
# GENETIC MODULATION OF CEREBRAL VASCULOPATHY IN CHILDREN WITH SICKLE CELL ANEMIA

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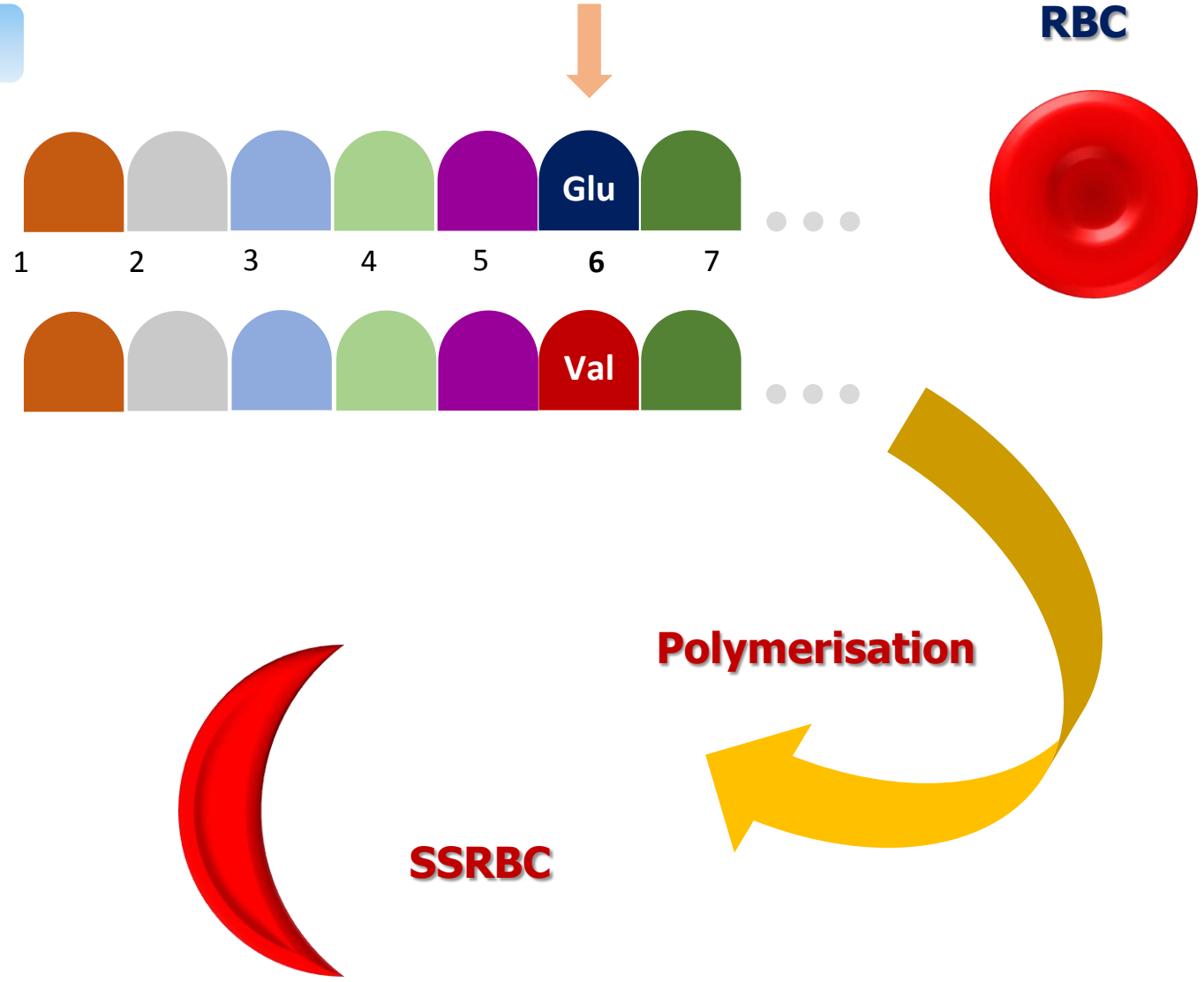
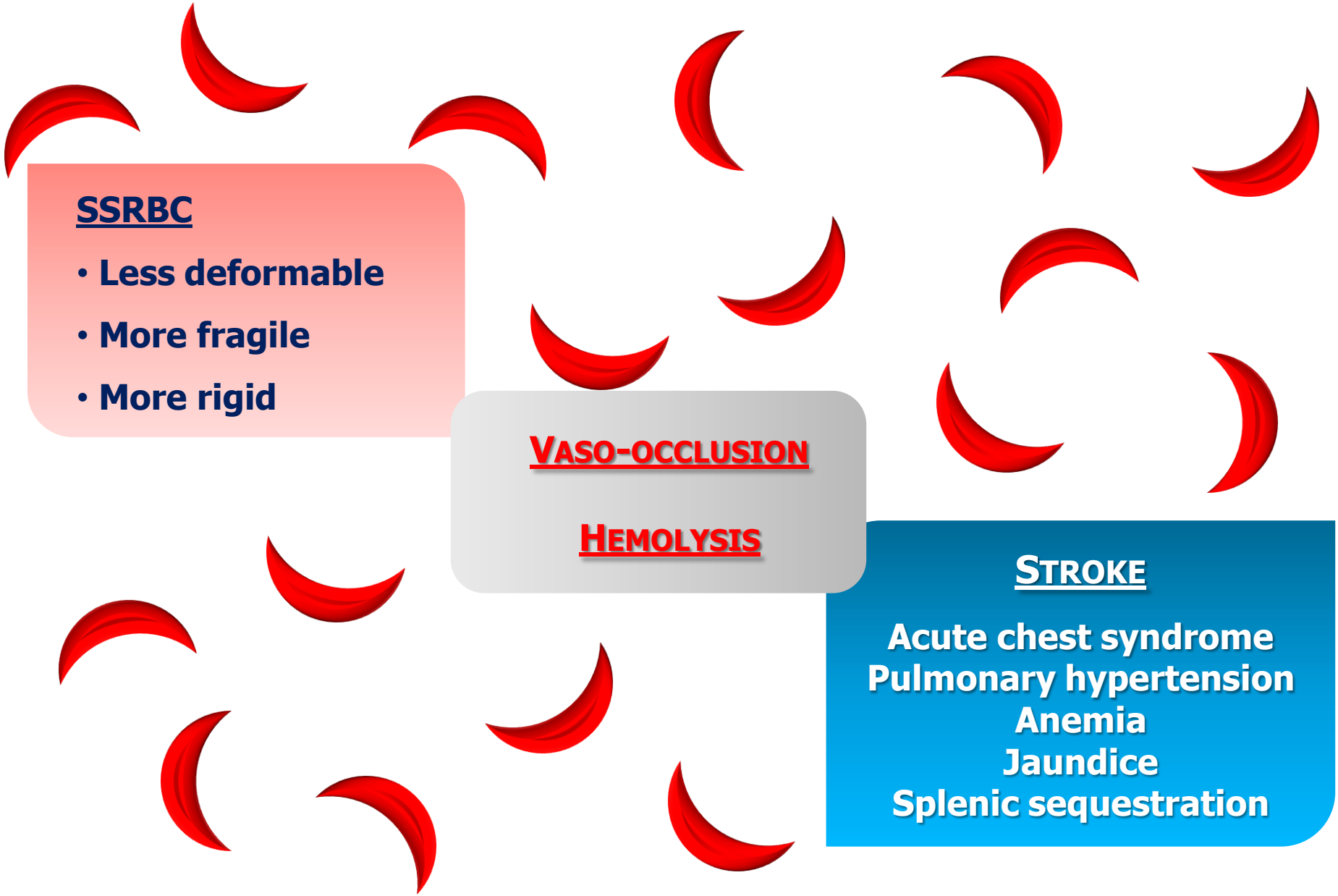
**Autosomal recessive**





