

## TO THE EDITOR:

## *GNE* variants causing autosomal recessive macrothrombocytopenia without associated muscle wasting

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We have identified 9 affected individuals from 3 unrelated families with macrothrombocytopenia and mild to moderate bleeding diathesis inherited due to missense mutations in the glucosamine (UDP-*N*-acetyl)-2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene. Previous reports have primarily described a myopathy associated with *GNE* variants. Eight of the 9 patients in our cohort had no evidence of myopathy, and the 1 patient with neuromuscular symptoms had a muscle biopsy inconsistent with *GNE* myopathy. Following is the description of our patients and the variants identified as well as the laboratory evidence supporting pathogenicity for the identified variants and describing our hypothesized mechanism for thrombocytopenia.

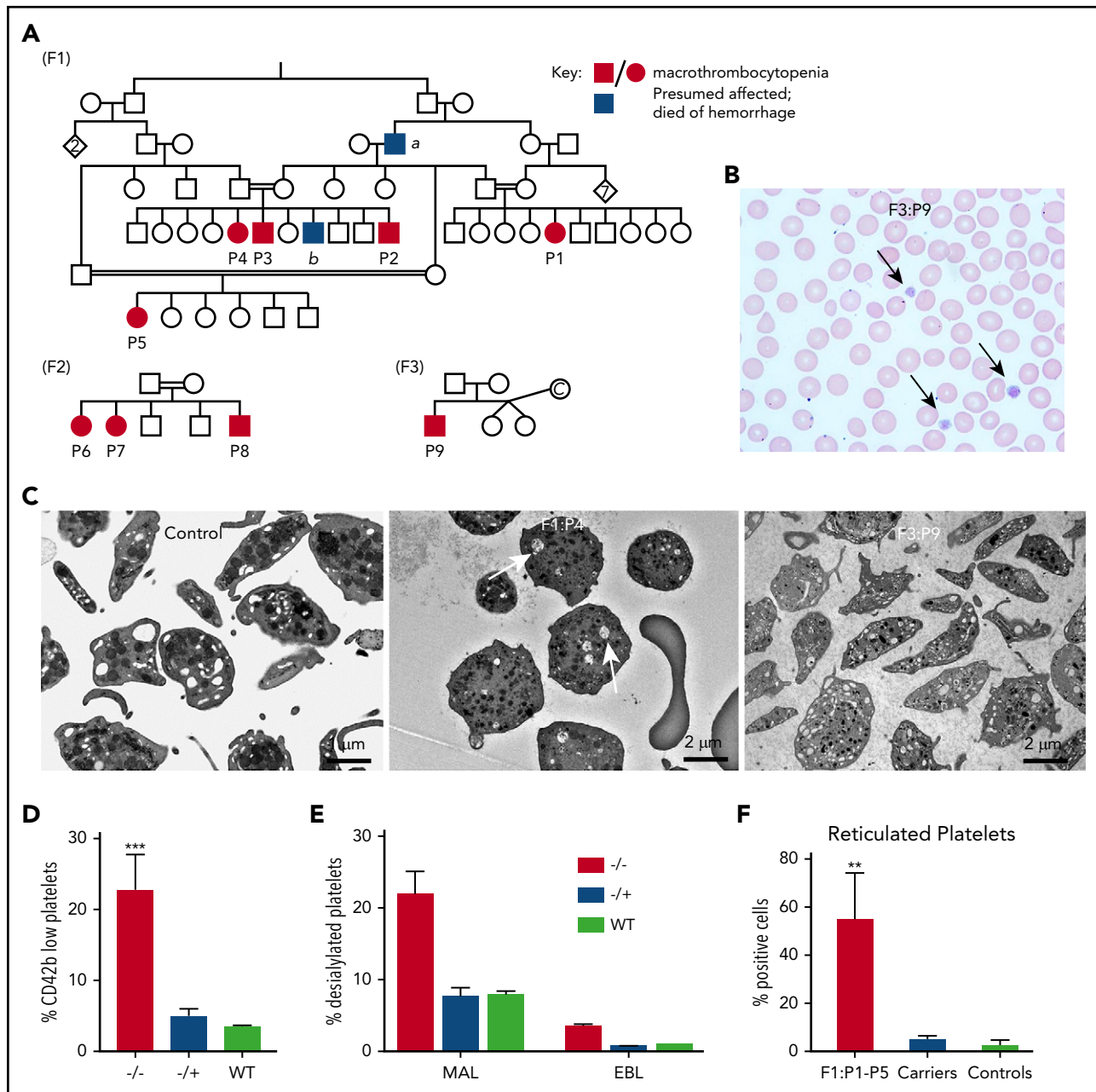
Platelets express the negatively charged, *N*-acetylneuraminic acid-containing pentasaccharide sialic acid, most of which resides on surface glycoprotein carbohydrate side chains.<sup>1</sup> A large proportion of the platelet sialic acid is neuraminidase labile and is implicated in platelet functional events, such as aggregation and adhesion, as well as contributing to the platelet's electrophoretic mobility.<sup>2</sup> Sialylation appears to affect the number of circulating platelets. Removal of sialic acid (desialylation) shortens platelet life span<sup>3</sup> and triggers clearance of senescent platelets.<sup>4</sup>

