Title

Diagnostic high-throughput sequencing of 2,390 patients with bleeding, thrombotic and platelet disorders

Authors

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Key points (each max 140 ch)

1. High-throughput sequencing (HTS) test reveals a molecular diagnosis for 38% of 2,390 patients with bleeding, thrombotic and platelet disorders.

2. ThromboGenomics HTS test validates recent gene discoveries and detects copy number and intronic variants.

Abstract

A targeted high-throughput sequencing (HTS) panel test for clinical diagnostics requires careful consideration of the inclusion of appropriate diagnostic-grade genes, the ability to detect multiple types of genomic variation with high levels of analytic sensitivity and reproducibility, and variant interpretation by a multi-disciplinary team (MDT) in the context of the clinical phenotype. We have sequenced 2,390 index patients using the ThromboGenomics HTS panel test of diagnostic-grade genes known to harbour variants associated with rare bleeding, thrombotic or platelet disorders (BPD). The diagnostic rate was determined by the clinical phenotype, with an overall rate of 50.4% for all thrombotic, coagulation, platelet count and function disorder patients and a rate of 6.2% for patients with unexplained bleeding disorders characterized by normal hemostasis test results. The MDT classified 756 unique variants, including copy number and intronic variants, as Pathogenic, Likely Pathogenic or Variants of Uncertain Significance. Almost half (49.7%) of these variants are novel and 41 unique variants were identified in 7 genes recently found to be implicated in BPD. Inspection of canonical hemostasis pathways identified 29 patients with evidence of oligogenic inheritance. A molecular diagnosis has been reported for 897 index patients providing evidence that introducing a HTS genetic test for BPD patients is meeting an important unmet clinical need.

Introduction

Inherited bleeding, thrombotic and platelet disorders (BPD) are a heterogeneous group of rare disorders caused by DNA variants in a large number of loci. The most common bleeding disorders are von Willebrand Disease (VWD), affecting up to 0.01% of the population and Hemophilia A and B, which together affect 0.01% of males.[1] There are no accurate estimates of the prevalence of the remaining rare inherited bleeding disorders, although registry data suggest the prevalence being <0.001%.[2] Venous thrombosis has an overall annual incidence of less than 1 in 1,000, however it is rare in the pediatric population, with rates of approximately 1 in 100,000, indicative of possible environmental and lifestyle effects in adult patients.[3] To obtain a conclusive molecular diagnosis requires attendance at multiple outpatient consultations for a large portion of patients with an assumed diagnosis of a rare inherited BPD.

The genetic architecture of inherited BPDs is well determined, however, new genes continue to be identified. To date there are 21, 11 and 63 diagnostic-grade genes (hereafter TIER1 genes) associated with coagulation, thrombotic and platelet disorders, respectively.

Since validation of the ThromboGenomics HTS test[4], 33 TIER1 genes, including recently discovered BPD genes, have been added to the HTS test, increasing the clinical utility. Others have reported on similar gene panel tests or used whole exome sequencing to identify DNA variants causing inherited BPDs[5-9]. However, all these studies have reported on relatively small numbers of patients (<160 index patients) preventing firm conclusions about the clinical utility of such tests. The diagnostic rates obtained in these studies cannot be compared as all focused on different sets of genes (with a subset of TIER1 genes, and also inclusion of 'research' genes), used different patient inclusion criteria and variant classification was not standardised as is now recommended.[10]

Here we report on the results obtained with the ThromboGenomics HTS test for 2,390 index patients categorised into five classes of disorders based on the appended human phenotype ontology (HPO) terms; [11] thrombotic, platelet count, platelet function,

coagulation and unexplained bleeding. Variant interpretation, by a multi-disciplinary team (MDT), determined the contribution of variants in TIER1 genes to the observed phenotypes thereby providing insights in the clinical utility of HTS testing for different categories of patients. We also comment on the standardisation of variant interpretation and how the reporting of a conclusive molecular diagnosis has immediately impacted on clinical management. Finally, due to the large number of patients tested, we are able to highlight the clinical importance of detecting copy number and deep intronic variants and possible oligogenic inheritance.

Methods

Patients

The 2,390 index patients were either referrals for the ThromboGenomics test or patients who joined the PANE and VIBB studies (Supplemental Table 1). Clinical and laboratory phenotypes were recorded using HPO terms as described.[4,12] Further details of the study participants, including institutional review board or research ethics committee information are in the Supplemental Information.

ThromboGenomics referrals for diagnostic testing of inherited BPDs

Samples and clinical/laboratory phenotype information from 1,602 index patients with a known or suspected diagnosis of inherited BPD according to criteria as described were referred by clinicians in 72 UK and 46 non-UK hospitals (Supplemental Figure 1).[4]

PANE: Preoperative screening for mild bleeding risk

A total of 212 patients, identified through preoperative assessment of bleeding risk at Maastricht University Medical Centre (MUMC), were recruited and underwent a full hematological assessment including extensive laboratory testing for hemostasis parameters (Supplemental Table 2).[13,14]

VIBB: Vienna Bleeding Biobank

A total of 594 patients referred to the Hematology and Hemostaseology specialist tertiary referral centre in Vienna for assessment of a mild to moderate bleeding disorder were recruited and subjected to a full hematological assessment, including extensive laboratory testing for hemostasis parameters (Supplemental Table 2).[15]

ThromboGenomics HTS test

The ThromboGenomics HTS test sample preparation and sequencing protocols are as described with minor modifications (Supplemental Information).[4] The content of the test has been reversioned twice since first described to include additional (mostly recently discovered) TIER1 genes. ThromboGenomics version 2 (TG.V2) and version 3 (TG.V3) include 80 and 96 genes, respectively (Supplemental Table 3). TIER1 genes are curated and approved by the Scientific and Standardization Committee on Genomics in Thrombosis and Hemostasis (SSC-GinTH) of the International Society on Thrombosis and Haemostasis (ISTH). For TG.V3, probes for 10,000 common single nucleotide variants (SNVs) were included to estimate relatedness and ancestry. Automated bioinformatics analysis pipeline methods, including variant calling, are described in the Supplemental Information.

