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An Oligogenic Case of Severe Neonatal Thrombocytopenia and a Purportedly Benign Variant in *GFI1B* Requiring Reinterpretation

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Abstract

Although thrombocytopenia in neonatal intensive care patients is rarely due to inherited disorders, the number of genetic variants implicated in platelet defects has grown dramatically with increasing genome-wide sequencing. Here we describe a case of severe, oligogenic neonatal thrombocytopenia and reinterpret a reportedly benign mutation that is likely pathogenic. Despite this patient's synonymous mutation (*GFI1B* 576 C>T, Phe192=) being annotated as benign, GFI1B is a well-known regulator of megakaryopoiesis, this variant alters splicing and megakaryocyte maturation, and our analysis of existing genome-wide associated studies demonstrate that it likely causes gray platelet syndrome. This variant has not been reported in a case of life-threatening thrombocytopenia. We propose the severity of this patient's phenotype is due to synergistic epistasis between the intrinsic platelet defect caused by this mutation and her concomitant inherited PMM2 congenital glycosylation disorder neither of which have been associated with such a severe phenotype. This case highlights the importance of whole exome/

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AUTHORS' CONTRIBUTIONS

MF initiated the inquiry, generated the hypotheses, performed analyses, interpreted data, reviewed the literature, and wrote the paper. SMM guided interpretation of data and edited the paper. CAD treated the patient, interpreted the data, and edited the paper. AH collected patient data, obtained consent, and edited the paper. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient's parents. Any information that could identify the subject or the parents has been withheld.

COMPETING INTERESTS

The authors declare that they have no competing interests.

genome sequencing for critically ill patients, re-examining variant interpretation when clinically indicated, and the need to study diverse genetic variation in hematopoiesis.

Keywords

Thrombocytopenia; GFI1B; PMM2 congenital disorder of glycosylation; Oligogenic; Case Report

BACKGROUND

Inherited disorders are a seemingly infrequent cause of severe thrombocytopenia in neonatal intensive care patients. However, genetic diseases are likely an underappreciated subset of platelet disorders given that 50–80% of variance in platelet counts is heritable[1,2] and that the cause of clinically significant thrombocytopenia is often not identified. We present an oligogenic case of severe thrombocytopenia in a neonate and reinterpret a reportedly benign synonymous substitution in *GFI1B* (576 C>T, Phe192=) as a causal variant. We argue that this patient's severe phenotype is a result of synergistic epistasis between the intrinsic platelet defect caused by *GFI1B* 576 C>T and consumptive coagulopathy caused by her inherited PMM2 congenital disorder of glycosylation (PMM2-CDG). Neither mutation has previously been associated with life threatening thrombocytopenia. This case illustrates the importance of unbiased deep sequencing in neonatal thrombocytopenia, the genetic heterogeneity of platelet disorders, the need to reevaluate known variants given new data, and the profound effect of epistasis among multiple causal variants.

CASE PRESENTATION

A baby girl was born prematurely at 28w6d to a 39 year-old G2P0 mother by Cesarean section because of difficulty with extraction in the setting of preeclampsia with severe features. The pregnancy was complicated by gestational diabetes mellitus and maternal Hashimoto's thyroiditis that was well-controlled with levothyroxine. The patient was diagnosed with hydrops fetalis on a 26-week fetal ultrasound and prenatal karyotyping was normal. After delivery, the patient was admitted to the neonatal intensive care unit (NICU) for management of severe thrombocytopenia, hydrops fetalis, and prematurity. Petechiae and bruising were noted at birth, and blood was seen during intubation. Her initial platelet count was ~6,000 /uL (reference 160,000 – 370,000 /uL). While platelets were sparse, most of those that were seen on peripheral smear were large, hypogranular, and were not aggregated (Figure 1). She was transfused, responded robustly (>100,000 platelets/uL), and was treated empirically with intravenous immunoglobulin (IVIG). However, all serologies returned negative, and her thrombocytopenia persisted over 72 hours.

