

Platelet-Related Variants Identified by Exomechip Meta-analysis in 157,293 Individuals

John D. Eicher,^{1,97} Nathalie Chami,^{2,3,97} Tim Kacprowski,^{4,5,97} Akihiro Nomura,^{6,7,8,9,10,97} Ming-Huei Chen,¹ Lisa R. Yanek,¹¹ Salman M. Tajuddin,¹² Ursula M. Schick,^{13,14} Andrew J. Slater,^{15,16} Nathan Pankratz,¹⁷ Linda Polfus,¹⁸ Claudia Schurmann,^{13,14} Ayush Giri,¹⁹ Jennifer A. Brody,²⁰ Leslie A. Lange,²¹ Ani Manichaikul,²² W. David Hill,^{23,24} Raha Pazoki,²⁵ Paul Elliot,²⁶ Evangelos Evangelou,^{26,27} Ioanna Tzoulaki,^{26,27} He Gao,²⁶ Anne-Claire Vergnaud,²⁶ Rasika A. Mathias,^{11,28} Diane M. Becker,¹¹ Lewis C. Becker,^{11,29} Amber Burt,³⁰ David R. Crosslin,³¹ Leo-Pekka Lyytikäinen,^{32,33} Kjell Nikus,^{34,35} Jussi Hernesniemi,^{32,33,34} Mika Kähönen,^{36,37} Emma Raitoharju,^{32,33} Nina Mononen,^{32,33} Olli T. Raitakari,^{38,39} Terho Lehtimäki,^{32,33} Mary Cushman,⁴⁰ Neil A. Zakai,⁴⁰ Deborah A. Nickerson,⁴¹ Laura M. Raffield,²¹ Rakale Quarells,⁴² Cristen J. Willer,^{43,44,45} Gina M. Peloso,^{6,7,46} Goncalo R. Abecasis,⁴⁷

(Author list continued on next page)

Platelet production, maintenance, and clearance are tightly controlled processes indicative of platelets' important roles in hemostasis and thrombosis. Platelets are common targets for primary and secondary prevention of several conditions. They are monitored clinically by complete blood counts, specifically with measurements of platelet count (PLT) and mean platelet volume (MPV). Identifying genetic effects on PLT and MPV can provide mechanistic insights into platelet biology and their role in disease. Therefore, we formed the Blood Cell Consortium (BCX) to perform a large-scale meta-analysis of Exomechip association results for PLT and MPV in 157,293 and 57,617 individuals, respectively. Using the low-frequency/rare coding variant-enriched Exomechip genotyping array, we sought to identify genetic variants associated with PLT and MPV. In addition to confirming 47 known PLT and 20 known MPV associations, we identified 32 PLT and 18 MPV associations not previously observed in the literature across the allele frequency spectrum, including rare large effect (*FCERIA*), low-frequency (*IQGAP2*, *MAP1A*, *LY75*), and common (*ZMIZ2*, *SMG6*, *PEAR1*, *ARFGAP3/PACSIN2*) variants. Several variants associated with PLT/MPV (*PEAR1*, *MRVII*, *PTGES3*) were also associated with platelet reactivity. In concurrent BCX analyses, there was overlap of platelet-associated variants with red (*MAP1A*, *TMPRSS6*, *ZMIZ2*) and white (*PEAR1*, *ZMIZ2*, *LY75*) blood cell traits, suggesting common regulatory pathways with shared genetic architecture among these hematopoietic lineages. Our large-scale Exomechip analyses identified previously undocumented associations with platelet traits and further indicate that several complex quantitative hematological, lipid, and cardiovascular traits share genetic factors.

Introduction

The number and size of circulating blood cells are determined by multiple genetic and environmental factors,

and abnormal values are a common manifestation of human disease. The three major cell types—red blood cells (RBCs), white blood cells (WBCs), and platelets—have distinct biological roles, with platelets serving as important

¹Population Sciences Branch, National Heart Lung and Blood Institute, The Framingham Heart Study, Framingham, MA 01702, USA; ²Department of Medicine, Université de Montréal, Montréal, QC H3T 1J4, Canada; ³Montreal Heart Institute, Montréal, QC H1T 1C8, Canada; ⁴Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald and Ernst-Mortiz-Arndt University Greifswald, Greifswald 17475, Germany; ⁵DZHK (German Centre for Cardiovascular Research), partner site Greifswald, Greifswald, Germany; ⁶Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA 02114, USA; ⁷Program in Medical and Population Genetics, Broad Institute, Cambridge, MA 02142, USA; ⁸Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA 02114, USA; ⁹Department of Medicine, Harvard Medical School, Boston, MA 02115, USA; ¹⁰Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa 9200942, Japan; ¹¹Department of Medicine, Division of General Internal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; ¹²Laboratory of Epidemiology and Population Sciences, National Institute on Aging, NIH, Baltimore, MD 21224, USA; ¹³The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; ¹⁴The Genetics of Obesity and Related Metabolic Traits Program, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; ¹⁵Genetics, Target Sciences, GlaxoSmithKline, Research Triangle Park, NC 27709, USA; ¹⁶OmicSoft Corporation, Cary, NC 27513, USA; ¹⁷Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55454, USA; ¹⁸Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, Houston, TX 77030, USA; ¹⁹Division of Epidemiology, Institute for Medicine and Public Health, Vanderbilt University, Nashville, TN 37235, USA; ²⁰Department of Medicine, University of Washington, Seattle, WA 98101, USA; ²¹Department of Genetics, University of North Carolina, Chapel Hill, NC 27514, USA; ²²Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908, USA; ²³Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh EH8 9JZ, UK; ²⁴Department of Psychology, University of Edinburgh, Edinburgh EH8 9JZ, UK; ²⁵Department of Epidemiology, Erasmus MC, Rotterdam 3000, the Netherlands; ²⁶Department of Epidemiology and Biostatistics, MRC-PHE Centre for Environment and Health, School of Public Health, Imperial College London, London W2 1PG, UK; ²⁷Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina 45110, Greece; ²⁸Divisions of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; ²⁹Divisions of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; ³⁰Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA 98195, USA; ³¹Department of Biomedical Informatics and Medical Education, University of Washington, Seattle, WA 98105, USA; ³²Department of Clinical Chemistry, Fimlab Laboratories, Tampere 33520,

(Affiliations continued on next page)

Dajiang J. Liu,⁴⁸ Global Lipids Genetics Consortium, Panos Deloukas,^{49,50} Nilesh J. Samani,^{51,52} Heribert Schunkert,^{53,54} Jeanette Erdmann,^{55,56} CARDIoGRAM Exome Consortium, Myocardial Infarction Genetics Consortium, Myriam Fornage,⁵⁷ Melissa Richard,⁵⁷ Jean-Claude Tardif,^{2,3} John D. Rioux,^{2,3} Marie-Pierre Dube,^{2,3} Simon de Denus,^{3,58} Yingchang Lu,¹³ Erwin P. Bottinger,¹³ Ruth J.F. Loos,¹³ Albert Vernon Smith,^{59,60} Tamara B. Harris,⁶¹ Lenore J. Launer,⁶¹ Vilmundur Gudnason,^{59,60} Digna R. Velez Edwards,⁶² Eric S. Torstenson,¹⁹ Yongmei Liu,⁶³ Russell P. Tracy,⁶⁴ Jerome I. Rotter,^{65,66} Stephen S. Rich,²² Heather M. Highland,^{67,68} Eric Boerwinkle,^{18,69} Jin Li,⁷⁰ Ethan Lange,^{21,71} James G. Wilson,⁷² Evelin Mihailov,⁷³ Reedik Mägi,⁷³ Joel Hirschhorn,^{7,74} Andres Metspalu,⁷³ Tõnu Esko,^{7,73} Caterina Vacchi-Suzzi,⁷⁵ Mike A. Nalls,⁷⁶ Alan B. Zonderman,¹² Michele K. Evans,¹² Gunnar Engström,^{77,78} Marju Orho-Melander,^{77,78} Olle Melander,^{77,78} Michelle L. O'Donoghue,⁷⁹ Dawn M. Waterworth,⁸⁰ Lars Wallentin,⁸¹ Harvey D. White,⁸² James S. Floyd,²⁰ Traci M. Bartz,⁸³ Kenneth M. Rice,⁸³ Bruce M. Psaty,^{84,85} J.M. Starr,^{23,86} David C.M. Liewald,^{23,24} Caroline Hayward,⁸⁷ Ian J. Deary,^{23,24} Andreas Greinacher,⁸⁸ Uwe Völker,^{4,5} Thomas Thiele,⁸⁸ Henry Völzke,^{5,89} Frank J.A. van Rooij,²⁵ André G. Uitterlinden,^{25,90,91} Oscar H. Franco,²⁵ Abbas Dehghan,²⁵ Todd L. Edwards,¹⁹ Santhi K. Ganesh,⁹² Sekar Kathiresan,^{6,7,8,9} Nauder Faraday,^{93,97} Paul L. Auer,^{94,97} Alex P. Reiner,^{95,96,97} Guillaume Lettre,^{2,3,97} and Andrew D. Johnson^{1,97,*}

mediators of hemostasis and wound healing. Platelet count (PLT) and mean platelet volume (MPV), a measure of platelet size, are clinical blood tests that are used to

screen for and diagnose disease. A number of well-described rare genetic disorders, including Bernard-Soulier syndrome (MIM: 231200), Glanzmann thrombasthenia

Finland;³³Department of Clinical Chemistry, University of Tampere School of Medicine, Tampere 33514, Finland;³⁴Department of Cardiology, Heart Center, Tampere University Hospital, Tampere 33521, Finland;³⁵University of Tampere, School of Medicine, Tampere 33514, Finland;³⁶Department of Clinical Physiology, Tampere University Hospital, Tampere 33521, Finland;³⁷Department of Clinical Physiology, University of Tampere, Tampere 33514, Finland;³⁸Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku 20521, Finland;³⁹Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku 20520, Finland;⁴⁰Departments of Medicine and Pathology, University of Vermont College of Medicine, Burlington, VT 05405, USA;⁴¹Department of Genome Sciences, University of Washington, Seattle, WA 98105, USA;⁴²Morehouse School of Medicine, Social Epidemiology Research Center, Cardiovascular Research Institute, Atlanta, GA 30310, USA;⁴³Department of Internal Medicine, Division of Cardiovascular Medicine, University of Michigan, Ann Arbor, MI 48108, USA;⁴⁴Department of Computational Medicine and Bioinformatics, Department of Human Genetics, University of Michigan, Ann Arbor, MI 48108, USA;⁴⁵Department of Biostatistics, University of Michigan, Ann Arbor, MI 48108, USA;⁴⁶Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA;⁴⁷Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI 48108, USA;⁴⁸Department of Public Health Sciences, College of Medicine, Pennsylvania State University, Hershey, PA 17033, USA;⁴⁹William Harvey Research Institute, Queen Mary University London, London E1 4NS, UK;⁵⁰Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders (PACER-HD), King Abdulaziz University, Jeddah 21589, Saudi Arabia;⁵¹Department of Cardiovascular Sciences, University of Leicester, Leicester LE1 7RH, UK;⁵²NIHR Leicester Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester LE3 9QP, UK;⁵³DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich 80333, Germany;⁵⁴Deutsches Herzzentrum München, Technische Universität München, Munich 80333, Germany;⁵⁵Institute for Integrative and Experimental Genomics, University of Lübeck, Lübeck 23562, Germany;⁵⁶DZHK (German Research Centre for Cardiovascular Research), partner site Hamburg/Lübeck/Kiel, Lübeck 23562, Germany;⁵⁷Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA;⁵⁸Faculty of Pharmacy, Université de Montréal, Montréal, QC H3T 1J4, Canada;⁵⁹Icelandic Heart Association, Kopavogur 201, Iceland;⁶⁰Faculty of Medicine, University of Iceland, Reykjavik 101, Iceland;⁶¹Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Intramural Research Program, NIH, Bethesda, MD 21224, USA;⁶²Vanderbilt Epidemiology Center, Department of Obstetrics & Gynecology, Institute for Medicine and Public Health, Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN 37203, USA;⁶³Center for Human Genetics, Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA;⁶⁴Departments of Pathology and Laboratory Medicine and Biochemistry, University of Vermont College of Medicine, Colchester, VT 05446, USA;⁶⁵Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute, Torrance, CA 90502, USA;⁶⁶Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA 90502, USA;⁶⁷The University of Texas School of Public Health, The University of Texas Graduate School of Biomedical Sciences at Houston, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA;⁶⁸Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27514, USA;⁶⁹Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA;⁷⁰Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Palo Alto, CA 94305, USA;⁷¹Department of Biostatistics, University of North Carolina, Chapel Hill, NC 27514, USA;⁷²Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS 39216, USA;⁷³Estonian Genome Center, University of Tartu, Tartu 51010, Estonia;⁷⁴Department of Endocrinology, Boston Children's Hospital, Boston, MA 02115, USA;⁷⁵Department of Family, Population and Preventive Medicine, Stony Brook University, Stony Brook, NY 11794, USA;⁷⁶Laboratory of Neurogenetics, National Institute on Aging, NIH, Bethesda, MD 21224, USA;⁷⁷Department of Clinical Sciences Malmö, Lund University, Malmö 221 00, Sweden;⁷⁸Skåne University Hospital, Malmö 222 41, Sweden;⁷⁹TIMI Study Group, Cardiovascular Division, Brigham and Women's Hospital, Boston, MA 02115, USA;⁸⁰Genetics, Target Sciences, GlaxoSmithKline, King of Prussia, PA 19406, USA;⁸¹Department of Medical Sciences, Cardiology, and Uppsala Clinical Research Center, Uppsala University, Uppsala 751 85, Sweden;⁸²Green Lane Cardiovascular Service, Auckland City Hospital and University of Auckland, Auckland 1142, New Zealand;⁸³Department of Biostatistics, University of Washington, Seattle, WA 98195, USA;⁸⁴Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology and Health Services, University of Washington, Seattle, WA 98101, USA;⁸⁵Group Health Research Institute, Group Health Cooperative, Seattle, WA 98101, USA;⁸⁶Alzheimer Scotland Research Centre, Edinburgh EH8 9JZ, UK;⁸⁷MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK;⁸⁸Institute for Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald 17475, Germany;⁸⁹Institute for Community Medicine, University Medicine Greifswald, Greifswald 13347, Germany;⁹⁰Department of Internal Medicine, Erasmus MC, Rotterdam 3000, the Netherlands;⁹¹Netherlands Consortium for Healthy Ageing (NCHA), Rotterdam 3015, the Netherlands;⁹²Departments of Internal and Human Genetics, University of Michigan, Ann Arbor, MI 48108, USA;⁹³Department of Anesthesiology & Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA;⁹⁴Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI 53205, USA;⁹⁵Department of Epidemiology, University of Washington, Seattle, WA 98105, USA;⁹⁶Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

*These authors contributed equally to this work

*Correspondence: johnsonad2@nhlbi.nih.gov
<http://dx.doi.org/10.1016/j.ajhg.2016.05.005>

(MIM: 273800), and Wiskott-Aldrich syndrome (MIM: 301000), as well as common conditions such as acute infection are characterized by abnormalities in the number, size, and/or reactivity of circulating blood platelets. MPV has also been reported to be an independent risk factor for myocardial infarction (MI) in population-based studies.¹ Accordingly, anti-platelet medications including aspirin and ADP/P2Y₁₂ receptor blockers such as clopidogrel and GIIb/IIIa inhibitors that reduce platelet reactivity are commonly used in the primary and secondary prevention of several cardiovascular conditions, including stroke and MI.^{2,3} Investigating the biological mechanisms that govern platelet number (PLT) and size (MPV) can provide insights into platelet development and clearance and has the potential to enhance our understanding of human diseases.

Genome-wide association studies (GWASs) have successfully identified numerous loci where variants are associated with PLT and MPV.^{4–13} To date, the largest GWAS of PLT ($n = 66,867$) and MPV ($n = 30,194$) identified 68 distinct loci.⁸ Subsequent functional experiments of several identified genes, including *ARHGEF3* (MIM: 612115), *DNM3* (MIM: 611445), *JMJD1C* (MIM: 604503), and *TPM1* (MIM: 191010), demonstrated their roles in hematopoiesis and megakaryopoiesis,^{8,14} as well as the potential of human genetic association methods to identify genetic factors that functionally contribute to platelet biology and dysfunction in disease.

