

REVIEW ARTICLE

Genomics and transcriptomics of megakaryocytes and platelets: Implications for health and disease

Marlie H. Fisher MS^{1,2} | Jorge Di Paola MD^{1,2}  

¹Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado

²Medical Scientist Training Program, University of Colorado School of Medicine, Aurora, Colorado

Correspondence

Jorge Di Paola, University of Colorado School of Medicine, 12800 East 19th Street, Aurora CO, 80015.
Email: jorge.dipaola@ucdenver.edu

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Summary

The field of megakaryocyte and platelet biology has been transformed with the implementation of high throughput sequencing. The use of modern sequencing technologies has led to the discovery of causative mutations in congenital platelet disorders and has been a useful tool in uncovering many other mechanisms of altered platelet formation and function. Although the understanding of the presence of RNA in platelets is relatively novel, mRNA and miRNA expression profiles are being shown to play an increasingly important role in megakaryopoiesis and platelet function in normal physiology as well as in disease states. Understanding the genetic perturbations underlying platelet dysfunction provides insight into normal megakaryopoiesis and thrombopoiesis, as well as guiding the development of novel therapeutics.

KEYWORDS

congenital platelet disorders, genomics, megakaryocytes, platelets, RNA

Essentials

- High throughput sequencing has significantly advanced the field of megakaryocyte and platelet biology.
- These sequencing methods have uncovered various causative mutations in congenital platelet disorders.
- Mechanisms of mRNA expression in megakaryocytes and platelets are altered in various states of inflammation and disease.
- Improved understanding of the genomics and transcriptomics of megakaryocytes and platelets will advance clinical diagnostics and therapeutics.

1 | INTRODUCTION

Platelets are not only pivotal in the process of normal hemostasis but also play a critical role in wound healing, inflammation, and vascular integrity.¹ Disorders of low platelet count (thrombocytopenia) or platelet dysfunction are often associated with bleeding and can be heritable; therefore, it is critical to understand the genomic landscape of megakaryocytes and platelets and its impact on megakaryopoiesis, thrombopoiesis, and platelet function. These genetic signatures might be poised in the future to serve as biomarkers in predicting bleeding risk and clinical outcomes.

Recently, modern genomics techniques have impacted megakaryocyte, platelet, and platelet disorders research, allowing for the discovery of several genes involved in platelet production and function as well as generating a deeper understanding of the ribonucleic acid (RNA) and microRNA (miRNA) networks that may govern platelet production and function. Since megakaryocytes invest platelets with the transcriptome they carry, it is important to better appreciate the transcriptional signature of platelets and their relationship with mechanisms of megakaryopoiesis and thrombopoiesis.² Defining the gene expression profiles of megakaryocytes and platelets will likely contribute to the understanding of basic

