Genetics and Molecular Biology, 31, 3, 621-625 (2008)

www.sbg.org.br

Research Article

Clinical and molecular characteristics of sickle cell anemia in the northeast of Brazil

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Abstract

Beta S-globin gene (β^S-globin) haplotypes, markers for severe sickle cell anemia (SCA), and the alpha-thalassemia 2 gene 3.7 kb deletion ($-\alpha_2^{3.7\ kb}$ thal) along with demographic and clinical data were investigated in SCA outpatients (n = 125, 63 female and 62 male) in the Brazilian state of Bahia, which has a high prevalence SCA. PCR-RFLP showed that the Central African Republic/Benin (CAR/BEN, 51.2%) haplotype was most frequent, followed by the Benin/Benin (Ben/Ben, 28.8%). At least one CAR haplotype was present in every outpatient with a history of cerebrovascular accident. The Cameroon (Cam), Senegal (Sen) and Arab-India haplotypes occurred in small numbers, as did atypical haplotypes. Fetal hemoglobin (HbF, %) was unevenly distributed. Compared to those > 18 y, those aged ≤ 18 y had had fewer erythrocyte transfusions and high HbF levels (12.3% ± 7.01 to 7.9% ± 4.36) but a higher frequency of spleen sequestration and pneumonia. Compared with normal α - genes carriers values, the outpatients with $-\alpha_2^{3.7\ kb}$ thal (determined by PCR analysis) had significantly higher mean hemoglobin concentration (Hb) (8.3 ± 1.34 g/dL, p = 0.018) and packed cell volume (PCV = 27.1% ± 4.26, p = 0.019) but low mean corpuscular volume (MCV = 86.1 fL = 10^{-15} L ± 9.56, p = 0.0004) and mean corpuscular hemoglobin (MCH = 26.6% ± 4.60, p = 0.039).

Key words: alpha-thalassemia 2 gene 3.7 kb deletion ($-\alpha_2^{3.7\ kb}$ thal), β^S-globin gene haplotypes, Fetal hemoglobin.

Received: April 27, 2007; Accepted: October 15, 2007.

Introduction

In sickle cell anemia (SCA), inheritance of the sickle cell trait involve two co-dominant beta-globin alleles, the normal A allele and the sickle-cell S allele. Individuals which are AS heterozygous produce both normal and abnormal hemoglobin and are usually healthy but may have some symptoms of sickle cell anemia at high altitudes when blood oxygen is low and may pass the S allele to their offspring, homozygous (SS) offspring manifesting SCA (Nagel and Steinberg, 2001a).

A wide spectrum of clinical manifestations and heterogeneous severity characterize SCA (Bunn and Forget, 1986; Nagel and Steinberg, 2001a). Fetal hemoglobin (HbF) levels have been associated with the modulation of SCA, decreased clinical severity, a decrease in the number of painful episodes, less need for blood therapy and a reduced number of hospital admissions (Steinberg, 2001; Nagel and Steinberg, 2001b). Individual HbF levels have been associated with specific beta S-globin gene (β^S-globin gene) haplotypes, usually recognized by a set of polymorphic restriction enzyme profiles distributed in the β^S-globin gene cluster region (Nagel and Ranney, 1990; Nagel and Steinberg, 2001b). The majors β-globin gene haplotypes are named according to the geographical origin and ethnic group in which they are frequently found, *i.e.*, Bantu or Central African Republic (abbreviated to BAN or CAR), Benin (Ben), Cameroon (Cam), Arab-India and Senegal (Sen) (Nagel and Ranney, 1990; Rahgozar *et al.*, 2000). The BAN or CAR haplotype being associated with the more severe forms of SCA (Powars and Hiti, 1993; Nagel and Steinberg, 2001a).

