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RECOMMENDATIONS AND GUIDELINES

Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH

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1 | INTRODUCTION

Inherited bleeding, thrombotic, and platelet disorders (BTPD) are a heterogeneous set of diseases. The most common inherited bleeding disorders are von Willebrand disease (VWD) and hemophilia, although all other BTPDs are globally very rare, with mostly an unknown prevalence. Over the past five decades, the genetic basis of some of these disorders has been identified. Most of the genes harboring variants responsible for BTPD have been identified through linkage studies across informative pedigrees or using candidate gene Sanger sequencing following thorough clinical and laboratory workup.[1] However, over the past decade, high-throughput sequencing has become the primary means of identifying disease-causing genetic variants.[2] Different diagnostic gene panel tests for BTPD have been developed using targeted or exome sequencing.[1,3–9] Interestingly, when comparing the gene content of these different genetic panel tests, significant differences were observed. A first level of difference was created by the choice of genes tested for BTPD. These included established genes, known for decades to play a role in many families with BTPD (e.g., *F8*, *F9*, *VWF*, *PROS1*, *PROC*, *ITGA2B*, *ITGB3*,

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amongst many others), genes with limited evidence from publications of single pedigrees, and, finally, genes identified through functional studies and/or knockout mice but without a known defined role in human pathology. The first group of genes are the diagnostic-grade (hereafter named TIER1) genes, whereas the others are referred to as TIER2 and TIER3 genes, respectively. A second level of difference is that some panels contain genes that are related to bleeding but are not considered classical coagulation or platelet regulatory genes, such as those for hereditary hemorrhagic telangiectasia (*ENG*, *SMAD4*, *ACVRL11*), Ehlers Danlos syndrome (e.g., *COL1A1*, *COL3A1*, *COL5A1*, *COL5A2*, *CHST14*), Gaucher syndrome (*GBA*), or Noonan syndrome (e.g., *PTPN11*). These genes are TIER1 genes for disorders that are often associated with primary phenotypes such as arteriovenous malformations, joint hypermobility, skin hyperextensibility, tissue fragility, and complex syndromic features that result in an increased bleeding tendency. These primary symptoms are typically recognized by a clinical expert, but systematic screening of these genes in a patient population with unexplained bleeding symptoms has not been performed; therefore, inclusion of an "extended" TIER1 BTPD gene list can be considered.

Providing a molecular diagnosis to BTPD patients is highly desirable because it aids prognostication, may alter therapy, and provides important information for counseling. Making incorrect assumptions about variants could be harmful, however.[10] There are potential pitfalls when interpreting the role of genetic variants in genes related to BTPD. The published literature, disease (Online Mendelian Inheritance in Man [OMIM]; http://omim.org) and variant databases (ClinVar; https://www.ncbi.nlm.nih.gov/clinvar/ and the Human Gene Mutation Database) are incomplete and littered with misinformation about gene-disease associations and erroneous interpretation of the pathogenicity of variants. It is essential when assigning pathogenicity that rigorous standards are applied to variants in fully evidenced TIER1 genes. The first step in providing diagnostic-grade genetic reports is gene curation. Gene curation is intended to help physicians and clinical geneticists decide a gene's role in a disease and provide information on the mode of inheritance and mutational disease mechanism. The process of selecting TIER1 core genes for BTPD was taken up by the Scientific and Standardization Committee (SSC) for Genetics in Thrombosis and Haemostasis (GinTH). The current study explains the different aspects related to this curation process and presents an up-to-date TIER1 gene-disease list for BTPD, useful for clinical genetic testing, the design of gene panel tests, or for filtering whole exome or whole genome sequencing data.