Common causes of persistent neonatal thrombocytopenia were ruled out with negative maternal infectious studies, blood cultures, and X-rays (excluding thrombocytopenia absent radius syndrome). Thrombopoietin (TPO) was severely elevated at 3,692 pg/mL (reference interval 7 – 99 pg/mL), but a bone marrow biopsy could not be performed given that the patient weighed ~2.5 kg. She was transfused dozens of times during the 5-month hospital course, which maintained an average platelet count of ~45,000 /uL (range 15,000 – 156,000 /uL). All other blood cell counts (including red blood cell counts) were

unremarkable and there were no episodes of clinically significant bleeding. Her PTT and INR were mildly elevated at averages of 53 (range 43.1 – 67.1) and 1.4 (range 1.1 – 1.8), respectively.

In addition to her severe thrombocytopenia, she had anasarca, required therapeutic paracentesis, bilateral chest tubes, and repeated albumin and furosemide infusions. Given these multisystem defects and thrombocytopenia of unknown etiology, whole exome sequencing was performed. This revealed compound heterozygosity for two known pathogenic mutations in *PMM2* (Phe119Leu and Phe157Ser) and heterozygosity for a synonymous substitution in *GFI1B* (576 C>T, Phe192=, ClinVar ID 711822), which was found to be inherited from her mother. The mother's CBC was normal with a platelet count of 227,000 /uL and mean platelet volume of 12.4 fL. The patient was diagnosed with PMM2-congenital disorder of glycosylation (PMM2-CDG), and she received several fresh frozen plasma infusions, bumetanide, and a two-day course of protein C concentrate for diffuse endothelial barrier dysfunction[3]. Her native protein C activity level was depressed at <10% normal pooled plasma (NPP). The normal adult range is 70–150% NPP. However, at birth, protein C activity is (on average) approximately 35% adult value making it difficult to interpret this patient's low activity level. After receiving two days of protein C concentrate, repeat measurement showed an increase to 45.2% NPP. Her hospital course was also complicated by an occlusive thrombus in her right lower leg at the site of insertion for a central catheter that was successfully treated with a 2-week heparin drip and resolved on repeat ultrasound. Unfortunately, she became septic in the 5th month of life, rapidly decompensated, interventions were withdrawn, and she died.

DISCUSSION

We present a case of severe, oligogenic neonatal thrombocytopenia and reinterpret a benign variant as pathogenic. PMM2-congenital disorder of glycosylation (CDG) is unlikely to be the sole cause of this patient's life-threatening thrombocytopenia given that PMM2-CDG is rarely associated with thrombocytopenia[4]. Moreover, there are no reports of patients with PMM2-CDG requiring near daily platelet transfusions to survive. While case reports exist of PMM2-congenital disorder of glycosylation (PMM2-CDG) with thrombocytopenia[5,6], the predominant defect in this disorder is that of secondary hemostasis. Further, these cases exist in the rare subset of patients with PMM2-CDG who survive into adulthood[6] (a phenotypically and likely biomolecularly distinct entity from the typical, severe PMM2-CDG), these cases did not use unbiased whole genome sequencing to exclude contributing genetic confounders, and pale macrothrombocytes have never been associated with the disorder. Theoretically, PMM2-CDG could cause a primary hemostatic defect either by platelet consumption secondary to endothelial barrier dysfunction or by impaired platelet surface protein glycosylation. Neither can account for the entirety of this patient's extreme phenotype. Endothelial barrier dysfunction and hydrops is common in PMM2-CDG whereas thrombocytopenia is not arguing against PMM2-CDG-mediated barrier dysfunction and platelet consumption as the sole cause of her platelet deficiency. The latter is possible given that platelets from patients with PMM2-CDG have reduced sialic acid content though there is no clear quantitative or qualitative glycosylation defect in platelets from patients with

PMM2-CDG[7]. Taken together, PMM2-CDG alone is unlikely to account for the entirety of this patient's severe macrothrombocytopenia.

