Sickle cell anemia: chronic hemolysis and cerebral vasculopathy

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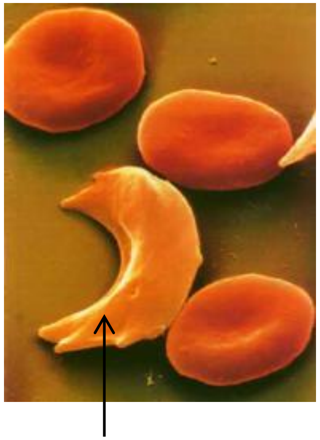
Sickle cell anemia – molecular basis

Beta-globin gene (*HBB*)

- **Gene variant**: *HBB* 6th codon, GAG>GTG; HBB:c.20A>T
- **Protein variant**: p.Glu6Val

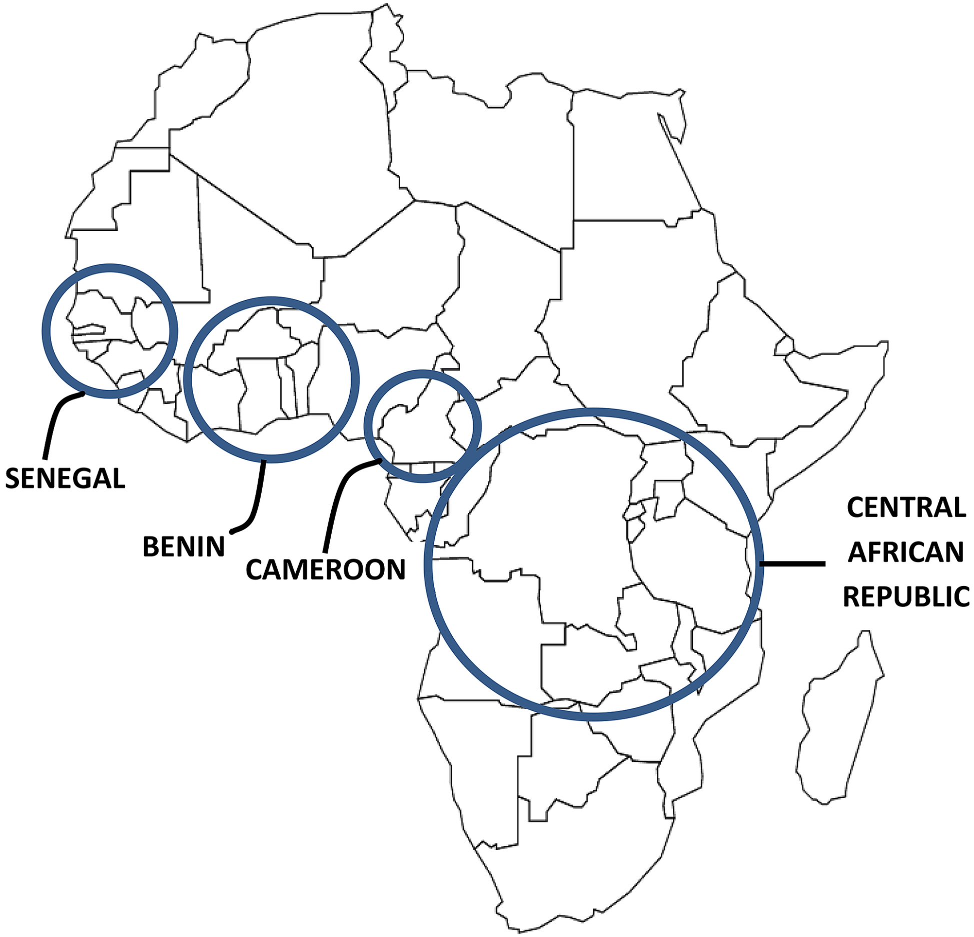
The mutation originates a hemoglobin variant named **Hemoglobin S (Hb S)**, as opposed to the normal adult Hb A.

Hb S ability to polymerize when deoxygenated gives rise to abnormal sickled red blood cells.



Sickled Red Blood Cell

Sickle Cell Anemia - prevalence



SCA is the most common autosomal recessive hereditary anemia in Africa

Multiple origin of SCA mutation (*HBB*:c.20A>T) in Africa

Positive selection – carrier advantage, protection against malaria

Sickle cell anemia in Portugal

- **Prevalence of sickle cell anemia carriers:**

≈ 0% - North of Portugal

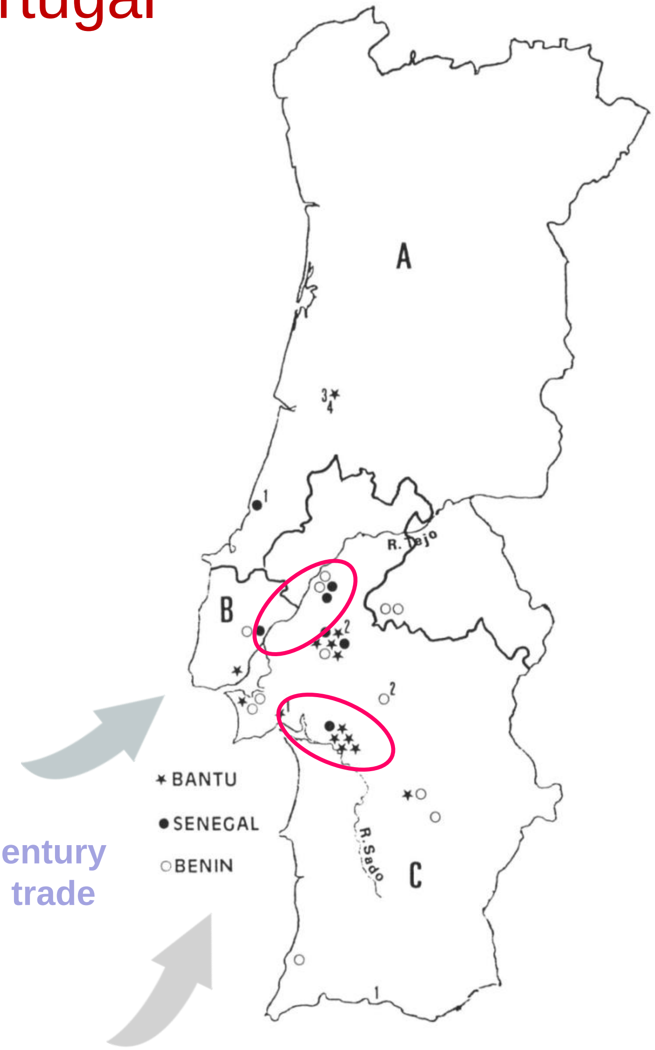
≈ 1.1% - South of Portugal

≈ 5-6% - High prevalence pockets ⬭

Positive selection – carrier advantage,
protection against malaria

- **Absence of Portuguese SCD patient registries:**

≈ 600 sickle cell disease patients.



**15th century
Slave trade**

**Current African
migraton**

Lavinha *et al*, Hum Biol 1992;
Martins *et al*, J Med Genet 1993;
Inez *et al*, Arq INSA 1993.

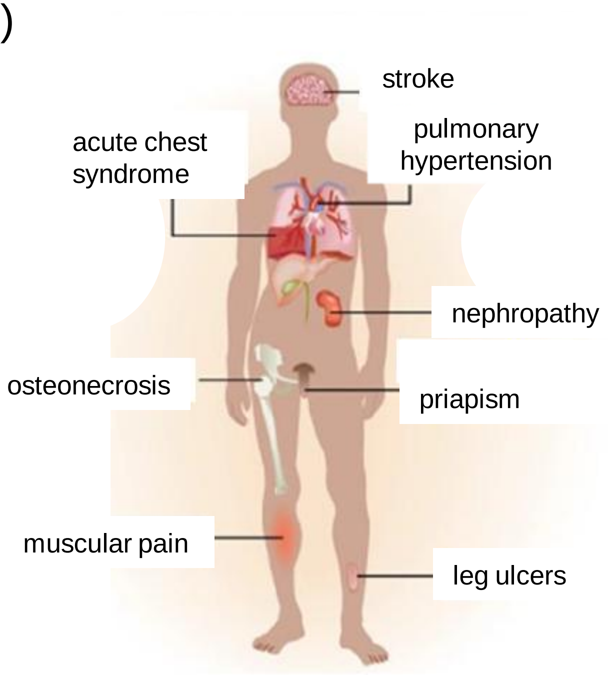
SCA – clinical manifestations

SCD is characterized by **recurrent episodes** of severe **vaso-occlusion**, **hemolysis** and **infection**.

