



Short Communication

## Prevalence of $\alpha$ -thalassemia 3.7 kb deletion in the adult population of Rio Grande do Norte, Brazil

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### Abstract

$\alpha$ -Thalassemia, arising from a defect in  $\alpha$ -globin chain synthesis, is often caused by deletions involving one or both of the  $\alpha$ -genes on the same allele. With the aim of investigating the prevalence of  $\alpha$ -thalassemia 3.7 kb deletion in the adult population of Rio Grande do Norte, 713 unrelated individuals, between 18 and 59 years-of-age, were analyzed. Red blood cell indices were electronically determined, and  $A_2$  and F hemoglobins evaluated by HPLC. PCR was applied to the molecular investigation of  $\alpha$ -thalassemia 3.7 kb deletion. Eighty (11.2%) of the 713 individuals investigated presented  $\alpha$ -thalassemia, of which 79 (11.1%) were heterozygous ( $-\alpha^{3.7}/\alpha\alpha$ ) deletions and 1 (0.1%) homozygous ( $-\alpha^{3.7}/-\alpha^{3.7}$ ). Ethnically, heterozygous deletions were higher (24.8%) in Afro-Brazilians. Comparison of hematological parameters between individuals with normal genotype and those with heterozygous  $\alpha^+$ -thalassemia showed a statistically significant difference in the number of erythrocytes ( $p < 0.001$ ), MCV ( $p < 0.001$ ), MCH ( $p < 0.001$ ) and Hb  $A_2$  ( $p = 0.007$ ). This study is one of the first dedicated to investigating  $\alpha$ -thalassemia 3.7 kb deletion in the population of the State Rio Grande do Norte state. Results obtained demonstrate the importance of investigating this condition in order to elucidate the causes of microcytosis and hypochromia.

*Key words:* alpha-thalassemia,  $-\alpha^{3.7}$  kb deletion, Brazilian population.

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Alpha ( $\alpha$ )-thalassemia results from a defect in  $\alpha$ -globin chain synthesis, often caused by deletions involving one ( $-\alpha$  haplotype,  $\alpha^+$  thalassemia) or both genes ( $--$  haplotype,  $\alpha^0$  thalassemia) of  $\alpha$ -globin (*HBA1* and *HBA2*), located in the  $\alpha$  cluster on chromosome 16 (16p13.3). Less frequently, it can also be caused by point mutations or oligonucleotide insertions and deletions involving the canonical sequences that control gene expression, also denominated non-deletion variants (Higgs, 1993).

Different  $\alpha$ -thalassemic haplotypes can be combined, thereby forming various genotypes, whose clinical phenotypes range from minimal or non-hematological alterations ( $-\alpha/\alpha\alpha$  genotype), to slight microcytosis and hypochromia

( $-\alpha/-\alpha$  or  $-/-\alpha\alpha$  genotypes), or even to hematological alterations with 5 to 30% Hb H in adulthood ( $-/-\alpha$  genotype). Homozygous  $\alpha^0$  thalassemia ( $-/-/-$ ) corresponds to Hb Bart's hydrops fetalis, which leads to intrauterine demise or death a few hours after birth (Harteveld and Higgs, 2010).

The most common  $\alpha^+$ -thalassemia deletions are  $-\alpha^{3.7}$  and  $-\alpha^{4.2}$  resulting from homologous recombination between misaligned chromosomes. Incorrect pairing of the *HBA2* and *HBA1* genes in  $\alpha^{3.7}$  deletion leads to the production of the *HBA2/HBA1* hybrid gene and a 3.7 kb deletion, whereas in  $\alpha^{4.2}$  deletion, the recombination removes 4.2 kb from the intervening sequence (Borg *et al.*, 2009).

The overall distribution of  $\alpha$ -thalassemias is similar to that of  $\beta$ -thalassemias, extending from sub-Saharan Africa, throughout the Mediterranean region and Middle East, to the Indian sub-continent and East and Southeast Asia (Higgs and Weatherall, 2009). Frequency of  $\alpha^+$ -thalas-

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semia ranges from 10 to 20% in some regions of Africa, 40% or more in some Middle Eastern countries and indigenous populations, and up to 80% in the north of Papua New Guinea and isolated groups in northeast India (Weatherall and Clegg, 2001).

