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THE EFFECT OF GENETIC POLYMORPHISMS IN *TRANS*-ACTING FACTOR GENES AS MODULATORS OF FETAL HEMOGLOBIN LEVEL IN SICKLE CELL DISEASE

Andreia Coelho[1], Armandina Miranda[2], Emanuel Ferreira[1], Paula Faustino[1]

[1]Departamento de Genética, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), Lisboa.
[2]Departamento de Promoção da Saúde e Doenças Crónicas, INSA, Lisboa.

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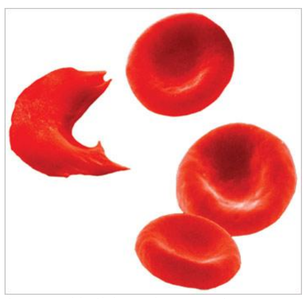
INTRODUCTION

Sickle Cell Anemia (SCA), one of the most common autosomal recessive hereditary anemia, is caused by a mutation in the β-globin gene (HBB:c.20A>T) on 11p15.5. This originates a hemoglobin variant named HbS, as opposed to the normal adult HbA. HbS ability to polymerize when deoxygenated gives rise to abnormal sickled red blood cells (Fig.1).

The two major SCA manifestations are chronic hemolysis and recurrent vaso-occlusive episodes[1]. However, the clinical phenotype of SCA is heterogeneous, ranging from relatively mild to severe due to the modifying effect of both environmental and genetic factors[2, 3].



(In www.abbotlab.wordpress.com)

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

The level of fetal hemoglobin (HbF) is a known modifier of the disease severity[reviewed in 4] and its variability among SCA patients has been associated with polymorphisms in both globinic *cis*-acting elements (e.g. the *Xmn*I SNP, upstream *HBG*2)[5, 6] and in non-globinic *trans*-acting factors (e.g. *BCL11A* and *HBS1L-MYB* intergenic region)[5].

In this study, we aimed to contribute to a better understanding of the non-globinic genetic factors modulating the expression level of HbF in SCA.

METHODS

We genotyped (by PCR-RFLP) 110 SCA patients for the SNP rs11886868 located in intron 2 of the *BCL11A* gene (2p16.1). Also, 79 SCA patients were screened for two other SNPs located in the *HBS1L-MYB* intergenic region on chromosome 6 (rs4895441 and rs6929404).

A normality test (K-S test) was applied to our sample and a normal statistical distribution of our dependent variable (%HbF) was not excluded. Therefore, we performed an ANOVA to access whether there were differences in %HbF distribution among the possible genotypes of all SNPs. Multiple comparisons were performed with the Bonferroni test.

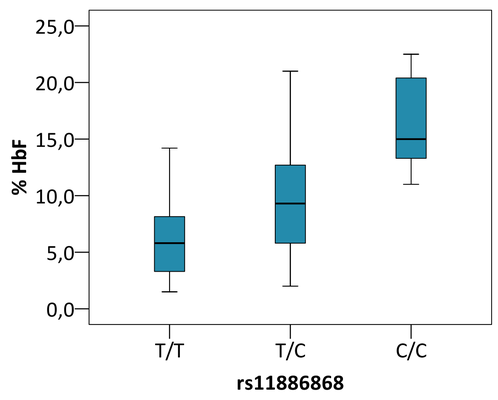
Patients were also divided in two groups, one with HbF<8% and other with HbF≥8%, and genotypic and allelic frequencies were compared between both using the Chi-square test of independence.

All statistical analysis were performed with SPSS v17 statistical software.

RESULTS

BCL11A – SNP rs11886868

1. We found statistical significant differences between the three genotypes of SNP rs11886868 regarding %HbF distribution. This result reveals **a strong association of this SNP with HbF levels.**



rs11886868	N	Mean HbF (%)	Median HbF (%)	p value*
T/T	51	6,1	5,8	
T/C	50	9,4	9,3	**<0.001**[1]
C/C	9	16,5	15,0	

* Statistical significance considered at p<0.05.
[1] ANOVA analysis.

2. The reciprocal analysis further confirms this result (genotypic and allelic frequencies are differentially distributed between the low and high HbF groups).