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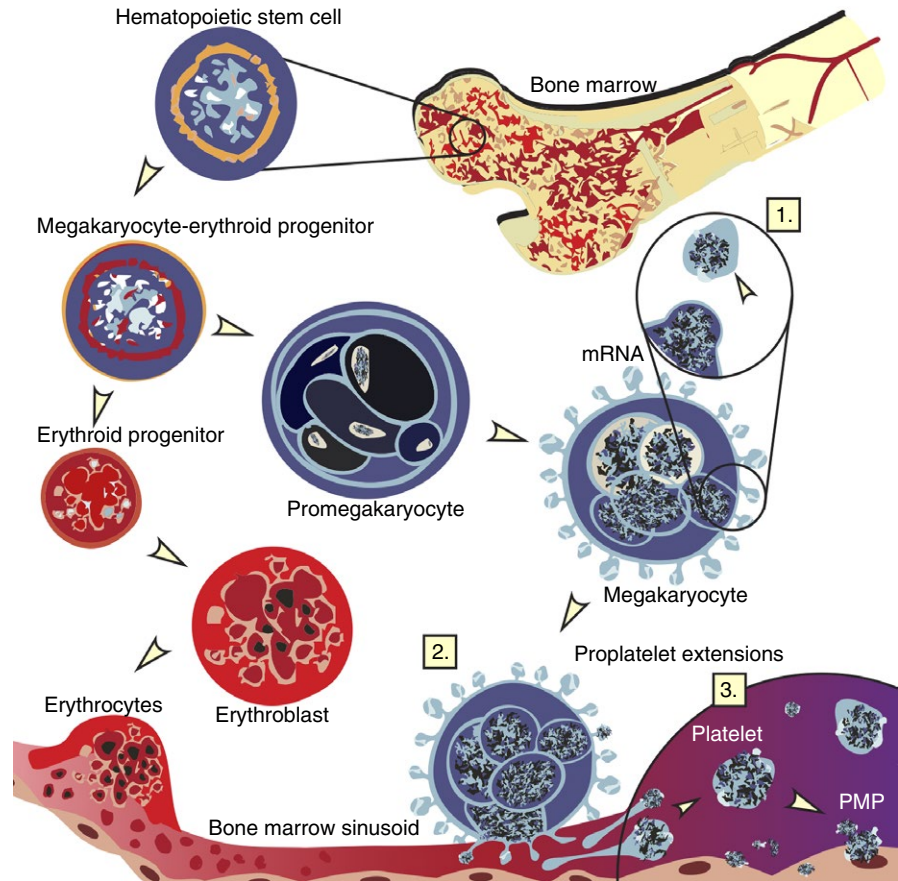


FIGURE 1 Megakaryopoiesis and the role of mRNA at various stages of thrombopoiesis. Simplified cartoon depicting megakaryopoiesis and thrombopoiesis with 1. mRNA being sorted into proplatelets 2. mRNA transferred to platelets released into circulation and 3. platelet microparticle (PMP) interaction with endothelial cells and release into circulation

hemostatic physiology and will help advance the knowledge of platelet response during stress, infection, and disease. In this short review we will focus on recent developments in the application of genomics to megakaryocyte and platelet biology. These new technologies have led to the discovery of causative mutations in several platelet disorders and have contributed to the molecular architecture that determines the “genetic profile” of platelets in health and disease.

2 | GENETIC REGULATION OF NORMAL MEGAKARYOPOIESIS

Platelets are shed into circulation by megakaryocytes, large polyploid cells that reside in the bone marrow and extend pro-platelet extensions into the bone marrow sinusoids.^{3,4} Once in the circulation, platelets survive for ~7 to 10 days.⁵ Megakaryocytes produce ~100 billion platelets daily to maintain a normal range of circulating platelets of 150 to 400 000 platelets per microliter of blood.³ During this process, megakaryocytes distribute their transcriptome and splicing machinery into platelets (see Figure 1.1-2).² This is a much more nuanced model of thrombopoiesis than was originally appreciated. What was once thought to be non-functional vestigial RNA in platelets is now understood to be a highly regulated sorting process by which megakaryocytes invest platelets with mRNA during thrombopoiesis.⁶

Despite the fact that they do not have a nucleus, platelets have been shown to have an active spliceosome and can process mRNA, which may play a role in platelet activation and thrombosis.^{7,8} Platelets have the ability to actively translate proteins required for thrombosis and inflammation with the mRNA and translation machinery derived from the megakaryocytes although the extent of these processes in normal hemostasis is not entirely clear.⁹ These unique mRNA signatures may account for the fact that platelet function is highly variable, even among healthy individuals. Common mRNA signatures and miRNA profiles have been associated with differences in platelet reactivity between races as well as hereditary platelet disorders.^{10,11}

Megakaryocytes are derived from pluripotent CD34+ hematopoietic stem cells (HSCs)¹² which give rise to common myeloid progenitors (CMP) and from them will differentiate into cells that are further committed down the lineage: the megakaryocyte-erythroid progenitor (MEP) which will give rise to megakaryocytes and/or erythroblasts (see Figure 1). Megakaryocytes mostly mature in the bone marrow, developing their characteristic polyploidy as they undergo endomitosis, a process by which the cells replicate their DNA without completing cell division.¹³ During this maturation process, megakaryocytes increase their cytoplasmic volume and generate pro-platelet extensions into the bone marrow sinusoids and shed platelets into the circulation.³ MEP lineages have been developmentally defined in human fetal liver and adult bone marrow. Prenatally, oligopotent progenitor cells residing in the fetal liver give rise to