Despite the high prevalence of  $\alpha$ -thalassemia in Brazil, few studies have been dedicated to examining its prevalence in the general population. On investigating  $\alpha^+$ -thalassemia in blood donors of African ancestry, in Campinas, southeast Brazil, 21.3% were found to be heterozygous and 2.1% homozygous for deletion  $\alpha^{3.7}$  (Sonati *et al.*, 1991). In the same region, Borges *et al.* (2001) studied 339 individuals with microcytosis and hypochromia without anemia, documenting a prevalence of 49.9% with  $\alpha$ -thalassemia. In the remaining individuals with  $\alpha$ -thalassemia, 1.5% were non-deletional heterozygous. In another study in northeastern Brazil, heterozygosity for  $\alpha$ -thalassemia ( $-\alpha^{3.7}/\alpha\alpha$ ) was 21.7% and 19.7%, respectively, in the study-population (Couto *et al.*, 2003; Adorno *et al.*, 2005), whereas in a recent research in the south of Brazil, involving 191 African and 201 European descendants, the frequencies of the  $-\alpha^{3.7}$  deletion were 23.1% and 4.5%, respectively (Wagner *et al.*, 2010). The remaining molecular forms of  $\alpha$ -thalassemia investigated in the same study ( $-\alpha^{4.2}$ ;  $-\alpha^{20.5}$ ;  $-\alpha_{SEA}$ ;  $-\alpha_{MED}$ ) were not detected. In the state of Rio Grande do Norte, Bezerra and Meissner (2010) observed the presence of the  $\alpha^+$ -thalassemia  $-\alpha^{3.7}$  deletion in 319 individuals with microcytosis and/or hypochromia, 29.1% of which heterozygous and 3.8% homozygous.

The aim in the present study was to establish the prevalence of the  $\alpha$ -thalassemia 3.7 kb deletion in the adult population of Rio Grande do Norte State, irrespective of microcytic or hypochromic conditions.

An analysis was undertaken of blood samples from 713 unrelated individuals, comprising 407 females and 306 males, between 18 and 59 years-of-age. Self-declared ethnic groups were classified as follows: 333 Caucasians, 308 mulattoes and 72 Afro-Brazilians. Sample size was calculated through stratified random sampling (SRS) based on the 2007 census carried out by the Brazilian Institute of Geography and Statistics (IBGE). The population of Rio Grande do Norte was considered within the age-range established for the study and 17 counties were selected for sample collection, according to population representativeness and ease of access to health services. Participants were randomly recruited from among individuals undergoing routine examination, in connivance with those in charge of the basic-health units of the county. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (protocol no. 243/08). All gave written informed consent prior to participation.

Blood samples were collected in vacuum tubes containing EDTA as anticoagulant, and hematological data obtained in an automated cell counter (ABX Micros 60,

Horiba Group, Japan). All the samples were submitted to hemoglobin electrophoresis on cellulose acetate, at pH 8.5 (Dacie and Lewis, 1995). Concentrations of hemoglobins A<sub>2</sub> and F were quantified by cation-exchange high-performance liquid chromatography (HPLC; Variant™ Bio-Rad Laboratories, USA). Genomic DNA isolation from peripheral blood leukocytes was with the Blood GenomicPrep Mini-Spin kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Molecular investigation of  $\alpha$ -thalassemia 3.7 kb deletion was done by PCR with a GeneAmp 9700 thermocycler (Applied Biosystem, Foster City, CA, USA), in accordance with the described methodology (Dodé *et al.*, 1992). PCR amplification products were visualized after 0.8% agarose gel electrophoresis and ethidium bromide staining. A sample with heterozygous deletion was applied to each gel as positive control and  $\lambda$  DNA/*Hind*III fragments (Invitrogen) were used as molecular-size standards. Serum ferritin levels were determined by an automated chemoluminescent immunoenzymatic method (Immulite, Diagnostic Products Co., Los Angeles, CA, USA) for all  $\alpha$ -thalassemic individuals. Statistical analysis of the data was by descriptive statistics (mean and standard deviation), and the Student *t*-test for relative risk and its confidence interval. "Statistica" 7.0 software was employed, and a significance level of 5% ( $p < 0.05$ ) established for all the tests.

Eighty (11.2%) of the 713 individuals investigated presented  $\alpha^+$ -thalassemia, 79 (11.1%) of which heterozygous ( $-\alpha^{3.7}/\alpha\alpha$ ) and 1 (0.1%) homozygous ( $-\alpha^{3.7}/-\alpha^{3.7}$ ) for the deletion. Genotype distribution among the studied participants remained within Hardy-Weinberg equilibrium ( $p = 0.365$  with 1 degree of freedom), while  $-\alpha^{3.7}$  allele frequency was 0.06 (81 in 1430 chromosomes).