Variant prioritisation and interpretation

Variants were annotated and prioritised for interpretation using the analytical process as reported[4] based on the predicted effect in the curated transcript, presence in the Human Gene Mutation Database (HGMD)[16] or in a curated set of known pathogenic variants (Supplemental Information) and the minor allele frequency (MAF) in the Exome Aggregation Consortium (ExAC) and Genome Aggregation (gnomAD) databases.[17] On a patient-by-patient basis, DNA variants passing filtering were prioritised and interpreted by a MDT in the context of the HPO terms and family history. Reported variants were characterised as Pathogenic, Likely Pathogenic and Variants of Uncertain Significance (VUS) alongside a decision of the likely contribution of each variant to the patients phenotype. The MDT made use of Congenica's diagnostic decision support platform Sapientia^{TM} (Cambridge, UK) to support the review process and record findings in the

form of research reports for return to referring clinicians. For all samples sequenced using TG.V2, variant interpretation was performed according to guidelines agreed by the members of the ThromboGenomics MDT (criteria in Supplemental Table 4). In 2017, the UK Association of Clinical Genomic Science published best practice guidelines for variant interpretation based on the earlier reported American College of Medical Genetics and Genomics (ACMG) guidelines.[10] Sapienta software implemented ACMG guidelines from release 1.7 (January 2018), allowing rapid variant interpretation following the guidelines. This updated system was applied for variant interpretation of all samples sequenced using TG.V3. Supplemental Table 5 summarises the main differences in panel content, methods, analysis and interpretation used for TG.V2 and TG.V3.

Results

Patient inclusion criteria and phenotypes

A total of 2,390 index patients and 156 samples from relatives and carriers were tested using the ThromboGenomics HTS test (Supplemental Table 1, Supplemental Figure 1). The largest group of 1,602 index patients and 18 referrals for hemophilia carrier status, were referred by specialist tertiary centres for diagnostic testing. To better appreciate the clinical utility of the HTS test we also included 193 and 594 samples from the PANE and VIBB single-centre studies. Both studies excluded patients with platelet counts $<100x10^9$/l.

Based on the clinical and laboratory phenotypes of all patients, a total of 7,340 HPO terms were appended, which were used to categorise all patients into five broad disorder classes: thrombotic (n=285), platelet count (n=329), platelet function (n=397), coagulation (n=685) and unexplained bleeding (n=698) (Figure 1A). The ThromboGenomics referrals included patients of all five classes, while the majority of the PANE (80.3%) and VIBB (60.1%) patients are classed as unexplained bleeding.

Most of the patients categorised to the thrombotic class were referred because of reduced Protein C or Protein S levels as indicated by the HPO term ‘Abnormality of the protein C anticoagulation pathway’ (Figure 1B). Patients with platelet count disorders

were generally referred due to (macro)thrombocytopenia. Platelet function abnormalities were diverse, including defects in aggregation, reduced platelet membrane protein expression (particularly GPIb/IX/V and GPIIb/IIIa) and reduced granule secretion. Coagulation defects included patients with reduced VWF levels or abnormal coagulation parameters of the intrinsic, extrinsic and common pathways. HPO terms coding abnormal phenotypes outside the blood system were appended to 41 (1.7%) of patients. These terms related to eyes (ocular albinism), hearing (deafness), skeletal (abnormal radius, joint disorders) and kidney (functional insufficiency). Across the three study groups 698 patients with bleeding symptoms and normal hemostasis test results were classed as unexplained bleeding (Figure 1B). Nearly half (49.1%) of the patients in this class were characterised by the presence of both spontaneous and trauma-related bleeding symptoms, a similar distribution as found for the coagulation and platelet function disease class (Supplemental Figure 2). Chronic mild thrombocytopenia is generally not accompanied by spontaneous bleeding.[18] However, 67.7% of the patients in the platelet count class presented with spontaneous bleeding, strongly suggesting an enrichment of this type of 'thrombocytopenia with bleeding' referrals for the ThromboGenomics test.

Performance of the ThromboGenomics HTS test

The previously reported validation of the ThromboGenomics HTS test (TG.V1) used 296 samples from patients, with and without previously known disease causing variants, sequenced for 63 TIER1 genes.[4] Here we sequenced 1,330 and 1,060 index patient samples with the TG.V2 and TG.V3 tests targeting 80 and 96 TIER1 genes, with a region of interest (ROI) of 0.222 Mb and 0.275 Mb respectively (Supplemental Table 3). Since validation, the analysis method for calling SNVs and short (<50 base pairs [bp]) insertion/deletions (indels) has undergone minor modifications, however, the detection of CNVs has substantially been improved.[19] In short, sequencing read depth is computed over 500 bp elements, to improve the sensitivity for the detection of shorter CNVs, and an optimised reference set of data obtained from 10 samples has been generated, using genetically unrelated individuals (Supplemental Information). Despite the increase of the ROI and increased multiplexing of samples, the read coverage has remained high, with 99.99% and 99.98% of the ROI with a read depth exceeding 30x for TG.V2 and TG.V3

tests, respectively. For each sample, an average of 146.7 and 190.9 SNVs, 9.4 and 11.2 indels and 0.20 and 0.21 CNVs were identified by the TG.V2 and TG.V3 tests, respectively. The proportions of variant types identified did not differ between test versions (Supplemental Figure 3). For all samples, on average 4.5 variants were prioritised for interpretation by the MDT (Supplemental Table 1).

Diagnostic rates and validation of recently discovered TIER1 genes

Prioritised variants were reviewed by the MDT in the context of the disease incidence, variant frequency, assigned HPO terms and family history. Variants were reported with pathogenicity and the contribution to the patients phenotype (full or partial). Variants for recessive BPDs were reported if present in the homozygous or compound heterozygous states. VUS were reported if the MDT predicted a future Likely Pathogenic status with additional evidence from cosegregation and functional studies. Screening of 2,390 index patients resulted in an overall diagnostic rate of 37.5% by reporting a total of 1,039 variants in 897 index patients (Figure 2A). Most reported variants (88.5%) are rare (<0.01%) or absent in gnomAD (Supplemental Figure 4, Supplemental Table 6). There was a marked difference in diagnostic yield between the five classes; thrombotic, 49.1%; platelet count 48.0%; platelet function 27.0%; coagulation disorders 65.4% and unexplained bleeding 6.2% (Figure 2A). For thrombotic and coagulation disorders, 68.0% and 69.1% of variants reported were known variants that had previously been associated with disease, while for the platelet count and function disorders this proportion was lower; 43.5% and 26.4% respectively (Figure 2B). We reason that this difference reflects the fact that cataloging of pathogenic variants for coagulation disorders (especially VWD and Hemophilia A and B) began over three decades ago, whilst the majority of TIER1 genes for platelet disorders have only been identified over the past decade. Of the 329 patients within the platelet count class, 29 were referred under a working diagnosis of ‘immune thrombocytopenia refractory to treatment’. In 7 of these patients, variants were reported in genes known to be associated with thrombocytopenia (*ANKRD26, ETV6, ITGA2B, TUBB1*).

