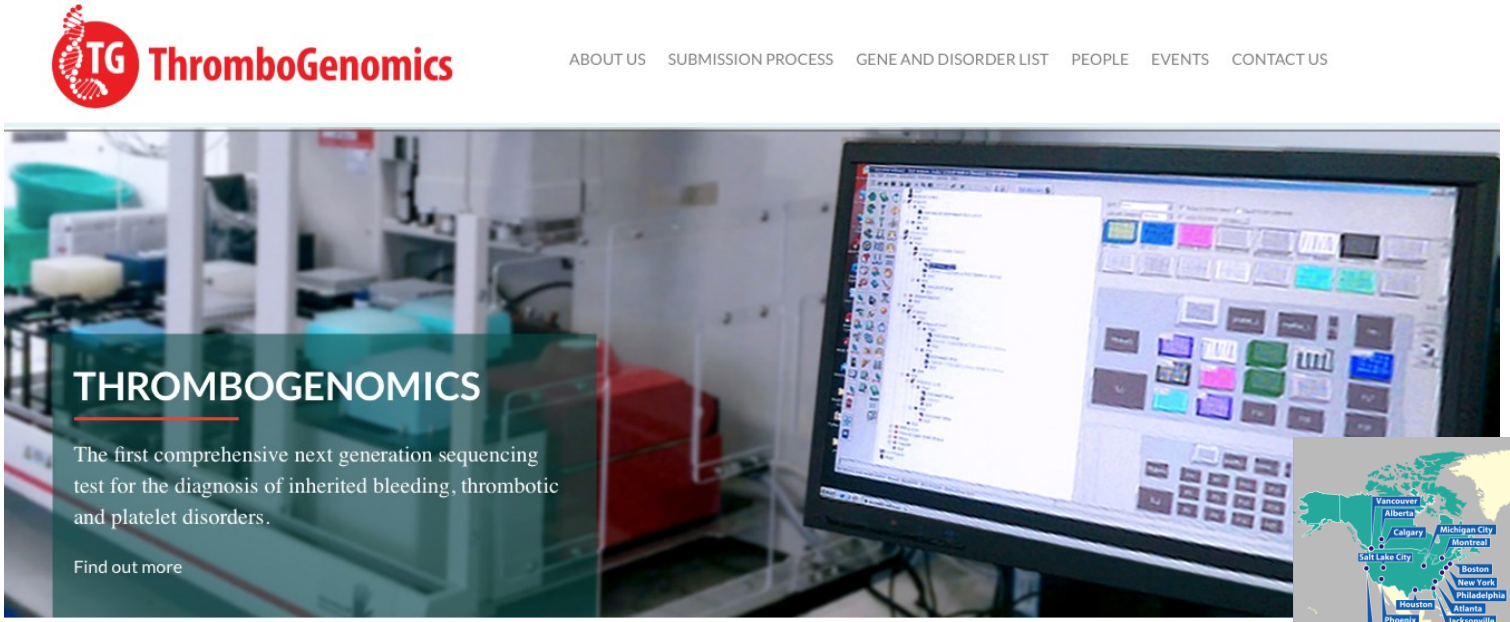
Thrombogenomics

Center for Molecular and Vascular Biology

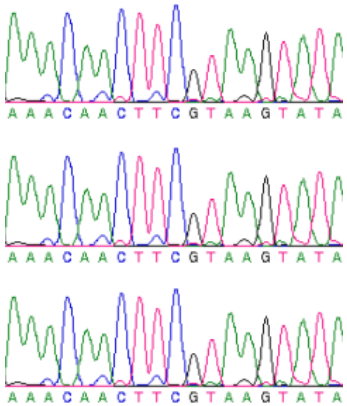
Kathleen Freson
University of Leuven

Implementation of multi-gene panel test : ThromboGenomics study



Simeoni I, et al. Blood. 2016

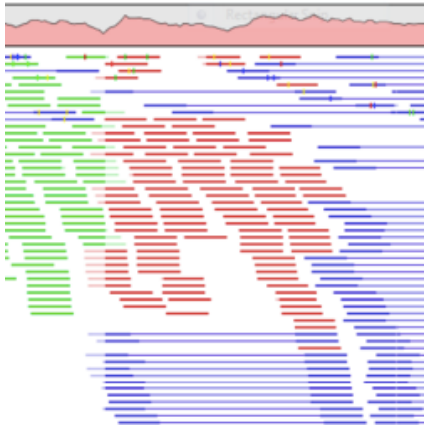
From Single gene analysis to Multi-gene panels