Mutation (Gene ID)*	Clinical features	Pattern of inheritance
GATA1 (2623)/2	Dyserythropoietic anemia, macrothrombocytopenia, thrombocytopenia/thalassemia ^{62,63}	X linked
FLI1 (2313)	Macrothrombocytopenia, dense granule deficiency, Paris-Trousseau thrombocytopenia ^{64,65}	Autosomal dominant and recessive
RUNX1 (861)/AML1 (861)	Mild thrombocytopenia, increased MDS/AML/T-ALL risk, ⁶⁶ familial platelet disorder/acute myeloid leukemia ⁶⁷	Autosomal dominant
ETV6 (2120)	Mild thrombocytopenia, slightly increased B-ALL risk ⁴⁰	Autosomal dominant
GFI1B (8328)	Autosomal dominant macrothrombocytopenia with decreased or absent alpha granules ^{68,69}	Autosomal dominant and recessive
THPO (7066)	Congenital amegakaryocytic thrombocytopenia ¹⁷	Autosomal recessive
HOXA11 (3207)	Amegakaryocytic thrombocytopenia with radioulnar synostosis ⁷⁰	Autosomal dominant
MECOM (2122)	Amegakaryocytic thrombocytopenia with radioulnar synostosis ⁴⁴	Autosomal dominant

*<https://www.ncbi.nlm.nih.gov/gene>

myeloid, erythroid, and megakaryocyte fates. Multipotent or unipotent progenitor classes predominate in the bone marrow, giving rise to the adult megakaryocyte and erythroid classes.¹⁴ Interestingly it has been recently shown that the murine lung is a reservoir for hematopoietic progenitors and megakaryocytes, and a site for platelet biogenesis,¹⁵ although the significance of these findings in human megakaryopoiesis is still unknown.

The commitment of MEPs toward differentiated megakaryocytes that produce platelets requires the cytokine thrombopoietin (TPO) and its receptor MPL, which signals downstream through JAK2, STAT3/5, and MAPK.¹⁶ Rare mutations of the genes encoding thrombopoietin (*THPO*) and its receptor (*MPL*) result in congenital thrombocytopenia.¹⁷ Several transcription factors have also been implicated in the differentiation process (see Table 1).¹⁸ The combinatorial effect of cell-specific and general hematopoietic transcription factors is crucial for characteristic gene expression profiles along the lineage pathway that defines the spectrum of immature, mature, and terminally differentiated cells. These transcription factors have also been studied in the context of malignancy, where the skewing of megakaryocyte commitment may play a role in pre-leukemic disorders and myeloproliferative neoplasms.¹⁸ There are several transcription factors that are shared by both the megakaryocyte and erythroid lineage (*GATA1*, *GATA2*, *FOG*, *NF-E2*, and *GFI1b*),¹⁹ as well as lineage-restricted transcription factors, such as *KLF1* (erythroid) and *ETS1/FLI1* (megakaryocyte).^{18,20} Additionally, *MYB* plays a key role in hematopoiesis by enhancing erythropoiesis through transactivation of *KLF1* and *LMO2*.²¹ A critical transcription factor for megakaryocyte development and thrombopoiesis is *RUNX1*, which has been shown to repress the erythroid gene expression program, thus facilitating megakaryocyte differentiation.²² Furthermore, *RUNX1* regulates cytoskeletal genes such as *MYH9* and *MYH10* and plays a

