Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br,adagmar.andriolo@gmail.com
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial

Herkenhoff, Marcos E.; Pitlovanciv, Ana Kelly; Remualdo, Vanessa R.
Prevalence of C282Y and H63D mutations in the HFE gene in patients from São Paulo and Southern Brazil
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 52, núm. 1, febrero, 2016, pp. 21-24
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

J Bras Patol Med Lab, v. 52, n. 1, p. 21-24, February 2016

10.5935/1676-2444.20160003

Prevalence of C282Y and H63D mutations in the *HFE* gene in patients from São Paulo and Southern Brazil

Prevalência das mutações C282Y e H63D no gene HFE *em pacientes de São Paulo e do Sul do Brasil*

Marcos E. Herkenhoff[1]; Ana Kelly Pitlovanciv[2]; Vanessa R. Remualdo[2]

1. Universidade Estadual Paulista (Unesp), São Paulo, Brasil. 2. Genolab, Santa Catarina, Brasil.

ABSTRACT

Hereditary hemochromatosis (HH) is an autosomal recessive disorder caused by mutations in the *HFE* gene; it is characterized by the risk of iron overload. C282Y and H63D are the most associated mutations in HH. This study aimed to determine the frequency of mutations in the south of Brazil and São Paulo. It used the real-time polymerase chain reaction (PCR) technique and the results collected from Genolab data. In 90 individuals, 46.67% had at least one of the mutations for HH. There is a high prevalence of these mutations in both populations, therefore searching for patients under clinical suspicion is recommended.

Key words: polymorphism single nucleotide; real-time polymerase chain reaction; hemochromatosis.

INTRODUCTION

Iron is an essential molecule for the structural and metabolic function of proteins in cells. There are several proteins, such as HFE, which act in the regulation of iron metabolism. Mutations in the *HFE* gene have been associated with the etiology of iron overload, known as hereditary hemochromatosis (HH), which causes increased intestinal iron absorption and progressive accumulation of iron in the body[1].

The HFE protein models iron absorption in erythrocytes forming a complex with β2-microglobulin, and this complex can interact with transferrin receptor 1 (TFR1), decreasing its affinity for transferring[2]. TFR2, which has high homology with TFR1, is expressed predominantly in the liver and is implicated, using a receptor-mediated endocytosis mechanism, in the uptake of iron by hepatocytes[3].

There are several mutations in the *HFE* gene associated with HH[4]. C282Y mutation is the most often found in patients with HH and is also frequent in a healthy population from Northern Europe (10%)[1]. Another mutation is characterized by substitutions of histidine to aspartic acid at position 63 (H63D)[5]. H63D is normally associated with low risk for HH. However, when simultaneous with C282Y, it presents elevated risk for the development of HH compared to individuals with the HFE 282YY genotype[6-8].

Some studies have reported the prevalence of these mutations in Brazil; at the same time, data still lacks from various regions of Brazil[9-17]. Considering that the population of Southern Brazil was colonized by Europeans, and that the state of São Paulo has a large percentage of Caucasians, this study aims to evaluate the prevalence of these two mutations in these populations.

MATERIAL AND METHODS

Sample collection

The study is the retrospective statistical analysis of data registered on 90 individuals who were subjected to molecular studies of the C282Y and H63D mutations at Genolab, Human Genetics Diseases Department, Blumenau, Santa Catarina state in 2013. The samples belong to patients from Southern Brazil (Paraná and Santa Catarina), and São Paulo. They were collected following the instructions: they should be refrigerated (at 4ºC, up to three days); no special conduct was necessary to prepare the

First submission on 31/07/15; last submission on 31/07/15; accepted for publication on 30/11/15; published on 20/02/16

patient (e.g. fasting); 5 ml of blood should be collected using tubes with ethylenediaminetetraacetic acid (EDTA), an anticoagulant.