**SSRBC**

- Less deformable
- More fragile
- More rigid

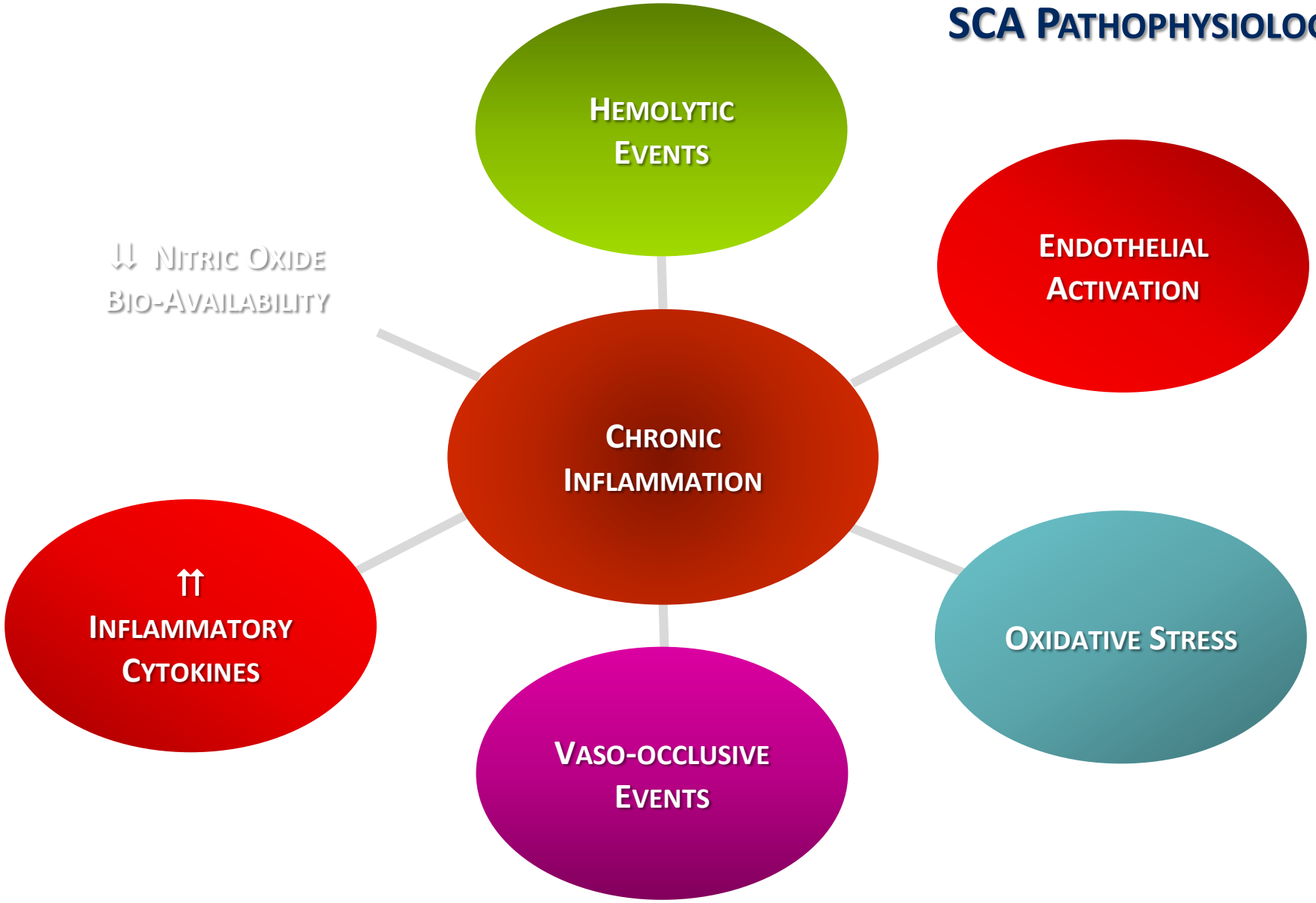
**VASO-OCCLUSION**

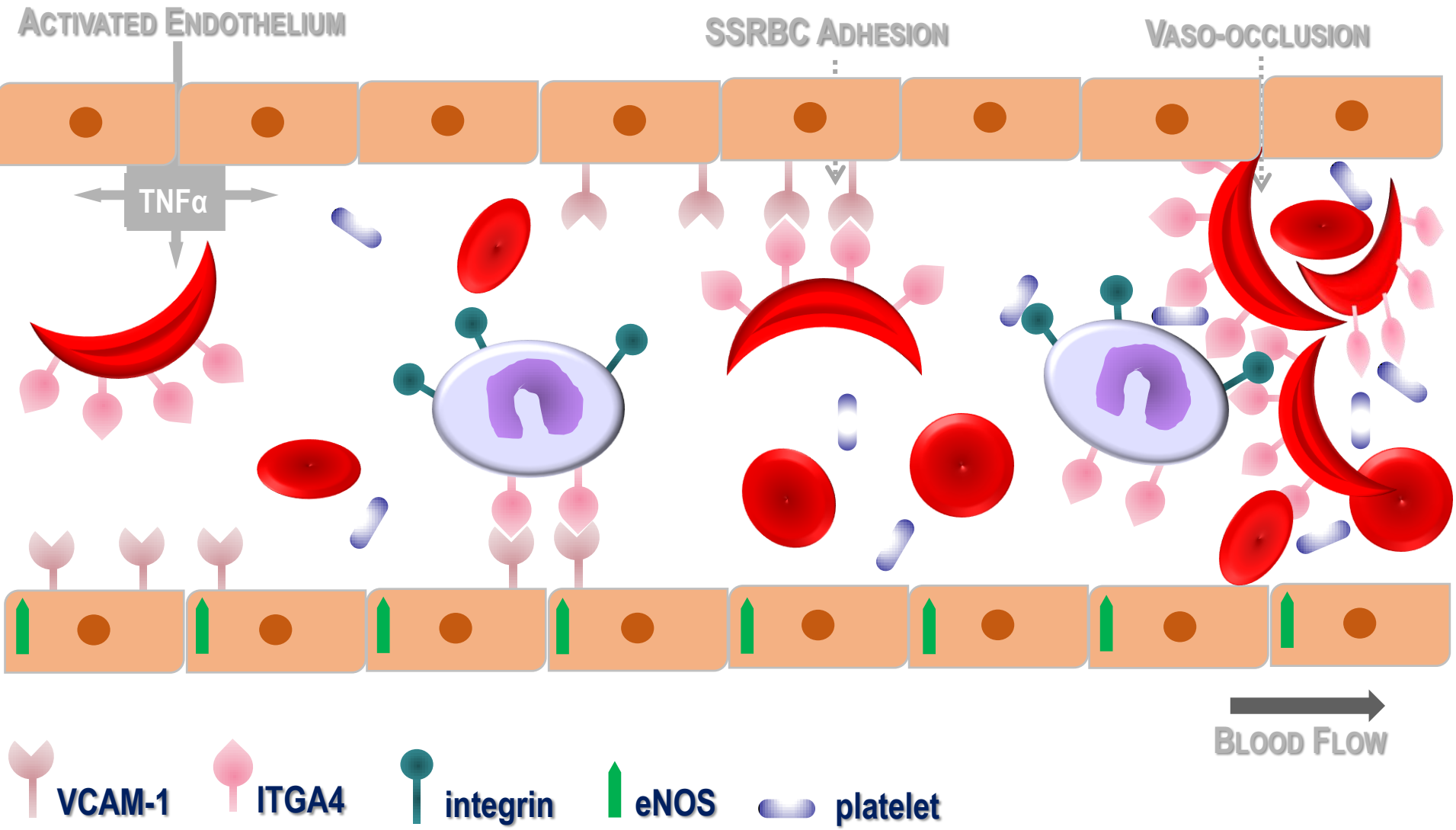
**HEMOLYSIS**

**STROKE**

Acute chest syndrome
Pulmonary hypertension
Anemia
Jaundice
Splenic sequestration

# SCA Pathophysiology




ACTIVATED ENDOTHELIUM
SSRBC ADHESION
VASO-OCCLUSION
TNFα
BLOOD FLOW
VCAM-1
ITGA4
integrin
eNOS
platelet

# CEREBRAL VASCULOPATHY

## 1 STROKE

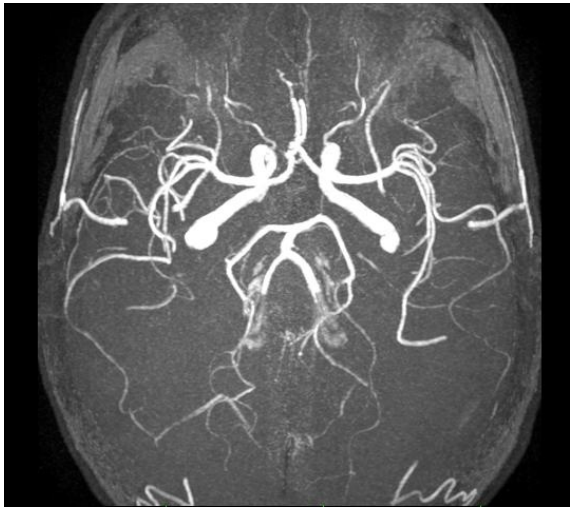
- Abrupt onset of neurological deficit
- Mostly ischemic
- Affects **large** and medium vessels
- High risk (fatal in 1/10 patients)
- Clinical features
    - Hemiparesis or focal seizures
    - Weakness, spasticity, epilepsy
    - Cognitive impairment
        - Compromised IQ
        - ↓ language, social and personal skills
- High recurrence risk
    - 2-3 yrs. after first stroke

## 2 SILENT CEREBRAL INFARCTION (SCI)

- Not clinically apparent
    - No neurological anomalies or not related to the MRI abnormalities
- 25% SCA children ≤ 6 years old
- 39% SCA children ≤18 years old
- Affects **small** vessels
- Rarely fatal
- High recurrence risk