TABLE 1 Mutations in transcription factors involved in megakaryopoiesis

role in megakaryocyte ploidy and proplatelet formation.²³⁻²⁵ *ETV6*, a member of the ETS transcription factor family, is required for late megakaryopoiesis and hematopoietic stem cell survival.²⁶ Over the last decade this traditional model of MEP to megakaryocyte and/or erythroblast has been challenged by new findings that suggest that there is a subset of HSCs that express von Willebrand factor (*VWF*), have a strong megakaryocyte bias and limited lymphoid potential.²⁷ More importantly, the *VWF* expressing HSCs can give rise to HSCs that do not express *VWF* while the opposite is not true, indicating that these cells are high in the hematopoietic hierarchy.^{20,28} Both of these models may not be mutually exclusive with megakaryocyte differentiation occurring through both the classic MEP pathway and by way of HSCs that exhibit an early megakaryocyte commitment in response to stress (ie, inflammation, chemotherapy, etc.).²⁹

While changes in germline DNA have been implicated in several platelet disorders, there is a large range of platelet function and reactivity traits that cannot be explained by germline variants alone. Classic mechanistic studies on hematopoietic stem cells and differentiation pathways have focused on the role of transcription factors. While they have been shown to play a crucial role, numerous recent studies have demonstrated further complexity in the system. miRNAs and ribonucleases (ie, *Dicer1*) which modulate gene expression appear to play an important role in the biology of hematopoiesis and hemostasis.^{23,30} Lu et al. demonstrate that miR-150, which targets the transcription factor *MYB*, regulates lineage fate in MEP's, driving megakaryocyte differentiation at the expense of the erythroid lineage.²⁴ Rowley et al. explored the impact of *Dicer1*, a ribonuclease that cleaves miRNA precursors into mature, active miRNAs, on platelet mRNA expression profiles and megakaryocyte function. While global ablation of *Dicer1* is embryonic lethal in mice, megakaryocyte specific deletion of

Dicer1 alters platelet miRNA and mRNA expression, specifically increased fibrinogen receptor subunits integrin α IIb and β 3, suggesting that miRNAs regulates the development and function of megakaryocytes and platelets.²⁵

miRNAs are short regulatory RNAs, about 21 to 23 nucleotides in length that regulate protein coding genes mostly through repression of gene expression, and numerous studies have begun to uncover the role they play in the regulation of megakaryopoiesis.³¹ Aberrant miRNA expression has been associated with a number of hematologic diseases, including leukemia, lymphoma, and myeloproliferative disorders. The miRNA signature of megakaryocytes derived from human CD34+ HSCs has been described suggesting that these miRNAs release the repression of target megakaryocytic transcription factors.²⁸ De-repression of these target genes theoretically allows the precursor cells to proceed in their differentiation program toward the megakaryocyte lineage. A recent study determined that miR-130a targets the transcription factor MAFB, an activator of the *GPIB* promoter, which encodes for the membrane receptor on platelets that binds VWF and stimulates platelet adhesion to subendothelial collagen. MAFB, a direct target of miR-130a, is upregulated as CD34+ HSCs become more terminally differentiated megakaryocytes, demonstrating a functional relationship between miRNA expression and megakaryocyte differentiation.³²

3 | GENOMICS APPROACHES TO PLATELET TRAITS

Over the last decades, these advances in high throughput sequencing and analytical techniques enabled the investigation and discovery of genetic polymorphisms associated with platelet traits in large cohorts of patients. The number of circulating platelets (platelet count) and the average size of the platelets (mean platelet volume) have been often associated with health and disease conditions and have been the target of several genome wide association studies (GWAS).

The first GWAS investigating genomic loci associated with platelet count and mean platelet volume (MPV) in 2009 identified 16 loci (*WDR66*, *ARHGEF3*, *TAOK1*, *PIK3CG*, *WTCCC*, *JMJD1C*, *SH2B3*, *BET1L*, *DNM3*, *EHD3*, *SIRPA*, *C226*, *TPM1*, *BAK1*).³³ This GWAS framework established by earlier studies in platelet diversity allowed for the discovery of 68 loci with significant associations with both platelet count and MPV. However, it is well known that the GWAS approach focuses on common variants rather than lower frequency rare variants. Using whole exome sequencing (WES), Auer et al. demonstrated association of rare variants in *BAK1*, *MPL*, *ITGB3*, *CD109*, and *CD36* with platelet count and MPV, which led to the development of the Exomechip, a quick genotyping method enriched in rare coding variants demonstrated in previous WES studies and common SNP variants identified in GWAS studies. The ability to increase sample size enabled the Exomechip technology to identify novel gene associations, including *JAK2*, *TUBB1*, *MAP1A*, *ZMIZ2*, *PEAR1*, and *PACSN2*.^{34,35}

