Mutations and haplotype diversity in 70 Portuguese G6PD-deficient individuals: an overview on the origin and evolution of mutated alleles

G6PD deficiency mutational profile and haplotype diversity using 6 RFLPs (FokI/PvuII/BspHI/PstI/BclI/NlaIII) and a (CTT)$_n$ microsatellite, were investigated in 70 G6PD-deficient Portuguese individuals. All but one G6PD A-$^{376G/202A}$ variants (44/45) have a single haplotype (+/+/–/+/–/+/195). G6PD Betica$^{376G/968C}$ alleles (n=10) have a single RFLP haplotype (+/–/–/+/–/+) and 4 different (CTT)$_n$ repeats. Age estimates based on microsatellite variation suggest that Betica mutation arose 900 generations ago. G6PD SantaMaria$^{376G/542T}$ allele was found on haplotype (+/–/–/+/–/+/201) and 10 G6PD variants on RFLP haplotypes (–/–/+/+/–/–), (–/–/+/+/–/+) and (–/–/+/+/+/+).

Haematologica 2007; 92:1713-1714. DOI: 10.3324/haematol.11670

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common human enzyme defect with a particularly high prevalence in tropical regions.[1] More than 140 different point mutations are responsible for the G6PD deficient variants.[2] The most widespread deficient alleles are G6PD A-$^{376G/202A}$, common in sub-Saharan Africa, and G6PD Med^{563T} predominantly in Mediterranean countries, the Middle East and India.[1] In Portugal, G6PD deficiency average frequency is less than 1%, unevenly distributed throughout the country.[3] G6PD A-$^{376G/202A}$ predominate, but other deficient alleles have also been found.[4] To better characterize the deficiency, we performed a molecular study of 70 unrelated G6PD-deficient individuals without known black ancestry, mainly from Central Portugal (n=32), and investigated the G6PD locus haplotype diversity. Blood samples from 54 hemizygous males, 14 heterozygous females and 1 homozygous female, diagnosed with G6PD deficiency based on the clinical history, hematologic data and demonstration of a reduced erythrocyte G6PD activity by quantitative spectrophotometer analysis, were collected for DNA analysis. G6PD gene exons 2 to 13 PCR amplification and mutation screening were carried out using primers and strategies previously reported.[5-7] Haplotype patterns in patients and in 66 unrelated normal individuals (41 males and 25 females) from Central Portugal were established with 6 intragenic RFLPs (Tables 1 and 2) and a (CTT)$_n$ microsatellite using primers and conditions previously reported.[5-7] Linkage phase from female diploid data was performed by analysis of the father's sample or by statistical inference. To estimate age to the most recent common ancestor (MRCA) of the original Betica$^{376G/968C}$ RFLP/microsatellite haplotype we used the algorithm described in Seixas *et al.*[8] A recombination fraction of 2.95×10^{-4} for this X chromosome region,[7] and the highest microsatellite mutation rate μ of 0.001 were drawn for iterations. Mutations and associated RFLP/microsatellite haplotypes from G6PD-deficient Portuguese patients are shown in Table 1. Thirteen different G6PD deficient alleles were found. The most common were G6PD A-$^{376G/202A}$ (63.4%) and G6PD Betica$^{376G/968C}$ (14.1%). From the remaining 11 G6PD deficient variants, 5 were previously reported to be restricted to the Portuguese population [Mira d'Aire^{1048A} (n=1), Anadia^{1193G} (n=1), Covão do Lobo^{1205A} (n=1), Figueira da Foz^{1366A} (n=1) and Coimbra^{592T} (n=3)][2,9] and 5 were reported in other populations [SantaMaria$^{376G/542T}$(n=1), Seattle^{844C} (n=3), Chatham^{1003A} (n=2), Kamiube^{1387T} (n=1) and Canton^{1376T} (n=1)].[2] G6PD Açores 595A→G (199Ile→Val) (n=1) is reported here for the first time. All the 45 G6PD A-$^{376G/202A}$ chromosomes were observed in the context of the RFLP haplotype (+/+/–/+/–/+), the same described in the great majority of A- alleles reported in sub-Saharan Africa and other populations.[5-7] As far as the CTT microsatellite is concerned, all but one of the A-$^{376G/202A}$ alleles (44/45) are associated with the 195-bp long repeat (Table 1), the same as found in sub-Saharan populations and Mexican Mestizos.[7,10] This is consistent with the hypothesis of a recent origin of the 202G→A mutation in Africa in the A haplotype (+/+/–/+/–/+/195) followed by a rapid increase in frequency and a significant gene flow to European populations and other geographic regions. The only A-$^{376G/202A}$ variant observed in association with a CTT 189-bp long allele is from an Azorean hemizygous patient, and may have resulted by forward slippage during DNA replication from the common A- 195-bp haplotype or by a recombination event. G6PD Betica$^{376G/968C}$ variant is present in the African A haplotype (+/–/–/+/–/+) associating (CTT)$_n$ repeats 195-, 198-, 204- and 207-bp long. This differs from the reports on Mexican Mestizos with all the 376G/968C alleles associating the CTT 204-bp allele.[10] The higher heterozygosity (H=0.78) for the CTT microsatellite observed in the Portuguese Betica$^{376G/968C}$ chromosomes suggests an old origin for the 968T→C change in the most common (40%) G6PD A haplotype (+/–/–/+/–/+/204). Betica allele age estimates, based on LD decay from the presumed original CTT 204-bp allele, gives a time to the MRCA of 900 generations (with a 95% CI of 300 to >2000), corresponding to 27,000 years if a mean value of 30 years per generation is assumed.