# SCREENING & DIAGNOSIS

- Transcranial Doppler ultrasonography (TCD)
- Cerebral Magnetic Resonance Imaging (MRI)
- Computer Tomography (CT)



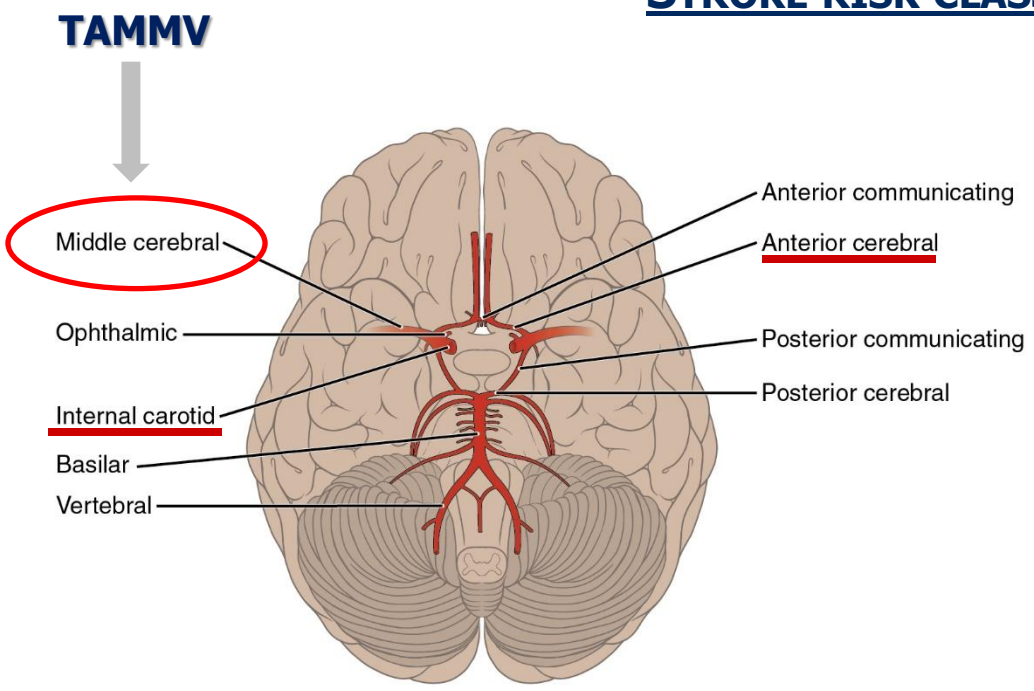
MRI


**TCD**

Measures **time-averaged mean of maximum velocity (TAMMV)** of blood flow in the middle cerebral artery

Sources (images): WikiCommons Licenses

# STROKE RISK CLASSIFICATION IN SCA




**High Risk** – TAMMV ≥ 200 cm/s

**Conditional** – 170 cm/s < TAMMV < 199 cm/s

**"Normal"** – TAMMV < 170 cm/s

# THERAPEUTIC STRATEGIES

**Regular blood transfusion therapy**

Alloimunization
Iron overload

**Hydroxyurea**

Bone Marrow or Human Stem Cell Transplant

Cerebral Revascularization Surgery

Genome Editing…

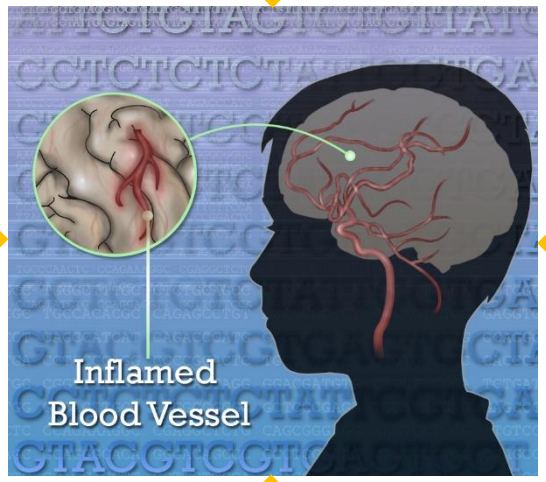
**Specific/sensitive Biomarkers of Cerebral Vasculopathy Risk**

# Risk Factors

↑ Cerebral Blood Flow
↓ Cerebrovascular reserve
- Low baseline Hb
- High %HbS (especially >40%)

- Hypertension
- Diabetes
- Hyperlipidemia
- Atrial fibrillation
- Renal disease

Anemia
(relative and acute)
- Low baseline Hb
- Relative acute drop in Hb (<5.5 g/dL)
- Parvovirus B19




CNS vasculopathy
(intra- or extracranial)
- Stenosis
- Occlusion
- Dissection
- Moyamoya

↓ Hb O2 saturation, ↑ O2 demand
- Acute chest syndrome
- Low baseline O2 saturation
- Fever (↑ O2 demand)
- Seizure (↑ O2 demand)

# PUTATIVE GENETIC MODULATORS OF PEDIATRIC STROKE IN SCA

**Endothelial Activation**

***VCAM-1***

**Cell-Endothelium Adhesion**

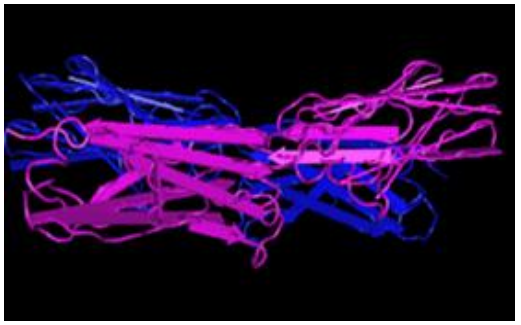
***ITGA4***

**Vascular Tone**

***NOS3***

# VCAM-1

- Vascular adhesion molecule 1
- Syaloglycoprotein (immunoglobullin family)
- Gene location: chr. 1 (q31-q32)
  - 25 kb
  - 9 exons
- Homodimeric protein
  - 739 aa