Despite these successes, much of the heritability of these traits remains unexplained.¹⁵ GWASs of PLT and MPV have largely focused on more common (minor allele frequency [MAF] > 0.05) genetic variation, with many of the associated markers located in intronic or intergenic regions. The examination of rare (MAF < 0.01) and low-frequency (MAF: 0.01–0.05) variants, particularly those in protein coding regions, has the potential to identify previously unidentified causal variants. Indeed, recent studies reaching sample sizes of 31,340 individuals have identified rare to low-frequency coding variants associated with PLT in *MPL* (MIM: 159530), *CD36* (MIM: 173510), and *JAK2* (MIM: 147796), among others.^{16,17} Studies with larger sample size are needed to further characterize the contribution of rare and low-frequency genetic variation to PLT and MPV.

To conduct such a study of platelet-related traits, we formed the Blood Cell Consortium (BCX) to perform a large-scale meta-analysis of Exomechip association results of blood cell traits. In this report, we describe results from a meta-analysis of Exomechip association data in 157,293 and 57,617 participants for PLT and MPV, respectively. The Exomechip is a custom genotyping array enriched for rare to low-frequency coding variants; in addition, the Exomechip contains a scaffold of nonsynonymous variants and common SNPs obtained from the NHGRI GWAS catalog of complex disorders and traits. With increased sample size and use of the Exomechip, our goal was to identify rare, low-frequency, and common variants associated with PLT and MPV.

Material and Methods

Study Participants

The Blood Cell Consortium (BCX) was formed to identify genetic variants associated with blood cell traits using the Exomechip genotyping array. As the BCX is interested in the genetics of common hematological measures, our collaborative group is divided into three main working groups: RBC, WBC, and platelet.^{18,19} For the platelet working group, our sample is comprised of 157,293 participants from 26 discovery and replication cohorts of five ancestries: European (EA), African American (AA), Hispanic, East Asian, and South Asian. Detailed descriptions of the participating cohorts are provided in the [Tables S1–S4](#). All participants provided informed consent, and all protocols were approved by the participating studies' respective institutional review boards. In the platelet working group, we analyzed two traits: PLT ($\times 10^9/L$ of whole blood) and MPV (fL) ([Table S3](#)).

Genotyping and Quality Control

Each participating study used one of the following Exomechip genotyping arrays: Illumina ExomeChip v.1.0, Illumina ExomeChip v.1.1_A, Illumina ExomeChip-12 v.1.1, Illumina ExomeChip-12 v.1.2, Affymetrix Axiom Biobank Plus GSKBB1, or Illumina HumanOmniExpress ExomeChip ([Table S2](#)). Genotypes were called using either (1) a combination of the Illumina GenomeStudio and zCall software or (2) the Exomechip joint calling plan developed by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium ([Table S2](#)).²⁰ Standard quality-control criteria were applied by each study. Exclusion criteria included: (1) sample call rates, (2) excess heterozygosity rate, (3) Hardy-Weinberg equilibrium p values < 1×10^{-6} , and (4) sex mismatch. Additionally, ancestry was confirmed through principal components or multi-dimensional scaling analyses using linkage disequilibrium (LD) pruned markers ($r^2 < 0.2$) with MAF > 1%. Scatterplots anchored using the 1000 Genomes Project populations were visually inspected and ancestry outliers excluded. We included only autosomal and X chromosome variants. All remaining variants (including monomorphic variants) were aligned to the forward strand and alleles checked to ensure that the correct reference allele was specified. We performed study-specific quality control on each trait association results using EasyQC.²¹ We plotted variant allele frequencies from each study against ethnicity-specific reference population allele frequencies to identify allele frequency deviations and presence of flipped alleles. After all quality-control procedures, each study generated an indexed variant call file (VCF) for subsequent analyses that was checked for allele alignment using the checkVCF package.

Association Analysis

To assess the association between the blood cell traits and Exomechip variants in the BCX, we considered blood cell traits measured in standard peripheral complete blood counts. When possible, we excluded individuals with blood cancer, leukemia, lymphoma, bone marrow transplant, congenital or hereditary anemia, HIV, end-stage kidney disease, dialysis, splenectomy, and cirrhosis, and those with extreme measurements of platelet traits. We also excluded individuals on erythropoietin treatment as well as those on chemotherapy. Additionally, we excluded women who were pregnant and individuals with acute medical illness at the time of complete blood count.

For platelet traits, we used raw values of PLT ($\times 10^9/L$) and MPV (fL). In each participating study, residuals for PLT and MPV were first calculated from linear regression models that adjusted for age, age², sex, study center (where applicable), and principal components of genotype data. We then transformed these residuals using the rank-based inverse normal transformation. To confirm proper implementation of this transformation in each cohort, a scatterplot of the median standard error versus study-specific sample size was visually inspected for deviations using EasyQC.²¹ Autosomal and X chromosome variants were then tested for association with each blood cell trait using either RvTests or RAREMETALWORKER. Within individual cohorts, we performed analyses in ancestry-stratified groups: EA, AA, Hispanic, East Asian, and South Asian. Both statistical packages generate single variant association score summary statistics, variance-covariance matrices containing LD relationships between variants within a 1 MB window, and variant-specific parameters including MAF, chromosome, position, strand, genotype call rate, and Hardy-Weinberg equilibrium p values.

Discovery Association Meta-analysis

We performed ancestry-stratified (EA and AA) and combined all ancestry (All) meta-analyses of single variant association results using the Cochran-Mantel-Haenszel approach implemented in RareMETALS.²² In the multi-ancestry meta-analyses (All), we combined individuals of EA, AA, Hispanic, South Asian, and East Asian ancestries. We included variants in the meta-analysis if the genotype call rate was $\geq 95\%$. For palindromic variants (i.e., A/T and C/G variants), we compared allele frequencies taken across the entire consortium in order to detect flipped alleles. We kept variants with an allele frequency difference < 0.30 or < 0.60 for race-specific (EA and AA) or combined all ancestry analyses, respectively.²¹ Heterogeneity metrics (I^2 and heterogeneity p value) were calculated using METAL.²³ Using single-variant score statistics and variance-covariance matrices of LD estimates, we performed two types of gene-based tests: (1) variable threshold (VT) burden test with greatest power when all rare variants in a gene are associated consistently with a trait²⁴ and (2) sequence kernel association test (SKAT)²⁵ with better power than the burden approach when rare variants in a gene have heterogeneous effects. For all gene-based tests, we considered only missense, nonsense, and splice site single-nucleotide variants (SNVs) with MAF $\leq 1\%$. Similar to the single variant meta-analyses, gene-based results were generated for each major ancestry group (EA and AA) and for the combined multi-ancestry (All) samples.

Conditional Analysis

To identify independent signals around significant associations, we performed stepwise conditional analyses conditioning on the most significant single variant in a 1 MB window in RareMETALS. This procedure was repeated until there was no additional SNP significantly associated with phenotype in each region, defined as a p value that accounts for the number of markers tested in each ancestry group. For discovery and conditional single variant analyses, the threshold was: AA $p < 3.03 \times 10^{-7}$, EA $p < 2.59 \times 10^{-7}$, and All $p < 2.20 \times 10^{-7}$. For gene-based tests, the significance threshold accounted for the number of genes tested: AA $p < 2.91 \times 10^{-6}$, EA $p < 2.90 \times 10^{-6}$, and All $p < 2.94 \times 10^{-6}$. In regions like chromosome 12q24 with known extended LD structure spanning more than 1 MB, we performed a stepwise conditional analysis in GCTA using the Montreal Heart Institute

Biobank cohort to disentangle seven independent PLT-associated SNVs (Table S9),²⁶ conditioning on the most significant variant in the region.

Replication Meta-analysis

We attempted to replicate PLT and MPV associations with independent SNVs that reached significance levels in six independent cohorts (Figure 1, Table S4). Single variant association results of the six independent cohorts were combined in RareMETALS. Contributing replication cohorts adhered to identical quality control and association analysis procedures described previously for the discovery phase. We combined results in EA (PLT $n = 19,939$, MPV $n = 15,519$) and All (PLT $n = 35,436$, MPV $n = 16,088$) ethnicity groupings (Table S4). The results of discovery and replication phases were further combined using fixed effects inverse variance weighted meta-analysis in METAL.²³

Platelet Function Exomechip

Two BCX cohorts, GeneSTAR and the Framingham Heart Study (FHS), measured platelet aggregation in a subset of genotyped participants. Platelet aggregation measures are described in detail elsewhere and briefly below (Table S18).²⁷ Both studies isolated platelet-rich plasma from fasting blood samples and measured platelet aggregation after addition of agonists using a four-channel light transmission aggregometer (Bio/Data Corporation). FHS (Offspring Exam 5) tested aggregation for periods of 4 min after administration of ADP (0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, and 15.0 μM) and 5 min after administration of epinephrine (0.01, 0.03, 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, and 10.0 μM), as well as lag time(s) to aggregation with 190 $\mu g/mL$ calf skin-derived type I collagen (Bio/Data Corporation). Threshold concentrations (EC_{50}) were determined as the minimal concentration of agonist required to produce a $>50\%$ aggregation. The maximal aggregation response (% aggregation) was also determined for each participant at each concentration tested. GeneSTAR recorded maximal aggregation (% aggregation) for periods of 5 min after ADP (2.0 and 10.0 μM) and 5 min after epinephrine administration (2.0 and 10.0 μM), as well as lag time(s) to aggregation with equine tendon-derived type I collagen (1, 2, 5, and 10 $\mu g/mL$). Exomechip genotyping, quality control, and association analyses adhered to methods described previously for PLT and MPV analysis. We queried independent SNVs associated with PLT ($n = 79$) and/or MPV ($n = 38$) in these platelet aggregation association results and report platelet aggregation associations with $p < 0.001$.

Further Variant Annotation

In addition to primary analyses completed in this investigation, we took advantage of several existing resources to annotate our associated SNVs. Associated variants were cross-referenced with Combined Annotation Dependent Depletion (CADD) scores for Exomechip.²⁸ The Global Lipids Genetics Consortium (GLGC), the CARDIOGRAM Exome Consortium, and Myocardial Infarction Genetics Consortium have each performed independent Exomechip analysis of lipids traits and coronary heart disease (CHD).^{29,30} The CHD phenotype reflected a composite endpoint that included MI, CHD, coronary artery bypass graft, and hospitalized angina, among others.²⁹ Similar to the platelet aggregation lookups, we queried our list of PLT- and/or MPV-associated SNVs against their Exomechip association results for lipids and CHD. We report lipid and CHD associations with $p < 0.0001$. From a curated collection of more than 100 separate expression

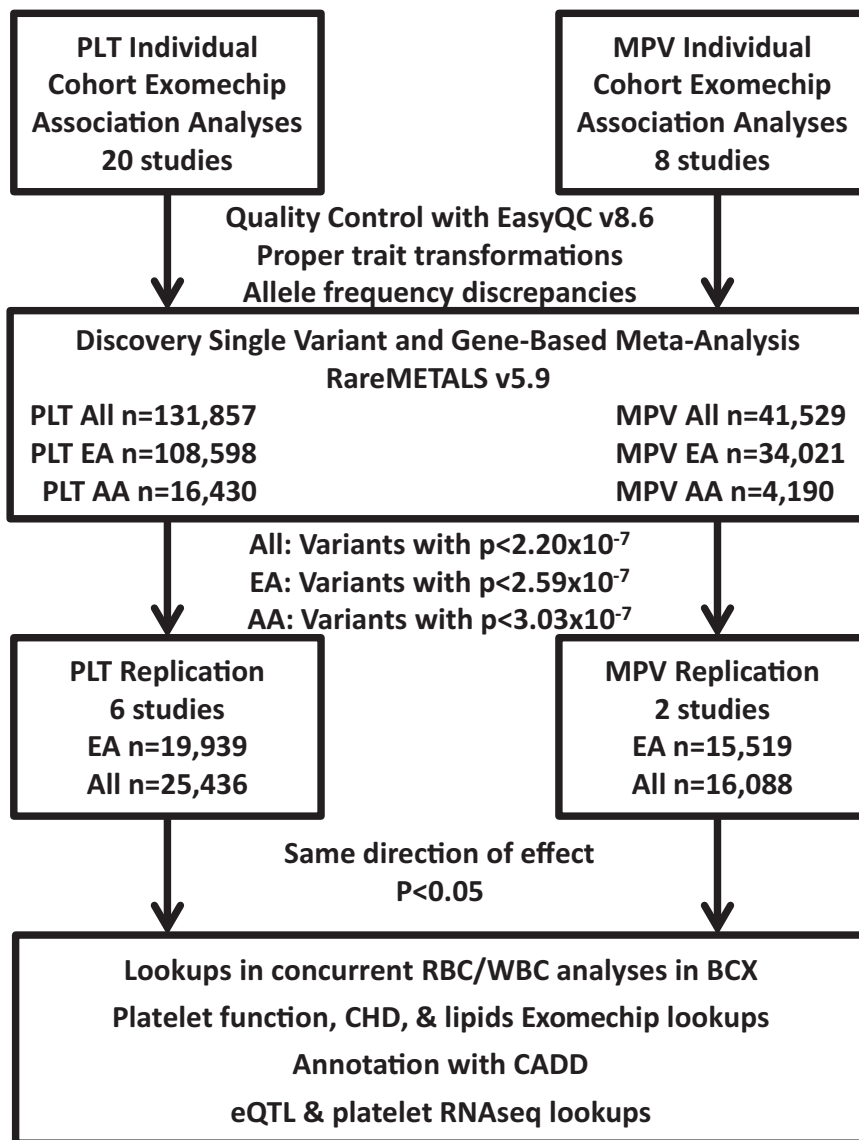


Figure 1. Study Design and Flow
Individual study-level association analyses were performed using RareMetalWorker or RVTests. To perform quality control of individual study association results, we used EasyQC v.8.6 to ensure proper trait transformations, to assess allele frequency discrepancies, and to evaluate other metrics. We then combined results in meta-analysis with RareMETALS v5.9 in three groups: African ancestry (AA), European ancestry (EA), and combined all five (AA, EA, Hispanic-Latino, East Asian, South Asian) ancestries (All). Independent variants identified by conditional analysis in RareMETALS with a p value less than the threshold corrected for multiple testing (All, $p < 2.20 \times 10^{-7}$; EA, $p < 2.59 \times 10^{-7}$; AA, $p < 3.03 \times 10^{-7}$) were carried forward for replication. Markers showed replication if they had $p < 0.05$ in the same direction of effect in the replication analyses. Associated markers were further annotated using various resources: (1) concurrent BCX Exomechip analyses of RBC and WBC traits, (2) on-going Exomechip analyses of platelet aggregation, quantitative lipids, and coronary heart disease (CHD) traits, (3) severity prediction by CADD, (4) an internal database of reported eQTL results, and (5) platelet RNA-seq data.

quantitative trait loci (eQTL) datasets, we conducted a more focused query of whether platelet loci were also associated with transcript expression in blood, arterial, and adipose-related tissues. A general overview of a subset of >50 eQTL studies has been published (Supplemental Data).³¹ Separately, we queried transcripts in loci corresponding to previously unreported associated variants and/or marginally associated variants showing further evidence of association in our replication analyses to assess their platelet expression levels using the largest platelet RNA-seq dataset to date ($n = 32$ patients with MI).³²

Results

Discovery Meta-Analysis

In our discovery phase, we performed a meta-analysis of the associations of 246,925 single-nucleotide variants (SNVs) with PLT and MPV in 131,857 and 41,529 individuals, respectively (Figures 1, S1, and S2; Tables S1–S4). After the initial meta-analyses, we ran conditional

analyses to identify independent loci and found 79 independent PLT and 38 independent MPV SNVs (Tables 1, 2, and S5–S8). One association, rs12692566 in *LY75-CD302*, with PLT in EA did not surpass the initial discovery statistical significance threshold but surpassed the threshold when conditioned on nearby rs78446341 ($p = 2.48 \times 10^{-7}$). There were no associations unique to the AA ancestry group, which had a limited sample size (Tables S10 and S11). Single variant meta-analysis results for each ancestry grouping that met our significance thresholds are available in the Supplement (Tables S10 and S11). Additionally, full discovery meta-analysis results are available online (Web Resources).