Thalassemia constitutes another group of inherited hemoglobin disorders commonly characterized by the reduction or absence of globin chain synthesis. The presence

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of the alpha-thalassemia 2 gene 3.7 kb deletion ($-\alpha_2^{3.7\ kb}$ thal) in individuals with SCA has been associated with an inhibitory effect on intracellular polymer formation of hemoglobin S (HbS), decreased mean corpuscular hemoglobin (MCH) and a decrease in the rate of hemolysis which results in an increase in the hemoglobin concentration (Hb) and an increased number of erythrocytes and a higher packed cell volume (PCV), leading to a decrease in the clinical severity of SCA (Steinberg and Embury, 1986).

Brazil is the largest South American country and has an heterogeneous population characterized by cultural, socioeconomic and ethnic diversity across its geographical regions. Bahia is a northeastern Brazilian state with a population of about 13 million and an important African genetic contribution due to the high level of slave trading which took place for more than three centuries (Azevêdo, 1980). In Bahia, the overall incidence of SCA in the general population has been estimated as 1:650 (Almeida *et al.*, 2006), with the AS genotype occurring at about 4.5% to 14.7% among different population groups (Azevêdo *et al.*, 1980). Regarding the presence of the $-\alpha_2^{3.7\ kb}$ thal has been identified in 20% to 25% of the Brazilian population of African descendent (Sonati *et al.*, 1991).

To identify the major pertinent molecular and clinical factors of SCA in Bahia we investigated the β^S-globin gene haplotypes in a group of SCA outpatients from Bahia and correlated these factors with the presence of $-\alpha_2^{3.7\ kb}$, HbF levels, other hematological data and phenotypic characteristics.

Materials and Methods

Study group

From 2002 to 2003 we conducted a cross-sectional transversal study of a group of outpatients with SCA from Bahia state who were attending the Hematology and Hemotherapy Foundation of Bahia (Fundação de Hematologia e Hemoterapia da Bahia (HEMOBA, Salvador, BA, Brazil). The group (n = 125) consisted of 63 (50.4%) females and 62 (49.6%) males aged between 1 year (y) to 73 y (median age = 19.1 ± 13.6 y), with 51.2% (n = 64, 24 females and 40 males) of the group being ≤ 18 y and 48.8% (n = 61, 39 females and 22 males) being > 18 y. The study was approved by the Human Subject Research Ethics Committee of the Oswaldo Cruz Research Foundation and informed consent was also obtained from the outpatients participating in the study or their legal guardians in accordance with the ethical principles and with the Helsinki Declaration of 1975, as revised in 2000.

The outpatients were divided into four groups according to their HbF levels: ≤ 5%, group I (n = 32, 25.6%); > 5% but ≤ 10%, group II (n = 37, 29.6%); > 10% but ≤ 15%, group III (n = 31, 24.8%); and > 15%, group IV with (n = 25, 20.0%).

Hematological and hemoglobin analyses

We assessed Hb (g/dL), HbF (%), PCV (%), MCH (%) and mean corpuscular volume (MCV, in femtoliters (1 fL = 10^{-15} L). Hematological analyses were carried out using a Coulter Count T-890 electronic cell counter (Coulter Corporation, FL, USA). The hemoglobin profile and HbF levels were investigated by high performance liquid chromatography (HPLC) using a Variant I HPLC (Bio-Rad Labs., CA, USA).

Assessment of β^S - globin haplotypes and $-\alpha_2^{3.7\ kb}$ thal

We used the GFX Genomic Blood DNA Purification kit (Amersham Pharmacia Biotech, NJ, USA) to isolate DNA from peripheral blood leukocytes taken from each of the outpatients involved in the study.

The β^S-globin Arab-India, Benin (Ben), Cameroon (Cam), Central African Republic (CAR) and Senegal (Sen) gene haplotypes were investigated using the polymerase chain reaction, PCR, and restriction fragment length polymorphism (RFLP) analysis using the, HindIII, HincII (Invitrogen Corporation, California, USA) and HinfI, HpaI and XmnI restriction enzymes (New England Biolabs, London, England) and PCR was used to investigate the $-\alpha_2^{3.7\ kb}$ thal in 110 outpatients (Sutton *et al.*, 1989; Dodé *et al.*, 1998). The β^S-globin haplotypes with fragment patterns which did not fit any of the above haplotypes were classified as Atypical (Aty).