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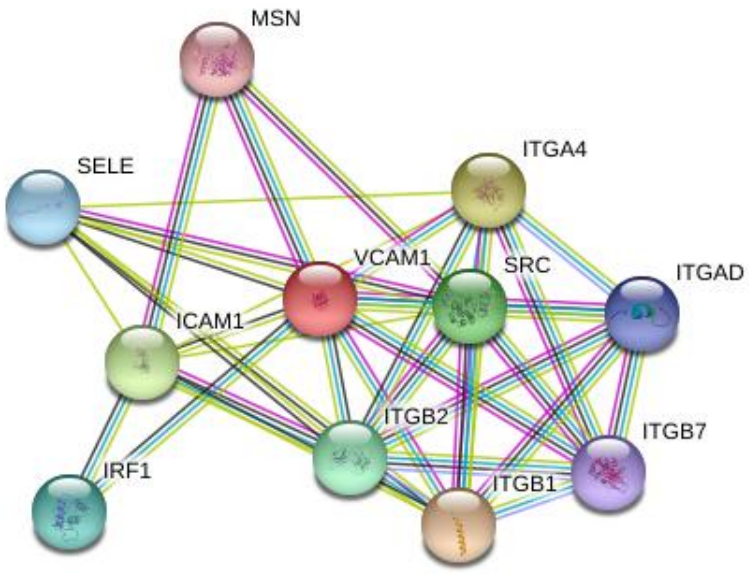


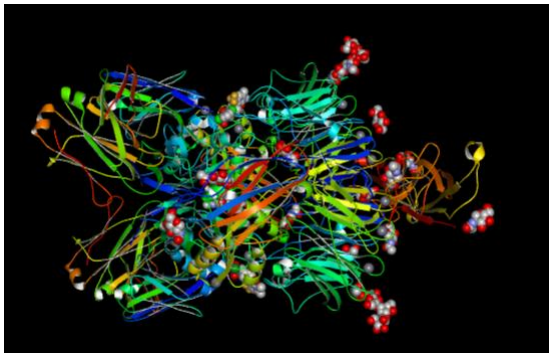

STRING v.10.5

- Cell adhesion
- Heterotypic cell-cell adhesion via plasma membrane cell adhesion molecules
- Cell adhesion molecule binding
- Response to hypoxia
- Acute inflammatory response
- Chronic inflammatory response
- Integrin binding
- Leukocyte cell-cell adhesion
- Cellular response to tumor necrosis factor
- Interferon-gamma-mediated signalling pathway
- Cell-matrix adhesion
- Leukocyte tethering or rolling
- Leukocyte migration
- Cellular response to vascular endothelial growth factor stimulus

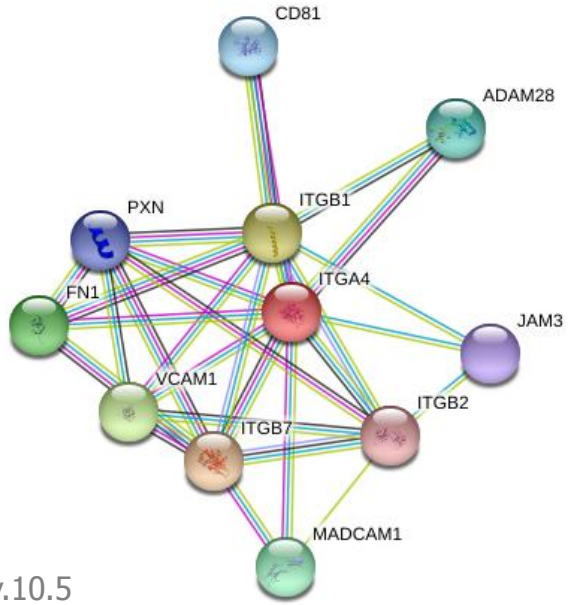
*Gene Ontology (GO)*

# ITGA4

- Integrin 4; very-late antigen 4 (VLA-4); integrin α4β1; CD49D
- Integrin alpha chain family
- Gene location: chr. 2 (q31.3)
  - 28 exons
- Heterodimeric protein
  - 1032 aa



