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Short Communication

Camperdown hemoglobin associated with β° thalassemia in a Brazilian child

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Abstract

We report the coexistence of Hb Camperdown [β104 (G6) Arg → Ser] and β°-thalassemia [β39 (Gln → stop codon)] in a nine-month-old Brazilian boy. He had a relatively more severe hypochromic and microcytic anemia in comparison to his mother's β-thalassemia trait. His Hb Camperdown heterozygous father was clinically and hematologically normal. To our knowledge, this is the first description of an association of β°-thalassemia with Hb Camperdown.

Key words: hemoglobin Camperdown, thalassemia.

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Inherited hemoglobin disorders are the most common single-gene diseases known, and approximately 1000 different mutant alleles have been identified, occurring widely throughout the tropics (Old, 2003; Weatherall and Clegg, 2001; Zago and Costa, 1985). The well-defined forms of hemoglobin disorders vary considerably within and between ethnic groups, and both ethnicity and environment are important factors in the analysis of genotype/phenotype correlations, as demonstrated by the clinical diversity of β-thalassemia (Thein, 2004).

Twenty-five years have elapsed since a mutation in the β-globin gene was first reported as the cause of β-thalassemia (Chang and Kan, 1979). Currently, almost 200 different mutations have been described which cause beta-globin imbalance, leading to anemia of variable severity (Fonseca *et al.*, 1998; Olivieri, 1999). The association of thalassemia and structural hemoglobin variants produces a wide spectrum of clinical and hematological outcomes, ranging from severe to moderate microcytic hypochromic anemia to no significant clinical alterations (Olivieri, 1999).

Although data on the extent of the diversity of hemoglobin disorders in Brazil are incomplete (Old, 2003; Zago and Costa, 1985; Fonseca *et al.,* 1998; Araújo *et al.,* 2003), many β-globin mutant alleles have been detected in patients from different Brazilian regions (Araújo *et al.,* 2003; Fonseca *et al.,* 1998; Grignoli *et al.,* 2000).

Herein we describe the co-inheritance of a rare hemoglobin variant and β-thalassemia in a Brazilian child.

A nine-month-old Brazilian boy was referred to the Department of Diagnosis of Hemoglobin Disorders at the "Hospital das Clínicas" of the São Paulo State University School of Medicine because his mother was a carrier of thalassemia minor. Blood samples of the child were submitted to routine hemoglobin electrophoresis and a subtle change in the electrophoretic pattern was observed on cellulose acetate, demonstrating a fast moving abnormality.

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Familial study was then undertaken. The propositus and his father presented abnormal hemoglobin migration, *i.e.*, faster than Hb A, on cellulose acetate, pH 8.9 (Figure 1), and their blood migration patterns on citrate agar, pH 6.1, were slightly different from that of Hb F. After isoelectrofocusing, the abnormal hemoglobin showed a migration pattern slightly more cathodic than Hb A, indicating a decrease in the number of positive charges. Electrophoresis of the globin chain revealed a β-chain variant with a slower migration rate than that of the normal one (Figure 1). High Pressure Liquid Chromatography (HPLC) analysis demonstrated a variant hemoglobin fraction characterized by concentration values of 83.5% and 49.6%, in the propositus and his father, respectively, as well as a retention time of 1.26 minutes. Additionally, the propositus' hematological profile - high RBC (red blood cell), low MCV (mean cell volume), and raised Hb A_2 - was strongly suggestive of an interaction with the thalassemia trait (Table 1).

The double mutation of β-globin genes in the propositus was subsequently confirmed by DNA sequencing of exon 2 of the β-globin. DNA samples were isolated from peripheral blood leucocytes. We used the primers described by Kimura *et al.* (2003). PCR (polymerase chain reaction) was performed with the upstream primer (P1) 5'-TCCTAAGCCAGTGCCAGAAG-3' and the downstream primer (P5) 5'-TCATTCGTCTGTTTCCCATTC-3'. Automatic backward and forward sequencing (ABI DNA sequencer, model 377, Applied Biosystems, Foster City, CA, USA) was performed with the upstream primer (β) 5'-TTTGCTTCTGACACAACTG-3' and the downstream primer (P5), using an ABI PRISM Big Dye Terminator Cycle sequencing Kit. The propositus and his mother were found to be heterozygous for a nonsense mutation: the C → T substitution at the first nucleotide position of the 39th codon, in exon 2 of the β-globin gene, creates a stop codon resulting in premature termination of the globin chain synthesis. This β-thalassemia mutation is common in the Mediterranean population and is also one of the most frequent β-globin mutations found in Brazil (Fonseca *et al.,* 1998). Another β-globin gene mutation was detected in the propositus and his father: the β104 (G6) arginine residue was replaced by serine [beta 104(G6) Arg → Ser], resulting in the variant Camperdown Hemoglobin (Hb Camperdown). Therefore, the DNA sequence analysis of the β-globin genes revealed the coexistence of β°-thalassemia and Hb Camperdown mutations in the propositus.

