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The genetics of platelet count and volume in humans

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Abstract

The last decade has witnessed an explosion in the depth, variety, and amount of human genetic data that can be generated. This revolution in technical and analytical capacities has enabled the genetic investigation of human traits and disease in thousands to now millions of participants. Investigators have taken advantage of these advancements to gain insight into platelet biology and the platelet's role in human disease. To do so, large human genetics studies have examined the association of genetic variation with two quantitative traits measured in many population and patient based cohorts: platelet count (PLT) and mean platelet volume (MPV). This article will review the many human genetic strategies—ranging from genome-wide association study (GWAS), Exomechip, whole exome sequencing (WES), to whole genome sequencing (WGS)—employed to identify genes and variants that contribute to platelet traits. Additionally, we will discuss how these investigations have examined and interpreted the functional implications of these newly identified genetic factors and whether they also impart risk to human disease. The depth and size of genetic, phenotypic, and other -omic data are primed to continue their growth in the coming years and provide unprecedented opportunities to gain critical insights into platelet biology and how platelets contribute to disease.

Platelets are circulating anucleate cells important in wound healing, thrombosis, hemostasis, and inflammation. Clinically, platelets contribute to severe bleeding and thrombotic disorders like idiopathic thrombocytopenic purpura and essential thrombocythemia as well as cardiovascular disease including myocardial infarction and atherosclerosis¹. In fact, platelets are among the most common targets in secondary prevention of cardiovascular disease via aspirin use and dual anti-platelet therapies^{2,3}. Derived from multinucleate megakaryocytes in bone marrow, a balance of platelet production, maintenance in the circulation, and ultimate clearance from the blood is necessary to ensure proper function.

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Declaration of Interest

No authors of the manuscript have any competing interests to declare in relation to the contents of the manuscript.

In clinical and research settings, measuring the number of circulating platelets—platelet count (PLT)—and the average size of circulating platelets—mean platelet volume (MPV)—can reveal insights into patient health as well as the underlying mechanisms of platelet biology and regulation. More practically, measuring PLT and MPV can be completed in a relatively inexpensive and high-throughput manner and is more commonly ascertained than other platelet indices such as plateletcrit, platelet distribution width, and platelet reactivity or other measures of platelet function. Many have also proposed MPV as a more convenient proxy for measuring platelet function^{1,4}. However, it is important to note that considerable variation in MPV can be due to pre-analytic factors including blood tube selection, processing and equipment used^{5–7}. *Platelets* recently released new editorial guidelines calling for careful consideration of such factors in new studies undertaken and submitted that involve MPV⁸. Measuring PLT is more standardized but can also face technical issues, particularly for PLT well outside normal ranges such as in extreme thrombocytopenia. Despite these potential limitations, the ability to generate data on both PLT and MPV on large numbers of individuals in various settings has made them attractive candidates to analyse in human studies of platelets.

With the use of PLT and MPV, many have sought to identify their environmental and genetic determinants. PLT and MPV have a strong negative correlation, with fewer number of platelets correlated with larger platelets and vice versa⁴. There are notable gender, age, and population differences in PLT and MPV, with women generally having higher PLT and PLT decreasing with age^{9–11}. However, heritability studies in twin and family-based cohorts have demonstrated PLT and MPV also have strong genetic components^{12–14}. Further supporting the importance of genetic effects, causative genes of several inherited thrombocytopenia (PLT < 150,000 platelets/ μ L), thrombocytosis (PLT > 450,000 platelets/ μ L), and platelet function disorders have been identified and characterized. For example, variants in the *WAS* gene cause Wiskott-Aldrich syndrome (OMIM #301000), a rare X-linked disorder characterized by eczema, thrombocytopenia, and infection¹⁵. Identification of causal genes and variants of rare platelet disorders improves proper diagnosis and can aid in understanding underlying pathobiology. As the goal of this review is to discuss the genetics of PLT and MPV in the general population, please see recent reviews for fuller discussion of the genetics of inherited platelet disorders^{16,17}.

While the benefits of identifying genetic variants that cause platelet disorders are intuitive, the purposes of characterizing genes contributing to inter-individual variability in PLT and MPV may be less so. However, human genetic association strategies can identify factors that play critical roles in platelet biology and inform experimental dissection of these pathways. Fully and appropriately leveraging genetic associations has the potential to disentangle the complicated regulation of platelets and to give insights of platelets' contribution to chronic and acute conditions.