2 | DESCRIPTION OF THE GENE AND TRANSCRIPT CURATION PROCESS

2.1 | Historical background and gene curation process

We have assembled a list of 91 TIER1 genes that are germline mutated (except one, *PIGA*) and are causally implicated in BTPD (Table 1; for the full version, see Table S1). The gene curation project was initiated by the SSC-GinTH in 2014 when 63 genes and transcripts were suggested by experts of the clinical and scientific community (Table S2) to be used for genetic testing of BTPDs. New genes and modes of inheritance have been curated and discussed at four subsequent SSC-GinTH meetings before designation to TIER1 status. These new genes required a status of strong evidence as specified later in more detail. Genes were grouped in three main categories: 21 genes mostly related to coagulation deficiencies implicated in bleeding, 9 genes known to be associated with thrombosis, and 61 genes involved in defects related to platelet function and their formation by blood stem cells. Four genes could have been assigned to multiple categories; *F2*, *F5*, and *THBD* to bleeding and thrombosis, and *VWF* to bleeding, but also VWD type 2B, which is considered a platelet disorder. Here the difference in clinical phenotype is caused by the variant type (inactivation vs activating) or location within the gene. This information is encoded in Table S1 as "Mutational mechanism for the disease." The predicted effect of a gene variant often indicates the impact of a disease; therefore, we have curated the categories of variants that occur in BTPD TIER1 genes that cause disease. Most BTPDs are caused by inactivating missense or loss-of-function (LoF) variants that are distributed throughout the gene, whereas others are exclusively caused by LoF variants (e.g., *PIGA*, *BLOC1S3*, *BLOC1S6*, *DTNBP1*, *FYB1*). In contrast, some BTPDs are the result of activating missense or LoF variants that mostly occur in specific protein domains (e.g., *THBD*, *DIAPH1*, *SRC*, *F5*, *F2*). Finally, noncoding variants have also been shown to cause BTPDs (e.g., 3′UTR variant in *F2*, 5′UTR variants in *ANKRD26*, variants in the noncoding gene *RNU4ATAC*). Genes with multiple disorders associated with different clinical or laboratory phenotypes (e.g., *GP1BA*, *GP1BB*, *ITGA2B*, *ITGB3*) have been represented as independent rows in Table S1, and multiple modes of inheritance (e.g., *VWF*, *FLI1*, *GFI1B*, *PROC*) are encoded within the "Inheritance" column.

To curate each gene-disease pair, three layers of evidence were collated that provide support for disease association, mode of inheritance and disease-causing "mutational mechanism." The first level of evidence was provided by reviewing the primary literature using PubMed searches, OMIM, and gene-specific databases (e.g., Medical College of Wisconsin-maintained database for Glanzmann thrombasthenia and European Association for Hemophilia and Allied Disorders-maintained databases for *F7*, *F8*, *F9*, and *VWF*) to evaluate the genetic confidence for a gene being disease-causing ("Level 1 evidence" in Table S1). For each gene-disease pair, genotype-phenotype cosegregation data, the mode of inheritance and the disease-causing mutation mechanism were reviewed in at least three independent families. For six genes (*AP3D1*, *BLOC1S3*, *FYB1*, *HOXA11*, *NBEA*, and *SRC*), only two unrelated families, whereas for *PLAU*, a single but very large pedigree with 28 affected patients and a significant linkage association signal (logarithm of odds score +11) for the *PLAU* locus, were reported.[11] The second layer of evidence was provided by knowledge from specific hemostasis, platelet, or molecular assays or phenotypes that support gene-disease associations ("Level 2 evidence" in Table S1). A third layer of evidence consisted of the existence of a mouse model affecting the ortholog of the human gene and presenting with the same phenotype as the associated human disease. This information was taken from the Mouse Genome Informatics (www.informatics.jax.org) database or a PubMed reference ("Level 3 evidence" in Table S1). Twenty of

TABLE 1 Curated gene-disease associations and transcripts for bleeding, thrombotic, and platelet disorders