Statistical analysis

The EPI info software version 6.04 (Centers for Disease Control and Prevention, Atlanta, USA) was used throughout to carry out all the statistical analyses. The ANOVA, Kruskal Wallis H, χ^2 and Fisher Exact test were used to test for significance between means at the probabilities stated.

Results

In our study of outpatients with SCA, those aged ≤ 18 y presented, on average, fewer leg ulcers (p = 0.0004) and had been submitted to erythrocyte transfusions less frequently (p = 0.02) than those > 18 y, although a higher frequency of both spleen sequestration (p = 0.03) and pneumonia (p = 0.0004) were observed in the younger group. We also found that 83 (66.4%) of the 125 outpatients investigated had a history of hospital admission, 71 (56.8%) had received blood therapy and 49 (39.2%) had had infections pneumonia (20%) and leg ulcers (16.8%) being the most frequent, 66 (52.8%) referred painful episodes, 15 (12.0%) presented spleen sequestration and six (4.8%) had had cerebrovascular accidents (CVA).

The HbF levels were unevenly distributed among the SCA outpatients, with those aged ≤ 18 y showing a smaller number of erythrocyte transfusions and high average relative HbF levels (12.3% ± 7.01) than those aged > 18 y

(7.9% ± 4.36) (p = 0.0004). The average relative HbF frequency by age-group being as follows: ≤ 5 y old, HbF = 16.6% ± 6.7%; 5 y to 10 y old, HbF = 10.9% ± 5.7%; 10 y to 15 y old, HbF = 8.9% ± 5.3%; and > 15 y old, HbF = 7.7% ± 4.5% (p < 0.00001). Hematological and clinical data, grouped by age and HbF levels, for our SCA outpatients are shown in the supplementary material given in the online version of this paper (Tables S1 and S2).

In our group of 125 outpatients with SCA the number of β^S-globin haplotypes and mean HbF levels were as follows: CAR/Ben 64 (51.2%), HbF 9.2% ± 5.7%; Ben/Ben 36 (28.8%), HbF 12.4% ± 6.5%; CAR/CAR 18 (14.4%), HbF 7.5% ± 4%; CAR/Aty 2 (1.6%), HbF 8.4% ± 1.8%; Ben/Cam 2 (1.6%), HbF 22.2% ± 7.3%; CAR/Cam 1 (0.8%), HbF 5%; CAR/Arab-India 1 (0.8%), HbF 26.4%; and Sen/Aty 1 (0.8%), HbF 0.8%. The CAR/Ben genotype was the most frequent haplotype in our group.

The distribution of clinical events among SCA outpatients with similar β^S- globin gene haplotypes and different HbF levels is shown in supplementary Table S3. At least one CAR haplotype was present in every outpatient with a history of CVA, which occurred in one CAR/Cam, two CAR/CAR and three CAR/Ben outpatients. The single Sen/Aty outpatient was 56 years old but presented no clinical complications and had never had an erythrocyte transfusion but the single CAR/Aty and one Ben/Cam outpatient had referred painful episodes, while the other Ben/Cam outpatient had a history of spleen sequestration and the CAR/Arab-India patient had a history of hospitalization and painful episodes.

Regarding thalassemia, the presence of $-\alpha_2^{3.7\ kb}$ thal influenced the hematological parameters, as previously described by other authors (Adams *et al.*, 1994; Steinberg, 2001). We found that of 110 outpatients two (1.8%) were $-\alpha_2^{3.7\ kb}$ homozygotes, both with the CAR/Ben β^S-globin haplotype, while 30 (27.3%) were $-\alpha_2^{3.7\ kb}$ heterozygous, with the following β^S-globin haplotypes: CAR/Ben, 18 (16.4%); Ben/Ben, 8 (7.3%); CAR/CAR, 3 (2.7%); and CAR/Arab-India, 1 (0.9%). The frequency of painful episodes was lower (p = 0.012) among outpatients with the CAR/Ben haplotype as compared to the other haplotypes. The pertinent hematological and phenotypic characteristics for the outpatients with the different α - genotypes are shown in supplementary Table S4.

