

Mutation Analysis of the HFE Gene in Brazilian Populations

Submitted 10/26/99; revised 11/02/99
(communicated by Ernest Beutler, M.D.)

Marcela F. Agostinho, Valder R. Arruda, Daniela S. Basseres, Silvana Bordin, Manoel C.P. Soares,¹ Raimundo C. Menezes,¹ Fernando F. Costa, Sara T.O. Saad

ABSTRACT: We analyzed the frequency of the C282Y and H63D mutations in the HFE gene in 227 individuals from Brazil comprising 71 Caucasians, 91 racially mixed Caucasian African-derived Amerindians (both populations from Southeast Brazil), 85 African-derived subjects (from Northeast Brazil) and 75 Parakanã Indians. Allelic frequency of the mutation C. 845G6A (C282Y) was 1.4% in the Caucasian population, 1.1% in the African-derived population, 1.1% in the racially mixed normal controls and 0% in the Parakanã Indians. In the African-derived population, the C282Y mutation was present on chromosomes bearing the haplotype 6/1h according to Beutler and West (1997). Allelic frequency of the mutation C. 187C6G (H63D) was 16.3% in the Caucasian population, 7.5% in the African-derived population, 9.8% in the racially mixed controls and 0% in the Amerindians. The presence of these mutations in the African-derived population reflects the fact that these subjects may have undergone a non-identified racial admixture in their past history. The absence of both defects in the Amerindians suggests that these mutations have emerged after the migration of Polynesians to America, or that they may not have reached the Polynesian population until after the migration to America had occurred.

Keywords: hemochromatosis, HFE gene, Brazilian population, Amerindians, Parakanã Indians

INTRODUCTION

Hemochromatosis is an autosomal recessive disorder, common among populations of Northern European origin, associated with progressive iron overload. Early diagnosis and treatment is essential to prevent irreversible tissue damage. Feder et al (1) described two mutations in the HLA-H gene, now designated HFE gene, which is located on chromosome 6 and is considered a candidate gene for bearing the primary defect responsible for hemochromatosis (2). The mutations detected were a G→A substitution at nucleotide 845, changing cysteine to tyrosine (C282Y) and a C→G substitution at nucleotide 187, changing histidine to aspartate (H63D). The C282Y mutation was homozygous in 83% of North American hemochromatosis patients (1) and in more than 90% of UK, French and Australian hemochromatosis patients (3,4,5). The second mutation (H63D) is widely distributed among many populations (3) and the homozygous state may be more prevalent in the hemochromatosis

population (6,7). Therefore, the compound heterozygote of the C282Y and H63D mutations seems at special risk for the development of hemochromatosis, nevertheless with very low penetrance (7,8).

The Brazilian population is unevenly distributed within a country of continental dimensions and its ethnic origin is heterogenous. Besides the native population, Brazil has received immigrants from Italy, Spain, Germany, Japan and the Middle East. During the slave-trading period, Brazil received about 4 million Africans, mainly from Angola, Congo and Mozambique, who settled in almost all regions of the country (9). All these different racial groups were subjected to an intense process of admixture.

The Amerindians are unlike any other population of similar size or antiquity, in which they have undergone evolution in virtual isolation for 15.000-20.000 years (10). The Parakanã Indians are derived from the Tupi tribe and first established contact with neo-Brazilians in 1971. These Indians constitute a very small population

Hemocentro and Departamento de Clínica Médica-Faculdade de Ciências Médicas- Universidade de Campinas, Campinas, São Paulo; Brazil.

¹Instituto Evandro Chagas, Belém, Pará, Brazil.

Reprint requests to: Sara T.O. Saad, Hemocentro UNICAMP, CP6198, Campinas, SP, Brazil, CEP 13083-970. Fax: + 55 19 289-1089. E-mail: sara@obelix.unicamp.br.

