بسم الله الرحمن الرحيم
"Association between BCL11A, HSB1L-MYB and Xmnl G-158 (C/T) genetic polymorphisms and hemoglobin F level in Egyptian sickle cell disease patients"

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• Sickle cell disease (SCD) is a Mendaliam disorder caused by genetic alteration in the beta globin gene leading to amino acid substitution and subsequently production of abnormal hemoglobin (HbS)

• Clinical severity of SCD is extremely variable, and reasons for this heterogeneity are not fully understood.

• As a consequence of these complications, SCD patients have increased mortality and mortality.
Although risk factors underlying these complications are not well characterized, higher expression of fetal hemoglobin (HbF) ameliorates morbidity and mortality in SCD.
Many studies suggested that disease complications most closely linked to sickle vaso-occlusion and blood viscosity were robustly related to HbF concentration while complications associated with the intensity of hemolysis were less affected.
• Increased levels of fetal hemoglobin (HbF, α2 γ2) may reduce the clinical severity of SCD due to its ability to inhibit HbS polymerization and reduce the mean corpuscular HbS concentration.

• Inter-individual variation in hemoglobin F (HbF) level is likely one of the main modifiers that contribute to the clinical heterogeneity observed in SCD patients.
• Extensive observations on the natural history of SCD have led to efforts to stimulate HbF expression to treat patients.
• Experimental and clinical work in this area have led to the use of Hydroxyurea, an agent that was found empirically to increase the production of HbF.
• As HbF levels vary considerably between individuals (populations) are a highly heritable trait, it is expected that identification of genetic polymorphisms that modulate HbF levels will shed light on molecular mechanisms that control HbF production.

• Such insights might ultimately identify novel drug targets for new treatments of SCD.
Recently, human genetic approaches have provided insight into previously unappreciated regulators of the fetal-to-adult hemoglobin switch and HbF silencing, revealing molecular targets to induce HbF.
Identification of regulators of HbF expression is extremely promising and suggests that rationally designed approaches targeting mechanisms mediating this switching process could lead to better, less toxic, and more effective strategies for HbF induction.
Studies were performed to find common genetic variants associated with variations in HbF levels and showed association of variants at 3 major genomic loci with HbF levels:

- The globin locus on chromosome 11
- A region between the genes HBS1L and MYB on chromosome 6
- A region within the BCL11A gene on chromosome 2
AIM OF WORK
Our study aimed at investigating the prevalence of BCL11A (rs11886868), HSB1L-MYB (rs9389268) and XmnI γG-158 (C/T) genetic polymorphisms in a cohort of Egyptian SCD patients and to clarify the possible association between this polymorphism and HbF level before and after Hydroxyurea (HU) therapy.
study population: 100 SCD patients & 100 controls

All patients were subjected to the following:

Patient history taking & clinical examination

Laboratory workup

I) Routine Workup  

ii) Special investigation
Sample collection:

From each patient, 4-5 ml venous blood were obtained by a sterile venipuncture into a sterile ethylene diamine tetraacetic acid (EDTA) vacutainer.

Samples were stored at -20°C till the analysis was performed.
I) DNA Extraction:

- Sample
- Lyse
- Bind
- Wash
- Elute

Pure Genomic DNA
II) Amplification of the extracted DNA:

- DNA
- Taq
- Primers
- dNTPs
- MgCl$_2$
- Buffer

Compared to Thermal cycler
Agarose gel electrophoresis
RESULTS
Baseline HBF

N = 100
Min = .00
Max = 37.90
Mean = 13.557
Std. Dev. = 8.8171

Steady state HbF

N = 100
Min = .00
Max = 39.10
Mean = 22.426
Std. Dev. = 9.2855
Genotyping of HSB11-MYB in SCD patients and controls

- Controls (n=100)
- SCD patients (n=100)

Wild genotype
Heteromutant
Homomutant
Genotyping of BCL11A in SCD patients and controls

- Controls (n=100)
- SCD patients (n=100)

- Wild genotype
- Heteromutant
- Homomutant
Genotyping of HSB11-MYB in SCD patients and controls

- Controls (n=100)
- SCD patients (n=100)
HbF level in HSB1L-MYB genotypes
Fold change for HbF level after HU therapy

<table>
<thead>
<tr>
<th>Genotyping of HSB1L</th>
<th>Wild genotype</th>
<th>mutant</th>
<th>P value</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Median</td>
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<tr>
<td>Baseline HBF</td>
<td>12.55</td>
<td>6.50</td>
<td>12.40</td>
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<td>Steady state HbF</td>
<td>21.06</td>
<td>8.79</td>
<td>20.10</td>
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<td>Count</td>
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<tr>
<td>HBF percent change</td>
<td>&gt;10%</td>
<td>41</td>
<td>87.2%</td>
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<tr>
<td></td>
<td>&lt;10%</td>
<td>6</td>
<td>12.8%</td>
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HbF level in BCL11A genotypes

- Baseline HBF: The boxplot shows a comparison between Wild genotype and Heteromutant genotypes. The Wild genotype has a lower median baseline HBF compared to the Heteromutant.

- Steady state HBF: The boxplot compares Wild genotype and Heteromutant genotypes. The Heteromutant genotype has a higher median steady state HBF compared to the Wild genotype.
Fold change in HbF level after HU therapy

<table>
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<th>Genotyping of BCL11A</th>
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<th>Heteromutant</th>
<th>P value</th>
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<td>Baseline HBF</td>
<td>12.88</td>
<td>6.09</td>
<td>13.15</td>
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<td>Steady state HBF</td>
<td>20.08</td>
<td>5.17</td>
<td>19.60</td>
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<td>&gt;10%</td>
<td>16</td>
<td>80%</td>
<td>65</td>
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<td>&lt;10%</td>
<td>4</td>
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HbF level in Xmn1 genotypes
Fold change for HbF level after HU therapy

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<td>Wild genotype</td>
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<tr>
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<td>SD</td>
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<td>Minimum</td>
<td>Maximum</td>
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<td>Baseline HBF</td>
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<td>Steady state HBF</td>
<td>22.27</td>
<td>9.34</td>
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<th>P value</th>
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<td>Count</td>
<td>%</td>
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<td>%</td>
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<tr>
<td>&gt;10%</td>
<td>75</td>
<td>80.6%</td>
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<td>&lt;10%</td>
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<td>19.4%</td>
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<td>16.7%</td>
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Combined genotypes analysis and HbF level
• The distribution of the studied SNPs did not differ between SCD patients and controls except for the heteromutant genotypes of *BCL11A* which was significantly higher in SCD patients.

• *HSB1L* and *BCL11A* genetic polymorphisms has no positive influence on HbF level.

• Xmn1 polymorphism has positive influence on baseline HbF level.
Research Team

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