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Artigo / Article

The identification of beta-thalassemia mutants in Brazilians with high Hb F levels

Identificação de mutantes de beta talassemia em grupo de indivíduos com Hb Fetal aumentada da população brasileira

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*Hemoglobinopathies are a heterogeneous group of genetic disorders which represent a public health problem, with significant morbidity, in countries where the prevalence is high. This study aimed at identifying molecular abnormalities that might explain the laboratorial profile obtained using electrophoresis and high performance liquid chromatography in a group of individuals without signs or clinical symptoms of anemia. Five different mutations for beta-thalassemia were found using PCR-ASO: three cases with CD 6 (-A), one CD 39, one IVI I-6, one -87 (mutations originating in the Mediterranean region) and one IVS II-654 (mutation originating in Asia). This is the first time that the CD 6 (-A), -87 and IVS II-654 mutations have been described in the Brazilian population. Rev. Bras. Hematol. Hemoter. 2010;**32**(3):215-218.*

Palavras-chave: *Beta-thalassemia; fetal hemoglobin; molecular biology.*

Introduction

Hemoglobin (Hb) is a tetramer of two α-like and two β-like globin chains. Each one is covalently linked to a prosthetic oxygen-binding heme group. The hemoglobinopathies refer to a diverse group of inherited disorders characterized by reduced synthesis of one or more globin chains, Thalassemia, or the synthesis of a structurally abnormal hemoglobin variant.[1,2,3] In Brazil, the colonization process had a great influence on the distribution of mutant globin genes. Therefore, the frequency of hemoglobinopathies is closely related to the ethnic groups that make up the population.[4]

The thalassemias are a heterogeneous group of genetic disorders caused by changes in the alpha/beta globin chain ratio due to partial or total reductions in the synthesis of one of the globin chains. They are classified according to the involved globin chain, as alpha, beta, delta or delta-beta. Thalassemias are very common in the Mediterranean region, Africa, the Middle East, India and southwest Asia. In Brazil, the most frequent forms are alpha and beta thalassemias due to heterozygosis.[4,5] Beta thalassemia is an inherited hemoglobin disorder characterized by hypochromic microcytic anemia and deficiency in the synthesis of beta globin chains. There is a relative increase in the alpha globin chains in this case which can combine with the delta and gamma chains, thereby raising the quantities of Hb A_2 and Hb F.[6] Molecular defects that cause beta thalassemia can be mutations affecting the transcription, the translation or the RNA stability of beta chains.[7] The commonest mutation described in the Brazilian population is CD 39 (C->T), which originated in the Mediterranean region and affects RNA translation creating a stop codon in position 39 of the polypeptidic chain.[7,8,9]

Fetal hemoglobin (Hb F), formed by two alpha and two gamma globin chains, is expressed at high levels during fetal

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development with the syntheses diminishing after birth.[10] The normal values of Hb F in hematologically healthy adults vary from 0% to 1%.[11] In some hereditary alterations, such as hereditary persistence of fetal hemoglobin (HPFH), or in some haplotypes of Hb S and beta thalassemia, the Hb F may remain at levels of greater than 1%.[12,13] The present study aimed at identifying molecular defects that may explain the increased level of Hb F identified by electrophoretical and chromatographic procedures in individuals without signs or clinical symptoms of anemia.

Material and Methods

After receiving written consent, blood samples of 65 asymptomatic adults from different Brazilian States were analyzed. The individuals, independent of ethnic background, did not present with signals or symptoms of anemia but had above normal levels of Hb F. The first analyses and phenotype definitions were established according to classical methods of identifying hemoglobinopathies and thalassemias.[4,14,15,16] The genomic DNA was isolated from whole blood using phenol-chloroform extraction and ethanol precipitation.[17]

In order to identify possible genetic defects involved in the elevation of Hb F, 15 mutations of beta thalassemia were analyzed by PCR-ASO, a methodology which, for the identification of mutants, uses allele-specific oligonucleotide probes provided in the *Betha Gene 1* and *Betha Gene 2* analysis kits (Bio-Rad Laboratories). From 15 mutations, eight originated in the Mediterranean region: CD 39 (C>T), IVSI-110 (G>A), IVSI-6 (T>C), IVSII-745 (C>G), IVSII-1 (G>A), -87 (C>G), CD 6 (-A), and seven had Asiatic origin: CD 41-42 (-TTCT), CD 17 (A>T), -28 (A>G), IVSII-654 (C>T), CD 19 (A>G), IVSI-5 (G>C), CD 71-72 (+A). The diagnostic procedures followed the instructions of the manufacturer.

