Co-inheritance of α-thalassemia dramatically decreases the risk of acute splenic sequestration in a large cohort of newborns with hemoglobin SC

Hemoglobin SC (HbSC) is the second most common variant of sickle cell disease worldwide after Hb SS. Hb C is caused by a mutation in the seventh codon of the *HBB* gene [HBB:c.19G>A(p.Glu7Lys)]. The β^S allele is associated with different β^S haplotypes that are named according to the region in which they originated,[1] although such multicentric origin of the β^S allele has recently been proven to be unreliable.[2] In addition to this anthropological importance, different β^S haplotypes can modulate the severity of sickle cell anemia.[3] β^C haplotypes I, II, and III were first described by Boehm *et al.*[4] There are no data in the literature on the association of β^C haplotypes with clinical and hematologic features. Likewise, the effect of co-inheritance of α-thalassemia (α-thal) in patients with HbSC is still unclear.

This study was based on a retrospective cohort of 461 newborns from Minas Gerais, Brazil (birth date: 01/01/1999 to 31/12/2012). Results on the clinical and hematologic profiles have been recently published.[5] Methods for the determination of α-thal and β-globin gene cluster haplotypes, as well as detailed statistical methods are available in the *Online Supplementary Appendix S1*. $P<0.01$ was considered significant. The study was approved by the institutional research ethics committee (*protocol 13327713.5.0000.5149*).

Co-inheritance of α-thal was determined in 389 children; 77 (19.8%) were $-\alpha^{3.7}/\alpha\alpha$ and one (0.26%) $-\alpha^{3.7}/-\alpha^{3.7}$; 311 (79.9%) had wild *HBA* alleles (αα/αα). The patients were analyzed for a follow up of a total of 3,522.72 patient-years. There was no difference in follow up between the two groups (8.78 and 9.13 years for patients with or without co-inheritance of α-thal, respectively; $P=0.51$). The incidence of pain crises for all those 389 children was 52.1 episodes per 100 patient-years (95%CI: 49.8-54.6). The incidence of infections was 60.1 events per 100 patient-years (95%CI: 57.6-62.7), and that of red blood cell (RBC) transfusions, 4.4 events per 100 patient-years (95%CI: 3.73-5.15).

The risks of pain crises, infections, and RBC transfusion were, respectively, 1.73 (95%CI: 1.5-1.99; $P<1\times10^{-7}$), 1.31 (95%CI: 1.16-1.47; $P=5\times10^{-6}$), and 2.88-fold (95%CI: 1.6-5.18; $P=9\times10^{-5}$) higher for αα/αα children compared with those with α-thal (Table 1). To the best of our knowledge, this is the first clear demonstration that co-inheritance of α-thal in children with HbSC decreases the incidence of these three events. In children with HbSS, the effect of α-thal on the incidence of pain crises before hydroxyurea was introduced in the management of patients with sickle cell anemia is controversial.[6,7] In a contemporary cohort of 250 children with HbSS, our group found that the incidence of pain crises before hydroxyurea was started was 82.3 (95%CI: 75.8-89.3) per 100 patient-years for those with α-thal compared with 76 (95%CI: 72.0-80.2) for those without α-thal ($P=0.11$) (*Rezende et al., 2019, unpublished data*).

Of the 78 children with co-inheritance of α-thal ($-\alpha^{3.7}/\alpha\alpha$ or $-\alpha^{3.7}/-\alpha^{3.7}$), only one (1.3%) had acute splenic sequestration crisis (ASSC) whereas 55 of 311 (17.7%) children without α-thal had at least one episode of ASSC (see *Online Supplementary Appendix S1* for a definition of phenotype). The cumulative probability of ASSC in the groups with and without co-inheritance of α-thal was

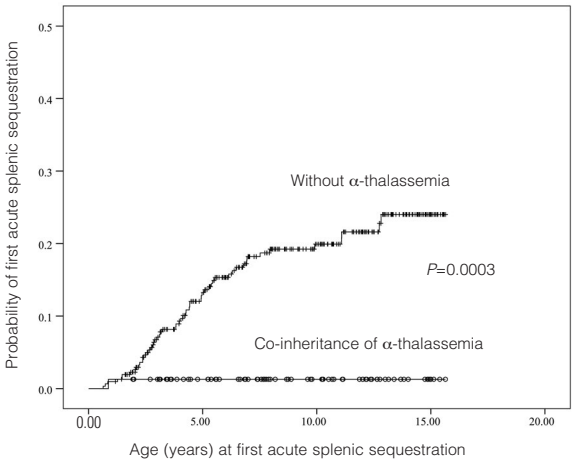

Figure 1. Kaplan-Meier probability curve of the occurrence of the first episode of acute splenic sequestration, according to the presence or absence of co-inheritance of $-\alpha^{3.7}$ thalassemia (one or two deleted genes).

Table 1. Association of co-inheritance of $-\alpha^{3.7}$ thalassemia (one or two deleted genes) with clinical data from 389 children with hemoglobin SC.