The implementation of the criteria of the ACMG guidelines, instead of our 'in-house' criteria (Supplemental Table 4), impacted on variant interpretation for TG.V3. On comparing the collection of ThromboGenomics samples sequenced and analysed using either TG.V2 or TG.V3 (PANE and VIBB samples were sequenced using only TG.V2 and TG.V3 respectively), there was minimal difference in the total number of reported variants for each of the five classes of patients (Supplemental Figure 5). However, for all classes, an interpretation shift from Likely Pathogenic to VUS was noted. This change in variant interpretation was mainly explained by novel missense variants (Supplemental Figure 6). For patients tested using TG.V2, variants that were not known as disease associated were often deemed as Likely Pathogenic by the MDT. On introduction of the ACMG guidelines, these novel missense variants did not reach the threshold for designation as Likely Pathogenic and thus were labeled as VUS.

The genes with reported variants in index patients are ranked in Figure 3. For the thrombotic, platelet count and coagulation disease classes, over a quarter of patients had variants reported in just one gene; *PROS1*, *MYH9* and *VWF*, respectively. The fourth quarter of patients for each patient class had reports spread across at least 7 genes. All reported variants are summarised in Supplemental Table 6 and have been submitted to the ClinVar database.[20]

Since TG.V1, 17 TIER1 genes associated with BPD have been included in TG.V2 and a further 16 more TIER1 genes in TG.V3. In addition to introducing known and recently discovered genes, also new modes of inheritance were added (detailed in Supplemental Table 3). Diagnostic reports for 41 patients have been issued with variants in one of the 19 recently discovered TIER1 genes or by applying a new mode of inheritance.

Copy number variation, deep intronic variants and oligogenic findings.

The analytical pipeline for the identification of CNVs has been modified with improved quality scoring and visualization tools. Overall, CNVs were reported in 40 patients, predicted to affect single exons (n=11), multiple exons (n=15) or whole genes

(n=14) (Supplemental Table 6). These heterozygous deletions and duplications would not generally be detected using PCR-based and Sanger sequencing testing strategies. Raw sequencing reads of all potentially pathogenic CNVs were visually inspected by the MDT. In some instances, this revealed the presence of a complex CNV, including one inversion with breakpoints in introns 26 and 27 of *DIAPH1* resulting in an in-frame deletion of exon 26 and an inversion flanked by two deletions within *F8* causing severe hemophilia A (Supplemental Figure 7).[21]

Aside from the core dinucleotide splice sites at the 5' and 3' of introns, the lack of reliable prediction tools makes it difficult to determine the likely functional consequences of potential splicing altering variants. Outside the SnpEff annotated splice regions (8 bp), intronic variants were only prioritised if previously associated with disease. A deep intronic homozygous *ITGA2B* variant identified in one index patient with Glanzmann Thrombasthenia was validated using platelet RNA expression studies confirming alternative splicing and the absence of normal ITGA2B transcript (Supplemental Figure 8). These functional data, together with co-segregation analysis, in the pedigree of the index patient, resulted in a reclassification of this *ITGA2B* variant from VUS to Likely Pathogenic.

Of the 897 index patients where variants were reported, 773 had a single reported variant while 124 had at least two reported variants. The latter category were mainly patients with recessive diseases, however for 28 patients the variants were reported in two or more genes. For most of these examples, the variants identified were within first or second order interactors in the known canonical hemostasis pathways (Supplemental Table 7). For the thrombotic and coagulation classes, we identified 11 (3.9%) and 13 (1.6%) patients with oligogenic variants, respectively (Figure 4).

Incidental findings

By sequencing a large number of patients for the TIER1 genes underlying known BPDs, we expected to observe incidental secondary findings. In four females, not referred for hemophilia carrier testing or known to have reduced factor levels, we identified

carriership of Likely Pathogenic variants in *F8* or *F9*. In addition, in two patients we identified a heterozygous deletion of the RBM8A gene. A heterozygous *RBM8A* loss-of function variant (generally a deletion), if accompanied by a low frequency non-coding regulatory variant on the alternate allele, results in thrombocytopenia with absent radius (TAR) syndrome.[22] These incidental secondary findings were reported to the referring clinician as they are actionable with respect to family planning. In contrast, sex chromosome aneuploidy, identified in three patients, was not reported in line with current guidance from the 100,000 Genomes Project.[23]

Discussion

We evaluated the performance of a targeted HTS panel test for TIER1 genes in over 2,500 patients drawn from three distinct groups: (1) patients with a high likelihood of having an inherited BPD, (2) patients undergoing an extended preoperative assessment for bleeding risk and (3) patients with a bleeding disorder of unknown aetiology referred to a tertiary referral centre. After HPO coding of the clinical and laboratory phenotypes, patients were assigned to one of five diseases classes: thrombotic, platelet count, platelet function, coagulation or unexplained bleeding. DNA samples were sequenced with the ThromboGenomics HTS test and prioritised variants reviewed and classified by an MDT and reported to referring clinicians. The resulting data were used to assess the effectiveness of the HTS test, analytical pipeline and MDT variant interpretation in generating a conclusive molecular diagnosis. For patients of the thrombotic, coagulation and platelet count or function disease class, variants were reported for half (50.4%) of the 1,692 index patients. In contrast, variants were reported for 6.2% of the 698 index unexplained bleeding patients with normal hemostasis test results. Overall 20.1% (215) of variants reported were VUS, that require additional evidence including estimation of variant odds ratios using the results from large genotyped cohort studies, functional testing and cosegregation analysis. These data illustrate the excellent diagnostic yield of applying the ThromboGenomics test for patients with a high likelihood of having an inherited BPD.