Table 1. Allelic Frequency of the C282Y and H63D Mutations in the HFE Gene in Four Brazilian Populations: Caucasians (Students and Members of the Staff of the University Hospital of Campinas), African-Derived (Non-miscegenated Individuals Living in Bahia), Miscegenated African-Derived-Caucasians-Amerindians (Newborns at the University Hospital of Campinas) and Amerindians (Parakanã Indians, Belonging to the Tupi Tribe)

Subjects	Chromosomes (n)	C282Y	H63D
Caucasians	142	1.4%	16.3%
African-derived	170	1.1%	7.5%
Racially mixed	182	1.1%	9.8%
Parakanã Indians	150	zero	zero

(about 347 individuals in 1984) and are scattered throughout three villages in the north of Pará State (Northern Brazil). In relation to the mating strategies, this group is polygamous and favors marriage of males to their sister's daughters. As a result, the population is very inbred (11). Therefore the bottleneck effect, to which this population has been submitted during its evolution, is not counteracted by the broadening of their gene pool through exogamous gene flow. Analysis of 13 biallelic systems in the Parakanã Indians show that they are more distant from the South American Indian founders and from Europeans than 40 other tribes (11).

Thus, we studied the frequency of the C282Y and H63A mutations in four Brazilian populations: Caucasians, African-derived, Amerindians, and in a racially mixed population.

MATERIAL AND METHODS

Populations Analyzed

We analyzed a total of 227 unrelated individuals during the development of this study: 71 Caucasians (students and members of the staff of the University Hospital of Campinas), 85 African-derived individuals (from Bahia state), and 91 racially mixed Caucasian African-derived Amerindians (newborns at the University Hospital of Campinas). We also included in this sample 75 Parakanã Indians, an Amazonian

Indian population belonging to the Tupi tribe, among whom it was possible to exclude first degree relationships, since this population is very inbred. The study was approved by the Ethical Committee of the University Hospital.

Detection of the HFE Mutations

The C282Y and H63D mutations were detected by restriction enzyme analysis of polymerase chain reaction-amplified DNA as already described (12). The digested products were run in 3% agarose gels and stained by ethidium bromide.

Analysis of HFE Haplotypes

A study of HFE haplotypes was carried out by the detection of polymorphisms in intron 2, 4 and 5 of the HFE gene by restriction enzyme digestion of PCR products as already described (13).

RESULTS AND DISCUSSION

The allelic frequency of the C282Y mutation was 1.4% in the Caucasian population, 1.1% in the African-derived population, 1.1% in the racially mixed normal controls and 0% in the Parakanã Indians (Table 1). In the African-derived population, the C282Y mutation was present on chromosomes bearing the haplotype 6h/1h (13,14) (Table 2). The allelic frequency of the mutation H63D was 16.3% in the Caucasian population, 7.5% in the African-derived population, 9.8% in the racially mixed controls and 0% in the Amerindians (Table 1). In the African-derived population, this mutation was observed in haplotypes 6h/1, 6h/2, 6h/6, 1/6h and 6h/8 (Table 2). Thus, the high frequency of this mutation in the African-derived population can also be explained by the intense racial mixture that occurs in the Brazilian population.

Studies in European descendants show that homozygosity for hemochromatosis occurs in 3 to 5 persons per 1000 and there is a carrier frequency of 1 in 10 to 1 in 15 (15,16). The

Table 2. HFE Haplotypes in the African-Derived Population with HFE Mutations

Sub-jects	845	187	IVS-2	IVS-4	IVS-5	Haplotype
01	g/g	c/g	t/c	t/t	a/a	6h/2
02	g/g	c/g	t/c	t/t	g/a	6h/1
03	g/g	c/g	c/c	t/t	a/a	6h/6
04	g/g	c/g	c/c	t/t	a/a	6h/6
05	g/g	c/g	c/c	t/c	a/a	6h/8
06	g/g	c/g	c/c	t/t	a/a	6h/6
07	g/g	c/g	t/c	t/t	g/a	6h/1
08	g/g	c/g	t/c	t/t	g/a	6h/1
09	g/g	c/g	t/c	t/t	g/a	6h/1
10	g/a	c/c	t/c	t/t	g/a	1h/6
11	g/a	c/c	t/c	t/t	g/a	1h/6

C282Y mutation is more frequent in the Northern European population, reaching 10% in Irish chromosomes, and being absent from African, Asian and Australasian chromosomes (3). As the Brazilian population is mainly composed by descendants of Europeans from Italy, Spain and Portugal, besides the native population and Africans, the lower frequency of the C282Y mutation observed in our population is in accordance with the proposal of a Celtic or Viking origin of this mutation (17,18). The fact that this mutation was found in the African-derived population suggests that some subjects may have undergone a non-identified Caucasian admixture sometime in their past history.

