original paper
Haematologica 1994; 79:319-321

MOLECULAR AND BIOCHEMICAL DATA ON SOME GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS FROM SOUTHERN SARDINIA

Roberto Frigerio*, Gabriella Sole*, Mario Lovicu*, Giuseppe Passiu°

**Research Institute on Thalassemias, Italian National Research Council, Cagliari; °Institute of Clinical Medicine, Cagliari University, Italy*

ABSTRACT

Background. **Glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49) deficiency is the most common human enzymopathy; nearly 400 different biochemical variants of the enzyme have been described. Sardinia is the Italian region with the highest frequency of this defect.**

Methods. **We examined genomic DNA of 16 subjects with G6PD Mediterranean, 2 with G6PD Athens-like, 1 with G6PD Ferrara 2 (all as biochemically defined).**

Results. **All G6PD Mediterranean subjects had a C→T mutation at nucleotide 563 and a C→T transition at nucleotide 1311; G6PD Athens-like and Ferrara 2 subjects had a G→C mutation at nucleotide 844 (the same mutation has been found in G6PD Seattle-like).**

Conclusions. **This study suggests that in Southern Sardinia G6PD mutations are relatively homogeneous and that the results of biochemical characterization studies must be carefully evaluated, because the same mutations might be responsible for different biochemical behavior.**

Key words: G6PD variants, G6PD characterization

Glucose-6-phosphate dehydrogenase (G6PD; D-glucose-6-phosphate: NADP oxydoreductase; E.C. 1.1.1.49) deficiency is one of the most common human genetic abnormalities: to date it affects more than 400 million people living in tropical and sub-tropical areas; it is very common among ethnical groups living along the sides of the Mediterranean basin. In this area Sardinia is not only the Italian region with the highest national frequency of the defect (12.61% of the male population affected), but it is also the one with the highest incidence, which in some places reaches 35%.[1]

G6PD deficiency is caused by a variant enzyme which differs from the wild one in its catalytical properties or in its stability. To date nearly 400 variants believed to be *unique* have been characterized on the basis of their biochemical behavior; they have been grouped into 5 classes according to the level of residual enzyme activity and clinical manifestations.[2]

The commonest variant in Sardinia is G6PD Mediterranean. Heterogeneity in the biochemical properties of subjects with a *Mediterranean* phenotype has been reported[3,4] but has not been confirmed at the DNA level. In order to identify the molecular lesions responsible for *putative Mediterranean* variant phenotypes and to verify the usefulness of data from biochemical characterization, we examined a little group of unrelated Sardinian males through biochemical and (whenever possible) molecular characterization.

Materials and methods

We examined 384 males coming from South-

Correspondence: Roberto Frigerio, Istituto di Ricerca sulle Talassemie, CNR, via Ospedale 107, 09124 Cagliari, Italy.
Tel. international +39.70.656937. Fax. international +39.70.655666.
Received March 22, 1994; accepted May 25, 1994.

Table 1. Biochemical characteristics of the G6PD variants examined.

G6PD	N.r of examined subjects	activity (% of normal)	electrophoretic mobility (TEB) (%)	Km G6P (M)	Km NADP (M)	Substrate analogue utilization (%) 2dG6P	dNADP	pH optimum
B	40	111.2±15.6	100	44.6±4.7	4.08±.77	5.24±1.2	65.14±5.42	biphasic
B[2]		100	100	50-70	2.9-4.4	<4	55-60	biphasic
Mediterr.	37	1.63±1.06	100	10.2±3.2	2.43±.63	47.8±6.2	353.1±38.9	6.5-9.5
Mediterr[3]		0-10	98-99	11.4±1.0	2.06±.26	50.3±6.1	312.8±27.6	6.5-9-5
Athens-like	1	9.08	97	23.08	4.76	12.68	122.21	7.0-9.5
Athens-like	1	17.09	97	21.96	2.67	13.82	143.82	7.0-9.5
Athens-like[3]		1-10	98-99	17.8±1.6	3.33±.29	15.4±3.9	152.7±11.8	biphasic
Ferrara 2	1	28.16	95	20.43	4.12	15.2	136.1	7.0-9.5
Ferrara 2[2]		18	95	28.29	2.3	10	121	7.0-9.5
Seattle[2]		8.21	80	15-25	2.4-2.8	7-11	154	biphasic

ern Sardinia. G6PD activity was determined for all of them on freeze-thawed hemolysates according to ICSH procedures.[5] G6PD was purified and characterized from the erythrocytes of 42 males with class 2 G6PD variants (residual activity < 10%) according to the methods recommended by the WHO,[6] except that DEAE-Sephadex A 50 was used instead of DEAE-cellulose in order to obtain a better yield. Genomic DNA was extracted from the whole blood leukocytes of 18 males with biochemically defined class 2 G6PD variants; DNA containing G6PD exons was amplified in two stages by polymerase chain reaction, using primers designed from intron sequences as previously published.[7] The amplified fragments were sequenced according to Sanger[8] and compared with the known sequences of G6PD cDNA.

Results

Of 384 males examined, 78 (20.31%) had a class 2, and 4 (1.04%) had a class 3 G6PD variant.