Screening patients with the TG.V2 and TG.V3 HTS test presented several improvements compared to the TG.V1 test. Adding more TIER1 genes on TG.V2 and TG.V3 supported the molecular diagnosis of 41 patients and important genotype-phenotype association were observed for recently discovered BPD genes such as *DIAPH1*[21], *ETV6*[24], *GFI1B*[25], *GNE*[26,27] and *RASGRP2*[28] or for the alternative mode of inheritance recently reported for *GP1BB.*[29] In comparison to TG.V1, the detection of CNVs was optimised, resulting in the detection of CNVs in 40 patients, including previously unobserved deletions in 11 genes, indicating CNVs being an important variant class for all categories of BPDs. In addition, 2 novel duplications, 1 novel inversion and a complex CNV were also reported. Nevertheless, the optimised ExomeDepth method is sensitive to variable read depths, has a minimum resolution and cannot detect inversions or predict the location of duplicated regions. The introduction of a split-read or read-pair CNV calling method, alongside the ExomeDepth analysis method, may further improve CNV detection in the future.

Previously, Sanger sequencing of most BPD genes was performed with primer sets flanking intron/exon boundaries and therefore, the frequency of non-coding or silent variants, aside from those disrupting the immediate splice site (<+/- 8 bp from the exon boundary), that are associated with BPDs is unknown. With the use of HTS, deep intronic variants have been identified and apparent silent variants in BPD genes have been shown to alter splicing.[30-33] Variants disrupting transcription regulatory motifs located in gene promoters and enhancer regions have also been associated with BPDs and with the recent mapping of endothelial and blood cell specific enhancers, it is likely that more variants located in these regions will be identified as associated with disease in the near future.[34-36] Nevertheless, due to the challenges in interpretation of non-coding variants we have only targeted and prioritised intronic and regulatory variants if previously associated with disease. Therefore, a research analysis of novel deep intronic, silent and likely regulatory variants detected is required and such studies are best performed using whole genome sequencing data from well characterised patient cohorts.

A diagnostic HTS platform requires careful selection of diagnostic-grade TIER1 genes. The decision to include a gene to the BPD TIER1 list is made by the GinTH SSC of the ISTH. The designation of genes for diagnostic reporting involved the review of associated literature to evaluate if a gene is associated with disease in more than three independent pedigrees with convincing cosegregation data or in less than three pedigrees, but with strong functional evidence (mouse models or cell/protein studies) in addition to cosegregation data. In the future, this task will also be coordinated by the NIH supported Hemostasis/Thrombosis Clinical Domain Working Group in close partnership with the ISTH and ASH expert working groups.[37]

Our results indicate that when screening large number of patients with a HTS test that approximately 41% of reported variants are novel (Figure 2B), and following current guidelines, the majority of novel missense variants are reported as VUS (Supplemental Figure 6). International initiatives for sharing of sequence data generated for BPD patients and the sequencing of the genomes or exomes of large prospective population cohorts, like the UK Biobank, the Million Veteran Program and the 100,000 Genomes Project will lead to statistically robust approaches for the functional labelling of DNA variants, including the VUS reported in this study.[38-39]

We obtained evidence of how having reached a molecular diagnosis has influenced clinical management and counselling of patients and their close relatives. In 30 patients, variants were reported in the ANKRD26, ETV6 and RUNX1 genes leading to counseling and follow up due to the increased risk of hematological malignancies. In 7 patients with treatment refractory immune thrombocytopenia evidence was obtained of rare germline variants likely causing their condition. In six of these cases the VUS finding has prompted follow-up studies in the probands and their relatives to obtain additional evidence for pathogenicity. Finally, in 24 patients with thrombotic or coagulation disorders, we reported two or more variants in relevant TIER1 genes. We postulate that defects in hemostasis are due to the disruption of two interacting proteins in the known canonical pathways. There have been case reports of possible oligogenic architecture for thrombotic and coagulation disorders that add to the findings of this large single study.

Together these justify further functional and genetic follow-up studies to provide patients with a molecular diagnosis and better estimates of risk to offspring.

All together we report on the results of the largest gene panel sequencing study of patients with possible inherited BPDs. We included 698 individuals with unexplained bleeding disorders with normal hemostasis test results and as per our hypothesis, we observed a low diagnostic rate for this group. It is possible that the propensity of bleeding in these patients is the result of the aggregation of a large number of small effects emanating from common variants at hundreds of loci which are modifying the overall effectiveness of the hemostasis system. Estimation of polygenic risk scores has recently been reported for several common diseases such as coronary artery disease, type 2 diabetes and breast cancer[40-43].

In conclusion the ThromboGenomics test is a valuable addition to the diagnostic algorithm for patients with a high likelihood of having an inherited BPD. The results provide clinicians with a molecular diagnosis for approximately half of patients allowing for more precise prognostication and management of disease and, with cascade testing, better informed counselling of patients and their close relatives.

Data availability

Variants reported as pathogenic, likely pathogenic and variants of uncertain significance have been deposited into ClinVar under accession number xxxx.

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Authorship

Contributions: D.D., J.S. and C.T. performed experiments; K.M., M.V., O.S., S.V.V.D., R.M., S.T., L.D., N.G., D.G., M.H., A.T., C.J.P., E.T. analysed data; J.G., S.H., M.W.B., N.C., S.P., S.R-V., E.S., S.S., E.S., W.T., I.S., Y.M.C.H. and I.P., collected samples and provided clinical support; N.A.H., H.M., and S.A. provided clinical support; K.D., W.H.O., M.A.L., A.D.M., K.G. and K.F. are members of the ThromboGenomics MDT and designed the study and wrote the manuscript, which was reviewed by all remaining authors.