Previous data in both human and animal models suggest a role for platelet sialylation in regulation of platelet count<sup>5-7</sup> and hint that genes that alter platelet glycoprotein expression may result in differences in baseline platelet numbers. In contrast, most previous reports of patients with *GNE* variants have described primarily a myopathy, although some patients with associated mild, asymptomatic thrombocytopenia have been described in 0% to 2.5% of *GNE* myopathy registry patients (Argov Avisohar, Hadassah-Hebrew University Medical Center, e-mail, 2017).<sup>8,9</sup> *GNE* regulates and initiates biosynthesis of *N*-acetylneuraminic acid, a precursor of sialic acids, which is required for normal sialylation in hematopoietic cells.<sup>10</sup>

The medical records of 9 patients from 3 distinct families (F1, F2, and F3), presenting with bleeding and macrothrombocytopenia, and found to have *GNE* gene variants, were reviewed. Detailed description of genetic analysis and other methods is given in the supplemental materials, available on the *Blood* Web site. Data concerning clinical presentation, platelet numbers and morphology, muscle weakness, and genetic findings were collected. All participants provided consent according to local institutional policies, including consent for DNA analysis.

F1 and F2 are of Palestinian Arab descent with 5 and 3 affected individuals (P1-P5 and P6-P8). F1 cases had a novel variant in *GNE* (c.1516\_1517delinsTT, p.Gly475Phe) (supplemental Figure 2). In F2, a homozygous variant in *GNE* (c.1457T>C, p.Leu486Pro) was found (supplemental Figure 2). Both variants in F1 and F2 occur in a highly conserved region of exon 9. F3 involves a 13-year-old male Caucasian (P9) born to nonconsanguineous parents of mixed European lineage (Figure 1A; Table 1). In F3, analysis revealed 2 rare variants: (c.1649A>G, p.Asn550Ser) previously reported in myopathy<sup>11</sup> and c.562C>T, p.His188Tyr occurring in a highly conserved region in exon 4 (supplemental Figure 2). All 4 variants were unobserved in several databases of healthy individuals. Clinically, all affected family members report bruising ± epistaxis. Affected women also reported severe menorrhagia and recurrent episodes of hemorrhagic corpus luteum requiring red cell and platelet transfusions. Three affected individuals had a history of surgery or trauma requiring red cell and platelet transfusions. Importantly, in all but 1 patient, there was no evidence of clinical myopathy (P1-P8), with exclusion of subclinical myopathy in 2 family members (P1 and P4) by magnetic resonance imaging.<sup>12</sup> P9 has hypotonia of the lower extremities, as well as autism spectrum disorder.<sup>13</sup> Muscle biopsy was not consistent with *GNE* myopathy (supplemental Figure 1).

