GNE-related thrombocytopenia: evidence for a mutational hotspot in the ADP/substrate domain of the GNE bifunctional enzyme

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Table 1S. List of IT-related genes and relative coverage from whole exome sequencing (WES).

Gene	Reference	P1					P2				
		Gene length (bp)	Coverage*	1X** (%)	5X*** (%)	Missing bases#	Gene length (bp)	Coverage*	1X** (%)	5X*** (%)	Missing bases#
ABCG5	Patel et al, 2018	2268	127	100.0	100.0	0	2268	170	100.0	100.0	0
ABCG8	Patel et al, 2018	2334	130	100.0	100.0	0	2334	205	100.0	100.0	0
ACTB	Pecci et al, 2020	1248	125	100.0	100.0	0	1248	180	100.0	100.0	0
ACTN1	Pecci et al, 2020	3273	83	100.0	100.0	0	3273	82	100.0	99.2	26
ADAMTS13	Galbusera et al, 2009	4980	119	96.7	96.7	164	4980	125	98.0	95.1	244
ANKRD26	Pecci et al, 2020	5949	106	100.0	100.0	0	5949	125	100.0	100.0	0
ANO6	Boisseau et al, 2018	3628	116	100.0	100.0	0	3628	157	100.0	100.0	0
ARPC1B	Pecci et al, 2020	1335	89	100.0	100.0	0	1335	62	99.0	95.5	60
CDC42	Pleines et al, 2010	810	93	100.0	100.0	0	810	96	100.0	100.0	0
CYCS	Pecci et al, 2020	366	148	100.0	100.0	0	366	161	100.0	100.0	0
DIAPH1	Pecci et al, 2020	4568	119	100.0	100.0	0	4568	191	99.8	96.6	155
EPHB2	Berrou et al, 2018	3585	103	97.6	97.6	86	3585	127	97.6	97.6	86
ETV6	Pecci et al, 2020	1551	90	100.0	100.0	0	1551	132	100.0	100.0	0
F2RL3	Tourdot et al, 2018	1206	67	100.0	100.0	0	1206	44	99.2	91.3	105
FLI1	Pecci et al, 2020	1631	108	100.0	100.0	0	1631	141	99.2	98.7	21
FLNA	Pecci et al, 2020	9072	59	100.0	100.0	0	9072	55	100.0	99.3	64
FYB1	Pecci et al, 2020	3003	91	99.1	99.1	27	3003	154	100.0	98.0	60
GALE	Pecci et al, 2020	1287	129	100.0	100.0	0	1287	203	100.0	100.0	0
GATA1	Pecci et al, 2020	1362	54	100.0	100.0	0	1362	68	100.0	96.4	49
GFI1B	Pecci et al, 2020	1203	129	100.0	100.0	0	1203	150	100.0	100.0	0
GNE	Pecci et al, 2020	2648	127	100.0	100.0	0	2648	171	100.0	100.0	0
GP1BA	Pecci et al, 2020	1983	100	100.0	99.6	0	1983	154	99.8	98.8	24
GP1BB	Pecci et al, 2020	669	14	69.5	56.0	204	669	14	57.8	39.1	407
GP9	Pecci et al, 2020	558	77	100.0	100.0	0	558	92	100.0	95.8	23
HOXA11	Pecci et al, 2020	990	85	100.0	97.4	0	990	94	96.2	89.6	103
IKZF5	Pecci et al, 2020	1332	89	100.0	100.0	0	1332	140	100.0	100.0	0
ITGA2B	Pecci et al, 2020	3840	114	100.0	100.0	0	3840	156	100.0	100.0	0
ITGB3	Pecci et al, 2020	2727	120	98.0	97.9	55	2727	181	98.4	98.1	52
KAT6A	Wiesel-Motiuk et al, 2020	6411	86	100.0	100.0	0	6411	127	99.7	98.0	128
KDSR	Pecci et al, 2020	1239	77	99.4	97.3	7	1239	87	100.0	93.0	87
MECOM	Pecci et al, 2020	4158	89	100.0	100.0	0	4158	128	100.0	100.0	0
MPIG6B	Pecci et al, 2020	918	63	100.0	100.0	0	918	68	99.4	94.7	49