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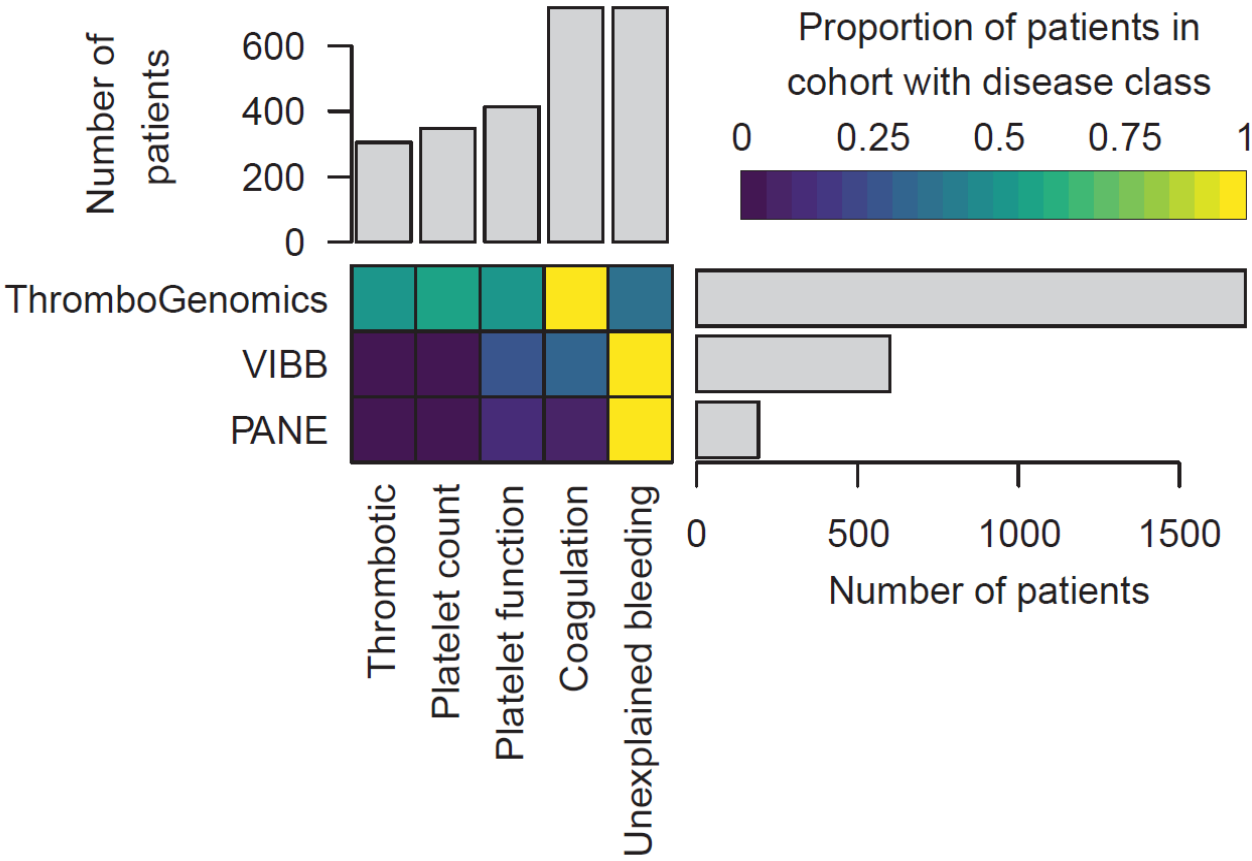
Figure 1

Classification of patients using clinical and laboratory phenotypes.

(A) Classification of all patients from the ThromboGenomics, VIBB and PANE cohorts into one of five disease classes; thrombotic, platelet count, platelet function, coagulation and unexplained bleeding.

(B) Representative HPO codes for patients characterised in each of the five disease classes.

A



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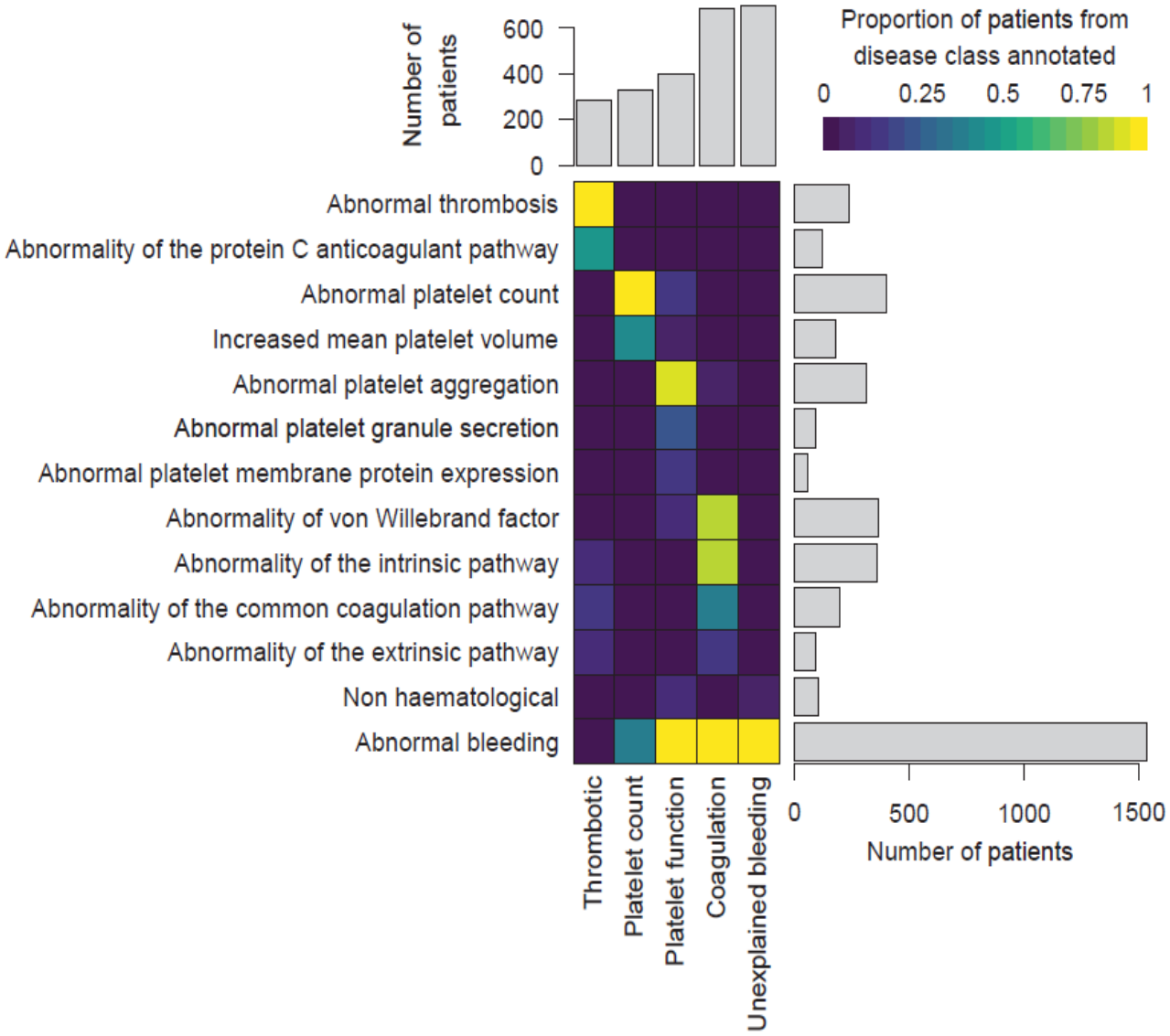