While essential for discovery of novel gene candidates to understand platelet biology in health and disease, genomic studies represent only one aspect of gene discovery and most of the associations require functional validation. As demonstrated by Gieger et al., a screen knocking down 68 GWAS associated loci in *Drosophila melanogaster* (fruit fly) and *Danio rerio* (zebrafish) resulted in 11 novel gene regulators of megakaryopoiesis and platelet formation, confirmed by hematopoietic phenotypes in the model organisms.³⁶ As the field of genomics continues to expand, appropriate curation of genetic mutations and polymorphisms is critical. The functional study of genes discovered by GWAS and WES is critical to better understand normal megakaryocyte and platelet biology. Current efforts by several groups including a newly developed Platelet Working Group at the Clinical Genomics Resource (<https://www.clinicalgenome.org>) sponsored by the National Institute of Health (NIH) and the American Society of Hematology (ASH), and in collaboration with the ThromboGenomics initiative (<http://thrombo.cambridgednadiagnosis.org.uk>) are underway to guarantee the curation of genetic mutations and polymorphisms associated with megakaryocytes and platelet phenotypes.

4 | GENOMIC APPROACHES TO CONGENITAL PLATELET DISORDERS

The introduction of high throughput sequencing technology has significantly advanced the discovery of novel genes. Germline mutations in the genes encoding for hematopoietic transcription factors have been associated with congenital thrombocytopenia (see Table 1). Aberrant expression of these transcription factors may skew cellular differentiation and produce abnormal megakaryocytes, which go on to shed abnormal quality and/or quantity of platelets.

One of the first examples of the use of next generation sequencing in megakaryocyte disorders was the discovery of the gene responsible for gray platelet syndrome (GPS) by three different research groups. Mutations in *NBEAL2* were determined to be the causative mutation in GPS by next-generation RNA sequencing of platelets, whole exome sequencing and traditional sequencing strategies, respectively.³⁷⁻³⁹ More recently, *ETV6* has been implicated in inherited thrombocytopenia using next-generation sequencing techniques.^{40,41}

The use of next generation sequencing has not only allowed for the discovery of highly penetrant mendelian thrombocytopenia such as the ones caused by *NBEAL2* and *ETV6* mutations but also helped to identify underlying mutations in more complex disorders such as the elusive recessive Thrombocytopenia Absent Radii syndrome (TAR). In 2007, Klopocki et al. reported the presence of a 1q21.1 microdeletion in the majority of patients from a cohort diagnosed with TAR syndrome.⁴² Interestingly, unaffected parents also carried the deletion indicating that the 1q21.1 mutation was required, but not sufficient to cause TAR syndrome. The 1q21.1 microdeletion encompasses at least 12 known genes including a gene known as *RBM8A*. In 2013, Albers et al., using high throughput sequencing, identified

