Deciphering the complexity of genotype-phenotype correlation in HbE-β-thalassemia disease

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Hemoglobin E

Beta26 [B8] Glu-Lys at external surface; GAG-AAG

Frequency in Thailand 13% (up to 50% in Northeast)

Moves like Hb A₂ on electrophoresis

Abnormal mRNA splicing: heterozygote has 30% Abn

HbE/HbA: normal, slight microcytosis (MCV 80 fl), 25-30% HbE

HbE/HbE: microcytosis, very mild anemia, >90% HbE

Hb E/β⁺-Thalassemia: mild anemia, hepatosplenomegaly, 40-60% Hb E, 10-20% HbF, 30-40% HbA

HbE/β⁰-Thalassemia: moderate to severe anemia, hepatosplenomegaly, variable Hb content 2.6 - 13.3 g/dl (av 7.7 g/dl), 40-70% HbE, 30-60% Hb F, no Hb A

Hemoglobin E

Hemoglobin E heterozygote

Homozygous hemoglobin E

Beta-thalassemia/hemoglobin E

Beta-thal/Hb E post-splenectomy

Starch Gel Electrophoresis

Hb E

Hb F

Hb E

Hb A

Hb E

Hb A2
Variable severity in β-thalassemia disorder

Normal Hemoglobin
12 to 14 g/dl

Intermediat

Mild

Severe

Number of Patients

Hemoglobin (g/dl) (mean ± SD = 7.7 ± 1.55 g/dl)

Fucharoen S et alBirth Defects 1988; 23(5A): 241-8
Thalassemia: Genotype-phenotype Interaction

1. Better understanding of gene-gene interaction, natural history, prognosis
2. Decision of therapeutic intervention eg. BM transplantation
3. Prenatal diagnosis and induced abortion
4. New therapeutic intervention
Anemia in β-Thalassemia

β-thalassemia gene

↓

Decrease β-globin chain synthesis

↑

Low hemoglobin production

Excess unbound α-globin chains

Precipitation of α-hemoglobin

Erythroblast

Apoptosis

Ineffective erythropoiesis

Erythrocyte

Hemolysis

Short RBC survival

ANEMIA

HbA (α₂β₂)

HbE (α₂β²)

MAHIDOL UNIVERSITY
Wisdom of the Land
Anemia in β-Thalassemia

Normal

HbE/β-thalassemia

Heterozygous β0-thalassemia

α β δ γ

No Clinical Symptom
Hb 11.2 ± 1.6 g/dL

Mild to Severe
Hb 3 – 12 g/dL
# HbE/β-Thalassemia Severity Scoring System

<table>
<thead>
<tr>
<th>Clinical Criteria</th>
<th>Points Scored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Hb at steady state &gt; 7.5 g/dL</td>
<td></td>
</tr>
<tr>
<td>Age at 1st transfusion &gt; 10 yr</td>
<td></td>
</tr>
<tr>
<td>Requirement for transfusion None/Rare</td>
<td></td>
</tr>
<tr>
<td>Size of spleen &lt; 3 cm</td>
<td></td>
</tr>
<tr>
<td>Age at presentation &gt; 10 yr</td>
<td></td>
</tr>
<tr>
<td>Growth development &gt; 25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;-25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity Group</th>
<th>Total Score Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>0 – 3.5</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 – 7</td>
</tr>
<tr>
<td>Severe</td>
<td>7.5 – 10</td>
</tr>
</tbody>
</table>

**Modifiers of HbE/β-Thalassemia**

**Heterozygous β^0-thalassemia**
No Clinical Symptom
Hb 11.2 ± 1.6 g/dL

**HbE/β^+ -thalassemia**
Mild
Hb 3 – 12 g/dL

**HbE/β^0-thalassemia**
Mild to Severe
Hb 3 – 12 g/dL
# HbE/β⁺-Thalassemias Display Mild Symptoms

<table>
<thead>
<tr>
<th>Race</th>
<th>No. (HbE/β⁺-thal)</th>
<th>Hb level (g/dL) Mean ± SD (range)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thai</td>
<td>32 (nt-28, A&gt;G)</td>
<td>9.7 ± 1.2 (7.6 – 12.1)</td>
<td></td>
</tr>
<tr>
<td>Thai</td>
<td>32</td>
<td>10.3 ± 1.8 (9.4 – 11.1)</td>
<td></td>
</tr>
</tbody>
</table>

Hb typing in non-transfusion HbE/β⁺-thalassemia:

- **HbA** = 19.0 ± 4.7 %
- **HbE** = 62.2 ± 6.2 %
- **HbF** = 15.4 ± 7.4 %

Modifiers of HbE/β-Thalassemia

Coinheritance of α-thalassemia

αα/αα  1 α-gene defect  2 α-genes defect  3 α-genes defect  ααα/αα

Mild to Severe Hb 3 – 12 g/dL  Mild (expected)  Severe
# HbE/β⁰-Thalassemias Coinherit with α-Thalassemia

<table>
<thead>
<tr>
<th>α-Globin Genotyping</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα (n=987)</td>
<td>268 (27.2%)</td>
<td>310 (31.4%)</td>
<td>409 (41.4%)</td>
</tr>
<tr>
<td><strong>α-Thalassemia (n=109)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−α³.7/αα (n=70)</td>
<td>65 (92.9%)</td>
<td>5 (7.1%)</td>
<td>0</td>
</tr>
<tr>
<td>−α⁴.2/αα (n=8)</td>
<td>6 (75.0%)</td>
<td>2 (25.0%)</td>
<td>0</td>
</tr>
<tr>
<td>αCSα /αα (n=19)</td>
<td>18 (94.7%)</td>
<td>1 (5.3%)</td>
<td>0</td>
</tr>
<tr>
<td>αPSα /αα (n=2)</td>
<td>2 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>−α³.7/−α³.7 (n=2)</td>
<td>2 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>−−SEA/αα (n=8)</td>
<td>7 (87.5%)</td>
<td>1 (12.5%)</td>
<td>0</td>
</tr>
<tr>
<td>αααanti³.7/αα (n=4)</td>
<td>0</td>
<td>0</td>
<td>4 (100%)</td>
</tr>
</tbody>
</table>

**HbE/β⁰-Thalassemias Coinherit with α-Thalassemia**

![Gene defects]

<table>
<thead>
<tr>
<th>No.</th>
<th>1 α-globin gene defect</th>
<th>2 α-globin genes defect</th>
<th>Triplicated α-globin gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>3</td>
<td>4 (splenec)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>10.9 ± 9.5 yr</th>
<th>7, 29, 35</th>
<th>10.8 ± 3.4 yr</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Transfusion requirement</th>
<th>None</th>
<th>Rare</th>
<th>Occasional</th>
<th>Frequent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at 1st transfusion</td>
<td>19.4 ± 13.2 yr</td>
<td></td>
<td>2.3 ± 1.2 yr</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Splenectomy</th>
<th>100%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Growth</th>
<th>86%</th>
<th>1st-degree retardation 50%</th>
<th>2nd-degree retardation 50% (Sripichai et al, 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100%</td>
<td>Normal 100%</td>
<td>1st-degree retardation 50%</td>
</tr>
<tr>
<td>86%</td>
<td>14%</td>
<td>1st-degree retardation 50%</td>
<td>2nd-degree retardation 50%</td>
</tr>
</tbody>
</table>
### HbE/β0-Thalassemias Coinherit with α-Thalassemia

<table>
<thead>
<tr>
<th></th>
<th>αα/αα</th>
<th>1 α-gene defect</th>
<th>2 α-genes defect</th>
<th>3 α-genes Defect (EFBart’s disease)</th>
<th>Triplicated α-gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
<td>350</td>
<td>78</td>
<td>3</td>
<td>6†</td>
<td>4 (splenec)</td>
</tr>
<tr>
<td><strong>Hb (g/dL)</strong></td>
<td>7.1 ± 1.4 (3.4 – 12.1)</td>
<td>8.1 ± 1.2 (5.4 – 10.8)</td>
<td>8.0, 9.2, 10.6</td>
<td>7.9 ± 2.1 (6.4 ± 0.3) (6.1 – 6.7)</td>
<td></td>
</tr>
<tr>
<td><strong>RBC (x12^{10}/L)</strong></td>
<td>3.8 ± 0.7 (2.0 – 5.9)</td>
<td>5.0 ± 0.6 (3.3 – 6.4)</td>
<td>4.7, 5.7, 6.4</td>
<td>2.6 ± 0.2 (2.4 – 2.8)</td>
<td></td>
</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>18.5 ± 2.1 (11.9 – 21.9)</td>
<td>17.0 ± 1.5 (14.5 – 21.6)</td>
<td>16.1, 17.0, 17.1</td>
<td>16.5 ± 0.3 (24.9 ± 1.0) (23.6–26.0)</td>
<td></td>
</tr>
</tbody>
</table>

Modifiers of HbE/β-Thalassemia

Induction of fetal hemoglobin (HbF)