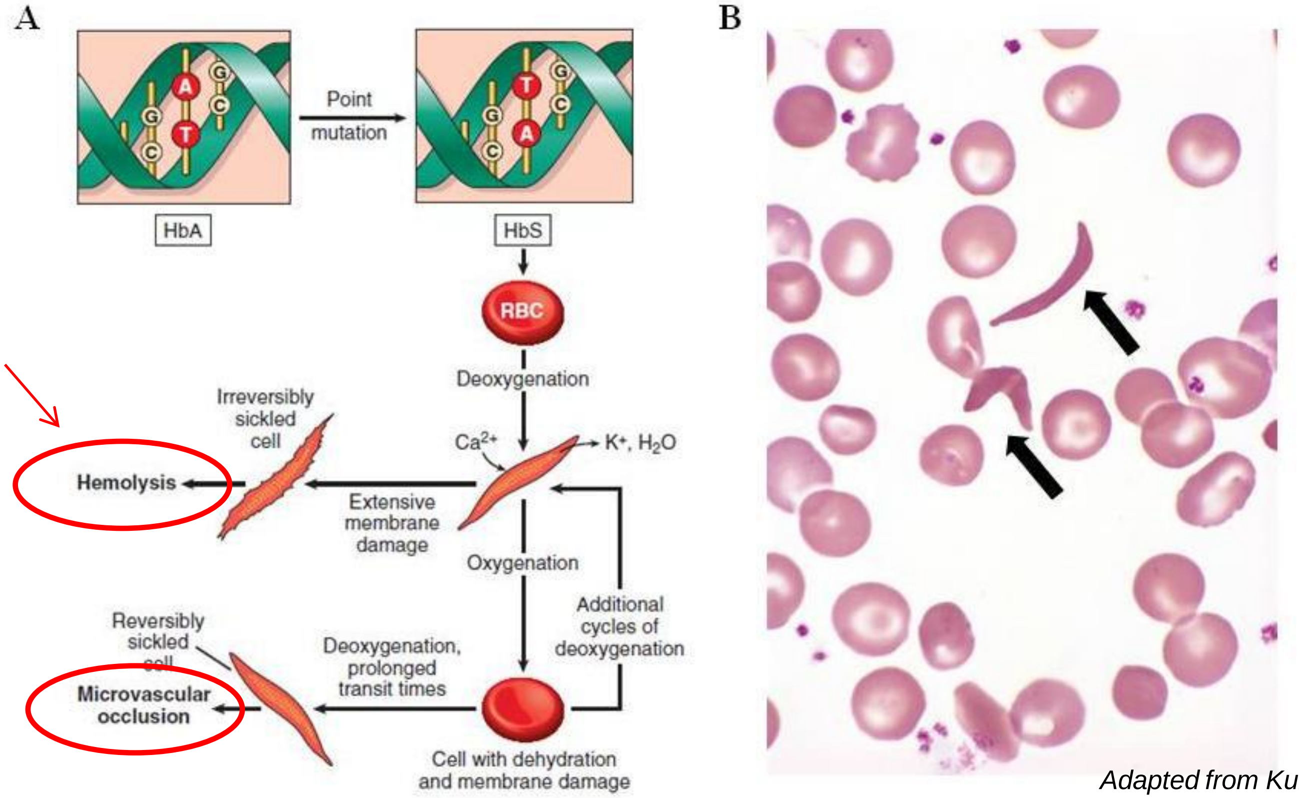
Sickle cells are destroyed rapidly (chronic hemolysis) causing anemia, jaundice and the formation of gallstones.

The sickle cells also block the flow of blood through vessels resulting in lung tissue damage (acute chest syndrome), pain episodes (arms, legs, chest and abdomen), stroke and priapism.

It also causes damage to most organs including the **spleen, kidneys** and **liver**. Damage to the spleen makes SCD patients easily overwhelmed by infections.



SCA – heterogeneity of clinical manifestations

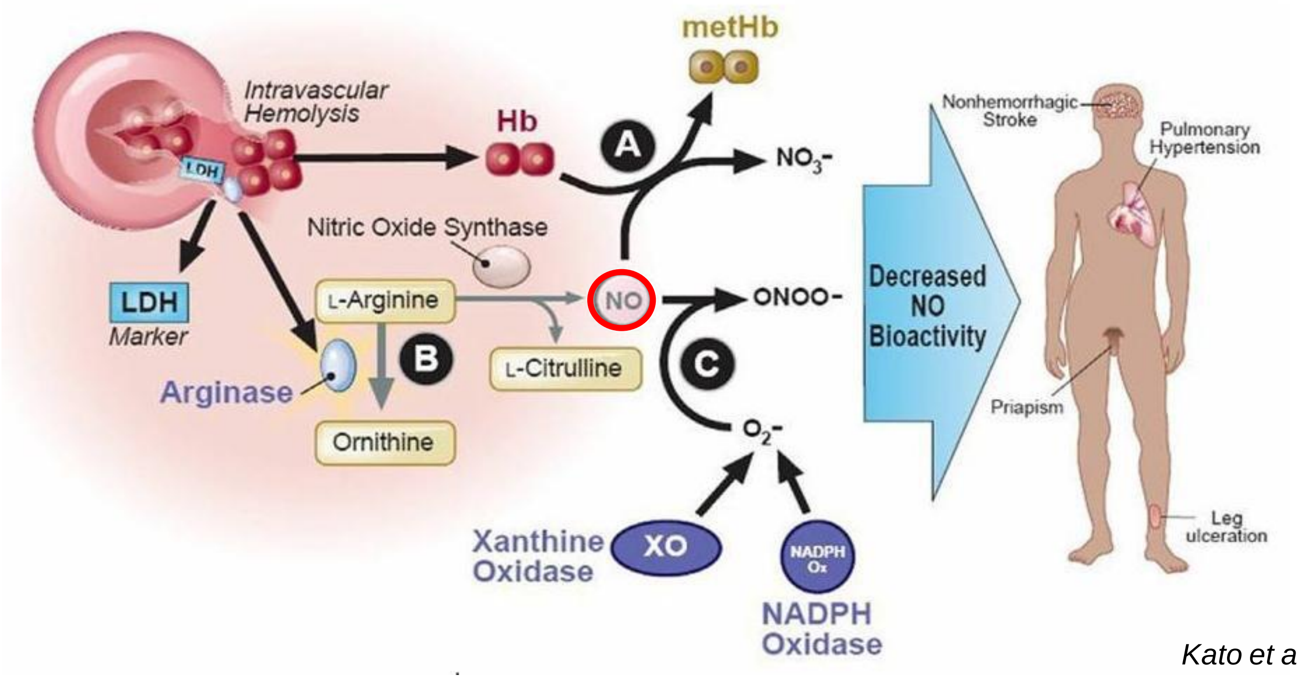


Adapted from Kumar et al., 2013.

SCA is a monogenic disorder with a multifatorial-like behaviour and a clinical phenotype heterogeneity inter- and intra-patients.

Several **genetic and environmental modifiers** have been suggested to modulate the onset and course of the disease.

SCA – consequences of intravascular hemolysis



Kato et al., Blood Rev,2007

Intravascular hemolysis reduces Nitric Oxide (NO) bioactivity

Cell-free plasma hemoglobin inactivates NO, generating metahemoglobin and inert nitrate **(A).**

Plasma **arginase** consumes plasma L-arginine to ornithine, depleting its availability for NO production **(B).**

NO is also consumed by reactions with **reactive oxygen species** (O_2) producing oxygen radicals like peroxynitrite (ONOO-) **(C).**

I - Study of the genetic modulators of intravascular hemolysis in SCA

Objective

- To study association between **intravascular hemolysis level** using commonly measured **hemolysis biomarkers** (biochemical and hematological parameters) and the **inheritance of genetic variants** of several candidate genes.

Genetic modulators of hemolysis in SCA

Patients clinical data at **steady-state** have been captured to a **database** in a **longitudinally observed series** of paediatric SCA patients.

SCA evaluated patients	
No. of patients (ethnicity)	**99** (61% Angolan; 97% of Sub-Saharan origin ancestry)
Male/Female ratio	**1.17**
Current age (year) median interquartile range total range	**9.9** 6.7-12.6 2.9 – 21.7
Entry age (year) median interquartile range total range	**2.3** 0.7-4.7 0.1 – 16.9
Total follow-up (person*year)	**557**
Follow-up/patient (median; year)	**5.0**

Genetic modulators of hemolysis in SCA

Hemolysis biomarkers:

Serum **LDH**, **total bilirubin** and **reticulocyte count**.

Candidate gene genotyping:

41 genetic variants within 13 candidate genes

- related with fetal hemoglobin level: *BCL11A, HBS1L-MYB; HBB* cluster (including *HBG)*
- **alpha-thalassaemia** (*HBA)*
- **red blood cell vascular adhesion** (*VCAM 1, THBS 1, CD36, EDN1, ITGA4)*
- **and vascular tonus:** (*NOS3, HMOX-1*)
- **inflammation** (*TNF α*)

Statistical analysis:

Association studies were performed using T test ANOVA parametric tests (LDH, total bilirrubin) or Mann-Whitney/Kuskal-Wallis non-parametric tests (reticulocyte count), all performed with SPSS v20.0 software.

A correction for multiple testing (false discovery rate) was done.

Genetic modulators of hemolysis in SCA

Results

Hematological and biochemical markers of hemolysis

vs

Genetic variants

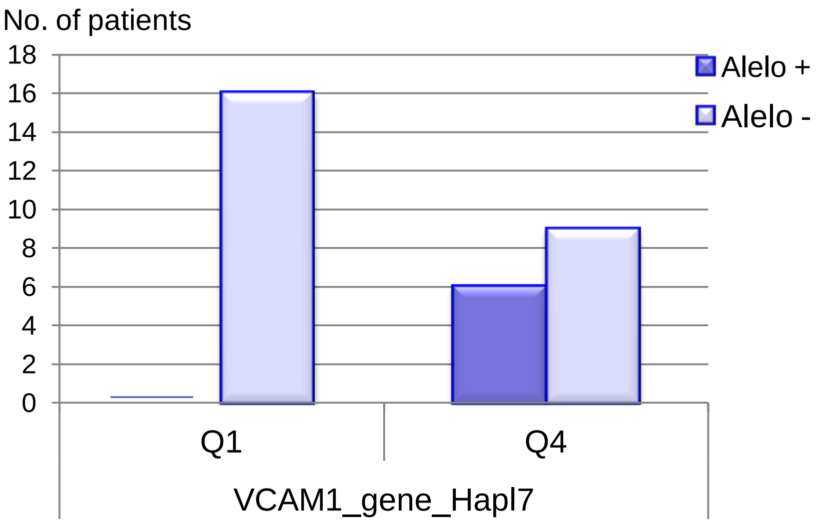
Genetic modulators of hemolysis in SCA

Gene	SNP reference	Allele[a]	Associated Allele or Haplotype		Presence of associated allele or haplotype	No. of patients	Haemolysis biomarkers[b]					
							LDH (U/L) mean±SD	*p*-value	Tot. Bilirubin (mg/dL) mean±SD	*p*-value	Reticulocyte count (%) mean±SD	*p*-value
VCAM1-gene	rs3783613	G/C	C	VCAM1_g_hapl 7	Yes	12	1270.6±279.3	*p*=0.002				
	rs3176878	C/T	C									
	rs3783615	A/T	A		No	49	926.6±348.4					
	rs3176879	A/G	A									
VCAM1-promoter	rs1409419	C/T	C	VCAM1_p_hapl 9								
	rs3917024	C/T	T									
	rs3917025	CT/delCT	delCT		Heter. (hapl 9/hapl X)[c]	4			1.65±0.05	*p*<0.001		
	rs3783597	C/G	G		Homoz. (hapl 9/hapl 9; hapl X/hapl X)	88			2.50±0.43			
	rs3783598	T/G	T									
	rs1041163	T/C	C									
	rs3783599	C/T	T									
CD36	rs1984112 5'UTR	A/G	G		Yes	45					13.06±0.80	*p*=0.001
					No	50					9.26±0.56	
NOS3	rs2070744 promoter	C/T	T		Yes	90			2.41±0.42	*p*<0.001		
					No	2			5.18±0.01			
HBA	del 3.7kb	Non-del/del	del		Yes	41			2.04±0.40	*p*=0.002		
					No	52			2.80±0.40			
					Yes	42					8.77±0.48	*p*=0.001
					No	53					13.21±0.73	

