Authorship

Contribution: G.M. and R.H. designed the research, analyzed data, and wrote the paper; C.H., P.B., K.B., A.S., J.-M.S., H.G., and L.D. provided the data; and all authors reviewed and approved the final draft.

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A complete list of the members of the Lymphoma Study Association appears in "Appendix."

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TO THE EDITOR:

A common *TCN1* loss-of-function variant is associated with lower vitamin B_{12} concentration in African Americans

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Vitamin B_{12} (VB_{12}), or cobalamin, is a water-soluble vitamin. It serves as an enzyme cofactor in the 1-carbon metabolism pathway, which plays a key role in a wide range of biological processes, including red blood cell (RBC) formation, DNA synthesis, and myelination of the central nervous system.[1-3] VB_{12} deficiency is clinically associated with megaloblastic anemia and neurodegenerative disorders and is also linked to cardiovascular diseases, which is thought to be mediated via hyperhomocysteinemia.[1,3] Uptake of VB_{12} from the diet in the gastrointestinal tract depends on intrinsic factor (encoded by *GIF*).[3,4] VB_{12} in blood is bound to haptocorrin (HC; encoded by *TCN1*) and transcobalamin (encoded by *TCN2*) and circulates as holohaptocorrin (holoHC) and holotranscobalamin, respectively.[3,5,6]

Although VB_{12} deficiency often results from poor gastrointestinal absorption,[7] genetic factors also contribute to phenotypic variation. Previous genome-wide association studies (GWASs), primarily focusing on populations of European and Asian ancestry, have identified 13 loci (*FUT2*, *CUBN*, *TCN1*, *MUT*, *MS4A3*, *FUT6*, *PRELID2*, *CLYBL*, *CD320*, *TCN2*, *ABCD4*, *MMAA*, and *MMACHC*) associated with VB_{12}.[8-15] GWASs in Chinese[11] and Indians[14] identified VB_{12}-associated shared genetic variants across ancestry groups, as well as population-specific variants. VB_{12} and holoHC concentrations are higher in blacks than in whites,[16] yet no GWAS has examined the genetic determinants of VB_{12} specifically in African Americans (AAs). In the current study, we used whole-genome sequencing (WGS) from the

Table 1. Association of *TCN1*-rs34530014 with VB_{12} concentration

Trait	Study	SNP	CHR:POS	N	EA/NEA	EAF	BETA (SE)	P*
VB_{12}	JHS	rs34530014	11:59631467	1280	A/AC	0.036	−0.817 (0.104)	6.48×10^{-15}
VB_{12}	BioVU	rs11822978	11:59626896	725	T/C	0.034	−0.158 (0.038)	3.28×10^{-5}
VB_{12}	BioMe	rs11822978	11:59626896	3199	T/C	0.032	−0.272 (0.037)	2.76×10^{-13}

CHR, chromosome; EA, effect allele; EAF, effect allele frequency; NEA, noneffect allele; POS, position; SE, standard error; SNP, single nucleotide polymorphism.
*Genome-wide significance was predefined as $P < 1.69 \times 10^{-9}$ or 0.05/29 665 030 variants tested.

National Heart, Lung, and Blood Institute (NHLBI) Trans-Omics for Precision Medicine (TOPMed) Program to assess genetic variants associated with VB_{12} in an unselected population-based sample of AAs from the Jackson Heart Study (JHS).[17]

The JHS is a population-based longitudinal study based in Jackson, Mississippi[17,18]; additional information is reported in supplemental Methods (available on the *Blood* Web site). The JHS was approved by the Institutional Review Board of the

