

## Molecular Identification of the Most Prevalent Mutations of Glucose-6-Phosphate Dehydrogenase (G6PD) in Fars and Isfahan of Iran

M.R. Noori-Dalooi,<sup>1,\*</sup> F. Hashemi-Gorji,<sup>2</sup> M.R. Alivand,<sup>2</sup> M. Hashemi,<sup>5</sup> R.  
Mirfakhraie,<sup>2</sup> P. Atef-vahid,<sup>3</sup> M. Salehi,<sup>4</sup> R. Salehi,<sup>4</sup> and C. Azimi<sup>1</sup>

<sup>1</sup>Department of Medical Genetics, Faculty of Medicine, Tehran University of  
Medical Sciences, Tehran, Islamic Republic of Iran

<sup>2</sup>Department of Medical Genetics, National Institute of Genetic Engineering and  
Biotechnology (NIGEB), Tehran, Islamic Republic of Iran

<sup>3</sup>Department of Basic Sciences, Science and Research Branch, Islamic Azad  
University, Tehran, Islamic Republic of Iran

<sup>4</sup>Department of Genetics, Medical School, Isfahan University of Medical  
Sciences, Isfahan, Islamic Republic of Iran

<sup>5</sup>Department of Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Islamic Republic of Iran

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### Abstract

Glucose-6-phosphate dehydrogenase (G6PD) in humans is in X-linked disorder, housekeeping enzyme and vital for the survival of every cell. It catalyses the oxidation of glucose-6-phosphate to 6-phospho Gluconat in the first committed step of the pentose phosphate pathway, which provides cells with pentoses and reducing power in the form of NADPH. NADPH is required to protect the cells against oxidative damage. The aim of this study was the molecular analysis of common G6PD mutations in the provinces of the Fars and Isfahan. The Study of G6PD deficiency was performed on 96 patients with a history of favism, consisted of 34 samples from Fars and 62 samples from Isfahan. Genomic DNA with specific oligonucleotide primers followed by digestion with restriction enzymes for known mutations such as; Mediterranean, Chatham, Cosenza, Aures, A-(202G-A/376A-G were carried out. The most common allele in Iran was found to be the G6PD Mediterranean (82.3%), followed by the G6PD Chatham (8.3%) and none of the samples had Cosenza or A-(G202A/A376G) mutation, and remained unknown (9.4%). Further sequencing required to search for the other mutations among the remained samples (9.4%).

**Keywords:** G6PD deficiency; Mediterranean; Chatham

### Introduction

Glucose-6-Phosphate Dehydrogenase (G6PD) defi-

ciency is the most common human enzyme deficiency affecting 10% of the world population which accounts for 400 million people [1]. More than 160 mutations

\* Corresponding author, Tel.: +98(21)88953005, Fax: +98(21)66958808, E-mail: nooridalooi@sina.tums.ac.ir

have been reported for G6PD deficiency [2]. G6PD deficiency is one of the most frequent hereditary abnormalities with a X-linked recessive pattern of inheritance. G6PD deficiency gives selective advantage against severe malaria [3]. G6PD is a housekeeping enzyme which catalyzes the first step in pentose phosphate pathway (PPP). Through a series of reactions, PPP converts glucose-6-phosphate to ribose-5-phosphate, a precursor of many important molecules like RNA, DNA, ATP, CoA, NAD, and FAD. The PPP also produces NADPH molecules which acts as an electron donor and thus reduces energy of the cell by maintaining the reduced glutathione in the cell. Reduced glutathione acts as an antioxidant and protects the cells against oxidative damage [4]. Different mutations are found, and have been characterized at the deoxyribonucleic acid (DNA) level. Interestingly, only few missense and small in-frame deletions are known. Therefore, most of the mutations identified so far are point mutations causing single amino acid substitution in the G6PD gene [5]. Mutations causing G6PD deficiency must have a serious enough effects on the enzyme to decrease its stability, ensuring decreased red cell activity, but it should not be so severe as to cause a decrease in efficiency of the enzyme that would drastically affect its activity in somatic cells [6]. Polymorphic G6PD variants (also known as WHO class II and III [7]) have gene frequencies 1–70% in particular populations. Different geographical areas have different sets of polymorphic variants. G6PD A– accounts for about 90% of G6PD deficiency [8]. G6PD A– is quite prevalent in Italy, the Canary Islands, Spain, Portugal, and in the Middle East, including, Egypt, and Lebanon [9]. G6PD A-<sup>202(G-A)/376(A-G)</sup> is the most common African mutation [3]. G6PD Mediterranean563T is widespread in the Mediterranean areas, the Middle East and the Indian subcontinent [10]. Statistical modeling indicates that the A- allele arose within the past 3840 to 11,760 years and the Med allele arose within the past 1600 to 6640 years [11].