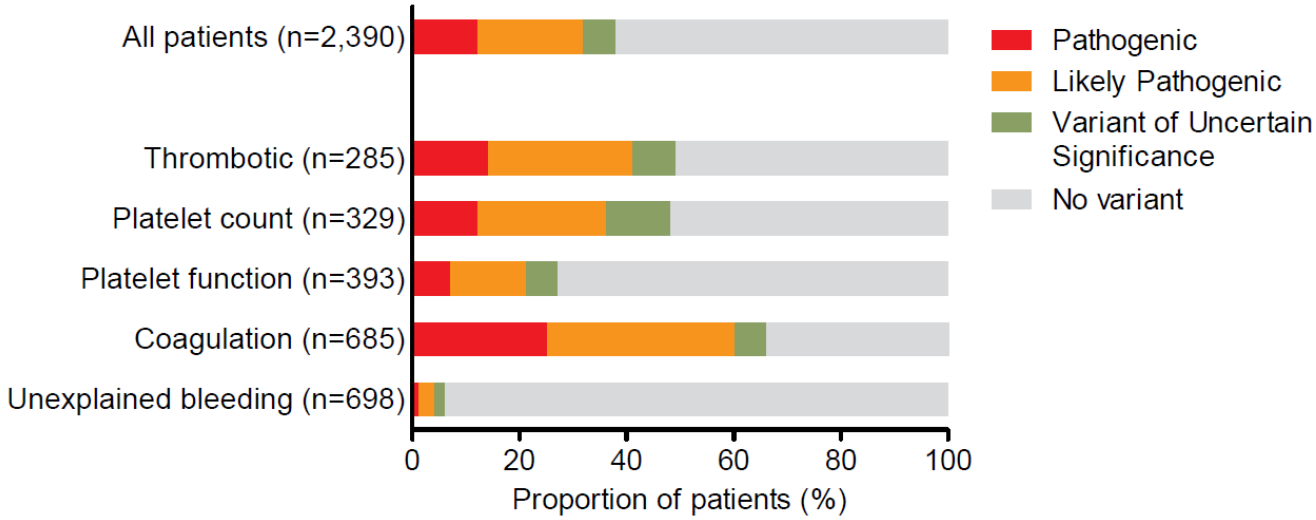
Figure 2

Diagnostic yield and proportion of novel variants by disease class.

(A) Diagnostic yield of reported variants for 2,390 index patients for each of the five disease classes; thrombotic, platelet count, platelet function, coagulation and unexplained bleeding. For patients with more than one reported variant, the most pathogenic variant was used in this analysis (n = number of index patients).

(B) Proportion of reported variants that were novel or known that were reported for patients in each disease class (n = number of variants).

A



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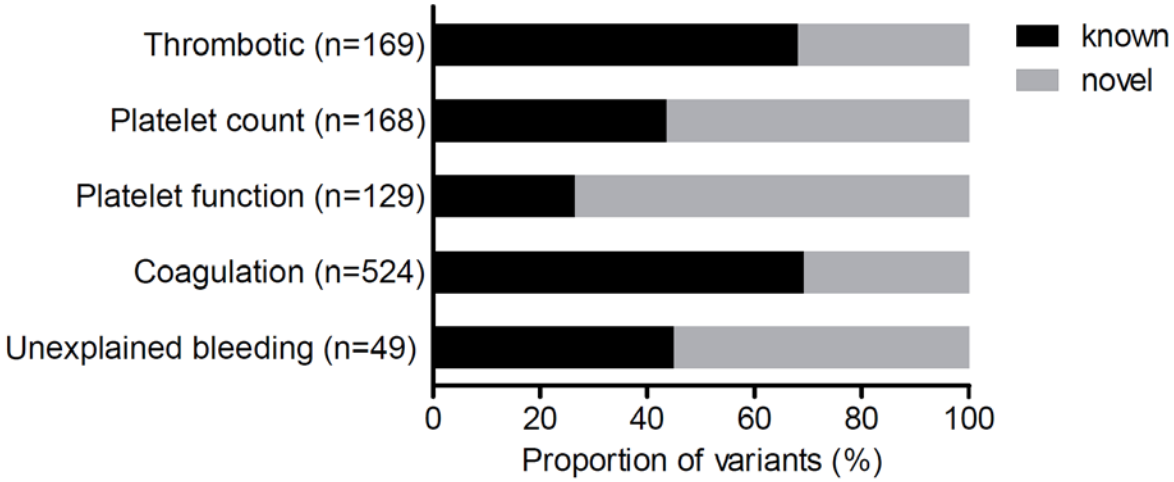


Figure 3

Gene ranking according to the number of reports per disease class.

For each disease class, genes were ranked according to number of times they were reported. Dashed lines represents, from top to bottom, the 25, 50 and 75 quantiles. Recently discovered genes and changes of mode of inheritance are in blue.

