Supplemental Methods:

Genetic Analysis

Genomic DNA was extracted from blood samples of affected children, parents and siblings. In F1, single nucleotide polymorphism (SNP) analysis was performed on DNA samples from the two most distant patients in the pedigree (P1,P5) using Affymetrix® CytoScan HD Array, according to the manufacturer's instructions. This platform harbors 750,000 markers and includes 200,000 gene-centric SNPs to enable 5 Mb ROH determination and regions identical-by-descent of > 3Mb (Affymetrix, Santa Clara, CA, USA). Exonic sequences were enriched in the DNA sample of P1 using Agilent SureSelect Target Enrichment Kit V.4 (Agilent Technologies). 140.7M paired-end sequences reads were generated by HiSeq2000 (Illumina). Reads alignment and variant calling were performed with DNAnexus Software (DNAnexus) using the default parameters against the human genome assembly Hg19 (GRCh37). Search for deleterious homozygous mutations was confined to the identical runs of homozygosity (ROH) obtained using the SNP analysis described above. Whole exome sequencing (WES) of P1 yielded a mean coverage of 149x. Following alignment and variant calling, we performed a series of variant filtering steps. These included removing variants which were called at sites with <7x coverage, were off-target, synonymous, heterozygous, predicted benign by MutationTaster or had minor allele frequency (MAF) <0.1% in dbSNP138 or a MAF <1% in the Hadassah in-house database. In F2, DNA samples from P7, were tested on the ThromboGenomics high-throughput sequencing (HTS) platform, which has been described previously (although this patient was analyzed using TG2 (the second generation panel with updated list of genes). For F3, whole-genome sequencing (WGS) on P9 was performed by Illumina Inc using a TruSeq DNA PCR-free protocol such that at least 95% of the reference was covered at >15x.

EM and Flow cytometry and Immunofluorescence analysis

EDTA-anticoagulated blood was used to count platelets and prepare blood smears according to each hospital laboratory's standard operating procedures. Electron microscopy of F1 and F3, isolated platelets were fixed as previously described and given in more detail in supplemental methods.[12] Flow cytometry was performed using monoclonal mouse anti-human fluorescein isothiocyanate, anti-CD41, phycoerythrin anti-CD42b, and pacific blue anti-CD42a antibodies (all from Beckman Coulter), each at 0.25 µg/20 µl of platelet rich plasma. Platelet sialylation was assessed using biotinylated maackai Amurensis Lectin II (MALII) and Sambucus Nigra Lectin (SNA) (both vector laboratories) with secondary APC-strepavidin (Beckman Coulter). Blood was fixed immediately with 300 µl cytofix (BD Biosciences) and samples were kept at 4˚C until analysis on an LSR Fortessa (BD Biosciences) and analyzed using FlowJo (FlowJo, LLC). Reticulated platelets were measured using the thiazole orange (TO; ReticCount, Becton Dickinson) at a final concentration of 1 µg/ml and analyzed on a FACS-calibur^{R} (Becton-Dickinson) within one hour of blood collection. Detailed description of immunofluorescence and intracellular protein analysis is given in supplemental methods.

For electron microscopy studies, EDTA collected platelets were resuspended in 2% paraformaldehyde, 0.2% glutaraldehyde in 0.1 mol/l phosphate buffer and were then infiltrated with epoxy resin and polymerized in the oven at 60°C for 24 hours. The blocks were sectioned using an ultramicrotome (LKB III), and 80 nm sections were placed on copper grids, and stained with uranyl acetate and lead citrate as described previously. Grids were micrographed with a JEM-1400 plus Transmission Electron Microscope (Jeol, Ltd). Blood smears of patients P1 and P5 generated from EDTA-anticoagulated blood were stained for platelet membrane, cytoskeleton, and alpha granule proteins as described.12 Immunofluorescence analysis of platelet membrane receptors (GPIbIX and GPIIbIIIa), alpha-granule markers (von Willebrand factor (VWF), thrombospondin, and P selectin), lysosome marker (LAMP1), dense-granule markers (LAMP2 and CD63), and platelet cytoskeleton markers (α and β-tubulin and non-muscular myosin IIa) were performed as described previously.