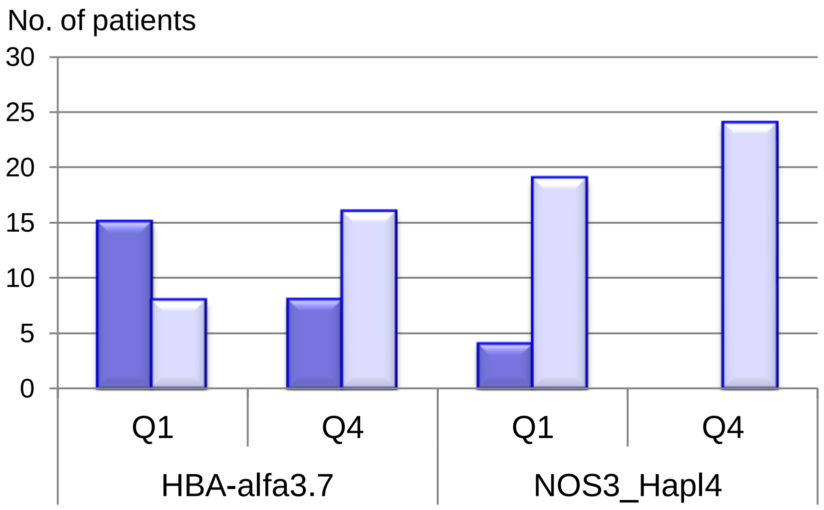
Coelho A *et al* . Eur J Haematol, 2014

Genetic modulators of hemolysis in SCA

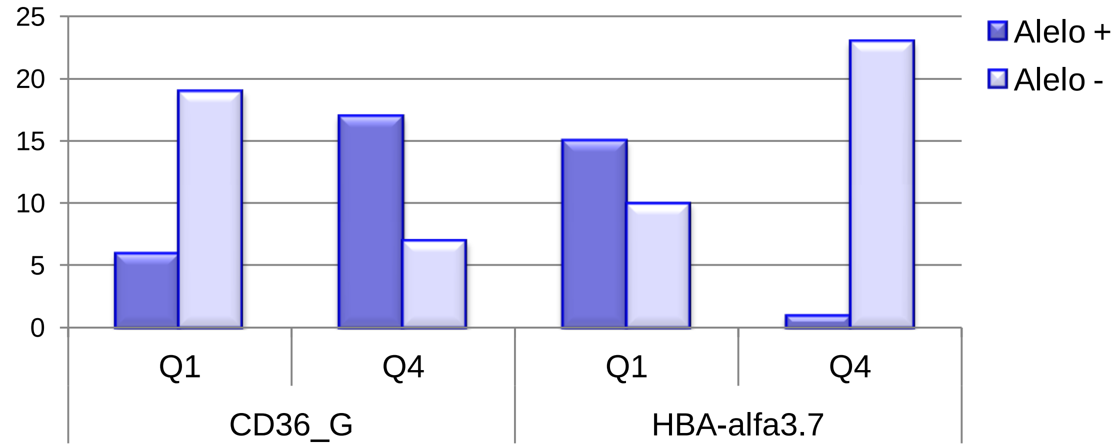
A. LDH



B. Total bilirubin



C. Reticulocyte count



Number of SCA patients with (+) or without (-) the genetic variant in the two extreme quartiles (Q1,Q4).

A diminished reticulocyte count was associated to the 3.7kb deletion at *HBA* , whereas an elevated count was associated to rs1984112_G allele at *CD36.*

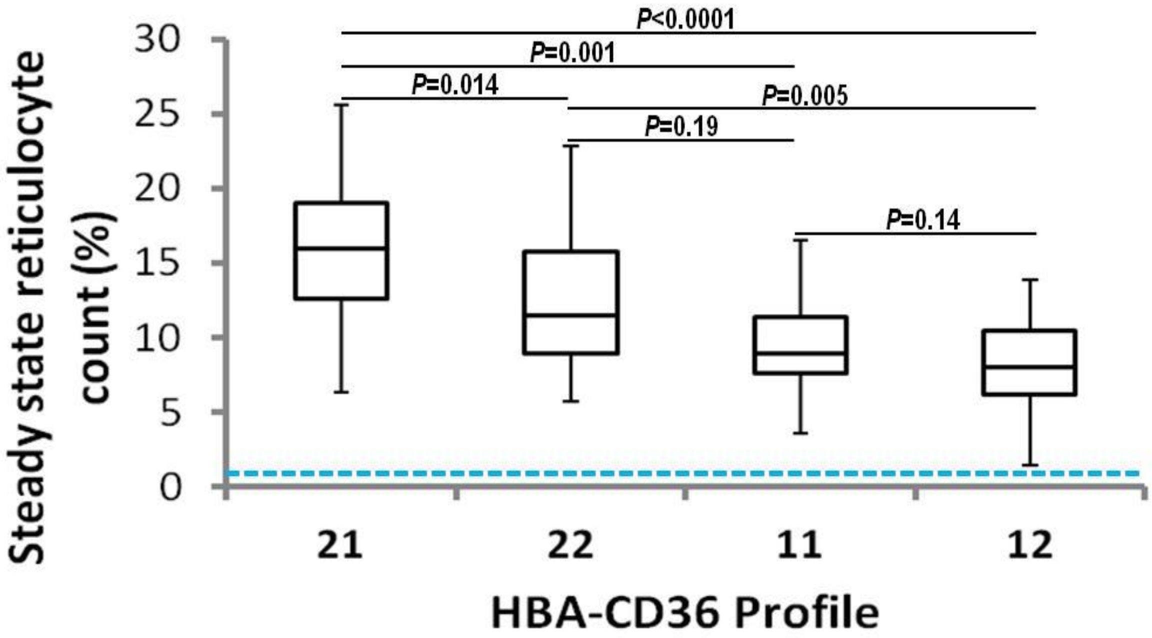
.

Toward a hemolytic genetic profile in sickle cell anemia

***HBA*_3.7del allele**	***CD36* (rs1984112)_G allele**	**Genetic profile identification**	**No. of SCA patients**
absence	presence	**2:1**	27
absence	absence	**2:2**	25
presence	presence	**1:1**	17
presence	absence	**1:2**	25

Categorisation of SCA patients into genetic profiles concerning theirs *HBA* and *CD36* variants, **assuming a dominant model of inheritance.**

Toward a hemolytic genetic profile in sickle cell anemia



Steady-state reticulocyte count as a function of the *HBA-CD36* profile

(median; interquartile range; minimum and maximum values).

P-values marked above each comparison (Mann-Whitney test)

Dashed horizontal line, reticulocyte count in the general population.

Our data are compatible with a synergetic interaction between *HBA*_3.7 del and *CD36* (rs1984112)_G for the assumed model of inheritance.

Hemolysis in SCA - genetic modifiers

1. Alpha-thalassaemia

The presence of the 3.7 kb deletion alpha-thalassaemia determinant at *HBA* gene were found associated with **low levels of haemolysis**, measured by low levels of total bilirubin and reticulocyte count.

SCA patients who co-inherited the deletion have reduced haemolysis owing to a lower intracellular concentration of HbS that in turn decreases HbS polymer-induced cellular damage.

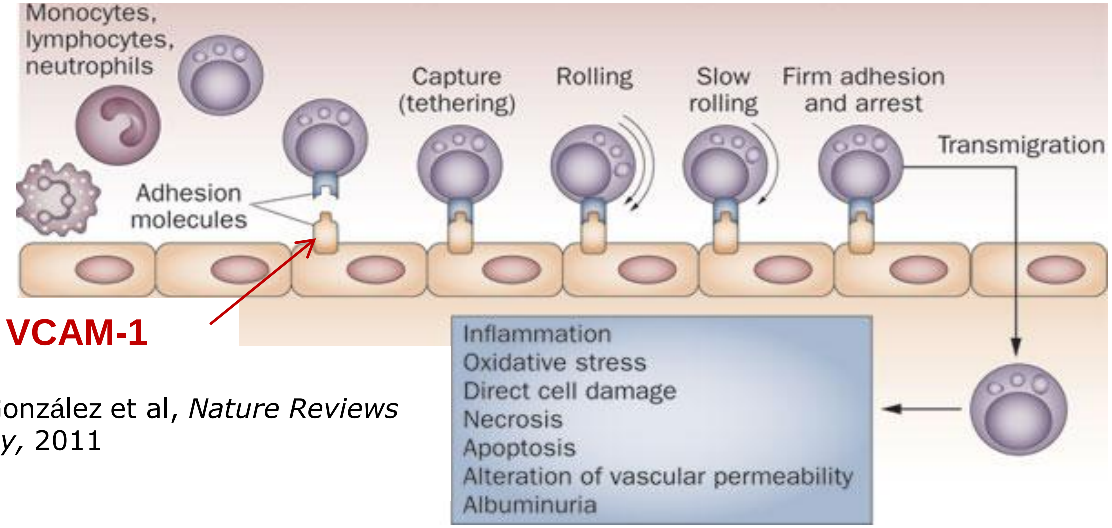
- Alpha-thalassaemia was one of the first idendified genetic modifier of SCD
- Due to the African ancestry of the SCA patients in this series, a **high frequency (0.22)** of the 3.7kb alpha-thalassaemia deletion was found.