TABLE 2 Other mutations that underlie inherited platelet defects

Mutation (Gene ID)*	Protein function	Clinical features	Pattern of inheritance
<i>LYST</i> (1130)	Platelet secretion ⁷¹	Chediak – Higashi syndrome	Autosomal recessive
<i>MPL</i> (4352)	Thrombopoietin receptor	Congenital amegakaryocytic thrombocytopenia ⁷²	Autosomal recessive
<i>HPS1</i> (3257) Other less common: <i>AP3B1</i> ; <i>HPS3</i> ; <i>HPS4</i> ; <i>HPS5</i> ; <i>HPS6</i> ; <i>DTNBP1</i> ; <i>BLOC153</i> ; <i>AP3D1</i>	Dense granule formation	Hermansky-Pudlak syndrome ⁷³	Autosomal recessive
<i>WAS</i> (7454)	Expressed in hematopoietic cells, activates actin polymerization ⁷⁴	Wiscott-Aldrich syndrome, thrombocytopenia ⁷⁵	X linked recessive
<i>MYH9</i> (4627)	Non-muscle myosin involved in cell motility and structure ⁷⁶	May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, Epstein syndrome ⁷⁷ Macrothrombocytopenia	Autosomal dominant
<i>TUBB1</i> (81027)	Tubulin beta chain, involved in platelet formation and various cell processes (mitosis, motility, etc.)	Macrothrombocytopenia ^{78,79}	Autosomal dominant
<i>ACTN1</i> (87)	Expressed in platelets and megakaryocytes, non-muscle actin that bundles actin	Macrothrombocytopenia ^{80,81}	Autosomal dominant
<i>FLNA</i> (2316)	Anchoring protein for integrin receptor	Periventricular nodular heterotopia ⁸²	X linked dominant
<i>DIAPH1</i> (1729)	Actin polymerization	Macrothrombocytopenia ⁸³	Autosomal dominant
<i>PRKACG</i> (5568)	PKA catalytic subunit, involved in cytoskeletal reorganization	Macrothrombocytopenia ⁸⁴	Autosomal recessive
<i>PLAU</i> (5328)	Duplication of urokinase plasminogen activator, accelerating clot breakdown	Quebec platelet disorder ⁸⁵	Autosomal dominant
<i>ANO6</i> (196527)	Calcium dependent exposure of PS on cell surface ⁸⁶	Scott syndrome ⁸⁷	Autosomal recessive
<i>GP1BA</i> (2811)	Platelet surface receptor for von Willebrand factor	Mediterranean macrothrombocytopenia, velocardiofacial syndrome (DiGeorge syndrome), Platelet VWD (type 2b VWD)	Autosomal dominant
<i>P2RY12</i> (64805)	Receptor for ADP, mediates platelet aggregation	Impaired platelet aggregation due to absence of receptor ⁷³	Autosomal recessive
<i>RASGRP2</i> (10235)	Guanine exchange factor, active in platelet $\alpha_2\beta_3$ inside-out signaling	Platelet aggregation defect in response to ADP stimulus ⁸⁸	Autosomal recessive
<i>ITGA2B</i> (3674) and <i>ITGB3</i> (3690)	Fibrinogen receptor	Glanzmann thrombasthenia (GT) ⁸⁹	Autosomal recessive
<i>GP1BA</i> (2811), <i>GP1BB</i> , <i>GP9</i>	Platelet surface receptor for von Willebrand factor	Bernard Soulier syndrome ⁹⁰	Autosomal recessive
<i>NBEAL2</i> (23218)	Alpha granule formation and secretion	Gray platelet syndrome ³⁷	Autosomal recessive
<i>TBXA2R</i> (6915)	G-protein coupled receptor for thromboxane	Impaired platelet aggregation ¹⁷	Autosomal recessive
<i>GP6</i> (51206)	GPVI deficiency (collagen receptor)	Impaired platelet aggregation ⁷³	Autosomal recessive
<i>CYCS</i> (54205)	Cytochrome c, involved in mitochondrial electron transport chain and cellular apoptosis	Thrombocytopenia ⁹¹	Autosomal dominant
<i>ANKRD26</i> (22852)	Component of the beta-catenin signaling pathway ⁹²	Thrombocytopenia ⁹³	Autosomal dominant

(Continued)

TABLE 2 (Continued)

Mutation (Gene ID)*	Protein function	Clinical features	Pattern of inheritance
<i>SLFN14</i> (342618)	Largely unknown, may play a role in RNA surveillance pathways ⁹⁴	Thrombocytopenia ⁹⁵	Autosomal dominant
<i>RBM8A</i> (9939)	RNA binding protein involved in protein production	TAR syndrome ⁹⁶	Autosomal recessive
<i>VIPAS39</i> (63894) and <i>VPS33B</i> (26276)	Lysosomal sorting protein	ARC syndrome ⁹⁷	Autosomal recessive
<i>STIM1</i> (6786)	Calcium sensing	Stormorken syndrome ⁹⁸	Autosomal dominant
<i>SRC</i> (6714)	Tyrosine kinase	Thrombocytopenia and myelofibrosis ⁹⁹	Autosomal dominant

