces in this area and Mazandaran province was selected first for this purpose. Due to the fact that fava beans are a common human food in this area, hemolytic anemia develops in subjects with G6PD deficiency after digestion of fave beans (Favism). G6PD Mediterranean (Gd-Med) is characterized by very low activity (less than 5% of normal), and is associated with favism and other acute hemolytic anemia caused by infection or the ingestion of certain drugs [22]. The mutation responsible for Mediterranean phenotype, a C→T transition of nucleotide 563 causing the amino acid replacement 188 Ser→Phe, has been determined for the first time by T. Vulliamy and *et al.* in a subject from Calabria, southern Italy [22,23]. In this study we have performed the Polymerase Chain Reaction-Restriction Enzyme (PCR-RE) analysis provided a rapid method for the diagnosis of the molecular defects in subjects with common forms of G6PD deficiency (Favism) found in one of the coastal provinces of the Caspian Sea.

Materials and Methods

We studied 74 G6PD deficient unrelated male subjects. Sample selection is based on hospital admissions. Male patients aged between 2 to 6 years, who already had a history of favism and hospitalized at the "Amircola Children's Hospital" (a medical center in Mazandaran province) and had received one or two blood units, were selected. Blood samples were then collected from these patients at least three months after the first blood reception when they recovered from their seasonal disease. All of the samples showed G6PD deficiency when the fluorescent spot test [24] was used.

DNA Analysis

Genomic DNA was extracted from peripheral blood leukocytes from normal and affected subjects by standard method [25]. Polymerase Chain Reaction (PCR) was performed according to T. Vulliamy's protocols. A Perkin-Elmer apparatus and Cinagen Taq DNA polymerase (an Iranian product) were used. Oligonucleotides 91(CCCCgAAgAggAATTCAAgggg gT), 92(gAAgAgTAgCCCTCgAgggTgACT), nB(CAg CCACTTCTAACCACACACCT), P4B(CCgAAgTTgg CCATgCTggg), A(CTgTCTgTgTgTCTgTCTgTCC) and M(ggCCAgCCTggCAggCgggAAgg), as well as Restriction Enzymes Mbo II, Bg1 II and Fok I were purchased from TIB MolBiol Syntheselabor, New England Biolabs and Roche Company respectively. All DNA samples were screened for the C→T mutation at nt 563, which is characteristic of G6PD Mediterranean, using 91 and 92 primers and PCR amplification and digestion by Mbo II restriction endonuclease [26]. Amplification was carried out for 30 cycles (one cycle consists of one minute at each of following temperatures: 95°C, 56C, 72°C) by using 2.5 unit of Taq polymerase in a final volume of 50 µl. The G6PD-Med mutation at base position 563 creates an Mbo II site in exon VI of the G6PD gene. The amplification product was digested with Mbo II for 4 h at 37°C, and the digestion products were analysed on 2.5% agarose gels (1:1 agarose and Nuseive agarose). Samples that were not G6PD-Med were then screened for T→C mutation at nt 143, which is characteristic of G6PD-Aures, using nB and P4B primers and PCR amplification under the same conditions mentioned above but at annealing temperature 58°C and digestion by Bg1 II. When this mutation was absent, samples were then screened for A→ G mutation at nt 376, characteristic of G6PD A^-, using A and M primers and PCR amplification at different condition and by Fok I digestion. If none of these mutations was present, the entire coding region must be sequenced using PCR-amplified DNA that the latter stage remained for near future.

Results

All 74 DNA samples from male children with severe G6PD deficiency (Favism) were first screened by PCR-RE analysis for the 563 C→T mutation (G6PD -Med). A 583 bp fragment encompassing exon 6 and 7 was amplified from genomic DNA by PCR with primers 91 and 92 (Fig. 1). After Mbo II digestion the fragment

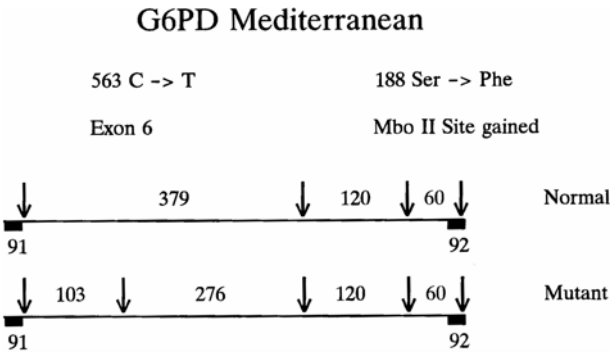

Figure 1. Oligonucleotide primers 91 and 92 amplify a 583 bp fragment across exon 6 and 7 of the G6PD gene. Explanations are in the text.