Of 40 males with a class 2 G6PD variant, 37 had the biochemical properties of G6PD Mediterranean, 2 had G6PD Athens-like, 1 had G6PD Ferrara 2. Results are listed in Table 1, along with the values reported for these same variants from the usual tabulations.[2]

Sixteen subjects with G6PD Mediterranean, 2 with G6PD Athens-like and 1 with G6PD Ferrara 2 (all as biochemically defined) were molecularly characterized. All G6PD Mediterranean subjects had the expected 563 C→T mutation, resulting in a phenylalanine for serine replacement at amino acid position 188.[9] They also all had the 1311 C→T silent mutation. The nucleotide sequence of the coding regions of the Athens-like and Ferrara 2 variants demonstrated the same 844 G→C mutation, resulting in a histidine for aspartic acid replacement at amino acid position 282; this mutation has already been described as being responsible for the Seattle-like phenotype.[9]

Table 2. Molecular data of the G6PD variants examined.

G6PD	*exon*	*mutation*	*amino acid substitution*
Mediterranean	6 11	563C→T 1311C→T	188 ser→phe
Athens-like	8	844G→C	205 asp→his
Ferrara 2	8	844G→C	205 asp→his

Discussion

G6PD deficiency, as biochemically defined, has been regarded as being quite heterogeneous; for a long time slight biochemical differences had been invoked to justify G6PD *Mediterranean* heterogeneity.[3,4] Molecular sequencing proved that the heterogeneity of G6PD Mediterranean-type variants was not as real[9,10] as previously thought. Molecular analysis confirmed that the Mediterranean mutation (563 C→T) accounts for all samples that we biochemically defined as G6PD Mediterranean (Table 2).

All subjects with the 563 C→T mutation had a second nucleotide difference in exon 11, a C→T transition that does not cause any amino acid change; the same silent mutation is present in almost all G6PD B subjects we examined. The high frequency of C→T mutations at nt 563 and nt 1311 is not surprising because C→T transition is a common event: cytosine is often methylated and 5'-methyl-cytosine can undergo spontaneous deamination to thymidine.

The G6PD variants biochemically defined as Athens-like and Ferrara 2 (to the best of our knowledge neither has been defined molecularly) had the Seattle-like mutation (844 G→C). All three of these variants differ from one another for a few slight biochemical characteristics; we defined as Ferrara 2 that variant which shared most biochemical characteristics with G6PD Athens-like, except for electrophoretic mobility (Table 1). An examination of the usual tabulation for G6PD variants[2] reveals that G6PD Ferrara 2, Athens-like and Seattle-like have a very moderate degree of variation in their biochemical properties. Molecular characterization of G6PD Ferrara 2 and G6PD Athens-like showed the same mutation already described as being responsible for G6PD Seattle-like, and this suggested that *two point mutations are responsible for G6PD polymorphism in Sardinia*.[9]

Our molecular data suggest that in Southern Sardinia G6PD mutations are relatively homogeneous; the biochemical heterogeneity of G6PD variants might only be due to the necessarily strict assay conditions. Despite the proposed standardization,[6] side-by-side comparison is still a difficult goal to achieve.

References

1. Carcassi UEF. The interaction between thalassemia, G6PD deficiency and favism. Ann NY Acad Sci USA 1974; 232:297-305.
2. Beutler E. The genetics of glucose-6-phosphate dehydrogenase deficiency. Semin Hematol 1990; 27:137-64.
3. Stamatoyannopoulos G, Voigtlander V, Kotsakis P, Akrivakis A. Genetic diversity of the "Mediterranean" glucose-6-phosphate dehydrogenase deficiency phenotype. J Clin Invest 1971; 50:1253-61.
4. Vives-Corrons JL, Pujades MA. Heterogeneity of "Mediterranean-type" glucose-6-phosphate dehydrogenase (G6PD) deficiency in Spain and description of two new variants associated with favism. Hum Genet 1980; 60:216-21.
5. Beutler E, Blume K, Kaplan PC, Lohr GW, Ramot B, Valentine WM. International Committee for Standardization in Haematology: recommended methods for red cell enzyme analysis. Br J Haematol 1977; 35:331-40.
6. Betke K, Beutler E, Brewer GJ, et al. Standardization of procedures for the study of glucose-6-phosphate dehydrogenase: report of a WHO scientific group. WHO Tech Rep Series No. 366, 1967.
7. Beutler E, Vives-Corrons JL, Hirono A, Prchal JT, Crader W. The molecular biology of variation in glucose-6-phosphate dehydrogenase. In: Brewer GJ, ed. The red cell: Seventh Ann Arbor Conference. New York: Alan Liss, 1989:39-54.
8. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
9. De Vita G, Alcalay M, Sampietro M, Cappellini MD, Fiorelli G, Toniolo D. Two point mutations are responsible for G6PD polymorphism in Sardinia. Am J Hum Genet 1989; 44:233-40.
10. Vulliamy TJ, D'Urso M, Battistuzzi G, et al. Diverse point mutations in the human glucose-6-phosphate dehydrogenase gene cause enzyme deficiency and mild or severe hemolytic anemia. Proc Natl Acad Sci USA 1988; 85:5171-5.