*<https://www.ncbi.nlm.nih.gov/gene>

low frequency noncoding single nucleotide polymorphisms (SNP) in the 5' UTR or first intron of *RBM8A* in 53 out of 55 TAR patients with the microdeletion.⁴³ The authors concluded unequivocally ($P < 5 \times 10^{-228}$) that the compound (bi-allelic) inheritance of these noncoding SNPs together with a null mutation in *RBM8A* causes TAR syndrome although the mechanism by which these coinherited molecular defects reduce *RBM8A* transcription and protein expression is not known.

Another example is the use of WES in the discovery of de novo missense mutations in the eighth zinc finger motif of the C-terminal EVI1 DNA binding domain of the *MECOM* gene.⁴⁴ Radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT) is an inherited bone marrow failure syndrome originally attributed to *HOXA11* mutations, however a number of individuals with RUSAT did not show mutations in *HOXA11*. Niihori et al. recently reported three individuals with RUSAT that harbored *MECOM* mutations, and the authors suggest transcriptional dysregulation by the mutant EVI1 DNA binding domain as the mechanism underlying this hematopoietic disorder.

The discovery of these genes as causative mutations for inherited platelet defects demonstrates the immense capability for sequencing technology in identifying genetic causes of human disease. Developing clinically useful applications of all these discoveries has been challenging because some of these genetic polymorphisms can be common and associated with only slightly increased or decreased disease risk.⁴⁵ However, using highly statistically powered studies, interpreting mechanisms and identifying causal alleles has become possible. As the ability to implement sequencing technologies in patient care became more readily accessible, clinicians are able now to screen and diagnose patients with inherited platelet disorders. Table 2 shows a current detailed description of inherited platelet disorders and their genetic causes. Furthermore, Astle et al. demonstrated that these rare mutations can be validated in high-powered GWAS studies.⁴⁵

5 | THE NOVEL ROLE OF PLATELET RNA IN HEALTH AND DISEASE

Modern genomic techniques have made RNA sequencing feasible and cost effective. Over the last decade the field of megakaryocyte

and platelet RNA has expanded significantly. For example, platelet reactivity has been associated with differential RNA expression profiles.⁴⁶ Platelet hyper-reactivity was associated with five-fold increase of *VAMP8* mRNA transcript and increased protein levels of *VAMP8*.⁴⁶ *VAMP8* is a v-SNARE protein that is involved in platelet granule secretion. The ability of platelets to aggregate is dependent on the release of the granule contents, and this process is facilitated by SNARE proteins at the plasma membrane.⁴⁷ *VAMP8* mRNA has been shown to be differentially expressed in patients with coronary artery disease due to a SNP in the *VAMP8* 3'-UTR (A->G rs1010). Furthermore, miR-96 is predicted to bind to 3'-UTR of *VAMP8* mRNA and has been shown to be expressed in platelets.⁴⁸ Patients with hyperreactive platelets had a 4.8-fold increase in *VAMP8* mRNA levels compared to patients with hyporeactive platelets. Interestingly, in a small number of study subjects, miR-96, which decreases *VAMP8* mRNA was expressed at higher levels in platelets that were defined to be hyporeactive. This finding demonstrates the potential for miRNA targeting in antiplatelet therapeutics.

Recently, it has been shown that platelet microparticles are capable of transferring miRNAs attracting hematopoietic cells and promoting their survival and proliferation by stimulating the release of cytokines, and inducing angiogenesis in the setting of cancer metastasis (see Figure 1.3).⁴⁹ Notably, these microparticles are enriched in miRNAs that can influence gene expression. These platelet microparticles have been shown to have a direct effect on tumor cell apoptosis via infiltration and transfer of platelet derived miRNA. MiR-24 has been implicated in this transfer mechanism and inhibits the growth of lung and colon ectopic tumors.⁵⁰ Platelet microparticles carrying MiR-24 inhibit tumor growth, whereas blockade of MiR-4 stimulated *in vivo* tumor growth.