MPL	Pecci et al, 2020	2196	124	100.0	100.0	0	2196	186	100.0	100.0	0
MYH9	Pecci et al, 2020	6843	120	100.0	100.0	0	6843	156	100.0	100.0	0
NBEA	Nuytens et al, 2013	10233	88	100.0	98.9	0	10233	106	98.9	98.8	123
NBEAL2	Pecci et al, 2020	9615	73	100.0	99.9	0	9615	77	99.8	96.2	365
P2RY12	Cattaneo, 2011	1053	96	100.0	100.0	0	1053	155	100.0	100.0	0
PLA2G4A	Adler et al, 2009	2658	128	100.0	100.0	0	2658	189	100.0	100.0	0
PLAU	Nurden et al, 2011	1594	88	100.0	100.0	0	1594	96	100.0	100.0	0
PRKACG	Pecci et al, 2020	1080	98	100.0	100.0	0	1080	141	100.0	100.0	0
PTGS1	Palma Barqueros et al, 2019	2107	94	100.0	100.0	0	2107	110	100.0	98.9	23
PTPRJ	Pecci et al, 2020	4643	90	99.9	97.9	5	4643	112	97.4	97.4	121
RASGRP2	Lozano et al, 2016	2190	146	100.0	100.0	0	2190	121	100.0	100.0	0
RBM8A	Pecci et al, 2020	669	113	100.0	100.0	0	669	128	100.0	100.0	0
RUNX1	Pecci et al, 2020	1704	82	100.0	100.0	0	1704	96	98.9	97.5	43
SLC35A1	Kauskot et al, 2018	1206	132	100.0	100.0	0	1206	162	100.0	96.6	41
SLFN14	Pecci et al, 2020	2835	93	100.0	100.0	0	2835	139	100.0	100.0	0
SRC	Pecci et al, 2020	1875	74	100.0	95.5	0	1875	55	99.3	95.0	94
STIM1	Pecci et al, 2020	2725	108	100.0	100.0	0	2725	164	100.0	100.0	0
TBXA2R	Mundell et al, 2018	1345	84	100.0	100.0	0	1345	91	99.9	97.3	36
THPO	Pecci et al, 2020	1626	82	92.4	81.6	124	1626	116	90.2	81.6	299
TPM4	Pecci et al, 2020	1571	65	89.3	84.5	168	1571	80	92.9	78.4	339
TRPM7	Pecci et al, 2020	6534	83	100.0	100.0	0	6534	91	100.0	100.0	0
TUBB1	Pecci et al, 2020	1452	127	100.0	100.0	0	1452	166	100.0	100.0	0
VWF	Sukumar et al, 2021	9666	126	100.0	99.4	0	9666	179	99.5	99.2	77
WAS	Pecci et al, 2020	1797	54	100.0	100.0	0	1797	74	99.7	91.8	147

*Coverage refers to the average number of times a nucleotide is read during sequencing.
**Percentage of gene bases sequenced at least once time (1X coverage).
***Percentage of gene bases sequenced at least five times (5X coverage).
#Number of gene bases not covered by the WES design.

Table 2S. List of homozygous variants found in ROH.

Gene		**GNE (NM_001128227)**		**PRRC2C (NM_015172)**		**PIK3R1 (NM_181523)**	**PRKDC (NM_001081640)**
Variant*		c.1724C>G	c.1546_1547delinsAG	c.328G>T	c.5776C>G	c.1118+11G>A	c.8599G>A
		p.Thr575Arg	p.Val516Arg	p.Ala110Ser	p.Pro1926Ala	NA	p.Ala2867Thr
Databases of Single Nucleotide Variations	gnomAD**	NA	NA	0.004216	0.004518	0.002331	NA
	dbSNP***	NA	rs1429946073 (c.1546G>A; p.Val516Met)	rs148272649	rs141768518	rs140892282	NA
			rs1168765605 (c.1547T>G; p.Val516Gly)				
Databases of diseases-related variants	HGMD†	NA	NA	NA	NA	NA	NA
	ClinVar††	NA	NA	NA	NA	NA	Uncertain significance
Pathogenicity prediction tools	SIFT†††	D	D	T	D	NA	NA
	PolyPhen-2§	P	P	B	B	NA	B
	CADD§§	32	NA	18.19	10.33	NA	18.98
Splicing prediction tool	NetGene2§§§	No effect	No effect	No effect	No effect	No effect	No effect

*Nucleotide A of the ATG translation initiation start site of cDNA of the respective gene accession numbers is indicated as nucleotide +1.
**Genome Aggregation Database (gnomAD) at https://gnomad.broadinstitute.org/
***Database of Single Nucleotide Polymorphism (dbSNP) at https://www.ncbi.nlm.nih.gov/snp/
†Human Gene Mutation Database (HGMD) at https://apps.ingenuity.com/
††Clinical Variation (ClinVar) database at https://www.ncbi.nlm.nih.gov/clinvar/
†††Sorting Intolerant From Tolerant (SIFT) tool at https://sift.bii.a-star.edu.sg/
§Polymorphism Phenotyping v2 (PolyPhen-2) tool at http://genetics.bwh.harvard.edu/pph2/
§§ Combined Annotation Dependent Depletion (CADD) tool at https://cadd.gs.washington.edu/
§§§ NetGene2 at http://www.cbs.dtu.dk/services/NetGene2/

Table 3S. Clinical e genetic features of patients affected by thrombocytopenia and GNE mutations