Past → Today

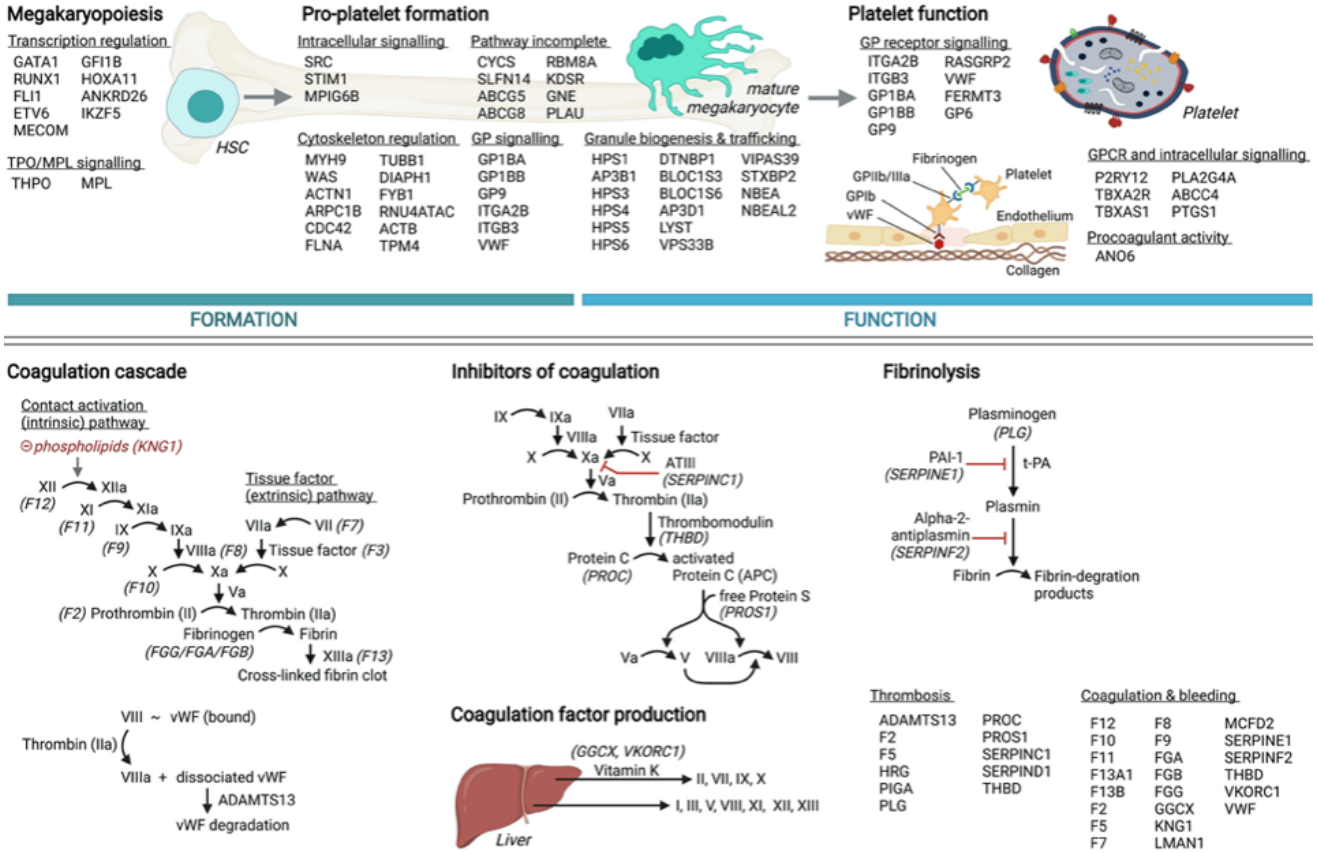
Laboratory assays
Clinical phenotype
Sanger sequencing, MLPA, CNV

Targeted, Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS)



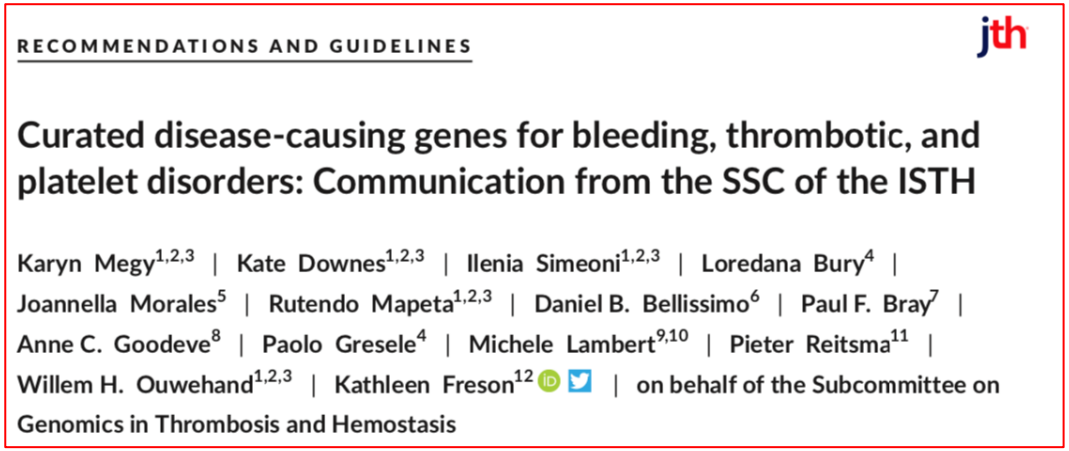
MULTI-GENE PANEL TEST (Virtual)

> 100 genes cause bleeding and thrombosis in humans



Ver Donck F et al. RPTH 2021

Gene curation to deliver diagnostic-grade genes (TIER1) for bleeding and platelet disorders



