HAEMOLYSIS IN SICKLE CELL ANAEMIA: A GENOTYPE/PHENOTYPE ASSOCIATION STUDY

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INTRODUCTION & OBJECTIVES

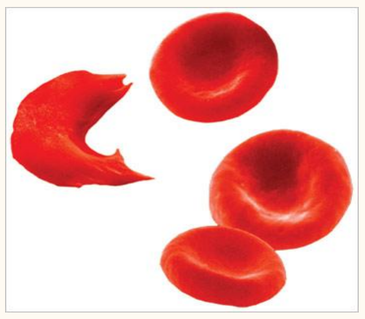

Fig. 1. Normal red blood cells and a sickled one .

Sickle Cell Anaemia (SCA), one of the most common autosomal recessive hereditary anemia, is caused by a mutation in the beta-globin gene (HBB:c.20A>T) on 11p15.5. This mutation originates a hemoglobin variant named **Hb S**, as opposed to the normal adult Hb A.

Hb S ability to polymerize when deoxygenated gives rise to abnormal sickled red blood cells (Fig.1).

SCA is characterized by recurrent episodes of severe vaso-occlusion, haemolysis and infection. Several genetic and environmental modifiers have been suggested to modulate the onset and course of this disease (1).

As part of a wider research on the development and validation of vaso-occlusion early predictors in SCA, we have studied the association between three haemolysis biomarkers (serum LDH, total bilirubin and reticulocyte count) and the inheritance of several genetic variants of candidate genes related to Hb Fetal level, red blood cell vascular adhesion and vascular tonus, as well as a common alpha-thalassaemia determinant, in a longitudinally observed series of paediatric SCA patients.

METHODS

Subjects: 99 paediatric SCA (SS) patients (median current age of 9.9 years) followed-up in two general hospitals in Greater Lisbon area (median follow-up/patient of 5.0 years).

Haemolysis biomarkers: LDH and total bilirrubin level and reticulocyte count.

Candidate gene genotyping: Forty-one genetic polymorphisms (34 SNP, 6 indel, 1 STR) in the following loci have been typed: *BCL11A, CD36, EDN1, HBA, HBB* cluster (including *HBG), HBS1L-MYB*, *ITGA4, HMOX1, NOS3*, *THBS1* and *VCAM1.*

Statistical analysis: Association studies were performed using T test ANOVA parametric tests (LDH, total bilirrubin) or Mann-Whitney/Kuskal-Wallis non-parametric tests (reticulocyte count), all performed with SPSS v20.0 software. A correction for multiple testing (false discovery rate) was done .

RESULTS

- Patients clinical data at steady-state have been captured to a database.
- Forty one genetic variants within 11 candidate genes were characterized.
- Association studies between candidate genotypes and haemolysis biomarkers were performed.
- The following significant associations were observed (Table I, Fig. 2)

Table I. Association between candidate gene variants and haemolysis markers in sickle cell anaemia

Gene	SNP reference	Allele[a]	Associated Allele or Haplotype		Presence of associated allele or haplotype	Number of patients	Haemolysis biomarkers[b]					
							LDH (U/L) mean±SD	*p*-value	Total Bilirubin (mg/dL) mean±SD	*p*-value	Reticulocyte count (%) mean±SD	*p*-value
VCAM1-gene	rs3783613	G/C	C	VCAM1_g_hapl 7	Yes	12	1270.6±279.3	*p*=0.002				
	rs3176878	C/T	C									
	rs3783615	A/T	A		No	49	926.6±348.4					
	rs3176879	A/G	A									
VCAM1-promoter	rs1409419	C/T	C	VCAM1_p_hapl 9	Heterozygosity (hapl 9/hapl X)[c]	4			1.65±0.05	*p*<0.001		
	rs3917024	C/T	T									
	rs3917025	CT/delCT	delCT		Homozygosity (hapl 9/hapl 9; hapl X/hapl X)	88			2.50±0.43			
	rs3783597	C/G	G									
	rs3783598	T/G	T									
	rs1041163	T/C	C									
	rs3783599	C/T	T									
CD36	rs1984112	A/G	G		Yes	45					13.06±0.80	*p*=0.001
					No	50					9.26±0.56	
NOS3	rs2070744	C/T	T		Yes	90			2.41±0.42	*p*<0.001		
					No	2			5.18±0.01			
HBA	del 3.7kb	Non-del/del	del		Yes	41			2.04±0.40	*p*=0.002		
					No	52			2.80±0.40			
					Yes	42					8.77±0.48	*p*=0.001
					No	53					13.21±0.73	

a) The first position represents the ancestral allele.
b) Individual patients levels measured in steady state and averaged for the whole corresponding follow-up period.
c) Hapl X = Non-hapl 9.

