Hemoglobin was determined in heparin-treated plasma of a total of 2,506 samples.4 Over a normal Hb range of 135-175 g/L and 120-155 g/L for men and women, respectively, the range of Epo was 4-24 IU/L without any significant difference between sex (Online Supplementary Table S1). A quadratic fit with Hb levels was found (Figure 1B), which may be explained by anemia-responsive Epo at low Hb levels and hormone-responsive Hb at high Epo levels. As shown in Table 2, Epo levels were significantly heritable, without significant sibling or marital components of variance, suggesting the absence of significant dominance variance and shared environmental variance.

We next performed a GWAS of 2.5x10^6 genotyped SNP and an additional 4.0x10^6 of imputed SNP with the mean of duplicate Epo measurements of 872 (79%) individuals, corrected for age, sex, center and familiarity. Figure 1C shows the level of significance of the association between each of the 6.5x10^6 markers and the normalized Epo levels. No signal reached P<5x10^{-5}, the commonly used level of genome-wide significance (Online Supplementary Table S2). However, a few SNP fell into the suggestive significance zone (P<10^{-5}) and the top hit, lying on chromosome 15, reached P=1.05x10^{-7} at rs413451. As shown in Figure 1D, the SNP identified on chromosome 15 are located within a linkage disequilibrium (LD) block comprising the last exons of mitogen-activated protein kinase kinase 5 (MAP2K5), the whole SKI family transcriptional corepressor 1 (SKOR1) gene and the 5’ upstream and promoter regions of protein inhibitor of activated STAT1 (PIAS1). PIAS1 is a SUMO E3 ligase affecting STAT1 and NFκB pathways. The PIAS1 locus has previously been associated with body mass index (BMI) and related phenotypes (weight, waist circumference, obesity, predicted visceral adipose tissue), as well as smoking-related phenotypes (initiation age, smoking status) and age at menarche.4-8 According to the MR-Base PHEWAS database in the UK Biobank cohort several RBC-related phenotypes were also associated with the MAP2K5-SKOR1-PIAS1 locus, further suggesting that it could be directly associated with Epo levels.

Figure 1E shows the significance of the association for the SNP present at the previously associated locus

Table 1. Heritability estimates of red blood cell indices in the SKIPOGH cohort.

| Trait          | Model 1 h² ± SEM | λ | P  | Model 2 h² ± SEM | λ | P  |
|----------------|------------------|---|----|------------------|---|----|
| Hemoglobin     | 0.40 ± 0.05      | 0.52 | <1.0x10^{-3} | 0.37 ± 0.06 | 0.52 | <1.0x10^{-7} |
| Hematocrit     | 0.37 ± 0.06      | 0.57 | 0.001 | 0.33 ± 0.07 | 0.57 | <1.0x10^{-7} |
| RBC count      | 0.50 ± 0.05      | 0.67 | <1.0x10^{-4} | 0.47 ± 0.06 | 0.66 | <1.0x10^{-7} |
| MCV            | 0.68 ± 0.04      | 0.59 | <1.0x10^{-5} | 0.68 ± 0.05 | 0.59 | <1.0x10^{-7} |
| MCH            | 0.63 ± 0.05      | 0.52 | <1.0x10^{-5} | 0.61 ± 0.06 | 0.51 | <1.0x10^{-7} |
| MCHC           | 0.60 ± 0.06      | 0.94 | <1.0x10^{-6} | 0.46 ± 0.06 | 0.93 | <1.0x10^{-7} |
| RDW            | 0.38 ± 0.07      | 0.20 | <0.001 | 0.36 ± 0.08 | 0.20 | <0.001 |

Models are adjusted for age, sex and center. Model 1, no sibship component of variance; model 2, including a sibship component of variance (which captures dominance genetic variance and shared environmental components between siblings). λ, power transformation; (λ = 0) and (λ = 1) correspond to log and no transformations, respectively. SKIPOGH: Swiss Kidney Project on Genes in Hypertension; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; h²: heritability; SEM: standard error of the mean.
HBS1L-MYB. Interestingly, the top common SNP of our study (rs9402685) and the associated HBS1L-MYB locus showed a robust association with Epo levels \( (P=1.46 \times 10^{-4}) \), confirming the previously published results. The SNP rs1617640 of the EPO locus itself has been reported to be associated with low Epo serum levels in predialysis chronic kidney disease patients, but neither this SNP nor any other SNP of the EPO locus (lowest \( P=0.17 \) at rs7789679) were associated with Epo levels in our study.