To identify genetic factors that influence PLT and MPV in the general population, investigators have employed diverse approaches (Table 1). One of the first studies took advantage of familial relatedness in a genome-wide linkage scan to identify a suggestive linkage peak on chromosome 19q13.13–19q13.31 for PLT and hypothesized that *glycoprotein VI (GP6)*—a platelet receptor for collagen—was responsible for this

association¹⁸. However, the genome-wide association study (GWAS) is now by far the most widely used strategy to identify PLT and MPV genetic associations (Table 1). In GWAS, investigators genotype hundreds of thousands to millions of single nucleotide polymorphisms (SNPs) designed to tag genomic variation and assess the association of each SNP with a trait¹⁹. GWAS can identify genomic regions where common variants (i.e., minor allele frequency [MAF] > 5%) influence traits. Individual common variants typically have small, yet critical regulatory effects. Taken together, common variants can have substantial effect on phenotypes and reflect the large number of such variants associated with complex traits like PLT and MPV²⁰. Disentangling these effects can reveal key players in how platelet traits are regulated and the genetic architecture underlying PLT and MPV.

In 2009, the first platelet GWASs identified 4 genomic loci (*WDR66*, *ARHGEF3*, *TAOK1*, *PIK3CG*) associated with MPV^{21;22}. Later that year, investigators in six European ancestry population-based cohorts formed the HaemGen Consortium²³. In addition to confirming the four previously identified loci, meta-analysing GWAS results (discovery n=4,627, replication n=9,316) identified 12 additional loci associated with PLT and/or MPV. This collaboration substantially increased sample size and thus statistical power to detect the small effects typical of common variants identified through GWAS. Additionally, the investigators built the collaborative and analytical framework to integrate GWAS results from independent studies. With this framework established, more groups joined to further increase discovery GWAS sample size (PLT n=48,666, MPV=18,600)²⁴. Doing so enabled the identification of 68 loci—52 not previously reported—associated with PLT and/or MPV. Of these 68 loci, 16 had genome-wide significant associations with both PLT and MPV ($p < 5.0 \times 10^{-8}$), indicative of their strong inverse phenotypic correlation⁴. The associated genetic factors explained 4.8% and 9.9% of the PLT and MPV phenotypic variance, respectively, indicating that common variants substantially contribute to these traits.

Although Gieger et al. further characterized their associations in samples of non-European ancestry, PLT and MPV discovery GWASs presented thus far were completed only in subjects of European ancestry. Epidemiologically, PLT and MPV differ among ethnic and population groups¹⁰. Such differences may indicate possible ethnic and population differences in genetic architecture and the specific SNPs associated with each. To begin to address this, several GWASs have been performed in samples of African, Hispanic, and Asian ancestries. Doing so has not only confirmed many associations observed in European ancestry cohorts, but also identified new loci associated with PLT and MPV. For example, a recent GWAS of PLT in the Hispanic Community Health Study/Study of Latinos (discovery n=12,491) identified 5 novel associations²⁵, at least partially due to differing MAFs of associated SNPs in *ACTN1*, *ETV7*, and *MEF2C*. These allele frequency differences enabled detection of effects that were not observed in more well-powered GWASs in European ancestry samples. Similarly, GWASs in Asian and African ancestry samples have identified novel associations including *KIAA0232*, *SLMO2*, *ACAD10*, and *EGF*^{26–30}. Several of these SNP associations (e.g., *EGF*, *SLMO2*) were subsequently detected in other populations, while others with more substantial allele frequency differences have not been observed (e.g., *KIAA0232*). GWASs in samples of non-European ancestry have identified novel genetic associations and demonstrated population similarities and differences in genetic associations. A larger investment and continued effort in studying platelet traits in non-

European ancestry groups are necessary to disentangle ethnic-specific and ethnic-independent genetic effects.

