

Molecular heterogeneity of glucose-6-phosphate dehydrogenase deficiency in Jordan

Molecular screening for glucose-6-phosphate (G6PD) mutations in two Jordanian populations revealed six different mutations and higher incidences of G6PD deficiency and G6PD A- (376A→G + 202G→A) mutation in Jordan Valley than in the Amman area. These observations may be explained by historically higher rates of malaria and African ancestral origins, respectively.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary disorder in humans and is found in people of Mediterranean, South-east Asian and African descent. The common clinical manifestations of G6PD deficiency are neonatal jaundice and acute hemolytic anemia triggered by certain drugs, infections or ingestion of fava beans.¹ G6PD deficiency is a heterogeneous enzyme abnormality. There are few published population-based studies on G6PD molecular heterogeneity in the Middle East.² In a recent pilot study, we found that individuals with G6PD Mediterranean mutation were more susceptible to hemolysis than were individuals with G6PD A- mutation in Jordan.³ The present study was performed on larger population samples from two regions, Amman area and Jordan Valley, to identify the different G6PD mutations in the Jordanian population.

Blood samples were collected from 875 Jordanian male donors in Amman area and 106 male subjects in Jordan Valley. Fluorescent spot screening for G6PD deficiency was carried out in all individuals.⁴ Quantitative G6PD assays were performed in individuals with positive screening test.⁴ DNA was extracted from whole blood G6PD-deficient individuals, after obtaining informed consent.

Polymerase chain reaction (PCR) products encompassing the twelve G6PD exons, including exon/intron boundaries, were amplified using AccuPrime Supermix according to manufacturer's instructions (Invitrogen Life Technologies) using primers previously described.⁵ The same PCR primers were used for the sequencing reaction with the ExoSAP-IT (USB Corporation) purified PCR product and an ABI Big Dye v3.1 terminator (Applied Biosystems) according to manufacturer's instructions. Samples were analyzed using an ABI 3100 genetic analyzer and Sequencer analysis software.

We found a 3.2% incidence rate of G6PD deficiency in the Amman area, as reported previously for the Jordanian community in Kuwait.⁶ Six different G6PD mutations were observed in our study of which 53.3% were Mediterranean mutation as compared to four different mutations (72.9% Mediterranean) in Kuwait, two different mutations (84% Mediterranean) in Saudi Arabia and four different mutations (84.6% Mediterranean) in Iran.² The lower prevalence of the G6PD

Table 1. G6PD mutations in Jordan.

G6PD variant	Mutation	Amman Area* (28 cases)		Jordan Valley° (6 cases)	
		No. of cases	(%)	No. of cases	(%)
G6PD Mediterranean	563C→T	15	53.6	2	33.3
G6PD A-	376A→G+202G→A	4	14.2	2	33.3
G6PD Chatham	1003G→A	1	3.6	2	33.3
G6PD Valladolid	406C→T	2	7.1	-	-
G6PD Aures	143T→C	1	3.6	-	-
G6PD Asahi	202G→A	1	3.6	-	-
Unknown		4	14.2	-	-

*Population of 2,000,000; °population of 110,000.

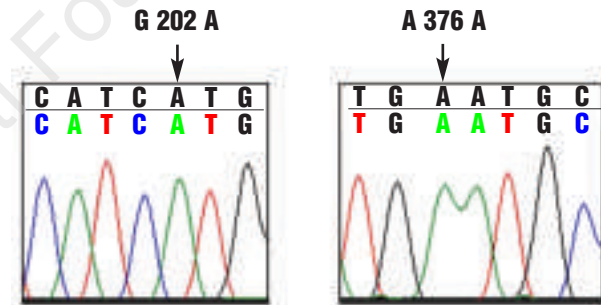


Figure 1. Partial nucleotide sequence of G6PD 202G→A. The G6PD gene has the 202G→A mutation (left) but not the 376A→G (right).

Mediterranean mutation and the finding of six different mutations in a relatively small population reflect the considerable genetic heterogeneity of the Jordanian population. Several unique mutations were observed, including the 202G→A and the 406C→T mutations (Table 1). The G6PD A- mutation, frequently found in people of African ancestry, consists of the 376A→G mutation with one of three additional mutations, mostly 202G→A1. Individuals with only the 376A→G mutation are asymptomatic with normal enzyme activity. This polymorphism is very common among Africans. The other mutation, 202G→A, has been seen by itself only once in a Japanese subject and was named G6PD Asahi.⁷ We detected the 202G→A mutation by itself in one subject in our study (Figure 1). We searched the entire coding sequence of the G6PD gene, the complete introns between exons 3 and 4, 9 and 10 and 11 and 13, the flanking adjacent introns of the remaining exons and the 3'-UTR and found them free of a second mutation. Our observation and those of Hirono *et al.*,⁷ contradict the results of the study of recombinant human

G6PD expression in *E.coli* which concluded that the 202G→A mutation and the 376A→G mutation are required to act together in a synergetic manner in order to produce G6PD deficiency.⁸ Our subject with the 202G→A mutation had 18% residual enzyme activity but was clinically normal with no history of hemolytic anemia and jaundice in spite of past ingestion of fava beans. Hirono *et al.*⁷ reported that their 3-year old patient with the Asahi variant had an attack of anemia and jaundice and a history of neonatal jaundice.

The G6PD Valladolid mutation, originally described in a single case with central Spanish ancestry,⁹ was detected in two cases in our study. One of them was examined clinically but was found to be normal with no history of any hemolytic crisis even after ingestion of fresh fava beans. Zara *et al.*,⁹ reported that the Valladolid mutation case suffered from acute hemolysis after ingestion of fava beans. Clinical observations of our subjects with G6PD Valladolid and Asahi illustrate that the clinical expression of G6PD deficiency results from an interaction of the molecular properties of each G6PD variant with exogenous factors and possibly with additional population-specific genetic factors.

The dwellers of Jordan Valley showed much less genetic heterogeneity than the residents of the Amman region. In recent history, there has been little immigration from other areas in Jordan to the Valley. Its people are thought to have some African ancestral origin. We found the incidence of G6PD deficiency in the Jordan Valley to be 8.5% (9/106). Six subjects underwent molecular studies, as shown in Table 1. The higher incidence of G6PD deficiency in Jordan Valley than in the Amman area may be explained by the fact that malaria was hyper-endemic in Jordan Valley with much higher prevalence than in other areas before eradication of the disease three decades ago. The selective advantage of malaria resistance which is found with G6PD deficiency¹ may have caused this higher prevalence in the Jordan Valley. The equal proportions of the G6PD A- and the Mediterranean mutations support the suggestion that the Jordan Valley population has a significant number of African ancestors, as has also recently been reported following an analysis of y-chromosome markers.¹⁰

No mutation was seen in the remaining four cases, although there is a remote possibility that a mutation was present in the intron regions with large sequences that we did not sequence completely. These samples are currently under investigation to test whether G6PD deficiency (1.3 to 4% of normal level) could be due to epigenetic changes in the G6PD promoter.¹

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