STROKE IN CHILDREN WITH SICKLE CELL DISEASE: Advances in understanding its molecular pathogenesis

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INTRODUCTION and OBJECTIVES

Sickle cell anemia (SCA) is an autosomal recessive disease caused by the mutation HBB:c.20A>T in beta-globin gene. This missense mutation gives rise to a hemoglobin variant (**Hb S**) that, upon deoxygenation, polymerises inside the red blood cells (RBCs) leading to abnormal sickle-shaped cells. Sickle RBCs are less deformable and stickier than normal, causing vessel obstruction and local ischemia. Also, the polymerization of HbS is accompanied by RBC membrane damage and dehydration, accelerating hemolysis. Thus, the clinical manifestations of the disease derive essentially from two phenomena - hemolysis and vaso-occlusion. However, their variability and severity are modulated by environmental and genetics factors (1).

One of the most devastating complication affecting children with SCA is **cerebral vasculopathy** (**overt stroke and silent infarcts**). Overt stroke occurs in about 11% of those children before the age of 20 (2, 3). However, its pathophysiology is complex and the underlying mechanisms remain largely unknown (**Fig. 1**). Therefore, in this study, the main objective was to identify genetic modulators of stroke in the context of SCA in paediatric patients.

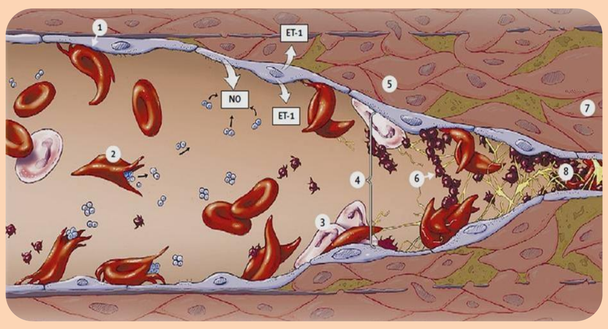

Fig. 1 - Pathophysiology of stroke in SCA.
The abnormal adherence (1) and high rate hemolysis (2) of the sickle RBCs are the basis for the development of cerebrovascular disease in patients with SCA. The activated endothelium expresses a great amount of endothelium-specific molecules, promoting leukocyte adhesion (3), platelet aggregation (6), and increased release of the vasoconstrictor endothelin (ET 1). The scavenging of NO by cell-free hemoglobin further increases vasomotor tone (4). Tissue remodeling due to smooth-muscle cells and fibroblasts proliferation in the intimal layer (5) leads to luminal narrowing, followed by vasculopathy (7) and occlusion (8).
Adapted from Switzer et al., 2006 (4).

MATERIALS and METHODS

Sixty six children with SCA, descendent of African families, were categorised according to their degree of cerebral vasculopathy:

- **Stroke group**, included 13 children with history of at least one stroke episode between ages 5 and 13.
- **Risk group**, included 29 children with high transcranial Doppler (TCD) velocities, either "conditional" (170 – 199 cm/s) or "high risk" (>200 cm/s), and children with silent infarcts or cerebral vasculopathy on magnetic resonance imaging (MRI) .
- **Control group**, included 24 children without previous history of stroke, normal TCD velocities and no abnormalities on MRI.

Relevant data were collected from patients' medical records.

Twenty three polymorphic regions were characterised in genes related to vascular cell adhesion (*VCAM 1*, *THBS 1, CD36*), vascular tonus (*NOS3, ET1*), and inflammation (*TNF α, HMOX 1*) as well as in known globin expression modulators (*HBB* cluster haplotype; *HBA* and *BCL11A* genotypes).

Data analysis was performed using R software.

Table I. Association between candidate gene variants and stroke risk in SCA

Gene	Genetic variant	Associated Allele	Mode of transmission	Association with phenotypic groups					
				Contingency Table			Association		Associated group
				Group	Presence n	Absence n	Fisher's exact test *p*	OR (95% CI)	
***VCAM 1* promoter**	**rs1409419** g.100717840 **T>C**	**T**	**Allele count (T)**	Stroke	14	12	**0.008**	4.33	**Stroke**
				Control	10	38	0.091*	(1.391 - 14.257)	
			Dominant (TT+CT)	Stroke	11	2	**0.014**	8.60	
				Control	9	15	0.091*	(1.407 – 97.351)	
NOS 3	**rs2070744** g.150992991 **C>T**	**C**	**Overdominant (TC)**	Stroke	6	7	**0.013**	8.75	**Stroke**
				Control	2	22	0.067*	(1.221 – 107.964)	
			Allele count (C)	Stroke	6	20	**0.019**	6.70	
				Control	2	46	0.073*	(1.081 – 73.323)	
	VNTR 27 bp 4a/4b/4c	**4a**	**Dominant (4a4a + 4x4a)**	Risk	23	6	**0.020**	4.89	**Risk**
				Control	11	13	0.1218*	(1.178 –18.321)	
			Allele count (4a)	Risk	26	32	**0.024**	2.71	
				Control	11	37	0.1218*	(1.088 – 7.088)	
		4b	**Dominant (4b4b)**	Risk	3	26	**0.005**	0.24	**Control**
				Control	8	16	0.3122*	(0.053 – 0.999)	
			Allele count (4b)	Risk	25	33	**0.033**	0.42	
				Control	31	17	0.3122*	(0.175 – 0.979)	
HMOX 1	**rs3074372** (STR – GT) **S/M/L**	**L**	**Dominant (L/L + other/L)**	Stroke	10	3	**0.019**	6.04	**Stroke**
				Risk	10	19	0.148*	(1.196 – 42.056)	
			Allele count (L)	Stroke	14	12	**0.012**	3.60	
				Risk	14	44	0.148*	(1.233 – 10.902)	

