Genetic Modifiers of Sickle Cell Disease Severity

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Outline

• Hb Molecule and Genetic control of globin synthesis
• Pathophysiology of SCD
• Complications of SCD
• Genetic modifiers of SCD severity
  • Haplotype
  • HbF
  • α-Thalassemia trait
• Other rare sickle compound heterozygotes
• Conclusions
α-globin genes

Chromosome 16

regulatory region

\(\zeta\) \(\alpha\) \(\alpha\)

α-LCR

Hemoglobin types

embryo \(\zeta_2\epsilon_2\) \n
fetus \(\alpha_2\gamma_2\)

adult \(\alpha_2\beta_2\)

β-LCR

Chromosome 11

regulatory regions

\(\epsilon\) \(\gamma_6\) \(\gamma_A\) \(\delta\) \(\beta\)

80 kb

β-globin genes
Sickle Cell Disease Spectrum

• HbSS – Sickle cell anemia
• Other rare genotypes
  • Sβ-thal
  • SC
  • SD
  • SO
  • SE
Figure 2 | Map of the estimated numbers of births with sickle cell anaemia. Estimated numbers of births with sickle cell anaemia per 100,000 births per country in 2015. Estimates are derived from prevalence data published in REF. 14. Birth data for 2015–2020 were extracted from the 2017 Revision of the United Nations World Population Prospects database. NA, not applicable.
Figure 3 | HbS polymerization and erythrocyte deformation. Long polymers of sickle haemoglobin (HbS) align into...

Kato et al. Nat Rev 2018;4:18010
Hypothetical Mechanisms of SCD Clinical Subphenotypes

Gladwin and Vichinsky NEJM 359:2254-2265, 2008
Genetic Modifiers of SCD Severity

- High Hb F determinants
  - Linked (β-globin haplotypes)
  - Unlinked (X-chromosome, others)
- β-globin haplotype
- α- thalassemia
- Other genetic factors
  - Coagulation related polymorphisms
  - Endothelial cell function
  - Inflammatory mediators
  - RBC membrane function
  - Others
Fig. 14.4 β gene cluster haplotypes linked to \( \beta^+ \) in Africa and the Middle East/Pakistan/India

At the top of the figure are shown the geographic distributions corresponding to the haplotypes described below. A haplotype is a particular array of polymorphic sites (that is, sites that vary among individuals), defined here by the capacity of endonuclease enzymes to recognize the short sequence and cut the DNA.
Beta S Haplotypes and Clinical Severity

• Target organ dysfunction is directly related to haplotype background
• The mildest disease is associated with the Arab/India (AI) and the Senegal (SEN) haplotypes
• The most severe disease is associated with the Central African Republic (CAR) haplotype
• Benin (BEN) and Cameroon (CAM) are intermediate
Hb F Levels and Clinical Severity

- The heterodimer ($\alpha_2\beta^S\gamma$) is more soluble than the homotetramer ($\alpha_2\beta^S_2$)
- Hb F of $\geq 20\%$ is associated with mild disease and decreased major organ complications
- Hb F $\geq 30\%$ is asymptomatic
Modifiers of Fetal Hemoglobin Levels

• Xmn-1
  • Polymorphism at -158C>T, in the $\gamma^G$-gene (Xmn1-HBG2 or rs782144, on chromosome 11p)
    • Accounts for 13 – 32% of the total F cell phenotypic variation in an on-anemic European population

• BCL11A
  • Transcriptional repressor of $\gamma$-globin gene; resides on a locus on chromosome 2
  • Discovered through GWAS studies showed an association between variants in BCL11A and higher HbF levels

• HBS1L-MYB
  • Variants in this intergenic region on chromosome 6 were also shown by GWAS to be associated with higher HbF levels
The Beta Globin Locus and Interacting Loci

Green and Barral, Pediatric Blood and Cancer 2010
Effects of Alpha Thalassemia on SCD

CELLULAR:
- Decreased MCHC
- Decreased Hb S polymer
- Decreased RBC cation loss
- Decreased RBC density
- Increased RBC deformability

HEMATOLOGICAL:
- Reduced Hemolysis
- Decreased reticulocyte count
- Increased Hb/Hct
- Decreased MCV
Effects of $\alpha$-Thal contd

**CLINICAL:**

- Increased:
  - Osteonecrosis
  - Splenic sequestration
  - Painful episodes (?)