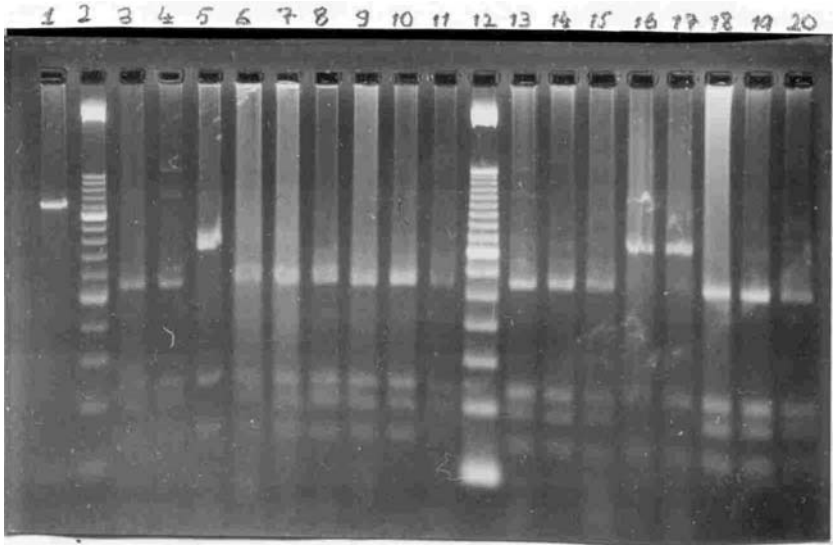

Figure 2. It indicates the Mbo II digestion of PCR products of 17 samples including G6PD Mediterranean positive control and it shows that 13 samples have the Mediterranean mutation on G6PD gene.

Lane 1=Uncut Sample. Lanes 2 and 12=50 bp DNA Ladder Marker. Lane 13=Gd-Med Positive Control.

sizes obtained are shown below. Mutant fragment of 276 bp and 103 bp are seen in place of the normal fragment of 379 bp. We found the Gd-Med genotype in 66.21% (49 cases of 74 subjects, Figure 2, as a sample of these results). The 25 remaining samples were then examined for G6PD Aures and G6PD A, and it revealed these mutations were not presented in all of samples.

Therefore, it requires to sequence the other coding region using advanced techniques such as DNA sequencing that we could be able to test and report it in near future.

Discussion

This paper reports the frequency of the most common molecular variant of G6PD in one of the coastal provinces of the Caspian Sea, Mazandaran, of Iran. Although the literature on the epidemiology of G6PD deficiency in the Middle-East is quite extensive, there are a few papers in this regard in Iranian population which indicates 9.8 percent for frequency of G6PD deficiency among different provinces and tribes (21,27). According to unpublished data about the frequencies of G6PD deficiency in some provinces of Iran, Favism is the most common form of G6PD deficiency in the coastal provinces of Caspian Sea. In this study we presented for the first time the results of the molecular analysis of DNA from 74 G6PD deficient subjects from the Mazandaran province of Caspian Sea. We used PCR-RE analysis providing a rapid method for detecting the known mutations. We found the G6PD Mediterranean (Gd-Med) genotype in 66.21% of the samples and this result confirmed the high frequency of Gd-Med in this province. As we were able to show, favism in people of Mazandaran province is simply due to mutation 563 C→T (G6PD-Med), it suggests a common origin of part of the north of Iran and Mediterranean population.

Acknowledgements

This work was supported by grants from the Tarbiat Modarres University, National Research Center for Genetic Engineering and Biotechnology (NRCGEB) and Ministry of Health, Treatment and Medical Education of Islamic Republic of Iran. We are indebted to T. J. Vulliamy from Department of Hematology, Hammersmith Hospital, London, UK For his comments, protocols and positive controls of Gd-Med variant and we would also like to thank to G. Joursaraee from Babol University and M. Bineshpajooh, director of Amircola Children's Hospital in Mazandaran province for their help on several occasions and to H. Abdul Tehrani from Sheffield University and S. Enayat from Birmingham University, UK for sending us the Mbo II restriction enzyme.

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