Of these independently associated single variants, 32 PLT and 18 MPV variants were in loci not previously reported (Tables 1 and 2). Of these 32 PLT loci, 4 had previously been identified as MPV loci (Table 1), and 10 of the 18 MPV loci had previously been identified with PLT (Table 2).^{8,9,17} Of the independent loci in our study, 23 SNVs showed association with both PLT and MPV (Table 3, Figure 2). All but one (rs6136489 intergenic to *SIRPA* [MIM: 602461] and *LOC727993*) had opposite directions of effect for PLT and MPV. Additionally, the observed effect sizes for PLT and MPV displayed strong negative correlations (Figure 2), indicative of the strong negative correlation between these traits.

Table 1. Previously Unreported Associations with PLT

rsID	Ref/Alt	Function	Gene	European Ancestry (EA)						Combined All Ancestry (All)					
				Discovery (n = 108,598)			Replication (n = 19,939)		Combined	Discovery (n = 131,857)			Replication (n = 25,436)		Combined
				EA F	Beta	p Value	Beta	p Value	p Value	EA F	Beta	p Value	Beta	p Value	p Value
rs3091242	C/T	intron	TMEM50A	0.54	-0.026	9.68×10^{-8}	-0.017	0.124	3.85×10^{-8}	0.50	-0.02	1.03×10^{-5}	-0.0084	0.390	1.24×10^{-5}
rs12566888	G/T	intron	PEAR1*	0.094	0.040	1.42×10^{-7}	0.061	1.26×10^{-3}	1.17×10^{-9}	0.16	0.034	2.09×10^{-8}	0.047	4.31×10^{-4}	5.71×10^{-11}
rs200731779	C/G	missense	FCERIA	1.5×10^{-5}	-2.96	2.48×10^{-7}	NA	NA	2.48×10^{-7}	1.2×10^{-5}	-2.96	2.48×10^{-7}	NA	NA	2.48×10^{-7}
rs6734238	A/G	intergenic	IL1F10/IL1RN	0.41	0.022	9.55×10^{-6}	0.0075	0.487	1.64×10^{-5}	0.41	0.026	7.19×10^{-9}	0.015	0.117	3.77×10^{-9}
rs12692566 ^a	C/A	missense	LY75-CD302*	0.82	-0.029	9.19×10^{-7}	-0.042	2.50×10^{-3}	1.23×10^{-8}	0.83	-0.026	2.27×10^{-6}	-0.05	7.84×10^{-5}	3.65×10^{-9}
rs78446341	G/A	missense	LY75-CD302*	0.02	0.092	4.16×10^{-9}	0.14	5.01×10^{-5}	1.98×10^{-12}	0.018	0.094	3.06×10^{-10}	0.13	9.23×10^{-5}	1.97×10^{-13}
rs56106611 ^b	T/G	missense	KALRN*	0.012	0.11	3.51×10^{-8}	0.11	7.14×10^{-3}	8.51×10^{-10}	0.01	0.11	8.59×10^{-8}	0.11	7.37×10^{-3}	2.14×10^{-9}
rs1470579	A/C	intron	IGF2BP2	0.32	-0.028	1.08×10^{-7}	-0.0073	0.562	2.82×10^{-7}	0.38	-0.023	6.07×10^{-7}	-0.012	0.272	5.15×10^{-7}
rs1126673	C/T	ncRNA	LOC100507053	0.69	0.026	6.38×10^{-8}	0.019	9.63×10^{-2}	1.81×10^{-8}	0.71	0.025	1.87×10^{-8}	0.014	0.168	1.12×10^{-8}
rs1473247 ^b	T/C	intron	RNF145*	0.27	-0.029	3.01×10^{-8}	-0.022	8.32×10^{-2}	7.28×10^{-9}	0.32	-0.026	1.32×10^{-8}	-0.025	1.85×10^{-2}	7.66×10^{-10}
rs2256183	A/G	intron	MICA	0.56	0.03	6.78×10^{-7}	-0.022	0.104	2.60×10^{-6}	0.59	0.028	2.13×10^{-7}	0.011	0.389	3.20×10^{-7}
rs1050331	T/G	3' UTR	ZMIZ2	0.47	0.037	1.32×10^{-15}	0.036	5.80×10^{-4}	3.28×10^{-18}	0.48	0.035	3.09×10^{-17}	0.031	8.80×10^{-4}	1.26×10^{-19}
rs755109	T/C	intron	HEMGN	0.37	0.028	2.87×10^{-9}	0.039	6.84×10^{-4}	1.17×10^{-11}	0.34	0.028	9.03×10^{-11}	0.044	2.18×10^{-5}	2.59×10^{-14}
rs2068888	G/A	nearGene-3	EXOC6	0.45	-0.023	2.81×10^{-7}	-0.012	0.266	2.47×10^{-7}	0.44	-0.022	1.19×10^{-7}	-0.012	0.212	8.61×10^{-8}
rs3794153	C/G	missense	ST5	0.45	-0.027	7.28×10^{-9}	-0.026	1.53×10^{-2}	3.57×10^{-10}	0.40	-0.027	2.19×10^{-9}	-0.023	2.47×10^{-2}	1.74×10^{-10}
rs174583	C/T	intron	FADS2	0.34	0.031	8.79×10^{-9}	0.048	1.22×10^{-4}	1.03×10^{-11}	0.34	0.028	4.72×10^{-9}	0.042	1.10×10^{-4}	4.42×10^{-12}
rs45535039	T/C	3' UTR	CCDC153	0.28	0.04	4.02×10^{-10}	0.071	5.31×10^{-2}	8.48×10^{-11}	0.28	0.04	2.5×10^{-12}	0.056	8.56×10^{-2}	6.25×10^{-13}
rs11616188	G/A	nearGene3	LTBR	0.42	-0.025	1.26×10^{-8}	-0.031	3.59×10^{-3}	1.81×10^{-10}	0.37	-0.025	7.57×10^{-9}	-0.033	1.07×10^{-3}	4.20×10^{-11}
rs10506328 ^b	A/C	intron	NFE2	0.64	0.033	5.63×10^{-11}	0.06	5.88×10^{-8}	2.01×10^{-16}	0.69	0.038	3.79×10^{-15}	0.059	2.33×10^{-8}	2.73×10^{-21}
rs2279574	C/A	missense	DUSP6	0.54	-0.023	2.47×10^{-7}	-0.0082	0.442	4.28×10^{-7}	0.50	-0.021	1.57×10^{-7}	-0.006	0.531	4.04×10^{-7}
rs61745424	G/A	missense	CUX2	0.025	-0.064	2.36×10^{-6}	-0.085	6.79×10^{-3}	6.49×10^{-8}	0.023	-0.068	1.37×10^{-7}	-0.073	1.43×10^{-2}	6.30×10^{-9}
rs2784521	A/G	nearGene-5	DDHD1	0.83	0.025	1.62×10^{-5}	0.0096	0.486	2.24×10^{-5}	0.76	0.028	2.92×10^{-8}	0.01	0.363	5.56×10^{-8}
rs55707100	C/T	missense	MAP1A*	0.032	0.095	7.03×10^{-14}	0.073	3.87×10^{-2}	9.53×10^{-15}	0.028	0.092	6.85×10^{-14}	0.082	1.62×10^{-2}	3.77×10^{-15}
rs10852932	G/T	intron	SMG6*	0.36	-0.024	1.82×10^{-6}	-0.042	8.93×10^{-4}	1.42×10^{-8}	0.39	-0.025	4.79×10^{-8}	-0.036	6.99×10^{-4}	2.15×10^{-10}
rs76066357	G/C	missense	ITGA2B*	0.014	-0.17	6.92×10^{-16}	-0.19	2.88×10^{-5}	1.05×10^{-19}	0.013	-0.16	1.92×10^{-15}	-0.18	6.00×10^{-5}	5.78×10^{-19}
rs1801689	A/C	missense	APOH*	0.036	0.083	6.34×10^{-12}	0.13	2.44×10^{-5}	1.82×10^{-15}	0.032	0.090	8.64×10^{-15}	0.12	2.03×10^{-5}	1.57×10^{-18}
rs892055	A/G	missense	RASGRP4	0.34	0.029	5.30×10^{-10}	0.018	9.87×10^{-2}	2.01×10^{-10}	0.38	0.025	3.49×10^{-9}	0.017	8.13×10^{-2}	9.96×10^{-10}

(Continued on next page)

Table 1. Continued

rsID	Ref/Alt	Function	Gene	European Ancestry (EA)						Combined All Ancestry (All)					
				Discovery (n = 108,598)			Replication (n = 19,939)			Discovery (n = 131,857)			Replication (n = 25,436)		
				EAF	Beta	p Value	Beta	p Value	EAF	Beta	p Value	Beta	p Value	Beta	p Value
rs3865444	C/A	5' UTR	CD33*	0.32	-0.026	1.11×10^{-6}	-0.034	2.52×10^{-3}	1.27×10^{-8}	0.29	-0.026	2.10×10^{-7}	-0.032	3.03×10^{-3}	2.59×10^{-9}
rs6136489 ^b	T/G	intergenic	SIRPA*	0.34	-0.033	8.69×10^{-13}	-0.028	1.24×10^{-2}	4.00×10^{-14}	0.39	-0.030	1.8×10^{-12}	-0.024	1.30×10^{-2}	8.78×10^{-14}
rs855791	A/G	missense	TMPRSS6*	0.56	-0.031	3.96×10^{-11}	-0.017	0.130	2.34×10^{-11}	0.60	-0.029	2.34×10^{-11}	-0.022	3.52×10^{-2}	2.97×10^{-12}
rs1018448	A/C	missense	ARFGAP3	0.54	-0.028	4.02×10^{-10}	-0.0053	0.618	2.62×10^{-9}	0.59	-0.025	1.55×10^{-9}	-0.0065	0.515	6.13×10^{-9}
rs738409	C/G	missense	PNPLA3*	0.23	-0.042	1.49×10^{-14}	-0.042	1.75×10^{-3}	1.03×10^{-16}	0.22	-0.044	1.33×10^{-18}	-0.038	1.61×10^{-3}	9.73×10^{-21}

We show variants in previously unreported loci (n = 32) and retained after conditional analyses in European ancestry (EA) ($p < 2.59 \times 10^{-7}$) and all ancestry (All) ($p < 2.20 \times 10^{-7}$) analyses. Associations in African ancestry (AA) had previously been reported in the literature (Table S10). Asterisks (*) indicate variants (20/32) showing evidence of replication ($p < 0.05$, same direction of effect). If multiple genes/transcripts were annotated to a variant, the transcript most expressed in Eicher et al.³² (Table S22) was selected. Full results and annotations are available in Table S5. Abbreviations are as follows: PLT, platelet count; MPV, mean platelet volume; REF, reference allele; ALT, alternate allele; EAF, effect allele frequency.

^aSurpasses significance threshold after conditioning on rs78446341 ($p = 2.48 \times 10^{-7}$).

^bPrevious association with MPV.

Associated variants ranged in allele frequency and included rare, low-frequency, and common SNVs. Most of the previously unreported associations were with common variants (PLT n = 25, MPV n = 15), although associations with low-frequency (PLT n = 6, MPV n = 2) and rare (PLT n = 1, MPV n = 1) variants were observed. Rare (PLT n = 6, MPV n = 1) SNVs associated with PLT and MPV had larger effects compared to common and low-frequency SNVs (Tables 1, 2, and S5–S8). A large majority of associated SNVs did not exhibit heterogeneous effects; however, one previously unreported association with *MRV1* and a few known associated loci (e.g., *MYL2/SH2B3/ATXN2*, *ARHGEF3*, *WDR66/HPD*, and *JAK2*) did show moderate to substantial heterogeneity across discovery studies (Table S23). Gene-based tests of missense, nonsense, and splice-site rare variants that found significant results largely reflected rare and low-frequency single variant results, with variants in *TUBB1* (MIM: 612901), *JAK2*, *LY75* (MIM: 604524), *IQGAP2* (MIM: 605401), and *FCER1A* (MIM: 147140) showing associations (Tables S12 and S13).

Replication Meta-Analysis

We attempted to replicate our associations in six independent cohorts (PLT n = 25,436, MPV n = 16,088) (Figure 1, Table S4). Of the loci not previously associated, 20/32 PLT and 11/18 MPV variants showed evidence of replication with $p < 0.05$ and the same direction of effect (Tables 1 and 2). In addition to the significant SNVs in our discovery analysis, we carried forward 13 PLT and 10 MPV sub-threshold variants that approached discovery significance thresholds with p values ranging from 2.47×10^{-7} to 1.99×10^{-6} (Tables S14 and S15). Of these, 7/13 PLT and 4/10 MPV showed associations in same direction of effect with $p < 0.05$ and surpassed significance thresholds when discovery and replication results were combined (Tables S14 and S15).

Intersection with Other Cardiovascular and Blood Traits

The BCX also completed analyses of RBC and WBC traits, so we cross-referenced our list of PLT- and MPV-associated SNVs with the results of the other blood cell traits.^{18,19} Of our replicated platelet loci previously unreported in the literature, six SNVs in *TMPRSS6* (MIM: 609862), *MAP1A* (MIM: 600178), *PNPLA3* (MIM: 609567), *FADS2* (MIM: 606149), *TMEM50A* (MIM: 605348), and *ZMIZ2* (MIM: 611196) showed association with RBC-related traits ($p < 0.0001$) (Table 4). Similarly, five replicated platelet SNVs previously unreported in the literature in *PEAR1* (MIM: 610278), *CD33* (MIM: 159590), *SIRPA*, *ZMIZ2*, and *LY75* showed association with WBC-related traits ($p < 0.0001$) (Table 4). To explore possible shared genetic associations of platelet size/number with platelet reactivity, we examined the association of PLT/MPV-associated SNVs with platelet reactivity to collagen, epinephrine, and ADP in GeneSTAR and FHS. Eight SNVs associated with PLT and/or MPV were also associated with platelet reactivity