On the other hand, the mutation *GFI1B* 576 C>T is a prime candidate for being causal despite being annotated as benign (ClinVar ID 711822). *GFI1B* is a transcriptional repressor critical for megakaryopoesis[8,9] and several pathogenic *GFI1B* variants have been associated with impaired platelet production and function[10–12]. *GFI1B* 576 C>T is a synonymous substitution (Phe192=) that alters splicing to produce a short isoform[13] that inhibits megakaryocyte differentiation (while sparring erythropoiesis) in a dominant-negative fashion[14] which explains this patient's phenotype despite being heterozygous. A megakaryocyte differentiate block also explains this patient's remarkably elevated thrombopoietin (3,692 pg/mL, reference interval 7 – 99 pg/mL). While we couldn't confirm a defect in her megakaryocyte lineage without a bone marrow aspirate, this patient's exceedingly high thrombopoietin (TPO) suggests defective differentiation is possible. Although elevated TPO could indicate increased platelet turnover in the setting of impaired platelet glycosylation, platelet glycosylation defects have never been definitively confirmed[7]. Additionally, such severely elevated TPO (>30 times the normal upper limit) has never been reported in CDG but is commonly observed in congenital amegakaryocytic thrombocytopenia (CAMT), a disorder of impaired megakaryopoesis. In CAMT, TPO is severely elevated given the lack of TPO-receptor (c-Mpl) expressing megakaryocytes which would otherwise remove TPO from circulation. It is likely that this patient's elevated TPO is due to combination of impaired megakaryocyte differentiation and possibly impaired c-Mpl glycosylation which could inhibit signaling through c-Mpl.

There is also significant population-scale data to implicate *GFI1B* 576 C>T in this patient's thrombocytopenia. Several genome-wide association studies (GWAS) in adult populations have found that this variant is highly associated with significant increased platelet volume and decreased platelet count but not associated with erythrocyte count[15–18] (Table 1). Indeed, this patient's sparse thrombocytes are large (Figure 1). The existing functional and population-level sequencing data are consistent with this patient's macrothrombocytopenia and normal RBC counts, and suggest that *GFI1B* 576 C>T be reclassified as pathogenic based on American College of Medical Genetics criteria (PVS1, PS3, PS4, PM1, PM4)[19].

In addition to her quantitative platelet defect, this patient's large and hypogranulated platelets are qualitatively reminiscent of gray platelet syndrome (GPS). Intriguingly, an autosomal-dominant GPS has been described where the causal defect is a heterozygous nonsense mutation in *GFI1B*[11]. That *GFI1B* 576 C>T is associated with macrothrombocytopenia (Table 1) and that this patient had hypogranulated macrothrombocytes leads to the suggestion that *GFI1B* 576 C>T might cause a disease that exists on a continuum with gray platelet syndrome. Alternatively, the pale granulations seen on this patient's peripheral smear may indicate that platelets have been activated. While this is likely contributing, we hypothesize that impaired megakaryocyte differentiation caused by *GFI1B* 576 C>T may result in a similar phenotype given the existing *in vitro* evidence that *GFI1B* 576 C>T inhibits megakaryocyte differentiation, that additional dominant-negative *GFI1B* variants alter megakaryocyte morphology[11], and given this patient's corroborating severely elevated TPO.

This is the first reported case of *GFI1B* 576 C>T in a neonate and the first report of the variant as pathogenic. This is also the most severe phenotype reported with this variant; notably, the patient's mother has the same *GFI1B* genotype but a normal platelet count, normal MPV, and no clinically apparent bleeding disorder. A mild or non-apparent hematologic defect with *GFI1B* 576 C>T alone is further supported by the many instances of this genotype in large cohorts of adults (Table 1). This patient's life-threatening thrombocytopenia is therefore likely of oligogenic origin: epistasis between the patient's intrinsic platelet defect driven by *GFI1B* 576 C>T and her concomitant glycosylation defect. We illustrate the importance of unbiased whole exome/genome sequencing in neonatal thrombocytopenia, the genetic heterogeneity of platelet disorders, the role of oligogenic causes in rare diseases, and the need to reevaluate known variants given new data. GFI1B alterations should be routinely included in the differential diagnosis for macrothrombocytopenia.