RECOMMENDATIONS AND GUIDELINES

jth

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

Karyn Megy[1,2,3] | Kate Downes[1,2,3] | Ilenia Simeoni[1,2,3] | Loredana Bury[4] | Joannella Morales[5] | Rutendo Mapeta[1,2,3] | Daniel B. Bellissimo[6] | Paul F. Bray[7] | Anne C. Goodeve[8] | Paolo Gresele[4] | Michele Lambert[9,10] | Pieter Reitsma[11] | Willem H. Ouwehand[1,2,3] | Kathleen Freson[12] | on behalf of the Subcommittee on Genomics in Thrombosis and Hemostasis

SSC Scientific and Standardization Committee

- www.isth.org/page/GinTh_GeneLists
- Yearly updates during the SSC session

Megy K, et al. J Thromb Haemost. 2019

P2RY12

TIER1

- **Proven disease-associated gene (DIAGNOSTIC-GRADE)**
- **3 unrelated pedigrees with co-segregation data**
- **Functional data (animal model and cell/protein studies)**

TIER2

- Evidence from 1 or 2 pedigrees with insufficient coseg data & without functional studies studies

P2RY1

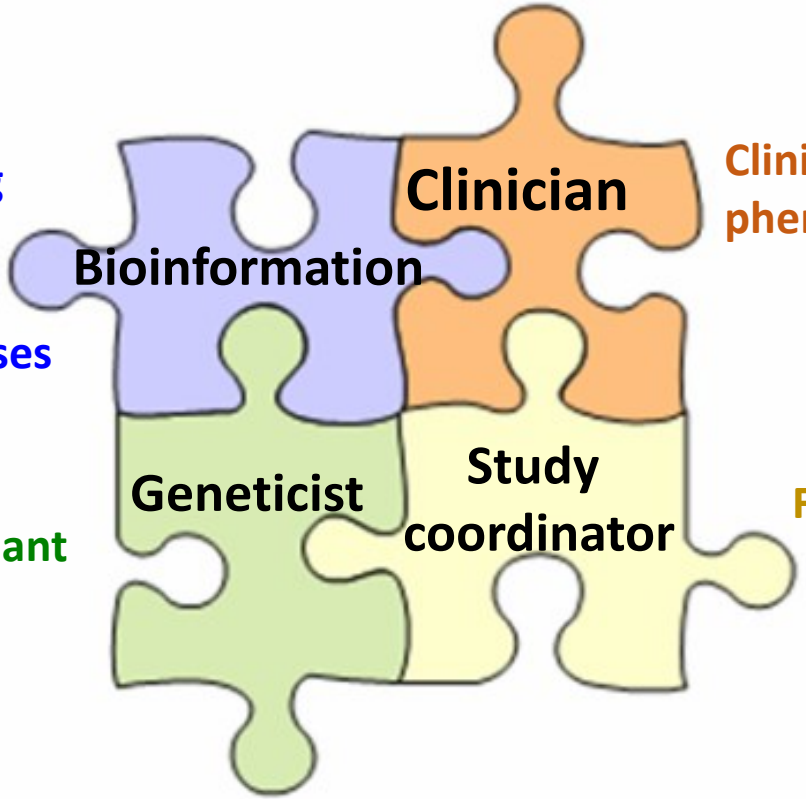
TIER3

- Evidence for role in platelet disorders (e.g. Functional studies or KO mice)

Variant Classification by Multi Disciplinary Team (MDT)

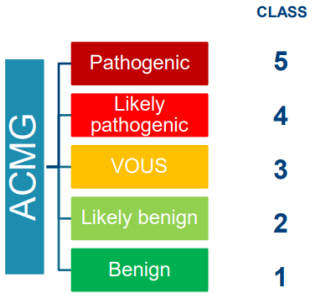
Variant filtering against control and disease databases