Category	Gene symbol	Associated disorder(s)	Inheritance	Transcript	Location
Bleeding/coagulation	F10	Factor X deficiency	AR; AD	NM_000504.3	13q34
Bleeding/coagulation	F11	Factor XI deficiency	AR; AD	NM_000128.3	4q35.2
Coagulation *Angioedema*	F12	Factor XII deficiency *Angioedema*	AR (coagulation) *AD (angioedema)*	NM_000505.3	5q35.3
Bleeding/coagulation	F13A1	Factor XIII deficiency	AR	NM_000129.3	6p25.1
Bleeding/coagulation	F13B	Factor XIII deficiency	AR	NM_001994.2	1q31.3
Bleeding/coagulation *Thrombosis*	F2	Prothrombin deficiency *Thrombophilia resulting from thrombin defect*	AR (bleeding/coagulation) *AD (thrombosis)*	NM_000506.4	11p11.2
Bleeding/coagulation *Thrombosis*	F5	Factor V deficiency *Thrombophilia resulting from activated protein C resistance*	AR (bleeding/coagulation) *AD (thrombosis)*	NM_000130.4	1q24.2
Bleeding/coagulation	F7	Factor VII deficiency	AR; AD	NM_000131.4	13q34
Bleeding/coagulation	F8	Hemophilia A	XLR	NM_000132.3	Xq28
Bleeding/coagulation	F9	Hemophilia B	XLR	NM_000133.3	Xq27.1
Bleeding	FGA	Fibrinogen deficiency	AR (afibrinogenemia) AD (hypo/dysfibrinogenemia)	NM_000508.3	4q31.3
Bleeding	FGB	Fibrinogen deficiency	AR (afibrinogenemia) AD (hypo/dysfibrinogenemia)	NM_005141.4	4q31.3
Bleeding	FGG	Fibrinogen deficiency	AR (afibrinogenemia) AD (hypo/dysfibrinogenemia)	NM_021870.2	4q32.1
Bleeding/coagulation	GGCX	Vitamin K-dependent clotting factors deficiency 1	AR	NM_000821.6	2p11.2
Coagulation	KNG1	Kininogen deficiency	AR	NM_000893.4	3q27.3
Bleeding/coagulation	LMAN1	Combined factor V and VIII deficiency	AR	NM_005570.3	18q21.32
Bleeding/coagulation	MCFD2	Combined factor V and VIII deficiency	AR	NM_139279.5	2p21
Bleeding	SERPINE1	Plasminogen activator inhibitor 1 deficiency	AR; AD	NM_000602.4	7q22.1
Bleeding	SERPINF2	Alpha 2 antiplasmin deficiency	AR	NM_000934.3	17p13.3
Bleeding/coagulation	VKORC1	Vitamin K-dependent clotting factors deficiency 2	AR	NM_024006.5	16p11.2
Bleeding *Platelet*	VWF	VWD	AD (VWD type 1 and 2) AR (VWD type 3) *AD (VWD type 2B)*	NM_000552.3	12p13.31
Thrombosis	ADAMTS13	Thrombotic thrombocytopenic purpura	AR	NM_139025.4	9q34.2
Thrombosis	HRG	Histidine-rich glycoprotein deficiency	AD	NM_000412.4	3q27.3
Thrombosis	PIGA	Paroxysmal nocturnal hemoglobinuria	Acquired (somatic)	NM_002641.3	Xp22.2

(Continues)

TABLE 1 (Continued)

Category	Gene symbol	Associated disorder(s)	Inheritance	Transcript	Location
Thrombosis	PLG	Plasminogen deficiency	AR	NM_000301.3	3q27.3
Thrombosis	PROC	Protein C deficiency	AR; AD	NM_000312.3	Xp22.2
Thrombosis	PROS1	Protein S deficiency	AR; AD	NM_000313.3	3q27.3
Thrombosis	SERPINC1	Antithrombin deficiency	AR; AD	NM_000488.3	1q25.1
Thrombosis	SERPIND1	Heparin cofactor 2 deficiency	AD	NM_000185.3	22q11.21
Thrombosis *Bleeding*	THBD	Thrombomodulin deficiency; *Bleeding resulting from high soluble thrombomodulin*	AD	NM_000361.2	20p11.21
Platelet	ABCG5	Sitosterolemia with macrothrombocytopenia	AR	NM_022436.2	2p21
Platelet	ABCG8	Sitosterolemia with macrothrombocytopenia	AR	NM_022437.2	2p21
Platelet	ACTB	Baraitser-Winter syndrome 1 with macrothrombocytopenia	AD	NM_001101.3	7p22.1
Platelet	ACTN1	Macrothrombocytopenia	AD	NM_001130004.1	14q24.1
Platelet	ANKRD26	AD thrombocytopenia 2	AD	NM_014915.2	10p12.1
Platelet	ANO6	Scott syndrome	AR	NM_001025356.2	12q12
Platelet	AP3B1	HPS	AR	NM_003664.4	5q14.1
Platelet	AP3D1	HPS	AR	NM_001261826.3	19p13.3
Platelet	ARPC1B	Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease	AR	NM_005720.4	7q22.1
Platelet	BLOC1S3	HPS	AR	NM_212550.4	19q13.32
Platelet	BLOC1S6	HPS	AR	NM_012388.3	15q21.1
Platelet	CDC42	Takenouchi-Kosaki syndrome with thrombocytopenia	AD	NM_001791.4	1p36.12
Platelet	CYCS	AD thrombocytopenia 4	AD	NM_018947.5	7p15.3
Platelet	DIAPH1	Macrothrombocytopenia and sensorineural hearing loss	AD	NM_001079812.2	5q31.3
Platelet	DTNBP1	HPS	AR	NM_032122.4	6p22.3
Platelet	ETV6	Thrombocytopenia and susceptibility to cancer	AD	NM_001987.4	12p13.2
Platelet	FERMT3	Leukocyte integrin adhesion deficiency, type 3	AR	NM_178443.2	11q13.1
Platelet	FLI1	Paris-Trousseau and Jacobson syndrome	AR; AD	NM_002017.4	11q24.3
Platelet	FLNA	Syndrome with macrothrombocytopenia	XLD; XLR	NM_001110556.2	Xq28
Platelet	FYB1	Thrombocytopenia 3	AR	NM_001465.6	5p13.1
Platelet	GATA1	X-linked thrombocytopenia with dyserythropoiesis	XR	NM_002049.3	Xp11.23

