

Brief report

The *HBS1L-MYB* intergenic region on chromosome 6q23.3 influences erythrocyte, platelet, and monocyte counts in humansStephan Menzel,¹ Jie Jiang,¹ Nicholas Silver,¹ Joy Gallagher,² Juliette Cunningham,¹ Gabriela Surdulescu,³ Mark Lathrop,⁴ Martin Farrall,⁵ Tim D. Spector,³ and Swee Lay Thein^{1,2}¹King's College London School of Medicine, Division of Gene and Cell Based Therapy, London, United Kingdom; ²King's College Hospital, Department of Haematological Medicine, London, United Kingdom; ³King's College London School of Medicine, Division of Genetics and Molecular Medicine, London, United Kingdom; ⁴Centre National de Génotypage, Institut Génomique, Commissariat à l'Energie Atomique, Evry, France; ⁵Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom

Common sequence variants situated between the *HBS1L* and *MYB* genes on chromosome 6q23.3 (*HMIP*) influence the proportion of F cells (erythrocytes that carry measurable amounts of fetal hemoglobin). Since the physiological processes underlying the F-cell variability are thought to be linked to kinetics of

erythrocyte maturation and differentiation, we have investigated the influence of the *HMIP* locus on other hematologic parameters. Here we show a significant impact of *HMIP* variability on several types of peripheral blood cells: erythrocyte, platelet, and monocyte counts as well as erythrocyte volume and hemoglobin con-

tent in healthy individuals of European ancestry. These results support the notion that changes of F-cell abundance can be an indicator of more general shifts in hematopoietic patterns in humans. (Blood. 2007;110:3624-3626)

© 2007 by The American Society of Hematology

Introduction

Hematologic variables that are routinely measured for clinical purposes are influenced by the individual genetic constitution of the patients^{1,2}: in an ethnically homogenous population, we found that 62% of the variance in white blood cell counts, 57% of that in platelet counts, and 42% of the red cell count variance were due to genetic factors.³ The identification of the underlying genes and sequence variants will not only help to explain the variability seen between patients, but should advance our understanding of hematopoiesis in human health and disease.

HMIP (*HBS1L-MYB* intergenic polymorphism) refers to an array of single-nucleotide polymorphisms (SNPs) situated in the interval between the gene for *HBS1L* (a G-protein/elongation factor) and the *MYB* oncogene, on chromosome 6q23.3. The physiological significance of this sequence variability was recently discovered through the investigation of persistent fetal hemoglobin synthesis in adults⁴: *HMIP* SNPs exist in 3 linkage disequilibrium (LD) blocks, *HMIP-1*, *HMIP-2*, and *HMIP-3*, and the genotype at each block influences the number of F cells and fetal hemoglobin (HbF) levels.⁴ Strong LD between the SNPs within blocks permits the selection of a single tag SNP for each block: *rs52090901* for *HMIP-1*, *rs9399137* for *HMIP-2*, and *rs6929404* for *HMIP-3*. The *HMIP* locus accounts for approximately 17% of the variation in F-cell numbers, with most of the effect contributed by the 22-kb haplotype block 2 (*HMIP-2*). *HMIP-2* has 2 common haplotypes, designated haplotype 1 and haplotype 2. Haplotype 2, with a frequency of 0.2 to 0.25, is associated with raised F-cell levels in white Europeans. Initial functional studies have provided evidence that the locus acts through the regulation of the flanking genes, *HBS1L* and *MYB*.⁴ The sequence between these genes⁵ and the *MYB* gene product itself⁶⁻⁸ have been shown to affect proliferation,

survival, and differentiation of hematopoietic progenitor cells as well as peripheral blood cell counts in animal studies. We therefore investigated the influence of *HMIP* genotypes on hematologic indices in a healthy human population.

Patients and methods

One thousand seven hundred ninety-four participants of North European ancestry including 740 same-sex dizygotic twin pairs, 114 monozygotic twin pairs, and 86 singletons from the St Thomas' Hospital Twin Register⁹ were studied. *HMIP* genotypes and full blood counts were available for all participants, and additional differential blood count data for 1420 of them. Due to the recruitment strategy of the Twin Register, most individuals (95%) were female. All subjects provided written informed consent in accordance with the Declaration of Helsinki and the study was approved by the local ethics committee of St Thomas' Hospital and King's College Hospital, London (LREC no. 00-245).