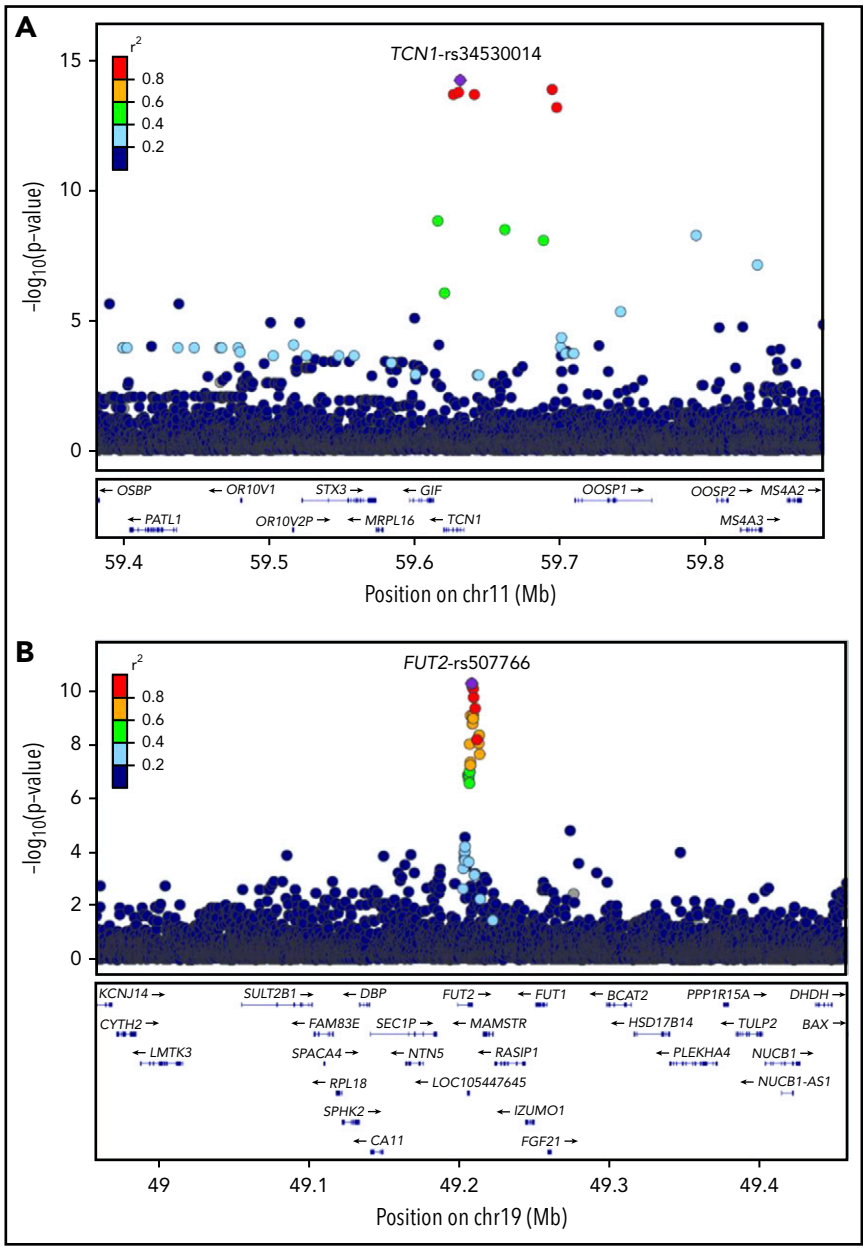

Figure 1. Locus-zoom plots. *TCN1*-rs34530014 (A) and *FUT2*-rs507766 (B). Genetic coordinates are displayed along the x-axis (Build 37/hg19), and genome-wide association significance level is plotted against the y-axis as $-\log_{10}(P \text{ value})$. The purple diamond indicates the top hit. LD is generated using JHS WGS data and is indicated by the color scale in relationship to the top hit, with red for strong LD ($r^2 > 0.8$) and navy blue for weak LD ($r^2 < 0.2$).