Hemolysis in SCD - genetic modifiers

2. Cell vascular adhesion

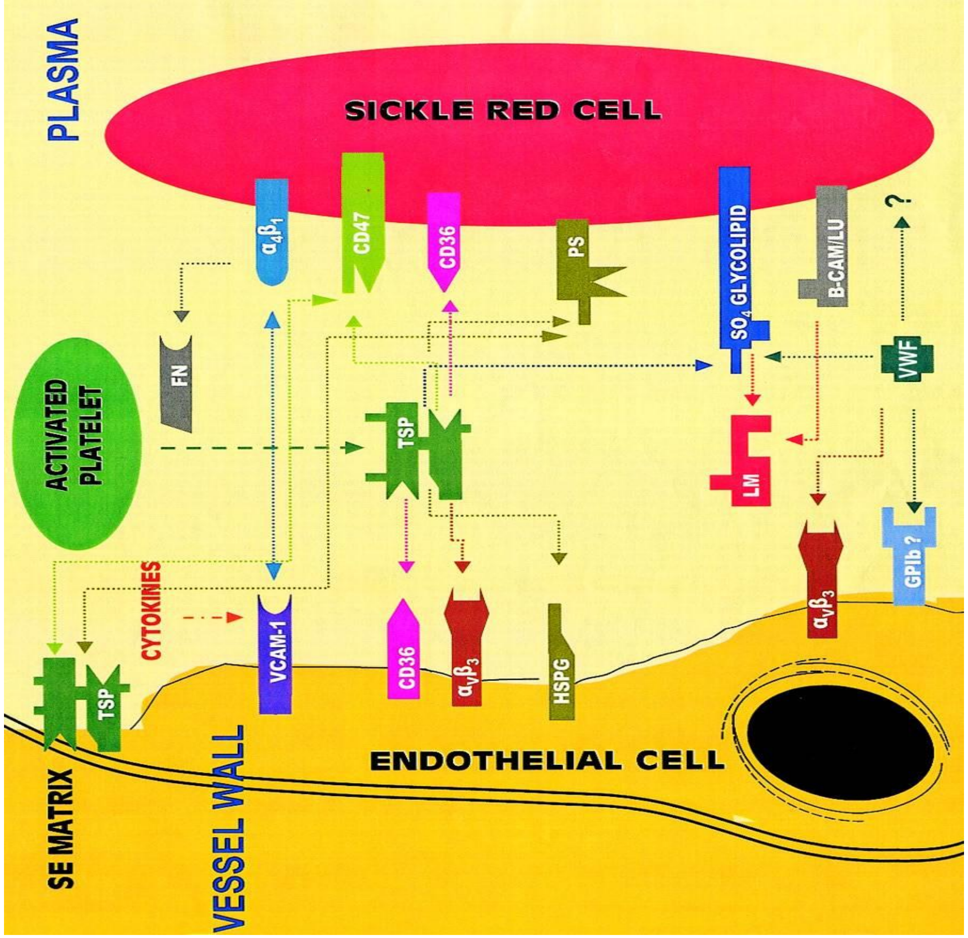
Vascular adhesion molecule-1 (VCAM-1) and Cluster of differentiation 36 (CD36)

Adhesion molecule: VCAM-1



Navarro-González et al, *Nature Reviews Nephrology*, 2011

Vascular adhesion molecule-1 (VCAM-1), is a glycoprotein present at surface of endothelial cells following cytokine stimulation that mediates the **adhesion of monocytes, lymphocytes and neutrophils** to the endothelium of both large and small blood vessels.



Setty *et al*, Blood, 2002

Stress reticulocytes and sickle erythrocytes have a propensity to adhere to VCAM-1 via the very late antigen-4 (VLA-4) or integrin (α4β1) expressed on their surface membrane.

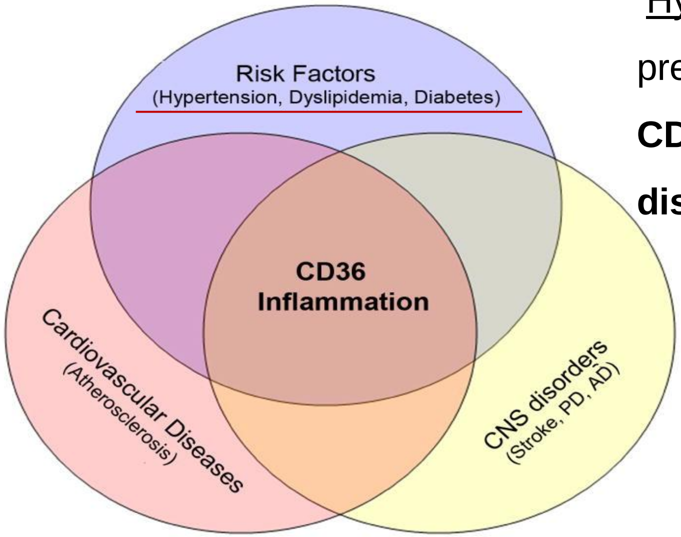
Hypoxia induces Integrin (α4β1)/VCAM-1 interaction to increase adhesion.

Adhesion molecule: CD36

Cluster of differentiation 36 (CD36) is expressed in many type of cells including microvascular endothelium cells, monocytos/macrophages, platelets, microglia, and erythroid precursors.

CD36 molecules are present at higher levels on **stress reticulocytes and sickle erythrocytes.**

CD36 is a common mediator for neurological and vascular diseases



Hypertension, Dyslipidemia and Diabetes present in common **elevated expression of CD36 and higher incidence of vascular diseases.**

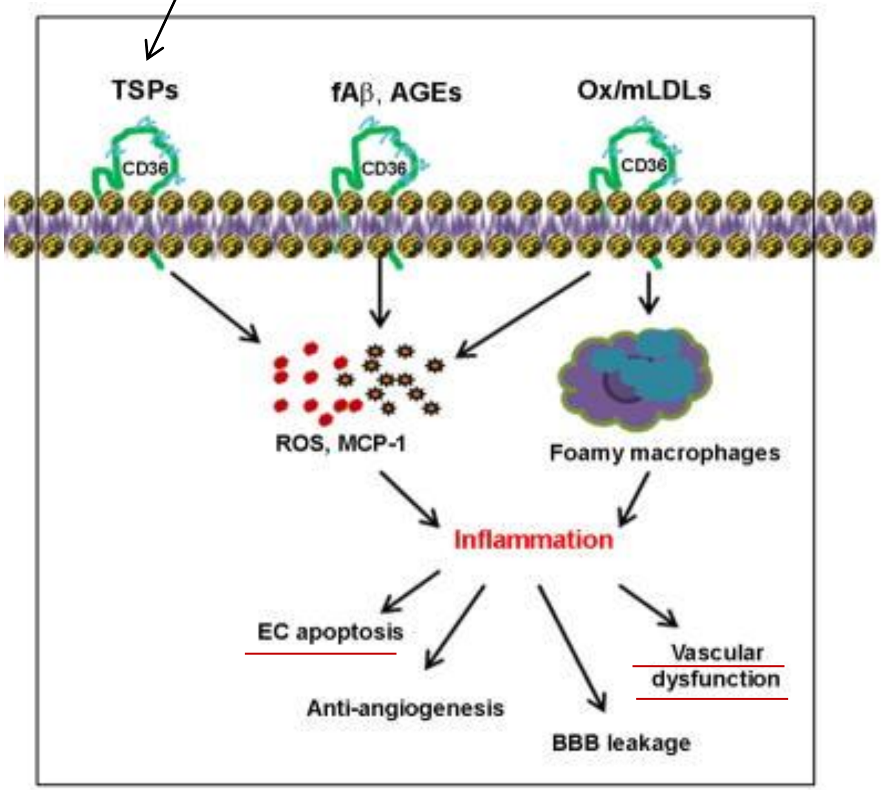
Cho S, Curr Pharm Des, 2012

Adhesion molecule: CD36

Thrombospondin are extracellular matrix proteins that mediate cell-cell interaction.
They promote greater adhesion of sickle **RBCs** to the microvascular endothelium

Several CD36 pathways activated by distinct ligands elicit inflammatory responses.

The binding of **thrombospondins** (TSPs) to CD 36 produces pro-inflammatory factors and causes vascular dysfunction and endothelial apoptosis.



Adhesion molecules - results

- ***VCAM*1_gene_haplotype 7** was found associated with **higher levels of LDH**, suggesting a relation between this variant and a sub-phenotype characterised by **more severe haemolysis (risk factor)**. Contrarily, heterozygosity for ***VCAM1*_promotor_haplotype 9** was found associated with **lower levels of total bilirubin** revealing a **protective effect against hemolysis.**

- The **rs1984112_G** allele located in the 5'UTR of the *CD36* gene revealed to be associated with higher levels of reticulocyte count. – **risk factor** of SCA hemolysis
- The rs1984112_G allele was already reported as a modulator of cholesterolemia and tissue vitamin E uptake.

Aims :

***In silico* studies – Transcriptional binding factor analyses**

Functional studies

Underlying mechanisms

Adhesion molecules

Target adhesion molecules to promote endothelial function ?