As to the prevalence of  $\alpha$ -thalassemia 3.7 kb deletion, according to ethnic group, among the 380 Afro-Brazilians and mulattoes, 47 (24.8%) were heterozygous for  $\alpha^+$ -thalassemia ( $-\alpha^{3.7}/\alpha\alpha$ ), in comparison to 32 (9.6%) among the 333 Caucasians (Table 1).

Comparison of hematological parameters between normal and heterozygous  $\alpha$ -thalassemia 3.7 kb deletion individuals showed a statistically significant difference ( $p < 0.05$ ), as to the number of erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and Hb A<sub>2</sub> for males and females, as well as hemoglobin concentration in females (Table 2). Although individuals heterozygous for deletion  $-\alpha^{3.7}$  presented lower MCV, MCH, and Hb A<sub>2</sub> values than those with the normal genotype ( $\alpha\alpha/\alpha\alpha$ ), the number of erythrocytes was higher.

Prior to determining the contribution of  $\alpha$ -thalassemia to microcytosis and/or hypochromia, the 701 individuals whose hematological parameters were recorded were divided into four groups: I) those with microcytosis and/or hypochromia with anemia; II) those with microcytosis and/or hypochromia without anemia; III) those

**Table 1** - Prevalence of  $\alpha^+$ -thalassemia 3.7 kb deletion among the 713 individuals, according to ethnic group.

Ethnic group (*)	Number of samples analyzed	Genotypes					
		$\alpha\alpha/\alpha\alpha$		$-\alpha^{3.7}/\alpha\alpha$		$-\alpha^{3.7}/-\alpha^{3.7}$	
		N	%	N	%	N	%
Caucasian	333	301	90.4	32	9.6	-	-
Mulatto	308	269	87.4	38	12.3	1	0.3
Afro-Brazilian	72	63	87.5	9	12.5	-	-
Total	713	633	88.8	79	11.1	1	0.1

(\*) Ethnicity was defined by self-declaration.

**Table 2** - Comparison of hematological parameters (mean  $\pm$  standard deviation) between individuals with normal genotype and those heterozygous for deletion  $-\alpha^{3.7}$ .

Genotype	Gender		Hematological parameters							
			Hemoglobin (g/dL)		Erythrocytes ( $\times 10^{12}/L$ )		MCV (fL)	MCH (pg)	Hb A <sub>2</sub> (%)	Hb F (%)
	M	F	M	F	M	F				
$\alpha\alpha/\alpha\alpha$ (n = 623*)	265	358	14.6 $\pm$ 1.1	12.8 $\pm$ 1.0	5.05 $\pm$ 0.41	4.46 $\pm$ 0.36	86.4 $\pm$ 4.8	28.8 $\pm$ 1.9	2.8 $\pm$ 0.5	0.4 $\pm$ 0.3
$-\alpha^{3.7}/\alpha\alpha$ (n = 77*)	38	39	14.4 $\pm$ 1.0	12.3 $\pm$ 0.8	5.46 $\pm$ 0.50	4.79 $\pm$ 0.33	80.5 $\pm$ 4.5	26.18 $\pm$ 1.7	2.6 $\pm$ 0.6	0.5 $\pm$ 0.3
p-value(**)			p = 0.211	p = 0.003	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p = 0.020	p = 0.750
Reference values <sup>†</sup>			15.0 $\pm$ 2.0	13.5 $\pm$ 1.5	5.00 $\pm$ 0.5	4.30 $\pm$ 0.5	92 $\pm$ 0.6	29.5 $\pm$ 2.5	2.4 $\pm$ 1.4	< 1.0

M: male; F: female; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; Hb = hemoglobin.

\*10 samples with normal genotype and 2 with  $\alpha^+$ -thalassemia were excluded due to hemolysis and/or coagulation.

\*\* p-value of the Student's t-test for independent samples.