University of Mississippi Medical Center, and participants provided written informed consent. At the baseline examination, VB_{12} was measured in plasma in a subset of 1851 JHS participants using a homogenous enzyme immunoassay system and Hitachi 911 equipment (Roche Diagnostics, Indianapolis, IN).[19] A total of 3406 JHS participants underwent 30× coverage WGS through the TOPMed project at the Northwest Genome Center at the University of Washington, and genotype calling was performed by the Informatics Resource Center at the University of Michigan, as previously described.[20] After quality control (supplemental Methods), a total of 29 665 030 variants (minor allele frequency > 0.001) and 1280 participants (20.6-91.6 years of age, 65.5% female, VB_{12} = 639.9 ± 289.3 pg/mL) were included in the discovery analysis (phs000964.v2.p1 for sequencing data and phs000286.v5.p1 for VB_{12} data on the Database of Genotypes and Phenotypes; https://www.ncbi.nlm.nih.gov/gap).

Association of each single variant with rank-based inverse-normal–transformed VB_{12} concentration was tested in JHS participants adjusted for age, sex, and the first 10 principal components (PCs) of genetic ancestry using a linear mixed model approach to account for familial relationships, as implemented in EPACTS 3.2.6 on the University of Michigan ENCORE server (https://encore.sph.umich.edu). Genome-wide significance was predefined as $P < 1.69 \times 10^{-9}$, or 0.05/29 665 030 variants tested; 33 variants across 2 genomic regions (*TCN1* and *FUT2*) reached genome-wide significance ($P < 1.69 \times 10^{-9}$) for VB_{12} (supplemental Figure 1).

The index variant rs34530014 on chromosome 11 is a 1-bp deletion of *TCN1* (minor allele frequency = 0.036; $P = 6.48 \times 10^{-15}$; Table 1; Figure 1A) associated with lower VB_{12}. It is a loss-of-function (LoF) frameshift mutation (p.Val58Cysfs) prevalent only among African ancestry populations, originally reported in 2 Afro-Caribbean pedigrees with HC deficiency.[21] Replication of the novel LoF variant *TCN1*-rs34530014 was performed using genomic data from 2 AA electronic medical record–linked biobanks: the Biobank of Vanderbilt University (BioVU)[22] and BioMe of Mount Sinai[23] (supplemental Methods). Because *TCN1*-rs34530014 was not directly genotyped or imputed in BioVU or BioMe, we used its linkage disequilibrium (LD) proxy rs11822978 (r^2 = 0.98 in JHS) and confirmed its association with lower VB_{12} in 3924 additional AAs from BioVU and BioMe (Table 1). *TCN1*-rs34530014 is distinct from other VB_{12}-associated *TCN1* variants (including 2 missense variants, rs34324219 and rs34528912) identified in Europeans and Indians.[9,12-14] Of the *TCN1* variants previously associated with lower VB_{12}, only rs34324219 was nominally associated with lower VB_{12} in the JHS (P = .009; supplemental Table 1). Together, these results demonstrate an ancestry-specific role for rs34530014 in lower VB_{12} concentration in African ancestry populations and substantial allelic heterogeneity in the genetic architecture of VB_{12} at the *TCN1* locus.

The identification of this naturally occurring LoF mutation prevalent in AAs allowed us to further investigate additional hematologic and clinical consequences of mild HC deficiency, beyond the association with lower VB_{12} concentration. The association of rs34530014 with additional quantitative traits, including RBC indices and homocysteine, was assessed in the JHS and the Women's Health Initiative SNP Health Association Resource[24] (supplemental Methods). An elevated concentration of methylmalonate is a more sensitive and specific marker for VB_{12} deficiency[1,3]; unfortunately, it was not available in our data sets. In addition, a phenome-wide association study (PheWAS) of *TCN1*-rs11822978 was performed in BioVU and BioMe. A total of 340 traits was scanned for association using logistic regression adjusted for age, sex, and the first 10 PCs, including metabolic, neurological, and hematologic outcomes and disorders. Individual study association results were combined using fixed-effect inverse-variance–weighted meta-analysis, and P values were corrected for multiple testing. Although marginal associations ($P < .05$) were observed for mean corpuscular hemoglobin and mean corpuscular volume, as well as diagnosis codes Vitamin Deficiency and Other Deficiency Anemia, none of these reached the Bonferroni-corrected threshold ($P > .006$, or 0.05/8 traits for RBC indices, supplemental Table 2; and $P > 1.47 \times 10^{-4}$, or 0.05/340 traits for PheWAS, supplemental Table 3).

