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Genetic variants at HbF-modifier loci moderate anemia and leukocytosis in sickle cell disease in Tanzania

Siana Nkya Mtatiro,1,2† Julie Makani,1,3 Bruno Mmbando,1 Swee Lay Thein,4,5 Stephan Menzel,4† and Sharon E. Cox1,6†

Fetal hemoglobin (HbF) is a recognized modulator of sickle cell disease (SCD) severity. HbF levels are strongly influenced by genetic variants at three major genetic loci, Xmn1-HBG2, HMIP-2, and BCL11A, but the effect of these loci on the hematological phenotype in SCD, has so far not been investigated. In a cohort of individuals with SCD in Tanzania (HbSS and HbS/βthalassemia, n = 726, aged 5 or older), HbF levels were positively correlated with hemoglobin, red blood cell (RBC) indices, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), and negatively with white blood cell (WBC) and platelet counts (all P < 0.0001). We subsequently assessed the contribution of the three HbF modifier loci and detected diverse effects, including a reduction in anemia, leukocytosis, and thrombocytosis associated with certain HbF-promoting alleles. The presence of the ‘T’ allele at Xmn1-HBG2 led to a significant increase in hemoglobin (P = 9.8 × 10⁻³) but no changes in cellular hemoglobin content. Xmn1-HBG2 ‘T’ also has a weak effect decreasing WBC (P = 0.06) and platelet (P = 0.06) counts. The BCL11A variant (rs11886668-C) increases hemoglobin (P = 2 × 10⁻⁵) and one of the HBSIL-MYB variants decreases WBC values selectively (P = 2.3 × 10⁻⁶). The distinct pattern of effects of each variant suggests that both, disease alleviation through increased HbF production, and ‘pleiotropic’ effects on blood cells, are involved, affecting a variety of pathways.

Multiple linear regression (STATA v.12, Stata Corp, College Station, TX) was used to test for association of genetic markers with hematological parameters and HbF, as well as for the influence of HbF on blood cell parameters. Alpha-thalassemia status (3.7 deletion), age (fitted as square or native value) and sex were included a priori in all regression models. WBC, MPV, and HbF were log-transformed. The genetic association analysis of the four tag markers with hematological parameters was repeated with ln[HbF] as a covariate, to test for the dependence of genetic effects on HbF levels.

Results

We detected a significant influence of HbF levels and of variants at three major HbF modifier loci, BCL11A, HMIP, and Xmn1-HBG2, on the hematological phenotype of Tanzanian SCD patients.

Influence of HbF levels on hematological parameters

HbF levels associated positively with hemoglobin (Hb Beta = 0.05, P = 5.49 × 10\(^{-5}\)), and negatively with WBC (lnWBC Beta = -0.01, P = 4.23 × 10\(^{-5}\)) and platelet counts (Beta = 7.62, P = 6.3 × 10\(^{-6}\)). Hb gains with higher HbF were accompanied by increases in MCV (Beta = 0.43, P = 9.9 × 10\(^{-5}\)) and MCH (Beta = 0.16, P = 2.95 × 10\(^{-6}\)), while RBC was unchanged (P = 0.97).

Influence of genetic HbF modifier variants on HbF and on hematological parameters

HbF levels were strongly influenced by all four variants tested, confirming previous findings [24] (Table II). The number of HbF-promoting alleles across all genotyped markers (‘Summary Score’) was positively associated with Hb, MCV, and MCH, similar to the pattern of effects exerted by HbF itself. Individual loci; however, had diverse effects (Table II). The rare HbF-promoting allele at Xmn1-HBG2 (rs7482144-'T') had by far the largest allelic effect on Hb (Beta = 0.79, P = 9.8 × 10\(^{-5}\)), with a tendency towards increased RBC (Beta = 0.28, P = 0.06), but no change in MCV and MCH values (P > 0.1). When adjusting for the influence of HbF levels, the Hb-increasing effect of rs7482144-'T' remained large and significant (Beta = 0.69, P = 0.03) suggesting independence of this relationship from HbF levels. BCL11A (rs11886868-'C') also had a significant effect on Hb (Beta = 0.19, P = 2 × 10\(^{-5}\)), but this ceased to be significant after adjusting for HbF levels (P > 0.1). The two HbF-increasing variants at HMIP-2 (rs66650371-'del', representing sub-locus HMIP-2A and rs9389269-'C', representing HMIP-2B) are uncommon in our population and no significant effect on Hb was detected. However, the HMIP-2B variant had a significant positive effect on MCV and MCH and the HMIP-2A variant had a negative effect on WBC (Beta = -0.19, P = 2.3 × 10\(^{-6}\)), which was not diminished when adjusting for HbF.