The presence of macrothrombocytopenia was confirmed on light microscopy (Figure 1B). Electron microscopy supported the



**Figure 1. Family and biology studies supporting GNE pathobiology.** (A) Family pedigrees. Each family is represented by the designation F1-3. Patients P1 through P9 are shown as black circles (female) and squares (males), whereas unaffected family members are shown as open symbols. Blue boxes refer to potentially affected individuals, but no blood counts were done. Red boxes/circles represent affected individuals. F1 pedigree: The common ancestor "a" died posttrauma bleeding, whereas "b" died at the age of 2 years from an intracranial hemorrhage. Parents of patients have normal platelet counts and morphology. F2 pedigree, F3 pedigree: "c" refers to an egg donor. (B) A representative hematology and eosin peripheral smear from a patient. Arrows indicate large platelets. (Original magnification,  $\times 60$ ; hematoxylin and eosin stain). (C) Transmission electron microscopy images of platelets isolated from control and indicated patients. Likely dilated open canalicular membrane system that engulfed some debris is shown by the arrows. Bar shown indicates image size. (D) Comparison of percentage of platelets (by FSC/SSC and expression of CD42) that are CD42b low expressing in patients vs heterozygous carriers and unaffected controls.  $n = 5$  patients, 4 heterozygous family members, and 4 unrelated controls.  $**P = .002$  vs control. (E) Fluorescence-activated cell sorter analysis of platelet sialylation levels demonstrating loss of sialylation in platelets from patients with GNE variants.  $***P < .001$  vs control for MAL. (F) Fluorescence-activated cell sorter analysis of total platelet population on F1 patients (P1 through P5), an obligate F1 carrier (father of P2-P4), and 3 nonaffected controls showing staining for thiazole orange. For panels D and F, graphs show mean  $\pm 1$  standard deviation.  $***P < .02$  comparing affected patients with control patients by Student t test. MAL, maackai amurensis lectin II; SNA/EBL, sambucus nigra lectin; WT, wild type.

presence of large platelets (Figure 1C). By flow cytometry, patient platelets were large and much more heterogeneous by forward and side scatter than control platelets. Costaining platelets for CD42a (GPIX) and its relative level to CD42b (GPIb) allow an estimate of degree of cleavage of the extracellular glycosialic acid domain of GPIb $\alpha$ .<sup>4</sup> Control platelets showed little to

no cleavage, whereas F1 and F3 patient platelets demonstrated a population of platelets with low CD42b expression (Figure 1D;  $P < .002$ ), suggesting loss of the glycosialic acid domain. In addition, patient platelets demonstrated decreased levels of sialic acid on platelets compared with similarly prepared control platelets (Figure 1E;  $P = .003$  compared with control). Finally,

**Table 1. Clinical and laboratory characteristics of patients in the 3 families**

	F1	F2	F3
Number of patients	3 F, 2 M (P1-P5);	2 F, 1 M (P6-P8);	1 M (P9);
Age, y	24-42	6-14	11
Bleeding symptoms	Easy bruising, epistaxis, menorrhagia, hemorrhagic corpus luteum	Easy bruising, menorrhagia	Easy bruising, epistaxis
Platelet count (N 150-450 × 10 <sup>9</sup> /L)	1-4 × 10 <sup>9</sup> /L	3-10 × 10 <sup>9</sup> /L	30-40 × 10 <sup>9</sup> /L
MPV	NA	NA	18.9-19.4 fL
Hemoglobin (N 12-16 g/dL)	10.7-14.4 g/dL	9.37 g/dL*	Normal
Blood smear	Macrothrombocytopenia; no pale platelets	Macrothrombocytopenia; no pale platelets	Macrothrombocytopenia; no pale platelets
Bone marrow	Not done	Mild increase in megakaryocytes with hypolobulated forms and focal mild increase reticulin fibers*	Not done
RBC transfusions	Yes	Yes	No
Platelet transfusions	Yes	Yes	Yes
Myopathy	No	No	Yes

P1 through P9 refers to patient designation in Figure 1.

F, female; M, male; NA, not available; RBC, red blood cell.

\*Available only for P7. Iron deficiency anemia was noted only in female patients secondary to menorrhagia.

increased levels of reticulated platelets were found in patients P1 through P5 compared with healthy controls, suggesting increased platelet turnover (Figure 1G). For patient P9, immature platelet fraction was markedly increased compared with healthy controls (55.2% vs reference interval: 1.6% to 4.7%), also consistent with increased platelet turnover. Based on the role of *GNE* in sialic acid formation and that removal of small amounts of sialic acid residues (8% to 10%) of neuraminidase results in increased platelet clearance,<sup>14</sup> we hypothesize that the thrombocytopenia is caused by increased platelet clearance. Although *GNE* is ubiquitously expressed in all hematologic cells, only platelets are dependent on levels of sialylation. Similarly, in the F2 family, bone marrow aspiration showed increased number of megakaryocytes (Table 1), compatible with increased platelet clearance.<sup>15</sup>