DNA samples were analyzed for HPFH-1, HPFH-2, HPFH-3 and δβ-thalassemia (Sicilian) by GAP-PCR, a methodology which utilizes three oligonucleotide primers in the same amplification reaction with the production of a unique deletion-specific product when a deletion is presence and a normal control band with the normal allele.[18]

Results

The individuals included in this study were evaluated in family studies by the National Neonatal Screening Program and considered without signs and symptoms clinical of anemia except for increased Hb F level. Most presented with normal osmotic globular resistance and red blood cell morphology varying between mild to moderate, with predominance of mild alterations that are explained as normal physiological variations in each individual.

All the analyzed individuals were considered normal for Hb A^2 with an average of 2.7% (standard deviation of ± 0.47). The average level of Hb F found in this group was 6.77% (standard deviation ± 9.16), a value higher than the perceptual interval described in publications for normal adults.

Seven (12.3%) of the 65 samples submitted to laboratorial and molecular analyses were positive for one of the analyzed mutations and 58 (87.7%) did not have any of these mutations. The results of the hemoglobinopathies and molecular analyses of these seven samples are described in better detail in Table 1.

Only one of the 65 samples analyzed by gap-PCR was heterozygous for HPFH-2. The other samples were not positive for any of the investigated mutations.

Table 1: Results obtained to hemoglobinopathies examination and molecular analyses for PCR-ASO with *Betha Gene 1* and *Betha Gene 2* kits (Bio-Rad Laboratories)

Samples	OGR	RBC	Electrop. pH 8,6	% Hb A2 (HPLC)	% Hb F (HPLC)	Mutations Found
1	Abnormal	Mild	Hb AF	2.6	2.7	Heterozygote CD 39
2	Abnormal	Mild	Hb AFH	2.6	2.0	Heterozygote IVS II-654
3	Normal	Normal	Hb AF	2.7	3.3	Heterozygote -87
4	Abnormal	Moderate	Hb AFH	2.5	5.0	Heterozygote CD6-A
5	Normal	Mild	Hb AFH	3.5	1.7	Heterozygote CD6-A
6	Normal	Moderate	Hb AFH	3.0	1.8	Heterozygote CD6-A
7	Normal	Mild	Hb AF	2.7	2.6	Heterozygote IVS I-6

OGR = osmotic globular resistance in 0.36% NaCl;RBC= red blood cell morphology; Electrop. pH 8.6 = electrophoresis alkaline; HPLC = High Pressure Liquid Chromatography

Discussion

The expression of beta thalassemia is variable; it may present a clinical phenotype of severe anemia, dependent on blood transfusions or be asymptomatic .[7,19] In the molecular analyses, five different mutations for beta thalassemia were found: three CD 6 (-A), one CD 39, one IVI I-6, one -87 (originating from the Mediterranean region) and one IVS II - 654 (from Asia). Fonseca *et al*. (1998), in a thalassemia research group from São Paulo state, found CD 39 (C→T), IVSI-6 (T→C), IVSI-110 (G→A) and IVSI-1 (G→T) mutations with the commonest being CD 39.

The different results between this study and data from Fonseca *et al.* are due to the origin of the different sample groups; in Brazil the original inhabitants were native Indians, but starting five hundred years ago Europeans, specifically the Portuguese with some Spanish and Italians, colonized the continent. Slaves were also brought from Africa. Thus, the population is an admixture of races: in the north there is a predominance native Indians, in the northeast there is a high

percentage of Negroes and their descendents and in the south and southeast there is a great influence of Caucasians. More recently there has been substantial immigration from Asia.[20]

The carriers of the CD 39 mutation normally have high levels of Hb A_2 (5.0% ± 0.5) and Hb F (2.7% ± 0.6); positive results for the osmotic globular resistance test in 0.36% NaCl and clinical and hematological signs and symptoms of anemia in heterozygotes. However, the cases described in this study had normal levels of Hb A_2 (2.6%) and no signs and symptoms of anemia which is divergent from published data.[21,22]

