Genetic Modifiers of the Intermediate Phenotypes in Sickle Cell Anemia

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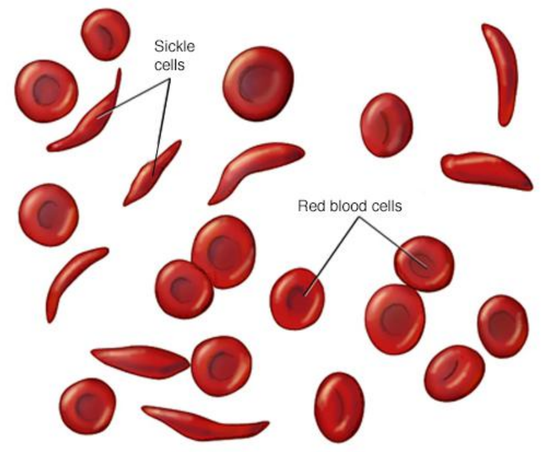
INTRODUCTION

Sickle cell anemia (SCA) is an inherited blood disorder characterized by the presence of hemoglobin S (HbS). This disease is caused by a mutation in the beta-globin gene (HBB:c.20A>T) on 11p15.5. The easily ability of Hb S to polymerize in deoxygenated conditions gives rise to abnormal sickled red blood cells (Figure 1) (Rees *et al*, 2010). Vaso-occlusion and hemolytic anemia are the major features of this disease, however SCA patients present clinical and hematologic variability that cannot be only explained by the single mutation in the beta-globin gene. Others genetic modifiers and environmental effects are important in the clinical phenotype (Steinberg & Sebastiani, 2012).



(In http://www.mayoclinic.org/diseases-conditions/sickle-cell-anemia/home/ovc-20303267)

Figure 1 - Normal and sickled red blood cells .

METHODS

- We studied the association between several hematological and biochemical parameters and some genetic variants in 26 pediatric SCA patients (mean age of 8.6 years) followed-up in Hospital de Dona Estefânia, in Lisbon.
- MPO and PlGF were determined by ELISA (R&D Systems Inc.). Amplification of DNA samples for the rs1050829 characterization, in the glucose-6-phosphate dehydrogenase (*G6PD*) gene, was performed by PCR followed by restriction fragment length analysis. A multiplex PCR assay was used for simultaneous amplification of glutathione S-transferases mu (*GSTM1*) and theta (*GSTT1*). All statistical tests were performed with SPSS 24.0 software.
- Association studies were performed using T test/ ANOVA parametric tests or Mann-Whitney/Kruskal-Wallis nonparametric tests, all performed with SPSS 24.0 software.

RESULTS

The following significant associations were observed (Table I, II, III and IV; Figure 2, 3, 4 and 5):

- We observed higher levels of MPO (p<0.001) and PlGF (p=0.048) in SCA patients.
- Moreover, in these patients we found associations between: 1) lower levels of total hemoglobin and the *GSTM1* null genotype (p=0.044); 2) higher levels of HbS with the rs1050829_G genotype (hemizygous males) in the *G6PD* gene (p=0.026).

Table I - Association between MPO concentration and the presence or absence of SCA

MPO (ng/mL)					
Population	**N**	**Median**	**Minimum**	**Maximum**	***p*-value***
SCA patients	14	29,56	14,14	78,63	<0.001
Controls	34	13,00	3,10	152,60	
*** Mann-Whitney test**					

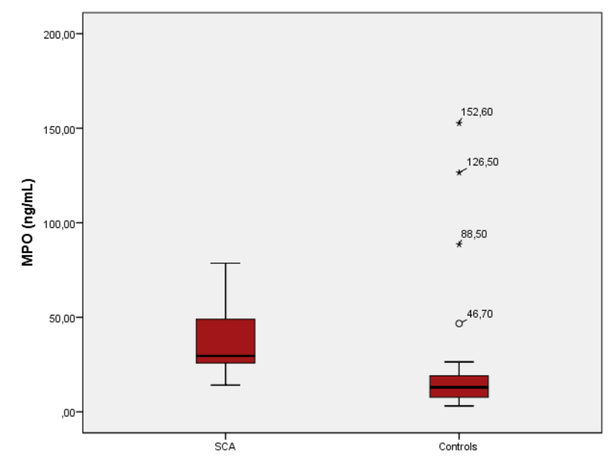


Figure 2 - Box plot showing distribution of MPO concentration among SCA patients and controls.