Gene-Disease-Variant interpretation

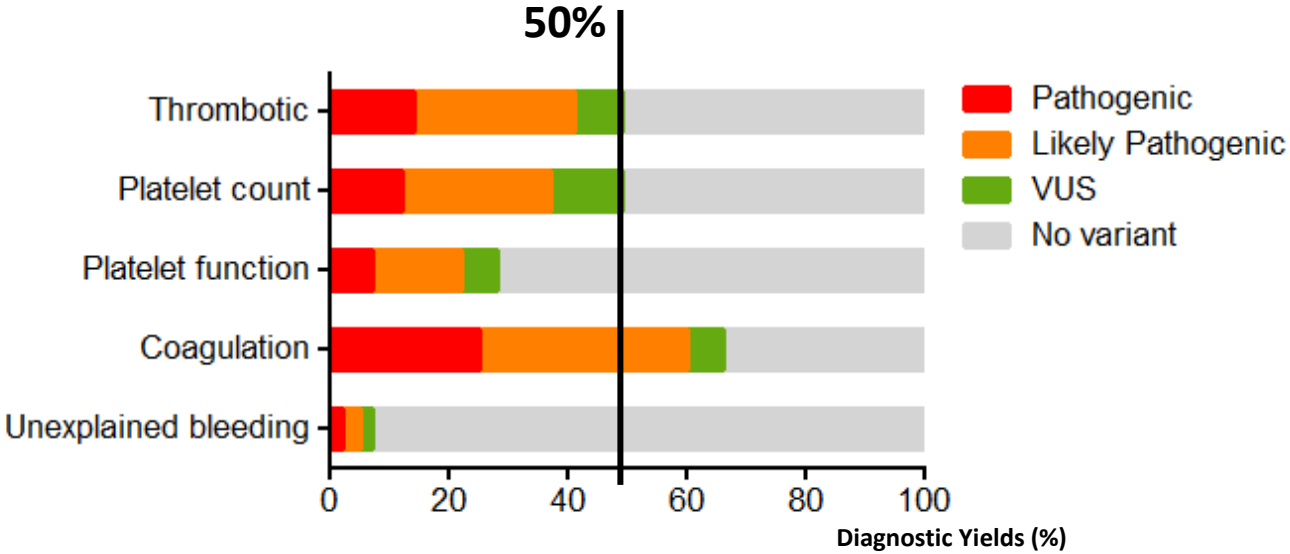


Clinical phenotyping

Final report



ThromboGenomics study: diagnostic rates for 2396 patients



Downes K, et al. Blood. 2019

Patient in- and exclusion criteria for gene panel testing

Inclusion criteria for HTS of BTPD

- Diagnosis of a rare bleeding, thrombotic and/or platelet disorder of known or unknown cause
- Early onset childhood case and/or with family history
- Can include syndromic features
- Bleeding of unknown etiology and normal laboratory test parameters but with evidence for Ehlers-Danlos Syndrome or Hereditary hemorrhagic Telangiectasia phenotypes
- Deficiency of (anti)coagulation factors
- (macro/micro)Thrombocytopenia, abnormal platelet morphology and/or function

Exclusion criteria for HTS of BTPD

- Acquired bleeding
- Thrombocytosis
- Thrombosis in single patient without family history or onset after 30Y
- Patients with platelet delta-storage pool disease (abnormal platelet ATP secretion and low dense granules) without Hermansky-Pudlak syndrome phenotypes
- Use of drugs known to be associated with abnormal platelet (function) phenotypes and/or bleeding disorders
- Patients with evidence of an autoimmune or systemic condition known to affect hemostasis & platelet homeostasis
- Medical conditions associated with abnormal platelet count and/or volume and/or abnormal platelet function
- Acute viral infection, HIV positivity and/or AIDS bone marrow aplasia, hepatic failure
- Malignancies, particularly those compromising hematopoiesis
- Splenomegaly, uremia

Adapted from Van Laer C et al, BJH 2020

Multi-gene panel test (WES) in an accredited genetic lab

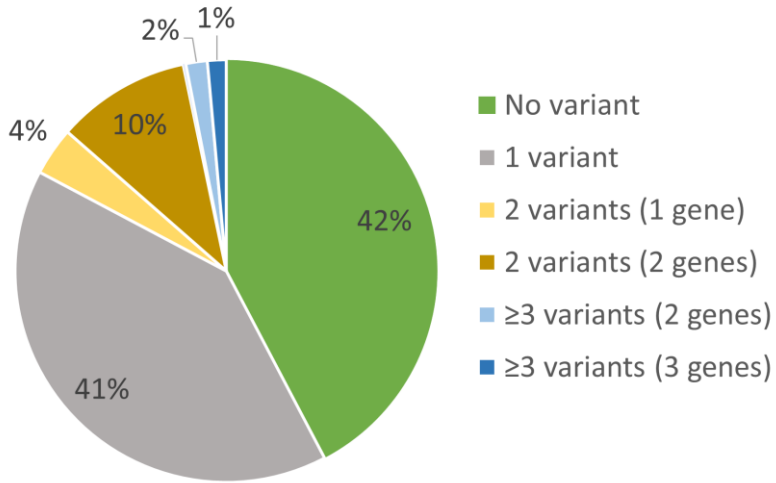
Christine Van Laer

3 subpanels

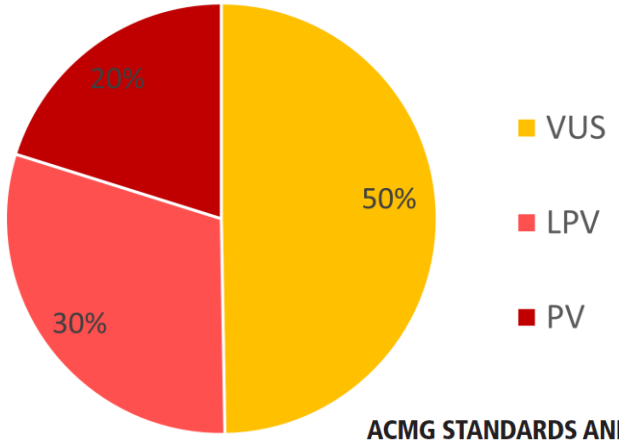
- Coagulation/anti-coagulation
- Platelet disorder panel
- Bleeding of unknown etiology panel

Results genetic testing Jan 2019 – May 2022

Detected variants per patient (n=520 from 26 referral centers)