Samples and data were collected over 10 years, between 1997 and 2006. All measurements were made in a routine clinical laboratory setting: dataset 1, from September 1997 to July 2000, using an H3 RTX automated blood cell analyzer (Bayer, Newbury, United Kingdom); dataset 2, February 2004 to March 2006, using an XE2100 fully automated hematology analyzer (Sysmex, Kobe, Japan). Hematologic indices (Table 1) were transformed (log, square-root, reciprocal), as appropriate, to induce approximate normality. Eosinophil and basophil counts were resistant to this procedure and were further analyzed with nonnormal distributions ($P < .001$). Repeat measurements were available for 551 individuals, and the mean of these results was analyzed.

SNP genotypes were generated by TaqMan assay (Applied Biosystems, Foster City, CA) at the Centre National de Génotypage (Evry, France) from

Submitted May 31, 2007; accepted August 11, 2007. Prepublished online as *Blood* First Edition paper, August 21, 2007; DOI 10.1182/blood-2007-05-093419.

The publication costs of this article were defrayed in part by page charge

payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2007 by The American Society of Hematology

Table 1. Hematologic variables associated with *HMIP-2* genotype

Variable	Median (interquartile range)	Individuals studied, no.	Significant* confounders	<i>HMIP-2</i> effect (β) of allele 2 (<i>P</i>)	$\Delta\text{VAR}_{\text{HMIP-2}}$, %
RBC	$4.5 \times 10^{12}/\text{L}$ (4.27-4.74)	1789	Sex	-0.07424 (<.001)	0.6
MCV	91.7 fL (88.7-95)	1761	Age	0.9202 (<.001)	1.7
MCH	29.9 pg (28.8-30.9)	1724	Age, sex, dataset	15.5833 (<.001)	2.1
WBC	$5.8 \times 10^9/\text{L}$ (4.8-7.1)	1788	Dataset	-0.02279 (.040)†	0.8
Plt	$232 \times 10^9/\text{L}$ (193-274)	1784	Sex, dataset	0.03908 (<.001)	0.6
Mon	$0.33 \times 10^9/\text{L}$ (0.26-0.41)	1416	Age, dataset	-0.04163 (.004)	1.6

The β parameter indicates the strength and direction of *HMIP-2* haplotype 2. No association was seen with traits hemoglobin concentration (Hb), packed red cell volume (PCV), mean red cell hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet distribution width (PDW), neutrophil, lymphocyte, eosinophil, and basophil counts.

$\Delta\text{VAR}_{\text{HMIP-2}}$ indicates decrease in trait variance after fitting the *HMIP-2* effect; RBC, red blood cell count; MCV, mean red cell volume; MCH, mean red cell hemoglobin; WBC, white blood cell count; Plt, platelet count; and Mon, monocyte count.

*Shown here when $P < .05$. Irrespective of their significance, the effects of all 3 potential confounders (age, sex, and dataset) were considered in the regression model.

†White cell counts are not significantly associated when considering the multiple statistical testing implications of examining 6 traits.

leukocyte DNA after genome-wide amplification (GenomiPhi; GE Healthcare, Little Chalfont, United Kingdom).

Data were initially processed in SPSS version 12.0 (SPSS, Chicago, IL) and then tested for association of hematologic variables with *HMIP* genotypes through a mixed-model ANOVA procedure (PROC MIXED) from SAS version 9 (SAS Institute, Cary, NC), as described.⁴ Sex, age, dataset, and *HMIP* genotypes were incorporated as fixed effects; random effects modeled the residual correlation between cotwins.

Results and discussion

The most functionally active haplotype block within the *HMIP* region, *HMIP-2*, was tested for association with our panel of hematologic indices, assuming an additive (codominant) genetic action. Haplotypes were strongly associated with several red-cell indices (RBC, MCV, MCH) and platelet counts, and showed some association ($P = .004$) with monocyte counts (Table 1). Small dominance effects (ie, a significant deviation of heterozygotes from the homozygote midpoint) were seen with MCV and MCH only (both $P = .04$). Additional small effects (eg, on WBC) might be present and perhaps detectable in larger or more homogeneous datasets. In addition, what influence the *HMIP-2* locus has outside of our mostly female European white study population will have to be shown separately. An independent association with the other *HMIP* blocks, 1 and 3, could not be detected (data not shown). The genetic association with *HMIP-2* explains 0.6% (RBC), 1.7% (MCV), 2.1% (MCH), 0.6% (Plt), and 1.6% (Mon) of the total variance of the traits studied (Table 1). These results confirm that *HMIP* variability leads to heritable diversity in general hematopoietic patterns. The effects can be directly observed in routine blood count parameters, but HbF and F-cell abundance are the most sensitive biologic indicators of the underlying genetic processes identified to date.

