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Thalassemia intermedia as a result of heterozygosis for ß^0-thalassemia and $\alpha\alpha\alpha^{anti-3.7}/\alpha\alpha$ genotype in a Brazilian patient

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Abstract

We report a case in which the interaction of heterozygosis for both the ß^0-IVS-II-1 (G→A) mutation and the $\alpha\alpha\alpha^{anti-3.7}$ allele was the probable cause for the clinical occurrence of thalassemia intermedia. The propositus, a 6-year-old Caucasian Brazilian boy of Portuguese descent, showed a moderately severe chronic anemia in spite of having the ß-thalassemia trait. Investigation of the α-globin gene status revealed heterozygosis for α-gene triplication ($\alpha\alpha\alpha/\alpha\alpha$). The patient's father, also presenting mild microcytic and hypochromic anemia, had the same α and ß genotypes as his son, while the mother, not related to the father and hematologically normal, was also a carrier of the $\alpha\alpha\alpha^{anti-3.7}$ allele. The present case emphasizes the need for considering the possibility of α-gene triplication in ß-thalassemia heterozygotes who display an unexpected severe phenotype. The ß-thalassemia mutation found here is being described for the first time in Brazil.

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- Triplicated α-globin genes
- Hemoglobinopathies

The pathophysiology and clinical severity of ß-thalassemia are associated with the degree of α/non-α-chain imbalance (1,2). A triplicated α-globin gene locus can exacerbate the effects of α-chain excess caused by a defective ß-globin gene, although this is not observed in all cases (1,2). We report here a patient in whom the interaction of heterozygosis for both the ß^0-IVS-II-1 (G→A) mutation and the $\alpha\alpha\alpha^{anti-3.7}$ allele in the globin genes was the probable cause of the clinical picture of thalassemia intermedia.

The propositus, a 6-year-old Caucasian Brazilian boy of Portuguese descent, showed a moderately severe chronic anemia with splenomegaly, microcytosis, hypochromia, target cells and a reticulocyte count of 6%. His father presented a mild microcytic and hypochromic anemia, while the mother, not related to the father, was clinically and hematologically normal. The hematological data of this family are shown in Table 1. Red blood cell indices were determined electronically with a Sysmex SE-9500 device (Sysmex Corporation, Kobe, Japan); hemoglobin (Hb) was analyzed by electrophoresis on cellulose acetate at alkaline pH (3), Hb A_2 was quantified by elution from cellulose acetate strips (3) and Hb F was estimated by alkali denaturation (4). DNA samples were isolated from peripheral blood leukocytes by an organic extraction method. The ß-globin gene was

amplified using the polymerase chain reaction (PCR) with primers P1 (5'-TCCTAAGC CAGTGCCAGAAG-3') and P5 (5'-TCATTC GTCTGTTTCCCATTC-3') (5). PCR products were purified with the Concert Rapid PCR Kit (Invitrogen Corporation, Carlsbad, CA, USA) and sequenced with an ABI PRISM-377-DNA Automated Sequencer (PE Applied Biosystems, Foster City, CA, USA) with primer P5. Alpha-globin gene genotype was investigated by PCR using primers C3 (5'-CCATTGTTGGCACATTCCGG-3') and C10 (5'-GATGCACCCACTGGACTCCT-3'), described by Dodé et al. (6).

The ß-thalassemia mutation found in the patient and in his father was located at the first position of the second intron of the ß-globin gene [$ß^0$-IVS-II-1 (G→A)] in heterozygosis. This mutation abolishes the 5' splicing site, and has been described in populations of different ethnic backgrounds such as Blacks, Japanese and Mediterraneans (7).

Table 1. Hematological data of the patient and his parents.

	Hb (g/dl)	Ht (%)	RBC (10^6/mm^3)	MCV (fl)	MCH (pg)	RDW (%)	Reticulocytes (%)	Hb A_2 (%)	Hb F (%)
Patient	9.05	30.0	5.17	57.9	17.5	18.7	6.0	5.6	2.1
Father	11.3	35.5	6.28	56.5	18.0	17.8	–	5.9	1.1
Mother	15.0	43.8	4.98	88.0	30.3	14.3	–	2.8	0.5

Hb = total hemoglobin level; Ht = hematocrit; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width.