Table II. Association between biochemical parameters and stroke risk in SCA

Hematological parameter	Groups	Wilcox-Mann-Whitney test for homogeneity *p*	Contingency Table			Association		Associated group
			Group	Low HbF n	High HbF n	Fisher's exact test *p*	OR (95% CI)	
Fetal hemoglobin (%)	Stroke Control	**0.008** 0.013*	Stroke Control	7 9	1 15	**0.037** 0.149*	10.82 (1.10 – 558.00)	**Stroke** (Lower HbF)
	Stroke Risk	**0.002** 0.007*						
LDH (U/L)	Risk Control	**0.0123** 0.0493*						**Risk** (Higher LDH)
	Risk Stroke	**0.0262**						

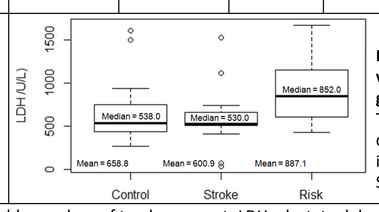

Fig. 3 - Distribution of LDH values in the three phenotype groups.
The boxplots revealed a clear difference between LDH values in Risk group when compared to Stroke or Control groups.

* False-discovery-rate corrected *p*-values; OR – Odds Ratio; VNTR – variable number of tandem repeat; LDH – lactate dehydrogenase; 4x – alleles 4b or 4c; S – Small nº of repeats (n≤ 26), M – medium nº of repeats (27≤ n ≤ 34), L – long nº of repeats (n≥ 35)

RESULTS and DISCUSSION

The genetic variants showed in Table I might modulate cerebral vasculopathy development (stroke/risk) due to their modifier effect on gene expression and on their corresponding protein product biological activities. Several mechanisms are involved:

- ***Cell vascular adhesion*** – genetic variants related with an increased synthesis of vascular cell adhesion molecules (i.e., rs1409419_allele T of ***VCAM 1*** gene promoter; Table I) promote RBCs and leukocyte adhesion to endothelium, as well platelet aggregation, and are associated with cerebral vasculopathy development and stroke events.
- ***Vascular tonus*** – The endothelial nitric oxide synthase has been implicated in vasculopathy and stroke. In fact, production of Nitric Oxide (NO) is of major importance to maintain a correct vascular tonus, which in SCA is skewed towards vasoconstriction, as a result of a net resistance to NO. Therefore, genetic variants in ***NOS 3*** (Table I) that further decrease basal NO levels can be very deleterious to SCA patients (Fig. 2).

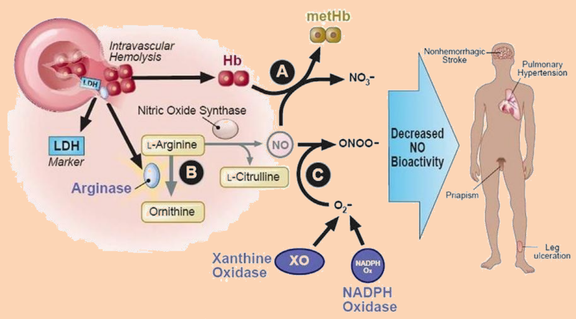

Fig. 2 - Decreased NO bioavailability in SCA.
Intravascular hemolysis reduces nitric oxide bioactivity by releasing hemoglobin and arginase, which inactivate NO and consume plasma L-arginine (NO precursor), respectively. Additionally , NO is consumed by reactions with reactive oxygen species highly produced in SCA. The resulting decrease in NO is associated with leg ulceration, priapism, pulmonary hypertension and possibly non-hemorrhagic stroke.
Adapted from Kato, et al., 2007 (5).

In addition, a decreased rate of transcription of ***HMOX 1*** due to the rs3074372_allele L (Table I) leads to lower circulating heme oxygenase 1 and consequently free heme is not adequately removed and further scavenges NO molecules.

- ***Hemolysis*** – Lactate dehydrogenase (LDH) is released from RBCs during the hemolytic process and constitutes a marker for the magnitude of hemoglobin and arginase release (marker of hemolysis). We have found that higher LDH levels are associated with Risk group (Table II; Fig. 3), which means that this proximal hemolytic marker is closely related with the initial stage of cerebral vasculopathy.

In **conclusion**, our findings reinforce the relevance of **vascular tonus**, **vascular cell adhesion**, **hemolysis rate** and ultimately **NO bioavailability** in modulating SCA stroke development and provide the first evidence of a protective role of **fetal hemoglobin** against stroke occurrence.

References:

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