Paediatric Cerebral Vasculopathy in Sickle Cell Anaemia - contribution of genetic modifiers

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1. Introduction

Sickle cell anaemia (SCA) arises from homozygosity for the mutation c.20A>T in the *HBB* gene. However, it shows a multifactorial-like behavior with high heterogeneity of clinical features[1]. Cerebral vasculopathy (CVA) constitutes a severe complication of SCA, especially in children, and comprises a spectrum of clinical manifestations that includes overt stroke, transient ischemic attacks, silent cerebral infarcts (SCIs), and frequent cognitive decline [2,3,4]. The risk of stroke development as well as of other CVA events, such as SCIs, may be modulated by underlying genetic modifiers, namely those affecting vascular homeostasis.

2. Aims

- Investigate the role of variants in **genes related with endothelial adhesion (*VCAM1* and *ITGA4)* and nitric oxide metabolism (*NOS3)*** on CVA events in SCA children;
- Study the effects of genetic variants on the levels of biochemical/haematological biomarkers of chronic haemolysis;
- Evaluate the putative additional modulating role of variants previously identified as stroke risk factors by genome-wide associated studies: *GOLGB1* Y1212C, *ENPP1* K173Q and *PON1* Q192R.

3. Methodology

- **Subjects**: 70 SCA children characterized according to their CVA degree, by transcranial Doppler ultrasound [time-averaged mean of maximum velocity (TAMMV) in the middle cerebral artery], magnetic resonance imaging (MRI) or CT scan;
- **Molecular analyses**: PCR, PCR-RFLP, Sanger sequencing and next-generation sequencing;
- **Haplotype reconstruction**: Haploview v.2.0,
- **Statistical analysis**: SPSS v.25.0.

4. Results and Discussion

Specific **genetic variants** and **haplotypes** in ***VCAM1*** (Table 1), ***ITGA4*** and ***NOS3*** (Table 2) were associated with **higher risk** or **protection** for the occurrence of overt ischemic stroke events or silent cerebral infarcts in children with SCA (Table 3).

Variant	*VCAM1* 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**

Variant	*NOS3* haplotypes						
	1	2	3	4	5	6	7
rs2070744 (C>T)	C	C	C	T	**T**	T	**T**
Intron 4 VNTR*	4a	4b	4b	4a	**4b**	4b	**4c**
rs1799983 (T>G)	G	G	T	G	**G**	T	**G**

*VNTR alleles: 4a = 4 repeats of 27bp; 4b = 5 repeats of 27bp; 4c = 6 repeats of 27bp

Table 1. (left) *VCAM1* gene promoter haplotypes.

Table 2. (above) *NOS3* haplotypes.

- ***VCAM1* promoter haplotype 7** as well as **rs1409419_T** alone were associated with **higher velocity of cerebral blood flow,** ↑TAMMV (>170 cm/s); the same was observed for ***ENPP1* K173Q (rs1044498_A)**;
- ***ITGA4* variants rs113276800_A** and **rs3770138_T** were associated with **stroke events**;
- ***NOS3* haplotype 5** as well as **VNTR 27bp _ 4b allele** showed a **protective** effect for **silent cerebral infarcts**; **haplotype 7** also showed a **protective** effect for **cerebral vasculopathy** globally.

Gene	Variant	Predicted Association	*P*	OR	$CI_{95\%}$
VCAM1	rs1409419_T	↑ TAMMV	0.010	4.705	1.566-14.132
	haplotype 7	↑ TAMMV	0.010	4.705	1.566-14.132
ITGA4	rs113276800_A	Stroke	0.025	7.619	1.394-41.653
	rs3770138_T	Stroke	0.045	5.571	1.122-27.665
		Cerebral vasculopathy	0.017	7.615	1.358-42.707
NOS3	VNTR 27 bp _ 4b allele	Silent cerebral infarcts Protective	0.041	0.114	0.016-0.808
	haplotype 5	Silent cerebral infarcts Protective	0.009	0.13	0.029-0.582
	haplotype 7	Cerebral vasculopathy Protective	0.015	0.084	0.010-0.691
ENPP1	rs1044498_A	↑ TAMMV	0.040	4.025	1.207-13.418

Table 3. *VCAM1*, *ITGA4*, *NOS3* and *ENPP1* variants with significant association to cerebral vasculopathy.

Chronic haemolysis biomarker levels also appear to be modulated by *VCAM1* and *ITGA4* variants (Table 4).

- **LDH** levels were **higher** in the presence of ***VCAM1*** promoter **rs1409419_T** alone as well as of **haplotype 7** but **lower** in patients with ***ITGA4* haplotype 1;**
- Total **bilirubin** levels, on the other hand, were **higher** in association with ***VCAM1* rs3783613_C** allele.

Gene	Variant	Predicted Effect	*p*
VCAM1	rs1409419_T	↑ LDH	<0.001
	haplotype 7	↑ LDH	<0.001
	rs3783613_C	↑ Bilirubin	0.026

Gene	Variant	Predicted Effect	*p*
ITGA4	**haplotype 1:** rs1375493_A rs35723031_insT g. 181459459_delTT	↓ LDH	0.003

Table 4. *VCAM1* and *ITGA4* variants' predicted effect on chronic hemolysis biomarkers (LDH and total bilirubin).

5. Conclusion

- We have found significant association between **specific variants in genes related with endothelial adhesion (*VCAM1* and *ITGA4)* and nitric oxide metabolism (*NOS3)*** with several cerebral vasculopathy outcomes;
- Those genetic factors may be used for the **early detection** of the SCA children presenting the highest CVA risk, thus allowing an intensification of **their preventive therapeutic strategies;**
- Our findings also provide additional clues on the SCA pathophysiology and uncover features in these genes that may prove to be crucial as potential therapeutic targets.

6. References

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