Although Iranian population consists of different ethnic groups, but the overall incidence of G6PD deficiency in Iranian population is estimated around 10%-14.9% [12]. Some studies were carried out on the molecular basis of G6PD deficiency in Iran and showed that the Mediterranean mutation have the highest frequency in Gilan, Mazandaran, Golestan [13], Khorasan, Hormozgan, Sistan & Baluchestan, Yazd and Kerman [12-17]. Chatham mutation is the next mutation in frequency. Cosenza mutation was detected only in Mazandaran and Kermanshah provinces and not in the other provinces [17,18]. The prevalence of G6PD deficiency has been reported to be around 12% in males

and 0.9% in females in the Fars province [19]. The aim of the present study was to characterize the mutations underlying G6PD deficiency such as Mediterranean, Chatham, Cosenza and identification of other variants such as Aures, A-(G202A/A376G) in Fars and Isfahan and other provinces such as Gilan, Mazandaran, Golestan, Hormozgan, Sistan & Baluchestan, Yazd, Kerman and Khuzestan, Iran which has been detected in the Arabic countries in the vicinity of Iran.

## Material and Method

Firstly, for the study of the mutations in G6PD deficient in Fars and Isfahan provinces, the samples of the 96 unrelated patients were collected (34 and 62 samples, respectively) and were tested for the G6PD deficiency, using the semi quantitative fluorescent spot test [1] (Table 1).

The blood samples in EDTA from none-related patients with G6PD deficiency were collected from hospital where they admitted. Genomic DNA was extracted and purified from the peripheral blood leukocytes using the standard phenol–chloroform extraction method.

To identify G6PD mutations, six point mutations in G6PD gene were selected and amplified by specific primers. First, all of the G6PD-deficient samples were screened for C to T mutation at nt 563, which is characteristic of G6PD Mediterranean.

Amplification was carried out and then Mediterranean PCR products were digested with MboII. In samples by which C to T mutation was absent looked for G to A mutation at nt 1003 in exon9, which is characteristic of G6PD Chatham. Chatham PCR product was digested with BstXI for overnight at 55c, and the digestion product were analyzed on acryl amide gel. In negative samples for Chatham, mutation was tested for Cosenza mutation (G-C mutation at nt 1376) and Cosenza PCR products were digested with *eco81I*. Then

**Table 1.** Prevalence of Mediterranean, Chatham, Cosenza, Aures and A - (G202A; A376G), in Fars and Isfahan

Provinces	Percent of mutations				
	Mediterranean	Chatham	Cosenza	Aures	A-(G202A; A376G)
Fars	79.46% 27/34 samples	8.82% 3/34 samples	-	-	-
Isfahan	83.87% 52/62 samples	8.06% 5/62 samples	-	-	-

PCR reaction for Aures mutation (T-C transition at nucleotide 143) was carried out. PCR product was digested with BglII and analyzed on 2.5% Agarose gel. PCR product was prepared for the point mutations A376 and A202. Then digestion was carried out with NlaIII and BseGI restriction enzymes for each of them, respectively. Primers were according to vulliamy's protocols [12,17] and Biotech apparatus was used for PCR reactions.

At the next step unknown samples from other provinces(Golestan, Sistan, Hormozgan, Gilan, Yazd and Kerman) that previously were reported as negative for three common mutations (Mediterranean, Chatham and Cosenza) were analyzed for the Aures and double mutations A-(G202A/A376G), respectively.

### Results

Firstly, the G6PD genotypes of the all samples were analyzed for the Med mutation (563 C-T). After digestion of the PCR product, the normal fragments showed 4 fragments (24, 60,120 and 379) on the acrylamide gel. 79samples produced a new restriction site (276 and 103in place of the normal fragment 379bp) for MboII in exon 6 as five fragment (276bp, 120bp, 103bp 60bp and 24bp) detected on acrylamide gel. 82.3% identified as Med mutation among these samples. Chatham mutation creates a new site for BstXI in exon9. 8.3% of the remaining samples on 12% acrylamide gel showed 100, 30 and 87bp fragments instead of 130and 78bp fragments in RFLP with BstXI. Then digestion for Cosenza PCR product was carried out but it was not found in our investigation. Search was also continued for the presence of Aures mutation in 9 remaining samples with BglII on PCR product but it was not found as well. Also Digestion with NlaIII and BseGI on the remaining negative samples did not show digestion for each of the double mutation A - (G202A/A376G), in our samples and the remained unknown.