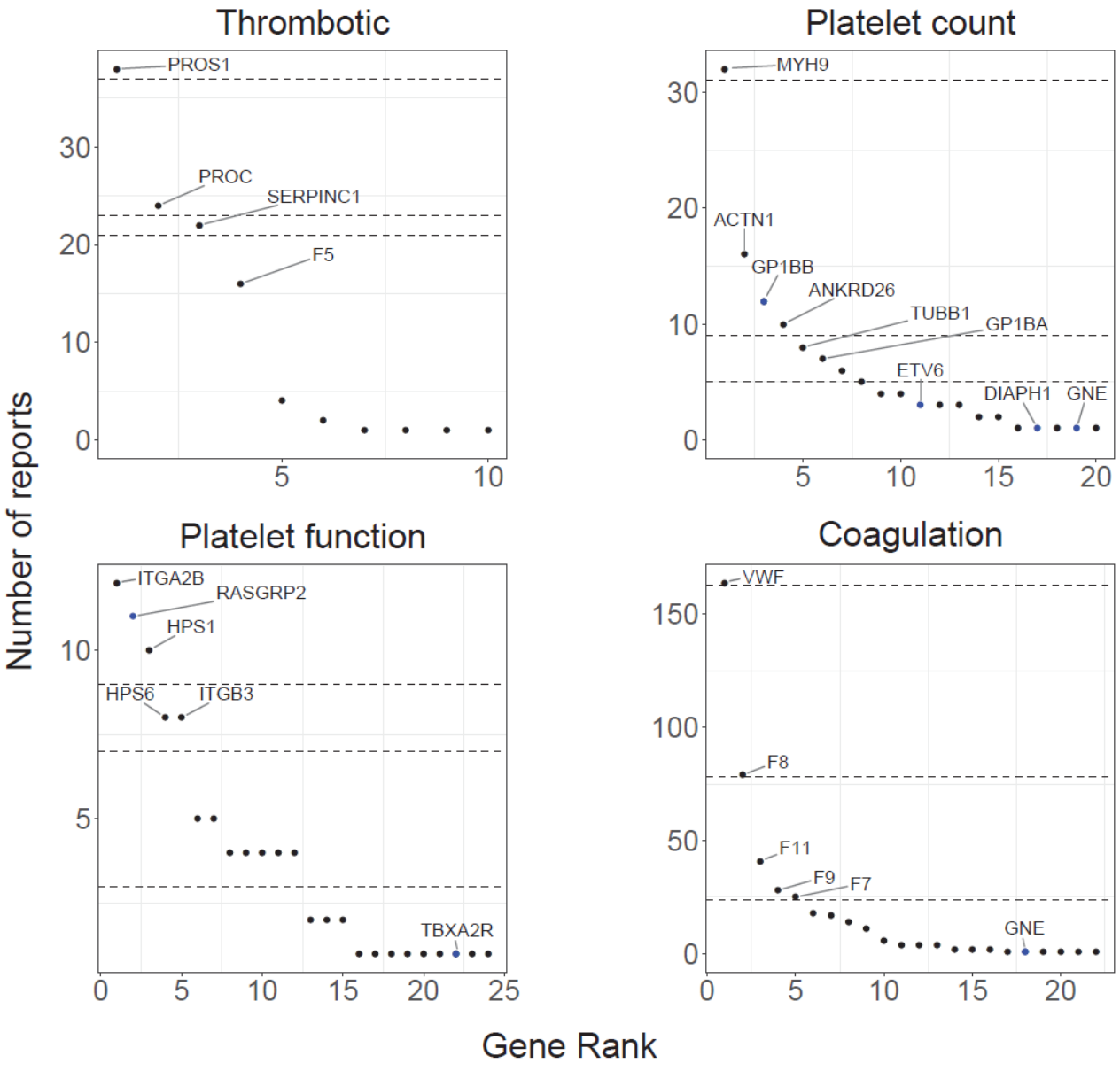
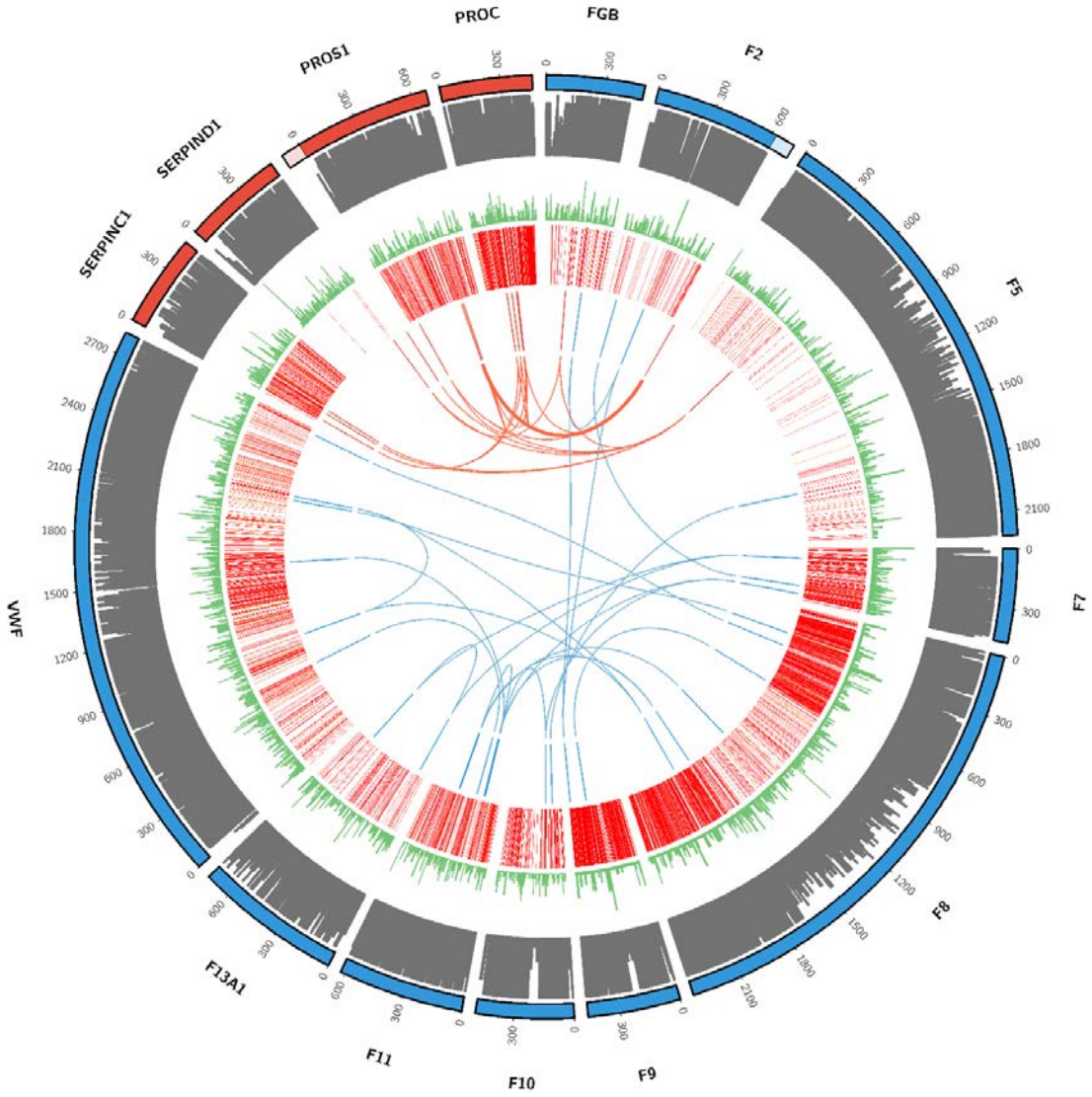