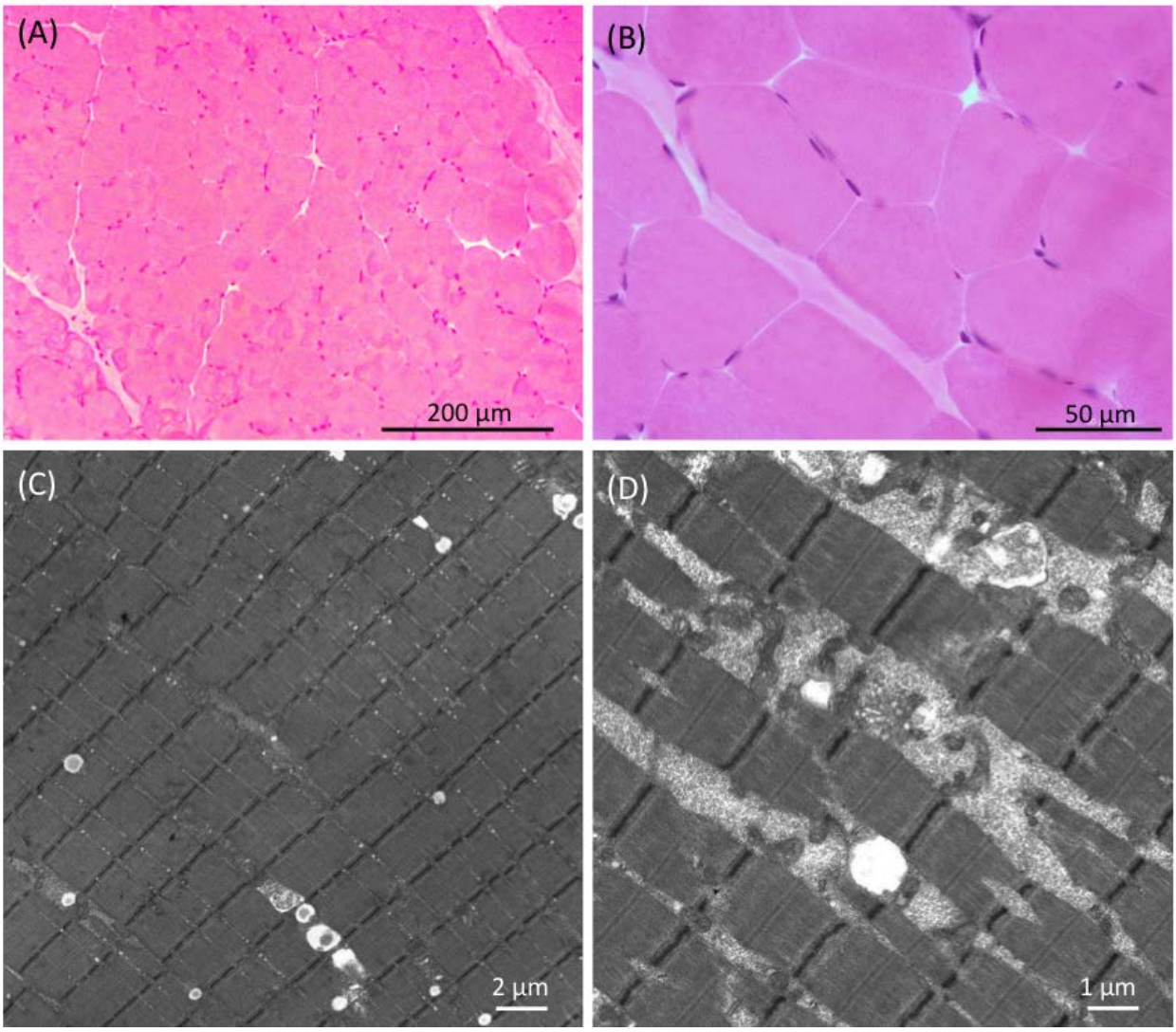
Protein analysis

Platelets mass spectrometry analysis was done for patients P1 through P5, all in F1, an obligate carrier in F1, and two unrelated healthy volunteers. Platelets were isolated from citrated blood, washed by re-suspension in a citrate wash buffer (11 mM glucose, 128 mM NaCl, 4.3 mM NaH2PO4, 7.5 mM Na2HPO4, 4.8 mM sodium citrate, 2.4 mM citric acid, pH 6.5) and pelleted by centrifugation at 1200 g for 10 min at room temperature, then digested with modified trypsin (Promega) at a 1:100 enzyme-to-substrate ratio, for 1 hour at 37°C and analyzed by liquid chromatography–mass spectrometry on an Q Exactive plus (Thermo). The data were analyzed with MaxQuant 1.5.2.8 with respect to the Human Uniprot Database. The peptide intensities were quantified by label-free analysis using the same software. The identifications were filtered for proteins identified with a false discovery rate <0.01 in at least 2 repeats in one group and at least 2 identified peptides in the project. Assignment of the proteins to signaling pathways was based on information available in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www. genome.jp/kegg/pathway.html).

