Genetic variants in endothelial nitric oxide synthase gene are modifiers of the hemolysis phenotype in Sickle Cell Anemia

Laura Aguiar[1,2,3] Andreia Matos[1,3] Ângela Gil[1,3] Conceição Afonso[1] Lígia Braga[4] João Lavinha[2] Paula Kjollerstrom[4], Paula Faustino[2], Manuel Bicho[1,3], Ângela Inácio[1,3]

[1]Laboratório de Genética, Faculdade de Medicina da Universidade de Lisboa (FMUL), Lisboa
[2]Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisboa
[3]Instituto De Investigação Científica Bento Da Rocha Cabral (IICBRC), Lisboa
[4]Unidade de Hematologia do Hospital de Dona Estefânia (HDE), Lisboa

INTRODUCTION

Sickle Cell Anemia (SCA) is an autosomal recessive hereditary anemia characterized by the presence of hemoglobin S (Hb S). This disease is caused by a single mutation in the beta-globin gene with a corresponding amino acid substitution at the sixth position of the beta-globin chain. 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).

The aim of this work was to determine the association between hematological or biochemical parameters and genetic variants from candidate genes, in SCA patients.

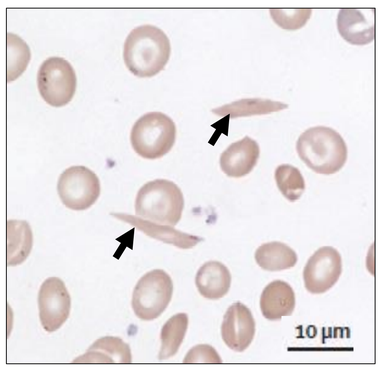

Figure 1 - Peripheral blood smear of a patient with SCA**.** The arrows point to two sickled erythrocytes (*Adapted from* Rees *et al*, 2010).

METHODS

Subjects: 26 paediatric SCA patients (mean age of 8.58 years) followed-up in Hospital de Dona Estefânia, in Lisbon.
Hematological or biochemical parameters: Hb S, total Hb, red cell distribution width (RDW), leukocytes, neutrophils, transmembrane reductase, methemoglobin reductase, serum lactate dehydrogenase (LDH), total bilirubin and reticulocyte count.
Candidate genes: *BCL11A, HBA, HBB* cluster*, HMOX1, eNOS*, *MTHFR* and *MPO.*
Statistical analysis: Association studies were performed using T test/ ANOVA parametric tests (Hb S, total Hb, RDW, neutrophils, transmembrane reductase, methemoglobin reductase and reticulocyte count) or Mann-Whitney/Kuskal-Wallis non-parametric tests (total bilirubin, leukocytes and LDH), all performed with SPSS 22.0 software.

RESULTS

- Association studies between candidate genotypes and hematological or biochemical parameters were performed.
- The following significant associations were observed (Table 1 and 2, Figure 2 and 3).
- Our results show a significant statistical association between two *eNOS* single nucleotide polymorphisms (SNPs) and two haemolysis parameters. Both the rs2070744_TT and the rs1799983_GG genotypes are associated with an increased reticulocyte count (p = 0.02 and 0.01, respectively) and higher serum LDH level (p = 0.04 and 0.04, respectively).

Table 1 - Association between the parameters reticulocyte count and LDH and rs2070744 genotypes (TT and CT) at *eNOS* gene

Parameters	TT	CT	p-value*
Reticulocyte count (%)	9.56 ± 3.43 (13)	6.12 ± 2.50 (10)	0.02[1]
LDH (U/L)	490.00; 410-793 (7)	371.50; 328-451 (4)	0.04[2]

[1]T test - Mean ± standard deviation (n – sample size)
[2]Mann-Whitney test - Median; minimum - maximum (n – sample size)

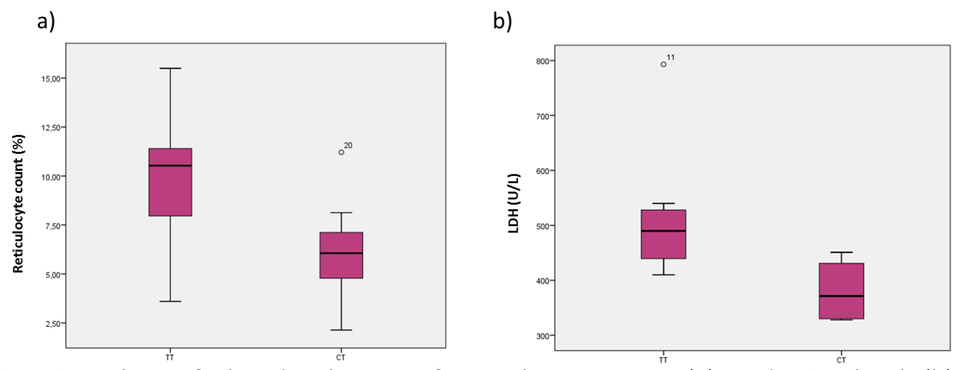

Figure 2 - Box plots of the distribution of reticulocyte count (a) and LDH level (b) in rs2070744 genotypes (TT and CT) at *eNOS* gene.

Table 2 - Association between the parameters reticulocyte count and LDH and rs1799983 genotypes (GG and GT/TT) at *eNOS* gene

Parameters	GG	GT/TT	p-value*
Reticulocyte count (%)	9.20 ± 3.21 (17)	4.53 ± 1.75 (5)	0.01[1]
LDH (U/L)	490.00; 410-793 (7)	371.50; 328-451 (4)	0.04[2]

[1]T test - Mean ± standard deviation (n – sample size)
[2]Mann-Whitney test - Median; minimum - maximum (n – sample size)

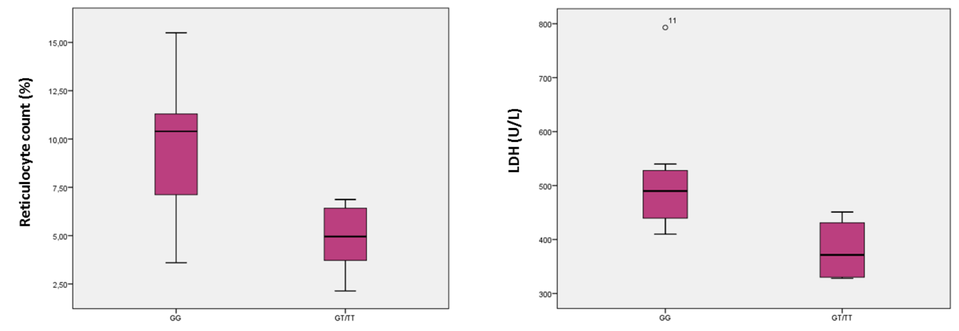

Figure 3 - Box plots of the distribution of reticulocyte count (a) and LDH level (b) in rs1799983 genotypes (GG and GT/TT) at *eNOS* gene.

CONCLUSIONS

Our findings suggest that polymorphisms in the *eNOS* gene may act as genetic modifiers of the haemolysis, which could provide utility for the prediction of increased susceptibility to haemolysis-related complications.
Furthermore, our results reinforce the importance of nitric oxide (NO) bioactivity in SCA. We presume that NO, and possible its precursors such as L-arginine or L-citrulline, might be used as pharmacological tools to improve the quality of life of these patients.

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