Discussion

Previous reports have shown an association between sickle cell anemia clinical heterogeneity and HbF levels, and individuals with high levels of HbF have been described as being less prone to hemolysis, having a high Hb concentration, exhibiting a milder clinical picture and a higher survival rate (Nagel and Steinberg, 2001a, 2001b). Salzano (1985) studied 409 individuals with SCA from southeast Brazil and observed a positive correlation between the levels of HbF, Hb and PCV, suggesting that high HbF values were directly associated with a decrease in the symptoms of SCA. In our study of a Brazilian population we detected a statistical correlation between HbF levels and PCV values, agreeing with the findings of a Nigerian study carried out by Falusi and Kulozik (1990).

Borba *et al.* (2003) found no association between the levels of HbF and Hb in Brazilians with SCA who were undergoing treatment with hydroxyurea, although their study did indicate a significant correlation between MCV and HbF levels. Buchanan *et al.* (2004) suggested an association between high HbF levels and a low frequency of leg ulcers, supporting our observation that in our group of Brazilians outpatients with high HbF values had milder SCA phenotypic characteristics and needed fewer sessions of erythrocyte transfusions. Our results also support those of Powars and Hiti (1993) and Nagel and Steinberg (2001b) who also showed that high levels of HbF were associated with milder SCA symptoms.

In our study, HbF levels seem to decrease with age, the highest levels being recorded among outpatients aged ≤ 5 years and the lowest in those older than 15 years. However, these results do not agree with those of previous reports, which describe lower HbF levels from childhood through adolescence and higher levels during adult life (Powars and Hiti, 1993; Nagel and Steinberg, 2001b).

We recorded a high frequency of the Ben β^S-globin gene haplotype, contrarily to findings obtained from samples from other regions of Brazil, such as the southeast and the north where there is a predominance of the CAR haplotype. This suggests an uneven β^S-globin gene haplotype distribution in Brazil (Costa *et al.*, 1994; Goncalves *et al.*, 1994; Figueiredo *et al.*, 1996; Pante-De-Souza *et al.*, 1999). In the present study, we report for the first time the presence of the Cam and Arabia-India haplotypes in Bahia, confirming the previously described African origin diversity of this population (Goncalves *et al.*, 2003; Adorno *et al.*, 2004).

Individuals with SCA and the CAR haplotype often have HbF levels below 5%, while those with the Ben haplotype show intermediary HbF levels of from 5 to 10% (Nagel and Steinberg, 2001a, 2001b; Steinberg, 2001). However, our results do not agree with some previous published data (Bunn and Forget, 1986; Costa *et al.*, 1994; Lugo *et al.*, 2003) on HbF levels and β^S-globin gene haplotypes but are consistant with some other reports (Falusi and Kulozik, 1990; Mouele *et al.*, 1999; Inati *et al.*, 2003). In our study, the presence of high HbF levels for the CAR/CAR haplotype could be due to sequence variations in regulatory regions, such as the 5'HS2 and flanking region of the γ gene (Lanclos *et al.*, 1991; Ofori-Acquah *et al.*, 2001). Furthermore, Patrinos *et al.* (2005) also described the heterogeneity of the Ben haplotype in Sicilian and North American individuals and associated this with

the presence of a T → A (-499) variation in the A γ-globin gene.