(Continues)

TABLE 1 (Continued)

Category	Gene symbol	Associated disorder(s)	Inheritance	Transcript	Location
Platelet	GFI1B	Platelet-type bleeding disorder 17	AD; AR	NM_004188.5	9q34.13
Platelet	GNE	Myopathy associated with Thrombocytopenia	AR	NM_005476.6	9p13.3
Platelet	GP1BA	BSS Mild macrothrombocytopenia Platelet-type VWD	AR (BSS) AD (mild macrothrombocytopenia) AD (platelet-type VWD)	NM_000173.5	17p13.2
Platelet	GP1BB	BSS Mild macroTP	AR (BSS) AD (mild macrothrombocytopenia)	NM_000407.4	22q11.21
Platelet	GP6	Bleeding diathesis resulting from glycoprotein VI deficiency	AR	NM_016363.5	19q13.42
Platelet	GP9	BSS	AR	NM_000174.4	3q21.3
Platelet	HOXA11	Amegakaryocytic thrombocytopenia with radioulnar synostosis	AD	NM_005523.5	7p15.2
Platelet	HPS1	HPS	AR	NM_000195.4	10q24.2
Platelet	HPS3	HPS	AR	NM_032383.4	3q24
Platelet	HPS4	HPS	AR	NM_022081.5	22q12.1
Platelet	HPS5	HPS	AR	NM_181507.1	11p15.1
Platelet	HPS6	HPS	AR	NM_024747.5	10q24.32
Platelet	ITGA2B	GT Platelet-type bleeding disorder 16	AR (GT) AD (bleeding disorder)	NM_000419.3	17q21.31
Platelet	ITGB3	GT Platelet-type bleeding disorder 16	AR (GT) AD (bleeding disorder)	NM_000212.2	17q21.32
Platelet	KDSR	Thrombocytopenia and erythrokeratoderma	AR	NM_002035.4	18q21.33
Platelet	LYST	Chediak-Higashi syndrome	AR	NM_000081.3	1q42.3
Platelet	MECOM	Amegakaryocytic thrombocytopenia with radioulnar synostosis 2	AD	NM_004991.3	3q26.2
Platelet	MPIG6B	Thrombocytopenia, anemia, and myelofibrosis	AR	NM_025260.3	6p21.33
Platelet	MPL	Congenital amegakaryocytic thrombocytopenia	AR	NM_005373.2	1p34.2
Platelet	MYH9	MYH9-related disorders	AD	NM_002473.5	22q12.3
Platelet	NBEA	Autism with platelet dense granule defect	AD	NM_015678.4	13q13.3
Platelet	NBEAL2	Gray platelet syndrome	AR	NM_015175.2	3p21.31
Platelet	P2RY12	ADP receptor defect	AR	NM_022788.4	3q25.1
Platelet	PLA2G4A	Deficiency of phospholipase A2, group IV A	AR	NM_024420.2	1q31.1
Platelet	PLAU	Quebec platelet disorder	AD	NM_002658.3	10q22.2
Platelet	RASGRP2	Platelet-type bleeding disorder 18	AR	NM_153819.1	11q13.1

(Continues)

TABLE 1 (Continued)