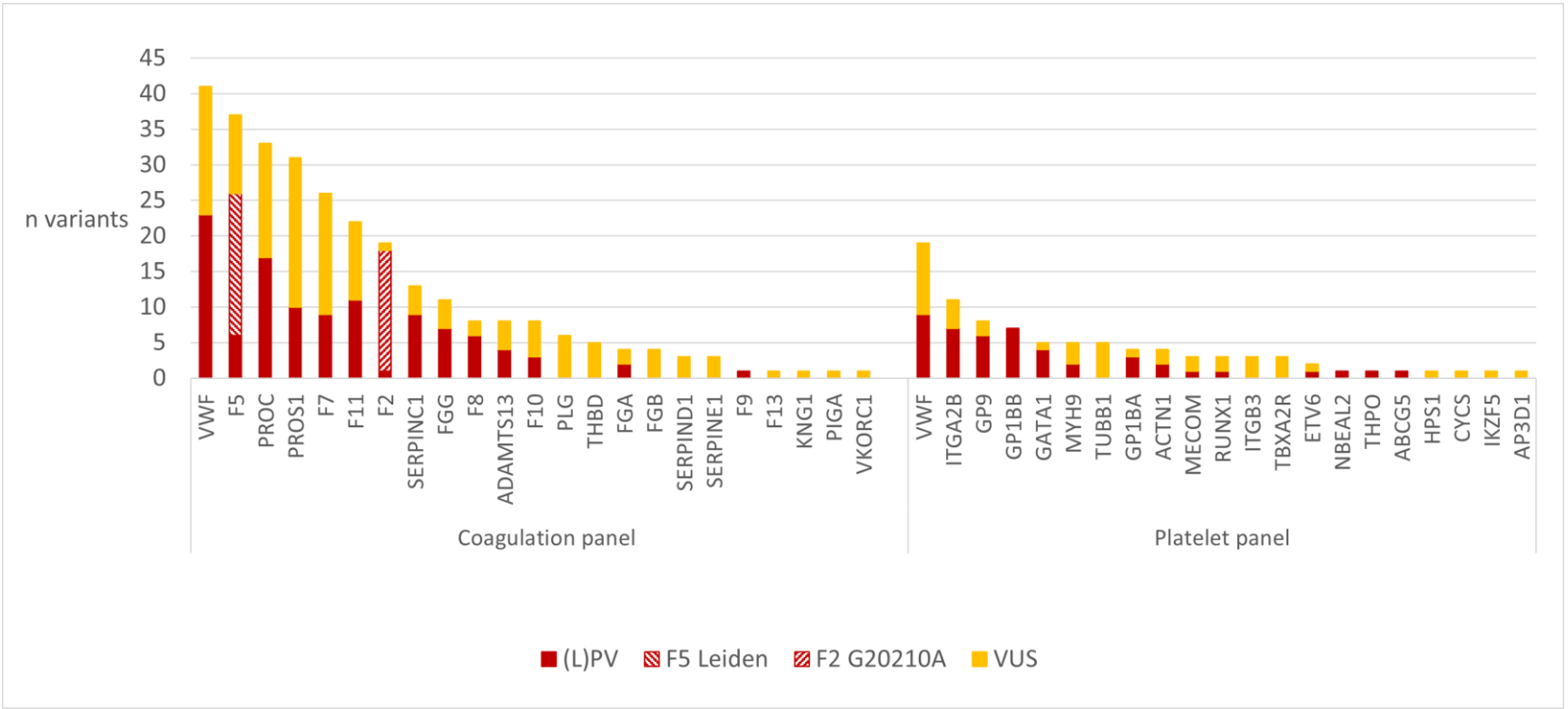
Classification of variants (n=382)