The absence of both defects in the Amerindians suggests that these mutations could have emerged after the migration of Polynesians to America, or that they may not have reached the Polynesian population until after the migration to America had occurred. Corroborating this hypothesis, the C282Y and the H63D mutations have a very low prevalence in populations of Asian origin (19). Therefore, the C282Y mutation may have been introduced into these populations by Caucasian admixture (19). Another possible explanation would be that the Parakanã Indians lost these mutations sometime during their evolution through a combination of founder effects and genetic drift.

ACKNOWLEDGMENTS

This study was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

REFERENCES

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 3:399–408, 1996.
2. Zhou XY, Tomatsu S, Fleming RE, et al. HFE gene knockout produces mouse of hereditary hemochromatosis. *Proc Natl Acad Sci USA* 5:2492–2497, 1998.
3. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJL. Global prevalence of putative haemochromatosis mutations. *J Med Genet* 34: 275–278, 1997.
4. Jouanolle AM, Gandon G, Jézéquel P, et al. Haemochromatosis and HLA-H. *Nat Genet* 14:251–252, 1996.
5. Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LW. Haemochromatosis and HLA-H. *Nat. Genet* 14:249–250, 1996.
6. Sham RL, Ou CY, Cappuccio J, Braggins C, Dunnigan K, Phatak PD. Correlation between genotype and phenotype in hereditary hemochromatosis: analysis of 61 cases. *Blood Cells Mol Dis* 23: 314–320, 1997.
7. Beutler E. The significance of the 187G(H63D) mutation in hemochromatosis. *Am J Hum Genet* 61:762–764, 1997.
8. Risch N. Haemochromatosis, HFE and genetic complexity. *Nat Genet* 1997;17:375–376.
9. Curtin PD. The Atlantic slave trade: a census. University of Wisconsin Press, Milwaukee, 1969.
10. Gibbons A. Geneticists trace the DNA trail of the 1st Americans. *Science* 259: 312–313, 1993.
11. Black FL, Salzano FM, Layrisse Z, Franco MHL, Harris NS and Weimer TA. Restriction and persistence of polymorphisms of HLA and other blood genetic traits in Parakanã Indians of Brazil. *Am J Phys Anthropol* 52: 119–132, 1980.
12. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. *Blood Cells Mol Dis* 22:187–194, 1996.
13. Beutler E and West C. New diallelic markers in the HLA region of chromosome 6. *Blood Cells Mol Dis* 30: 219–229, 1997.
14. Aguilar-Martinez P, Thelcide C, Jeanjean P, Masméjean C, Giansily M and Schved J. Haplotype analysis of the HFE gene: implications for the origins

- for hemochromatosis related mutations. *Blood Cells Mol Dis* 15: 166–169, 1999.
15. Edwards CQ, Griffin LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11065 presumably healthy blood donors. *N Eng J Med* 318: 1355–1362, 1988.
 16. Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of haemochromatosis amongst asymptomatic Australians. *Br J Haematol* 74: 525–530, 1990.
 17. Mercier G, Bathelier C, Lucotte G. Frequency of the C282Y mutation of hemochromatosis in five French populations. *Blood Cells Mol Dis* 24: 165–166, 1998.
 18. Lucotte G. Celtic origin of the C282Y mutation of hemochromatosis. *Blood Cells Mol Dis* 24: 433–438, 1998.
 19. Cullen LM, Gao X, Eastal S, Jazwinska EC. The Hemochromatosis 845 G→A and 187 C→G mutations: Prevalence in Non-Caucasian Populations. *Am J Hum Genet* 62:1403–1407, 1998.