	With α thalassemia		Without α thalassemia
Painful vaso-occlusive crises			
Incidence per 100 patient-years (95% CI)	32.86 (28.71 – 37.45)		56.77 (54.03 – 59.61)
Estimated risk (95% CI)		1.73 (1.5 – 1.99)	
P value*		< 0.0000001	
Transfusions of packed red cells			
Incidence per 100 patient-years (95% CI)	1.75 (0.9 – 3.06)		5.04 (4.25 – 5.94)
Estimated risk (95% CI)		2.88 (1.6 – 5.18)	
P value*		0.00009	
Infections			
Incidence per 100 patient-years (95% CI)	48.19 (43.13 – 53.69)		63 (60.12 – 65.99)
Estimated risk (95% CI)		1.31 (1.16 – 1.47)	
P value*		0.000005	

95% CI: confidence interval set at 95%; * Fisher's exact test (Clopper-Pearson).

Table 2. Association of co-inheritance of -$\alpha^{3.7}$ thalassemia (one or two deleted genes) and mean laboratory results in 389 children with hemoglobin SC.

	With α thalassemia (SEM; n)	Without α thalassemia (SEM; n)	P*
Baseline hemoglobin (g/dL)	10.55 (0.08; 78)	10.48 (0.04; 309)	0.48
Platelet count (x10^9/L)	329. 9 (7.1; 78)	333.7 (4.9; 311)	0.7
Hemoglobin S (%)	47.4 (0.3; 69)	46.7 (0.2; 280)	0.052
Hemoglobin F (%)	5.1 (0.4; 68)	6.0 (0.3; 279)	0.07
White blood cell count (x10^9/L)	10.3 (0.2; 78)	11.4 (0.2; 311)	<0.001
Reticulocytes (%)	2.9 (0.1; 76)	3.6 (0.1; 308)	<0.001
Mean corpuscular volume (fL)	71 (0.5; 78)	76.9 (0.3; 311)	<0.001
Mean corpuscular hemoglobin (pg)	23.1 (0.3; 70)	25.4 (0.1; 299)	<0.001

SEM: standard error of the mean; * Student *t*-test.

1.3% (95%CI: 0-3.85%) and 24% (95%CI: 17.7-30.3%), respectively (*P*=0.0003) (Figure 1). As expected, a statistically significant association between co-inheritance of α-thal and lower levels of mean corpuscular volume (MCV), mean cell Hb (MCH), reticulocyte, and white blood cell (WBC) counts was found (*P*<0.001) (Table 2). In children with Hb SS, the rates of events seem to be similar in those with or without α-thal.[6] In our cohort of 250 HbSS children (*Rezende et al., 2019, unpublished data*), there was no significant difference in the cumulative probability of ASSC between children with or without α-thal (42% and 38%, respectively; *P*=0.51).

In the present study, one-fifth of children with HbSC co-inherited α-thalassemia.[3.7] The frequency of this co-inheritance in São Paulo, Brazil, was recently shown to be 26.7%,[8] but was as high as 35.2% in other reports.[9] In a clinical study of 179 adults with HbSC in France, the prevalence was 27%.[10] In Minas Gerais, the proportion of α-thal[3.7] co-inheritance in children with Hb SS was 26.6%.[11] Studies suggest that α-thal would limit the number of cells with high intracellular Hb concentration, with consequent attenuation of the clinical events, as observed in the present study and that of others.[12] The most remarkable clinical effect in the present study was the protection against ASSC in HbSC-possessing children with co-inheritance of α-thal, but rates of painful crises, infections, and RBC transfusion were also significantly lower in children with α-thal. In 1991, Platt *et al.* attributed the slight increase in the pain rate among HbSS patients with α-thal to their higher hematocrits.[7] In the present study, the mean Hb concentration for HbSC children with and without α-thal was 10.55 g/L and 10.48 g/L, respectively (*P*=0.50). In a multivariate regression model, the decrease of the pain rate in children who co-inherited α-thal was statistically significant (*P*=0.001), and independent of the non-significant positive effect of the Hb concentration level on the pain rate (*P*=0.14).

Haplotype determination was performed in 387 children (*Online Supplementary Table S1*): 211 (54.5%) were β^S CAR and 162 (41.9%) β^S Benin. Regarding β^C alleles, type I was predominant (305 cases; 78.8%), as usually reported.[4,10,13] The most common genotypes were CAR/I (42.6%) and Benin/I (34.9%). The HpaI restriction site (rs4426157) was "–/–" when the β^S haplotype was Benin (n=34) and "+/–" when it was CAR (n=50).

As expected, the prevalence of β^S CAR and Benin haplotypes in SC children was very similar to that already reported for our SS children in Minas Gerais.[11] Many studies indicate a gradually less severe clinical outcome in patients with the Arab-Indian, Senegal, Benin, and CAR haplotypes,[3] probably related to the observed gradual decline of the fetal Hb concentration. In the present study, there was a statistically significantly higher risk of pain crises and transfusion rate in children with the CAR haplotype, but the difference was modest (*Online Supplementary Table S2*). There was no difference in baseline hematologic laboratory results when the β^S CAR and β^S Benin haplotype groups were compared to each other (*Online Supplementary Table S3*). Some statistically significant clinical differences between β^C-I and β^C-II haplotype were observed (*Online Supplementary Table S2*), but the usefulness of this as a prognostic clinical tool seems to be irrelevant.

Another interesting result of the present study, corroborating the unicentric origin of HbC allele in West-Central Africa, was that the HpaI restriction site, characteristically negative for β^C,[4] was equally negative when associated with β^S-Benin and positive when associated to β^S-CAR. Nowadays, the Benin haplotype is predominant in West-Central Africa.[1,4]

In conclusion, the present study adds new and relevant information regarding the mitigating influence of co-inheritance of α-thal on the clinical and laboratory course of HbSC disease in a large cohort of newborns.

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