Deoxyribonucleic acid (DNA) extraction and real-time polymerase chain reaction (PCR)

We used the phenol-chloroform method to isolate DNA[18], which was eluted in 200 µl of elution buffer and stored at -20ºC. PCR was performed in a final reaction volume of 25 µl, which contained 12.5 µl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems), 450 nM each primer, 200 nM each probe, and 5 µl of DNA solution. Real-time PCR is a method that allows quantification of the products while reactions are following step by step in all cycles. For this reason, it dispenses with the use of electrophoresis, what makes this method nimble. The advantage of using real-time PCR is that this technique is much more responsive than conventional PCR, enabling the detection of low nucleic acid concentrations in the sample.

Reports and statistical analysis

We consulted the results of the reports for *HH* gene, derived from the Genolab database for one year. The reports were analyzed, the allele and genotype frequencies were calculated and then compared with the frequencies from other studies. The clinical and molecular characteristics of the studied individuals were analyzed using the chi-square test. The Hardy-Weinberg equilibrium was also analyzed. Values for $p < 0.05$ were considered significant.

RESULTS

Prevalence of mutations

This study evaluated the registered data for 90 individuals with clinical suspicion of HH, who were subjected to genotyping analysis for C282Y and H63D mutations. Fifty percent of the individuals showed at least one of these mutations. Forty-two point one and 46.67% individuals have at least one of these mutations in São Paulo and Southern Brazil, respectively.

The C282Y mutation was presented as homozygous in 4.44% of the individuals and as heterozygous in 7.78%, while the H63D mutation was homozygous in 3.33% and heterozygous in 33.33%. The allele frequency was 0.1999 (19.99%) for H63D and 0.0833 (8.33%) for C282Y (**Figure**). Compound heterozygosity (C282Y/H63D) was observed in 1.11% of the cases, and compound homozygosity (282YY/63DD) was also observed in 1.11% of the cases.

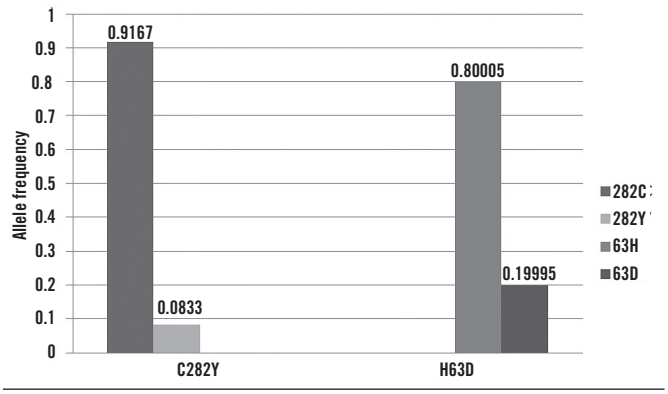

FIGURE – *Allele frequencies for C282Y and H63D mutations*

The C282Y mutation is not in the Hardy-Weinberg equilibrium (p = high significance) while the H63D mutation is in equilibrium ($p = 1.3058$).

DISCUSSION

The frequencies of C282Y and H63D mutations found in this study are the same described by Ferreira *et al*. (2008)[13], higher than those reported for the general Brazilian population[9-12, 14-17] and lower than the genotypic frequencies among patients with HH[8, 19]. The C282Y mutation is prevalent in more than 80% of the individuals with HH in the world, consequently it is the main responsible for HH in all studied populations[10, 20, 21], while the H63D is prevalent in 5.3% of the cases[21]. For this reason, diagnostic guides are based on the result of testing for C282Y mutation[11, 12, 19].

Individuals who are homozygous for C282Y mutation present an earlier onset of pathological aspects compared to heterozygous individuals; the heterozygous, earlier than the homozygous considering the wild allele[11]. The C282Y mutation is responsible for about 80% of the cases of HH at least in Northern European populations[22]. The allele frequency of 282Y in the present study was 8.33%, considered similar as that of the study by Ferreira *et al*. (2008)[13] (7.9%) and higher than that of other Brazilian studies, which is around 2% in general population[16], indicating a relationship between this mutation and clinical suspicion of HH.

