

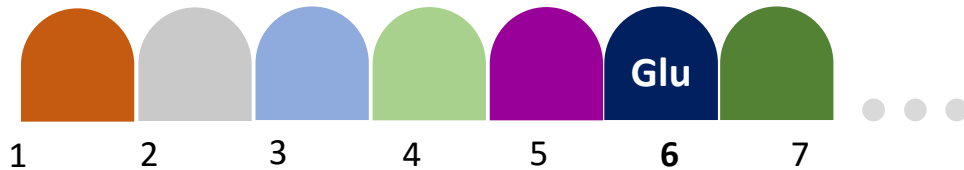
# Endothelial Factors and Stroke Risk in Pediatric Sickle Cell Anemia Patients

## Insights from *VCAM1* and *ITGA4* Variants

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**Autosomal recessive**

Hemoglobin A



**RBC**



**Hemoglobin S**



**SSRBC**



**Polymerisation**

**SSRBC**

- Less deformable
- More fragile
- More rigid

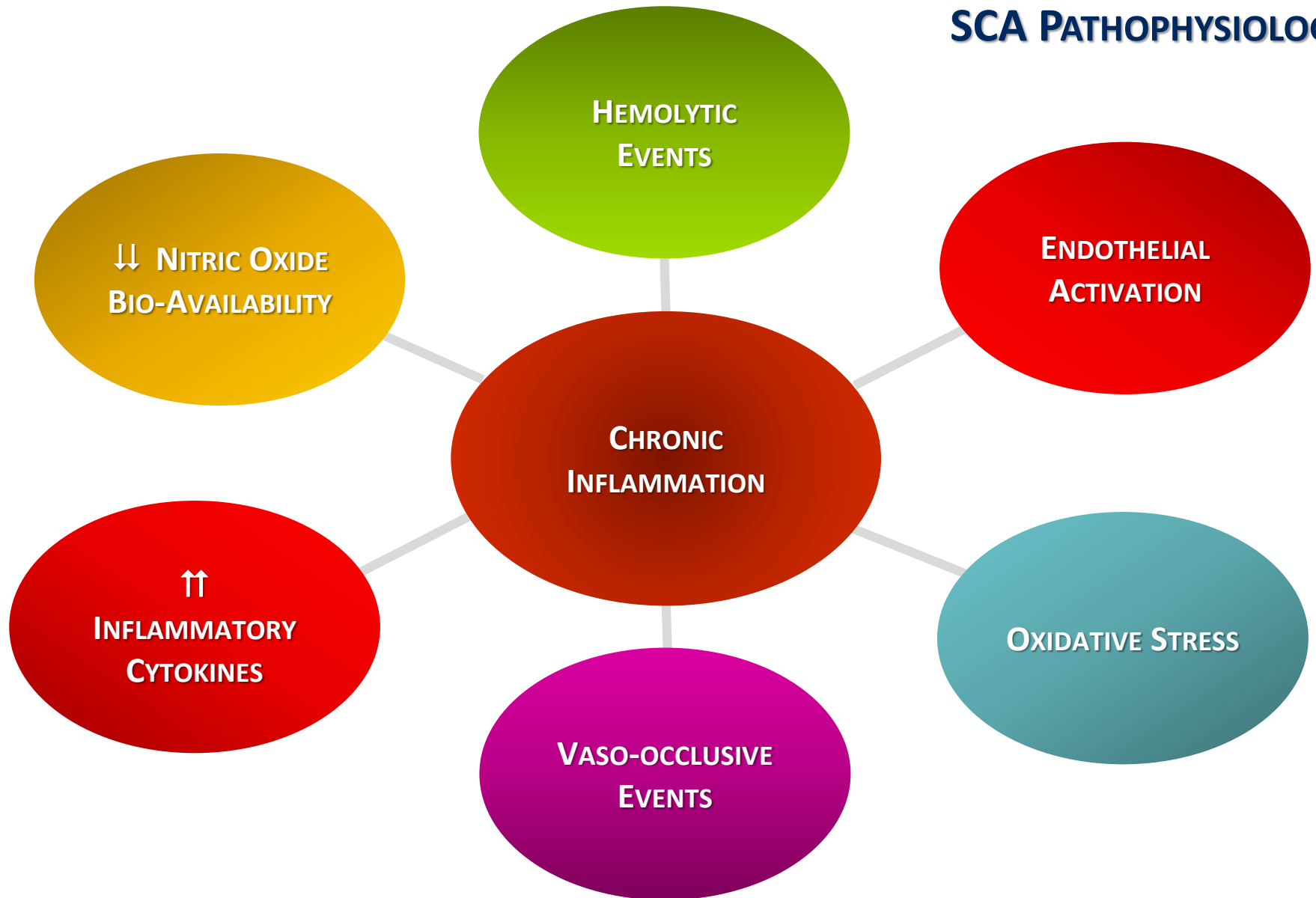
**VASO-OCCLUSION**

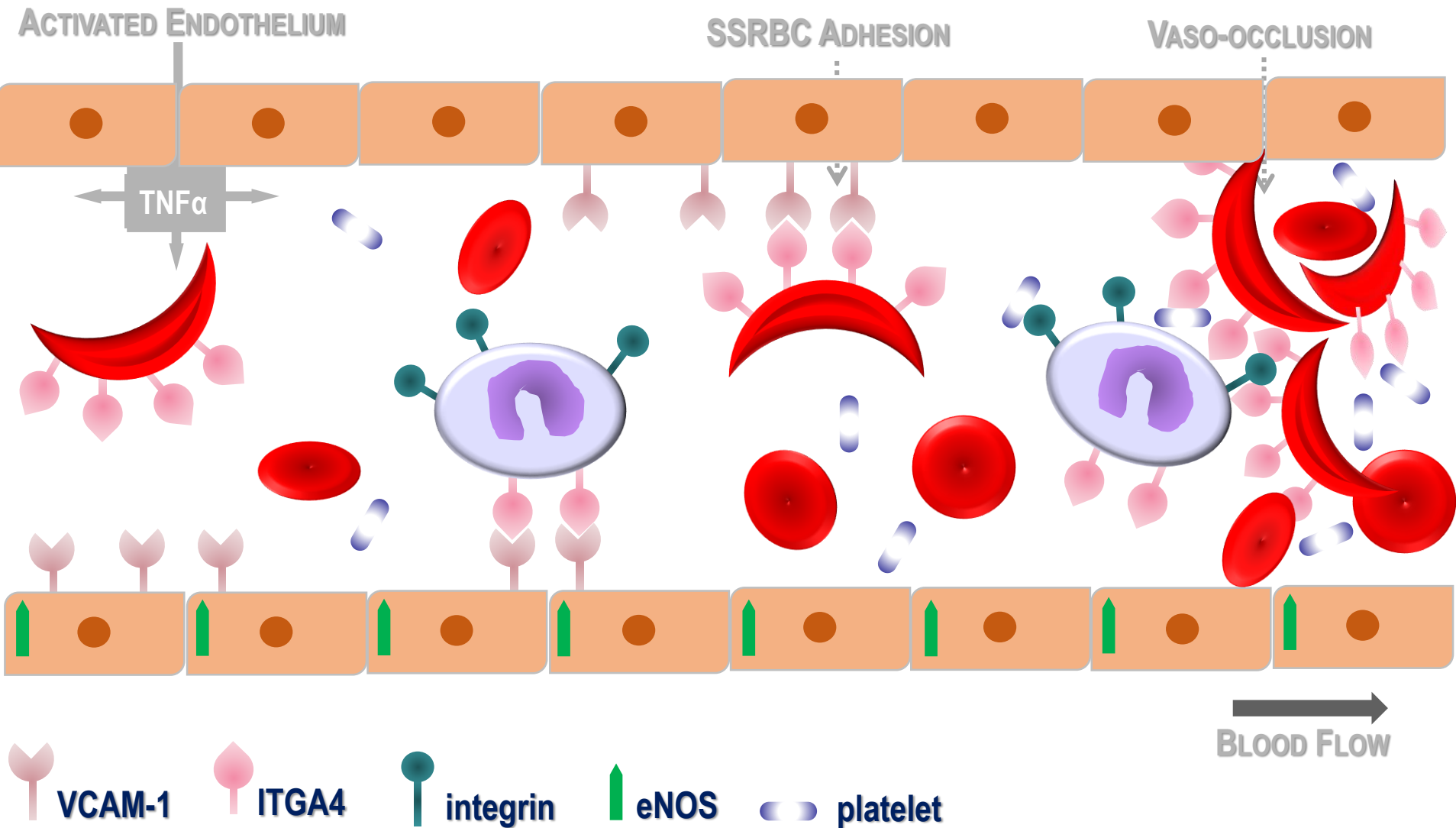
**HEMOLYSIS**

**STROKE**

Acute chest syndrome  
Pulmonary hypertension  