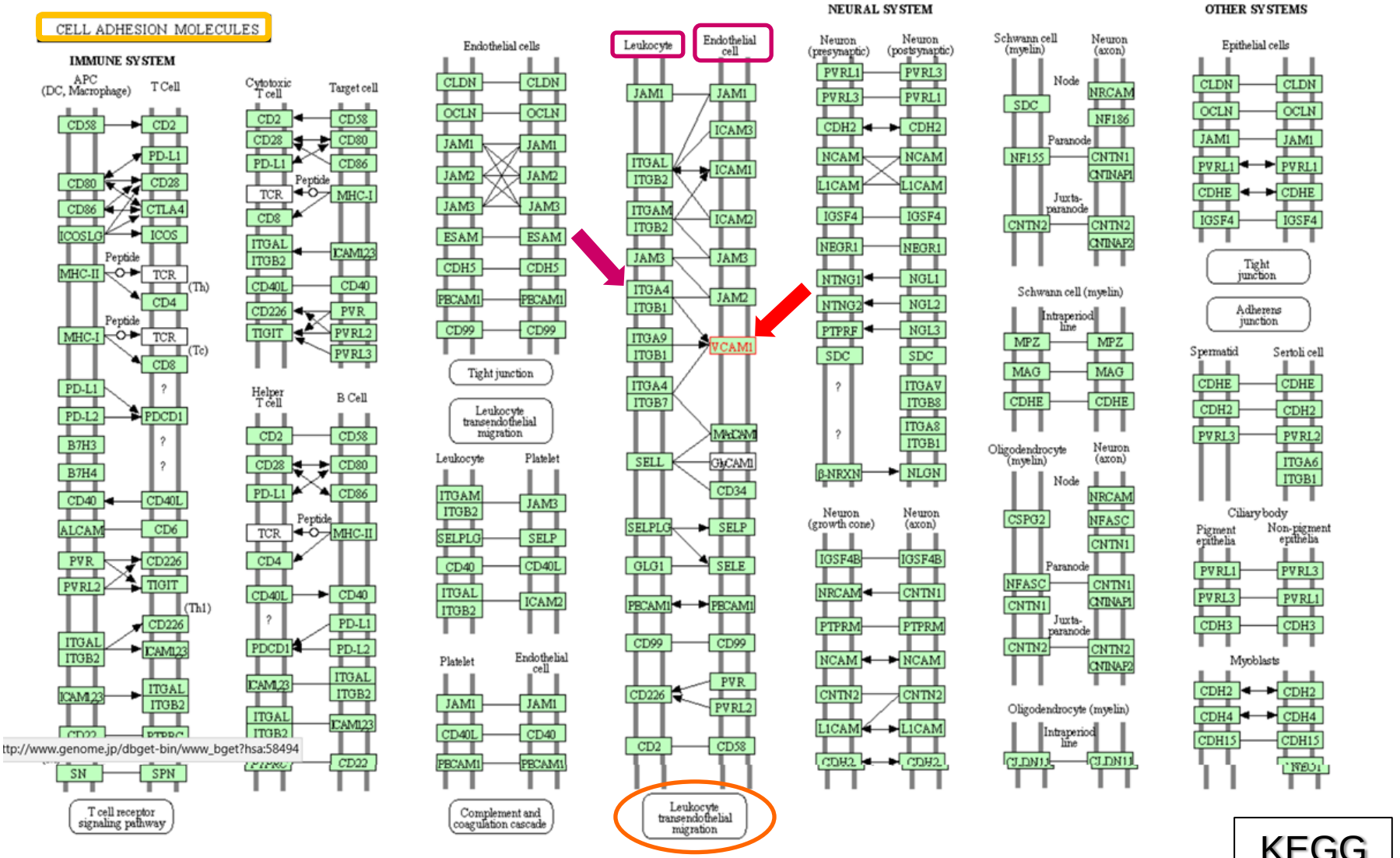
PDBJ: 3v4v




STRING v.10.5

- Cell-matrix adhesion involved in ameboidal cell migration
- Fibronectin binding
- Focal adhesion
- Leukocyte cell-cell adhesion
- Cell-matrix adhesion
- Integrin mediated signalling pathway
- Heterotypic cell-cell adhesion
- Positive regulation of leukocyte tethering or rolling
- Leukocyte migration
- Cell adhesion molecule binding
- Cellular response to cytokine stimulus
- Substrate adhesion-dependent cell spreading

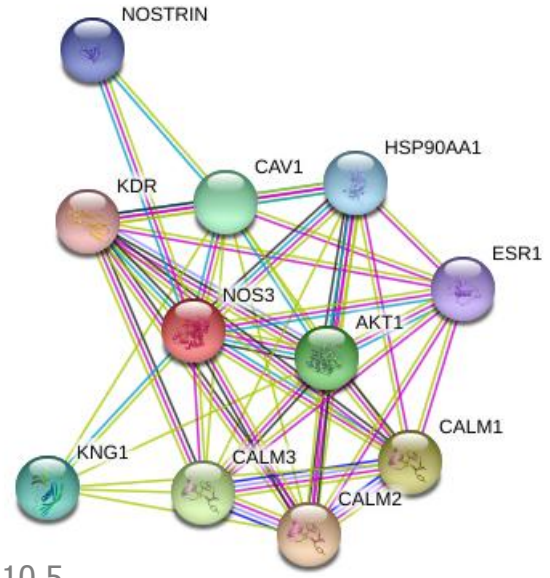
*Gene Ontology (GO)*


CELL ADHESION MOLECULES
IMMUNE SYSTEM
APC (DC, Macrophage)
T Cell
Cytotoxic T cell
Target cell
Helper T cell
B Cell
Peptide
(Th)
(Tc)
(Th1)
T cell receptor signaling pathway
Endothelial cells
Tight junction
Leukocyte transendothelial migration
Leukocyte
Platelet
Endothelial cell
Complement and coagulation cascade
Leukocyte
Endothelial cell
VCAM1
Leukocyte transendothelial migration
NEURAL SYSTEM
Neuron (presynaptic)
Neuron (postsynaptic)
Neuron (growth cone)
Neuron (axon)
Schwann cell (myelin)
Neuron (axon)
Node
Paranode
Juxta-paranode
Schwann cell (myelin)
Intraperiod line
Oligodendrocyte (myelin)
Neuron (axon)
Oligodendrocyte (myelin)
Intraperiod line
OTHER SYSTEMS
Epithelial cells
Tight junction
Adherens junction
Spermatid
Sertoli cell
Ciliary body
Pigment epithelia
Non-pigment epithelia
Myoblasts
KEGG

# eNOS

- Endothelial nitric oxide synthase (constitutive)
- Gene location: chr. 7 (q35)
  - 21 kb, 26 exons
- Homodimeric protein (1203 a.a.; 133,289 Da)
- Co-factors:
  - 5,6,7,8-tetrahydrobiopterin (BH4)
  - Flavin mononucleotide (FMN)
  - Flavin adenine nucleotide (FAD)
  - HEME
- Stimulated by Calcium/Calmodulin
- Inhibited by NOSIP and NOSTRIN