This allele has a prevalence of 4.4% in Germany[23], 3.69% in Italian Celtic population[24], 2.5% in five Italian regions[25], 4.7% in an Italian population of Northern European ancestry[26], 3.2% in North Italy[27] and 1.89% in Central Italy[28]. Although the Southern Region of Brazil was settled by Germans and Italians, and São Paulo was colonized by Italians, the frequency of this allele in this population is higher than that in Italy and Germany, being

more similar to the frequency found in France (7.6%)[29], Norway (7.8%)[30] and UK (8.5%)[31]. This allele was also more frequent in this study than in a study from the state of Paraná, which belongs to the Southern Region of Brazil, and showed a frequency of 1.9%[14].

Therefore, the homozygous and heterozygous genotypes for the H63D mutation may show minor penetration at the onset of HH clinical manifestations. This can also be supported by Hardy-Weinberg equilibrium. The fact that C282Y mutation was not in Hardy-Weinberg equilibrium may indicate the existence of natural selection of homozygous individuals, what may mean that this mutation has greater influence over the phenotype than the H63D mutation. That is why, the Hardy-Weinberg relationship is very useful for predicting risks in genetic counseling[32]. The H63D mutation obeys the Hardy-Weinberg equilibrium and does not present with a heterozygous deficit, probably because this mutation is common in our population.

Although presenting lesser penetration, H63D has also great importance in the development of HH, whether homozygous or simultaneously with C282Y, and because of that it is considered an essential part of genetic tests which investigate the cause of HH[8].

In the present study, the allele frequency of H63D was 19.99%, in accordance with the majority of the other world populations, whose frequency ranges from 10% to 20%. Southern Brazil was colonized mostly by German and Italian people. The German prevalence of this mutation is 19% in the control group[33], however, Portugal and Spain also present frequencies around 20% in the general population[16, 34-37]. Italy showed a prevalence ranging from 12.6% to 14.7%[25-28, 38], which, contrary to expectations, is slightly lower than the one in this study.

The frequency of 1.1% for C282Y/H63D compound heterozygotes is in accordance with Brazilian data[11, 12, 16, 17] and also with Europe pool data (1.3%)[21], although not in accordance with data reported for the American population[39]. Compound heterozygosity for C282Y and H63D was found in 5.3% of hemochromatosis patients in Europe[21]. One percent to 2% of compound heterozygous individuals are predisposed to the expression of HH[40].

CONCLUSION

São Paulo and Southern Brazil have the highest rates of Caucasians in Brazil. Thus, the frequency of C282Y and H63D mutations was expected to have high prevalence in those places. Even so, the frequency of C282Y is higher than that in Italy and Germany, and even in Paraná (Southern Brazil), and the H63D allele prevalence is higher than that in Italy. These data show that it is highly recommended to do the analysis of these two mutations in the studied populations. Further research is needed to enrich the database of these mutations in Brazil, mainly in the south. These data will serve as an additional resource to be consulted on the distribution of these mutations in Brazil.

RESUMO

A hemocromatose hereditária (HH) é uma desordem autossômica recessiva provocada por mutações no gene HFE *e caracterizada pelo risco de uma sobrecarga de ferro. As mutações mais conhecidas associadas à HH são a C282Y e a H63D. Este estudo teve como objetivo determinar a frequência das mutações no sul do Brasil e em São Paulo. Ele utilizou a técnica de reação em cadeia da polimerase (PCR) em tempo real e o resultado coletado dos dados do Genolab. De 90 indivíduos, 46,67% possuíam pelo menos uma das mutações para HH. Existe alta prevalência dessas mutações nas duas populações, portanto recomenda-se a busca em pacientes sob suspeita clínica.*

Unitermos: *polimorfismo de nucleotídeo único; reação em cadeia da polimerase em tempo real; hemocromatose.*

REFERENCES

1. Swinkels DW, Janssen MC, Bergmans J, Marx JJ. Hereditary hemochromatosis: genetic complexity and new diagnostic approaches. Clin Chem. 2006; 52: 950-68.