Mild to Severe
Hb 3 – 12 g/dL
**HbE/β⁰-Thalassemia Cohort (β⁰E/β⁰; αα/αα, n = 968)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>236 (24%)</td>
<td>317 (33%)</td>
<td>415 (43%)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>20.8 ± 14.6</td>
<td>22.6 ± 15.5</td>
<td>14.7 ± 9.7</td>
</tr>
<tr>
<td>Requirement for transfusion</td>
<td>None 49% Regular 5%</td>
<td>None 1% Regular 45%</td>
<td>None 0% Regular 93%</td>
</tr>
<tr>
<td>Age at 1ˢᵗ transfusion</td>
<td>0 – 4 yr 5%</td>
<td>0 – 4 yr 32%</td>
<td>0 – 4 yr 80%</td>
</tr>
<tr>
<td>Size of spleen</td>
<td>&lt; 3 cm 30% Splenectomy 2%</td>
<td>&lt; 3 cm 14% Splenectomy 32%</td>
<td>&lt; 3 cm 3% Splenectomy 59%</td>
</tr>
<tr>
<td>Growth development</td>
<td>&lt; 3ʳᵈ percentile 10%</td>
<td>&lt; 3ʳᵈ percentile 21%</td>
<td>&lt; 3ʳᵈ percentile 50%</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>7.7 ± 1.3</td>
<td>5.8 ± 1.5</td>
<td>4.5 ± 1.4</td>
</tr>
<tr>
<td>Fetal hemoglobin (HbF)</td>
<td>3.1 ± 1.2 g/dL 41 ± 11%</td>
<td>2.0 ± 0.9 g/dL 35 ± 11%</td>
<td>1.4 ± 0.7 g/dL 31 ± 11%</td>
</tr>
</tbody>
</table>

HbF-QTLs (Fetal Hemoglobin-Quantitative Trait Loci)

β-Thalassemia/HbE cohort displayed a normal distribution of HbF level.

baseline HbF = 10.5 - 77.7%
mean±SD = 34.4 ± 12.2%
(n = 1330)


HbF-QTLs

Chromosome 11p15; β-globin gene cluster
Chromosome 2p16.1; BCL11A
Chromosome 6q23; HBS1L-MYB intergenic region
Etc.
Association Studies

- Detect association between genetic variants and phenotype across families
  - Case-Control designs
  - Cohort designs

**Single Nucleotide Polymorphisms: SNPs**

- Biallelic polymorphism
- Accounting for ~90% of human genetic variants
- Occurring on coding and non-coding chromosome region

SNP markers associated with modifier alleles
'Omic Data from beta-thalassemia/HbE Study

GWAS

Data Analysis

Microarray

Bioinformatics & Biomarker

Candidate Genes

Functional Study
Genomewide Association in β-Thalassemia/HbE

1300 β-Thalassemia/HbE

425 MILD
N = 235
HbF 43±12 %
3.4±1.2 g/dL

380 MODERATE

495 SEVERE
N = 383
HbF 32±12 %
1.9±0.9 g/dL

- 110,000 gene-based SNPs (Sequenom MassARRAY)
- 548,094 SNPs (Illumina 610-Quad BeadChips)

GWAS Mild vs. Severe β-Thalassemia/HbE

HbF-QTLs ; β-Globin Gene Cluster

BCL11A
Chromosome 2p16.1

HBS1L-MYB
Chromosome 6q23

OR gene cluster
Chromosome 11p15

HBB gene cluster
Chromosome 11p15

-\log_{10} (P \text{ minimum})

\text{XmnI}-^{G\gamma} \text{ polymorphism}

β-globin mutations
**HbE/β⁰-Thalassemias & XmnI-Gγ Polymorphism**

<table>
<thead>
<tr>
<th>XmnI-Gγ polymorphism</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patient = 1040</td>
<td>347 (33%)</td>
<td>290 (29%)</td>
<td>403 (38%)</td>
</tr>
<tr>
<td>TT, +/+ (6%)</td>
<td>41 (69%)</td>
<td>10 (17%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>TC, +/- (70%)</td>
<td>250 (34%)</td>
<td>216 (30%)</td>
<td>261 (36%)</td>
</tr>
<tr>
<td>CC, +/- (24%)</td>
<td>56 (22%)</td>
<td>64 (25%)</td>
<td>134 (53%)</td>
</tr>
</tbody>
</table>

HbF-QTLs; β-Globin Gene Cluster

Polymorphisms on β-globin gene cluster associated with β-hemoglobinopathies clinical severity and HbF levels, but its regulating molecular mechanisms still unknown.