The IVSI-6 mutation reduces the efficacy at the splicing site, thus modifying the consensus sequence. In the literature this mutation is described as being associated with high levels of Hb A_2 (4.0 % ± 0.4) and Hb F (2.2 % ± 0.5).[21] The -87 is a mutation in the region close to the CACCC box and thus modifies the location site of the erythroid Krüppel-like factor, providing a DNA transcription defect. The carriers' hematological manifestations are mild, and the values of Hb A_2 (5.2 % ± 0.5) and Hb F (3.3 % ± 1.1) are increased.[21] Both mutations presented intermediate phenotypes of beta thalassemia. In this study, alterations in the results of laboratorial analyses of individuals with IVSI-6 and -87 mutations were not found (Table 1). This phenotype confirm the results of Rosatelli *et al.*,[23] who reported that Hb A_2 is often normal with no alterations in the hematimetric parameters of heterozygotes.

The CD6 (-A) mutation affects the reading frame and presents well-defined phenotypic characteristics, such as above normal levels of Hb A_2 and Hb F, increased osmotic globular resistance and mild red blood cell alterations. The frequency of this mutation is around 2.5% in Italy and 40.0% in the northeast of Portugal.[21] This was the commonest mutation in the current study sample and thus, although concordant with the ethnic background of the population, the results are in disagreement with published results for the Brazilian population, which do not describe this beta thalassemia mutant.[7]

The IVSII-654 mutation that originated in Asia has a high frequency (42.4%) in the Taiwanese population.[21] This mutation creates a new splicing site which affects RNA processing.[24] Even though it is rare in Brazil (1.75%), the frequency of this mutation in the analyzed group shows the influence of Asiatic components in the formation of the Brazilian population.

The HPFH-2 (Ghanaian, Africa) is the result of extensive deletions of nearly identical sizes of approximately 105 Kb of DNA. Heterozygotes have normal hematological parameters except with Hb F levels averaging 24.4% (± 2.8%).[25]

Conclusions

The results obtained in this study show the importance of molecular investigations of hemoglobin defects in individuals without clinical signals of anemia, in order to the diagnose disorders and provide genetic counseling, as this condition is not a clinical form of thalassemia. This latter aspect demonstrates the importance of population studies as many carriers do not have signs or symptoms of anemia.

The CD 6 (-A) and -87 mutations originated from the Mediterranean region, population groups that greatly influenced the make up of the Brazilian population. The IVS II-654 mutation demonstrates an Asiatic component in the formation of the Brazilian population, thus highlighting the great miscegenation and the need to amplify molecular studies.

Resumo

*As hemoglobinopatias são um grupo de afecções genéticas que representam problema de saúde pública em muitos países em que sua incidência é alta, com significativa morbidade. Objetivamos identificar defeitos moleculares que pudessem explicar o perfil laboratorial obtido por eletroforese e HPLC com Hb F elevada, em um grupo de indivíduos adultos sem sinais ou sintomas de anemia. Encontramos cinco diferentes mutações que originam beta talassemia por PCR-ASO: três casos com CD 6 (-A), um CD 39, um IVS 1-5, um -87 todas de origem mediterrânea, e um IVS II-654 de origem asiática. As mutações CD 6 (-A), -87 e IVS II-654 foram descritas pela primeira vez na população brasileira. Rev. Bras. Hematol. Hemoter. 2010;**32**(3):215-218.*

Palavras-chave: *Talassemia beta; hemoglobina fetal; biologia molecular.*

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References

1. Clark BE, Thein SL. Molecular diagnosis of haemoglobin disorders. Clin Lab Haem 2004;26(3):159-76.
2. Bunn HF, Forget BG. Hemoglobin: molecular genetic and clinical aspects. Philadelphia: Saunders, 1986.
3. Lehmann H, Huntsman RG. Man's haemoglobins. Amsterdan: North Holland; 1974. 478 p.
4. Bonini-Domingos CR. Metodologias laboratoriais para o diagnóstico de hemoglobinopatias e talassemias. Editora HN, São José do Rio Preto, SP, 2006.
5. Huisman TH, Carver MF, Baysal E. A syllabus of thalassemia mutations. The Sickle Cell Anemia Foundation, Augusta, GA. 1997.
6. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000; 46(8 Pt 2):1284-90.
7. Fonseca SF, *et al.* Genetics analysis of beta-thalassemia major and beta-thalassemia intermedia in Brazil. Hemoglobin. 1998;22:197-207.