ACMG STANDARDS AND GUIDELINES

Paper in preparation

Genes with variants



Paper in preparation

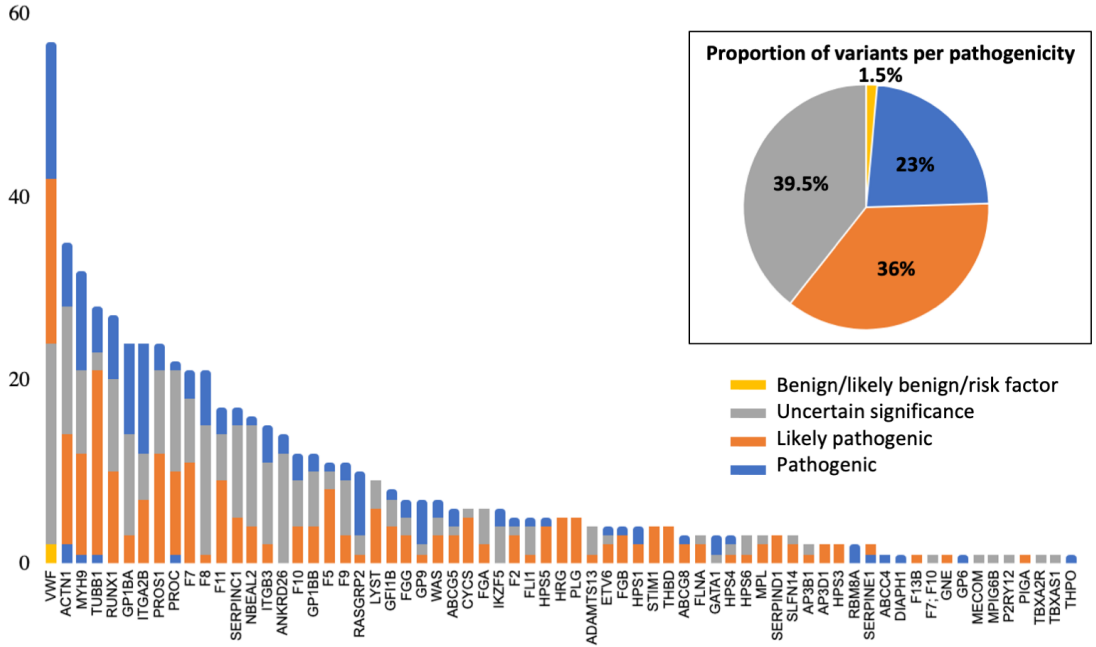
VUS reclassification

Additional functional coagulation assays	Additional genetic testing	Check databases & literature
31 index patients coagulation factors, protein S, protein C, antithrombin, RNA splicing assay, …	35 index patients 64 candidate variants 83 family members	ISTH database ‘GoldVariants’

Reclassification of 10 VUS in 25 index patients

Resource for Variant Capture to improve classification

814 Variants have been submitted from 30 diagnostic labs from 14 countries



GoldVariants

SSC Scientific and Standardization Committee

ISTH SSC-GinTH

ClinVar

Clinically relevant variation

Variant reclassification by ClinGen working groups for thrombosis & hemostasis

https://www.isth.org/page/GinTh_GeneLists

Megy et al, JTH 2021

NM_019616.4(F7):c.400G>A (p.Gly134Ser)

Interpretation:	**Conflicting interpretations of pathogenicity** **Likely pathogenic(1); Uncertain significance(1)**
Review status:	★ ☆ ☆ ☆ criteria provided, conflicting interpretations
Submissions:	2
First in ClinVar:	Nov 6, 2021
Most recent Submission:	Apr 23, 2022
Last evaluated:	Nov 26, 2019
Accession:	VCV001315977.2
Variation ID:	1315977
Description:	single nucleotide variant

Submitted interpretations and evidence

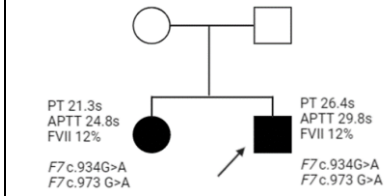
Interpretation (Last evaluated)	Review status (Assertion criteria)	Condition (Inheritance)	Submitter
Uncertain significance (Nov 26, 2019)	criteria provided, single submitter (GeneDX Variant Classification (06012015)) Method: clinical testing	Not Provided Affected status: yes Allele origin: germline	GeneDx Accession: SCV002007598.1 First in ClinVar: Nov 06, 2021 Last updated: Nov 06, 2021
Likely pathogenic (-)	criteria provided, single submitter (ACMG Guidelines, 2015) Method: clinical testing	Congenital factor VII deficiency Affected status: yes Allele origin: unknown	ISTH-SSC Genomics in Thrombosis and Hemostasis, KU Leuven, Center for Molecular and Vascular Biology Accession: SCV002500859.1 First in ClinVar: Apr 23, 2022 Last updated: Apr 23, 2022 Comment: GoldVariant submitter: Bilal Jradeh, Katharine Dormandy Haemophilia and Thrombosis Centre, Royal Free Hospital, London, UK and Dr Karyn Mégy, NIHR Bioresource - Cambridge University, UK

VUS reclassification

Gene	Variant DNA	Variant Protein	Conclusion
Downgrade			
F2	c.1298+19G>A	INTRONIC	VUS → Benign
F8	c.396A>C	p.Glu132Asp	LPV → VUS
MYH9	c.651C>A	p.Ile217=	VUS → LB
Upgrade			
F7	c.934G>A	p.Val312Met	VUS → LPV
F11	c.616C>T	p.Pro206Ser	VUS → LPV
PROC	c.1106C>T	p.Pro369Leu	VUS→LPV
VWF	c.4195C>T	p.Arg1399Cys	VUS → LPV
VWF	c.4751A>G	p.Tyr1584Cys	VUS → LPV
VWF	c.3569G>A	p.Cys1190Tyr	VUS → LPV

Paper in preparation

F7, Val312Met

Sex, age, phenotype	Laboratory results	Segregation study
F, 28y, Low FVII, no bleeding	FVII 24%, 31%	nd
M, 63y, Low FVII	FVII 2% *2nd F7 LPV: c.1271 G>A*	nd
M, 20y, Low FVII, no bleeding	FVII 12% *2nd F7 VUS: c.973G>A*	
M, 54y, Low FVII	FVII 25%	nd

What do I tell my patient about panel testing?