Anemia  
Jaundice  
Splenic sequestration

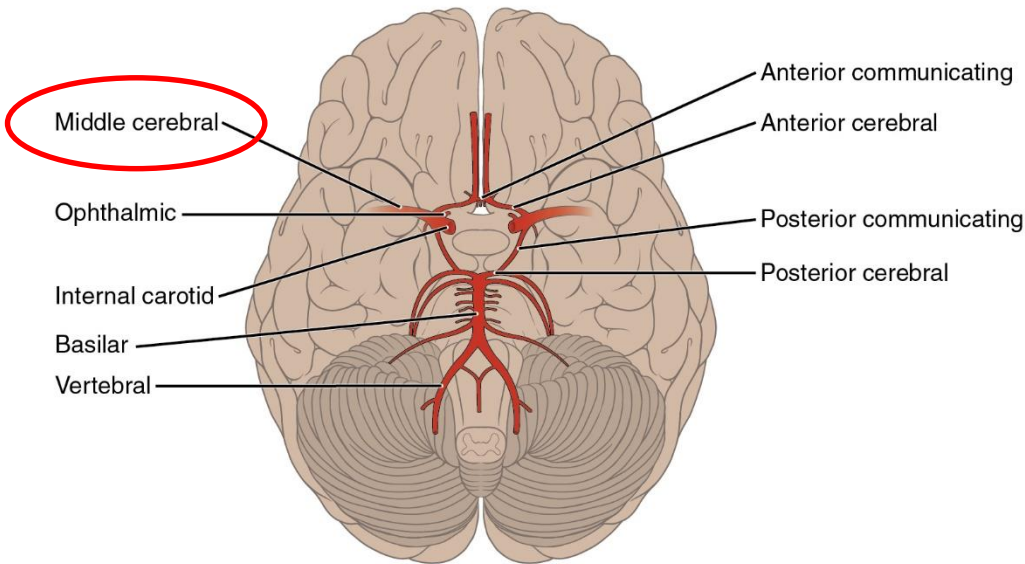
# SCA PATHOPHYSIOLOGY





(Transcranial Doppler - TCD)

(TAMMV)



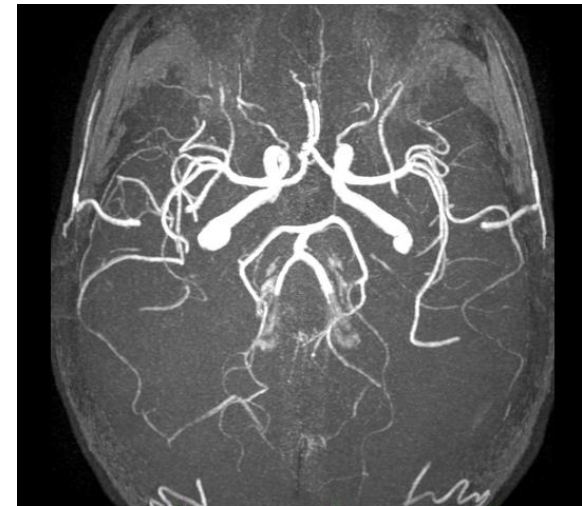
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**Stroke** (and SCI)