Previous reports have suggested that the CAR haplotype is associated with a higher incidence of organ damage and the Ben haplotype with a milder form of SCA (Powars and Hiti, 1993; Nagel and Steinberg, 2001a) but our results showed no association between severe clinical SCA and the CAR/CAR genotype (HbF = 7.5% ± 4.00), while the CAR/Arab-India and CAR/Aty genotypes exhibited higher HbF levels (26.4% and 8.4% ± 1.8, respectively) and a mild clinical picture. However, Sarnaik and Ballas (2001) have reported that CVA are associated with the CAR haplotype, suggesting that, compared with other haplotypes, the presence of the CAR/CAR, CAR/Ben or Atypical haplotypes may be associated with a higher risk of CVA. In our study we found significant differences in the use of erythrocyte therapy for CAR/Ben and Ben/Ben outpatients with uneven HbF levels but this was not observed for CAR/CAR patients, suggesting that high HbF levels per se do not influence the clinical picture of sickle-cell anemia. However, since there was only a small number of CAR/CAR haplotypes the associations involving the CAR/CAR genotype with hematological or clinical findings seen in our study and discussed above should be interpreted with caution because some of these associations could be due to chance sampling events. It is interesting to note that we found that the outpatient with the Sen/Atypical haplotype presented low HbF levels and had no history of erythrocyte therapy, possibly suggesting that the Senegal haplotype is associated with mild clinical manifestations, as previously reported by Diop *et al.* (1999).

In our study we found that the presence of the alpha-thalassemia 2 gene 3.7 kb deletion ($-\alpha_2^{3.7\ kb}$ thal) was associated with an increase in Hb concentration and PCV and a reduction in MCV and MCH values, as described previously (Steinberg and Embury, 1986; Adams *et al.*, 1994). Sickle-cell anemia individuals with $-\alpha_2^{3.7\ kb}$ thal and a CAR/Ben genotype seem to have a smaller number of painful episodes. Mukherjee *et al.* (1997) reported a low incidence of painful crisis in individuals with $-\alpha_2^{3.7\ kb}$ thalassemia and the CAR/CAR genotype, conflicting with an earlier report of Billett *et al.* (1995) which suggest that the presence of a-thalassemia and others epistatic effects, most likely involving vascular response, should be influence the capacity of the sickle cells to adhere to the vascular endothelium. In our study, HbF levels did not show a significant difference among α-thalassemia genotypes (*i.e.*, those with and without the $\alpha_2^{3.7\ kb}$ deletion), this observation being consistent with previous reports (Figueiredo *et al.*, 1996).

Our study confirms the high diversity of β^S-globin gene haplotypes and the high level of phenotypic heterogeneity among sickle cell anemia outpatients from northeastern Brazil.

Acknowledgments

This study was supported by CNPq, contract grant number: 306524/2004-0; FAPESB/SECTI/SESAB/Gestão Saúde, contract grant number: 143104005306; FIOCRUZ/ Brazilian Minister of Health; project code: MDTP 1.

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Supplementary Material

The following online material is available for this article:

- Table S1. Hematological and clinical characteristics of sickle cell anemia outpatients from Bahia (Brazil) who were ≤ 18 y and had fetal hemoglobin (HbF) levels = 10.0 and > 10.0%.

- Table S2. Hematological and clinical characteristics of sickle cell anemia outpatients from Bahia (Brazil) who were > 18 y and had fetal hemoglobin (HbF) levels levels = 10.0 and > 10.0%.

- Table S3. Age and phenotypic characteristics of sickle cell anemia outpatients from Bahia (Brazil) with different beta S-globin gene (β^S-globin) haplotypes.

- Table S4. Gender, hematological and phenotypic characteristics of sickle cell anemia outpatients from Bahia (Brazil) with different alpha-thalassemia 2 gene 3.7 kb deletion ($-\alpha_2^{3.7\ kb}$) genotypes.

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Associate Editor: Paulo A. Otto

Table S1. Hematologic and clinical characteristics among sickle cell anemia patients from Bahia – Brazil, with age ≤ 18.0 years and HbF levels ≤10.0 and > 10.0%. Significant results of statistical tests (at the 5% level) are shown in bold face types.