Table 2. Previously Unreported Associations with MPV

rsID	Ref/Alt	Function	Gene	European Ancestry (EA)						Combined All Ancestry (All)					
				Discovery (n = 34,021)			Replication (n = 15,519)		Combined	Discovery (n = 41,529)			Replication (n = 16,088)		Combined
				EAF	Beta	p Value	Beta	p Value	p Value	EAF	Beta	p Value	Beta	p Value	p Value
rs6687605	T/C	missense	<i>LDLRAP1*</i>	0.53	0.046	8.27×10^{-12}	0.025	3.74×10^{-2}	1.80×10^{-9}	0.51	0.046	9.92×10^{-11}	0.024	3.58×10^{-2}	3.80×10^{-11}
rs56043070 ^a	G/A	splice	<i>GCSAML*</i>	0.069	0.094	1.30×10^{-9}	0.19	4.48×10^{-16}	1.12×10^{-21}	0.064	0.092	2.25×10^{-10}	0.19	3.66×10^{-16}	2.42×10^{-22}
rs1339847 ^a	G/A	missense	<i>TRIM58</i>	0.10	-0.10	1.47×10^{-13}	-0.037	5.44×10^{-2}	9.31×10^{-13}	0.10	-0.11	2.18×10^{-17}	-0.032	9.77×10^{-2}	1.06×10^{-15}
rs34968964 ^a	G/C	missense	<i>IQGAP2</i>	0.0049	0.32	7.65×10^{-9}	0.12	9.18×10^{-2}	1.99×10^{-8}	0.004	0.32	2.11×10^{-9}	0.11	0.106	8.18×10^{-9}
rs34950321 ^a	C/T	missense	<i>IQGAP2*</i>	0.018	0.18	7.80×10^{-10}	0.14	1.49×10^{-3}	6.03×10^{-12}	0.016	0.17	2.61×10^{-9}	0.14	1.59×10^{-3}	1.86×10^{-11}
rs34592828 ^a	G/A	missense	<i>IQGAP2*</i>	0.037	0.22	1.72×10^{-27}	0.16	2.73×10^{-9}	1.61×10^{-34}	0.032	0.23	1.68×10^{-31}	0.16	2.95×10^{-9}	2.98×10^{-38}
rs1012899 ^a	G/A	missense	<i>LRRC16A</i>	0.77	0.051	1.40×10^{-7}	0.012	0.417	1.24×10^{-6}	0.77	0.042	1.32×10^{-6}	0.016	0.273	2.50×10^{-6}
rs664370	A/G	missense	<i>PXT1*</i>	0.30	-0.034	8.03×10^{-5}	-0.025	5.61×10^{-2}	1.39×10^{-5}	0.35	-0.042	5.77×10^{-8}	-0.028	2.78×10^{-2}	7.23×10^{-9}
rs2343596 ^a	C/A	intron	<i>ZFPM2</i>	0.31	0.062	2.02×10^{-13}	0.012	0.357	3.32×10^{-11}	0.38	0.052	1.59×10^{-11}	0.012	0.339	4.35×10^{-10}
rs55895668 ^a	T/C	missense	<i>PLEC</i>	0.43	-0.042	5.94×10^{-7}	-0.013	0.350	2.19×10^{-6}	0.47	-0.041	1.23×10^{-7}	-0.011	0.409	5.97×10^{-7}
rs4909945	T/C	missense	<i>MRVII*</i>	0.68	-0.048	1.25×10^{-8}	-0.035	8.41×10^{-3}	5.19×10^{-10}	0.71	-0.041	3.96×10^{-7}	-0.035	7.42×10^{-3}	1.06×10^{-8}
rs11125	A/T	missense	<i>LGALS3</i>	0.078	-0.091	1.55×10^{-8}	-0.037	0.117	2.76×10^{-8}	0.07	-0.09	4.22×10^{-9}	-0.037	0.117	7.21×10^{-9}
rs2010875 ^a	C/T	missense	<i>PLEKHO2*</i>	0.14	-0.076	1.33×10^{-7}	-0.042	1.62×10^{-2}	2.10×10^{-8}	0.15	-0.063	3.01×10^{-7}	-0.042	1.62×10^{-2}	2.43×10^{-8}
rs10512472 ^a	T/C	missense	<i>SLFN14*</i>	0.18	-0.059	1.37×10^{-8}	-0.059	1.96×10^{-4}	1.12×10^{-11}	0.18	-0.058	3.15×10^{-10}	-0.059	1.20×10^{-4}	1.67×10^{-13}
rs35385129	C/A	missense	<i>PVR*</i>	0.16	-0.058	6.24×10^{-8}	-0.044	7.36×10^{-3}	2.01×10^{-9}	0.15	-0.055	3.00×10^{-8}	-0.043	7.13×10^{-3}	8.79×10^{-10}
rs2243603	C/G	missense	<i>SIRPB1</i>	0.77	0.044	5.89×10^{-6}	0.077	0.167	2.62×10^{-6}	0.79	0.049	4.58×10^{-8}	0.088	7.78×10^{-2}	1.25×10^{-8}
rs1018448	A/C	missense	<i>ARFGAP3*</i>	0.55	0.056	1.13×10^{-12}	0.051	1.78×10^{-5}	1.04×10^{-16}	0.60	0.055	1.52×10^{-13}	0.05	2.16×10^{-5}	1.68×10^{-17}
rs1997715	G/A	3' UTR	<i>ZXDB*</i>	0.26	0.048	1.93×10^{-9}	0.084	5.83×10^{-2}	4.26×10^{-10}	0.35	0.04	4.58×10^{-8}	0.08	3.99×10^{-2}	8.88×10^{-9}

We show variants in previously unreported MPV loci (n = 18) and retained after conditional analyses in European ancestry (EA) ($p < 2.59 \times 10^{-7}$) and all ancestry (All) ($p < 2.20 \times 10^{-7}$) analyses. Associations in African ancestry (AA) had previously been reported in the literature (Table S11). Asterisk (*) indicates variants (11/18) that showed evidence of replication ($p < 0.05$, same direction of effect). If multiple genes/transcripts were annotated to a variant, the transcript more expressed in Eicher et al.³² (Table S22) was selected. Full results and annotations are available in Table S7. Abbreviations are as follows: MPV, mean platelet volume; PLT, platelet count; REF, reference allele; ALT, alternate allele; EAF, effect allele frequency.

^aPrevious association with PLT.

Table 3. Variants Associated with Both PLT and MPV

rsID	Gene	PLT	MPV
rs12566888	<i>PEAR1</i>	↑	↓
rs1668873	<i>TMCC2</i>	↑	↓
rs56043070	<i>GCSAML</i>	↓	↑
rs12485738	<i>ARHGEF3</i>	↑	↓
rs56106611	<i>KALRN</i>	↑	↓
rs34592828	<i>IQGAP2</i>	↓	↑
rs1012899	<i>LRRC16A</i>	↓	↑
rs342293	<i>PIK3CG</i>	↓	↑
rs2343596	<i>ZFPM2</i>	↓	↑
rs10761731	<i>JMJD1C</i>	↑	↓
rs11602954	<i>BET1L</i>	↑	↓
rs10506328	<i>NFE2</i>	↑	↓
rs2958154	<i>PTGES3</i>	↓	↑
rs7961894	<i>WDR66</i>	↓	↑
rs1465788	<i>ZFP36L1</i>	↑	↓
rs2297067	<i>EXOC3L4</i>	↑	↓
rs2138852	<i>TAOK1</i>	↓	↑
rs10512472	<i>SLFN14</i>	↑	↓
rs11082304	<i>CABLES1</i>	↓	↑
rs6136489*	<i>SIRPA/LOC727993</i>	↓	↓
rs41303899	<i>TUBB1</i>	↓	↑
rs6070697	<i>TUBB1</i>	↑	↓
rs1018448	<i>ARFGAP3</i>	↓	↑

All variants listed here showed association with both PLT and MPV in the opposite direction of effect as indicated by the arrows, except for rs6136489 (denoted by asterisk), which showed association with decreased PLT and decreased MPV. Abbreviations are as follows: PLT, platelet count; MPV, mean platelet volume.

($p < 0.001$) (Tables 5, S18, and S19). The most strongly associated SNVs were located in genes implicated with platelet reactivity in prior GWASs, including *PEAR1*, *MRVI1* (MIM: 604673), *JMJD1C*, and *PIK3CG* (MIM: 601232).²⁷ However, we did observe new suggestive relationships between platelet reactivity and SNVs in *PTGES* (MIM: 607061), *LINC00523*, and *RASGRP4* (MIM: 607320) (Table 5).

In addition to examining possibly shared genetic associations with blood cell-specific traits, we queried our list of associated platelet SNVs against independent Exomechip genotyping efforts in lipids and CHD by the GLGC, CARDIoGRAM Exome Consortium, and Myocardial Infarction Genetics Consortium Exomechip studies.^{29,30} Numerous platelet-associated SNVs ($n = 37$), including those in *GCKR* (MIM: 600842), *FADS1* (MIM: 606148), *FADS2*, *MAP1A*, *APOH* (MIM: 138700), and *JMJD1C*, showed association with one or more lipids traits ($p < 0.0001$) (Table S20). Far fewer ($n = 4$; *MYL2*

[MIM: 160781], *SH2B3* [MIM: 605093], *BRAP* [MIM: 604986], *APOH*) showed association with CHD ($p < 0.0001$) (Table S20).

Annotation of Associated Variants

We used various resources to annotate our platelet-associated variants. First, we used CADD to predict the putative functional severity of associated variants.²⁸ As expected, rare and low-frequency coding SNVs were predicted to be more severe than common, non-coding variation (Tables 1, 2, and S5–S8). To assess potential impact on gene expression, we queried our list of platelet-associated SNVs against a collection of results from existing eQTL datasets.³¹ Many ($n = 67$) platelet-associated SNVs were also associated with gene expression in blood, arterial, or adipose tissues (Table S21). These included the reported *trans*-eQTL rs12485738 in *ARHGEF3* with several platelet-related transcript targets (e.g., *GP1BA*, *GP6*, *ITGA2B*, *MPL*, *TUBB1*, and *VWF*),³³ as well as eQTLs in newly identified PLT/MPV loci (e.g., rs1018448 with *ARFGAP3/PACSIN2*, rs1050331 with *ZMIZ2*, and rs174546 with *FADS1/FADS2/TMEM258* expression). Using platelet RNA-seq data from 32 subjects with MI, we found that almost all of the genes closest to our previously unreported associated SNVs or marginal SNVs with evidence of replication were expressed in platelets, indicating the feasibility of potential functional roles in the relevant target cell type (Table S22).³²

Discussion

Here, we present a large-scale meta-analysis of Exomechip association data with two clinical platelet measurements, PLT and MPV. By combining Exomechip association results in 157,293 and 57,617 participants, respectively, we detected numerous associations with rare, low-frequency, and common variants. There was substantial overlap of our platelet associations with concurrent Exomechip association findings for RBC and WBC traits, indicating shared genetic influence on regulatory and functional mechanisms among the three different blood cell lineages.^{18,19} More surprisingly, we observed shared associations of platelet and lipids loci. The identification of shared blood cell and lipids associations as well as identifying genes with entirely new associations reveals candidates for further examination in order to elucidate the mechanisms underlying platelet development and function.

Using Exomechip to Identify Previously Unreported Genetic Associations

Using the Exomechip that has an emphasis on rare and infrequent coding variation, we found associations with variants that ranged from common to rare in allele frequency. We attempted to replicate independent associations, although our replication cohorts were underpowered to associations of rare variants. To inform our replication criteria, we conducted a power analysis by using a

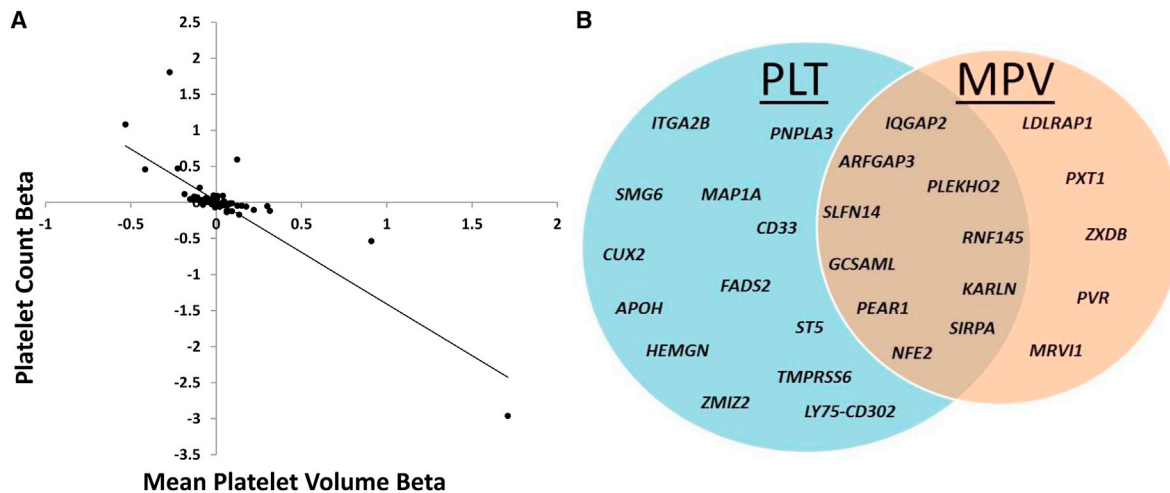


Figure 2. Shared PLT and MPV Genetic Associations

(A) Comparing PLT and MPV effects sizes ($r = -0.84$) in European ancestry (EA) analyses of all identified SNVs identified ($n = 124$). Examined SNPs include all those from Tables 1, 2, S5–S9, S14, and S15.

(B) 56 independent SNVs showed association to PLT only, and 15 independent SNVs were associated with MPV only. 23 independent SNVs were associated with both PLT and MPV. Named genes indicate that the association was not previously reported in the literature.

sample size of 20,000 and considering multiple combinations of allele frequencies and effect sizes. Based on allele frequency and effect size, our most difficult to replicate variant was rs56106611 (MAF = 0.012, Beta = 0.11). However, we still had approximately 80% power to detect this association in the replication stage. Despite this, replication of extremely rare variants remains a challenge. For example, there were associations with rare coding variants with large effect sizes in *FCER1A*, *MPL*, *JAK2*, *SH2B3*, *TUBB1*, and *IQGAP2*.^{16,17} The overall effect size of these rare variants must be validated in independent studies. The PLT-associated and predicted deleterious variant rs200731779 in *FCER1A* (p.Leu114Val) had a large effect ($\beta = -2.96$) in discovery analyses, but could not be replicated in available samples due to its extremely rare allele frequency (MAF = 1.48×10^{-5} in EA). The affected amino acid is extracellularly positioned near the interface of two Ig-like domains, an area of the protein critical for FC-IgE interaction as shown through its crystal structure, biochemical data, and mutagenesis studies.^{34–37} Other variants in *FCER1A*, a subunit of the allergy response IgE receptor and basophil differentiation factor, have previously been associated with IgE levels and monocyte counts.^{38,39} Increased platelet activation has been postulated to contribute to or be a consequence of allergic and inflammatory responses.⁴⁰ Our association of rare deleterious variation in *FCER1A* to reduced PLT provides a further link between platelet biology and allergy response.

Although SNVs in *IQGAP2* have previously been associated with PLT, we detected independent *IQGAP2* low-frequency and rare missense variants associated with increased MPV (Table 2, Figures S3 and S4).^{8,17} Located proximal to thrombin receptor *F2R* (MIM: 187930), *IQGAP2* functions in the cytoskeletal dynamics in response to thrombin-induced platelet aggregation.⁴¹ We

did not observe *IQGAP2* associations with platelet aggregation, which may be due to the rare/low-frequency nature of the SNVs and the absence of thrombin-induced aggregation data in the available cohorts. Nonetheless, the associations of rare and low-frequency variants in *IQGAP2* further strengthen its contribution to platelet biology. In addition to *IQGAP2*, we observed other low-frequency associations, including nonsynonymous coding variants in *ITGA2B* (MIM: 607759), *LY75*, *MAP1A*, and *APOH*. The SNV rs76066357 in *ITGA2B*, a gene implicated in Glanzmann's thrombasthenia (MIM: 273800), was associated with decreased PLT (Table 1). Moreover, *ITGA2B* codes for the platelet glycoprotein alpha-IIb, which is part of the target receptor of GIIb/IIIa inhibitors (e.g., eptifibatid and abciximab) used in the acute management of acute coronary syndromes. Although ClinVar lists rs76066357 as pathogenic (ID: 216944) with limited evidence, rs76066357 is a non-rare, predicted benign variant that contributes to population variability in PLT in our study as opposed to a severe Mendelian disorder of platelet reactivity.⁴² Previous studies do suggest a potential role for variants in *ITGA2B* and *ITGB3* (MIM: 173470) leading to thrombocytopenia as well as abnormalities in platelet reactivity.⁴³

In addition to rare and low-frequency variant associations, we detected previously unreported associations for PLT and MPV at 25 and 15 common loci, respectively. For example, a common missense SNV rs1018489 in *ARFGAP3* (MIM: 612439) showed association with decreased PLT and increased MPV. This variant is an eQTL for both *ARFGAP3* and neighboring gene *PACSIN2* (MIM: 604960) in blood tissues (Table S21, Figures S5 and S6). Although the possible role of the androgen receptor (AR) gene target and cellular secretory factor *ARFGAP3* is unknown in platelets,^{44–46} *PACSIN2* functions in the

Table 4. Intersection of Platelet-Associated Variants with RBC and WBC Traits

SNP	MarkerName	Gene	PLT	Trait	Other Blood Cell
rs855791	chr22: 37,462,936	<i>TMPRSS6</i>	↓	MCH, MCV, HGB MCHC, HCT	↑
rs855791	chr22: 37,462,936	<i>TMPRSS6</i>	↓	RDW	↓
rs55707100	chr15: 43,820,717	<i>MAP1A</i>	↑	HGB, MCH, HCT, MCHC	↓
rs174583	chr11: 61,609,750	<i>FADS2</i>	↑	RDW	↓
rs174583	chr11: 61,609,750	<i>FADS2</i>	↑	HGB, RBC, HCT, MCHC	↑
rs738409	chr22: 44,324,727	<i>PNPLA3</i>	↓	HCT, HGB	↑
rs3091242	chr1: 25,674,785	<i>TMEM50A</i>	↓	RDW	↑
rs1050331	chr7: 44,808,091	<i>ZMIZ2</i>	↑	MCH, MCV	↓
rs1050331	chr7: 44,808,091	<i>ZMIZ2</i>	↑	WBC	↑
rs6734238 ^a	chr2: 113,841,030	<i>IL1F10/IL1RN</i>	↑	MCH	↓
rs6734238 ^a	chr2: 113,841,030	<i>IL1F10/IL1RN</i>	↑	WBC, NEU	↑
rs12566888	chr1: 156,869,047	<i>PEAR1</i>	↑	WBC, NEU, MON	↓
rs3865444	chr19: 51,727,962	<i>CD33</i>	↓	WBC	↓
rs6136489	chr20: 1,923,734	<i>SIRPA/LOC727993</i>	↓	WBC, LYM	↓
rs2256183 ^a	chr6: 31,380,529	<i>MICA</i>	↑	BAS	↑
rs12692566	chr2: 160,676,427	<i>LY75-CD302</i>	↓	WBC	↓

We cross-referenced novel variants associated with platelet count (PLT) and/or mean platelet volume (MPV) in RBC and WBC association analyses in the Blood Cell Consortium (BCX). Here, we show RBC/WBC-associated platelet variants with $p < 0.0001$. Full details of RBC/WBC associations are shown in Tables S16 and S17. Arrows denote direction of effect for the platelet and other blood cell trait(s). Abbreviations are as follows: BCX, Blood Cell Consortium; RBC, red blood cell; WBC, white blood cell; PLT, platelet count; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HGB, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; RDW, red blood cell distribution width; PLT, platelet count; NEU, neutrophil; MON, monocyte; LYM, lymphocyte; BAS, basophil.