While the recently reported SNP rs1130864 of the C-reactive protein (CRP) locus did not associate with Epo levels in our study \( (P=0.69) \), it associated with altered Epo levels in dried neonatal blood spots. In the same study, EPO SNP heritability was approximately zero. However, at this age blood volume and hematocrit are different from the adult stage, the liver-to-kidney switch of Epo synthesis is still ongoing, and the method for Epo determination was much less precise, altogether explaining the discrepancy.

The result obtained with our top SNP of the HBS1L-MYB locus (rs9402685) was meta-analyzed with results publicly available from Beverborg et al. The combined \( P \)-value reached \( 1.78 \times 10^{-23} \), which was more significant than in any of the two individual studies (1.46x10^{-4} and 1.09x10^{-20}, respectively). The intergenic locus between the HBS1L (GTP-binding elongation factor) and MYB (myeloblastosis oncogene) genes had previously been reported to be associated with deregulated HbF in a
Chinese β-thalassemia anemia population, a favorable genetic environment for the selection of otherwise erythrocytosis-causing mutations.11 Several erythropoietic transcription factors have been shown to be prevented from binding to the mutant locus, resulting in lowered Myb gene activation and increased HbF synthesis.12 A link to increased Epo levels, suggesting secondary (Epo-dependent) rather than primary (Epo-independent) erythrocytosis, has not been made in these original reports. GWAS performed in a UK Biobank cohort and a Japanese population showed significant associations between the HBS1L-MYB locus and RBC-related phenotypes.13,14 Together with our direct replication of the association with circulating Epo levels in a Swiss cohort, these results further confirm the implication of this locus in erythrocytosis, maybe both upstream as well as downstream of Epo. However, it is currently not known whether the HBS1L-MYB locus also contributes to the heritable genetic determinants triggering Epo levels.

A gene score and a pathway analysis, run with the PASCAL algorithm based on our GWAS results, failed to show any significant pathway after applying multiple testing corrections.

For a candidate-based approach, we selected 33 genes known to influence EPO gene expression. The association gene scores from PASCAL could be retrieved from 30 of these 33 genes. Bonferroni correction applied to the number of genes observed led to a significance threshold of 1.67x10⁻³. The OS9 gene was significantly associated with a gene score association P-value of 1.47x10⁻² (Online Supplementary Table S3). OS-9 is known to interact with both HIF-1α and HIF prolyl-4-hydroxylases, promoting HIF-1α degradation. Interestingly, a OS9 gene variant has previously been reported to be associated with erythrocytosis in a single patient.15

The top SNP of the MAP2K3-SKOR1-PIAS1 locus (rs413451) was subjected to a phenome-wide association study (PHEWAS) using the MR-Base database of the UK Biobank cohort. SNP rs413451 was most significantly associated with BMI-related phenotypes. Interestingly, Hb concentration (P=6.35x10⁻⁴), reticulocyte count (P=7.28x10⁻⁴), hematocrit (P=1.16x10⁻³), reticulocyte fraction of RBC (P=2.04x10⁻³) and RBC count (P=2.23x10⁻⁴) were also highly associated with rs413451. Circulatory Epo levels were not available in the UK Biobank. However, the GWAS Atlas database showed a preponderance of BMI-related phenotypes for most significant studies in the database of published GWAS.

In summary, our study revealed the heritability of circulating Epo levels, validated a previously published association with the HBS1L-MYB locus, and identified an association with the MAP2K3-SKOR1-PIAS1 locus. From the list of candidate Epo-regulatory genes, OS9 showed the highest association with circulating Epo levels. However, the two latter associations require replication, and the functional implication of all three loci in Epo regulation needs to be further investigated. Regarding the idiopathic nature of the majority of erythrocytosis cases, we suggest that especially in patients with high Epo levels, indicative of secondary erythrocytosis, these loci should be considered for further investigation.

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