- Decreased:
  - Cerebrovascular accidents
  - Leg ulcers

- Longevity
UDP-GLUCURONOSYL TRANSFERASE-1 (UGT1A1)

- UDP-Glucuronosyl transferase mediates the glucuronidation of bilirubin
- A common promoter polymorphism in the TATAA box is associated with decreased enzyme activity and indirect hyperbilirubinemia (Gilbert’s syndrome)
- A(TA)7TAA is associated with Gilbert’s
  - A(TA)6TAA: wild type
- Co-inheritance of (TA)7 polymorphism with hereditary hemolytic anemias (HS, β-thal, G-6-PD deficiency) causes marked hyperbilirubinemia and increased incidence of gallstones
# Genetic Modifiers Associated with SCD Complications

<table>
<thead>
<tr>
<th>Pain Events</th>
<th>Stroke</th>
<th>Avascular Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>• MBL2</td>
<td>• VCAM1</td>
<td>• KL</td>
</tr>
<tr>
<td>• VEGF</td>
<td>• TNFα</td>
<td>• BMP6</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>• ANXA</td>
<td>• BMP6</td>
</tr>
<tr>
<td><strong>Angiogenesis</strong></td>
<td>• TGFRP3</td>
<td><strong>Vit D Regulation</strong></td>
</tr>
<tr>
<td><strong>T. bru resistance</strong></td>
<td>• ENPP1</td>
<td><strong>Inflammation &amp; Bone formation</strong></td>
</tr>
<tr>
<td><strong>Cell integrity</strong></td>
<td><strong>Inflammation</strong></td>
<td><strong>Signal transduction</strong></td>
</tr>
<tr>
<td><strong>Cartilage formation</strong></td>
<td><strong>Hypercoagulability</strong></td>
<td><strong>Signal transduction</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nephropathy</th>
<th><strong>Cell adhesion</strong></th>
<th><strong>Transmembrane glycoprotein</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• APOL1</td>
<td>• VCAM1</td>
<td><strong>Inflammation &amp; Bone formation</strong></td>
</tr>
<tr>
<td>• MYH9</td>
<td>• TNFα</td>
<td><strong>Signal transduction</strong></td>
</tr>
<tr>
<td>• BMP</td>
<td>• ANXA</td>
<td><strong>Signal transduction</strong></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pulm. Hypertension</th>
<th><strong>Vit D Regulation</strong></th>
<th><strong>Signal transduction</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• ACVR</td>
<td>• KL</td>
<td><strong>Signal transduction</strong></td>
</tr>
<tr>
<td>• eNOS</td>
<td>• BMP6</td>
<td><strong>Signal transduction</strong></td>
</tr>
<tr>
<td>• ADRB1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td><strong>Cell adhesion</strong></td>
<td></td>
</tr>
<tr>
<td><strong>NO synthesis</strong></td>
<td><strong>Hypercoagulability</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Signal transduction</strong></td>
<td><strong>Transmembrane glycoprotein</strong></td>
<td></td>
</tr>
</tbody>
</table>
Other Rare Sickle Genotypes
HbSβ-Thalassemia

• Phenotype depends on the nature of the β-thalassemia mutation
  • Sβ0
    • No production of β-globin chain i.e. HbA
    • Usually associated with RNA processing, RNA translation and frameshift mutations
  • Sβ+ and Sβ++
    • Mild to moderate decrease in β-globin chain production
    • Usually associated with transcriptional, splice site and RNA cleavage mutations

• Wide variation in phenotypes
HbSC

• HbC: β6 Glu → Lys
• Prevalent in West Africa
• HbSC tends to be moderate – severe phenotype
• 16% of SCD patients in SW Nigeria
• Up to 47% in Mali
• May have a higher incidence of retinopathy and avascular necrosis
Distribution of hemoglobin level among HbSC population.

François Lionnet et al. Haematologica 2012;97:1136-1141
Hb D-Punjab or D-Los Angeles

- HbD: $\beta_{121}$ Glu $\rightarrow$ Gln
- Electrophoretic mobility similar to HbS on alkaline medium
- Separates easily on anion or cation HPLC
- Widely distributed but most frequent in Asia
- Hetero and homozygotes are clinically and hematologically normal
- HbSD tends to be quite severe because HbS polymerization is facilitated by the presence of HbD
## HbSD Phenotype

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation, months</td>
<td>24</td>
<td>24</td>
<td>11</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Hb F, %</td>
<td>24.6</td>
<td>24.2</td>
<td>22.0</td>
<td>26.5</td>
<td>23.3</td>
</tr>
<tr>
<td>Hb S, %</td>
<td>24.7</td>
<td>28.2</td>
<td>30.7</td>
<td>26.3</td>
<td>27.9</td>
</tr>
<tr>
<td>Hb D, %</td>
<td>48.5</td>
<td>45.5</td>
<td>45.9</td>
<td>44.5</td>
<td>46.3</td>
</tr>
<tr>
<td>Mode of presentation</td>
<td>pallor</td>
<td>LUQ pain</td>
<td>pallor</td>
<td>pallor</td>
<td>pallor</td>
</tr>
<tr>
<td>Acute splenic sequestration</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>VOC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acute osteomyelitis</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acute splenic infarction</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACS</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Avascular bone necrosis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

LUQ = Left upper quadrant.

Adekile et al, Acta Haemat 123(3), 2010
Conclusions

• SCD is very heterogeneous in phenotype
• There are important environmental factors (lifestyle, climate, infections, diet etc)
• Genetic factors (genotype, haplotype, HbF, α-thal trait)
• Polymorphisms in different genetic loci modulate the different sub-phenotypes
• More SNPs are being described with newer whole genome-wide techniques
Thank you