Category	Gene symbol	Associated disorder(s)	Inheritance	Transcript	Location
Platelet	RBM8A	Thrombocytopenia-absent radius syndrome	AR	NM_005105.4	1q21.1
Platelet	RNU4ATAC	Roifman syndrome	AR	NR_023343.1	2q14.2
Platelet	RUNX1	Familial platelet disorder with predisposition to AML	AD	NM_001754.4	21q22.12
Platelet	SLFN14	Platelet-type bleeding disorder 20	AD	NM_001129820.1	17q12
Platelet	SRC	Thrombocytopenia 6	AD	NM_198291.2	20q11.23
Platelet	STIM1	Stormorken syndrome (York platelet syndrome)	AD	NM_003156.3	11p15.4
Platelet	STXBP2	Familial hemophagocytic lymphohistiocytosis type 5	AR	NM_006949.2	19p13.2
Platelet	TBXA2R	Thromboxane A2 receptor defect	AR; AD (partial phenotype)	NM_001060.5	19p13.3
Platelet	TBXAS1	Ghosal syndrome	AR	NM_030984.3	7q34
Platelet	THPO	Thrombocytopenia progressing to trilineage bone marrow failure	AR	NM_000460.4	3q27.1
Platelet	TUBB1	Macrothrombocytopenia	AD	NM_030773.3	20q13.32
Platelet	VIPAS39	Arthrogryposis, renal dysfunction, and cholestasis 1	AR	NM_001193315.1	14q24.3
Platelet	VPS33B	Arthrogryposis, renal dysfunction, and cholestasis 2	AR	NM_018668.4	15q26.1
Platelet	WAS	Wiskott-Aldrich syndrome	XLR	NM_000377.2	Xp11.23

Note: For each gene is indicated the HGNC symbol, OMIM associated disorder(s), mode(s) of inheritance, LRG reference transcript, and cytogenetic location.
Categories in italics indicate a rarer occurrence for a specific gene.
Abbreviations: AD, autosomal dominant; AR, autosomal recessive; BSS, Bernard-Soulier syndrome; GT, Glanzmann thrombasthenia; HPS, Hermansky-Pudlak syndrome; VWD, von Willebrand Disease; XLD, X-linked dominant; XLR, X-linked recessive.

the genes had a mouse model that did not mimic the human disease, whereas for five genes, no model has been developed.

In summary, evidence-based curation resulted in a total of 91 genes that reached a TIER1 status (Table 1). These were gene-disease association identified in at least three genetically independent families with supportive genotype-phenotype cosegregation data or with robust support from functional studies and/or a mouse phenocopy matching the human disease where less than three families are known in combination with linkage analysis data for large pedigrees. The list is versioned and will be reassessed by the SSC-GinTH at the yearly International Society on Thrombosis and Haemostasis meeting.

2.2 | Transcript curation process

When reporting likely pathogenic and pathogenic variants, it is essential to report on a fixed, evidenced-based transcript. For each TIER1 gene, the curated transcript was selected, in collaboration with the Locus Reference Genomic project (LRG; http://www.lrg-sequence.org/),[12] based on recommendations by members of the SSC-GinTH community, previously reported causal variants in Human Gene Mutation Database and ClinVar, transcript and protein lengths, and considering RNA-sequencing expression data in blood cells, other relevant tissues, and cap analysis gene expression data for defining the most common transcription start site (Table 1 and Table S1). For some genes, more than one transcript was included in the LRG record. In general, these transcripts include additional and well-supported protein-coding exons not present in the transcript highlighted in the tables. The TIER1 BTPD gene and transcript list is accessible at https://www.isth.org/page/GinTh_GeneLists.

3 | CONCLUSION

Although specific guidelines for variant interpretation in TIER1 genes have been published by the American College of Medical Genetics and Genomics,[13] guidelines for assessing the association of a specific gene with a specific disease are still nascent. The Clinical Genome Resource, ClinGen, is coordinating expert analysis of gene-disease associations using a comprehensive and publicly available criteria using evidence including the number of reported patients with variants in the gene and supporting experimental data for all rare diseases.[14] A ClinGen clinical domain working group for thrombosis and hemostasis has been initiated (https://www.clinicalgenome.org/working-groups/clinical-domain/hemostasis-thrombosis-clinical-domain-working-group/) in 2017. Curating the links between genes and disease is a complex and demanding task. ClinGen gene curation efforts for different disease working groups (e.g., epilepsy, RASopathies) have applied detailed scoring system using association's strength classified as definitive, strong, moderate, limited, disputed, or no evidence to systematically evaluate gene-disease relationships.[15,16] Because of the urgent need in diagnostic genetic laboratories, the SSC-GinTH has already applied a simplified scoring system to specify the definitive gene-disease pairs relevant for BTPD. Our experience highlights the importance of careful literature curation and evaluation by experts in the field. Our scoring system is simple enough to be quickly implemented while updating the TIER1 gene database with the latest findings and it can specifically guide diagnostic laboratories. Further, we find that review of previous cases while updating clinical validity of gene-disease relationships can contribute to increased diagnostic rates resulting in improved patient care.