HbF-QTLs; BCL11A

BCL11A
(B-cell CLL/lymphoma 11A)
zinc finger protein
HbF Repressor

BCL11A shRNA

Control  shRNA 1  shRNA 2

BCL11A SNP rs766432

Percentage HbF

Absolute HbF (g/dL)

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>520</td>
<td>207</td>
<td>23</td>
</tr>
</tbody>
</table>

HbF-QTLs ; HMIR

HBS1L
(HBS1-like translational GTPase)
Unknown function in erythroid

MYB
(MYB proto-oncogene)
Control erythroid differentiation

HBS1L-MYB intergenic region (HMIR)
### Fetal Hemoglobin Analysis

<table>
<thead>
<tr>
<th>Locus</th>
<th>rs ID</th>
<th>SNP allele</th>
<th>High-HbF allele</th>
<th>Linear regression analysis (Additive model, P value)</th>
<th>Number of high-HbF allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL11A</td>
<td>rs766432</td>
<td>A/C</td>
<td>C</td>
<td>HbF (%) 2.33E-07, Abs HbF (g/dL) 1.20E-09</td>
<td>AA  AC  CC</td>
</tr>
<tr>
<td>HBS1L-MYB</td>
<td>rs9399137</td>
<td>A/G</td>
<td>G</td>
<td>HbF (%) 2.76E-18, Abs HbF (g/dL) 5.93E-19</td>
<td>AA  AG  GG</td>
</tr>
<tr>
<td>HBG2(XmnI)</td>
<td>rs7482144</td>
<td>C/T</td>
<td>T</td>
<td>HbF (%) 2.52E-19, Abs HbF (g/dL) 1.90E-23</td>
<td>CC  CT  TT</td>
</tr>
</tbody>
</table>
Regulation of Globin Gene Expression
Regulation of Erythropoiesis

Transcription factors, small RNAs, and DNA-binding factors:

- miR-150
- Myc
- Myb
- Pu.1
- Dot1L
- GATA2
- HiF1α
- GC
- Smad5
- SCL/Tal1
- GATA2
- GATA1
- SCL/Tal1
- FOX
- Lmo2
- Ldb1
- miR-223
- miR-144
- miR-451
- Rek3
- Mxi1
- miR-191

Growth Factors:

- IL3, SCF, GM-CSF
- IL3, SCF, GM-CSF, BMP4, +/-Epo
- Epo

Stem Cells → CFU-GEMM → BFU-Es → CFU-Es → Erythroblasts → Extruded nuclei
Conclusion: Genotype-phenotype Interaction HbE/β-Thal

1. Three known factors namely: β-thalassemia mutation, α-thalassemia gene interaction, genes/factors regulate Hb F production

2. There may be some other genes/factors affect disease severity in β-thalassemia

3. Understanding these modifiers are very important for decision of therapeutic intervention such as PND and termination of pregnancy

4. Future manipulation of such modifiers may lead novel treatment for β-thalassemia
HbE/β-Thalassemia Disease Heterogeneity

~1985 versus 2005

HbE/β-Thalassemia Disease Modifying Factors

**Primary Modifying Factors**
- β-thalassemia mutation
- β^E^-hemoglobin synthesis (level of β-globin)

**Secondary Modifying Factors**
- Coinheritance of α-thalassemia (level of α-globin)
- Level of Fetal hemoglobin (level of γ-globin)

**Tertiary Modifying Factors**
- ? UGT1A
- ? HFE
- ? APOE
- ? DM
- etc.
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Dr. Pranee Fucharoen

Thalassemia Research Center Staff

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Nittaya Wisanuyothin, MD
Kesada Chandsawang, MD
Soraya Dhamarakf, MD
Ladda Sriborriboon, MD
Chuchat Koosirirat, MD
Thalassemia Conference
October 31- Nov2, 2018
Guangxi Medical University, Nanning

- To inaugurate Asian Thalassemia Training Center in Nanning, Guangxi
- No registration fee
- Call for abstract for poster presentation

- Contact Email:
  Dr. Chen Ping: ping [cping62@hotmail.com]
  Dr. Suthat Fucharoen: [suthat.fuc@mahidol.ac.th]
ASEAN Thalassemia Federation Conference
May, 2019
Kuching, Sarawak, Malaysia

The conference will compose of 3 major activities:

- Scientific program for professional people
- Lecture for patients and parents
- Social program for patients

- Contact Email:
  Dunstan Chan [dunstan.desee@gmail.com]
  Khoo Swee Hong [sweehong_khoo@hotmail.com]