Family (Ethnicity)	GNE mutations	Patient/ Gender (Age at observation)	Thrombocytopenia features							Myopathy (Age at diagnosis)	References
			Age at diagnosis	Bleeding	Platelet count (Normal range 150–450× 10^9/L)	MPV* (Normal range 7-12 fL) (blood smear)	IPF** (Normal range 1.1-<7%)	Megakaryocyte	Treatment		
F1 (Egypt)	c.1546_1547delGTinsAG (**p.Val516Arg**)§	P1/M (1 month)	Neonatal	Petechiae, bruising	3-7	11.9	50-80	Expansion of MKs, several immature MKs	PLT transfusion, romiplostim, no response to HD-IgIV and steroids	Absent	This paper
F2 (Moroccan)	c.1724C>G (**p.Thr575Arg**)§	P2/M (3 years)	Neonatal	Petechiae, bruising	4-10	10.8	39-89	Expansion of MKs, several immature MKs	PLT transfusion, romiplostim, no response to HD-IgIV and steroids	Absent	This paper
F3 (Chinese)	c.1351C>T (p. Arg451*)# c.1330G>T (**p.Asp444Tyr**)	P3/F (22 days)	Neonatal	Mild self-limiting hematochezia	1	10.6–13.2	na	Immature MKs	PLT transfusion, no response to HD-IgIV	Absent	Li et al. 2020
		P4/F (22 days)	Neonatal	no	6	10.6–13.2	na	Immature MKs	PLT transfusion, no response to HD-IgIV	Absent	
F4 (Thai)	c.1732G>A **(p.Gly578Ser)**§ reported as c.1417G>A (p.Gly473Ser)	P5/F (24 years)	Childhood	Bruising, hematochezia, menorrhagia	17	na (large PLTs)	na	na	PLT transfusion, no response to HD-IgIV and steroids	Absent	Mekchay et al. 2020
F5 (Pakistani)	c.1339G>A (**p.Gly447Arg**)§ reported as c.1246G>A (p.Gly416Arg)	P6/M (3 years)	Neonatal	Neonatal bilateral intraventricular hemorrhages, cutaneous bleeding	10	15	87	normal	PLT transfusion	Absent	Johnson et al. 2016; Futterer et al. 2018
		P7/F (7 years)	7 years	Epistaxis, bruising	15.0-20.0	10.4	83	na	PLT transfusion	Absent	
F6 (Palestinian Arab)	c.1516_1517delinsTT (**p.Gly506Phe**)§	P8-12 2M-3F (24-42 years)	na	Epistaxis, bruising, menorrhagia, hemorrhagic corpus luteum	1.0-4.0	na (large PLTs)	53	na	PLT transfusion	All asymptomatic Signs of myopathy on muscle MRI in P8 and P11	Revel-Vilk et al. 2018
F7 (Palestinian Arab)	c.1550T>C **(p.Leu517Pro)**§	P13-P15 1M-2F (6-14 years)	na	Bruising, menorrhagia	3.0-10.0	na (large PLTs)	na	Mild expansion of MKs, immature MKs, focal mild increase in reticulin fibers	PLT transfusion	Absent	Revel-Vilk et al. 2018
F8 (Caucasian)	c.562C>T **p.His188Tyr)**§ c.1649A>G (p.Asn550Ser)§#	P16/M (11 years)	na	Epistaxis, easy bruising	30.0-40.0	18.9-19.4 (large PLTs)	55.25	na	PLT transfusion	Clinical signs of myopathy biopsy not consistent with GNE myopathy	Revel-Vilk et al. 2018
F9 (West India)	c.1768G>A (p.Gly590Arg)^#	P17/F (23 years)	4 years	Epistaxis, spontaneous hematomas, menorrhagias inducing anemia, 3 life-threatening bleedings	5	na (90% giant)	na	Clusters of nomal MKs	PLT transfusion	Absent	Manchev et al. 2014
		P18/M (Sibling)	2 years	Epistaxis, cutaneous hematomas	8	na (90% giant)	na	Clusters of nomal MKs	na	Absent	
F10 (Chinese)	c.649T>C (p.Tyr217His)# c.1636_1637delGA (p.Asp546Glnfs*2)#	P19/F (29 years)	29 years	no	36	na	na	Expansion of MKs	na	Present (29 years)	Zhen et al. 2014
		P20/M (26 years)	26 years	no	45	na	na	Expansion of MKs	na	Present (26 years)	
F11 (Japanese)	c.1807G>C (p.Val603Leu)#; c.2215G>A (p.Gly739Ser)#	P21/M (32 years)	Neonatal	no	1.7-16.2	10.0-11.0	na	Expansion of Mks	na	Present (adolescence)	Izumi et al. 2014
		P22/F (29 years)	2 years	Epistaxis	1.1-9.0	10.0-13.0	na	Normal	na	Present (18 years)	

Nucleotide A of the ATG translation initiation start site of the GNE cDNA in GenBank sequence NM_001128227.2 is indicated as nucleotide +1. Some mutations are also reported using NM_005476.6.

§Homozygous variant.

^The p.G590R variant was not regarded as a thrombocytopenia-causing mutation because the affected individuals did not presented myopathy and another, in linkage disequilibrium, mutation (c.222C>G; p.I74M) of the PRKACG gene was considered a better disease-causing mutation.[14]

#Mutation identified in patients with myopathy.

*Mean Platelet Volume.

**Immature Platelet Fraction