Table II - Association between PlGF concentration and the presence or absence of SCA

PlGF (pg/mL)				
Population	**N**	**Mean**	**Standard deviation**	***p*-value***
SCA patients	13	6,24	3,95	0.048
Controls	23	3,69	2,35	
*** T test**				

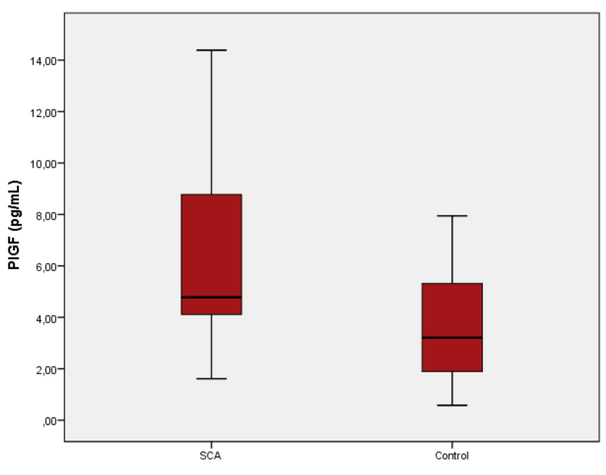


Figure3 - Box plot showing distribution of PlGF concentration among SCA patients and controls.

Table III - Association between total haemoglobin and *GSTM1* genotypes

Total Hb (g/dL)				
***GSTM1* genotype**	**N**	**Mean**	**Standard deviation**	***p*-value***
positive	17	8,89	1,34	0.044
null	3	7,17	0,42	
*** T test**				

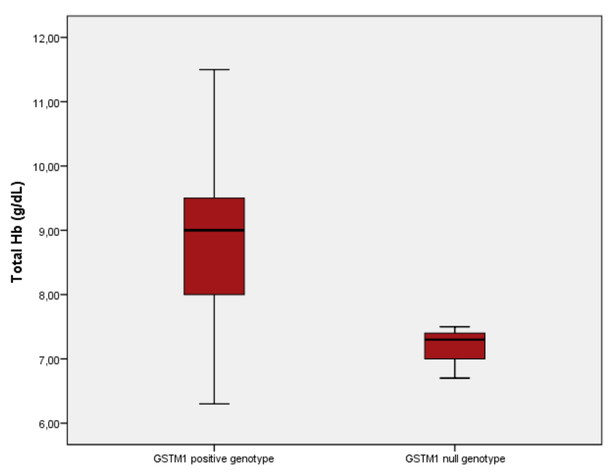


Figure 4 - Box plot showing distribution of total haemoglobin among positive and null genotypes at *GSTM1* gene.

Table IV - Association between HbS and *G6PD* genotypes

HbS (%)				
***G6PD* genotype**	**N**	**Mean**	**Standard deviation**	***p*-value***
A/AA	10	71,90	5,60	0.026
AG	3	70,73	6,11	
G	5	81,32	6,90	
*** ANOVA**				

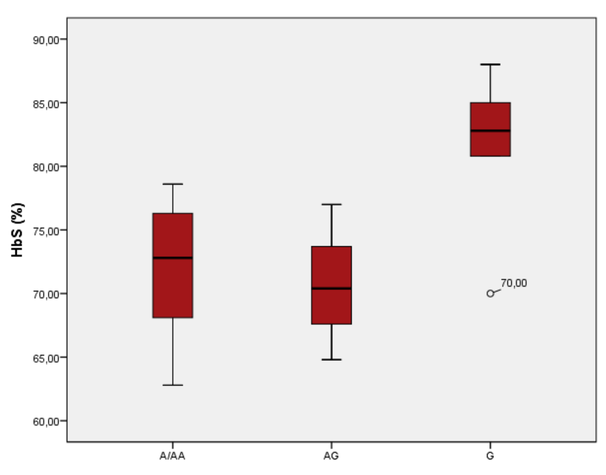


Figure 5 - Box plot showing distribution of HbS in rs1050829 genotypes at *G6PD* gene.

CONCLUSIONS

We suggest that the mentioned polymorphisms in *GSTM1* and *G6PD* genes may act as genetic modifiers in SCA, which could be useful for the prediction of increased susceptibility to complications. Furthermore, our results reinforce the importance to study biochemical parameters for a better understanding of the clinical outcome of this disease.

References:

Rees, D.C., Williams, T.N. & Gladwin, M.T. (2010) Sickle-cell disease. *The Lancet*, **376,** 2018–2031

Steinberg, M.H. & Sebastiani, P. (2012) Genetic modifiers of sickle cell disease. *American Journal of Hematology*, **87,** 795–803