The 3 *HMIP-2* genotype groups (2/2, 1/2, and 1/1) show distinct trait distributions for associated parameters, with 2/2 individuals, which make up approximately 8% of the European population under study, having markedly smaller numbers of red blood cells with larger cell volumes and higher hemoglobin content per cell, more platelets, and fewer monocytes (Figure 1). The behavior of erythrocytes and platelets in subjects with *HMIP-2* 2/2 phenotype in humans mirrors a mild *MYB* knock-down phenotype,^{6,8} or findings in mice with a disruption of *HBSIL-MYB* intergenic sequence.⁵ Similarly, we have previously shown that individuals with high HbF (higher than 0.8%) have a depressed expansion of erythroblasts in a 2-phase erythroid culture, and that high HbF is associated with larger erythrocyte volumes and higher platelet counts in vivo.¹⁰ *HMIP-2* haplotype 2 (ie, the high-F genotype) increased the *HBSIL* expression in erythroblasts, but showed no detectable effect on *MYB* expression.⁴ *HMIP-2* 2/2 monocyte counts are

low, which contrasts with our findings in the erythroid culture experiments¹⁰ and might reflect the polygenic nature of HbF regulation. The fact that *HMIP-2* 2/2 erythrocytes are larger and include a larger proportion of F cells⁴ could indicate that they are derived from earlier progenitors, similar to the situation encountered in stress erythropoiesis.¹¹ We speculate that the locus may influence the stage of maturity of the progenitor pools from which the erythroid cells are derived; the less mature progenitors leading to higher F-cell levels. Overall, the hemoglobin concentration was unaffected by the opposing effects of the “2” haplotype on the erythrocytes (ie, lower numbers but higher hemoglobin content [MCH]).

Our study shows that common genetic factors influencing blood cell indices can be detected in humans, reaching genome-wide significance ($P = 3.1 \times 10^{-8}$, for RBC) with a sample size of approximately 1800 individuals. Some loci may be pleiotropic (ie, show multiple effects across different cell types). While we have used a candidate gene approach, systematic, genome-wide searches might uncover loci with stronger effects. Linkage-type studies in mice and humans have detected a series of loci for hematologic indices,¹²⁻¹⁵ with one¹⁵ detecting linkage with MCV near the *HMIP* locus. High-resolution mapping in heterogeneous stock mice,¹⁶ which has a resolution comparable with linkage disequilibrium mapping studies in humans, found significant peaks at a region

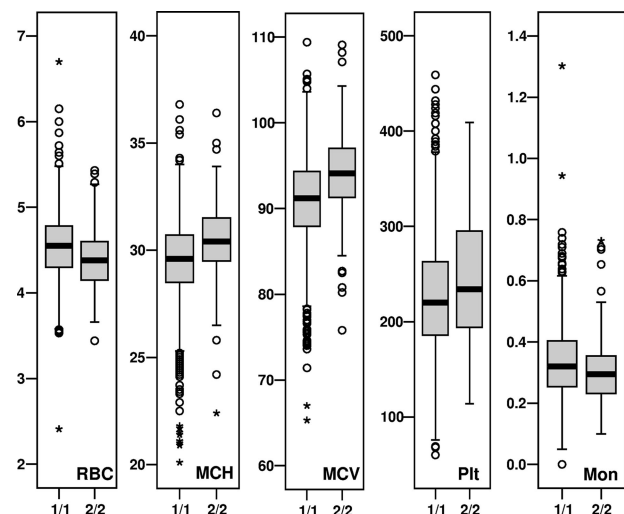


Figure 1. Median and range of *HMIP-2*-associated variables for individuals with homozygous *HMIP-2* genotypes (1/1 and 2/2). Plotted are individual measurements from dataset 1: median (■), quartiles (□), overall trait distribution (I), outliers (○), and extremes (+).

homologous to *HMIP* for white cell counts, MCH, RDW, and other parameters.

We hope that this and similar studies will lead to an understanding of the genetic basis of normal blood phenotypes and will help to uncover molecular mechanisms of the regulation of hematopoiesis.

Acknowledgments

This work was supported by Medical Research Council (MRC) grant (G0000111, ID51640) to S.L.T. The TwinsUK project is funded by the Wellcome Trust.

