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Molecular identification of Sicilian (δß)°-thalassemia associated with ß-thalassemia and hemoglobin S in Brazil

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Abstract

We describe the clinical and molecular characteristics of two unrelated Brazilian families with an association of the Sicilian form of (δß)°-thalassemia with hemoglobin S and ß-thalassemia. Direct sequencing of the ß-globin gene showed only the hemoglobin S mutation in patient 1 and the ß-thalassemia IVS1-110 in patient 2. The other allele was deleted in both patients and PCR of DNA samples of the breakpoint region of both patients showed a band of approximately 1,150 bp, expected to be observed in the DNA of carriers of Sicilian (δß)°-thalassemia. The nucleotide sequence of this fragment confirmed the Sicilian deletion. There are few reports concerning the Hb S/(δß)°-thalassemia association and patient 2 is the first reported case of Sicilian type of (δß)°-thalassemia in association with ß-thalassemia documented at the molecular level.

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- Sicilian thalassemia

The (δß)°-thalassemias are a rare group of disorders characterized, in the heterozygous state, by increased levels of fetal hemoglobin ranging from 5 to 15% during adulthood. This condition preserves some of the thalassemic phenotype, such as microcytic and hypochromic red blood cells, and is caused by extensive deletions involving a variable extent of the DNA segment in the ß-globin gene cluster (1).

Heterozygous compound states for both (δß)°- and ß-thalassemia have been described in Italian (2,3), Greek (2,4,5) and Yugoslavian (6) populations. Although the clinical manifestations of these conditions are variable, the disorder is, in general, milder than homozygous ß-thalassemia major. The association of sickle-cell hemoglobinopathy and (δß)°-thalassemia is described as a relatively mild sickling disorder and has been observed in Italian (1), Greek (7), Arab (8) and Afro-American (9,10) individuals. Most of the reported cases have not been submitted to detailed DNA analysis and, to our knowledge, there is no description of these associations in the Brazilian population.

We describe here the clinical and molecular characteristics of two unrelated Brazilian families with an association of the Sicilian form of (δß)°-thalassemia with hemoglobin S and ß-thalassemia.

Hematological data and red blood cell

(RBC) levels were determined electronically with a Coulter Counter S. Sr. (Miami, FL, USA). Hemoglobin electrophoresis was performed on cellulose acetate with Tris-EDTA-boric acid buffer, pH 8.9. Hb A_2 was quantitated spectrophotometrically after elution of the Hb fraction from cellulose strips (11); the Hb F level was determined by alkali denaturation (12), its distribution in RBC was evaluated by the Kleihauer technique (13). Globin analysis was performed on urea-Triton-acrylamide gel (14). The Gγ/Aγ ratio was estimated by densitometric analysis. DNA was obtained from peripheral blood buffy coats for molecular analysis, as previously reported (15). Point mutations were identified by sequencing the complete ß-globin gene, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit and the ABI Prism 377 DNA Automatic Sequencer (Perkin Elmer, Foster City, CA, USA). To screen for the Sicilian deletion, we used three oligonucleotide primers in the same amplification reaction: F1 (TTGG GTTTCTGATAGGCACTG), F2 (GTGTCA CCCATTAATGCCTTGTAC) and F3 (TAGATCCCTTTGCCATTATG). These primers were designed to flank the deletion breakpoints and led to the production of a single deletion-specific product in the presence of the deletion (primers F1 and F3) and a normal control band of different size in the presence of the normal allele (primers F1 and F2), visualized on ethidium bromide-stained agarose gel (16) (Figure 1A). The deletion-specific product was also sequenced as described above.

Patient 1 was a 40-year-old male of Italian and African descent and presented mild sickling disease with moderate hypochromia and microcytic anemia. He was splenectomized at the age of 28 years, and had a history of a few vaso-occlusive crises which required hospitalization. Two sisters of the patient presented similar laboratory and clinical findings (Table 1). Electrophoretic hemoglobin examination demonstrated Hb A_2, Hb F and Hb S.

Patient 2 was a male of Italian descent who presented a typical thalassemia intermedia state, with moderate hypochromia and microcytic anemia. He also was splenectomized. This patient died at the age of 42 years, probably as a consequence of anaphylactic shock. He had a sister with similar laboratory and clinical findings. Electrophoretic hemoglobin examination demonstrated Hb A_2 and Hb F.

The hematological data of the patients and their families are shown in Table 1. Direct sequencing of the ß-globin gene of patient 1 showed only the hemoglobin S mutation (A→T in the triplet codon for the sixth residue of the ß-globin chain). The same procedure for patient 2 showed only the ß-thalassemia IVS1-110 (a G→A substitution in the 110 nucleotide of the first intron).

Sicilian (δß)^o-thalassemia presents a deletion of 13,379-bp spanning δ-IVS2 to a region located 3' from the ß-globin gene within an L1 repeat (17). The PCR analyses of the samples from both patients with the primers described above showed a band of approximately 1,150 bp, expected to be observed in the DNA of carriers of Sicilian (δß)^o-thalassemia (Figure 1B). In order to confirm that the fragment corresponded to a breakpoint region, we directly sequenced the amplified fragment in both directions. The nucleotide sequence matched exactly that reported by Henthorn et al. (17) and Esposito et al. (18). There are two "orphan" nucleotides at the specific locus of the deletion junction region and two single base substitutions, G→A and T→G, 15 and 35 nucleotides downstream from the breakpoint, respectively (Figure 1C). These data indicate the probable single origin of the Sicilian deletion.