<sup>†</sup>Dacie and Lewis (1995).

without microcytosis and/or hypochromia with anemia; and IV) those without any hematological alteration (Table 3). Significant relative risks to  $\alpha$ -thalassemia were noted, on comparing individuals with microcytosis and/or hypochromia (groups I and II) to individuals without microcytosis and/or hypochromia (groups III and IV). As no significant risks were found on comparing group I to group II, neither group III to group IV (Table 3),  $\alpha$ -thalassemia can be considered as strongly associated with the presence of microcytosis and/or hypochromia. Serum ferritin levels determined for cases of  $\alpha$ -thalassemia were

all above the lower normal limits (10 ng/mL for women and 28 ng/mL for men). The exception was 4 individuals with microcytosis and/or hypochromia presenting lower levels of ferritin, a characteristic of iron deficiency associated with thalassemia.

Alpha<sup>+</sup> thalassemia with 3.7 kb deletion has been observed worldwide, with higher frequencies in some African and Mediterranean populations (Higgs and Weatherall, 2009). In studies, such as those by Peres *et al.* (1995) in Portugal, and Mouélé *et al.*, (2000) in Africa, the  $-\alpha^{3.7}$  allele was detected in 7% and 59.7% of newborns, respectively.

**Table 3** - Distribution of the 701 individuals<sup>§</sup> according to the presence of microcytosis, hypochromia and/or anemia.

Group	N	Genotype	
		$\alpha\alpha/\alpha\alpha$	$\alpha^{3.7}/\alpha\alpha$ or $-\alpha^{3.7}/-\alpha^{3.7}$
(I) With microcytosis and/or hypochromia with anemia	33	20 (60.6%)	13 (39.4%)
(II) With microcytosis and/or hypochromia without anemia	101	56 (55.4%)	45 (44.6%)
(III) Without microcytosis and/or hypochromia with anemia	49	48 (98.0%)	1 (2.0%)
(IV) Without hematological alterations	518	499 (96.3%)	19 (3.7%)

<sup>§</sup>10 samples with normal genotype and 2 with  $\alpha^+$ -thalassemia were excluded due to hemolysis and/or coagulation.

Risk relative (RR) to alpha-thalassemia among the groups: [RR(Confidence Interval), p value].

Group I x Group II: [0.86 (0.46-1.57), p = 0.6054] Group II x Group III: [1.82 (1.51-2.18), p < 0.0001].

Group I x Group III: [3.16 (2.13-4.69), p < 0.0001] Group II x Group IV: [6.97 (5.19-9.34), p < 0.0001].

Group I x Group IV: [10.54 (5.79-19.21), p < 0.0001] Group III x Group IV: [0.57 (0.08-3.92), p = 0.5677].

In the few studies undertaken in Brazilian populations,  $-\alpha^{3.7}$  deletion was found to be the most common. Sonati *et al.* (1991) found  $\alpha$ -thalassemia 3.7 kb deletion in 23.4% of Afro-Brazilian blood donors from Campinas, São Paulo State. In the Northeast,  $\alpha$ -thalassemia 3.7 kb deletion was investigated by Adorno *et al.* (2005) in 514 newborns from Salvador, Bahia, whereupon it was observed that 114 (22.2%) presented  $\alpha^{3.7}$  thalassemia, of which 101 (19.7%) were heterozygous and 13 (2.5%) homozygous. Borges *et al.* (2001), on studying 339 adult outpatients with microcytosis and hypochromia without anemia at the Unicamp University Hospital, found 169 (49.9%) with  $\alpha$ -thalassemia, of which 145 (42.8%) were heterozygous ( $-\alpha^{3.7}/\alpha\alpha$ ) and 18 (5.3%) homozygous ( $-\alpha^{3.7}/-\alpha^{3.7}$ ). In a recent study in a north Brazilian population, the frequency of  $\alpha$ -thalassemia 3.7 kb deletion among 103 patients with microcytic hypochromic anemia was 19.4% (Souza *et al.*, 2009).

The present study detected  $\alpha$ -thalassemia 3.7 kb deletion in 11.2% (80/713) of an adult population from the state of Rio Grande do Norte. Few studies have been dedicated to investigating  $\alpha^+$ -thalassemia in the general population. These usually involve defined population groups with a greater likelihood of encountering deletion, such as individuals with microcytosis and hypochromia, or African descendants (Sonati *et al.*, 1991; Borges *et al.*, 2001; Souza *et al.*, 2009; Bezerra and Meissner, 2010).

Data from the Brazilian Institute of Geography and Statistics (IBGE) (2010), in which inclusion into a distinct ethnic group was based on self-declaration, showed that the population of Rio Grande do Norte was composed of 41.15% Caucasians, 52.48% mulattoes, 5.24% Afro-Brazilians, 1.04% yellow-skinned, 0.08% indigenous people, and 0.01 individuals with no declared color or race, to a total of 3,168,027 inhabitants.