2. Feder JN, Penny DM, Irrinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci USA. 1998; 95: 1472-7.

3. Fleming RE, Ahmann JR, Migas MC, et al. Targeted mutagenesis of the murine transferrin receptor-2 gene produces hemochromatosis. Proc Natl Acad Sci USA. 2002; 99: 10653-8.

4. Camaschella C, Roetto A, De Gobbi M. Genetic haemochromatosis: genes and mutations associated with iron loading. Best Pract Res Clin Haematol. 2002; 15: 261-76.

5. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996; 13: 399-408.

6. Beutler E. The significance of the 187G (H63D) mutation in hemochromatosis. Am J Hum Genet. 1997; 61: 762-4.

7. Carella M, D'Ambrosio L, Totaro A, et al. Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet. 1997; 60: 828-32.

8. Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med. 2004; 350(23): 2383-97.

9. Agostinho MF, Arruda VR, Basseres DS, et al. Mutation analysis of the HFE gene in Brazilian populations. Blood Cells Mol Dis. 1999; 25(5-6): 324-7.

10. Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood. 2004; 103(8): 2914-9.

11. Bittencourt PL, Palácios SA, Couto CA, et al. Analysis of HLA-A antigens and C282Y and H63D mutations of the HFE gene in Brazilian patients with hemochromatosis. Braz J Med Biol Res. 2002; 35(3): 329-35.

12. Calado RT, Franco RF, Pazin-Filho A, Simões MV, Marin-Neto JA, Zago MA. HFE gene mutations in coronary atherothrombotic disease. Braz J Med Biol Res. 2000; 33(3): 301-6.

13. Ferreira ACS, Oliveira VC, Caxito FA, Gomes KB, Castro AM, Pardini VC. Prevalence of C282Y and H63D mutations in the HFE gene of Brazilian individuals with clinical suspicion of hereditary hemochromatosis. Rev Bras Hematol Hemoter. 2008; 30(5): 379-83.

14. Jackowski D, Rebello ES, Faucz FR. Análise da frequência da mutação C282Y na população paranaense. Revista Estudos de Biologia. 2004; 26(55): 11-8.

15. Martinelli AL, Filho R, Cruz S, et al. Hereditary hemochromatosis in a Brazilian university hospital in São Paulo State (1990-2000). Genet Mol Res. 2005; 4(1): 31-8.

16. Oliveira TM, Souza FP, Jardim AC, et al. HFE gene mutations in Brazilian thalassemic patients. Braz J Med Biol Res. 2006; 39(12): 1575-80.

17. Perícole FV, Alves MAVR, Saad STO, Costa FF. Hemochromatosis (HFE) gene mutations in Brazilian chronic hemodialysis patients. Braz J Med Biol Res. 2005; 38(9): 1321-4.

18. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning a laboratory manual. 2 ed. USA: Cold Spring Harbor Laboratory Press; 1989. 1886 p.

19. Limdi JK, Crampton JR. Hereditary haemochromatosis. QJM. 2004; 97(6): 315-24.

20. Acton RT, Barton JC, Passmore LV, et al. Relationships of serum ferritin, transferrin saturation, and HFE mutations and self-reported diabetes in the Hemochromatosis and Iron Overload Screening (HEIRS) study. Diabetes Care. 2006; 29(9): 2084-9.

