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

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Table S1. Genotypic and allelic frequencies of β-globin gene cluster haplotypes in 387 children with hemoglobin SC

Genotype		Number of children	Frequency (%)
β^S haplotypes	CAR	211	54.5
	BEN	162	41.9
	Ind*	14	3.6
	Total	387	100
β^C haplotypes	I	305	78.8
	II	54	14.0
	III	5	1.3
	Ind*	23	5.9
	Total	387	100
Groups (β^S/β^C)	BEN/I	135	34.9
	BEN/II	15	3.9
	BEN/III	3	0.8
	CAR/I	165	42.6
	CAR/II	38	9.8
	CAR/III	2	0.5
	ATP/I	5	1.3
	ATP/II	1	0.3
	BEN/ATP	9	2.3
	CAR/ATP	6	1.6
	Ind*	8	2.0
	Total	387	100

* It was not possible to characterize by the method; ATP: atypical.

Table S2. Association of β^S and β^C haplotypes with clinical data in a cohort of 387 children with hemoglobin SC

	β^S haplotype		**β^C haplotype***		**β^C haplotype stratified for β^S haplotype CAR**		**β^C haplotype stratified for β^S haplotype Benin**	
	CAR	Benin	I	II	CAR/I	CAR/II	Benin/I	Benin/II
Painful vaso-occlusive crises								
Incidence per 100 patient-years (95% CI)	55.5 (52.12 – 59.1)	48.6 (45.21 – 52.21)	49.61 (47.04 – 52.3)	66.5 (58.8 – 74.9)	53.05 (49.34 – 56.7)	72.36 (63.13 – 82.6)	45.99 (42.43 – 49.76)	53.23 (39.25 – 70.6)
Estimated risk (95% CI)	1.14 (1.04 – 1.26)		1.34 (1.18 – 1.53)		1.36 (1.17 – 1.58)		1.16 (0.86 – 1.55)	
P Value†	0.006		0.000002		0.000046		0.33	
Transfusions								
Incidence per 100 patient-years (95% CI)	5.26 (4.26 – 6.43)	3.22 (2.39 – 4.24)	4.45 (3.70 – 5.30)	5.16 (3.19 – 7.89)	5.25 (4.13 – 6.58)	6.55 (4.0 – 10.1)	3.65 (2.7 – 4.8)	1.1 (0.01 – 6.2)
Estimated risk (95% CI)	1.64 (1.16 – 2.3)		1.16 (0.73 – 1.84)		1.25 (0.76 – 2.0)		0.3 (0.04 – 2.2)	
P Value†	0.005		0.60		0.38		0.21	
Acute splenic sequestration								
Incidence per 100 patient-years (95% CI)	1.73 (1.15 – 2.5)	1.74 (1.13 – 2.57)	1.63 (1.17 – 2.2)	2.97 (1.42 – 5.47)	17.0 (10.7 – 25.7)	23.7 (8.7 – 51.6)	16.1 (9.8 – 24.8)	55.8 (15.0 – 142.8)
Estimated risk (95% CI)	0.99 (0.58 – 1.71)		1.83 (0.92 – 3.64)	1.4 (0.57 – 3.4)			3.47 (1.19 – 10.2)	
P Value†	0.98		0.14	0.47			0.015	
Infections								
Incidence per 100 patient-years (95% CI)	60.4 (56.84 – 64.1)	59.7 (55.9 – 63.7)	59.3 (56.5 – 62.2)	67.0 (59.3 – 75.5)	59.35 (55.4 – 63.5)	67.12 (58.2 – 76.9)	58.8 (54.8 – 63.1)	67.7 (51.7 – 86.9)
Estimated risk (95% CI)	1.01 (0.93 – 1.1)		1.13 (0.99 – 1.29)		1.13 (0.97 – 1.32)		1.15 (0.88 – 1.49)	
P Value†	0.8		0.07		0.11		0.29	

***** Statistical analysis for β^C haplotypes was performed only in those children with types I and II due to the small number with type III (5/387).

† In the non-stratified analyses, P values were obtained by the Fisher's test (Clopper-Pearson); in the stratified analyses, the z score was used instead.