According to ethnic distribution, prevalence of  $\alpha^+$ -thalassemia was greater among African descendants (24.8%), while among Caucasians this was 9.6%. Since in Brazil the degree of ethnic miscegenation is high, it appears that even in the non-African population, prevalence is also high.

African influence in the ethnic composition of the state of Rio Grande do Norte was not so high as that in other states, since fewer Africans were sent there as slaves during the colonization period, mainly due to the lack of adequate ports (Cascardo, 1984).

In spite of the different ancestral influence between the populations of Santarem (PA) and Rio Grande do Norte, the prevalence of heterozygotes (12.7%) was found to be similar to that of our study (11.1%) (Souza *et al.*, 2009), thereby revealing the elevated frequency and wide distribution of  $-\alpha^{3.7}$  deletion in our environment.

In southern Brazil, Wagner *et al.*, (2010) studied the prevalence of  $\alpha$ -thalassemia in various ethnic groups. On dividing the studied population into African and European

descendants, they observed greater prevalence in the former (23.1%) than in the latter (4.5%), thus corroborating the strong correlation between thalassemia and ethnic group.

$\alpha$ -Thalassemia is characterized by slight microcytosis and hypochromia, reduced levels of hemoglobin, and an increased number of erythrocytes, when compared to parameters of normal individuals. These modifications are more marked in homozygous  $\alpha$ -thalassemia individuals than in their heterozygous counterparts (Harteveld and Higgs, 2010).

As observed in studies by Adorno *et al.*, (2005) and Souza *et al.*, (2009), there was a statistically significant difference ( $p < 0.05$ ) in the number of erythrocytes, MCV, MCH and HbA<sub>2</sub> in  $\alpha$ -thalassemia carriers ( $-\alpha^{3.7}/\alpha\alpha$ ), compared to individuals with normal  $\alpha$ -genes ( $\alpha\alpha/\alpha\alpha$ ). This was to be expected, since the unbalanced globin synthesis in  $\alpha$ -thalassemia gives rise to deficiency in the amount of Hb (accounts for 30%-35% of the red cell content) per cell, thereby leading to the production of hypochromic and microcytic cells (Higgs, 2009). The lower level of HbA<sub>2</sub> can be explained by the preferential formation of HbA in relation to HbA<sub>2</sub>. Under normal conditions of  $\alpha$ -globin chain sufficiency or slight excess,  $\alpha\beta$  dimers assemble more readily than  $\alpha\delta$  dimers. When supply of the  $\alpha$ -globin chain is limited, as in  $\alpha$ -thalassemia, there is a compatible increase in dimer formation, with  $\beta$  chains competing more effectively than  $\delta$  chains for scarce  $\alpha$ -globin (Steinberg and Nagel, 2009).

The high prevalence of  $-\alpha^{3.7}$  deletion in individuals with microcytosis and/or hypochromia has already been observed (Borges *et al.*, 2001; Sankar *et al.*, 2006; Rahim, 2009; Bezerra and Meissner, 2010; Wagner *et al.*, 2010). In the present study, 44.6% of those with microcytosis and/or hypochromia without anemia had  $\alpha^+$ -thalassemia, thus confirming similar findings in a southeastern Brazilian population, in which 48.1% of individuals under the same conditions were homozygous or heterozygous for the  $-\alpha^{3.7}$  deletion (Borges *et al.*, 2001). Elevated percentages of  $\alpha^+$ -thalassemia in individuals with this disorder give emphasis to the importance of  $-\alpha^{3.7}$  deletion as a possible cause of hematological alterations.

Notwithstanding, alterations in the hematological parameters evident in  $\alpha^+$  heterozygotes, through being minimal, just below the lower limits, or sometimes even normal, can hinder the detection of  $-\alpha^{3.7}$  deletion carriers during molecular screening (Harteveld and Higgs, 2010). In the present study, and corroborating findings in the literature, and according to Souza *et al.* (2009), the  $-\alpha^{3.7}$  deletion was detected in 3.7% of the individuals with hematological indices within reference limits.

Although heterozygous  $\alpha$ -thalassemia individuals are clinically normal, and thus dispense with treatment, it is im-



portant to recognize the condition in order to elucidate the etiology of microcytosis and/or hypochromia, often confounded with and treated as iron deficiency anemia.

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## Internet Resources

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