In mice, genetic ablation of CD36 is associated with a less inflammatory state and delays in vascular disease progression (Qin, J Neurosci. 2011)

Several pharmacological agents have been identified to reduce CD36 expression and functions (reviewed in Sunghee, Curr Pharm Des, 2012)

CD36 antagonists:

- Small-molecules based on a CD36-binding peptide sequence from TSP1 are being tested.
- Statins – they suppress oxLDL uptake and down-regulate CD36 expression

Hemolysis in SCA - genetic modifiers

3. Vascular tonus

Endothelial Nitric Oxide Synthase (**eNOS**), also known as **nitric oxide synthase 3** (**NOS3**) is an enzyme encoded by the ***NOS3 gene.***

NOS3 in endothelial cells generates **nitric oxide (NO)**, a gas with potent **vasodilation and antiadhesive properties**. NO is a critical molecule for proper endothelial function and maintain of a patent vascular lumen.

NO also plays a role in cellular proliferation, leukocyte adhesion, and platelet aggregation. It also presents **antioxidative activities**, such us superoxide scavenging and heme oxygenase induction.

➢ In our study, the **rs2070744_T allele at *NOS3* promoter** seems to have a **protective effect on SCA haemolysis** as it was found associated with **lower bilirubin levels**.

On the other hand, the **rs2070744_C allele** has already been considerate a genetic **risk factor** of coronary heart disease and rheumatoid arthritis.

(Cattaruzza *et al*, Circ Res 2004; Melchers *et al*, Arthritis Rheum 2006).

NOS3 promoter rs2070744 – A modulator of *NOS3* transcription?

- ***In silico* studies** – Transcriptional binding factor analyses
- **In vitro expression studies**
- **Underlying mechanism**

- **NO donors (citrulline)**

II - Hemolysis in SCD and Cerebral Vasculopathy

Objectives:

- To study association between **cerebral vasculopathy** and **intravascular hemolysis level** using commonly measured **haemolysis biomarkers** (biochemical and haematological parameters).
- To search for associations between **putative genetic modifiers of vascular tonus, vascular cell adhesion and inflammation**, and the **risk for stroke**, in the context of SCD in pediatric patients.

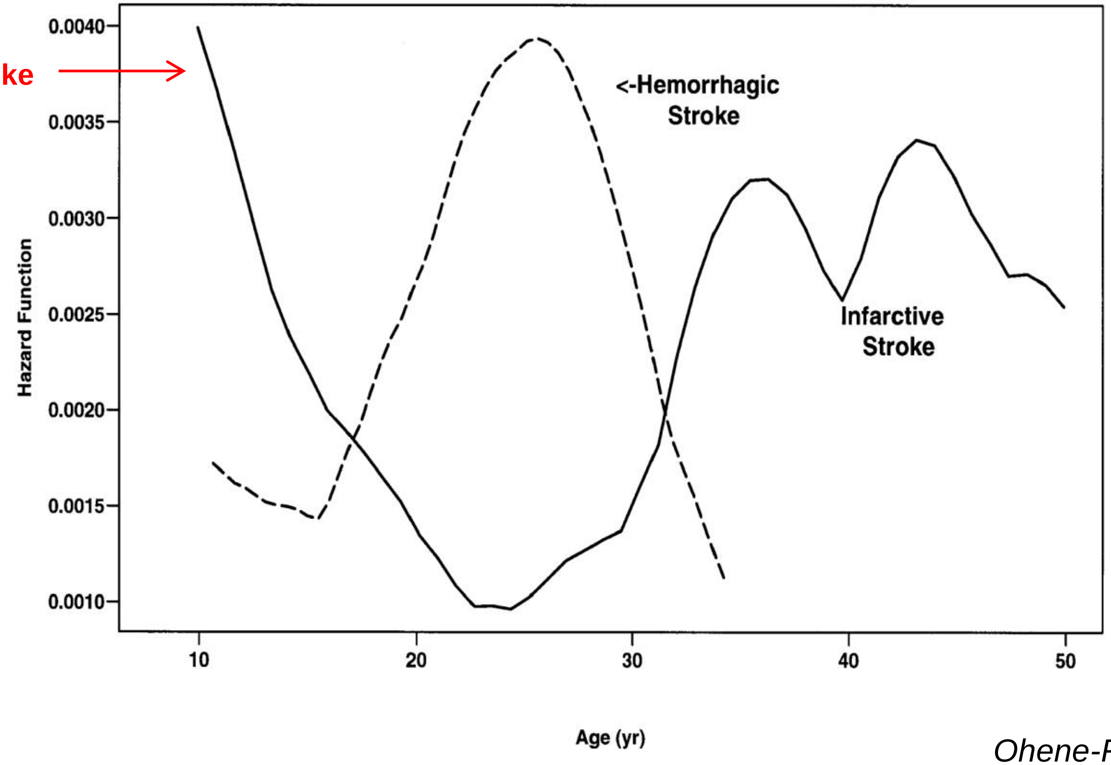
Cerebral Vasculopathy in SCD

- **Overt stroke (ischemic)** = abrupt focal neurological deficit with corresponding evidence of cerebral infarct on neuroimaging (Magnetic Resonance Imaging, MRI); locates in the cortex and deep in the white matter, with large dimension.

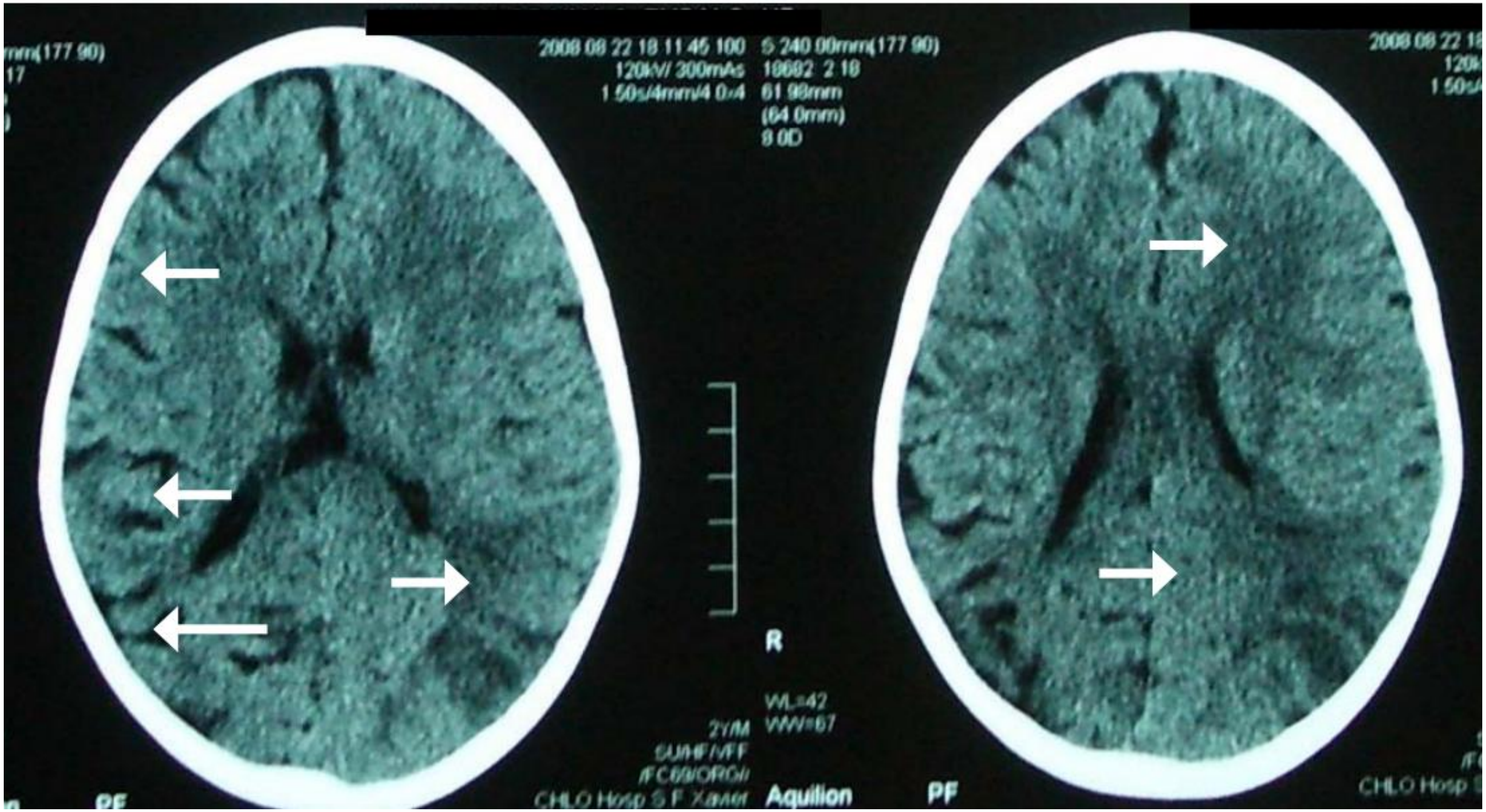
  May result in permanent physical or neucognitive impairment**.**

SCD children have 221-times increased risk for the occurrence of overt stroke than other children.

Ischemic stroke



Ohene-Frempong et al.,Blood 1998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A **head computed tomography scan** in a **2 years old Portuguese child with sickle cell anemia**: extensive acute infarction of the left middle and posterior cerebral arteries; atrophy of the right brain hemisphere.

Simões *et al*. Acta Med Port. 2011

Cerebral Vasculopathy in SCD

➢ **Silent infarct** = area of intensified signal on cerebral MRI, without history or physical findings associated with focal deficit; deep in the white matter of (mostly) frontal and parietal lobes, with smaller size.

Associated to **congnitive function impairment**. Most common cause of **neurological disease** in children with SCD;

SCD children have 410-times increased risk to develop cerebral infarcts than other children.