HbF Groups	Hb, g/dL (Mean ± SD)	PCV, % (Mean ± SD)	MCV, fL (Mean ± SD)	MCH, pg (Mean ± SD)	MCHC, g/dL (Mean ± SD)	Hospitalization, n/total (%)	RBC Transfusion, n/total (%)	Pneumonia, n/total (%)	Leg ulcers, n /total (%)	Spleen sequestration, n/total (%)	CVA, n/total (%)	Painful episodes, n/total (%)
Group I (HbF≤10.0%) (n=26)	7.0 ± 1.27	22.9 ± 3.77	92.6 ± 11.58	28.4 ± 5.13	30.6 ± 3.0	19 / 26 (73.1)	17 / 26 (65.4)	07 / 35 (34.2)	01 / 26 (35.9)	04 / 26 (15.4)	04 / 26 (15.4)	10 / 26 (38.5)
Group II (HbF>10.0%) (n=38)	8.1 ± 1.57	26.7 ± 4.10	88.2 ± 9.8	26.4 ± 4.10	29.8± 2.4	25 / 38 (65.8)	13 / 38 (34.2)	14 / 39 (35.9)	02 / 38 (5.3)	08 / 38 (21.1)	01 / 38 (2.6)	19 / 38 (50.0)
p-Value	0.063**	**0.011****	0.260**	0.230**	0.440**	0.730†	**0.0028**†	0.570†	1.00††	0.747††	0.149††	0.512†

**ANOVA † X^2 Test †† Fisher Exact Test

Table S2. Hematological and clinical characteristics in sickle cell anemia patients from Bahia, Brazil, with age > 18.0 years and and HbF levels ≤10.0 and > 10.0%. Significant results of statistical tests (at the 5% level) are shown in bold face types.

HbF Groups	Hb, g/dL (Mean ± SD)	PCV, % (Mean ± SD)	MCV, fL (Mean ± SD)	MCH, pg (Mean ± SD)	MCHC, g/dL (Mean ± SD)	Hospitalization, n/total (%)	RBC Transfusion, n/total (%)	Pneumonia, n/total (%)	Leg ulcers, n /total (%)	Spleen sequestration, n/total (%)	CVA, n/total (%)	Painful episodes, n/total (%)
Group I (HbF≤10.0%) (n=43)	7.4 ± 1.77	23.9 ± 5.72	95.1 ± 9.20	29.5 ± 4.15	31.0 ± 3.17	25 / 43 (58.1)	30 / 43 (69.8)	04 / 43 (9.30)	14 / 43 (32.6)	00 / 43 (0.0)	00 / 43 (0.0)	25 / 43 (58.1)
Group II (HbF>10.0%) (n=18)	8.3 ± 1.47	25.4 ± 3.57	102.6 ± 13.30	33.5 ± 4.89	32.6 ± 2.23	14 / 18 (77.8)	11 / 18 (61.1)	00 / 18 (0.0)	04 / 18 (22.2)	00 / 18 (0.0)	01 / 18 (5.6)	12 / 18 (66.7)
p-Value	0.180**	0.483 **	0.08 **	**0.029** **	0.175**	0.244 †	0.720†	0.309 ††	0.617 †	1.00 ††	0.295†	0.817 †

**ANOVA † X^2 Test †† Fisher Exact Test

Table S3. Age and phenotypic characteristics among sickle cell anemia patients from Bahia, Brazil with similar β^S- globin gene haplotypes and different HbF levels. Significant results of statistical tests (at the 5% level) are shown in bold face types.

