

## DELETION TYPE $\alpha$ -THALASSEMIA AMONG BRAZILIAN PATIENTS WITH SICKLE CELL ANEMIA

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### ABSTRACT

The genotype  $\alpha$ -/ $\alpha$   $\alpha$  (heterozygous  $\alpha^+$ -thalassemia) was demonstrated through restriction enzyme DNA analysis for 9/41 (22%) of sickle cell anemia patients which indicates a gene frequency of 0.126. MCV for the group with three  $\alpha$  genes was lower than that for patients with four  $\alpha$  genes. No difference was observed for HbA<sub>2</sub>, HbF and total hemoglobin. The data indicate that the deletion type  $\alpha$ -thalassemia has a high prevalence among Brazilian Blacks.

### INTRODUCTION

Functional human hemoglobins are tetramers formed by the combination of two alpha-like ( $\alpha$ ,  $\xi$ ) and five beta-like ( $\beta$ ,  $\delta$ ,  $\epsilon$ ,  $A\gamma$ ,  $G\gamma$ ) globin chains, whose synthesis is under the control of separate genes (Weatherall and Clegg, 1981; Higgs *et al.*, 1989). The  $\alpha$  globin gene cluster resides on the short arm of chromosome 16. The  $\alpha$  globin genes are duplicated and separated by 4 kb of DNA, so that each diploid cell contains four copies of  $\alpha$  globin genes that code for identical globin chains (Weatherall and Clegg, 1981). The  $\alpha$ -thalassemia syndromes comprise a heterogenous group of hereditary disorders of hemoglobin, characterized by a decrease of  $\alpha$  globin chain synthesis caused by mutation or deletion of one to four of the  $\alpha$  globin genes (Weatherall and Clegg, 1981; Higgs *et al.*, 1989). The presence of one inactive globin gene (-  $\alpha$ /haplotype) is called  $\alpha^+$ -thalassemia whereas the defect produced by two inactive  $\alpha$  globin genes in *cis* is known as  $\alpha^0$ -thalassemia (--/haplotype) (Weatherall and Clegg, 1981). Individuals with the - $\alpha$ / $\alpha\alpha$  genotype are asymptomatic and usually have a normal mean corpuscular volume (MCV) of erythrocytes. The patients with

$-\alpha/-\alpha$  or  $--/\alpha\alpha$  genotypes (homozygous for  $\alpha^+$ -thalassemia or heterozygous for  $\alpha^0$ -thalassemia) have microcytic erythrocytes and may (rarely) show a mild anemia. Double heterozygosity for  $\alpha^0$ - and  $\alpha^+$ -thalassemia ( $--/\alpha-$ ) results in Hb H disease, a moderately severe anemia which sometimes requires lifelong blood transfusions. *Hydrops fetalis* with Hb Bart's, causing stillbirth or last-trimester abortion, is due to  $\alpha^0$ -thalassemia homozygosity ( $--/--$ ) (Weatherall and Clegg, 1981; Higgs *et al.*, 1989).

The deletion of a single globin gene from one or both chromosomes ( $-\alpha/\alpha\alpha$  or  $-\alpha/-\alpha$  genotypes) accounts for most forms of  $\alpha$ -thalassemia in Blacks, while non-deletion forms of the disease or the  $\alpha^0$ -thalassemia haplotype are rare. The prevalence of  $\alpha$ -thalassemia is high among different Black populations thus far studied, with a heterozygote ( $-\alpha/\alpha\alpha$ ) frequency as high as 30% and approximately 2% homozygotes (Dozy *et al.*, 1979). In addition, the interaction between  $\alpha$ -thalassemia and sickle cell anemia may have significant effects upon the clinical and hematological features of sickle cell diseases, possibly by reducing the intracellular concentration of hemoglobin S (HbS) (Embury, 1985; Steinberg and Embury, 1986).

Available information about the prevalence of  $\alpha$ -thalassemia in Brazilian populations are based on the detection of Hb Bart's in cord blood, an approach which underestimates the frequency of heterozygous  $\alpha$ -thalassemia (Zago *et al.*, 1983; Sonati, 1986). In order to establish the prevalence of  $\alpha$ -thalassemia in a Black Brazilian population and to evaluate the effects of the interaction between  $\alpha$ -thalassemia and sickle cell anemia upon some hematological data, we carried out a DNA analysis of the globin gene cluster among patients with sickle cell anemia.