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AVAILABILITY OF DATA AND MATERIALS

Data sharing is not applicable to this article as no datasets were generated during the current study.

LIST OF ABBREVIATIONS

PMM2	Phosphomannomutase 2
PMM2-CDG	PMM2 – Phosphomannomutase 2 congenital disorder of glycosylation
pLoF	Predicted loss of function
GPS	Gray platelet syndrome
GFI1B	Growth factor independent 1B transcriptional repressor
NBEAL2	Neurobeachin-like 2
TPO	Thrombopoietin
PTT	Partial thromboplastin time
INR	International normalized ratio
AST	Aspartate aminotransferase
ALT	Alanine transaminase
IVIG	Intravenous immunoglobulin
NICU	Neonatal intensive care unit

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PLAIN LANGUAGE SUMMARY

What is the context?

Low platelets (thrombocytopenia) in the neonatal population isn't frequently inherited. As we perform unbiased DNA sequencing in more patients, the number of inherited platelet disorders and implicated variants are growing.

The gene *GFI1B* encodes for a transcription factor that regulates megakaryocytes, the cell type that produces platelets. A synonymous substitution in *GFI1B* (576 C>T, Phe192=) is annotated as benign; however, experimental studies have shown that it inhibits megakaryocyte production.

There is growing appreciation for oligogenic inheritance where multiple causal variants contribute to clinical phenotypes.

What is new?

We present a case of life-threatening neonatal macrothrombocytopenia (large, hypogranulated sparse platelets) that has an oligogenic cause. We reinterpret the synonymous substitution *GFI1B* 576 C>T as pathogenic.

This patient's severe phenotype was likely due to the combined effect of *GFI1B* 576 C>T and her inherited glycosylation disorder (PMM2-CDG). Neither variant alone causes severe thrombocytopenia, but the combined intrinsic platelet defect (*GFI1B* mutation) and consumption (PMM2-CDG) likely produced her life-threatening phenotype.

What is the impact?

GFI1B is a critical regulator of megakaryocyte production. The purportedly benign mutation 576 C>T is likely pathogenic causing thrombocytopenia by impairing megakaryocyte maturation.

As more patients have unbiased genome sequencing, oligogenic and polygenic inheritance will become increasingly appreciated as causes of platelet disorders.

NICU providers should consider whole genome or exome sequencing of neonates with severe thrombocytopenia after reversible causes are ruled out.