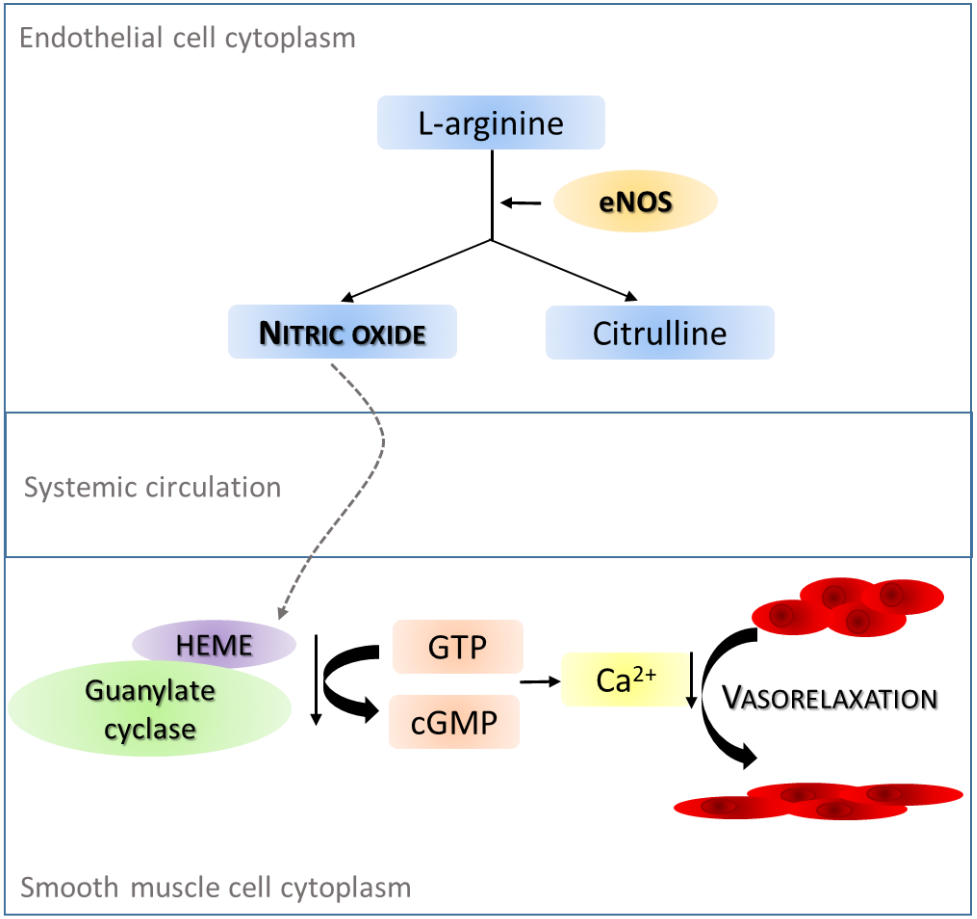
RCSB-PDB: 3NOS




STRING v.10.5

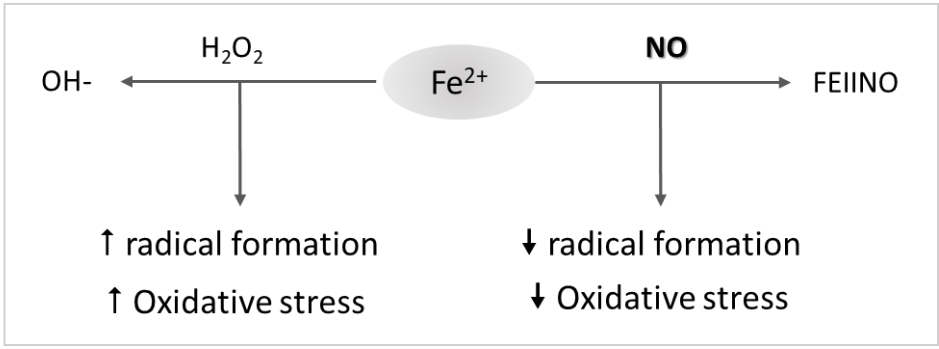
- Nitric oxide synthase activity
- Arginine binding
- Heme binding
- Blood vessel remodeling
- Cell redox homeostasis
- Endothelial cell migration
- Nitric oxide biosynthetic process
- Positive regulation of blood vessel diameter
- Regulation of blood pressure
- Regulation of blood vessel size
- Removal of superoxide radicals
- Response of fluid shear stress
- Vasodilation

*Gene Ontology (GO)*




## eNOS-DEPENDENT GENERATION OF NO

## CYTOPROTECTIVE EFFECT OF NO



# POTENTIAL GENETIC MODULATION IN SCA

**VCAM-1**

- Highly expressed on the surface of endothelial cells of blood vessels, following cytokine stimulation (inflammatory response)
- Sickle cells are prone to adhere to VCAM-1

**ITGA4**

- Expressed on the surface of leukocytes, stress reticulocytes and SSRBCs
- Surface cell ligand to VCAM-1
- Cell-endothelium and cell-cell adhesion (cell adhesion)

**eNOS**

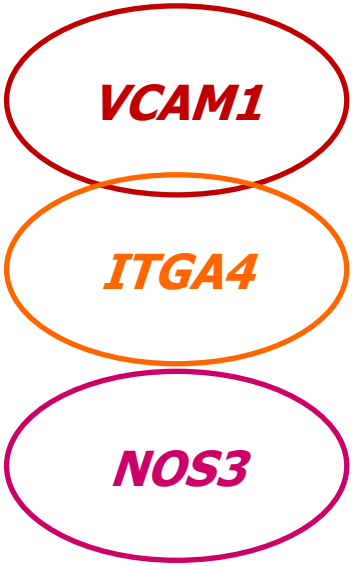
- produces nitric oxide (NO) in endotelial cells (bio-availability of NO)
- vasodilation (resting tonus) (arterial blood pressure regulation)
- Involved in platelet activation ⇢ hypercoagulation (vaso-occlusion)

# POPULATION SAMPLE

- 70 pediatric SCA patients (age ≥ 3 years)
- Cerebral vasculopathy - 3 main groups:
  - **Stroke:** ≥ 1 stroke event (n=14)
  - **Risk:** abnormal TCD and or MRI scan results (n=42)
  - **Normal**: no history of abnormalities in TCD or MRI scans (n=14)
- Database with clinical, imaging and laboratory data

# METHODOLOGY

- **PCR, PCR–RFLP**
- **Sanger sequencing**
- **Next-generation sequencing (NGS)**
  - LR-PCR (FailSafe – Epicentre)
  - Nextera XT® (Illumina)
  - MiSeq® (Illumina)