When implementing the BTPD gene list for diagnostics, good practice suggests that gene panels are applied, either through the testing of specific genes using targeted panels or through the application of virtual panels to whole genome and exome data, limiting the number of potentially pathogenic variants to those in genes relevant to a patient's condition, and reducing the possibility of identifying incidental pathogenic variants. Incidental findings associated with BTPD can include the identification of variants known to be associated with hemophilia in unaffected female carriers and variants associated with mild to moderate thrombocytopenia but also causing an increased risk of malignancy (RUNX1, ANKRD26, and ETV6). Concerns regarding these findings have recently been discussed and solutions include the necessity of discussing these risks with patients before consenting and performing a genetic test or that virtual subpanels of genes are created (with or without genes with risk for incidental findings) that would allow a patient to choose.[17,18] Future discussion must center on the consent process that must also consider the local laws of the country, the risks of discrimination, the policy of the genetic testing service, and the age of the individual being tested. Our main goal was to deliver a curated BTPD disease-causing gene list for use by diagnostic laboratories; however, as genetic testing becomes more common through biobanking studies and direct-to-consumer testing, this list may also be used in research studies and to aid appropriate feedback of genetic information to participants.

The rapid pace of gene discovery using whole exome sequencing or whole genome sequencing approaches also emphasizes a need for data sharing. Many recent putative discoveries were made for single small pedigrees, sometimes accompanied by limited functional studies and no mouse model; therefore, without further evidence, these genes are designated TIER2. These include macrothrombocytopenia resulting from a recessive missense variant in *PRKACG*,[19] macrothrombocytopenia from dominant loss-of-function variant in *TPM4*,[20] macrothrombocytopenia from a dominant missense variant in *TRPM7*,[21] a platelet function defect from recessive *EPHB2* variants,[22] and thrombocytopenia from a recessive *PTPRJ* LoF variants.[23] Such TIER2 genes are relevant for BPTD diagnostics but still require confirmation studies in independent pedigrees and therefore, the SSC-GinTH encourages the publication of such short confirmation reports.

In conclusion, recent curation efforts by membership of the SSC-GinTH now provide a well-curated and evidence-based catalog of TIER1 gene-disease associations that can be used for diagnostic genetic screening of BTPD patients.

CONFLICT OF INTERESTS

All authors reviewed the manuscript and have no conflict of interest. All authors have curated the gene and transcript list and participated in the writing of this manuscript.