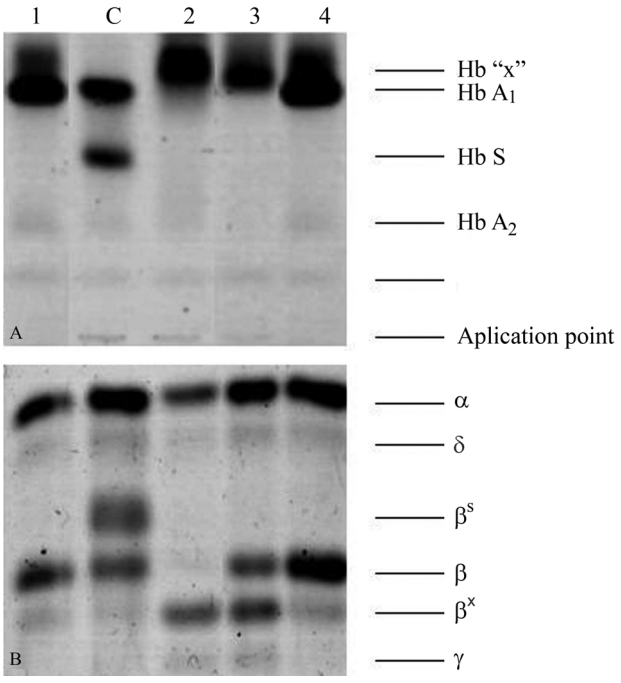

Figure 1 - Electrophoresis: (A) Alkaline electrophoresis: faster HbA migration in the propositus and his father. (B) Globin chain electrophoresis: slower β-chain migration (β^x) in the propositus and his father. Lanes: (1) mother; (2) propositus; (3) father; (4) normal brother; (C) control, heterozygous for sickle cell anemia.

This is the first report of the co-occurrence of Hb Camperdown and β°-thalassemia. β-thalassemia heterozygotes manifest a typical clinical picture, characterized by mild anemia, reduction in mean cell volumes and in mean cell hemoglobin concentrations, elevated concentrations of HbA_2, altered erythrocyte morphology, and imbalance of globin-chain synthesis with an alpha/beta ratio > 1 (Bianco *et al.,* 1977). Hb Camperdown is considered slightly unstable, resulting in a benign clinical course in heterozygous individuals (Wilkinson *et al.*; 1975; Blouquit *et al.,* 1984; Kister *et al.*, 1989; Miranda *et al.,* 1996). This hemoglobin mutation leads to a slightly decreased molecular stability, due to the positively charged residues lining the β1β2 interface, where anionic cofactors bind to tetrameric hemoglobin (Arnone, 1972). It was first described by Wilkinson *et al.* (1975) in a woman of Maltese origin, and several other carriers of this mutant hemoglobin were later described originating from countries bordering the Mediterranean Sea (Blouquit *et al.,* 1984). Miranda *et al.* (1996) reported the first case of Hb Camperdown in South America, in a Brazilian woman of Italian descent. Hb Camperdown carriers do not show significant clinical alterations.

The doubly heterozygous child described herein presented a relatively more pronounced hypochromic and microcytic anemia in comparison with his mother's β-thalassemia trait, while his Hb Camperdown heterozygous father was clinically and hematologically normal. The propositus also presented lower values of MCV and MCH than those obtained by Bertuzzo *et al.* (1997) in 45 β-thalassemia carriers with the β° 39 mutation, ranging in age from 16 to 66 years. The child's follow-up will provide information for a better understanding of the clinical impact of the interaction of heterozygosis for both β-thalassemia and Hb Camperdown.

Table 1 - Molecular, biochemical and hematological data.

DNA sequence variation β globin gene (exon 2)[1]		Propositus [g. 115 C > T]; [g. 312 G > T]	Mother [g. 115 C > T]	Father [g. 312 G > T]	Brother No mutation
Age		9 m	22 y	24 y	nd[2]
Ht	%	38.1	37.0	45.4	38.1
Hb	g/dL	11.3	11.5	16.1	13.7
RBC	10^{12}/L	6.81	5.90	5.63	4.94
MCV	Fl	55.9	62.7	80.7	77.1
MCH	Pg	16.6	19.5	28.5	27.7
HbA2[3]	%	5.2	4.4	2.6	2.4
Hb x: Hb Camperdown[3]	%	83.5	absent	49.6	Absent
Hb F[3]	%	4.4	1.3	0	0
Reticulocytes	%	1.3	2.2	1.3	1.9

[1]Analysis by ABI DNA sequencer, model 377, Applied Biosystems, Foster City, CA, USA. Nomenclature based on Human Database Mutation.
[2]n.d. - not determined data
[3]Quantification by HPLC, β-thalassemia Short Program (Bio-Rad Laboratories, Hercules, CA, USA).

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