## MATERIAL AND METHODS

### *Patients*

Blood samples for hematological and DNA analysis were obtained from 41 patients with sickle cell anemia. In every case, the diagnosis was based on clinical and laboratory findings (Zago *et al.*, 1983). Hemoglobin and red cell count were determined in an electronic counter and the packed-cell volume was measured as the micro hematocrit. Hemoglobin electrophoresis was performed on cellulose acetate strips with Tris-EDTA-boric acid buffer at pH 8.9 and on agar gel with citrate buffer at pH 6.1 (Weatherall and Clegg, 1981). The presence of HbS was confirmed by a low solubility in 2.35 M phosphate buffer containing dithionite and a positive sickling test. HbA<sub>2</sub> was measured quantitatively by elution from acetate strips following electrophoresis, and HbF was measured by alkali denaturation (Weatherall and Clegg, 1981).

### DNA analysis

High molecular weight DNA was isolated from peripheral blood leukocytes with proteinase K or urea using standard procedures (Davies *et al.*, 1986). Ten  $\mu\text{g}$  of DNA from each sample was digested with Bam HI (Pharmacia, Sweden) according to the manufacturer's suggestions, subjected to horizontal electrophoresis in 0.8% agarose, and transferred to nitrocellulose or nylon filter as described by Southern (1975). The  $\alpha$  probe was a 1.5 Kb PstI genomic fragment containing the  $\alpha_1$  globin gene extracted from pSVO1 vector (Mellon *et al.*, 1981).

The probe was labelled by nick-translation to a specific activity of  $5.0 \times 10^7$  cpm/ $\mu\text{g}$  using ( $\alpha^{32}$ -P) adenosine triphosphate as precursor. Hybridization was performed for 18 hours at  $42^\circ\text{C}$  in a solution containing 50% formamide, 1% SDS, 5 x SSC, 0.02 M Tris-HCl pH 7.5, 1% Denhardt's, 5% dextran-sulphate and 100 mg/ml salmon sperm DNA. After hybridization, the filters were washed under stringent conditions, dried and exposed to a regular X-ray film for 2 to 10 days with Dupont Lightning-Plus intensifying screens at  $-70^\circ\text{C}$  (Davies *et al.*, 1986).

## RESULTS

The restriction enzyme Bam HI cuts the DNA outside the duplicate  $\alpha$ -globin genes to produce a DNA fragment 14 kb in length which contains the two normal  $\alpha$  genes. Two of the most common  $\alpha$ -thalassemia determinants are those associated with the deletion of one or both  $\alpha$  globin genes, which produce a fragment of 10.5 kb ( $-\alpha$ /haplotype) or no detectable fragment ( $-$ /haplotype), respectively, Lane 2 of Figure 1 illustrates a heterozygote for the  $\alpha^+$ -thalassemia (fragments of 14 kb and 10.5 kb), while the other lanes represent the pattern obtained from individuals with a normal  $\alpha$  globin gene cluster (only the normal 14 kb fragment).

DNA analysis showed that 9 out of 41 (22%) of the sickle cell anemia patients were simultaneously heterozygous for the deletion form of  $\alpha^+$ -thalassemia. No  $\alpha^+$ -thalassemia homozygote was observed in the sample studied.

The relevant hematological data of the two groups of sickle cell anemia patients (with or without concomitant  $\alpha$ -thalassemia) are summarized in Table I. The values for MCV were significantly different between the two groups ( $P < 0.02$ , Wilcoxon rank sum test).

## DISCUSSION

Our results demonstrate that the prevalence of heterozygous  $\alpha^+$ -thalassemia among Brazilian Blacks with sickle cell anemia is 22%, which indicates that about 1.6% of this population should be homozygous for this condition, corresponding to a gene

Figure 1 - Autoradiograph of Southern blot of DNA from five sickle cell anemia patients, digested with Bam HI and hybridized with an  $\alpha$ -specific gene probe. Patients 1 and 3-5 have a normal  $\alpha$  gene pattern, with a single 14.0-kb fragment which contains the two normal  $\alpha$  genes. Patient 2 is an  $\alpha^+$ -thalassemia heterozygote, with the deleted  $\alpha$  gene cluster located within the 10.5-kb fragment.

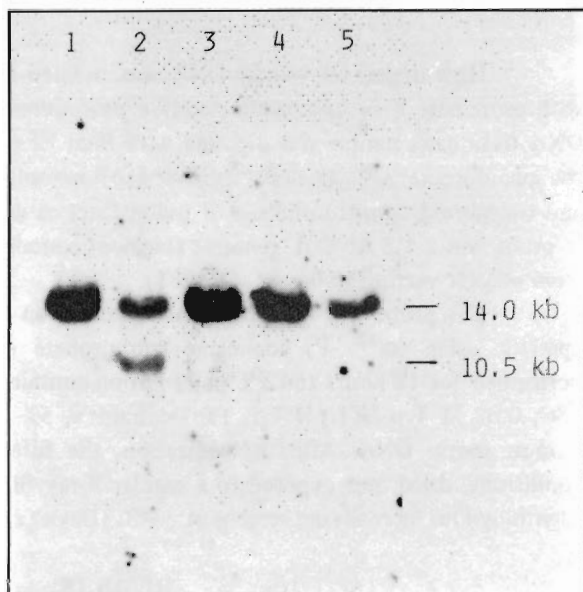


Table I - Comparison of hematological data obtained for sickle cell anemia patients with four or with three  $\alpha$  globin genes.