21. European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol. 2010; 53: 3-22.

22. Bomford A. Genetics of haemochromatosis. Lancet. 2002; 360: 1673-81.

23. Meier P, Schuff-Werner P, Steiner M. Hemochromatosis gene HFE Cys282Tyr mutation analysis in a cohort of Northeast German hospitalized patients supports assumption of a North to South allele frequency gradient throughout Germany. Clin Lab. 2005; 51(9-10): 539-43.

24. Pozzato G, Zorat F, Nascimben F. Haemochromatosis gene mutations in a clustered Italian population: evidence of high prevalence in people of Celtic ancestry. Eur J Hum Genet. 2001; 9(6): 445-51.

25. Candore G, Mantovani V, Balistreri CR, et al. Frequency of the HFE gene mutations in five Italian populations. Blood Cells Mol Dis. 2002; 29(3): 267-73.

26. Salvioni A, Mariani R, Oberkanins C, et al. Prevalence of C282Y and E168X HFE mutations in an Italian population of Northern European ancestry. Haematologica. 2003; 88(3): 250-5.

27. Mariani R, Salvioni A, Corengia C, et al. Prevalence of HFE mutations in upper Northern Italy: study of 1132 unrelated blood donors. Dig Liver Dis. 2003; 35(7): 479-81.

28. Floreani A, Rosa Rizzotto E, Basso D, et al. An open population screening study for HFE gene major mutations proves the low prevalence of C282Y mutation in Central Italy. Aliment Pharmacol Ther. 2007; 26(4): 577-86.

29. Deugnier Y, Jouanolle AM, Chaperon J, et al. Gender-specific phenotypic expression and screening strategies in C282Ylinked haemochromatosis: a study of 9396 French people. Br J Haematol. 2002; 118: 1170-8.

30. Distante S, Berg JP, Lande K, Haug E, Bell H. High prevalence of the hemochromatosis-associated Cys282Tyr HFE gene mutation in a healthy Norwegian population in the city of Oslo, and its phenotypic expression. Scand J Gastroenterol. 1999; 34: 529-34.

31. Jones DC, Young NT, Pigott C, et al. Comprehensive hereditary hemochromatosis genotyping. Tissue Antigens. 2002; 60: 481-8.

32. Strachan T, Read AP. Human molecular genetics. 4 ed. USA: Garland Science; 2010. 781p.

33. Frank J, Poblete-Gutiérrez P, Weiskirchen R, Gressner O, Merk HF, Lammert F. Hemochromatosis gene sequence deviations in German patients with porphyria cutanea tarda. Physiol Res. 2006; 55: S75-S83, 2006.

34. Altes A, Ruiz A, Barceló MJ, et al. Prevalence of the C282Y, H63D, and S65C mutations of the HFE gene in 1146 newborns from a region of Northern Spain. Genet Test. 2004; 8(4): 407-10.

35. Aranda N, Viteri FE, Fernández-Ballart J, Murphy M, Arija V. Frequency of the hemochromatosis gene (HFE) 282C → Y, 63H → D, and 65S → C mutations in a general Mediterranean population from Tarragona, Spain. Ann Hematol. 2007; 86(1): 17-21.

36. Guix P, Picornell A, Parera M, et al. Distribution of HFE C282Y and H63D mutations in the Balearic Islands (NE Spain). Clin Genet. 2002; 61(1): 43-8.

37. Sanchez M, Villa M, Ingelmo M, et al. Population screening for hemochromatosis: a study in 5370 Spanish blood donors. J Hepatol. 2003; 38(6): 745-50.

38. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J Med Genet. 1997; 34(4): 275-8.

39. Beutler E. Hemochromatosis: genetics and pathophysiology. Annu Rev Med. 2006; 57: 331-47.

40. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. Gastroenterology. 2010; 139: 393.

CORRESPONDING AUTHOR

Marcos Edgar Herkenhoff

Laboratório Genolab; Rua Marechal Floriano Peixoto, 425; Centro; CEP: 89010-325; Blumenau-SC, Brasil; e-mail: marcos.herkenhoff@gmail.com.