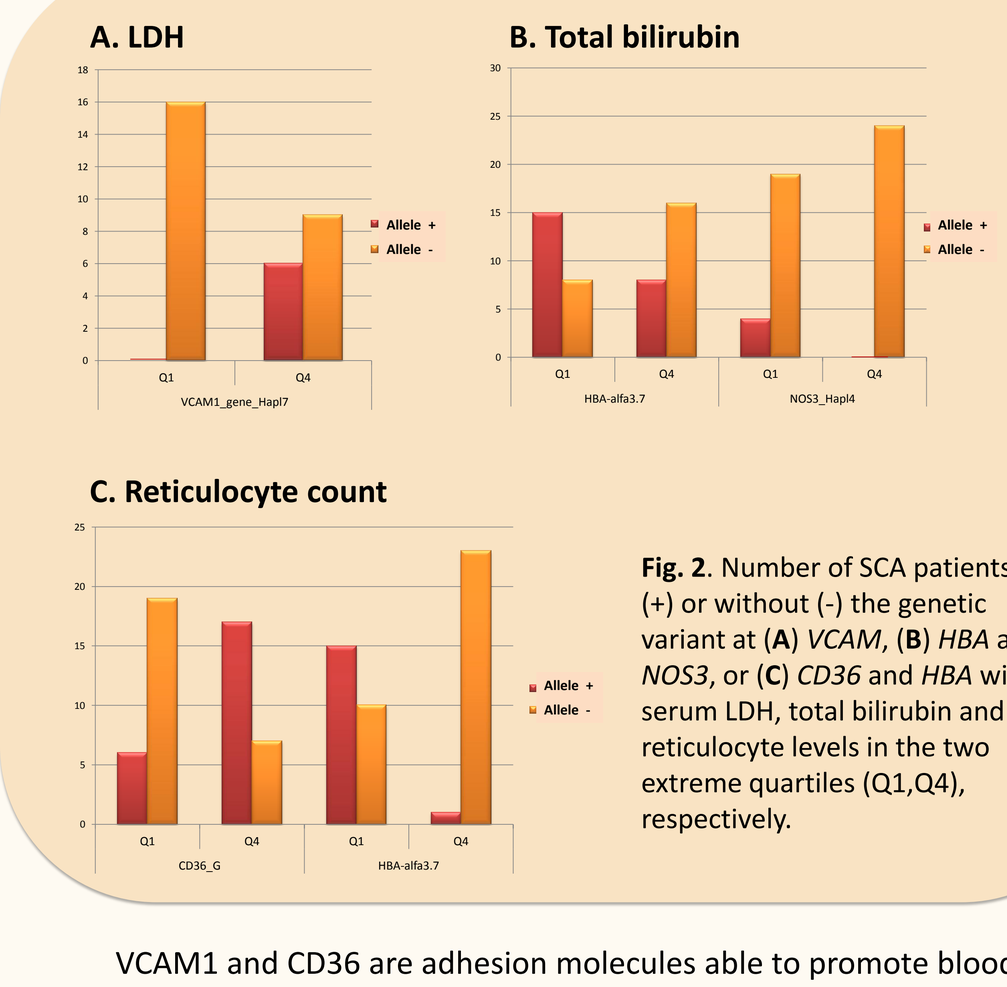

Fig. 2. Number of SCA patients with (+) or without (-) the genetic variant at (**A**) *VCAM*, (**B**) *HBA* and *NOS3*, or (**C**) *CD36* and *HBA* with serum LDH, total bilirubin and reticulocyte levels in the two extreme quartiles (Q1,Q4), respectively.

CONCLUSIONS

The lifelong haemolytic anaemia is known to be a distinct hallmark of SCA clinical course. In this study a statistically significant association was found between biochemical or cellular correlates of different stages of the haemolytic phenotype and the following genetic determinants:

Cell vascular adhesion

VCAM1 and CD36 are adhesion molecules able to promote blood cells adhesion to vascular endothelium. Some genetic variants of their corresponding genes were found associated with SCA haemolysis severity. VCAM1_gene_haplotype 7 was found associated with higher levels of LDH, suggesting a relation between this variant and a sub-phenotype characterised by more severe haemolysis. Contrarely, heterozygosity for *VCAM1*_promotor_haplotype 9 was found associated with lower levels of total bilirubin revealing a protective effect against haemolysis. Also the rs1984112_G allele at *CD36* gene revealed to be associated with higher levels of reticulocyte count, a likely more distal consequence of an increased haemolysis status.

Vascular tonus

NOS3 encodes nitric oxide synthase 3, which in endothelial cells generates NO, a gas with potent vasodilation and antiadhesive properties. The rs2070744_T allele at *NOS3* seems to have a protective effect on SCA haemolysis as it was found associated with lower bilirubin levels.

Alpha-thalassaemia

Low levels of haemolysis, measured by low levels of total bilirubin and reticulocyte count were found associated with the presence of the 3.7 kb deletion alpha-thalassaemia determinant at *HBA* gene. SCA patients who co-inherited the deletion have reduced haemolysis owing to a lower intracellular concentration of HbS that in turn decreases HbS polymer-induced cellular damage.

On the whole, our findings suggest a complex genetic architecture for the haemolytic endophenotype in SCA involving multiple pathways, namely control of vascular cell adhesion, NO synthesis and erythrocyte volume and haemoglobinisation.

Reference

1. Steinberg MH and Sebastiani P. Genetic modifiers of sickle cell disease. Am J Hematol 87:795-803, 2012.

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