Pharmacogenomics for hemoglobinopathies

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Hemoglobinopathies

Most commonly used therapeutic modalities for β-thalassemia are frequent blood transfusions, bone marrow transplantation and pharmacological reactivation of fetal hemoglobin (Hb F) production.
Human β-globin gene switching in humans and mice
Developmental regulation of the human β-globin locus

Primitive cells
Embryo

Definitive cells
Fetus

Definitive cells
Adult

LCR
Model depicting human ε- / γ-globin genes silencing

Genetic etiology for the persistent fetal hemoglobin production in the adult

1. Hereditary Persistence of Fetal Hemoglobin (HPFH)
   α. Deletional type: Large deletions in the β-globin cluster
   β. Non-deletional type: Point mutations in the human fetal globin (HBG2 and HBG1) genes.
   Hb F: 5-30%, Hb A₂<2%, pancellular Hb F distribution

2. δβ-Thalassemia
   Large deletions in the human β-like globin genes.
   Hb F: 8-20%, Hb A₂<2%, heterocellular Hb F distribution

3. Quantitative trait loci (QTLs), outside the human β-globin gene cluster (HBS1L, MYB, BCL11A).
   Hb F: 5-25%, Hb A₂ normal, heterocellular Hb F distribution
Identification of genes involved in human fetal globin gene regulation

1. Pharmacogenomic analysis of β-hemoglobinopathies patients responding or not to fetal hemoglobin augmenting therapy.

2. Genomic analysis of tissues expressing or not fetal hemoglobin, such as fetal liver, umbilical cord blood or adult peripheral blood, respectively.

3. Genome-wide association studies to identify genes or genomic loci correlated with increased fetal hemoglobin levels using large families or large groups of unrelated individuals.
Pharmacogenomics analysis rationale

1. A substantial number of β-thalassemia patients present with very mild or no symptoms without any transfusions, contrary to the majority of β-thalassemia patients, bearing the SAME β-thalassemia-causing mutation, and occasionally under an IDENTICAL chromosomal background.

2. Similar observations are made for sickle cell anemia patients bearing a different chromosomal background.
Multicentric origin of sickle cell anemia

Patients with mild phenotype and high Hb F levels

Patients with severe phenotype and low Hb F

Patrinos GP, Antonarakis SE. Human Genetics, 4th ed., 2010
Pharmacogenomics analysis rationale

1. A substantial number of β-thalassemia patients present with very mild or no symptoms without any transfusions, contrary to the majority of β-thalassemia patients, bearing the SAME β-thalassemia-causing mutation, and occasionally under an IDENTICAL chromosomal background.

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3. 25% of HU- and butyrate-treated β-thalassemia patients and 20% of sickle cell anemia patients fail to respond to their pharmacological treatment with Hb F inducing drugs.

AIM of the study: To correlate individual genetic constitution with response and tolerance to commonly used Hb F inducing agents, i.e., HU and butyrate.
Study design

1. Recruitment of β-thalassemia patients, categorized as “Responders” and “Non-responders” to HU, based on the levels of Hb F induction.

2a. Correlation of pharmacological induction of Hb F with SNPs in (a) the human β-globin gene cluster, and (b) the entire genome.

2b. Whole-genome transcription profiling of (a) responders and non-responders to HU treatment and (b) human Hb F expressing or non-expressing tissues.

3. Confirmation of those genes found to be differentially expressed using functional assays, including reporter cell lines transduced with shRNAs.
Study design

(a) Recruitment of β-thalassemia and SCD patients that do or do not respond to HU treatment
(b) Isolation of human tissues expressing (fetal liver, umbilical cord blood) or not (peripheral adult blood) Hb \( F \)

1. Determination of the growth curve and cell morphology
2. Total hemoglobin measurement
3. CX-HPLC of hemoglobin fractions
4. Genome-wide transcription profiling and SNP scoring
Differences in cell growth

Normal | Responder | Non-responder

- HU

+ HU
Differences in cell growth

Hematopoietic progenitor cells from HU-responding patients grow BETTER in the presence of HU.
Hierarchical clustering of β-thalassemia patients under HU-treatment
Transcription profiling of tissues that do or do not express fetal hemoglobin

Adult blood vs Fetal liver

Adult vs Umbilical cord blood
Transcription profiling of tissues that do or do not express fetal hemoglobin
**Previous pharmacogenetic studies for HU treatment**

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A putative pharmacogenetic marker in the human β-globin gene cluster

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**In vitro functional assays**

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Supershift GATA-1 →
GATA-1 →

Persistent high HbF levels (3.3-20%) in a consanguineous Maltese family

Joseph Borg, Alex Felice
Mapping of the candidate region on chrom. 19

Joseph Borg, Annemieke Verkerk
KLF1 is found to be correlated with high HbF levels
Carriers for the KLF1 mutation cluster separately from their wild-type relatives
Confirmation of differentially expressed genes based on qPCR
Knockdown of KFL1 gene results in increased fetal hemoglobin levels in erythroid cells

Joseph Borg*, Petros Papadopoulos*, Marianna Georgitsi* et al., Submitted, 2010
Overexpression of the full-length but not the truncated KLF1 in erythroid cells grown from the Maltese family members results in a sharp decrease of HbF levels.

Joseph Borg*, Petros Papadopoulos*, Marianna Georgitsi* et al., Submitted, 2010
Interestingly, there is an inverse correlation between BCL11A gene levels, a known suppressor of fetal globin gene transcription and fetal globin gene levels.
KLF1 binds directly to the *BCL11A* gene promoter.
KLF1 has a dual regulatory role in β-like globin gene transcription

(a) KLF1 directly activates $HBB$ gene transcription; 
(b) KLF1 indirectly suppresses fetal-globin genes by activating BCL11A

Joseph Borg*, Petros Papadopoulos*, Marianna Georgitsi* et al., Submitted, 2010
Conclusions

1. Proerythroblast cell cultures from responders to HU treatment grow better in the presence of HU, compared to non-responders.

2. Responders to HU treatment display a distinct transcription profile compared to non-responders.

3. Similarly, human tissues expressing Hb F (fetal liver, umbilical cord blood) also display a distinct transcription profile compared to tissues that do not express Hb F (adult peripheral blood).

4. Linkage analysis in members of the Maltese family revealed that KLF1 is a key regulator of human fetal globin gene transcriptional silencing.

5. Ongoing functional studies are expected to identify novel genes that modulate HbF levels that would possibly yield novel therapeutic modalities for Hb-pathies.
Acknowledgements

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