Not only is there a need to further increase sample diversity, but the need to increase sample size and depth of data collected continues to grow. Despite the on-going challenge to translate genetic associations into clinical or biological mechanism, increasing sample size, diversity, and depth offers the opportunity to refine the implications of genetic associations into contributing pathways. Building upon these studies will further inform the necessary experiments to interrogate associated loci and pathways both at the bench and in the clinic. The emergence of biobank and electronic health record (EHR) cohorts epitomizes the mobilization of mass resources to fuel genetic research. An example of utilizing EHR-based cohorts is the recent study of PLT and MPV in the Electronic Medical Records and Genomics (eMERGE) network. Although all observed PLT and MPV associations had been previously reported, the use of EHRs enabled investigators to perform a phenome-wide association study (PheWAS) of associated SNPs. By doing so, they examined the relationships of PLT/MPV associated SNPs across all measured phenotypes in the EHR system and observed associations with myocardial infarction and auto-immune disease³¹. Such a PheWAS strategy can tie genetic associations of quantitative traits like PLT and MPV with human disease. The largest reported GWAS of PLT and MPV (n=166,066) at the time of this review took advantage of similar EHR and biobank resources in the UK Biobank and INTERVAL studies³². Such a vast resource enabled detection of hundreds of platelet associated loci that explained 18% and 30% of PLT and MPV variation, respectively. As sample size, participant diversity, and phenotype depth increases through national biobank and registry, EHR, and other initiatives, the opportunity to identify genetic factors associated with PLT/MPV and their relationships with clinical traits and disease will continue to grow.

Despite their success in identifying PLT and MPV associated loci, GWAS results do not fully account for their genetic components. As GWAS largely interrogate common variants (i.e., MAF > 5%), the field has further examined the contribution of rare (MAF < 1%) and low-frequency (MAF: 1–5%) variants to population variation in PLT and MPV. One such strategy is to target the protein coding portion of the genome through whole exome sequencing (WES), where exons are specifically sequenced and coding variants examined (Table 1). WES has successfully identified causal genetic variants for several platelet disorders and other rare inherited conditions^{16;17}. However, there is evidence that rare variation also contributes to population variation of complex traits³³. These associated rare, coding variants typically have larger effects than common variants examined in GWAS and can offer relatively easier functional interrogation in follow-up experiments.

Two WES population-based studies of PLT have been reported. The first study presented the results of WES in a sample of 761 African-American subjects. Realizing this sample size gave inadequate statistical power to detect associations of rare coding variants, the authors used these exome data to impute genotypes in a larger sample of over 13,000 African-American individuals. With this larger sample size, they found associations of rare variants in *BAK1* and *MPL* as well as suggestive associations with *ITGB3*, *CD109*, and *CD36*³⁴. Another study employed a different strategy by generating WES data on over 15,000 individuals from six population-based cohorts and then performing *in silico* replication in

over 52,000 participants³⁵. There, they found replicated associations of rare variants in *GFI1B* and *CPS1* with PLT. WES studies provided an orthogonal means to GWAS of identifying genomic loci important in platelet traits. Specifically, these studies have identified new genetic factors (e.g., *CPS1*) and putatively functional variants in known genes (e.g., *BAK1*, *MPL*, *ITGB3*, *CD109*, *CD36*, *GFI1B*), which would have been difficult to detect with GWAS.

With more WES studies completed, the need for a fast and inexpensive method to increase sample size for discovery and replication emerged. With that in mind, a new tool called Exomechip was developed. Enriched with rare coding variants previously observed in WES studies and SNPs identified in previous GWASs, Exomechip allowed for quick genotyping of the same rare variants in large numbers that would have taken years and substantial resources otherwise³⁶. Two investigations used Exomechip to identify novel genetic associations with increased sample sizes of PLT and MPV to over 150,000 and 57,000 individuals, respectively. This expansion of sample size and use of Exomechip enabled the detection of many novel associations, including *JAK2*, *TUBB1*, *MAP1A*, *ZMIZ2*, *PEAR1*, and *PACSIN2*^{37,38}. A small proportion of these novel loci contained associated rare variants as intended with the Exomechip design. The relative lack of novel rare variant associations in Exomechip studies may indicate that increased sample size and unbiased genetic data from WES and whole genome sequencing (WGS) platforms may be necessary to detect many such associations.

As sequencing technology has evolved, the prospect of performing WGS in large studies is quickly becoming feasible. Generating WGS data in these cohorts will allow for improved resolution of relevant genomic elements in identified loci, but also direct interrogation of rare non-coding variants. However, before these data are generated in large enough numbers, investigators can use WGS in smaller samples to infer genotypes in other individuals. For example, Iotchkova et al. used WGS data from the UK10K (n=3,781) and 1000 Genomes projects to infer over 17 million variants in approximately 40,000 individuals³⁹. This increase in genomic coverage allowed them to confirm many and detect several novel associations with PLT (e.g., *S1PR3*, *TP53BP1*, and *TRABC-MOV10L1*). As more WGS data are generated, the ability to impute genotypes and to increase resolution of genetic associations will grow, particularly with the recent advent of readily accessible imputation servers (e.g., imputationserver.sph.umich.edu/index.html and imputation.sanger.ac.uk)^{40,41}.