8. Ragusa A, Amata S, Lombardo T, Castiglia L, Maier-Redelsperger M, Labie D, *et al.* Asymptomatic and mild beta-thalassemia in homozygotes and compound heterozygotes for the IVS2+1G>A mutation: role the beta-globin gene haplotype. Haematologica. 2003;88:1099-105.
9. Moreira HW, de Oliveira CM, Martins CS, de Sales TS, Costa FF. Determination of neutral polymorphisms (frameworks) of the human beta globin gene in beta-thalassemias by PCR/DGGE. Sangre. 1997;42(1):21-4.
10. Papadakis MN, Patrinos GP, Tsaftaridis P, Loutradi-Anagnostou A. A comparative study of Greek nondeletional hereditary persistence of fetal hemoglobin and beta-thalassemia compound heterozygotes. J Mol Med. 2002;80(4):243-7.
11. Nagel RL, Labie D. DNA haplotypes and the β^S globin gene. Hemoglobin Switching. p. 371-393, 1989.
12. Shimizu K, Keino H, Terasawa T, Shichishima T, Ikuta K, Hayashi Y. Elevated haemoglobin F in juvenile and adult chronic myelogenous leukaemia. Acta Haematol. 1988;80(1):28-33.
13. Steinberg MH. Sickle cell anemia and fetal hemoglobin. Am J Med Sci. 1994;308(5):259-65.
14. Silvestroni E, Bianco I. Screening for microcytemia in Italy: analysis of data collected in the past 30 years. Am J Hum Genet. 1975; 27(2):198-212.
15. Marengo-Rowe AJ. Rapid electrophoresis and quantification of haemoglobin on cellulose acetate. J Clin Path. 1965;18(6):790-2.
16. Eastman JW, Wong R, Liao CL, Morales DR. Automated HPLC screening of newborns for sickle cell anemia and other hemoglobinopathies. Clin Chem. 1996;42(5):704-10.
17. Old JM, Higgs DR. Gene analysis. In: DJ Weatherall (ed) The Thalassemias: Methods in hematology. Churchill Livingstone. Edinburg; 1983. 74-102 p.
18. Craig JE, Barnetson RA, Prior J, Raven JL, Thein SL. Rapid detection of deletions causing delta beta-thalassemia and hereditary persistence of fetal hemoglobin by enzymatic amplification. Blood. 1994; 83(6):1673-82.
19. de Sousa SM, Khater L, Peroni LA, Miranda K, Murai MJ, Albuquerque DM, *et al.* Beta-thalassemia intermedia in Brazilian patient with-101 (C>T) and codon 39 (C>T) mutations. São Paulo Med J. 2003;121(1):28-30.
20. Bonini-Domingos CR. Thalassemia screening in Brazil - Results for 20 years. Rev Bras Hematol Hemoter. 2004;26(4):288-9.
21. Silvestroni IB. Le Talassemie. Um problema medico-sociale: ieri e oggi. Instituto Italiano di Medicina Sociale, Roma, 1998. 147 p.
22. Lemsaddek W, Picanço I, Seuanes F, Nogueira P, Mahmal L, Benchekroun S, *et al.* The beta-thalassemia mutation/haplotype distribution in the moroccan population. Hemoglobin. 2004;28(1): 25-37.
23. Rosatelli C, Leoni GB, Tuveri T, Scalas MT, Mosca A, Galanello R, *et al.* Heterozygous beta-thalassemia: relationship between the hematological phenotype and type of beta-thalassemia mutation. Am J Hematol. 1992;39(1):1-4.
24. Tan Jin Ai MA, Yap SF, Tan KL, Wong YC, Wee YC, Kok JL. Mild beta-thalassemia intermedia caused by compound heterozygosity for (G) gamma ((A)gammadeltabeta)(o)/beta-thalassemia and molecular characterization of the defect in four Chinese families. Acta Haematol. 2003;109(4):169-75.
25. Forget BG. Molecular basis of hereditary persistence of fetal hemoglobin. Ann N Y Acad Sci. 1998;850:38-44.

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