SSC Scientific and Standardization Committee

RECOMMENDATIONS AND GUIDELINES | Open Access

Clinical management, ethics and informed consent related to multi-gene panel-based high throughput sequencing testing for platelet disorders: Communication from the SSC of the ISTH

Kate Downes, Pascal Borry, Katrin Ericson, Keith Gomez, Andreas Greinacher, Michele Lambert, Eva Leinoe, Patrizia Noris, Chris Van Geet, Kathleen Freson ✉, Subcommittee on Genomics in Thrombosis, Hemostasis ... See fewer authors

First published: 08 July 2020 | https://doi.org/10.1111/jth.14993

Downes K, et al. J Thromb Haemost 2020

Risk for unsolicited findings using a panel test for IPD

Unsolicited findings: refer to variants in disease-causing genes that are unrelated to the original rationale for testing and that are identified inadvertently

Examples:

RUNX1, ETV6 and ANKRD26 variants that are risk factors for leukaemia when testing for platelet disorders

Carriership of variants in recessive genes

Downes K, et al. J Thromb Haemost 2020

- Index case, 35 y
- Mucocutanous bleeding symptoms
- Platelet count 145- 161 K, normal size
- Platelet delta storage pool disease

- RUNX1 p.Glu5ValfsTer5

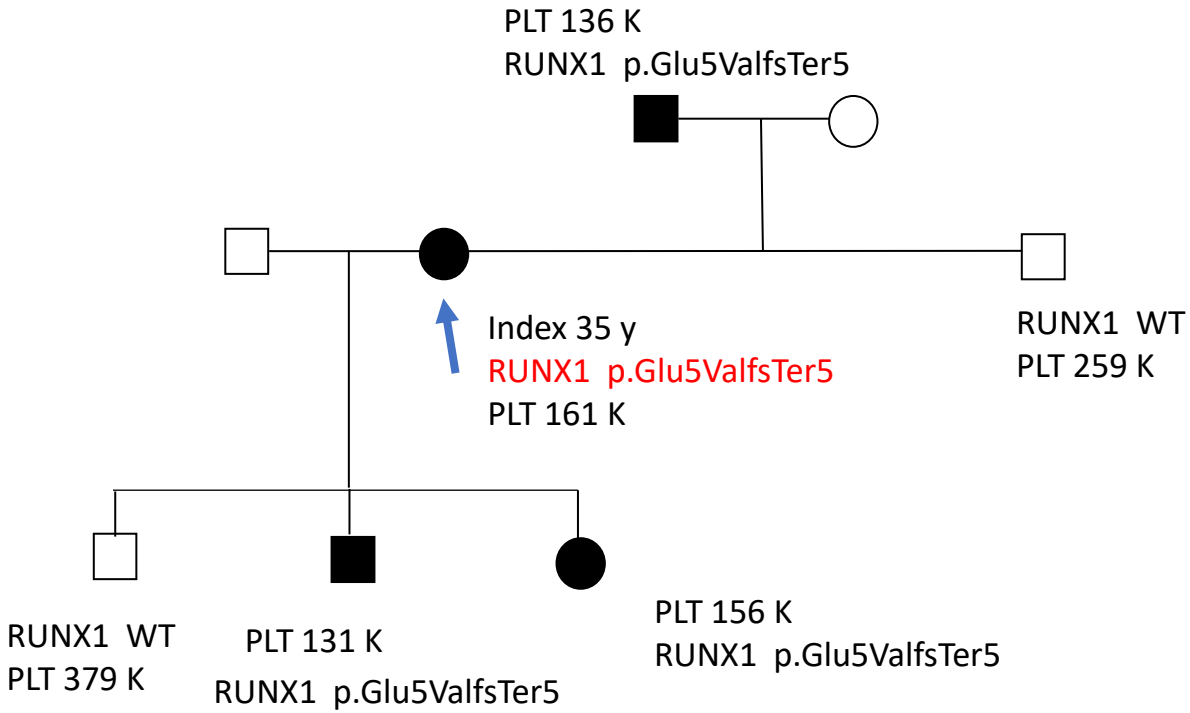
BRIEF REPORT | DECEMBER 12, 2013

Enrichment of *FLI1* and *RUNX1* mutations in families with excessive bleeding and platelet dense granule secretion defects

Brief Report

Jacqueline Stockley, Neil V. Morgan, Danai Bem, Gillian C. Lowe, Marie Lordkipanidzé, Ban Dawood, Michael A. Simpson, Kirsty Macfarlane, Kevin Horner, Vincenzo C. Leo, Katherine Talks, Jayashree Motwani, Jonathan T. Wilde, Peter W. Collins, Michael Makris, Steve P. Watson,
Martina E. Daly on behalf of the UK Genotyping and Phenotyping of Platelets Study Group

Blood (2013) 122 (25): 4090–4093.