We thank Helen Rooks, Ayrin Nessa, and Dimitrios Paximada for expert technical assistance.

References

- Buckley MF, James JW, Brown DE, et al. A novel approach to the assessment of variations in the human platelet count. *Thromb Haemost*. 2000;83:480-484.
- Evans DM, Frazer IH, Martin NG. Genetic and environmental causes of variation in basal levels of blood cells. *Twin Res*. 1999;2:250-257.
- Garner C, Tatu T, Reittie JE, et al. Genetic influences on F cells and other hematologic variables: a twin heritability study. *Blood*. 2000;95:342-346.
- Thein SL, Menzel S, Peng X, et al. Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetal hemoglobin levels in adults. *Proc Natl Acad Sci U S A*. 2007;104:11346-11351.
- Mukai HY, Motohashi H, Ohneda O, Suzuki N, Nagano M, Yamamoto M. Transgene insertion in proximity to the c-myb gene disrupts erythroid-megakaryocytic lineage bifurcation. *Mol Cell Biol*. 2006;26:7953-7965.
- Sandberg ML, Sutton SE, Pletcher MT, et al. c-Myb and p300 regulate hematopoietic stem cell proliferation and differentiation. *Dev Cell*. 2005;8:153-166.
- Mucenski ML, McLain K, Kier AB, et al. A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. *Cell*. 1991;65:677-689.
- Emambokus N, Vegiopoulos A, Harman B, Jenkinson E, Anderson G, Frampton J. Progression through key stages of haemopoiesis is dependent on distinct threshold levels of c-Myb. *EMBO J*. 2003;22:4478-4488.
- Spector TD, MacGregor AJ. The St. Thomas' UK Adult Twin Registry. *Twin Res*. 2002;5:440-443.
- Jiang J, Best S, Menzel S, et al. cMYB is involved in the regulation of fetal hemoglobin production in adults. *Blood*. 2006;108:1077-1083.
- Stamatoyannopoulos G. Control of globin gene expression during development and erythroid differentiation. *Exp Hematol*. 2005;33:259-271.
- Peters LL, Zhang W, Lambert AJ, Brugnara C, Churchill GA, Platt OS. Quantitative trait loci for baseline white blood cell count, platelet count, and mean platelet volume. *Mamm Genome*. 2005;16:749-763.
- Peters LL, Lambert AJ, Zhang W, Churchill GA, Brugnara C, Platt OS. Quantitative trait loci for baseline erythroid traits. *Mamm Genome*. 2006;17:298-309.
- Evans DM, Zhu G, Duffy DL, Montgomery GW, Frazer IH, Martin NG. Multivariate QTL linkage analysis suggests a QTL for platelet count on chromosome 19q. *Eur J Hum Genet*. 2004;12:835-842.
- Lin JP, O'Donnell CJ, Jin L, Fox C, Yang Q, Cupples LA. Evidence for linkage of red blood cell size and count: genome-wide scans in the Framingham Heart Study. *Am J Hematol*. 2007;82:605-610.
- Valdar W, Solberg LC, Gauguier D, et al. Genome-wide genetic association of complex traits in heterogeneous stock mice. *Nat Genet*. 2006;38:879-887.

Authorship

Contribution: S.M. performed research, analyzed data, and wrote the paper; J.J., N.S., J.G., J.C., and G.S. performed research; M.F. designed the analysis approach and corrected the draft; M.L. directed research; T.D.S. contributed valuable samples; S.L.T. designed and performed research as well as wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Swee Lay Thein, Department of Haematological Medicine, King's College London School of Medicine, James Black Centre, 125 Coldharbour Lane, London, SE5 9NU, United Kingdom; e-mail: sl.thein@kcl.ac.uk.



blood

2007 110: 3624-3626

doi:10.1182/blood-2007-05-093419 originally published
online August 21, 2007

The *HBS1L-MYB* intergenic region on chromosome 6q23.3 influences erythrocyte, platelet, and monocyte counts in humans

Stephan Menzel, Jie Jiang, Nicholas Silver, Joy Gallagher, Juliette Cunningham, Gabriela Surdulescu, Mark Lathrop, Martin Farrall, Tim D. Spector and Swee Lay Thein

Updated information and services can be found at:

<http://www.bloodjournal.org/content/110/10/3624.full.html>

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:

http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:

<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:

<http://www.bloodjournal.org/site/subscriptions/index.xhtml>