^aMarker not replicated in platelet analyses.

formation of the megakaryocyte demarcation membrane system during platelet production through interactions with FlnA.⁴⁷ Genetic variation that influences *PACSIN2* expression may hinder the formation of the megakaryocyte demarcation membrane system and lead to the production of fewer but larger and potentially more reactive platelets. We also observed several other novel associations with common variants, including those in *SMG6* (MIM: 610963), a mediator of embryonic stem cell differentiation through nonsense-mediated decay, and *LY75*, an endocytotic immunity-related receptor highly expressed on dendritic cells where it is involved in recognition of apoptotic and necrotic cells.^{48–50}

Overlap with Other Platelet and Blood Cell Traits

There was substantial overlap of variants associated with both PLT and MPV ($n = 23$) as well as a strong negative correlation in effect sizes, consistent with the documented negative correlation between the two traits in population studies (Figure 2).⁵¹ Only rs6136489, a reported eQTL for *SIRPA*, showed the same direction of effect for both PLT and MPV. *SIRPA* directly interacts with CD47, and *SIRPA/CD47* signaling plays an important role in platelet clearance and the etiology of immune thrombocytopenia purpura.^{52–54} Knockout *Sirpa* mice exhibit thrombocytopenia phenotypes, although they have similar MPV to control

animals.⁵⁴ How genetic variation in *SIRPA* influences MPV in addition to its demonstrated contribution to PLT remains to be characterized. In addition to shared associations of PLT and MPV, there was overlap in the parallel Exomechip analyses of platelet reactivity. Largely mirroring results from previous GWASs, markers within *PEAR1*, *JMJD1C*, *PIK3CG*, and *MRVII* showed the strongest associations with PLT/MPV and platelet reactivity.^{27,55–57} Other PLT/MPV-associated markers in *PTGES3*, *LINC00523*, and *RASGRP4* showed marginal associations. Notably, *PTGES3* is linked to prostaglandin synthesis and the RasGRP family has been shown to have functional roles in blood cells including in platelet adhesion.⁵⁸ The association of platelet reactivity genes, particularly *PEAR1* and *MRVII*, with PLT/MPV further supports a biological relationship between processes that control platelet function, megakaryopoiesis, and clearance.^{51,59,60} However, these large-scale association analyses are unable to demonstrate whether these shared associations indicate shared biological mechanisms or simply reflect the epidemiological correlations among these traits.

In addition to platelet traits, there was substantial overlap of genetic associations with RBC and WBC traits examined by the BCX.^{18,19} The shared genetic associations with the two other primary blood cell lineages further supports other studies proposing that mechanisms that govern

Table 5. Overlap of Associations of Platelet Count and Mean Platelet Volume Variants with Platelet Reactivity

rsID	Gene	PLT	MPV	Agonist(s) ^a	Direction of Effects ^b
rs12566886	<i>PEAR1</i>	↑	↓	epi, ADP, collagen	↓↓↓
rs10761731	<i>JMJD1C</i>	↑	↓	epi, ADP	↑↑
rs12355784	<i>JMJD1C</i>	↑	ns	epi	↑
rs342293	<i>PIK3CG</i>	↓	↑	epi	↓
rs4909945	<i>MRV11</i>	ns	↓	epi, ADP	↓↓
rs2958154	<i>PTGES3</i>	↓	↑	collagen	↑
rs12883126	<i>LINC00523</i>	↑	ns	epi	↑
rs892055	<i>RASGRP4</i>	↑	ns	epi	↓

Variants were examined using platelet reactivity phenotypes (Table S18) in GeneSTAR and the Framingham Heart Study (FHS). Arrows denote direction of effect for platelet count (PLT), mean platelet volume (MPV), and platelet reactivity ($p < 0.001$). Multiple arrows refer to direction for respective agonist for platelet reactivity. Detailed association results for platelet reactivity are given in Table S19. Abbreviations are as follows: PLT, platelet count; MPV, mean platelet volume; ns, not significant ($p > 0.05$); epi, epinephrine.

^aPlatelet reactivity associations with $p < 0.001$.

^bCollagen measurements reflect lag time to aggregation, so direction of effect has been flipped to denote a negative direction of effect as less reactive and positive direction of effect as more reactive.

platelet size and number also influence RBC and WBC traits.⁶¹ In BCX analyses, rs1050331 in the 3' UTR of *ZMIZ2* was associated with increased PLT, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), as well as with decreased WBC count.^{18,19} rs1050331 is also an eQTL for *ZMIZ2* expression in whole blood (Table S21).⁶² There are known sex differences in cell counts, with females consistently having higher PLT and mixed results on MPV.^{63,64} Similar to well-established PLT- and MPV-associated transcriptional regulator *JMJD1C*, *ZMIZ2* directly interacts with AR to modulate AR-mediated transcription and influences mesodermal development, and thus genetic variation in *ZMIZ2* could potentially contribute to hormonally mediate differences in PLT across genders.^{65–67} Also associated with increased PLT and decreased RBC indices was rs55707100 in *MAP1A*.¹⁸ Though typically examined in a neurological context, *MAP1A* is involved in microtubule assembly, a process important in blood cell development and function.⁶⁸ Our observed association of *MAP1A* and its expression in platelets and RBCs suggests that the known role of *MAP1A* in developmental and cytoskeletal processes in neural tissues may extend to blood cells (Table S22). How these shared genetic factors specifically influence the development, maintenance, or clearance of multiple blood cell types remains to be determined.

Overlap with Non-Blood Cell Traits

Although the overlap with other blood cell traits may be intuitive, we also observed overlap with quantitative lipids traits. In cross-trait lookups, several known PLT/MPV loci confirmed in this study (e.g., *JMJD1C*, *GCKR*, and *SH2B3*) showed associations with lipids traits, and several known

lipids loci showed association to PLT/MPV (e.g., *FADS1*, *FADS2*, *APOH*, and *TMEM50A*). Moreover, *SH2B3*, which is also expressed in human vascular endothelial cells where it modulates inflammation, has been associated with blood pressure and the risk of MI.^{69–71} Our study further suggests that a regulation of platelets could also contribute to potential implication of *SH2B3* in the development of cardiovascular diseases. The associated SNVs in the *FADS1/FADS2* locus (rs174546 and rs174583) are eQTLs for multiple lipid-related transcripts in blood-related tissues, including *TMEM258*, *FADS1*, *FADS2*, and *LDLR* (Table S21).⁶² Intriguingly, expression of *TMEM258* has also been shown to be a transcriptional regulatory target of cardiovascular disease implicated *CDKN2B-AS1* (MIM: 613149), a region marginally associated with PLT (discovery EA $p = 1.00 \times 10^{-6}$, replication EA $p = 0.0577$, combined EA $p = 1.56 \times 10^{-7}$) (Table S14).^{72,73} Our genetic association results link the underlying genetic architecture of platelet and lipids traits as suggested by previous epidemiological, genetic, and animal studies.^{63,74–77} However, these observed shared genetic associations do not demonstrate whether these reflect direct genetic pleiotropy or indirect relationships. Several variants previously implicated in lipids (e.g., *FADS1*, *FADS2*, *SH2B3*, *TMEM50A*, and *GCKR*) have stronger associations with lipids traits relative to our platelet associations, suggesting that their primary effects are on lipids pathways (Table S20). Determining the directionality and causality among genetic variants, lipids, and platelets remains an important future step in dissecting which genetic variants may reveal new insights into platelet biology.

Conclusions

By performing a large meta-analysis of Exomechip association results, we identified rare, low-frequency, and common variants that influence PLT and MPV. Despite our ability to detect numerous associations with SNVs across a wide range of allele frequencies, the Exomechip interrogated a limited fraction of genomic variation. Sequencing-based studies across the genome in large sample sizes will be necessary to fully assess the contribution of variants across the allele frequency spectrum, particularly of rare variants in intergenic regions. Nonetheless, our results identify several intriguing genes and genetic mechanisms of platelet biology. Many of these associations overlapped with related blood cell and lipids traits, pointing to common mechanisms underlying their development and maintenance. Because blood cells share developmental lineages and several of our platelet-associated genes have known developmental or transcriptional regulatory functions, we hypothesize that the origins of these shared genetic associations are mainly in blood cell development in the bone marrow. How these genes function and interact in RBC, WBC, and platelet development will need to be tested in future experiments in both functional and human-based studies. Advances in these domains could provide key insights into genes that influence

human blood disorders and reveal new mechanisms for the development of novel therapeutic applications.

Supplemental Data

Supplemental Data include a note on eQTL analyses and additional funding information, 6 figures, and 23 tables and can be found with this article online at <http://dx.doi.org/10.1016/j.ajhg.2016.05.005>.

Acknowledgments

We thank all participants and study coordinating centers. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute, the NIH, or the U.S. Department of Health and Human Services. The Framingham Heart Study (FHS) authors acknowledge that the computational work reported on in this paper was performed on the Shared Computing Cluster, which is administered by Boston University's Research Computing Services. The MHI Biobank acknowledges the technical support of the Beaulieu-Saucier MHI Pharmacogenomic Center. We would like to thank Liling Warren for contributions to the genetic analysis of the SOLID-TIMI-52 and STABILITY datasets. The University Medicine Greifswald is a member of the Caché Campus program of the InterSystems GmbH. The SHIP and SHIP-TREND samples were genotyped at the Helmholtz Zentrum München. Estonian Genome Center, University of Tartu (EGCUT) would like to acknowledge Mr. V. Soo, Mr. S. Smith, and Dr. L. Milani. The Airwave Health Monitoring Study thanks Louisa Cavaliero who assisted in data collection and management as well as Peter McFarlane and the Glasgow CARE, Patricia Munroe at Queen Mary University of London, and Joanna Sarnecka and Ania Zawodniak at Northwick Park. FINCAVAS thanks the staff of the Department of Clinical Physiology for collecting the exercise test data. Young Finns Study (YFS) acknowledges the expert technical assistance in statistical analyses by Irina Lisinen.

Received: February 18, 2016

Accepted: May 3, 2016

Published: June 23, 2016

Web Resources

1000 Genomes, <http://www.1000genomes.org>
BCX ExomeChip association results, <http://www.mhi-humangenetics.org/en/resources>
CheckVCF, <https://github.com/zhanxw/checkVCF>
ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar/>
OMIM, <http://www.omim.org/>
RareMETALS, <http://genome.sph.umich.edu/wiki/RareMETALS>
RareMetalWorker, <http://genome.sph.umich.edu/wiki/RAREMETALWORKER>
Research Computing Services, <http://www.bu.edu/tech/support/research/>
RvTests, <http://genome.sph.umich.edu/wiki/RvTests>

References

1. Chu, S.G., Becker, R.C., Berger, P.B., Bhatt, D.L., Eikelboom, J.W., Konkle, B., Mohler, E.R., Reilly, M.P., and Berger, J.S. (2010). Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. *J. Thromb. Haemost.* 8, 148–156.
2. Sutcliffe, P., Connock, M., Gurung, T., Freeman, K., Johnson, S., Kandala, N.B., Grove, A., Gurung, B., Morrow, S., and Clarke, A. (2013). Aspirin for prophylactic use in the primary prevention of cardiovascular disease and cancer: a systematic review and overview of reviews. *Health Technol. Assess.* 17, 1–253.
3. Hennekens, C.H., Dyken, M.L., and Fuster, V. (1997). Aspirin as a therapeutic agent in cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* 96, 2751–2753.
4. Schick, U.M., Jain, D., Hodonsky, C.J., Morrison, J.V., Davis, J.P., Brown, L., Sofer, T., Conomos, M.P., Schurmann, C., McHugh, C.P., et al. (2016). Genome-wide association study of platelet count identifies ancestry-specific loci in Hispanic/Latino Americans. *Am. J. Hum. Genet.* 98, 229–242.
5. Soranzo, N., Rendon, A., Gieger, C., Jones, C.I., Watkins, N.A., Menzel, S., Döring, A., Stephens, J., Prokisch, H., Erber, W., et al. (2009). A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function. *Blood* 113, 3831–3837.
6. Shameer, K., Denny, J.C., Ding, K., Jouni, H., Crosslin, D.R., de Andrade, M., Chute, C.G., Peissig, P., Pacheco, J.A., Li, R., et al. (2014). A genome- and phenome-wide association study to identify genetic variants influencing platelet count and volume and their pleiotropic effects. *Hum. Genet.* 133, 95–109.
7. Qayyum, R., Snively, B.M., Ziv, E., Nalls, M.A., Liu, Y., Tang, W., Yanek, L.R., Lange, L., Evans, M.K., Ganesh, S., et al. (2012). A meta-analysis and genome-wide association study of platelet count and mean platelet volume in African Americans. *PLoS Genet.* 8, e1002491.
8. Gieger, C., Radhakrishnan, A., Cvejic, A., Tang, W., Porcu, E., Pistis, G., Serbanovic-Canic, J., Elling, U., Goodall, A.H., Labruno, Y., et al. (2011). New gene functions in megakaryopoiesis and platelet formation. *Nature* 480, 201–208.
9. Soranzo, N., Spector, T.D., Mangino, M., Kühnel, B., Rendon, A., Teumer, A., Willenborg, C., Wright, B., Chen, L., Li, M., et al. (2009). A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. *Nat. Genet.* 41, 1182–1190.
10. Kim, Y.K., Oh, J.H., Kim, Y.J., Hwang, M.Y., Moon, S., Low, S.K., Takahashi, A., Matsuda, K., Kubo, M., Lee, J., and Kim, B.J. (2015). Influence of genetic variants in EGF and other genes on hematological traits in Korean populations by a genome-wide approach. *BioMed Res. Int.* 2015, 914965.
11. Oh, J.H., Kim, Y.K., Moon, S., Kim, Y.J., and Kim, B.J. (2014). Genome-wide association study identifies candidate loci associated with platelet count in Koreans. *Genomics Inform.* 12, 225–230.
12. Li, J., Glessner, J.T., Zhang, H., Hou, C., Wei, Z., Bradfield, J.P., Mentch, F.D., Guo, Y., Kim, C., Xia, Q., et al. (2013). GWAS of blood cell traits identifies novel associated loci and epistatic interactions in Caucasian and African-American children. *Hum. Mol. Genet.* 22, 1457–1464.
13. Guerrero, J.A., Rivera, J., Quiroga, T., Martínez-Perez, A., Antón, A.I., Martínez, C., Panes, O., Vicente, V., Mezzano, D., Soria, J.M., and Corral, J. (2011). Novel loci involved in platelet function and platelet count identified by a genome-wide study performed in children. *Haematologica* 96, 1335–1343.

