

cations due to iron overload and on desferrioxamine therapy was not increased among those with *TFR2* polymorphisms. Our results show that the *TFR2* polymorphisms, I238M and IVS16 +251 CA deletion, while prevalent in Chinese patients, do not influence the degree of iron loading in transfusion-independent β -thalassaemia intermedia. These *TFR2* polymorphisms are therefore not useful in explaining the severe iron overload that may be encountered in our patients. This agrees with findings on I238M polymorphism in normal Asian subjects.¹⁰ Furthermore, detection of common *HFE* polymorphisms is also not expected to be fruitful, given the low prevalence of these in our area. Nevertheless, the presence of other, hitherto unidentified genetic determinant(s) of iron overload in the Chinese population cannot be excluded and may need to be unraveled in the future.

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Key words: *TFR2* polymorphism, iron overload, β -thalassaemia intermedia, genetic hemochromatosis.

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Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Fars province of Iran

We investigated 78 glucose-6-phosphate dehydrogenase (G6PD)-deficient alleles from the Fars province of Iran by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and direct sequencing. The frequency of G6PD Mediterranean in Fars was 84.6%, G6PD Chatham was found to be highly polymorphic and two other sporadic variants (G6PD A- and G6PD Canton) were detected in single cases.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme disorder in humans and is characterized by considerable biochemical and molecular heterogeneity.¹ The prevalence of G6PD deficiency in the Middle East varies greatly, ranging from 1% among Egyptians to 11.55% among Iranians.^{2,3} G6PD Mediterranean (563 C→T) mutation is probably the most common G6PD variant in the world; it has been widely reported in Europe but also in the Middle-East and in neighboring countries not bordering the Mediterranean sea.^{4,5} Among the known variants, the relative frequency of this mutation ranges from 70% among Egyptians to 97% for Kurdish Jews.⁶ A recent study carried out on the population of the Mazandaran state of North Iran near the Caspian sea showed a frequency for the G6PD Mediterranean mutation of 66.2% and the presence of two other polymorphic mutations: G6PD Chatham^{1003A} (27%) and G6PD Cosenza^{1376C} (6.7%).⁷ We report here a study performed on 78 G6PD-deficient alleles from a different population of Iran, originating from the Fars province, located in the South of the country. The incidence of G6PD deficiency in this area is estimated to be about 12% in males and 0.9% in females.³

The study was carried out on 74 unrelated G6PD deficient subjects (66 males, 8 females) aged between 10 days to 20 years (mean 8±5 years) all originating from the Fars province of Iran. The subjects were recruited from neonatal and school screening. The diagnosis of G6PD deficiency was based on the fluorescent spot test. Clinical data were recorded considering neonatal jaundice, favism or drug-related hemolysis.

As preliminary screening, the following polymorphic G6PD molecular variants were tested by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): G6PD Mediterranean^{563T}, G6PD A-^{376G/202A}, G6PD Seattle^{844C}, G6PD Aures^{143C} and G6PD Santamaria^{376G/542T}.

The G6PD Mediterranean mutation was detected in 62/74 (83.8%) samples. Four females were homozygous for this mutation, leading to an overall allele frequency of 84.6%. Among the other variants screened for, we identified one subject with G6PD A- (1.3%) whereas G6PD Seattle, G6PD Aures and G6PD Santamaria were absent from all our samples. The 11 negative samples were submitted to SSCP analysis of the entire G6PD coding region that allowed us to identify two different abnormal patterns in exon 9 and 12, respectively. Nucleotide sequencing of exon 9 revealed a G to A substitution at nt 1003 responsible for

Table 1. Frequencies of G6PD mutations in the Fars province of Iran.

Molecular variant	Cases		Alleles	
	N.	(%)	N.	(%)
Mediterranean (563 C→T)	62	83.78	66	84.62
Chatham (1003 G→A)	10	13.51	10	12.82
A- (202 G→A/376 A→G)	1	1.35	1	1.28
Canton (1376 G→T)	1	1.35	1	1.28
Total	74	100	78	100

Table 2. Clinical data of the 74 G6PD-deficient subjects.

Clinical manifestation	Total	Med	Chatham	A-	Canton
Favism	33	28/33 (84.9%)	4/33 (12.1%)	1/33 (3.0%)	0/33
Acute hemolytic anemia	35	29/35 (82.9%)	5/35 (14.3%)	1/35 (2.8%)	0/33
Neonatal jaundice	24	18/24 (75.0%)	5/24 (20.8%)	0/24	1/24 (4.2%)
Hyperbilirubinemia	41	35/41 (85.4%)	5/41 (12.2%)	1/41 (2.4%)	0/41

G6PD Chatham in 10/74 samples (13.5%). Nucleotide sequencing of exon 12 revealed the substitution C→T at position 1376 which is responsible for G6PD Canton variant (allele frequency: 1.3%). All mutations and frequencies are summarized in Table 1. G6PD Chatham was reported for the first time in an Indian boy but it is now recognized as one of the most common variants worldwide, being present in several populations.⁷⁻⁹ The medical records of all the G6PD-deficient subjects showed that neonatal jaundice occurred in 32.4% of the cases (24/74), favism in 44.6% (33/74) and moderate to severe hyperbilirubinemia in 55.4% (41/74). The incidence of clinical manifestations related to the different molecular variants are reported in Table 2. This is the first report on the molecular basis of G6PD deficiency in the Fars province of Iran showing that the allele frequency of G6PD Mediterranean mutation in Fars (84.6%) is similar to that described for other neighboring countries^{9,10} and for the Mediterranean region, but higher than that observed in Mazandaran (66.2%), located on the South coast of the Caspian sea, in the North of Iran.⁷ Furthermore, the absence of mutations that are polymorphic in North Africa and the Arabian peninsula (G6PD Aures, Seattle and Santamaria)^{8,10} suggests a different origin and spread of G6PD variants in the South of Iran.

The G6PD Chatham mutation (1003 G→A) is highly polymorphic in the Fars region (13%), although its frequency does not reach the values recently observed in the Mazandaran province (27%). However, G6PD Mediterranean and Chatham variants are by far the most commonly observed in both areas. It is noteworthy that G6PD Cosenza and Canton, found in two different regions of Iran, affect the same nucleotide position in the G6PD gene, but the origin of the two mutations is likely to

be different. In fact, G6PD Cosenza, identified for the first time in Italy, is a known variant already described in the Middle-East and in the Mediterranean probably migrating from Western countries to Iran. By contrast, G6PD Canton is one of the most common variants in the South East of Asia⁹ and is likely to have been spread during the agricultural migration from China.

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