Table I. SNP Markers Used to Tag the Main HbF Modifier Loci

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Chr. 2</th>
<th>Chr. 6</th>
<th>HMIP-2A</th>
<th>HMIP-2B</th>
<th>Xmn1-HBG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>rs11886868</td>
<td>rs66650371</td>
<td>rs9389269</td>
<td>rs7482144</td>
<td></td>
</tr>
<tr>
<td>Position</td>
<td>60,720,496</td>
<td>135,418,633</td>
<td>135,427,159</td>
<td>5,276,419</td>
<td></td>
</tr>
<tr>
<td>Allele change</td>
<td>T→C</td>
<td>In→Del</td>
<td>T→C</td>
<td>C→T</td>
<td></td>
</tr>
<tr>
<td>N/MAF</td>
<td>764/0.29</td>
<td>727/0.03</td>
<td>764/0.03</td>
<td>723/0.01</td>
<td></td>
</tr>
<tr>
<td>H/W(_p) (IDF)</td>
<td>0.29</td>
<td>0.85</td>
<td>-0.80</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>G. success (%)</td>
<td>99</td>
<td>94.29</td>
<td>99</td>
<td>95.71</td>
<td></td>
</tr>
</tbody>
</table>

In all regression models, WBC, MPV, and HbF were log-transformed. The genetic association analysis of the four tag markers with hematological parameters was repeated with ln[HbF] as a covariate, to test for the dependence of genetic effects on HbF levels.

Table II. Regression Analysis Testing the Influence of HbF and HbF Modifier Loci on Hematological Parameters in Tanzanian Patients with Sickle Cell Disease

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>HbF (lnHbF%)</th>
<th>rs11886868 (BCL11A)</th>
<th>rs66650371 (HMIP-2A)</th>
<th>rs9389269 (HMIP-2B)</th>
<th>rs7482144 (Xmn1-HBG2)</th>
<th>Summary score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbF%</td>
<td>0.05 (5.5 × 10(^{-6}))</td>
<td>0.37 (5.23 × 10(^{-2}))</td>
<td>0.51 (2.88 × 10(^{-5}))</td>
<td>0.44 (3.86 × 10(^{-5}))</td>
<td>0.55 (4.35 × 10(^{-5}))</td>
<td>0.40 (8.57 × 10(^{-3}))</td>
</tr>
<tr>
<td>Hb</td>
<td>-0.0002 (0.97)</td>
<td>0.03 (0.36)</td>
<td>0.004 (0.96)</td>
<td>-0.05 (0.49)</td>
<td>0.28 (0.06)</td>
<td>0.03 (0.35)</td>
</tr>
<tr>
<td>MCV</td>
<td>0.43 (9.9 × 10(^{-3}))</td>
<td>0.72 (0.10)</td>
<td>1.82 (0.18)</td>
<td>2.74 (0.02)</td>
<td>2.15 (0.32)</td>
<td>1.05 (5.5 × 10(^{-3}))</td>
</tr>
<tr>
<td>MCH</td>
<td>0.16 (3.0 × 10(^{-5}))</td>
<td>0.26 (0.11)</td>
<td>0.74 (0.14)</td>
<td>0.96 (0.02)</td>
<td>0.12 (0.88)</td>
<td>0.36 (0.01)</td>
</tr>
<tr>
<td>MCHC</td>
<td>0.02 (0.14)</td>
<td>0.07 (0.44)</td>
<td>0.13 (0.61)</td>
<td>0.06 (0.78)</td>
<td>-0.29 (0.48)</td>
<td>0.05 (0.49)</td>
</tr>
<tr>
<td>lnWBC</td>
<td>-0.01 (4.2 × 10(^{-5}))</td>
<td>5.7 × 10(^{-3})</td>
<td>-0.19 (2.3 × 10(^{-5}))</td>
<td>0.02 (0.67)</td>
<td>-0.149 (0.06)</td>
<td>-0.02 (0.21)</td>
</tr>
<tr>
<td>PLT</td>
<td>7.62 (6.3 × 10(^{-5}))</td>
<td>-9.82 (0.31)</td>
<td>33.29 (0.27)</td>
<td>22.36 (0.39)</td>
<td>-9.21 (0.96)</td>
<td>-6.43 (0.48)</td>
</tr>
<tr>
<td>lnMPV</td>
<td>0.001 (0.57)</td>
<td>0.0002 (0.98)</td>
<td>-0.018 (0.28)</td>
<td>-0.0007 (0.97)</td>
<td>-0.009 (0.73)</td>
<td>-0.002 (0.73)</td>
</tr>
</tbody>
</table>

Shown are regression coefficient (Beta) estimates and significance (in brackets). Age, sex, and alpha globin status were included as covariates. For the genetic data, Beta serves as a measure of the effect of an allele change from low-HbF to high-HbF allele. Nominally significant effects are in bold font. N = 664–721.