Table S3. Association of β^S and β^C haplotypes with laboratory data in a cohort of 387 children with hemoglobin SC

	β^S haplotypes			β^C haplotypes*		
	CAR (n)	**BEN (n)**	**P Value†**	**I (n)**	**II (n)**	**P Value†**
Baseline hemoglobin (g/dL)	10.4 (210)	10.5 (161)	0.27	10.5 (303)	10.6 (54)	0.34
Platelet count (x10^9/L)	335.6 (211)	331.7 (162)	0.65	332.1 (305)	344.5 (54)	0.3
Hemoglobin S (%)	46.8 (185)	46.7 (148)	0.75	46.9 (275)	46.2 (45)	0.3
Hemoglobin F (%)	5.9 (184)	5.8 (148)	0.93	5.6 (274)	7.4 (45)	0.04
White blood cell count (x10^9/L)	11.2 (211)	11.13(162)	0.85	11.2 (305)	11.4 (54)	0.58
Reticulocytes (%)	3.5 (207)	3.3 (161)	0.2	3.4 (301)	3.6 (53)	0.53
Mean corpuscular volume (fL)	75.3 (211)	76.2 (162)	0.17	75.8 (305)	75.3 (54)	0.6
Mean corpuscular hemoglobin (pg)	25 (197)	24.9 (156)	0.9	25 (290)	25 (49)	0.99

* Statistical analysis for β^C haplotypes was performed only in those children with types I and II due to the small number with type III (5/387)

† Student t test

Appendix S1. Methods

Detection of seven gene deletions underlying alpha thalassemia was carried out using multiplex gap-PCR reactions.[1] Identification of β-globin gene cluster haplotypes was performed by PCR-RFLP (restriction fragment length polymorphism) at five known restriction sites (rs113425530, rs28440105, rs10128556, rs968857, and rs16911905), using a previously published protocol.[2] The *HincII* restriction site (5' of the *HBE* gene; rs3834466) was determined by gene sequencing. The *HpaI* restriction site (rs4426157) was determined by PCR-RFLP using a "long-distance" amplification (Qiagen LongRange PCR kit) with primers 1 and 2 devised by Sheth *et al*[3] in 34 randomized β^S Benin and 50 β^S CAR subsamples from children.

The information about episodes of acute splenic sequestration crisis (ASSC) was retrospectively retrieved by the investigators from the children's medical files and was based on hospital discharge reports made by the attending pediatrician or pediatric hematologist. The definition of ASSC[4] included a tender rapidly enlarging spleen and a sudden drop of basal hemoglobin concentration that required red blood cell transfusion. Reticulocyte count on admission was not available in all cases. The attending physician excluded alternative causes of the clinical manifestations.

Quantitative results were expressed as the mean ± SD, or as the median and interquartile range when the variable exhibited non-Gaussian distribution. Prevalence was expressed in rates and 95% confidence intervals (95% CI).

Differences in hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), percentages of S and fetal hemoglobin, baseline white blood cell counts, as well as platelets and reticulocytes between groups (for example, with or without alpha thalassemia) were analyzed by Student's t-test. Statistical values corresponding to homoscedastic (homogeneous variance) or heteroscedastic distributions (heterogeneous variance) were reported based on the Levene test.

The incidence of clinical events, such as ASSC, painful vaso-occlusive crises, infections, and blood transfusions were expressed in rates of 100 patient-years, with 95% confidence interval. Incidence rates for the two distinct groups of children were

compared using Fisher's test (Clopper-Pearson) using the open source program available at http://www.openepi.com.

Probability survival curves were constructed using the Kaplan-Meier model. The probability curve for the occurrence of the first episode of ASSC was extracted from the "One minus Survival function" chart, which represents the function (1 – Survival function). The logrank test was used to compare curves derived for children of different groups.

Statistical analyses were performed using the SPSS 20.0 software. *P*-values < 0.01 were considered to be significant.

This study was approved by the Research Ethics Committee of the Institution (Protocol # 13327713.5.0000.5149). Patients and parents/guardians were asked to sign a free and informed consent form prior to study initiation.

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