Risk factor for overt stroke occurrence (14-fold)

Risk factors	
Overt Stroke	**Silent Cerebral Infarcts**
• Elevated WBC counts • Low Hb • Relative hypertension • Increased frequency of ACS • Nocturnal hypoxemia • Presence of SCI	• Seizures • Low Hb • Systolic hypertension (adults) • Male gender • Elevated RBC counts • Elevated WBC counts

Cerebral Vasculopathy in SCD

- **Diagnosis of the risk to develop Stroke –**

Blood flow at the medial cerebral artery

Transcranial Doppler (TCD) → time-averaged mean of maximum velocity (TAMMV)

< 170 cm/s: “normal” = average risk
170 – 199 cm/s : “conditional” = moderate risk
> 200 cm/s = **high risk**

Magnetic resonance imaging (MRI) → assessment of SCIs

DATABASE, containing all relevant demographic, clinical, hematological, biochemical and imaging information retrospectively collected, from hospital records of SCD children.

POPULATION SAMPLE:

- **66 children (4-16 years) with SCD**: 65 $\beta^S\beta^S$ + 1 $\beta^S\beta^0$-thal
- 29 females (43.9%) and 37 males (56.1%)
- All with African ancestry: Angola, Cape Verde, Guinea-Bissau, São Tomé and Príncipe and Nigeria.
- 4 Hospitals: HDE (n=25); HSM (n=23); HFF (n=17); HGO (n=1).

GROUP CRITERIA:

✓ **Stroke (n=13)** → at least one episode of stroke (5 – 13 yrs)

✓ **Risk (n=29)** → “conditional” or high risk (TAMMV > 170 cm/s) and/or SCI on MRI

✓ **Control (n=24)** → without stroke, without SCI, TCD velocities < 170 cm/s

MOLECULAR CHARACTERISATION OF GENETIC VARIANTS:

- **Molecular confirmation of the sickle cell anemia mutation** (homozygosity or compound heterozygosity)

- **Molecular characterisation 22 polymorphic regions** (SNPs, indels, STRs) in genes related to

  vascular cell adhesion (*VCAM 1*, *THBS 1, CD36*),

  vascular tonus (*NOS 3, EDN 1, HMOX 1*)

  inflammation (*TNF α,*)

as well as in known globin expression modulators:

***HBB* cluster haplotype**
***HBA* genotype**
***BCL11A* genotype**

DATA ANALYSIS.

Association studies between **severity groups (stroke, risk, control)**

and the **genetic variants** and **hematological and biochemical data** of patients.

1. Allelic and genotypic counts and frequencies for the overall population sample
2. Checking of Hardy-Weinberg Equilibrium (USING R SOFTWARE)
3. Association studies (contingency tables for Fisher's exact test and Odds Ratio
4. False Discovery Rate
5. Clinical/hematological/biochemical parameters analysis with Wilcox-Mann-Whitney test and boxplots visualisation

Stroke risk in SCD

Hematological and Biochemical markers

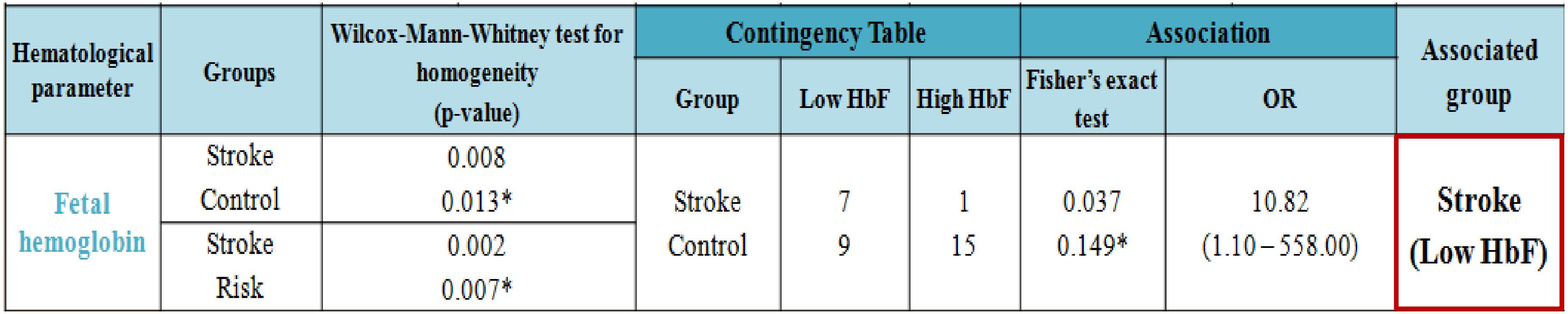
VS

Stroke risk

Lower Hb F levels associate with stroke

HbSF subphenotype → >10% HbF

Mean HbS (66 patients) = 80.59 %
Mean HbS (High HbF – 36 patients) = 74.95 %
Mean HbS (Low HbF – 30 patients) = 87.30 %

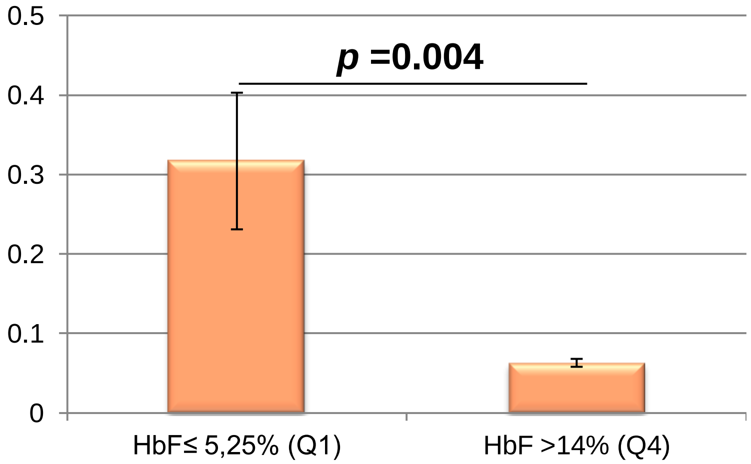


Hematological parameter	Groups	Wilcox-Mann-Whitney test for homogeneity (p-value)	Contingency Table			Association		Associated group
			Group	Low HbF	High HbF	Fisher's exact test	OR	
Fetal hemoglobin	Stroke Control	0.008 0.013*	Stroke Control	7 9	1 15	0.037 0.149*	10.82 (1.10 – 558.00)	Stroke (Low HbF)
	Stroke Risk	0.002 0.007*						

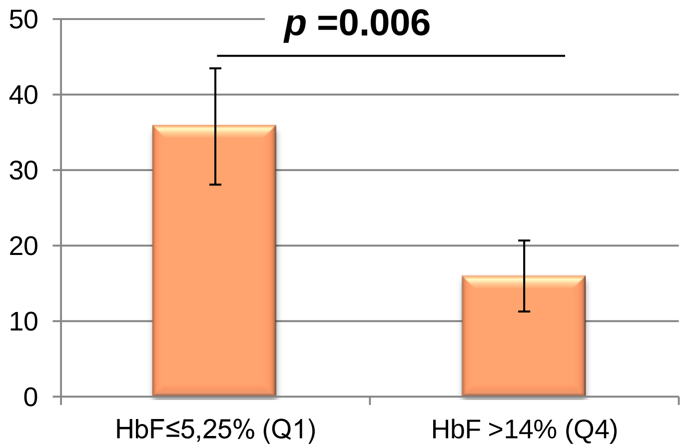
Risk factor

Disease severity scores and HbF

Disease Severity Score (DSS)



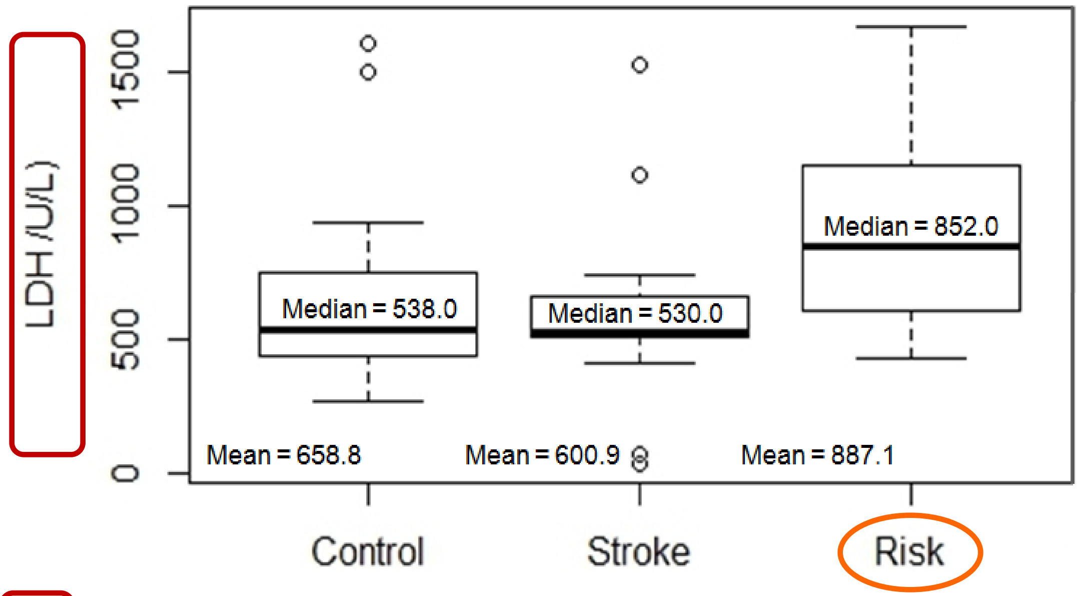
Pediatric Severity Score (PSS)