* The total number of high-HbF alleles present in a patient.
Additive effect of Xmn1-HBG2 and BCL11A alleles

When the impact of both loci was analyzed in a joint regression model, estimates of allelic effects were similar to those obtained in separate analysis (rs1188668: Beta = 0.19, P = 0.002, rs7482144: Beta = 0.79, P = 9.8 × 10^{-3}), suggesting that they contribute independently and therefore beneficial effects might add their effects when occurring in the same individual. Accordingly, patients with HbF-promoting alleles at both loci (one at Xmn1-HBG2 and either one or two at BCL11A) had Hb levels of up to 8.5 g/dl on average, compared with 7.3 g/dl for patients lacking any such allele (Fig. 1).

Discussion

We report that both, increased HbF levels and HbF-promoting alleles at HbF modifier loci significantly reduce anemia, leukocytosis, and thrombocytosis in Tanzanian patients with SCD.

The beneficial effects of higher HbF on hematological parameters, such as a higher Hb, lower WBC, and platelet counts, have previously been described in Jamaica [27–30], but might be less evident in a setting where the most-severely anemic patients are transfused regularly. The reduction in anemia we saw in patients with higher HbF levels was paralleled by an increase in two RBC indices (MCV and MCH), while RBC numbers were unchanged. Possibly, this indicates the presence of a larger F-cell fraction in such patients, a hypothesis that will be investigated in further studies. It should be noted that iron status, an independent cause of variability in red blood cell indices particularly MCV, has only been assessed in a sub-set of the patients.

Effects of the known genetic HbF modifiers (HbF-increasing variants at Xmn1-HBG2, BCL11A, and HMIP-2) were, in general, similar to that of HbF itself: Hb, MCV, and MCH were increased when the loci were analyzed jointly, using a summary score. However, individual analysis of the four variants revealed distinct patterns of effects, suggesting that diverse biological mechanisms are involved. The ‘T’ allele at Xmn1-HBG2 is a component of the ‘Arab-Indian’ β globin gene cluster haplotype [31–33], known to be associated with higher HbF values and milder sickle disease. In the seven patients carrying a Xmn1-HBG2-T allele, Hb was raised, but MCV and MCH were not, probably due to the direct [34], ‘pancellular’ effect of β globin cluster variants on HbF production.

BCL11A (rs11886686-C) had a significant effect on Hb, which was HbF-dependent. The HbF increase due to this allele (Beta) was small, but as it is highly prevalent in this population (29% allele frequency), it created an overall significant impact. Five patients had HbF increasing alleles at both BCL11A and Xmn1-HBG2 loci, resulting in maximum Hb levels (Fig. 1). Joint regression analysis of both loci supports a model of independence of their effects on Hb levels, and an additive contribution to overall hemoglobin variability. However, this will have to be confirmed in a larger population. HbF-promoting alleles at HMIP are infrequent in Tanzanian patients (frequency of <0.20) and we detected no effect on Hb. HMIP-2B (rs9389269) does influence MCV and MCH, a finding that resembles pleiotropic HMIP-2 effects observed in nonanemic individuals [4,5,7,8,10]. HMIP-2A, but not HMIP-2B, has a striking effect on WBC, independent of Hb. HMIP-2 variants have been reported to influence the WBC count in healthy populations [9], but a possible influence of population stratification, given the ethnic diversity of Tanzania, will be evaluated in further studies.

We believe that the significant effects of the three modifier loci on general blood traits we have shown represent a combination of both, disease amelioration through HbF modification and pleiotropic effects, and that the mechanisms underlying both phenomena are diverse and gene-specific. To explore this further, we will increase the power provided by our cohort by recruiting more patients and by broadening the scope of biological systems tested. Inclusion of additional hematological data in future analysis is expected to account for part of the background variability, thus improving our ability to detect more subtle genetic effects.

Acknowledgments

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Author Contributions

S.L.T., S.M., S.N.M., S.E.C., and J.M. designed the study. S. M and S.N.M. designed and performed the genotyping assays. B.M. performed the initial analysis. S.N.M., S.M., S.L.T., and S.E.C. wrote the manuscript and all authors commented on the drafts of the manuscript.

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