Genetic association studies are typically just a first step in examining the role of PLT/MPV associated genes and variants. Further analytical, animal, and molecular work is necessary to fully examine the biological mechanisms underlying associations. Most simply, cross-referencing genetic association results has shown large overlap among blood cells (i.e., red blood cells, white blood cells, and platelets) as well as between platelets and lipids³⁸ (Figure 1). More formally, genetic correlations using GWAS has confirmed the shared genetic architecture of PLT and MPV, as well as suggested shared genetic factors of platelets with LDL and height⁴² (Figure 1). Such analyses can reveal relationships that platelets share with seemingly disparate traits. In addition, identifying genetic factors that strongly influence platelet traits can enable other analyses including Mendelian Randomization to assess causal relationships between platelets and other clinical traits⁴³. Mendelian Randomization

analyses can tease apart whether platelets causally impact disease and risk factors or whether epidemiological associations are simply correlative. For example, using a polygenic risk score of associated variants, Astle and colleagues examined the causal effects of PLT and MPV on several auto-immune, cardiometabolic, and neuropsychiatric disorders³². They observed a weak inverse relationship between MPV and coronary heart disease (Figure 1). Observational studies of MPV and CHD are mixed, with the largest systematic meta-analysis finding a positive relationship, opposite of what Astle et al. observed using Mendelian Randomization^{44;45}. Though controversies remain regarding the standardized measurement of MPV and its correlation with other platelet and cardiometabolic indices, the use of Mendelian Randomization and selection of proper genetic instruments may present a new strategy to disentangle relationships of platelets with disease⁴⁶.

Functional insights are more typically ascertained through interrogation of individual variants and genes. Most commonly, associated variants are annotated using gene expression, epigenetic, and evolutionary resources as well as variant prediction tools like CADD, SIFT, and PolyPhen⁴⁷. Such annotations can give clues to whether an associated variant may be causal and how it exerts its effects. For example, predicted deleterious rare coding variants in evolutionary conserved regions may affect protein function; while variants within enhancers also associated with gene expression likely have regulatory effects. Recent manuscripts have shown many PLT/MPV associated SNPs are also expression (eQTLs) or splicing (sQTLs) quantitative trait loci that influence gene expression and/or isoform of gene expressed^{32;35;38}. Such overlap suggests regulatory effects mediate much of the genetic effects on platelet traits in the general population. Taking advantage of growing resources integrating transcriptomic, epigenomic, proteomic, and metabolomic with genomic data will be vital in translating associations to biological mechanism.

Despite their implicit relevance to clinical outcomes, human-based genetic and -omic studies cannot entirely untangle the effects of associated genes and variants. To specifically interrogate these missing links, a number of functional investigations in animal and cellular models have examined associated genes. A common approach is to knockout or knockdown expression of a candidate gene in model systems. For example, Gieger et al. further examined their GWAS findings by knocking down expression of 15 prioritized genes in the fruitfly *D. melanogaster* and zebrafish *D. rerio*. These experiments recapitulated many hematopoietic phenotypes observed in rodents and revealed a degree of conservation in platelet biology²⁴. Other investigations have shown effects of GWAS associated genes (e.g., *PEAR1*, *NFE2*, *PIK3CG*, *JMJD1C*) on platelet development, maintenance, and clearance in animal and cellular systems^{48–53}. Human genetic studies of platelet reactivity have also indicated a role for some of these loci in affecting platelet aggregation^{54;55}. Together, these studies provide stronger evidence that genes implicated in human association studies do in fact play a role in platelet biology.

In addition to studying the functions of entire genes, other investigations have examined the functional consequences of specific variants and how they may yield their effects. Overlap with reported eQTLs only provides limited evidence that different SNP alleles causally influence gene expression. Experimental evidence functionally linking genetic variation to molecular phenotypes is fundamental to solidifying regulatory effects. In a recent WES

study, a PLT association variant (rs150813342) in *GFI1B* was shown to promote production of a short GFI1B protein isoform as opposed to a long isoform³⁵. Further experiments showed that this long GFI1B isoform promoted megakaryocyte differentiation and platelet production as compared to the short isoform, proposing the molecular mechanism of how a PLT associated SNP could influence platelet number. Similarly, other studies have demonstrated how SNPs can introduce alternate promoters in *DNM3* and shown allelic differences in transcription factor binding in *ACTN1*^{25;56;57}. These functional interrogations of regulatory implicated genetic variants are vital to tying statistical associations to biology.