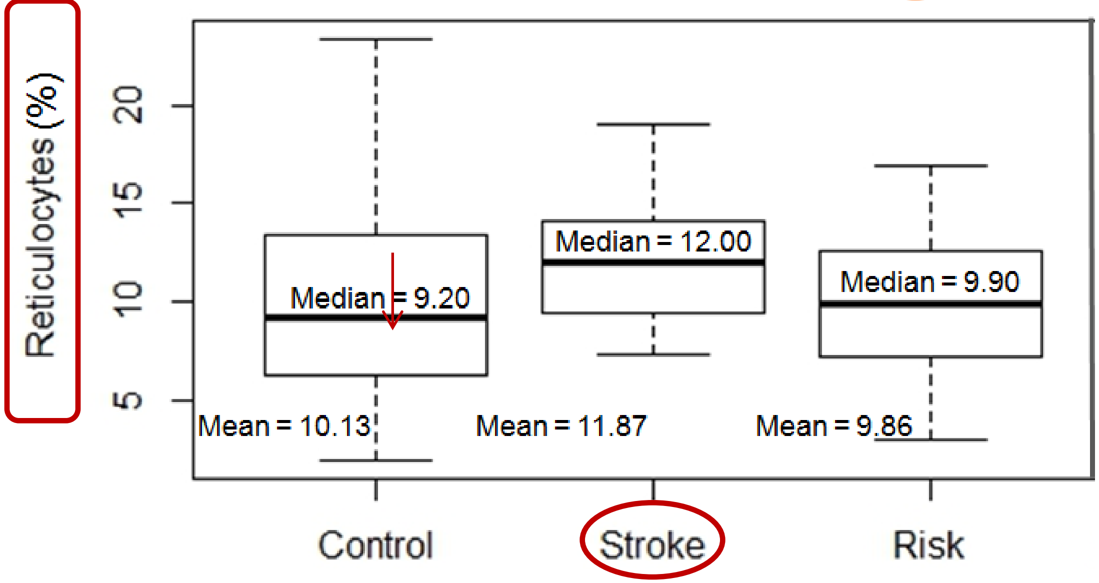


DSS and PSS are both negatively associated with HbF.

Hb F↓ - Risk factor - disease severity
- stroke

Stroke risk assotiation with **level of hemolysis**





Hematological parameter	Groups	Wilcox-Mann-whitney test for homogeneity (p-value)
LDH	Risk *vs* Control	0.0123 0.0493*
	Risk *vs* Stroke	0.0262

Higher LDH levels are associated with the Risk group which probably means that this proximal hemolytic marker is closely related with the initial stage of cerebral vasculopathy.

Reticulocyte count difference between groups are not significant but suggest a tendency.

Hemolysis levels↑

Risk factor - stroke

Stroke risk in SCD

Genetic variants

VS

Stroke risk

Association between candidate gene variants and stroke risk in SCD

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 (1.391 - 14.257)	**Stroke**
				Control	10	38	0.091*		
			Dominant (TT+CT)	Stroke	11	2	**0.014**	8.60 (1.407 – 97.351)	
				Control	9	15	0.091*		
NOS 3	**rs2070744** g.150992991 **C>T** **promoter**	**C**	**Overdominant (TC)**	Stroke	6	7	**0.013**	8.75 (1.221 – 107.964)	**Stroke**
				Control	2	22	0.067*		
			Allele count (C)	Stroke	6	20	**0.019**	6.70 (1.081 – 73.323)	
				Control	2	46	0.073*		
	VNTR 27 bp **4a/4b/4c** **Intron 4**	**4a**	**Dominant (4a4a + 4x4a)**	Risk	23	6	**0.020**	4.89 (1.178 –18.321)	**Risk**
				Control	11	13	0.1218*		
			Allele count (4a)	Risk	26	32	**0.024**	2.71 (1.088 – 7.088)	
				Control	11	37	0.1218*		
		4b	**Dominant (4b4b)**	Risk	3	26	**0.005**	0.24 (0.053 – 0.999)	**Control**
				Control	8	16	0.3122*		
			Allele count (4b)	Risk	25	33	**0.033**	0.42 (0.175 – 0.979)	
				Control	31	17	0.3122*		
***HMOX 1* promoter**	**rs3074372** (STR – GT) **S/M/L**	**L**	**Dominant (L/L + other/L)**	Stroke	10	3	**0.019**	6.04 (1.196 – 42.056)	**Stroke**
				Risk	10	19	0.148*		
			Allele count (L)	Stroke	14	12	**0.012**	3.60 (1.233 – 10.902)	
				Risk	14	44	0.148*		

NOS 3 VNTR 4a=4x 27 bp; 4b=5x 27bp; *HMOX* 1 STR-L = (GT)n, n>35

VCAM-1 promoter SNPs haplotypes

Haplotype	rs1409419	rs3917024	rs3917025	rs3978598	rs1041163	rs3783599	Frequency
#1	C	C	CT	T	T	C	0.341
#2	C	C	CT	T	T	**T**	0.008
#3	C	C	CT	T	**C**	C	0.144
#4	C	C	CT	T	**C**	**T**	0.106
#5	C	C	**delCT**	T	T	C	0.038
#6	C	**T**	**delCT**	**G**	T	C	0.030
#7	**T**	C	CT	T	T	C	0.333

Gene	Genetic variant	Associated Allele	Mode of transmission	Association to phenotypic groups					
				Contingency Table			Association		Associated group
				Group	Presence	Absence	Fisher's exact test	OR (95% CI)	
VCAM1-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)	

Risk allele (not yet reported)

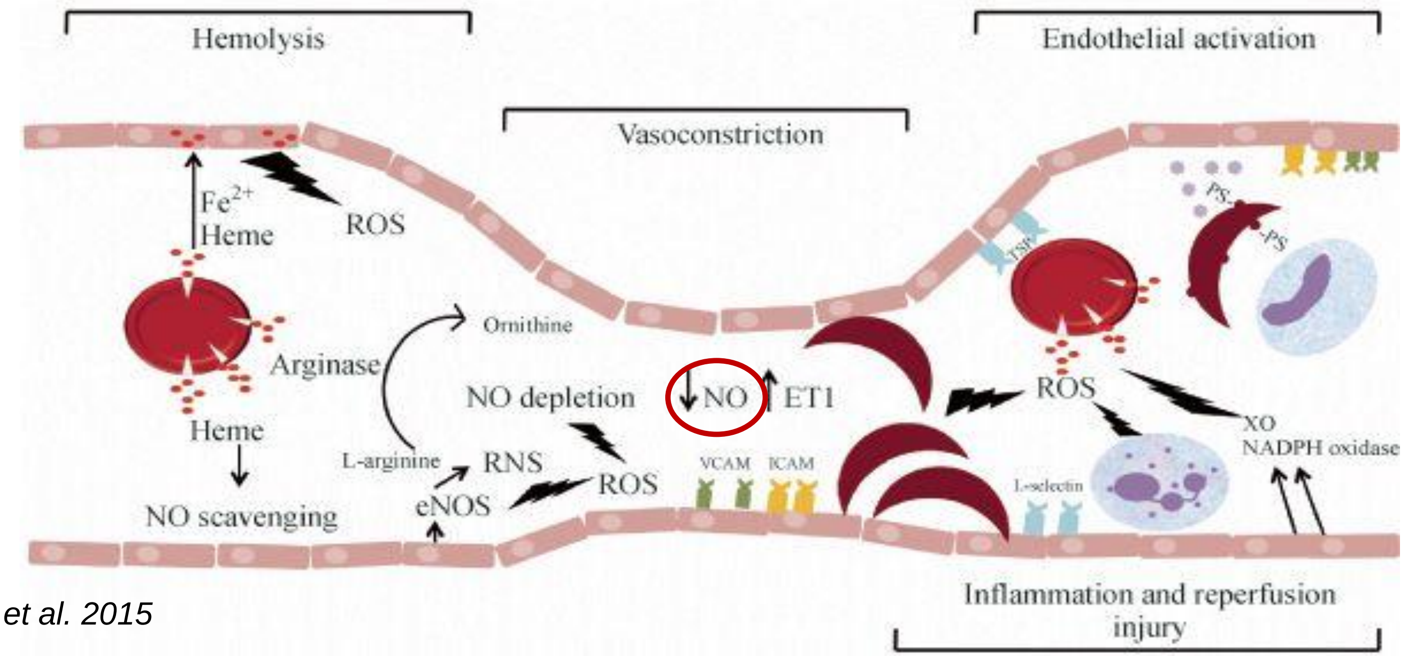
- On the other hand, rs1409419_**C** was found associated with lower levels of bilirubin (1st study) ☑

NOS3 polymorphisms haplotypes

Haplotype	rs2070744	VNTR 27 bp	rs1799983	Frequency
#1	**C**	4a	G	0.08
#2	**C**	4b	G	0.02
#3	**C**	4b	**T**	0.03
#4	T	4a	G	0.27
#5	T	4b	G	0.45
#6	T	4b	**T**	0.05
#7	T	4c	G	0.10

Gene	Genetic variant	Associated Allele	Mode of transmission	Association to phenotypic groups					
				Contingency Table			Association		Associated group
				Group	Presence	Absence	Fisher's exact test	OR (95% CI)	
NOS3	**rs2070744** g.150992991 **C>T** promoter	**C**	**Overdominant (TC)**	Stroke	6	7	0.013	8.75 (1.221 – 107.964)	**Stroke**
				Control	2	22	0.067*		
			Allele count (C)	Stroke	6	20	0.019	6.70 (1.081 – 73.323)	
				Control	2	46	0.073*		
	VNTR 27 bp **4a/4b/4c** Intron 4	**4a** 4x 27bp	**Dominant (4a4a + 4x4a)**	Risk	23	6	0.020	4.89 (1.178 –18.321)	**Risk**
				Control	11	13	0.1218*		
			Allele count (4a)	Risk	26	32	0.024	2.71 (1.088 – 7.088)	
				Control	11	37	0.1218*		
		4b 5x 27bp	**Dominant (4b4b)**	Risk	3	26	0.005	0.24 (0.053 – 0.999)	**Control**
				Control	8	16	0.3122*		
			Allele count (4b)	Risk	25	33	0.033	0.42 (0.175 – 0.979)	
				Control	31	17	0.3122*		

