Sickle Cell Anaemia (SCA), one of the most common autosomal recessive hereditary anemias, 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.

**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).

**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 bilirubin 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.

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

**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:

**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 T was found associated with higher levels of LDH, suggesting a relation between this variant and a sub-phenotype characterised by more severe haemolysis. Contrarily, heterozygosity for VCAM1_promoter_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 with potent vasodilation and antiadhesives 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.