Macro- and Microvascular Endothelial Dysfunction Modulation by *VCAM1* Haplotypes of Pediatric Sickle Cell Anemia Patients

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INTRODUCTION

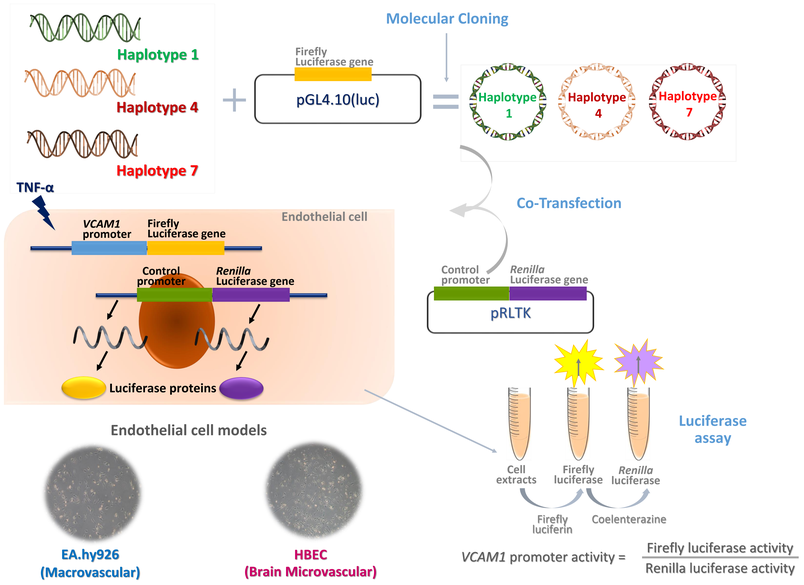
- Sickle cell anemia (SCA) is caused by homozygosity for the c.20A>T mutation in *HBB* gene, resulting in abnormal hemoglobin S (HbS);
- It is a multifactorial-like monogenic disease. Pediatric subphenotypes include ***cerebral vasculopathy (CV)***, pain crisis, frequent infections and renal disease[1,2];
- Genetic modulation has been described to affect disease progression, namely by involvement on pathophysiological mechanisms[3,4], such as endothelial dysfunction[3];
- Variants of the vascular cell adhesion molecule gene, *VCAM1*, have already been reported in association with stroke risk[3].

OBJECTIVES

- To assess the role of *VCAM1* variants, together with imaging, serological and hematological parameters as potential biomarkers of CV in SCA children;
- To evaluate the functional effects of the *VCAM1* promoter haplotypes on endothelial cell response, following endothelial activation by TNF-α stimulation.

METHODS

- Genotyping of 70 SCA children with CV or stroke risk (measured by transcranial Doppler ultrasound)
- *VCAM1* promoter haplotype reconstruction using PHASE2 software;
- Statistical analyses were performed using SPSS (v.25.0);
- The haplotypes identified were then cloned and transfected to endothelial cell lines for luciferase reporter assays, as depicted below.



RESULTS

- Six *VCAM1* promoter variants and 7 haplotypes showed potential modulating effect;
- rs1409419_T allele and haplotype 7 (Hap7) were positively associated with stroke, stroke risk, and high levels of LDH, while haplotype 1 (Hap1) was negatively associated with stroke (Fig. 1)

Variant	*VCAM1* promoter haplotypes						
	1	2	3	4	5	6	7
rs1409419 (T>C)	C	C	C	C	C	C	T
rs3917024 (C>T)	C	C	C	C	C	T	C
rs3917025 (CT>delCT)	CT	CT	CT	CT	--	--	CT
rs3783598 (T>G)	T	T	T	T	T	G	T
rs1041163 (T>C)	T	T	C	C	T	T	T
rs3783599 (C>T)	C	T	C	T	C	C	C

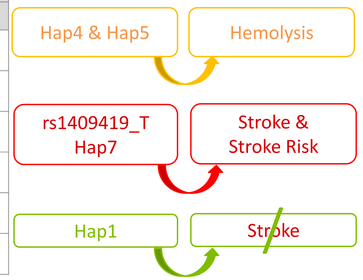


***Fig 1**. VCAM1 haplotypes and their association with SCA subphenotypes; Hap: haplotype*

- Stimulation with TNF-α leads to a ≈2-fold increase in promoter activity in cells transfected with haplotypes 4 or 7, when compared with non-stimulated cells;
- The presence of haplotype 7 results the highest promoter activity, especially in brain microvascular cells (Fig. 2, right);
- The inductive effect of haplotype 4 is more significant in macrovascular cells, despite ≈2-fold increase in stimulated cells.

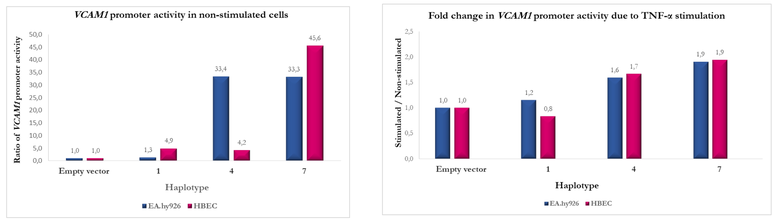


***Figure 2**. VCAM1 Promoter activity ratios, as measured by luciferase assay. normalised to empty vector construct; Left: Ratios in non-stimulated cells; Right: fold change of promoter activity in cells after TNF-α (8h, 20 ng/mL) stimulus when compared with non-stimulated cells.*

- In a pro-inflammatory milieu, haplotype 1 leads to a less active *VCAM1* promoter in brain microvascular cells, suggestive of a protective effect;
- Basal *VCAM1* promoter activity is affected by all *VCAM1* haplotypes tested, both in micro and macrovascular endothelial cells (Fig.2, left);

CONCLUSIONS

- Functional studies show increased *VCAM1* expression on cytokine-induced endothelial cells;
- Promoter haplotypes, previously associated with CVA, show different effects which further enhances the modifier effect of *VCAM1*;
- The results on macrovascular and microvascular endothelial cell models enhance the possibility of this effect extending beyond cerebral to systemic vasculopathy;
- Furthermore, we suggest that those haplotypes, together with the imaging, biochemical and hematological parameters, may be used to design a sensitive and specific biomarker panel for SCA vasculopathy risk, severity and prognosis.

ACKNOWLEDGEMENTS

This work was partially supported by INSA_202DGH720 project.

REFERENCES

1. Kato *et al*. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes . *Blood rev 2007; 21: 37–47*
2. Ohene-Frempong *et al*. Cerebrovascular accidents in sickle cell disease: Rates and risk factors . *Blood 1998; 91: 288–294*
3. Taylor JG *et al*. Variants in the VCAM1 gene and risk for symptomatic stroke in sickle cell disease. *Blood*. 2002;100: 4303-4309
4. Flanagan *et al*. Genetic mapping and exome sequencing identify 2 mutations associated with stroke protection in pediatric patients with sickle cell anemia. *Blood 2013, 121: 3237–3245*

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