14. Nürnberg, S.T., Rendon, A., Smethurst, P.A., Paul, D.S., Voss, K., Thon, J.N., Lloyd-Jones, H., Sambrook, J.G., Tijssen, M.R., Italiano, J.E., Jr., et al.; HaemGen Consortium (2012). A GWAS sequence variant for platelet volume marks an alternative DNMT3 promoter in megakaryocytes near a MEIS1 binding site. *Blood* 120, 4859–4868.
15. Johnson, A.D. (2011). The genetics of common variation affecting platelet development, function and pharmaceutical targeting. *J. Thromb. Haemost.* 9 (Suppl 1), 246–257.
16. Auer, P.L., Johnsen, J.M., Johnson, A.D., Logsdon, B.A., Lange, L.A., Nalls, M.A., Zhang, G., Franceschini, N., Fox, K., Lange, E.M., et al. (2012). Imputation of exome sequence variants into population-based samples and blood-cell-trait-associated loci in African Americans: NHLBI GO Exome Sequencing Project. *Am. J. Hum. Genet.* 91, 794–808.
17. Auer, P.L., Teumer, A., Schick, U., O’Shaughnessy, A., Lo, K.S., Chami, N., Carlson, C., de Denu, S., Dubé, M.P., Haessler, J., et al. (2014). Rare and low-frequency coding variants in CXCR2 and other genes are associated with hematological traits. *Nat. Genet.* 46, 629–634.
18. Chami, N., Chen, M.-H., Slater, A.J., Eicher, J.D., Evangelou, E., Tajuddin, S.M., Love-Gregory, L., Kacprowski, T., Schick, U.M., Nomura, A., et al. (2016). Exome genotyping identifies pleiotropic variants associated with red blood cell traits. *Am. J. Hum. Genet.* 99, this issue, 8–21.
19. Tajuddin, S.M., Schick, U.M., Eicher, J.D., Chami, N., Giri, A., Brody, J.A., Hill, W.D., Kacprowski, T., Li, J., Lyytikäinen, L.-P., et al. (2016). Large-scale exome-wide association analysis identifies loci for white blood cell traits and pleiotropy with immune-mediated diseases. *Am. J. Hum. Genet.* 99, this issue, 22–39.
20. Grove, M.L., Yu, B., Cochran, B.J., Haritunians, T., Bis, J.C., Taylor, K.D., Hansen, M., Borecki, I.B., Cupples, L.A., Fornage, M., et al. (2013). Best practices and joint calling of the HumanExome BeadChip: the CHARGE Consortium. *PLoS ONE* 8, e68095.
21. Winkler, T.W., Day, F.R., Croteau-Chonka, D.C., Wood, A.R., Locke, A.E., Mägi, R., Ferreira, T., Fall, T., Graff, M., Justice, A.E., et al.; Genetic Investigation of Anthropometric Traits (GIANT) Consortium (2014). Quality control and conduct of genome-wide association meta-analyses. *Nat. Protoc.* 9, 1192–1212.
22. Liu, D.J., Peloso, G.M., Zhan, X., Holmen, O.L., Zawistowski, M., Feng, S., Nikpay, M., Auer, P.L., Goel, A., Zhang, H., et al. (2014). Meta-analysis of gene-level tests for rare variant association. *Nat. Genet.* 46, 200–204.
23. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26, 2190–2191.
24. Price, A.L., Kryukov, G.V., de Bakker, P.I., Purcell, S.M., Staples, J., Wei, L.J., and Sunyaev, S.R. (2010). Pooled association tests for rare variants in exon-resequencing studies. *Am. J. Hum. Genet.* 86, 832–838.
25. Wu, M.C., Lee, S., Cai, T., Li, Y., Boehnke, M., and Lin, X. (2011). Rare-variant association testing for sequencing data with the sequence kernel association test. *Am. J. Hum. Genet.* 89, 82–93.
26. Yang, J., Lee, S.H., Goddard, M.E., and Visscher, P.M. (2011). GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* 88, 76–82.
27. Johnson, A.D., Yanek, L.R., Chen, M.H., Faraday, N., Larson, M.G., Tofler, G., Lin, S.J., Kraja, A.T., Province, M.A., Yang, Q., et al. (2010). Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. *Nat. Genet.* 42, 608–613.
28. Kircher, M., Witten, D.M., Jain, P., O’Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. *Nat. Genet.* 46, 310–315.
29. (2016). Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary disease. *N. Engl. J. Med.* 374, 1898.
30. Willer, C.J., Schmidt, E.M., Sengupta, S., Peloso, G.M., Gustafsson, S., Kanoni, S., Ganna, A., Chen, J., Buchkovich, M.L., Mora, S., et al.; Global Lipids Genetics Consortium (2013). Discovery and refinement of loci associated with lipid levels. *Nat. Genet.* 45, 1274–1283.
31. Zhang, X., Gierman, H.J., Levy, D., Plump, A., Dobrin, R., Goring, H.H., Curran, J.E., Johnson, M.P., Blangero, J., Kim, S.K., et al. (2014). Synthesis of 53 tissue and cell line expression QTL datasets reveals master eQTLs. *BMC Genomics* 15, 532.
32. Eicher, J.D., Wakabayashi, Y., Vitseva, O., Esa, N., Yang, Y., Zhu, J., Freedman, J.E., McManus, D.D., and Johnson, A.D. (2016). Characterization of the platelet transcriptome by RNA sequencing in patients with acute myocardial infarction. *Platelets* 27, 230–239.
33. Fehrmann, R.S., Jansen, R.C., Veldink, J.H., Westra, H.J., Arends, D., Bonder, M.J., Fu, J., Deelen, P., Groen, H.J., Smolonska, A., et al. (2011). Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. *PLoS Genet.* 7, e1002197.
34. Sandomenico, A., Monti, S.M., Marasco, D., Dathan, N., Palumbo, R., Saviano, M., and Ruvo, M. (2009). IgE-binding properties and selectivity of peptide mimics of the Fc ϵ 2A binding site. *Mol. Immunol.* 46, 3300–3309.
35. Mackay, G.A., Hulett, M.D., Cook, J.P., Trist, H.M., Henry, A.J., McDonnell, J.M., Beavil, A.J., Beavil, R.L., Sutton, B.J., Hogarth, P.M., and Gould, H.J. (2002). Mutagenesis within human Fc ϵ 2A1 differentially affects human and murine IgE binding. *J. Immunol.* 168, 1787–1795.
36. Cook, J.P., Henry, A.J., McDonnell, J.M., Owens, R.J., Sutton, B.J., and Gould, H.J. (1997). Identification of contact residues in the IgE binding site of human Fc ϵ 2A1. *Biochemistry* 36, 15579–15588.
37. Garman, S.C., Kinet, J.P., and Jardetzky, T.S. (1999). The crystal structure of the human high-affinity IgE receptor (Fc ϵ 2A1). *Annu. Rev. Immunol.* 17, 973–976.
38. Granada, M., Wilk, J.B., Tuzova, M., Strachan, D.P., Weidinger, S., Albrecht, E., Gieger, C., Heinrich, J., Himes, B.E., Hunninghake, G.M., et al. (2012). A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. *J. Allergy Clin. Immunol.* 129, 840–845.e21.
39. Reiner, A.P., Lettre, G., Nalls, M.A., Ganesh, S.K., Mathias, R., Austin, M.A., Dean, E., Arepalli, S., Britton, A., Chen, Z., et al. (2011). Genome-wide association study of white blood cell count in 16,388 African Americans: the continental origins and genetic epidemiology network (COGENT). *PLoS Genet.* 7, e1002108.
40. Page, C., and Pitchford, S. (2014). Platelets and allergic inflammation. *Clin. Exp. Allergy* 44, 901–913.
41. Schmidt, V.A., Scudder, L., Devoe, C.E., Bernards, A., Cupit, L.D., and Bahou, W.F. (2003). IQGAP2 functions as a

- GTP-dependent effector protein in thrombin-induced platelet cytoskeletal reorganization. *Blood* *101*, 3021–3028.
42. Lee, H., Deignan, J.L., Dorrani, N., Strom, S.P., Kantarci, S., Quintero-Rivera, F., Das, K., Toy, T., Harry, B., Yourshaw, M., et al. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA* *312*, 1880–1887.
 43. Nurden, A.T., Pillois, X., Fiore, M., Heilig, R., and Nurden, P. (2011). Glanzmann thrombasthenia-like syndromes associated with macrothrombocytopenias and mutations in the genes encoding the α IIb β 3 integrin. *Semin. Thromb. Hemost.* *37*, 698–706.
 44. Obinata, D., Takayama, K., Urano, T., Murata, T., Ikeda, K., Horie-Inoue, K., Ouchi, Y., Takahashi, S., and Inoue, S. (2012). ARFGAP3, an androgen target gene, promotes prostate cancer cell proliferation and migration. *Int. J. Cancer* *130*, 2240–2248.
 45. Kartberg, F., Asp, L., Dejgaard, S.Y., Smedh, M., Fernandez-Rodríguez, J., Nilsson, T., and Presley, J.F. (2010). ARFGAP2 and ARFGAP3 are essential for COPI coat assembly on the Golgi membrane of living cells. *J. Biol. Chem.* *285*, 36709–36720.
 46. Weimer, C., Beck, R., Eckert, P., Reckmann, I., Moelleken, J., Brügger, B., and Wieland, F. (2008). Differential roles of ArfGAP1, ArfGAP2, and ArfGAP3 in COPI trafficking. *J. Cell Biol.* *183*, 725–735.
 47. Begonja, A.J., Pluthero, E.G., Suphamungmee, W., Giannini, S., Christensen, H., Leung, R., Lo, R.W., Nakamura, F., Lehman, W., Plomann, M., et al. (2015). FlnA binding to PACSIN2 F-BAR domain regulates membrane tubulation in megakaryocytes and platelets. *Blood* *126*, 80–88.
 48. Li, T., Shi, Y., Wang, P., Guachalla, L.M., Sun, B., Joerss, T., Chen, Y.S., Groth, M., Krueger, A., Platzer, M., et al. (2015). Smg6/Est1 licenses embryonic stem cell differentiation via nonsense-mediated mRNA decay. *EMBO J.* *34*, 1630–1647.
 49. Butler, M., Morel, A.S., Jordan, W.J., Eren, E., Hue, S., Shrimpton, R.E., and Ritter, M.A. (2007). Altered expression and endocytic function of CD205 in human dendritic cells, and detection of a CD205-DCL-1 fusion protein upon dendritic cell maturation. *Immunology* *120*, 362–371.
 50. Cao, L., Shi, X., Chang, H., Zhang, Q., and He, Y. (2015). pH-dependent recognition of apoptotic and necrotic cells by the human dendritic cell receptor DEC205. *Proc. Natl. Acad. Sci. USA* *112*, 7237–7242.
 51. Karparkin, S. (1978). Heterogeneity of human platelets. VI. Correlation of platelet function with platelet volume. *Blood* *51*, 307–316.
 52. Catani, L., Sollazzo, D., Ricci, F., Polverelli, N., Palandri, F., Baccarani, M., Vianelli, N., and Lemoli, R.M. (2011). The CD47 pathway is deregulated in human immune thrombocytopenia. *Exp. Hematol.* *39*, 486–494.
 53. Olsson, M., Bruhns, P., Frazier, W.A., Ravetch, J.V., and Oldenborg, P.A. (2005). Platelet homeostasis is regulated by platelet expression of CD47 under normal conditions and in passive immune thrombocytopenia. *Blood* *105*, 3577–3582.
 54. Yamao, T., Noguchi, T., Takeuchi, O., Nishiyama, U., Morita, H., Hagiwara, T., Akahori, H., Kato, T., Inagaki, K., Okazawa, H., et al. (2002). Negative regulation of platelet clearance and of the macrophage phagocytic response by the transmembrane glycoprotein SHPS-1. *J. Biol. Chem.* *277*, 39833–39839.
 55. Qayyum, R., Becker, L.C., Becker, D.M., Faraday, N., Yanek, L.R., Leal, S.M., Shaw, C., Mathias, R., Suktitipat, B., and Bray, P.F. (2015). Genome-wide association study of platelet aggregation in African Americans. *BMC Genet.* *16*, 58.
 56. Lewis, J.P., Ryan, K., O’Connell, J.R., Horenstein, R.B., Damcott, C.M., Gibson, Q., Pollin, T.I., Mitchell, B.D., Beitelshes, A.L., Pakzy, R., et al. (2013). Genetic variation in PEAR1 is associated with platelet aggregation and cardiovascular outcomes. *Circ Cardiovasc Genet* *6*, 184–192.
 57. Eicher, J.D., Xue, L., Ben-Shlomo, Y., Beswick, A.D., and Johnson, A.D. (2016). Replication and hematological characterization of human platelet reactivity genetic associations in men from the Caerphilly Prospective Study (CaPS). *J. Thromb. Thrombolysis* *41*, 343–350.
 58. Stone, J.C. (2011). Regulation and function of the RasGRP family of Ras activators in blood cells. *Genes Cancer* *2*, 320–334.
 59. van der Loo, B., and Martin, J.F. (1999). A role for changes in platelet production in the cause of acute coronary syndromes. *Arterioscler. Thromb. Vasc. Biol.* *19*, 672–679.
 60. Kauskot, A., Vandenbrielle, C., Louwette, S., Gijsbers, R., Tousseyn, T., Freson, K., Verhamme, P., and Hoylaerts, M.F. (2013). PEAR1 attenuates megakaryopoiesis via control of the PI3K/PTEN pathway. *Blood* *121*, 5208–5217.
 61. Bertin, A., Mahaney, M.C., Cox, L.A., Rogers, J., VandeBerg, J.L., Brugnara, C., and Platt, O.S. (2007). Quantitative trait loci for peripheral blood cell counts: a study in baboons. *Mamm. Genome* *18*, 361–372.
 62. Westra, H.J., Peters, M.J., Esko, T., Yaghootkar, H., Schurmann, C., Kettunen, J., Christiansen, M.W., Fairfax, B.P., Schramm, K., Powell, J.E., et al. (2013). Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* *45*, 1238–1243.
 63. Sloan, A., Gona, P., and Johnson, A.D. (2015). Cardiovascular correlates of platelet count and volume in the Framingham Heart Study. *Ann. Epidemiol.* *25*, 492–498.
 64. Panova-Noeva, M., Schulz, A., Hermanns, M.I., Grossmann, V., Pefani, E., Spronk, H.M., Laubert-Reh, D., Binder, H., Beutel, M., Pfeiffer, N., et al. (2016). Sex-specific differences in genetic and nongenetic determinants of mean platelet volume: results from the Gutenberg Health Study. *Blood* *127*, 251–259.
 65. Daly, M.E. (2011). Determinants of platelet count in humans. *Haematologica* *96*, 10–13.
 66. Moreno-Ayala, R., Schnabel, D., Salas-Vidal, E., and Lomeli, H. (2015). PIAS-like protein Zimp7 is required for the restriction of the zebrafish organizer and mesoderm development. *Dev. Biol.* *403*, 89–100.
 67. Peng, Y., Lee, J., Zhu, C., and Sun, Z. (2010). A novel role for protein inhibitor of activated STAT (PIAS) proteins in modulating the activity of Zimp7, a novel PIAS-like protein, in androgen receptor-mediated transcription. *J. Biol. Chem.* *285*, 11465–11475.
 68. Liu, Y., Lee, J.W., and Ackerman, S.L. (2015). Mutations in the microtubule-associated protein 1A (Map1a) gene cause Purkinje cell degeneration. *J. Neurosci.* *35*, 4587–4598.
 69. Ganesh, S.K., Tragante, V., Guo, W., Guo, Y., Lanktree, M.B., Smith, E.N., Johnson, T., Castillo, B.A., Barnard, J., Baumert, J., et al.; CARDIOGRAM, METASTROKE; LifeLines Cohort Study (2013). Loci influencing blood pressure identified using a cardiovascular gene-centric array. *Hum. Mol. Genet.* *22*, 1663–1678.
 70. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L., Najjar, S.S., Zhao, J.H., Heath, S.C., Eyheramendy, S., et al.; Wellcome Trust Case Control Consortium (2009). Genome-wide association study identifies