In contrast to mutations of *TCN2*, which result in transcobalamin II deficiency (OMIM #275350) with severe clinical manifestations,[25] the clinical impact of HC deficiency is poorly defined. The distinct consequences of *TCN1* and *TCN2* mutations are consistent with the fact that holotranscobalamin is the bioactive form of VB_{12} that is taken up by all tissues, whereas the biological role of holoHC is unclear.[6] Despite the relatively large overall sample, the small number of homozygotes (4 in JHS) limited our ability to accurately assess the recessive effect of the *TCN1*-rs34530014 on VB_{12} and related traits and disorders. Therefore, further investigation, including very large phenotypic data sets of African ancestry individuals, will be needed to draw firm conclusions about the clinical impact of HC deficiency.

The index single nucleotide polymorphism at the other genome-wide significant association peak in the JHS on chromosome 19 was rs507766 (located in the 3′ untranslated region of *FUT2*; $P = 5.51 \times 10^{-11}$) (Figure 1B). This noncoding variant is in only moderate LD (r^2 = 0.55 in JHS) with the common secretor variant *FUT2*-rs601338 ($P = 1.49 \times 10^{-7}$ for association with VB_{12} in JHS), which was previously identified in Europeans and Indians.[8,9,14] Thus, we found evidence for 2 functional alleles involving *FUT2*: 1 with an additive genetic effect (rs507766) and 1 with a dominant effect (rs601338) on VB_{12} (supplemental Table 4). We confirmed 4 previously identified loci (*MUT*, *MMACHC*, *FUT6*, and *CD320*; supplemental Table 1) at $P < .05$ with consistent association directions of effects.

We report a novel association between the African ancestry–specific LoF variant *TCN1*-rs34530014 and VB_{12} concentration and an additional *FUT2* variant rs507766 associated with VB_{12} specific to AAs, highlighting the ancestry heterogeneity of genetic factors that influence VB_{12}. Another population-specific VB_{12}-associated LoF variant, *CLYBL*-rs41281112 (p.Arg259Ter), is found in ~3% of apparently healthy non-African individuals (but is rare in Africans).[11] Further elucidation of the genetics and role of HC-bound VB_{12} may lead to improved diagnosis and differentiation of true bioavailable VB_{12} deficiency from total VB_{12} deficiency.

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Authorship

Contribution: A.P.R. and P.L.A. were responsible for the study concept and design; J.G.W. and A.C. acquired the phenotypic and genotypic data in the JHS; A.P.R., P.L.A., Y.H., L.M.R., and L.M.P. performed the statistical analyses in the JHS and the Women's Health Initiative SNP Health Association Resource, the meta-analysis, and the PheWAS; A.M., G.N., M.H.P., and R.J.F.L. performed replication analysis in BioMe; X.Z., Q.W., and B.L. performed replication analysis in BioVU; Y.H. drafted the manuscript; and all authors contributed to the interpretation of the results and critical revision of the manuscript.

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A complete list of the members of the NHLBI Trans-Omics for Precision Medicine Consortium appears in the online appendix.

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Footnote

The online version of this article contains a data supplement.