Genotype	No.	Hb (g/dl)	MCV (fl)	HbA <sub>2</sub> (%)	HbF (%)
$\alpha\alpha/\alpha\alpha$	32	7.4 $\pm$ 1.2	98.6 $\pm$ 13.0	2.36 $\pm$ 0.45	8.39 $\pm$ 6.45
$\alpha/\alpha\alpha$	9	6.7 $\pm$ 2.1	81.4 $\pm$ 16.2	2.45 $\pm$ 0.48	5.51 $\pm$ 3.54
		n.s.*	P < 0.02*	n.s.*	n.s.

\*Wilcoxon rank sum test; n.s. = not significant.

frequency of 0.126. These results represent the first data on prevalence of  $\alpha$ -thalassemia in Brazil, employing DNA analysis. The data are in agreement with those obtained for Black populations of the U.S.A., Jamaica and Africa (Dozy *et al.*, 1979; Steinberg and Embury, 1986; Higgs *et al.*, 1989). The reported data on the prevalence of Hb Bart's among Brazilian newborns disclosed, as expected, lower frequencies of  $\alpha$ -thalassemia (Sonati, 1986), since it has been demonstrated that this frequency is underestimated if the diagnosis is based solely on Hb Bart's detection (Higgs *et al.*, 1982).

Although digestion with Bam HI does not allow the identification of the deletion type involved, all cases are probably examples of the deletion which removes

about 3.7 kb of DNA ( $-\alpha^{3.7}$ , "rightward deletion"), since this is almost the only form of thalassemia found among Blacks (Steinberg and Embury, 1986). The single alpha gene left in the deleted chromosome may be an  $\alpha_1 - \alpha_2$  hybrid or the intact  $\alpha_2$  gene. The "leftward" deletion ( $-\alpha^{4.2}$ ) is uncommon in Blacks and is found mainly in Asians (Higgs *et al.*, 1989). Chromosomes that have lost both genes or thalassemia caused by a point mutation are uncommon among Blacks. Because the haplotype  $\alpha^0$ -thalassemia is so infrequent among Blacks, the clinically important forms of  $\alpha$ -thalassemia, such as HbH disease, are rarely observed, and *hydrops fetalis* has not yet been described in this race. The coexistence of  $\alpha$ -thalassemia affects both the red cell indices and the clinical evolution of the sickle cell disease. The mean MCV for the group of patients with the  $\alpha^-/\alpha$  genotype was lower than for the patients with four  $\alpha$  globin genes. This is in agreement with previously reported results, which indicate a reduction of the MCV and of the estimated mean cell hemoglobin concentration (MCHC) parallel to the decreasing  $\alpha$  globin gene number (Serjeant, 1985). The decreased MCHC inhibits the rate of intracellular HbS polymerization and intravascular sickling, consistent with a less rapid hemolysis and a milder anemia observed in patients who have an association of  $\alpha$  thalassemia and sickle cell anemia (de Cuelaeer, 1983). Influence of  $\alpha$ -thalassemia on HbF, HbA and the total hemoglobin concentration was not detected in the present study, probably because the alterations are better observed in  $\alpha^+$ -thalassemia homozygotes, who present higher concentrations of HbA<sub>2</sub> and total hemoglobin (Serjeant, 1985). There are conflicting data on the effect of  $\alpha$ -thalassemia on Hb F concentrations, but the results obtained in the largest series of cases studied disclosed significantly lower HbF levels in patients with both homozygous  $\alpha^+$ -thalassemia and sickle cell anemia (Serjeant, 1985).

The results of this study provide a clear indication that the deletion type of  $\alpha^+$ -thalassemia has a high prevalence among Brazilian Blacks. Thus, coexistence of  $\alpha$ -thalassemia could be an important factor affecting the hematological data and clinical variability of patients with sickle cell anemia.

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### RESUMO

A frequência de  $\alpha$ -talassemia determinada entre 41 pacientes com anemia falciforme, empregando análise de DNA com enzimas de restrição, demonstrou 9 (22%) heterozigotos  $\alpha^+$ -

talassêmicos (genótipo  $\alpha\text{-}/\alpha\alpha$ ), o que indica uma frequência gênica de 0,126. A comparação dos dois grupos de pacientes com anemia falciforme, com três ou com quatro genes de globina  $\alpha$ , mostrou VCM menor no primeiro grupo, ao passo que não foram detectadas diferenças nos valores de HbF, HbA<sub>2</sub> e hemoglobina total. Estes dados indicam uma elevada prevalência de  $\alpha$ -talassemia na população negróide brasileira.

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