### Discussion

G6PD deficiency is highly prevalent among Iranians. So far five variant detected in Iran at the molecular level (Mediterranean, Chatham, Cosenza, Canton, A-mutations). G6PD Mediterranean mutation is located in exon 6 of the G6PD gene and is the most common variant in many parts of the world. It has widespread distribution in countries around the Mediterranean Sea and also in the Middle East and Iran [20]. In this report G6PD Mediterranean (82.3%) was found to have a widespread distribution and the most common variant in the Fars and Isfahan, two provinces of Iran. The Table 1

shows the frequency of five variants in Fars and Isfahan. This mutation was found to be present in all the G6PD-deficient cases amongst Hormozgan, Khorasan, Kurdistan, Sistan & Baluchestan, Gilan, Mazandaran, Golestan, Zanzan, Yazd, Kerman and Khuzestan provinces, and the data have been published before, except for Khuzestan (Table 2) [12-16,18]. The highest and lowest frequencies for Mediterranean reached to 91.2% in Kermanshah and to 63% in Kerman provinces [14,18]. This mutation was also present in the neighbor countries of Iran such as Saudi Arabia , UAE, Turkey, India and also Italy at Mediterranean region [6], but in the South of Asia countries such as China and Japan, G6PD Mahidol (487 G>A) is the most common mutation [21]. The Mediterranean mutation in Europe and Middle East is associated with a silent C→T transition at nucleotide position 1311 [22]. Such a Med<sup>+</sup>/BcII<sup>+</sup> haplotype is typical for the Mediterranean regions and the Middle East, while the most cases from India and South East Asia are Med<sup>+</sup>/BcII<sup>-</sup>, suggesting independent origin of Mediterranean mutation [23]. Previous study on Kermanshah province with the Mediterranean mutation reported that 90% of Mediterranean cases were polymorphic1311T-C(BcII<sup>+</sup>) [18].

G6PD Chatham mutation is located in exon 9 in the G6PD gene. Missense mutation Chatham (1003G-A) is the next common variants and accounts for 8.3%. The highest frequency for Chatham (27%) reported from Mazandaran and Golestan, two provinces in the North of Iran, and the lowest frequency for G6PD Chatham in the world has been reported from Brazil (0.66%) [12]. The Chatham mutation has much wider in ethnic

**Table 2.** Prevalence of Mediterranean, Chatham and Cosenza in north, center and south of Iran

Area's group	Provinces	Percent of mutations		
		Mediterranean	Chatham	Cosenza
<i>Northern</i>				
	Gilan	86.5	9.7	-
	Mazandaran	66.2	27	6.7
	Golestan	69	26.7	-
	Khorasan	66	12	-
<i>Central</i>				
	Yazd	64	3.6	-
	Kerman	63	-	-
<i>Southern</i>				
	Sistan & Baluchestan	80	2.1	-
	Hormozgan	79	8	-

distribution, and firstly identified among English of Indian descent [24]. Most case of the G6PD deficiency Chatham had the *Bc/I* haplotype [25].

Cosenza mutation first reported from Italy and recently, it was reported from Mazandaran in north costal province and Kermanshah in Kermanshah province in western region of Iran, but in Fars and Isfahan as well as Hormozgan, Khorasan, Kurdistan, Sistan & Baluchestan, Gilan, Zanjan, Khuzestan, Yazd, Kerman it has not been found yet. It seems that Cosenza allele does not exist in this area (Table 2).

We investigated Aures mutation in two provinces (Fars and Isfahan) and also among unknown samples from Gilan, Golestan, Sistan & Baluchestan and Hormozgan province in Iran. The Aures mutation was mainly limited to Arabs and it was firstly found in Algeria, and then it was identified later in Saudi Arabia, Spain, Yemen, Kuwait and UAE [24,26]. In this study none of the sample had Aures mutation. It seems that Aures mutation does not exist among the Iranian population as well.

In our study we could not detect any double mutation A- in the remaining samples from all provinces. A-(G202A/A376G), variant has been reported as genetically heterogeneous that located mainly in Africa, Mediterranean region and the Middle East, such as in Arabic countries around the Persian Gulf such as Oman, Saudi Arabia, Kuwait and Iraq [19,23]. In previous study by Karimi *et al.* only one sporadic variant A- was detected in Fars province [19].

In summary, it can be seen that the two polymorphic mutations, G6PD Mediterranean, G6PD Chatham account for the most of the G6PD deficiencies found in the Fars and Isfahan in Iran. From the present investigation as well as other studies, it can be presumed that G6PD Mediterranean is older than the other because this mutation has been found mainly in all the provinces of Iran. Although the origin of the Iranian population is uncertain but this experiment as well as previous study showed that the Iranian origin differ from the African or Arabic countries in the vicinity of Iran and are more close to the Mediterranean countries.

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