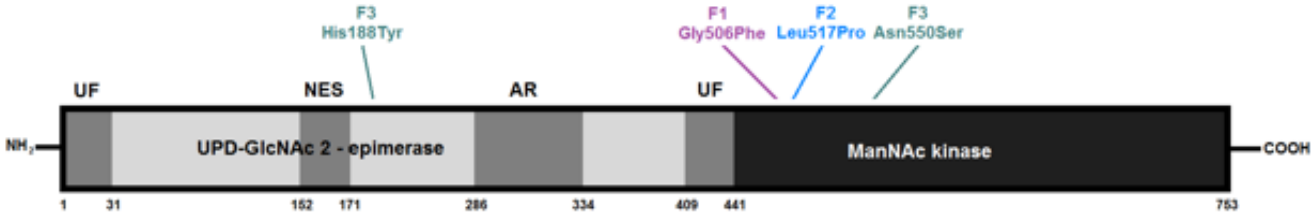
Supplemental Table 1: Mass spectroscopy analysis of platelet pathways in patients.

	Pathway	**Matching proteins**
Upregulated		
	Ribosome	RPL11, RPL12, RPL18A, RPL23A, RPL30, RPL4, RPLP0, RPLP1, RPLP2, RPS10, RPS12, RPS21, RPS24, RPS26, RPS3A, RPS4X, RPS7
	Spliceosome	DDX39B, HNRNPA3, HNRNPK, HSPA1A, RBM25, SF3B1, SRSF1, SRSF3, SRSF7
	Pentose phosphate pathway	GPI, PGD, RPIA, TKT
	Phagosome	ATP6V1G1, ATP6V1H, CD14, CORO1A, CTSS, DYNC1I2, NCF1
	Regulation of actin cytoskeleton	ARHGEF1, CD14, EZR, IQGAP1, PAK2, PXN, RAC2, RDX
Downregulated		
	Platelet activation	ADCY5, ADCY6, AKT2, BTK, FCER1G, FERMT3, GNAQ, GP1BA, GP1BB, GP5, GP6, GP9, ITGA2, ITGA2B, LYN, MYLK, P2RX1, PRKACB
	Focal adhesion	ACTN1, AKT2, FLNA, ILK, ITGA2, ITGA2B, ITGA6, MYLK, PARVB, RAC1, RAC3, RAP1B, ROCK2, SRC, THBS1, ZYX
	ECM-receptor interaction	CD36, GP1BA, GP1BB, GP5, GP6, GP9, ITGA2,ITGA2B,ITGA6,THBS1
	Rap1 signaling pathway	ADCY5,ADCY6,AKT2,FYB,GNAQ,ITGA2B,RAC1,RAC3,RALB,RAP1B,RASGRP2,SRC,THBS1
	Chemokine signaling pathway	ADCY5,ADCY6,AKT2,LYN,PF4,PPBP,PRKACB,RAC1,RAP1B,RASGRP2, ROCK2,SRC