PLT 136 K
RUNX1 p.Glu5ValfsTer5
Index 35 y
RUNX1 p.Glu5ValfsTer5
PLT 161 K
RUNX1 WT
PLT 259 K
RUNX1 WT
PLT 379 K
PLT 131 K
RUNX1 p.Glu5ValfsTer5
PLT 156 K
RUNX1 p.Glu5ValfsTer5

Detection of a missed diagnosis

- Index case, 22 y
- Bleeding after trauma, menorrhagia
- Mild macrothrombocytopenia (PLT 125K, MPV >13fL)
- Normal aggregations and ATP secretion
- FACS normal CD61, CD41 and CD42

- GP1BB p.Leu16Pro
- TUBB1 p.Gly109Glu

GP1BB p.Leu16Pro

BRIEF REPORT | JANUARY 26, 2017

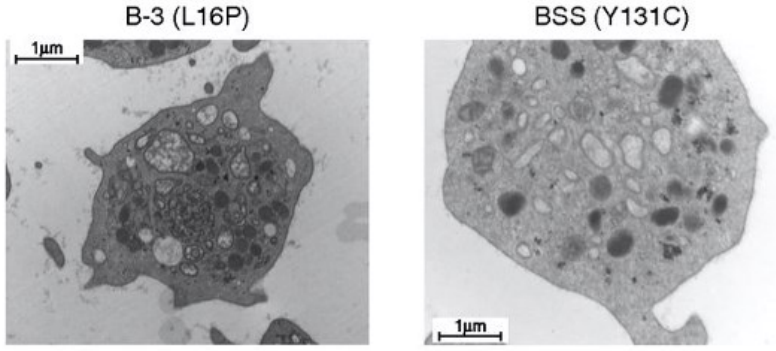
Rare variants in *GP1BB* are responsible for autosomal dominant macrothrombocytopenia

Brief Report

Suthesh Sivapalaratnam, Sarah K. Westbury, Jonathan C. Stephens, Daniel Greene, Kate Downes, Anne M. Kelly, Claire Lentaigne, William J. Astle, Eric G. Huizinga, Paquita Nurden, Sofia Papadia, Kathelijne Peerlinck, Christopher J. Penkett, David J. Perry, Catherine Roughley, Ilenia Simeoni, Kathleen Stirrups, Daniel P. Hart, R. Campbell Tait, Andrew D. Mumford, NIHR BioResource, Michael A. Laffan, Kathleen Freson, Willem H. Ouwehand, Shinji Kunishima, Ernest Turro

Check for updates

Blood (2017) 129 (4): 520–524.



TUBB1 p.Gly109Glu

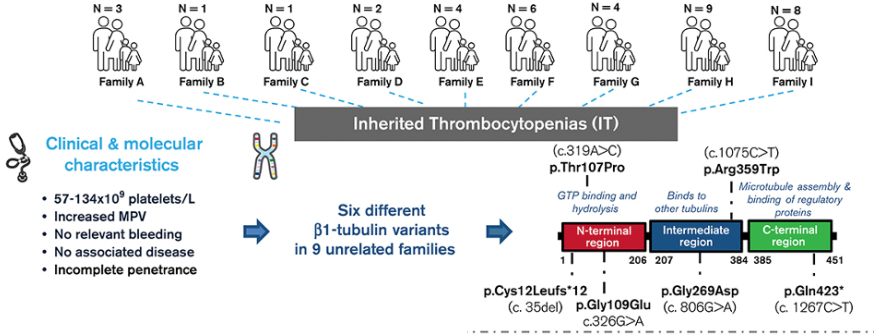
PLATELETS AND THROMBOPOIESIS | DECEMBER 16, 2021

Expanding the genetic spectrum of *TUBB1*-related thrombocytopenia

Verónica Palma-Barqueros, Loredana Bury, Shinji Kunishima, María Luisa Lozano, Augustín Rodríguez-Alen, Nuria Revilla, Natalia Bohdan, José Padilla, María P. Fernández-Pérez, María Eugenia de la Morena-Barrio, Ana Marín-Quiles, Rocío Benito, María F. López-Fernández, Shally Marcellini, Ana Zamora-Cánovas, Vicente Vicente, Constantino Martínez, Paolo Gresele, José M. Bastida, José Rivera on behalf of the Inherited Platelet Disorders Project, Grupo Español de Alteraciones Plaquetarias Congénitas (GEAPC), Spanish Society of Thrombosis and Haemostasis (SETH)

Check for updates

Blood Adv (2021) 5 (24): 5453–5467.



Key messages for use of a panel test for diagnostics of bleeding and thrombosis

- ✓ A (virtual) panel test is fast (TAT 4-6 months) and cheap
- ✓ It detects unexpected phenotype-genotype associations (including unsolicitated findings)
- ✓ Panel test is typically ordered by specialist with knowledge of the complexity of such test and its inclusion/exclusion criteria. Patients should be aware of what this test means (opt-out for RUNX1, ETV6, ANKRD26 and consenting).
- ✓ Sufficient phenotype information should be provide to allow variant classification
- ✓ Variants of Unknown clinical Significance need further research (improved variant databases) before they can be used in the clinic
- ✓ Report needs sufficient details to clearly explain the findings

- **ThromboGenomics**: Kate Downes, Karyn Megy, Keith Gomez, Mike Laffan, Andrew Mumford, Willem Ouwehand

- **Trombose-Hemostase Leuven panel**: Anniek Corveleyn

- **ISTH-SSC for genomics in thrombosis and hemostasis**

- **Center for Molecular and Vascular Biology**

  Chantal Thys
  Christine Van Laer
  Marc Jacquemin
  Chris Van Geet
  Veerle Labarque
  Peter Verhamme
  Kathelijne Peerlinck

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