To our knowledge, patient 2 is the first case of the Sicilian type of (δß)^o-thalassemia in association with ß-thalassemia to be characterized using molecular methods. The pa-

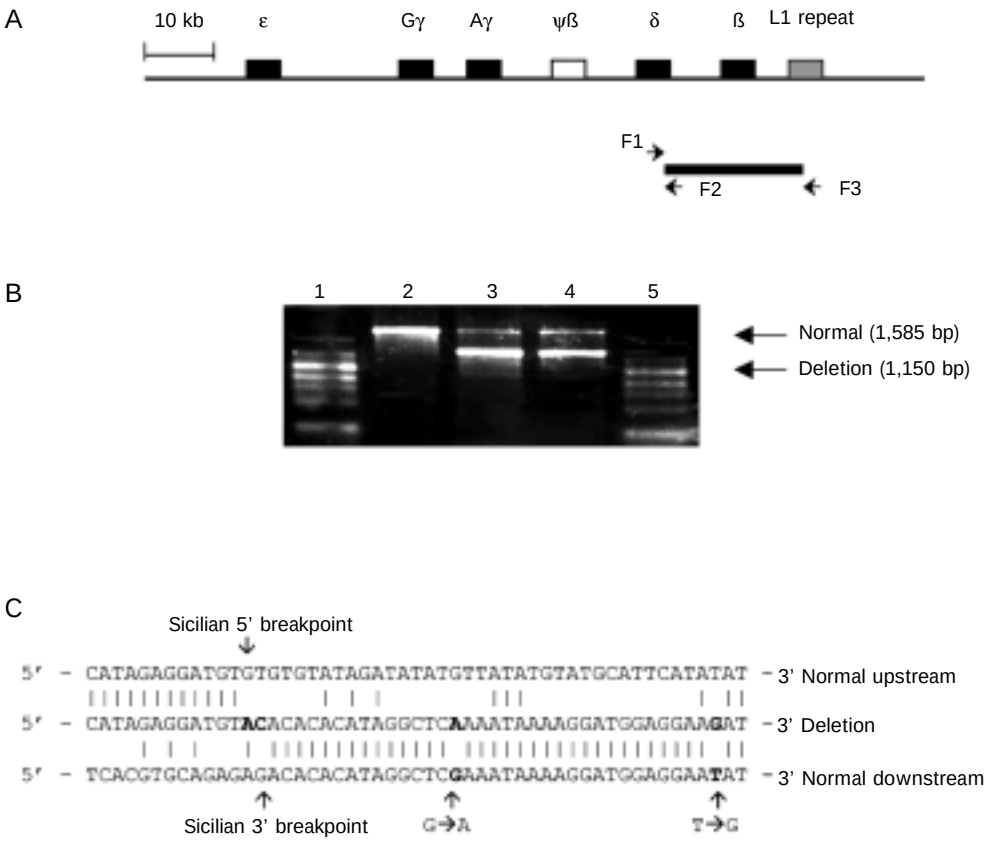

Figure 1. Analysis of the Sicilian (δß)°-thalassemia deletion. *A*, Representation of the human ß-globin gene cluster and location of the Sicilian 13,379-bp deletion (black bar). The distribution of the three oligonucleotides used is also included (arrows). *B*, Ethidium bromide-stained agarose gel (1.2%) with the PCR products for the deletions. The upper band (1,585 bp) is the normal allele and the lower band (1,150 bp) the mutant allele. *Lane 1*, DNA marker ϕX174/*Hae*III; *lane 2*, control; *lane 3*, patient 1; *lane 4*, patient 2; *lane 5*, DNA marker ϕX174/*Hae*III. *C*, Mutant fragment sequence compared to the normal upstream and downstream sequences. Vertical lines connect identical bases. The arrows indicate the Sicilian breakpoints and the base substitutions. The two orphan nucleotides are also shown.

Table 1. Hematological data of the patients' families.

	RBC (x 10^6 cells/µl)	Hb (g/dl)	PCV (l/l)	MCV (fl)	MCH (pg)	Hb A_2 (%)	Hb F (%)	Hb S (%)
Family 1								
Patient 1	4.3	10.5	0.31	72	24	1.1	12.1	86.8
Sister 1	4.3	10.5	0.31	71	24	1.3	20.7	78.0
Sister 2	3.8	10.8	0.31	80	27	1.5	17.4	81.1
Sister 3	4.7	15.3	0.44	93	32	2.2	0.50	40.0
Brother	5.1	15.2	0.44	87	30	2.5	0.30	-
Son	5.5	11.1	0.33	61	20	1.5	23.0	-
Daughter 1	5.4	12.2	0.39	72	22	1.4	21.0	-
Daughter 2	5.5	11.1	0.36	64	20	1.7	20.0	-
Family 2								
Patient 2	3.4	8.40	0.27	80	24	2.8	66.4	-
Sister	3.8	8.70	0.27	72	22	1.6	72.5	-
Brother	6.6	14.3	0.44	65	23	2.1	9.80	-
Father	6.2	12.8	0.40	65	23	2.5	7.30	-
Mother	7.0	13.2	0.41	61	21	4.9	0.60	-

RBC - Red blood cells; Hb - hemoglobin; PCV - packed cell volume; MCV - mean cell volume; MCH - mean cell hemoglobin.

tient presented here had thalassemia intermedia with no necessity for regular transfusion, as previously reported for patients with this association.

There are few molecular analysis reports concerning the Hb S/(δß)°-thalassemia association (19,20). Although the patient described in this report also has a milder clinical state, he had experienced several episodes of vaso-occlusive crisis, which apparently was not frequent in the other cases described.

Taken together, the cases described herein represent the first descriptions of the association of Sicilian (δß)°-thalassemia with ß-thalassemia with a complete molecular characterization and highlight the heterogeneity of the genetic background of the Brazilian population.

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