NOS3: rs2070744 and VNTR 27 bp



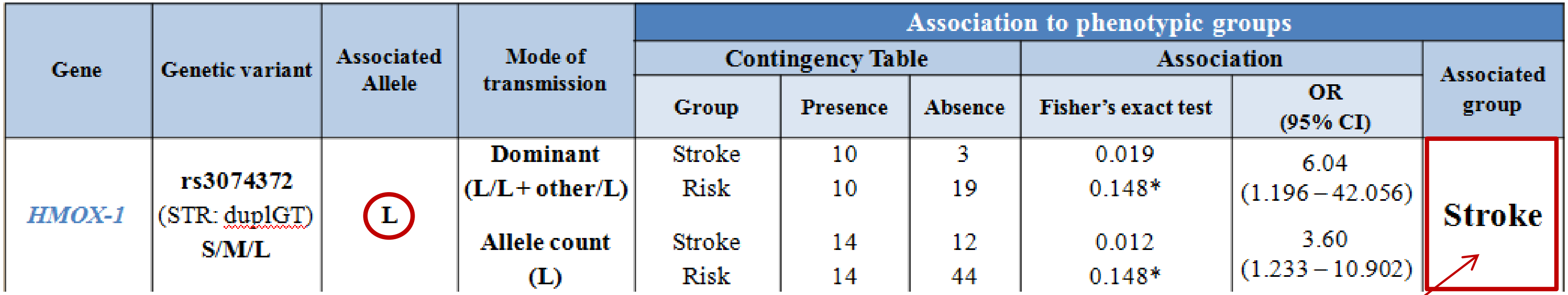
Owusu-Ansah A, et al. 2015

NOS3, Haplotype #1

(rs2070744_C / VNTR 4a) NOS3 expression↓ NO↓ → Risk Haplotype

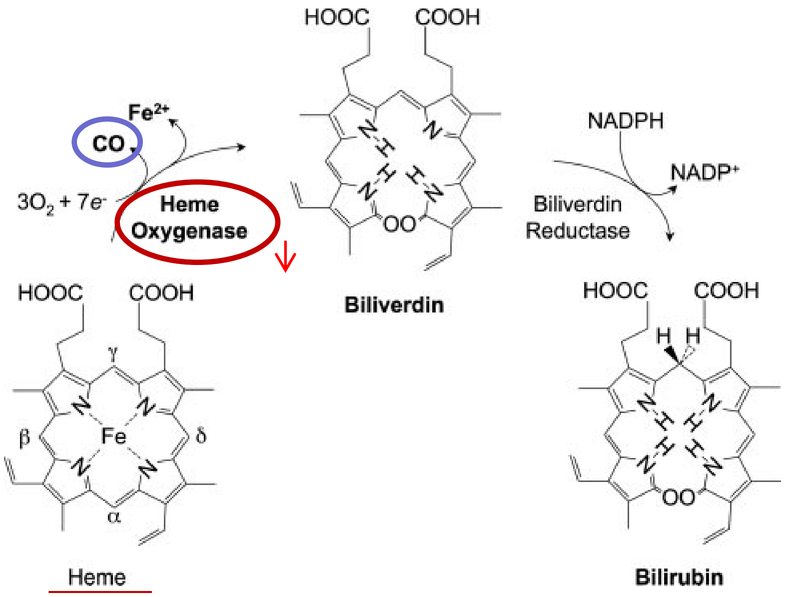
- The **rs2070744_C** allele has already been considered a genetic increased **risk factor of coronary heart disease and rheumatoid arthritis** ☑
- On the other hand, rs2070744_**T** was found associated with lower levels of bilirubin (1st study) Protective for hemolysis ☑

HMOX-1: rs3074372



Gene	Genetic variant	Associated Allele	Mode of transmission	Association to phenotypic groups					
				Contingency Table			Association		Associated group
				Group	Presence	Absence	Fisher's exact test	OR (95% CI)	
HMOX-1	**rs3074372** (STR: duplGT) S/M/L	L	**Dominant (L/L+other/L)**	Stroke	10	3	0.019	6.04 (1.196 – 42.056)	**Stroke**
				Risk	10	19	0.148*		
			Allele count (L)	Stroke	14	12	0.012	3.60 (1.233 – 10.902)	
				Risk	14	44	0.148*		

rs3074372 is a highly polymorphic (GT)n microssatelite in gene **promoter;** Long allele n≥35



Longer STR

↓

Decreased *HMOX-1* transcription

↓

Lower levels of HO-1

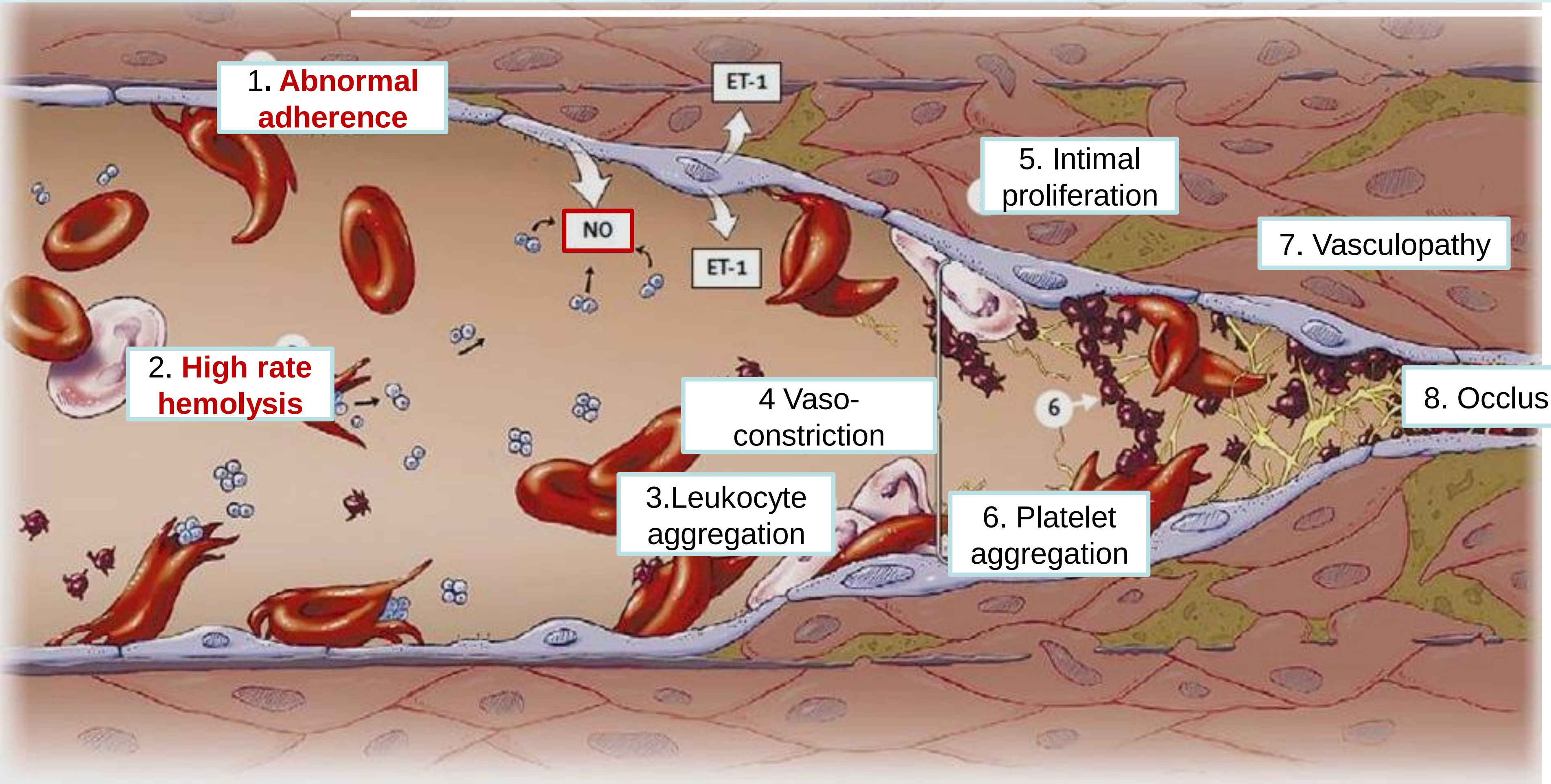
↓

Free heme is not adequately removed and further scavenges NO molecules.

→ **Functional studies should be done.**

Adapted from Stocker and Perrella, 2006

Cerebral Vasculopathy in SCD



1. **Abnormal adherence**

2. **High rate hemolysis**

3.Leukocyte aggregation

4 Vaso-constriction

5. Intimal proliferation

6. Platelet aggregation

7. Vasculopathy

8. Occlusi

Strokes in children with SCD
progressive stenosis of arteries due to intimal proliferation

Adapted from Switzer et al., 2006.

Sickle cell anemia: chronic hemolysis and cerebral vasculopathy

Conclusions

- This study **contributed to the line of evidence** that stroke is a consequence of the hemolysis rate – endothelial dysfunction in SCD.

- This was the first study to evidence a protective role of HbF in stroke occurrence.

- Beginning to delineate a **profile of genetic biomarkers able to predict the risk of stroke** occurrence in SCD pediatric patients. It will include:

➢ genetic variants related with **an increased synthesis of vascular cell adhesion molecules** (i.e., rs1409419_allele T of *VCAM 1* gene promoter)

➢ *genetic variants related with* ***a*** **decreased** rate of transcription of ***NOS3*** (i.e., rs2070744_C / VNTR 4a) and *HMOX 1* (rs3074372_allele L) that give rise to lower levels of NO and heme oxygenase.

Future perspectives:

- To **validate** the results in a larger patient cohort with a longer follow-up.
- A number of mechanistic hypotheses compatible with the observed genotype/phenotype were proposed but **further mechanistic studies** are needed to a better understanding of the inter- and intra-individual clinical variability.
- **Functional studies** are crucial in understanding the role of genetic variants in **disease pathophysiology and evaluate potential therapy targets.**

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