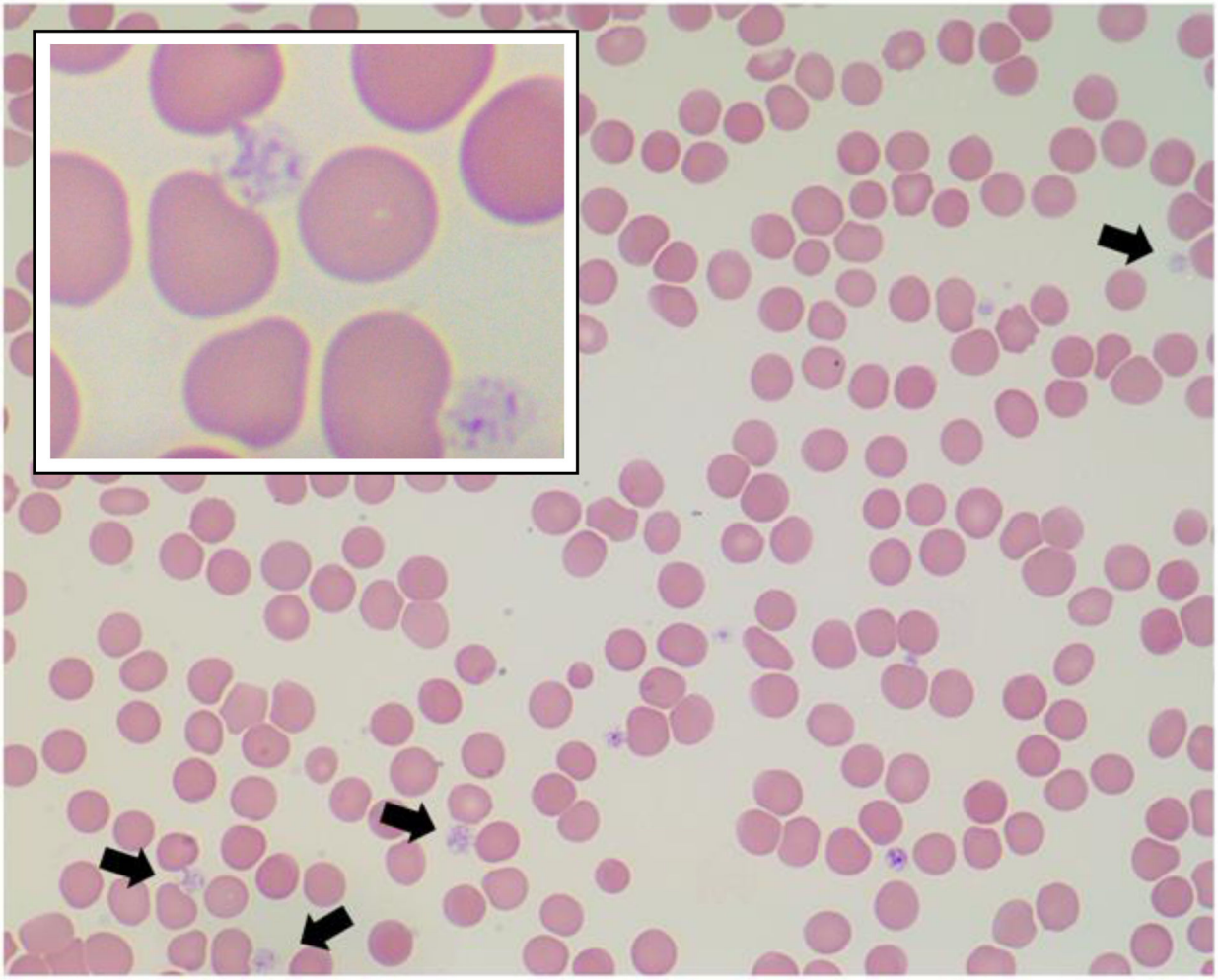

Figure 1:
Peripheral blood smear from the patient at the time of birth demonstrating sparse and hypogranulated macrothrombocytes (black arrows). Insert: magnified hypogranular macrothrombocytes from bottom left of widefield microscopy. RBC volume (from CBC) and morphology are within normal limits. Her RBC diameter therefore is also normal (~8 μm). Platelets shown are often 50% or more of RBC diameter making the platelet diameters ~4 μm or larger and therefore above the normal 1.5–3 μm range.

Table 1:

GFI1B 576 C>T is highly associated with quantitative platelet traits in diverse adult populations. Data listed is derived from previously published genome-wide association studies.

Trait	Direction	p-value	Beta (SD)	CI	Study
Platelet Count	Decrease	2×10^{-292}	0.41	0.39 – 0.43	Chen et al. *Cell*. 2020
Platelet Count	Decrease	4×10^{-232}	0.43	0.41 – 0.46	Sakaue et al. *Nat Gen*. 2021
Platelet Count	Decrease	5×10^{-198}	0.41	0.38 – 0.43	Vuckovic et al. *Cell*. 2020
Platelet Count	Decrease	6×10^{-88}	0.42	0.38 – 0.46	Astle et al. *Cell*. 2016
Platelet Count	Decrease	1.8×10^{-27}	0.43	0.34 – 0.52	Polfus et al. *AJHG*. 2016
Platelet Volume	Increase	1×10^{-255}	0.46	0.44 – 0.49	Vuckovic et al. *Cell*. 2020
Platelet Volume	Increase	2×10^{-107}	0.47	0.43 – 0.51	Astle et al. *Cell*. 2016
Platelet Distribution Width	Increase	3×10^{-74}	0.25	0.22 – 0.27	Vuckovic et al. *Cell*. 2020
Platelet Distribution Width	Increase	4×10^{-40}	0.28	0.24 – 0.32	Astle et al. *Cell*. 2016