- eight loci associated with blood pressure. *Nat. Genet.* *41*, 666–676.
71. Gudbjartsson, D.F., Bjornsdottir, U.S., Halapi, E., Helgadottir, A., Sulem, P., Jonsdottir, G.M., Thorleifsson, G., Helgadottir, H., Steinthorsdottir, V., Stefansson, H., et al. (2009). Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat. Genet.* *41*, 342–347.
72. Bochenek, G., Häsler, R., El Mokhtari, N.E., König, I.R., Loos, B.G., Jepsen, S., Rosenstiel, P., Schreiber, S., and Schaefer, A.S. (2013). The large non-coding RNA ANRIL, which is associated with atherosclerosis, periodontitis and several forms of cancer, regulates ADIPOR1, VAMP3 and C11ORF10. *Hum. Mol. Genet.* *22*, 4516–4527.
73. Deloukas, P., Kanoni, S., Willenborg, C., Farrall, M., Assimes, T.L., Thompson, J.R., Ingelsson, E., Saleheen, D., Erdmann, J., Goldstein, B.A., et al.; CARDIoGRAMplusC4D Consortium; DIAGRAM Consortium; CARDIOGENICS Consortium; MuTHER Consortium; Wellcome Trust Case Control Consortium (2013). Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat. Genet.* *45*, 25–33.
74. Gomes, A.L., Carvalho, T., Serpa, J., Torre, C., and Dias, S. (2010). Hypercholesterolemia promotes bone marrow cell mobilization by perturbing the SDF-1: CXCR4 axis. *Blood* *115*, 3886–3894.
75. Su, Y., Wang, Z., Yang, H., Cao, L., Liu, F., Bai, X., and Ruan, C. (2006). Clinical and molecular genetic analysis of a family with sitosterolemia and co-existing erythrocyte and platelet abnormalities. *Haematologica* *91*, 1392–1395.
76. Wang, Z., Cao, L., Su, Y., Wang, G., Wang, R., Yu, Z., Bai, X., and Ruan, C. (2014). Specific macrothrombocytopenia/hemolytic anemia associated with sitosterolemia. *Am. J. Hematol.* *89*, 320–324.
77. Murphy, A.J., Bijl, N., Yvan-Charvet, L., Welch, C.B., Bhagwat, N., Rehemian, A., Wang, Y., Shaw, J.A., Levine, R.L., Ni, H., et al. (2013). Cholesterol efflux in megakaryocyte progenitors suppresses platelet production and thrombocytosis. *Nat. Med.* *19*, 586–594.

Supplemental Data

Platelet-Related Variants Identified by Exomechip

Meta-analysis in 157,293 Individuals

John D. Eicher, Nathalie Chami, Tim Kacprowski, Akihiro Nomura, Ming-Huei Chen, Lisa R. Yanek, Salman M. Tajuddin, Ursula M. Schick, Andrew J. Slater, Nathan Pankratz, Linda Polfus, Claudia Schurmann, Ayush Giri, Jennifer A. Brody, Leslie A. Lange, Ani Manichaikul, W. David Hill, Raha Pazoki, Paul Elliot, Evangelos Evangelou, Ioanna Tzoulaki, He Gao, Anne-Claire Vergnaud, Rasika A. Mathias, Diane M. Becker, Lewis C. Becker, Amber Burt, David R. Crosslin, Leo-Pekka Lyytikäinen, Kjell Nikus, Jussi Hernesniemi, Mika Kähönen, Emma Raitoharju, Nina Mononen, Olli T. Raitakari, Terho Lehtimäki, Mary Cushman, Neil A. Zakai, Deborah A. Nickerson, Laura M. Raffield, Rakale Quarells, Cristen J. Willer, Gina M. Peloso, Goncalo R. Abecasis, Dajiang J. Liu, Global Lipids Genetics Consortium, Panos Deloukas, Nilesh J. Samani, Heribert Schunkert, Jeanette Erdmann, CARDIoGRAM Exome Consortium, Myocardial Infarction Genetics Consortium, Myriam Fornage, Melissa Richard, Jean-Claude Tardif, John D. Rioux, Marie-Pierre Dube, Simon de Denus, Yingchang Lu, Erwin P. Bottinger, Ruth J.F. Loos, Albert Vernon Smith, Tamara B. Harris, Lenore J. Launer, Vilmundur Gudnason, Digna R. Velez Edwards, Eric S. Torstenson, Yongmei Liu, Russell P. Tracy, Jerome I. Rotter, Stephen S. Rich, Heather M. Highland, Eric Boerwinkle, Jin Li, Ethan Lange, James G. Wilson, Evelin Mihailov, Reedik Mägi, Joel Hirschhorn, Andres Metspalu, Tõnu Esko, Caterina Vacchi-Suzzi, Mike A. Nalls, Alan B. Zonderman, Michele K. Evans, Gunnar Engström, Marju Orho-Melander, Olle Melander, Michelle L. O'Donoghue, Dawn M. Waterworth, Lars Wallentin, Harvey D. White, James S. Floyd, Traci M. Bartz, Kenneth M. Rice, Bruce M. Psaty, J.M. Starr, David C.M. Liewald, Caroline Hayward, Ian J. Deary, Andreas Greinacher, Uwe Völker, Thomas Thiele, Henry Völzke, Frank J.A. van Rooij, André G. Uitterlinden, Oscar H. Franco, Abbas Dehghan, Todd L. Edwards, Santhi K. Ganesh, Sekar Kathiresan, Nauder Faraday, Paul L. Auer, Alex P. Reiner, Guillaume Lettre, and Andrew D. Johnson

Supplemental Note

1. Datasets examined in expression quantitative trait loci (eQTL) analyses

We queried PLT and MPV loci in over 100 separate expression quantitative trait loci (eQTL) datasets in a wide range of tissues. Datasets were collected through publications, publically available sources, or private collaboration. A general overview of a subset of >50 eQTL studies has been published (PMID: 24973796), with specific citations for >100 datasets included in the current query following here. As our investigation focused primarily on a blood cell and secondarily and overlap of genetic associations with lipids, we only present eQTLs in blood and adipose related tissues.

Blood cell related eQTL studies included fresh lymphocytes (17873875), fresh leukocytes (19966804), leukocyte samples in individuals with Celiac disease (19128478), whole blood samples (18344981, 21829388, 22692066, 23818875, 23359819, 23880221, 24013639, 23157493, 23715323, 24092820, 24314549, 24956270, 24592274, 24728292, 24740359, 25609184, 22563384, 25474530, 25816334, 25578447), lymphoblastoid cell lines (LCL) derived from asthmatic children (17873877, 23345460), HapMap LCL from 3 populations (17873874), a separate study on HapMap CEU LCL (18193047), additional LCL population samples (19644074, 22286170, 22941192, 23755361, 23995691, 25010687, 25951796), neutrophils (26151758, 26259071), CD19+ B cells (22446964), primary PHA-stimulated T cells (19644074, 23755361), CD4+ T cells (20833654), peripheral blood monocytes (19222302, 20502693, 22446964, 23300628, 25951796, 26019233), long non-coding RNAs in monocytes (25025429) and CD14+ monocytes before and after stimulation with LPS or interferon-gamma (24604202). Micro-RNA QTLs (21691150, 26020509), DNase-I QTLs (22307276), histone acetylation QTLs

(25799442), and ribosomal occupancy QTLs (25657249) were also queried for LCL. Splicing QTLs (25685889) and micro-RNA QTLs (25791433) were queried in whole blood.

Non-blood cell tissue eQTLs searched included omental and subcutaneous adipose (18344981, 21602305, 22941192, 23715323, 25578447), visceral fat (25578447) stomach (21602305), arterial wall (25578447) and heart tissue from left ventricles (23715323, 24846176) and left and right atria (24177373). Micro-RNA QTLs were also queried for gluteal and abdominal adipose (22102887).

Additional eQTL data was integrated from online sources including the Broad Institute GTEx Portal, and the Pritchard Lab (eqtl.uchicago.edu). Results for GTEx Analysis V4 for 13 tissues were downloaded from the GTEx Portal and then additionally filtered as described below [www.gtexportal.org: aortic artery, tibial artery, skeletal muscle, heart (left ventricle), stomach, whole blood, and subcutaneous adipose (23715323)]. Splicing QTL (sQTL) results generated with sQTLseeker with false discovery rate $P \leq 0.05$ were retained. For all gene-level eQTLs, if at least 1 SNP passed the tissue-specific empirical threshold in GTEx, the best SNP for that eQTL was always retained. All gene-level eQTL SNPs with $P < 1.67E-11$ were also retained, reflecting a global threshold correction of $P = 0.05 / (30,000 \text{ genes} \times 1,000,000 \text{ tests})$.

Figure S1: Q-Q plots for discovery meta-analysis of PLT in African (AA), European (EA), and combined all (All) ancestry groups and inflation metrics as measured by lambda.

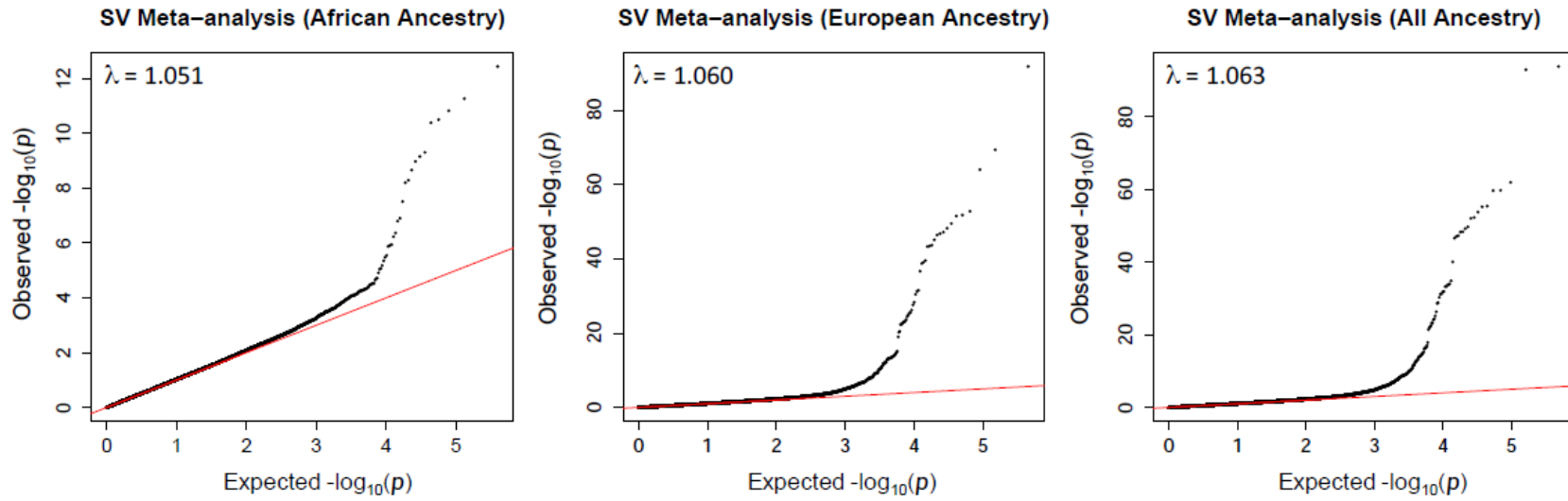


Figure S2: Q-Q plots for discovery meta-analysis of MPV in African (AA), European (EA), and combined all (All) ancestry groups and inflation metrics as measured by lambda.

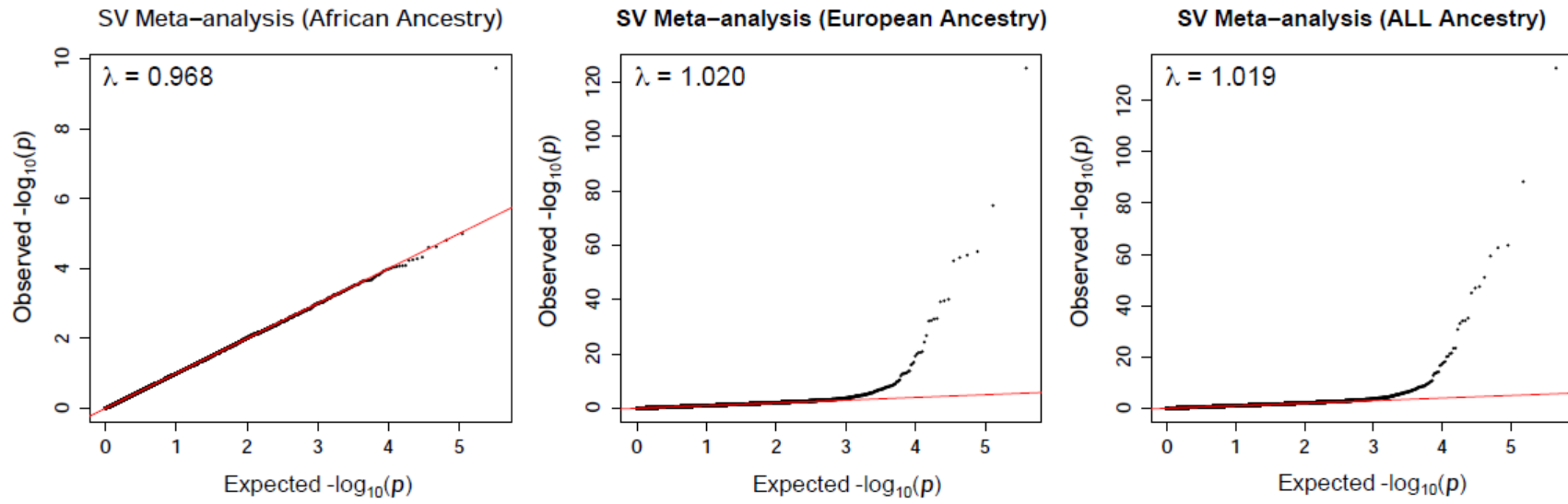


Figure S4: LocusZoom plot of *IQGAP2* locus for MPV in European ancestry (EA). rs34592828, rs34968964, and rs34950321 all showed significant independent association with MPV.