**High Risk** – TAMMV  $\geq 200$  cm/s

**Conditional** –  $170$  cm/s  $<$  TAMMV  $<$   $200$  cm/s

**Normal** – TAMMV  $<$   $170$  cm/s



(Magnetic Resonance Imaging - MRI)

## PUTATIVE GENETIC MODULATORS OF PEDIATRIC STROKE IN SCA

### Endothelial Activation

*VCAM-1*

*CD-36*

*THBS-1*

### Vascular Tone Balance

*NOS3*

*ET-1*

### Systemic Inflammation

*TNF- $\alpha$*

*HMOX-1*



- 70 pediatric SCA patients (age  $\geq 3$  years)
- Cerebral vasculopathy - 3 main groups:
  - **Stroke:**  $\geq 1$  stroke event
  - **Risk:** abnormal TCD and or MRI scan results
  - **Normal:** no history of abnormalities in TCD or MRI scans
- Database with clinical, imaging and laboratory data

METHODOLOGY

**VCAM1**  
**ITGA4**

**NOS3**

- **PCR, Gap-PCR, PCR –RFLP, ARMS, FFLA**
- **Sanger sequencing**
- **Next-generation sequencing**
  - **LR-PCR (FailSafe – Epicentre)**
  - **Nextera XT® (Illumina)**
  - **MiSeq® (Illumina)**
  - **MiSeq® Reporter; BW; GATK; FastQC; IGV; VEP**
- **R v. and SPSS v.24 ( $\chi^2$  and Fisher; Bonferroni c.)**
- **MatInspector; TFbind; Human Splicing Finder; Polyphen2**

**VCAM1 (promoter)**

**Stroke** → rs1409419\_T [p=0.008 ; OR=4.330 (CI: 1.391-14.259)]

**ITGA4**

**Stroke** { rs113276800\_C [p=0.021; OR=9.714 (CI: 1.479-63.806)]  
rs74850478\_A [p=0.035; OR=13.125 (CI:1.203-143.233)]  
rs3770138\_C [p=0.042; OR=6.286 (CI:1.143-34.570)]

**Stroke + SCI** → rs3770138\_C [p=0.045; OR=6.176 (CI:1.126-33.876)]

**Risk** { rs1375493\_G [p=0.050; OR=8.750 (CI: 0.993-2.992)]  
rs35723031\_GA [p=0.050; OR=8.75 (CI: 0.99-2.99)]  
g.181459459\_TT [p=0.050; OR=8.75 (CI: 0.99-2.99)]

**NOS3**

**Stroke** → (promoter) rs2070744\_C [p=0.019 ; OR=6.700 (CI: 1.081 – 73.323)]

**Risk** → intron 4 VNTR 27 bp (=4 repeats) [p=0.020 ; OR=2.710 (CI:1.088-7.088)]



## POTENTIAL MODULATION OF PEDIATRIC STROKE IN SCA

### *VCAM1 variant*

- Altered TF binding site (position -1592) (RXRF→PRDF and FHXB loss)
- Increased VCAM1 inducible expression

ENDOTHELIAL DYSFUNCTION

### **VASCULOPATHY**

### *ITGA4 variants*

- Altered enhancers/silencers → expression
- Increased cell adhesion to activated endothelium

CELL – ENDOTHELIAL ADHESION

### *NOS3 variants*

- Decreased NO bio-availability
- Decreased vascular tone

VASOCONSTRICTION

### **VASO-OCCLUSION**

## FUTURE PROSPECTS

*In vitro* functional studies

biological link between the *VCAM-1* and *ITGA4* variants with cerebral vasculopathy

Gene panel (NGS) (diagnostic/prognostic)

*VCAM1* and *ITGA4* – potential therapeutic targets



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