Genotypes Groups	HbF Groups	Age (years), median	Hospitalization, n / total (%)	RBC Transfusion, n / total (%)	Pneumonia, n / total (%)	Leg ulcers, n / total (%)	Spleen sequestration, n / total (%)	CVA, n / total (%)	Painful episodes, n / total (%)
CAR/Ben	Hb F>10.0% (n=26)	14.5	18 / 26 (69.2)	10 / 26 (38.5)	07 / 26 (26.9)	01 / 26 (3.8)	04 / 26 (15.4)	02 / 26 (7.7)	13 / 26 (50.0)
	Hb F≤10.0% (n=38)	21.0	27 / 38 (71.0)	25 / 38 (65.8)	05 /.38 (13.2)	07 / 38 (18.4)	05 / 38 (13.2)	01 / 38 (2.6)	22 / 38 (57.9)
	P Value	**0.047**∗	0.90†	**0.047**†	0.20††	0.13††	1.00††	0.56††	0.71†
CAR/CAR	Hb F> 10.0% (n=04)	18.0	03 / 04 (75.0)	03 / 04 (75.0)	01 / 04 (25.0)	01 / 04 (25.0)	00 / 04 (0.0)	00 / 04 (0.0)	03 / 04 (75.0)
	Hb F≤10.0% (n=14)	16.0	08 / 14 (57.1)	08 / 14 (57.1)	02 / 14 (14.3)	03 / 14 (21.4)	01 / 14 (7.1)	02 / 14 (14.3)	06 / 14 (42.9)
	P Value	0.95∗	1.00††	1.00††	1.00††	1.00††	1.00††	1.00††	0.58††
Ben/Ben	Hb F>10.0% (n=23)	7.0	16 / 23 (69.6)	10 / 23 (43.5)	05 / 23 (21.7)	04 / 23 (17.4)	04 / 23 (17.4)	00 / 23 (0.0)	12 / 23 (52.2)
	Hb F≤10.0% (n=13)	23.0	06 / 13 (46.2)	11 / 13 (84.6)	03 / 13 (23.1)	05 / 13 (38.5)	00 / 13 (0.0)	00 / 13 (0.0)	05 / 13 (38.5)
	P Value	**0.004**∗∗	0.30††	**0.04**††	1.00††	0.23††	0.27††	--------	0.81 †

∗ Kruskal-Wallis H ∗∗ANOVA † X^2 Test †† Fisher Exact Test

Table S4. Gender, hematological and phenotypic characteristics among sickle cell anemia patients from Bahia, Brazil, with different α - genes genotype. Significant results of statistical tests (at the 5% level) are shown in bold face types.

	αα / αα	$\alpha_2^{3.7Kb}$ - thalassemia	P value
Gender			
Male, n (%)	44 (77.2%)	13 (22.8%)	0.20†
Female, n (%)	34 (64.2%)	19 (35.8%)	
HbF, % (Mean ± SD)	9.9 ± 6.25	10.7 ± 6.97	0.56**
Hb, g/dL (Mean ± SD)	7.3 ± 1.58	8.3 ± 1.34	**0.018** **
PCV, % (Mean ± SD)	24.0± 4.55	27.1 ± 4.26	**0.019** **
MCV, fL (Mean ± SD)	96.8± 9.90	86.1 ± 9.56	**0.0004** **
MCH, pg (Mean ± SD)	29.4 ± 4.40	26.6 ± 4.60	**0.039** **
Hospitalization, n / total (%)	50 / 78 (64.1%)	22 / 32 (68.8%)	0.81††
RBC Transfusion, n / total (%)	44 / 78 (56.4%)	15 / 32 (46.9%)	0.48††
Pneumonia, n / total (%)	20 / 78 (25.6%)	03 / 32 (9.4%)	0.10††
Leg ulcers, n / total (%)	13 / 78 (16.7%)	04 / 32 (12.5%)	0.77††
Spleen sequestration, n / total (%)	06 / 78 (7.7%)	06 / 32 (18.8%)	0.10††
CVA, n / total (%)	04 / 78 (5.1%)	01 / 32 (3.1%)	1.00††
Painful episodes, n / total (%)	38 / 78 (48.7%)	20 / 32 (62.5%)	0.20†

** ANOVA † X^2 Test †† Fisher Exact Test