Despite the successes of the above studies, this translation of associated genetic variants to underlying functional mechanism remains a major hurdle. Genetic association studies have revealed shared genetic factors among numerous traits, suggesting common regulatory pathways (Figure 1). Additionally, the accumulation of genetic associations in cytoskeletal-related genes (e.g., *ACTN1*, *PACSIN2*) has further confirmed the importance of cytoskeletal dynamics in megakaryocytes with effects on PLT and MPV^{58;59}. Identification of more associated loci has the prospect of revealing new pathways that contribute to PLT/MPV. However, to confirm the roles of these pathways in PLT and MPV, direct experimentation at the bench is necessary. Only through the communication of genetic association findings and collaborative translation to appropriate experimental systems will the genetic factors that govern PLT and MPV be identified, confirmed, and properly interrogated.

Human genetic studies leveraging large clinical samples and the emergence of high-throughput genomic technologies have enabled the detection of many genetic contributors to PLT and MPV. Increasing the number, depth, and complexity of platelet and genetic data will further reveal more genetic factors that influence platelet traits and their relationships with human disease. As WGS and massive nation-/healthcare-based cohorts grow through programs such as Genomics England, the NIH's Precision Medicine Initiative, and the VA's Million Veterans Project, PLT and MPV can serve as model complex traits to refine genetic analyses. Identifying associated genetic factors is only the beginning of identifying the mechanisms that govern platelet biology, their relationships with human disease, and possible platelet therapeutics targets. Connecting PLT/MPV associated genetic factors with biological function is vital in understanding platelet mechanisms and their contributions to human disease. Future studies taking advantage of the single base pair resolution of WGS need to focus on the translation of such association signals to both their functional and clinical implications. It is only through this translation and cooperation between clinical and bench scientists that new insights into platelet biology and therapeutic development can occur and fulfill the potential of genetically informed medicine.

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Figure 1.

Connections among genome-wide significant PLT and MPV associated loci and other traits and diseases via different evidence (shared association, PheWAS, Mendelian Randomization).

Table 1:
Various genetic methods used to identify and utilize genetic associations in the study of PLT and MPV

Method	Abbreviation	Description	Type of variation	Studies
Genome-wide association study	GWAS	Genotyping of 100,000s to millions of common single nucleotide polymorphisms (SNPs) across the genome to identify regions associated with trait of interest	Common	Meisinger et al. 2009 PMID: 19110211
			Non-coding Regulatory	Soranzo et al. 2009 PMID: 19221038 Soranzo et al. 2009 PMID: 19820697 Gieger et al. 2011 PMID: 22139419 Schick et al. 2016 PMID: 26805783 Qayyum et al. 2012 PMID: 22423221 Li et al. 2013 PMID: 23263863 Oh et al. 2014 PMID: 25705162 Kim et al. 2015 PMID: 26064965 Kamatani et al. 2010 PMID: 20139978 Shameer et al. 2014 PMID: 24026423 Astle et al. 2016 PMID: 27863252
Whole exome sequencing	WES	Direct sequencing of coding regions of the genome to identify non-synonymous and synonymous variants associated with trait of interest	Rare Coding	Auer et al. 2012 PMID: 23103231 Polfus et al. 2016 PMID: 27486782
Exomechip		Genotyping of ~250,000 previously observed rare to low frequency coding variants and a small fraction of previously associated SNPs from GWAS to examine contribution of exonic variants to trait of interest and to replicate previous findings, respectively	Mostly Rare Mostly Coding	Auer et al. 2014 PMID: 24777453 Eicher et al. 2016 PMID: 27346686
Whole genome sequencing	WGS	Direct sequencing of the entire genome to identify individual SNPs, genomic regions, or other types of variation associated with trait of interest. Sometimes such data are used as a reference panel to impute or infer genotypes in larger samples.	All	Lotchkova et al. 2016 PMID: 27668658
Phenome-wide association study	PheWAS	Examining the association of a single genetic variant across hundreds of available traits to determine possible effects of a variant across phenotypes	Usually common	Shameer et al. 2014 PMID: 24026423
Mendelian Randomization	MIR	Using the random assortment of genetic alleles and SNPs as strong instrument variables for prospective intermediate phenotypes to determine causal relationships between different traits	N/A	Astle et al. 2016 PMID: 27863252