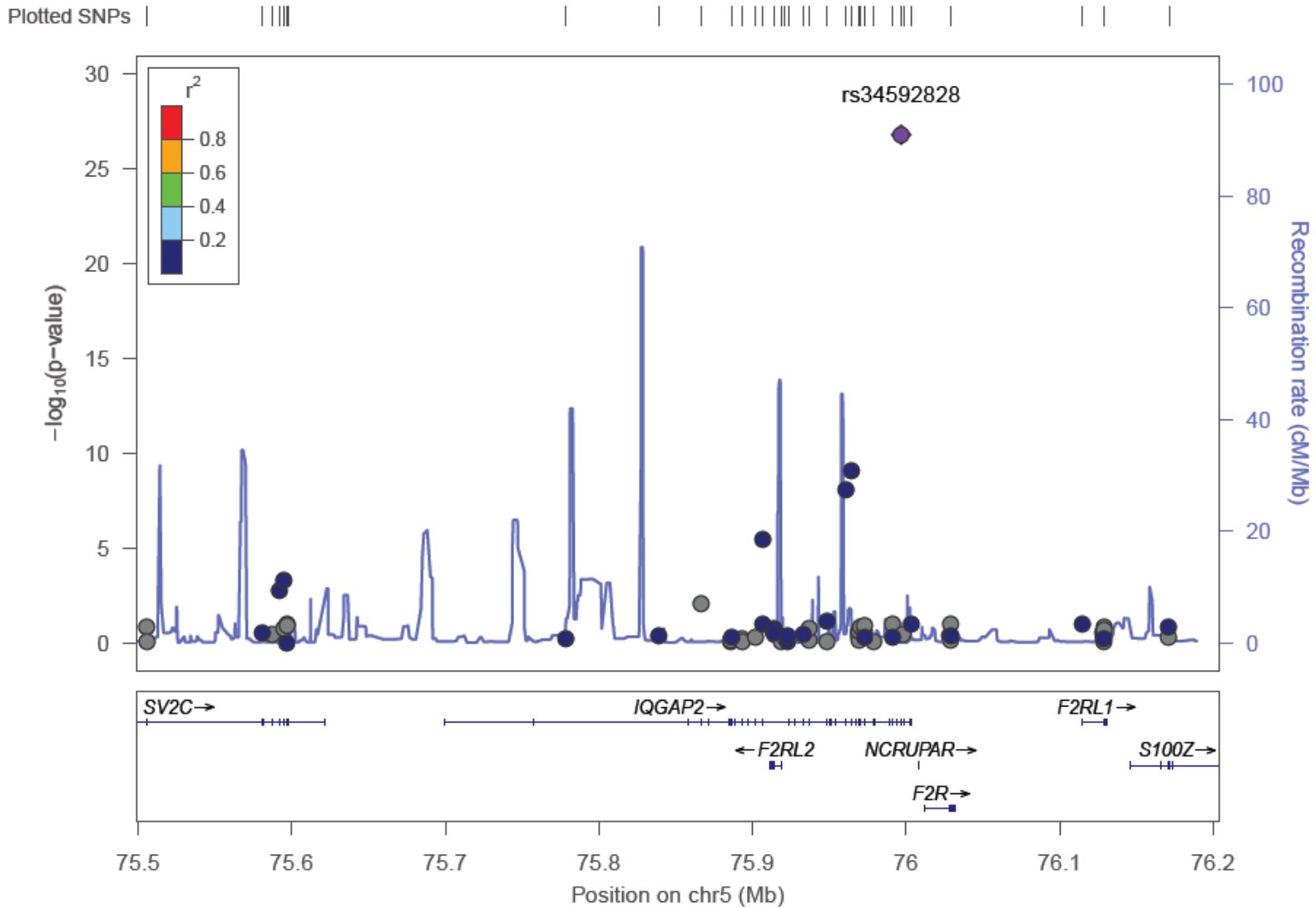
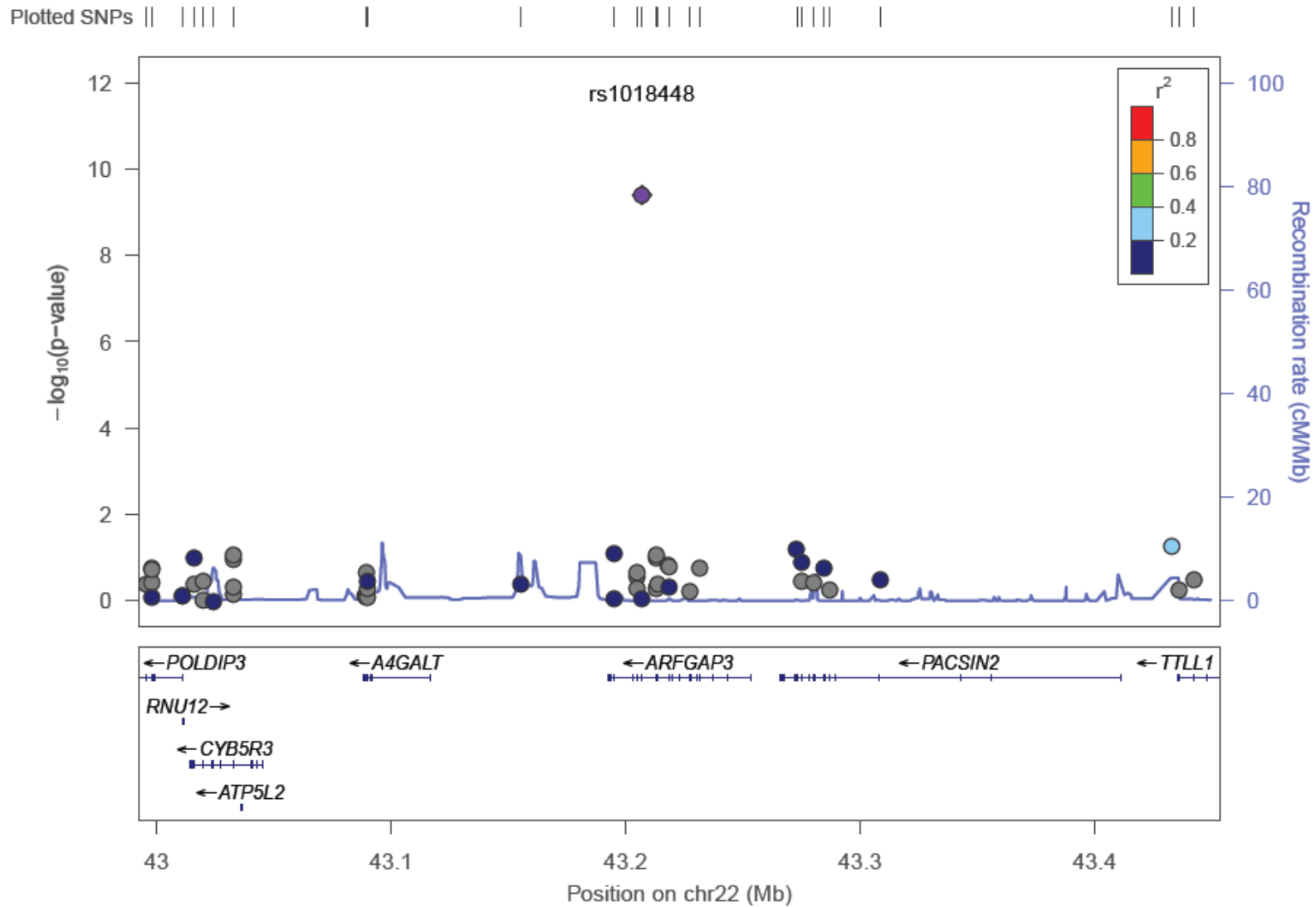


Figure S5: LocusZoom plot of *ARFGAP3/PACSIN2* locus for PLT in European ancestry (EA). rs1018448 showed association with PLT. However, rs1018448 is a reported eQTL for both *ARFGAP3* and *PACSIN2* gene expression.



Additional Funding Information

AGES

ARIC

The authors thank the staff of the ARIC study for their important contributions. The Atherosclerosis Risk in Communities (ARIC) Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. The meta-analysis and meta-regression analyses were funded by grant R01 HL086694 from the National Heart, Lung, and Blood Institute.

BioMe

The Mount Sinai IPM Biobank Program is supported by The Andrea and Charles Bronfman Philanthropies.

BIOVU

The dataset used in the analyses described were obtained from Vanderbilt University Medical Center's BioVU which is supported by institutional funding and by the Vanderbilt CTSA grant UL1 TR000445 from NCATS/NIH. Genome-wide genotyping was funded by NIH grants RC2GM092618 from NIGMS/OD and U01HG004603 from NHGRI/NIGMS. Funding for TLE and DRVE was provided by 1R21HL12142902 from NHLBI/NIH. Funding for the BioVU replication cohort was provided by 5R01HD074711 from NICHD/NIH.

CARDIA

The CARDIA Study is conducted and supported by the National Heart, Lung, and Blood Institute in collaboration with the University of Alabama at Birmingham (HHSN268201300025C & HHSN268201300026C), Northwestern University (HHSN268201300027C), University of Minnesota (HHSN268201300028C), Kaiser Foundation Research Institute (HHSN268201300029C), and Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging. Exome Chip genotyping was supported from grants R01-HL093029 and U01- HG004729 to MF. This manuscript has been reviewed and approved by CARDIA for scientific content.

CHS

A full list of CHS investigators and institutions can be found at www.chs-nhlbi.org. This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants HL080295, HL087652, HL103612, HL105756, HL120393, and R01HL068986 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through AG023629 from the

National Institute on Aging (NIA). The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

ECGUT

EGCUT would like to thank co-workers at Estonian Biobank. This study was supported by EU H2020 grants 692145, 676550, 654248, Estonian Research Council Grant IUT20-60, NIASC, EIT – Health and NIH-BMI grant 2R01DK075787-06A1.

Framingham Heart Study (FHS)

Genotyping, quality control, and calling of the Illumina HumanExome BeadChip in the Framingham Heart Study were supported by funding from the National Heart, Lung and Blood Institute Division of Intramural Research (Daniel Levy and Christopher J. O'Donnell, Principle Investigators). Support for the centralized genotype calling was provided by Building on GWAS for NHLBI-diseases: the U.S. CHARGE consortium through the National Institutes of Health (NIH) American Recovery and Reinvestment Act of 2009 (5RC2HL102419). The NHLBI's Framingham Heart Study is a joint project of the National Institutes of Health and Boston University School of Medicine and was supported by contract N01-HC-25195.

HANDLS

The Healthy Aging in Neighborhoods of Diversity across the Life Span Study (HANDLS) research was supported by the Intramural Research Program of the NIH, National Institute on Aging and the National Center on Minority Health and Health Disparities (project # Z01-AG000513 and human subjects protocol # 2009-149). Data analyses for the HANDLS study utilized the computational resources of the NIH HPC Biowulf cluster at the National Institutes of Health, Bethesda, MD (<http://hpc.nih.gov>).

JHS

The JHS is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities.

LBC1921 and LBC1936

The Lothian Birth Cohorts thank the team members who contributed to these studies. Phenotype collection in the Lothian Birth Cohort 1921 was supported by the UK's Biotechnology and Biological Sciences Research Council (BBSRC), The Royal Society, and The Chief Scientist Office of the Scottish Government. Phenotype collection in the Lothian Birth Cohort 1936 was supported by Age UK (The Disconnected Mind project). Genotyping was supported by Centre for Cognitive Ageing and Cognitive Epidemiology (Pilot Fund award), Age UK, and the Royal Society of Edinburgh. The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (MR/K026992/1). Funding from the BBSRC and Medical Research Council (MRC) is gratefully acknowledged. WDH is supported by a grant from Age UK (Disconnected Mind Project).

MDCS-CC

The Malmö Diet and Cancer cohort acknowledges the contributions of all the study investigators and field staff of Malmo Diet and Cancer Study. The Malmö Diet and Cancer cohort studies were supported by grants from the Swedish Research Council, the Swedish Heart and Lung Foundation, the Pålsson Foundation, the Novo Nordic Foundation and European Research Council starting grant StG-282255. AN was supported by the Yoshida Scholarship Foundation. SK was supported by a Research Scholar award from the Massachusetts General Hospital (MGH), the Howard Goodman Fellowship from MGH, the Donovan Family Foundation, R01HL107816, and a grant from Fondation Leducq.

MESA

MESA thanks its Coordinating Center, MESA investigators, and study staff for their valuable contributions. A full list of participating MESA investigators and institutions can be found at www.mesa-nhlbi.org. MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, UL1-TR-000040, and DK063491. MESA Family is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support is provided by grants and contracts R01HL071051, R01HL071205, R01HL071250, R01HL071251, R01HL071258, R01HL071259, by the National Center for Research Resources, Grant UL1RR033176, and the National Center for Advancing Translational Sciences, Grant UL1TR000124. Funding support for the inflammation dataset was provided by grant HL077449. The MESA Epigenomics & Transcriptomics Study was funded by NIA grant 1R01HL101250-01 to Wake Forest University Health Sciences.

MHIBB

This work was supported by the Fonds de Recherche du Québec–Santé (FRQS, scholarship to NC), the Canadian Institute of Health Research (Banting-CIHR, scholarship to SL and operating grant MOP#123382 to GL), the Canada Research Chair program (to GL, JDR, and JCT), and the MHI Foundation. PLA was supported by NHLBI R21 HL121422-02. JCT holds the Canada Research Chair in translational and personalized medicine and the Université de Montréal endowed research chair in atherosclerosis.

RS

The generation and management of the Illumina exome chip v1.0 array data for the Rotterdam Study (RS-I) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The Exome chip array data set was funded by the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, from the Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO)-sponsored Netherlands Consortium for Healthy Aging (NCHA; project nr. 050-060-810); the Netherlands Organization for Scientific Research (NWO; project number 184021007) and by the Rainbow Project (RP10; Netherlands Exome Chip Project) of the Biobanking and Biomolecular Research Infrastructure Netherlands (BBMRI-NL; www.bbMRI.nl). We thank Ms. Mila Jhamai, Ms. Sarah Higgins, and Mr. Marijn Verkerk for their help in creating the exome chip database, and Carolina Medina-Gomez, MSc, Lennard Karsten, MSc, and Linda

Broer PhD for QC and variant calling. Variants were called using the best practice protocol developed by Grove et al. as part of the CHARGE consortium exome chip central calling effort. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

SHIP and SHIP-TREND

SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany (www.community-medicine.de), which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Siemens AG, the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). ExomeChip data have been supported by the Federal Ministry of Education and Research (grant no. 03Z1CN22) and the Federal State of Mecklenburg-West Pomerania.

STABILITY and SOLID TIMI-52

The STABILITY and SOLID TIMI-52 studies were funded by GlaxoSmithKline.

WHI

WHI thanks investigators and staff for their dedication in making the program possible. The WHI program is funded by the National Heart, Lung, and Blood Institute, the US National Institutes of Health and the US Department of Health and Human Services (HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C and HHSN271201100004C). The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at:

<http://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf>. Exome chip data and analysis were supported through the Exome Sequencing Project (NHLBI RC2 HL-102924, RC2 HL-102925 and RC2 HL-102926), the Genetics and Epidemiology of Colorectal Cancer Consortium (NCI CA137088), and the Genomics and Randomized Trials Network (NHGRI U01-HG005152). PLA was supported by NHLBI R21 HL121422-02.

Airwave

The Airwave Study is funded by the Home Office (grant number 780-TETRA) with additional support from the National Institute for Health Research (NIHR) Imperial College Healthcare NHS Trust (ICHNT) and Imperial College Biomedical Research Centre (BRC) (Grant number BRC-P38084). Paul Elliott is an NIHR Senior Investigator and is supported by the ICHNT and Imperial College BRC, the MRC-PHE Centre for Environment and Health and the NIHR Health Protection Research Unit on Health Impact of Environmental Hazards.

FINCAVAS

This work was supported by the Competitive Research Funding of the Tampere University Hospital (Grant 9M048 and 9N035), the Finnish Cultural Foundation, the Finnish Foundation for Cardiovascular Research, the Emil Aaltonen Foundation, Finland, and the Tampere Tuberculosis Foundation.

GeneSTAR

GeneSTAR was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute (U01 HL72518, HL087698, and HL112064) and by a grant from the National Institutes of Health/National Center for Research Resources (M01-RR000052) to the Johns Hopkins General Clinical Research Center. Genotyping services were provided through the RS&G Service by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number HHSN268201100037C from the National Heart, Lung, and Blood Institute.

NWIGM

This phase of the eMERGE Network was initiated and funded by the NHGRI through the following grants: U01HG8657 (Group Health Cooperative/University of Washington); U01HG8685 (Brigham and Women's Hospital); U01HG8672 (Vanderbilt University Medical Center); U01HG8666 (Cincinnati Children's Hospital Medical Center); U01HG6379 (Mayo Clinic); U01HG8679 (Geisinger Clinic); U01HG8680 (Columbia University Health Sciences); U01HG8684 (Children's Hospital of Philadelphia); U01HG8673 (Northwestern University); U01HG8701 (Vanderbilt University Medical Center serving as the Coordinating Center); U01HG8676 (Partners Healthcare/Broad Institute); and U01HG8664 (Baylor College of Medicine). NWIGM dataset please also add "Additional support was provided by the University of Washington's Northwest Institute of Genetic Medicine from Washington State Life Sciences Discovery funds (Grant 265508).

REGARDS

We thank the investigators, staff and participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at <http://www.regardsstudy.org>. The genotyping for this project was provided by NIH/NCCR center grant 5U54RR026137-03. This research project is supported by a cooperative agreement U01 NS041588 from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Department of Health and Human Service. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health.

YFS

The Young Finns Study has been financially supported by the Academy of Finland: grants 285902, 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Kuopio, Tampere and Turku University Hospital Medical Funds (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation of Cardiovascular Research ; Finnish Cultural Foundation; Tampere Tuberculosis Foundation ; Emil Aaltonen Foundation; and Yrjö Jahnsson Foundation.