Immunofluorescence studies of platelets from P5 compared with an unaffected individual's platelets showed enlarged platelets with normal distribution of surface receptors, cytoskeletal proteins, as well as dense granule, lysosome, and  $\alpha$ -granule markers (supplemental Figure 3). The normal distribution of surface receptors by immunofluorescence but decreased expression by flow cytometry is not surprising given that flow cytometry is able to give more information about relative expression levels. The bleeding phenotype in our patients is out of proportion to the degree of thrombocytopenia, suggesting a defect in platelet activation. Therefore, we performed protein analysis of platelet extracts, which demonstrated differential bands in affected individuals by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and mass spectrometry–confirmed downregulation of platelet activation, adhesion, and wound-healing pathways (supplemental Table 1).

There are 2 large registries of *GNE* myopathy, a Japanese Nationwide patient registry with 121 patients, reporting 3 patients with thrombocytopenia (2.5%), and the Israel Nationwide *GNE* registry with >200 individuals and no associated thrombocytopenia (Argov Avisohar, Hadassah-Hebrew University Medical Center, e-mail, 2017). In the literature, alterations in platelet count were previously reported in patients with myopathy as an incidental finding, but not as the primary condition.<sup>15,16</sup> Isolated thrombocytopenia without associated myopathy due to *GNE* variants has not been previously reported. Our communication with the UK GAPP investigators identified simultaneously a family of Pakistani origin with homozygous *GNE* variant.<sup>17</sup> We describe 3 additional kindreds with isolated thrombocytopenia and, in the F1 and F2 families reported here, >20 years of follow-up with affected patients with macrothrombocytopenia (up to age 24-42 years) without myopathy. The 1 patient with neurologic findings has a muscle biopsy inconsistent with *GNE* myopathy (supplemental Figure 1). Thus, we believe that mutations in *GNE* should be added to the list of diseases associated with isolated macrothrombocytopenia with a bleeding diathesis. It is not clear why some patients have isolated thrombocytopenia, whereas others have myopathy, and further studies are needed to better understand why variants in *GNE* are associated with 3 distinct clinical phenotypes: myopathy, sialuria,<sup>18</sup> and now isolated thrombocytopenia.

In summary, we have defined 3 families with autosomal recessive inheritance of macrothrombocytopenia and mild to life-threatening bleeding histories due to defects in *GNE*. Notably,

these families did not have concurrent muscle wasting phenotypes. The patients appear to have rapid clearance of their platelets associated with loss of their surface GPIb/IX receptors and changes in surface sialylation. Our finding suggests a strong link between sialylation, alteration of surface GPIb/IX expression with reduced GPIX:GPIb expression, increased platelet size, and platelet clearance. Further characterization of the pathways leading to abnormal platelets and/or abnormal muscle biology needs to be defined, and genetic determinants of the observed phenotypic heterogeneity needs to be better understood.

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This study makes use of data generated by the NIHR BioResource. A full list of investigators who contributed to the generation of the data is available at <https://bioresource.nihr.ac.uk/researchers/researchers/acknowledgement/>.

## Authorship

Contribution: S.R.-V., E.S., M.P., D.V., and M.P.L. were involved in study design, interpretation of results, and preparation of the manuscript; E.T. initially identified *GNE* as the leading candidate gene in F1 and F3; S.R.-V., E.S., D.V., N.J., E.H.-A., G.S., H.D., and Y.K. provided clinical, laboratory, and genetic data of F1; D.J. performed sialylation flow cytometry and interpreted results; K.A. and A.G. provided the immunofluorescence analysis and assisted with interpretation of results; C.K. and S.I. provided clinical, laboratory, and genetic data of F2; M.P.L. provided clinical, laboratory, and genetic data of F3; E.T., K.D., S.V.V.D., R.M., and W.H.O. are members of the NIHR BioResource, who provided clinical and genetic data and played key roles in with interpretation of results. All authors reviewed the manuscript and provided input in manuscript preparation and approved the final draft for submission. The NIHR BioResource–Rare Disease Study is a multicenter whole-genome sequencing study of ~13 000 patients.

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## Footnote

The online version of this article contains a data supplement.

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