- **Bioinformatic analyses**
  - MiSeq® Reporter; BW; GATK; FastQC;
  - IGV; VEP;
- **Statistical analysis**
  - SPSS v.24 ($\chi^2$ and Fisher tests; FDR correction)
- **In silico analyses**
  - Haploview v.4.2
  - MatInspector; TFbind;
  - Human Splicing Finder v.3.0
  - Polyphen2

- 7 variants
  - 6 SNPs; 1 deletion
- 7 haplotypes (6 promoter variants)

***VCAM1***

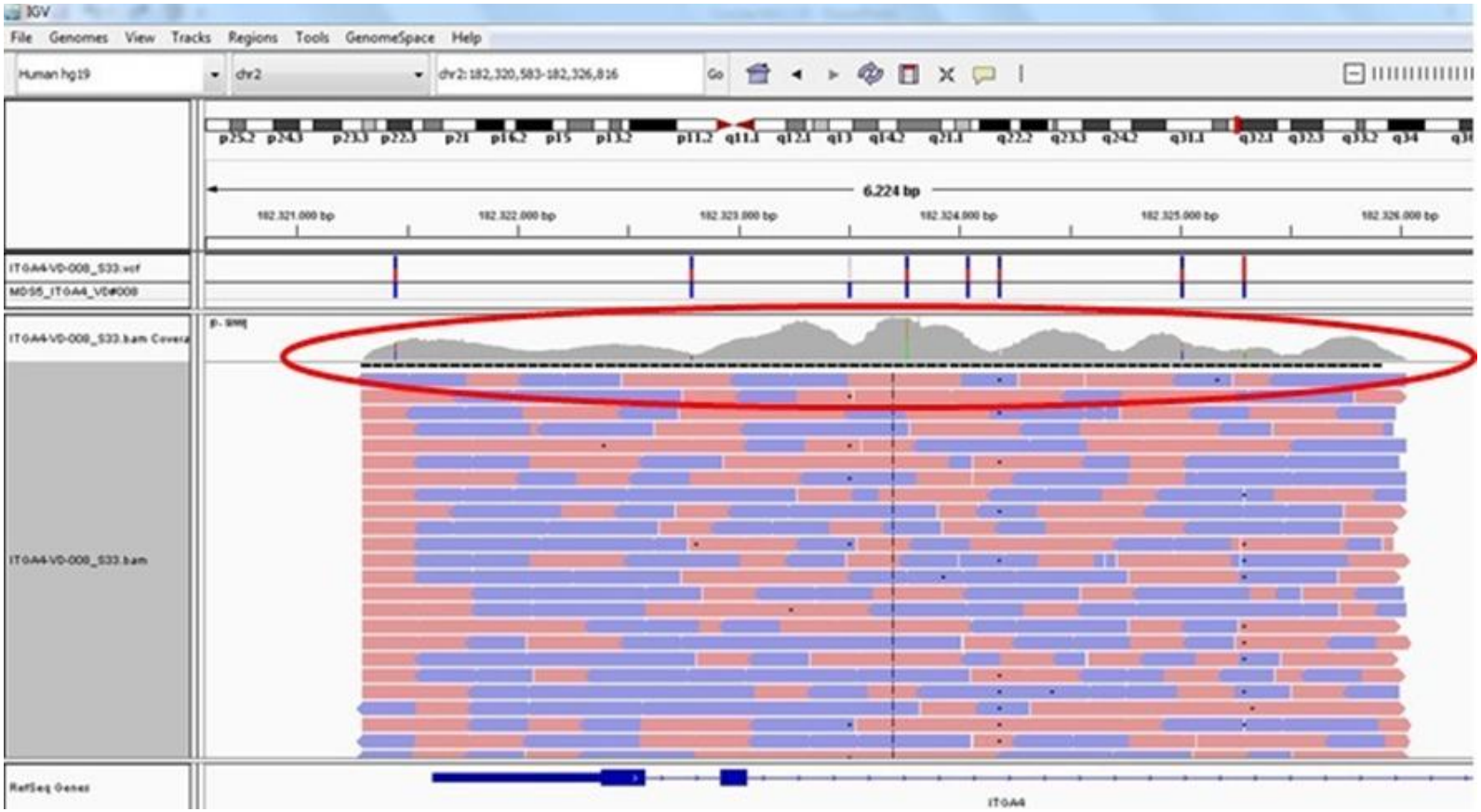
- 40 variants
  - 4 novel
  - 34 SNPs; 1 insertion; 5 deletions
  - 2 haplotypes
  - 32 validated

***ITGA4***

- 3 variants
  - 2 SNPs; 1 VNTR
- 6 haplotypes

***NOS3***

## *ITGA4* (NGS)


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
Human hg19
chr2
chr2:182,320,583-182,326,816
Go
6.224 bp
182.321.000 bp
182.322.000 bp
182.323.000 bp
182.324.000 bp
182.325.000 bp
182.326.000 bp
ITGA4-VD-008_S33.vcf
MOSS_ITGA4_VD#008
ITGA4-VD-008_S33.bam Covera
ITGA4-VD-008_S33.bam
RefSeq Genes
ITGA4

# ITGA4 VARIANTS (NGS)

# STATISTICAL ASSOCIATION

# Potential Modulation of Pediatric Stroke in SCA

***VCAM1 variant***

- **Altered TF binding site (position -1592; RXRF→PRDF)**
- **Increased *VCAM1* inducible expression**

→ Endothelial Dysfunction

***ITGA4 variants***

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

→ Cell – Endothelial Adhesion

→ **Vasculopathy**

***NOS3 variants***

- **Decreased NO bio-availability**
- **Decreased vascular tone**

→ Vasoconstriction

→ **Vaso-occlusion**

# FUTURE PROSPECTS

***In vitro* functional studies**

**Biomarker panel (diagnosis/prognosis)**

**e.g.: Gene Panel (NGS)**

***VCAM1, ITGA4, NOS3* – potential therapeutic targets**

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