Platelets are also capable of transferring RNA molecules while in the circulation or interacting with other normal cells.⁵¹ Labeled RNA from platelet-derived particles can be transferred to other immune cells and endothelial cells. Risitano et al. described a system in which cytoplasmic labeled RNA from platelets can be visually transferred to THP-1 AML cells, monocytes, and endothelial cells.⁵² The transfer of genetic material subsequently alters the expression profile of the recipient cells. Furthermore, the RNA that is transferred by the particles can be actively translated by the recipient cells. This has

implication for a previously unappreciated role of platelets in the regulation of inflammation and vascular homeostasis.

Platelets play a role in the interface of hemostasis and systemic inflammation. Platelet RNA in patients with a higher BMI was enriched for several inflammatory transcripts, including among others *ICAM1*, *IL1R*, *IFNG*, *IL6*, and cultured megakaryocytes exposed to c-reactive protein (CRP) and IL-6 resulted in altered expression of the same genes seen in the patient dataset (ie, *ICAM1*, *IL1R*, *IFNG*, *IL6*).⁵³ IL-6, among other inflammatory cytokines, triggers the production of CRP and other acute phase reactants that were found to be associated with several platelet derived genes (*ALOX5*, *PTGER2*, *S100A9*, *SELENBP1*, *TLR4*, and *TNFRSF1B*) in obese patients.⁵⁴ Furthermore, a strong association between BMI, coronary heart disease, and specific platelet transcripts, particularly those involved in the NF- κ B pathway, was reported in the Framingham study.⁵⁵

Finally, it has been known for decades that black individuals are at an increased risk for thrombotic events (ie, myocardial infarction, stroke) compared to white individuals.⁵⁶ These racial differences are dependent in part, on thrombin protease-activated receptors (PARs), most specifically PAR4 activation.⁵⁷ Black individuals were shown to have a 4-fold higher expression of phosphatidylcholine transfer protein (PCTP) mRNA correlating with increased PAR4 mediated aggregation. PAR4 surface expression was not different between white and black individuals, meaning that the difference in aggregation and reactivity is due to PAR4 mediated signaling rather than variable surface expression of PAR4. Further studies demonstrate that the downstream Gq signaling axis is altered in black individuals upon PAR4-mediated platelet stimulation.⁵⁸ Interestingly, miR-376c, which regulates the expression of PCTP mRNA in human megakaryocytes, was differentially expressed by race. An inverse relationship between PCTP mRNA expression, PCTP protein levels, and PAR4 reactivity as compared to miR-376c levels was demonstrated by Edelstein et al., indicating a critical racial difference in the genomics of platelet function.⁵⁹

6 | CONCLUSIONS

The use of high throughput sequencing has significantly improved the ability to characterize genetic mechanisms of megakaryopoiesis and platelet formation and function, as well as to identify candidate genes for inherited platelet disorders and traits.⁶⁰ Initiatives like the 1000 Genomes Project, the International HapMap project, ENCODE project database, and other large-scale whole genome sequencing projects that link genotypes with health and social care records will be invaluable tools in understanding the link between gene mutations and corresponding disease.⁶¹

While understanding the genetic variations that underlie platelet dysfunction is crucial for screening patients and developing a clear picture of the genetic landscape of normal megakaryopoiesis, sequencing alone does not always explain the molecular mechanism by which these genetic changes result in clinical

phenotypes. More research into the functional consequence of these mutations is required to better understand the role that these genetic changes play. Functional studies will begin to elucidate the complex biology of megakaryopoiesis and thrombopoiesis and put these genetic mutations into the context of the larger hematopoietic system.

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None of the authors have any disclosures relevant to this paper.

AUTHOR CONTRIBUTIONS

Marlie Fisher and Jorge Di Paola wrote the manuscript.

ORCID

Jorge Di Paola  <http://orcid.org/0000-0002-7871-6143>

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