BCL11A (rs11886868) [110 SCA patients]							
Low HbF (<8,0%) [55 patients]				High HbF (≥8,0 %) [55 patients]			
Mean	Median	Max.	Min.	Mean	Median	Max.	Min.
4,6	4,7	7,7	1,5	12,3	11,0	22,5	8,0

rs11886868	Low HbF (<8%)	High HbF (≥8%)	p value*
Genotypic frequencies			
TT	35	16	**<0.001**[1]
TC	20	30	
CC	0	9	
Allelic frequencies			
T	90	62	**<0.001**[1]
C	20	48	

* Statistical significance considered at p<0.05.
[1] Chi-Square test of independance

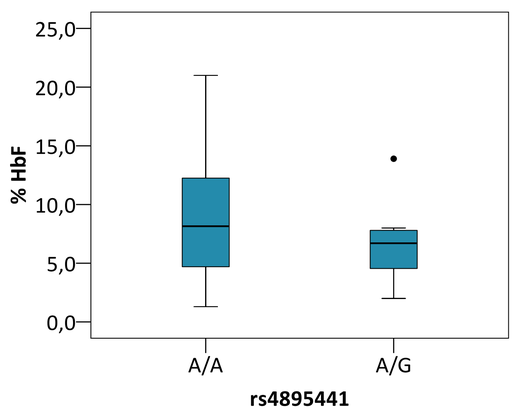
3. After multiple comparison analysis we were able to determine that **the genotypes with at least one C allele were strongly associated with higher HbF percentages.**

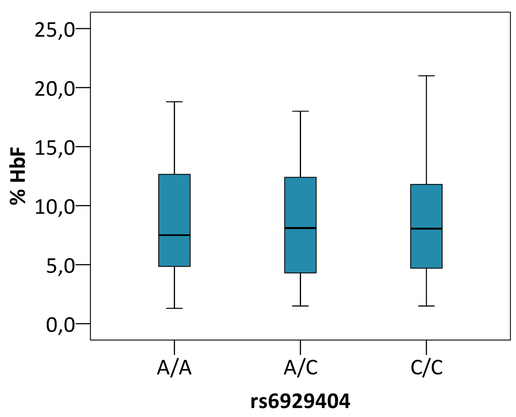
Genotypes		Mean difference (HbF %)	p value*
T/T	C/T	-3,3	**<0.001**[1]
T/T	C/C	-10,4	**<0.001**[1]
C/T	C/C	-7,1	**<0.001**[1]

* Statistical significance considered at p<0.05.
[1] Bonferroni Test

HBS1L-MYB – SNPs rs4895441 and rs6929404

1. No association was found between genotypes and HbF level distribuition.





rs4895441	N	Mean HbF (%)	Median HbF (%)	p value*
A/A	72	8,8	8,2	
A/G	7	6,8	6,7	0.306[1]
G/G	0	-	-	
rs6929404	**N**	**Mean HbF (%)**	**Median HbF (%)**	**p value***
A/A	11	8,9	7,5	
A/C	38	8,7	8,1	0.964[3]
C/C	30	8,5	8,0	

* Statistical significance considered at p<0.05.
[1] T Test; [2] ANOVA analysis

CONCLUSIONS

• We observed, in this group of SCA patients, a strong association of SNP rs11886868 in *BCL11A* gene with higher levels of HbF ($\alpha_2\gamma_2$).
BCL11A factor functions as a repressor of γ-globin genes expression, in cooperation with other co-factors[7] (Fig.2). The SNP rs11886868 probably impairs normal *BCL11A* gene expression, decreasing its ability to silence γ-globin genes, which leads to increased HbF levels in the adult life.

• MYB factor is also thought to be a potent negative regulator of HbF expression (Fig. 2). However, we didn't observe association of SNPs rs4895441 and rs6929404 located within the *HBS1L-MYB* intergenic region with the level of HbF.

• The results gathered in this study confirm that genetic polymorphisms in some *trans*-acting factor genes can modulate the HbF level in SCA, with consequences in its pathophysiology. This knowledge may provide new insights for the development of new therapeutic strategies for this pathology.

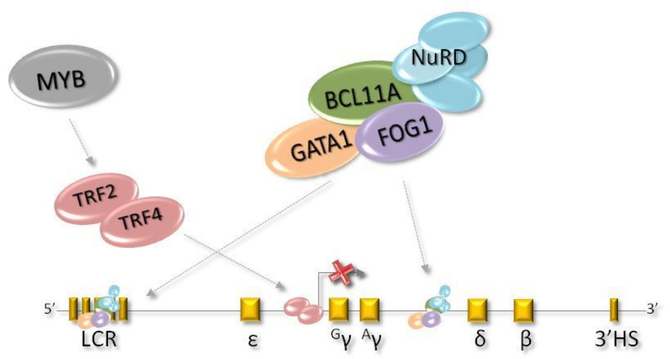


Fig. 2. Schematic representation of BCL11A/MYB mediated silencing of γ-globin genes. Adapted from Xu J el al. (2010) [7].

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