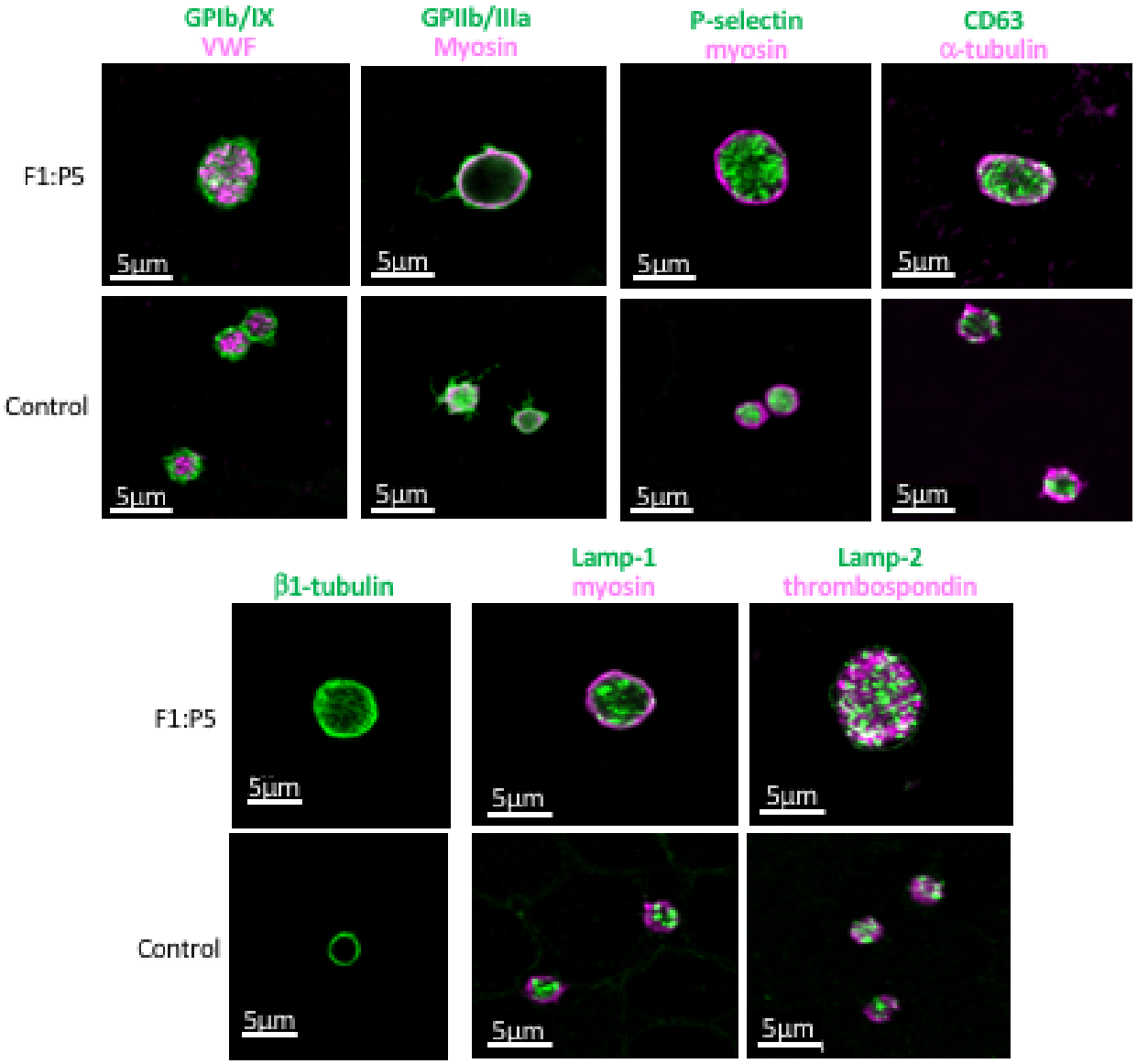
Supplemental Figures:



Supplemental Figure 1: Muscle biopsy of F3:P9. (A) & (B) Hemotoxylin and eosin staining of muscle biopsy from gastrocnemius muscle demonstrating mild, non-specific myopathic change with enlarged muscle bodies and inflammatory changes. (C) & (D) Electron microscopy demonstrating absence of tubulofilamentous inclusion bodies reported in GNE myopathy. Images represent characteristic images. Bars represented denoted size.



Supplemental Figure 2: *GNE* variants in F1 through F3.
Diagram of the functional domains of GNE based on [35] with notation of the location of the variants described. F1 affected individuals were homozygous for a G506F substitution in *GNE* variant in exon 9. F2 affected individuals were homozygous for a *GNE* variant in exon 9, resulting in a L517P substitution. The F3 individual P9 was compound heterozygous for a H188Y substitution in *GNE* exon 4 and a N550S substitution in exon 11. UF = unknown function domain; NES = putative nuclear export signal. AR = experimental allosteric region. Modified from Celeste FV et al 2014 Hum Mutat.



Supplemental Figure 3. Immunofluorescence staining of affected platelets (F1:P5). Representative resting platelet immunofluorescence studies from an affected patient (top in each row) and unaffected control (bottom in each row) are shown for a series of surface receptors (GPIb/IX, stained with an anti-GPIX antibody; GPIIb/IIIa, stained with a complex specific antibody), cytoskeletal proteins (myosin, and α- and β1-tubulin), granule proteins (P-selectin and thrombospondin, CD36, and (lamp-1 and -2). Size bars are included.