G6PD SantaMaria$^{376G/542T}$ variant observed in one chromosome occurs on the haplotype (+/–/–/+/–/+/201), as do 3 chromosomes described in Mexico Mestizos, which supports a unique origin for this African deleterious mutation. Eight different rare variants were observed on the B haplotype (–/–/+/+/–/–), the most prevalent among the Portuguese control population (Table 2). Only G6PD Anadia^{1193G} and G6PD Mira d'Aire^{1048A} variants were associated with 2 less frequent Portuguese B haplotypes

Table 1. Mutations and RFLP/microsatellite haplotypes found in 70 Portuguese G6PD deficient patients. Alleles were nominated (+) and (–) according to the restriction site presence or absence.

G6PD variant	*RFLP haplotype*						*(CTT)n Allele size*	*n*
	E-4 376A/G FokI	*I-5 611C/G PvuII*	*I-8 163C/T BspHI*	*E-10 1116A/G PstI*	*E-11 1311C/T BclI*	*I-11 93T/C NlaIII*		
A-$^{376G/202A}$	+	+	-	+	-	+	195	43
							189	1
Betica$^{376G/968C}$							204	4
							195	3
	+	-	-	+	-	+	207	2
							198	1
Santa Maria$^{376G/542T}$	+	-	-	+	-	+	201	1
Covão Lobo^{1205A}	-	-	+	+	-	-	198	1
Figueira da Foz^{1366A}	-	-	+	+	-	-	192	1
Coimbra^{592T}	-	-	+	+	-	-	198	3
Chatham^{1003A}	-	-	+	+	-	-	198	2
Açores^{595A}	-	-	+	+	-	-	198	1
Kamiube^{1387T}	-	-	+	+	-	-	207	1
Seattle^{844C}	-	-	+	+	-	-	201	3
Canton^{1376T}	-	-	+	+	-	-	201	1
Anadia^{1193G}	-	-	+	+	-	+	198	1
Mira d'Aire^{1048A}	-	-	+	+	+	+	204	1

n: number of X chromosomes; E: exon; I: intron.

Table 2. RFLP (n=93) and RFLP/microsatellite haplotypes (n=87) in the Portuguese control population (G6PD B) and frequencies of the RFLP[a] and RFLP/microsatellite[b] haplotypes. Alleles were nominated (+) and (–) according to the restriction site presence or absence.

G6PD variant	RFLP haplotype E-4 376A/G FokI	I-5 611C/G PvuII	I-8 163C/T BspHI	E-10 1116A/G PstI	E-11 1311C/T BclI	I-11 93T/C NlaIII	(%)[a]	(CTT)n Allele size	(%)[b]
B		–	+	+	–	–	0.77	192	0.012
								195	0.022
								198	0.620
								201	0.012
								204	0.012
								207	0.057
								210	0.034
B	–	–	+	+	–	+	0.13	198	0.080
								207	0.012
								210	0.023
								213	0.012
B	–	–	+	+	+	+	0.10	201	0.012
								198	0.012
								210	0.057
								213	0.023

E: exon; I: intron.

(–/–/+/+/–/+) and (–/–/+/+/+/+) respectively (Tables 1 and 2). Each of the recurrent G6PD mutations Coimbra[592T] (n=3), Chatham[1003A] (n=2) and Seattle[844C] (n=3) has been found in the context of a same RFLP/CTT haplotype, (–/–/+/+/–/–/198), (–/–/+/+/–/–/198) and (–/–/+/+/–/–/201) respectively (Table 1), suggesting a single origin for each variant.

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Funding: this work was performed in part with the support of Forum Hematológico and Centro de Investigação em Antropologia, Universidade de Coimbra.

Acknowledgments: we thank Prof. Jorge Rocha from IPATIMUP for his help in estimating mutation age.

Key words: G6PD deficiency in Portugal, G6PD mutations, haplotype analysis, G6PD microsatellites.

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