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TO THE EDITOR:

Plerixafor effectively mobilizes CD56^{bright} NK cells in blood, providing an allograft predicted to protect against GVHD

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The recent *Blood* article by Schroeder et al demonstrates that plerixafor mobilizes a unique hematopoietic stem and progenitor cell (HSPC) product that is enriched in plasmacytoid dendritic cell (pDC) precursors (pre-pDCs).[1] This study further reports that enrichment of these cells in plerixafor-mobilized allografts is associated with lower cumulative incidence of acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD). In an earlier study, Waller et al reported a protective advantage against aGVHD of pDCs derived from bone marrow (BM) but not granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB) graft.[2] Studies have shown that allogeneic transplantation with a BM allograft results in lower rates of aGVHD and cGVHD when compared with G-CSF–mobilized PB graft. In a commentary to the Schroeder study, Waller proposes that the relatively rapid onset of mobilization by plerixafor (hours vs days by G-CSF) generates a novel PB graft that immunologically and phenotypically resembles cells harvested from bone marrow.[3] He hypothesizes that this unique allograft contains immune subsets, including pre-pDCs and pDCs, which favorably modulate alloreactivity post-transplantation thus resulting in lower GVHD rates.

Recently, the Canadian Blood and Marrow Transplant Group (CBMTG) conducted a phase 3 randomized trial and compared the impact of G-CSF–mobilized PB to BM. In this trial, G-CSF–treated BM donors had a significantly lower frequency of cGVHD,[4] similar to that seen in the Schroeder study. A further comprehensive investigation of the donor graft composition found that the lower rate of cGVHD in G-BM correlated most closely with a higher proportion of CD56^{bright} regulatory NK cells (NK$_{reg}$) represented as the percentage of CD56^{bright} NK$_{reg}$ cells.[5] Additional evidence of the importance of this immune-regulatory population is our recent observation of a correlation of low numbers of CXCR3^{+}CD56^{bright} NK$_{reg}$ cells with the onset of cGVHD in adults.[6] Based on these observations, we hypothesized that an alternate donor cell population, with a selective increase in NK$_{reg}$ cells by plerixafor, may contribute to the lower rate of cGVHD seen in the recent Schroeder study.

To address this possible mechanism, we recruited 9 healthy adult human volunteers after full consent. This study was approved by the Dalhousie University and University of British Columbia (UBC) research ethical boards. All subjects were between 18 and 60 years of age and were enrolled at the Dalhousie University. All biological analyses were performed at the UBC-affiliated BC Children's Hospital Research Institute. Five subjects received 1 dose of plerixafor at 240 μg/kg per day subcutaneously and PB and BM samples were harvested prior to administration and at 4 and 24 hours after plerixafor administration. The subsequent 4 participants each received 4 daily doses of G-CSF at 5 μg/kg per day for 4 days, followed by a dose of plerixafor on day 5. To minimize the impact of circadian rhythm, PB and BM samples were collected between 9 AM and noon, prior to G-CSF administration, after G-CSF completion, and at 4 and 24 hours after plerixafor administration. We performed a focused analysis on CD56^{bright} NK$_{reg}$ cells in these samples using multiparametric flow cytometry. Nucleated cells isolated from peripheral blood and bone marrow samples were stained with CD3, CD8, perforin, granzyme B, CD56 antibodies conjugated to fluorescein isothiocyanate, phycoerythrin, PB, Flow-Check 770 fluorospheres, and allophycocyanin, respectively. Stained cells were acquired by LSR II cytometer (BD) and the data were analyzed by FlowJo v10. The immunophenotype of NK$_{reg}$ cells is CD3^{-}, CD56^{bright}, perforin^{-} and granzyme-B^{-}.

Plerixafor significantly mobilized nucleated cells to PB after 4 hours of treatment, whereas no significant difference was observed after 24 hours of treatment (Figure 1A). Furthermore, longer treatment time resulted in a significantly lower cell number indicating that plerixafor's action peaked at 4 hours after administration. The magnitude of mobilization observed 4 hours after plerixafor administration was similar to that seen in the previous phase II clinical study.[7] Interestingly, plerixafor did not alter the nucleated cell number in the bone marrow (Figure 1A). Four daily injections of G-CSF followed by plerixafor administration significantly mobilized more nucleated cells to PB than either treatment alone (Figure 1B). However, the two agents mobilized similar number of nucleated cells to PB separately. Therefore, plerixafor mobilized nucleated cells to peripheral blood more rapidly than G-CSF.