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REFERENCES

1. Heremans J, Freson K. High-throughput sequencing for diagnosing platelet disorders: lessons learned from exploring the causes of bleeding disorders. Int J Lab Hematol. 2018;40:89–96.
2. Freson K, Turro E. High-throughput sequencing approaches for diagnosing hereditary bleeding and platelet disorders. J Thromb Haemost. 2017;15:1262–72.
3. Simeoni I, Stephens JC, Hu F, Deevi SV, Megy K, Bariana TK, et al. A high-throughput sequencing test for diagnosing inherited bleeding, thrombotic, and platelet disorders. Blood. 2016;127:2791–803.
4. Johnson B, Lowe GC, Futterer J, Lordkipanidzé M, MacDonald D, Simpson MA, et al.; UK GAPP Study Group. Whole exome sequencing identifies genetic variants in inherited thrombocytopenia with secondary qualitative function defects. Haematologica. 2016;101:1170–9.
5. Bastida JM, Lozano ML, Benito R, Janusz K, Palma-Barqueros V, Del Rey M, et al. Introducing high-throughput sequencing into mainstream genetic diagnosis practice in inherited platelet disorders. Haematologica. 2018;103:148–62.
6. Leinøe E, Zetterberg E, Kinalis S, Østrup O, Kampmann P, Norström E, et al. Application of whole-exome sequencing to direct the specific functional testing and diagnosis of rare inherited bleeding disorders in patients from the Öresund Region, Scandinavia. Br J Haematol. 2017;179:308–22.
7. Lee EJ, Dykas DJ, Leavitt AD, Camire RM, Ebberink E, García de Frutos P, et al. Whole- exome sequencing in evaluation of patients with venous thromboembolism. Blood Adv. 2017;1:1224–37.
8. Saes JL, Simons A, de Munnik SA, Nijziel MR, Blijlevens NMA, Jongmans MC, et al. Whole exome sequencing in the diagnostic workup of patients with a bleeding diathesis. Haemophilia. 2019;25:127–35.
9. Andres Oliver, König Eva-Maria, Althaus Karina, Bakchoul Tamam, Bugert Peter, Eber Stefan, et al. ; on behalf of the THROMKIDplus Study Group of the Society of Paediatric Oncology Haematology (Gesellschaft für Pädiatrische Onkologie und Hämatologie, GPOH) and the Society of Thrombosis Haemostasis Research (Gesellschaft für Thrombose- und Hämostaseforschung, GTH). Use of targeted high-throughput sequencing for genetic classification of patients with bleeding diathesis and suspected platelet disorder. TH Open. 2018;02:e445–54.
10. Lentaigne C, Freson K, Laffan MA, Turro E, Ouwehand WH; BRIDGE-BPD Consortium and the ThromboGenomics Consortium. Inherited platelet disorders: toward DNA-based diagnosis. Blood. 2016;127:2814–23.
11. Diamandis M, Paterson AD, Rommens JM, Veljkovic DK, Blavignac J, Bulman DE, et al. Quebec platelet disorder is linked to the urokinase plasminogen activator gene (PLAU) and increases expression of the linked allele in megakaryocytes. Blood. 2009;113:1543–6.
12. MacArthur JA, Morales J, Tully RE, Astashyn A, Gil L, Bruford EA, et al. Locus Reference Genomic: reference sequences for the reporting of clinically relevant sequence variants. Nucleic Acids Res. 2014;42:D873–8.
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
14. Strande NT, Riggs ER, Buchanan AH, Ceyhan-Birsoy O, DiStefano M, Dwight SS, et al. Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the clinical genome resource. Am J Hum Genet. 2017;100:895–906.
15. Helbig I, Riggs ER, Barry CA, Klein KM, Dyment D, Thaxton C, et al. The ClinGen Epilepsy Gene Curation Expert Panel-Bridging the divide between clinical domain knowledge and formal gene curation criteria. Hum Mutat. 2018;39:1476–84.
16. Grant AR, Cushman BJ, Cavé H, Dillon MW, Gelb BD, Gripp KW, et al. Assessing the gene-disease association of 19 genes with the RASopathies using the ClinGen gene curation framework. Hum Mutat. 2018;39:1485–93.
17. Greinacher A, Eekels JJM. Diagnosis of hereditary platelet disorders in the era of next-generation sequencing: "primum non nocere". J Thromb Haemost. 2019;17:551–4.
18. Greinacher A, Eekels JJM. Simplifying the diagnosis of inherited platelet disorders? The new tools do not make it any easier. Blood. 2019. https://doi.org/10.1182/blood-2019-01-852350. [Epub ahead of print]
19. Manchev VT, Hilpert M, Berrou E, Elaib Z, Aouba A, Boukour S, et al. A new form of macrothrombocytopenia induced by a germline mutation in the PRKACG gene. Blood. 2014;124:2554–63.
20. Pleines I, Woods J, Chappaz S, Kew V, Foad N, Ballester-Beltrán J, et al. Mutations in tropomyosin 4 underlie a rare form of human macrothrombocytopenia. J Clin Invest. 2017;127:814–29.
21. Stritt S, Nurden P, Favier R, Favier M, Ferioli S, Gotru SK, et al. Defects in TRPM7 channel function deregulate thrombopoiesis through altered cellular Mg(2+) homeostasis and cytoskeletal architecture. Nat Commun. 2016;7:11097.
22. Berrou E, Soukaseum C, Favier R, Adam F, Elaib Z, Kauskot A, et al. A mutation of the human EPHB2 gene leads to a major platelet functional defect. Blood. 2018;132:2067–77.
23. Marconi C, Di Buduo CA, LeVine K, Barozzi S, Faleschini M, Bozzi V, et al. A new form of inherited thrombocytopenia caused by loss-of-function mutations in PTPRJ. Blood. 2019;133:1346–57.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.