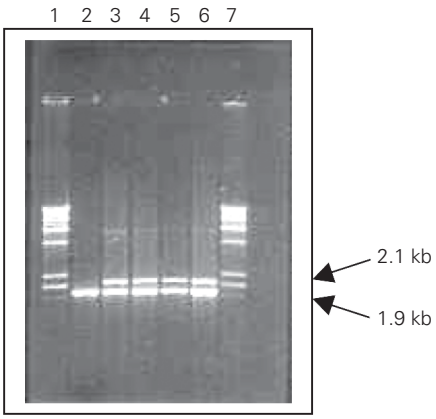

Figura 1. α-Globin gene analysis by PCR. The heterozygosis of the $\alpha\alpha\alpha^{anti\text{-}3.7}$ allele is indicated by the 2.1-kb fragment and the normal allele (αα/αα) is indicated by the 1.9-kb fragment. *Lanes 1* and *7*, DNA size marker (λ *Hin*dIII); lane 2, normal control (αα/αα); lane 3, patient (ααα/αα); lane 4, patient's father (ααα/αα); lane 5, patient's mother (ααα/αα); lane 6, triplicated α-gene heterozygous control (ααα/αα).

In South America, this mutation was found in Guadeloupe and Argentina and is being described for the first time in Brazil in this report.

Investigation of the α genotype revealed the presence of an $\alpha\alpha\alpha^{anti\text{-}3.7}$ allele in addition to the normal αα allele, in the patient, his father and his mother. The presence of an extra α-gene results in a PCR fragment of 2.1 kb, while the normal haplotype (αα) corresponds to a PCR fragment of 1.9 kb (Figure 1).

The clinical and hematological picture of ß-thalassemia heterozygotes with a triplicated α-globin gene arrangement is variable, ranging from an asymptomatic presentation to a mild to moderate thalassemia intermedia phenotype (1,2,8,9). Camaschella et al. (2) reported a group of 17 patients who were heterozygous for both the $\alpha\alpha\alpha^{anti\text{-}3.7}$ allele and a mutation in the ß-globin gene. Their clinical phenotypes varied: six had mild anemia with microcytosis and hypochromia while 11 had more severe anemia with splenomegaly requiring splenectomy (3 cases) and blood transfusions (4 cases). Different phenotypes were also evident in the presence of the same ß-thalassemia mutation: in one family, two individuals had the same α- and ß-globin genotypes but presented different hematological manifestations. Ma et al. (10) in 2001 described the clinical phenotype of eight Chinese subjects from Hong Kong with heterozygosity for both the triplicated α-globin gene and a $ß^0$-thalassemia allele. Although genotypically identical, six subjects showed a ß-thalassemia intermedia phenotype, while two were clinically indistinguishable from ß-thalassemia minor, implying the presence of genetic modifying factors that remained undefined. Similar data have been reported by others (1,11-15).

In contrast, Galanello et al. (16) reported a Sardinian family in whom the combination of heterozygous ß-thalassemia with the heterozygous state for the triplicated α-globin gene loci produced no clinical manifestations and showed a hematological pheno-

type indistinguishable from that of heterozygous ß-thalassemia with a normal α genotype (αα/αα). Only the homozygous state for the triplicated α-globin gene loci, associated with the heterozygous state for ß-thalassemia, produced a clinical picture of thalassemia intermedia with a very mild clinical course. Yet, Kanavakis et al. (17), in a study on five families with both ß-thalassemia and triplicated α-genes, detected no phenotypic effect of the triplicated α-gene clinically or at the hematological level among the ß-thalassemia heterozygotes. However, four of five ß-thalassemia homozygotes with an ααα/αα gene complement had the milder clinical condition of thalassemia intermedia and, in at least one case, there was evidence to suggest that this might be due to the α-gene arrangement acting as an α-thalassemia allele.

The additional α-gene in otherwise normal individuals seems to have no phenotypic effect on red cells, but its expression can be detected by slightly higher than normal α/ß-globin synthesis and mRNA levels and an increase in the α2/α1 mRNA ratios. It has been suggested that the effect of the additional α-chain production might be more readily detected in ß-thalassemia heterozygotes, who have an excess of α-chain synthesis over ß-chains as a result of the deficit in ß-chain production, and that the combination of ααα/αα with heterozygous ß-thalassemia may be responsible for the occasional reports of apparent ß-thalassemia heterozygotes with unusually severe hematological features (17).

The genetic and phenotypic characteristics of the patients described here indicate the need to consider the possibility of a triplicated α-gene allele in patients with heterozygosis for ß-thalassemia who show an unexpected severe phenotype.

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