Figure 4

Oligogenic variants in patients with thrombotic (red) and coagulation (blue) disorders.

From outside to in, track 1: Amino acid (AA) numbering with pro- and anti-coagulant proteins in blue and red, respectively; lighter shades denote sections of the F2 gene 3' UTR or the PROS1 gene 5' UTR; track 2: AA conservation scores calculated using ConSurf (http://consurf.tau.ac.il/2016/); track 3: variant frequency in gnomAD (minor allele frequency normalized scale to 1/106); track 4: Disease causing (DM, red) and potentially disease causing (DM?, orange) HGMD Pro variants; track 5 and arcs: reported variants in the 11 thrombotic (red) and 13 coagulation (blue) patients with oligogenic findings.



Supplemental Information

Diagnostic high-throughput sequencing of 2,390 patients with bleeding, thrombotic and platelet disorders

Patient cohorts

ThromboGenomics cohort

International recruitment of 1602 index patients with mostly known or suspected inherited bleeding, thrombotic or platelet disorders following previously described criteria used for the validation of the ThromboGenomics HTS test (Supplemental Table 1). [1] Patients were recruited or referred to this study through two routes.

Patients recruited to the bleeding and platelet disorder arm of the NIHR-BioResource Rare Diseases study with a suspected disease etiology were sequenced using the HTS test as a pre screen prior to whole genome sequencing. Patients provided written consent according to the study ethical approval (East of England Cambridge South national research ethics committee (REC) reference 13/EE/0325). Obtaining consent from overseas patients was the responsibility of the respective principal investigators at the enrolling hospitals. Material and transfer agreements were applied to regulate the exchange of samples and data between the donor institutions and the University of Cambridge.

Patients were referred for clinical testing and consented by the referring clinician, according to local clinical practice. For UK patients, a UKHCDO approved patient information leaflet and consent form was provided for use. Consent for genetic testing was also obtained from patients and a small number of relatives and carriers (Supplemental Table 1).

Patients referred for clinical testing with severe hemophilia A were tested for the pathogenic intron 1 and intron 22 inversions in the F8 gene using inverse shifting-polymerase chain reaction prior to testing using the ThromboGenomics test.[2]

All likely pathogenic and pathogenic variants in patients referred for clinical testing have been confirmed using Sanger sequencing or Multiplex ligation-dependent probe amplification (MLPA) copy number variation (CNV) analysis in the Cambridge University Hospitals Genetic Laboratories.

Preoperative screening for mild bleeding disorders cohort (PANE)

Individuals were identified using responses to a pre operative anesthesiology bleeding questionnaire when admitted for elective surgery at Maastricht University Medical Centre (MUMC). Patients were invited to join the PANE study if they reported one or more bleeding symptoms and were over the age of 18, had no anaemia and were not using drugs that could interfere with hemostasis.[3,4] Ethical approval was obtained from the Medical Ethics Committee of MUMC (NL38767.068.11/METC11-2-096). Written informed consent was obtained from all patients. During the study visit, bleeding symptoms of the participants were evaluated by an experienced hematologist. Blood was drawn and subjected to a panel of 30 laboratory tests (Supplemental Table 2). Using local reference values, HPO codes were appended to patients with abnormal test parameters and clinical bleeding symptoms. HTS was performed for 212 unrelated study subjects that included 193 index patients and 19 subjects that did not have any bleeding symptoms or clinical test results that indicated a BPD-related HPO code (Supplemental Table 1).

Vienna Bleeding Biobank cohort (VIBB)

The VIBB study was established in collaboration with the MedUni Wien Biobank (Department of Laboratory Medicine, Medical University of Vienna, Austria, www.biobank.at) as a single-centre study.[5] Patients who were referred to the hemostasis outpatient department for investigation of a mild to moderate bleeding disorder were recruited into the The Vienna Bleeding Biobank (VIBB) study. The Ethics Committee of the Medical University of Vienna approved the project (EK No 603/2009 and 039/2006). All patients gave written informed consent before inclusion in the VIBB study. On recruitment to the study, blood was drawn and subjected to a panel of 42 laboratory tests (Supplemental Table 2). Using local reference values, HPO codes were appended to patients with abnormal test parameters and clinical bleeding symptoms. HTS was performed for 594 index patients (Supplemental Table 1).

ThromboGenomics HTS BPD test design

ROCHE NimbleGen SeqCap capture baits (ROCHE NimbleGen, Inc. Madison, WI USA) were designed to target all consensus coding sequences (CCDS) of the ThromboGenomics genes, the first and last 100 bp of introns, 5’ and 3’ UTRs, regions 1,000bp upstream of the transcription start site and the position of known pathogenic variants (see below). Regions of four allosome genes (SRY, TSPY1, AMELY, AMELX) were included to assign genomics sex. For TG.V3, capture baits were also included for a panel of 10,000 common SNVs to calculate ethnicity and relatedness estimates.

Known variants

A curated list of known disease associated variants were included in the HTS test design and for variant prioritisation.

At the time of design, all known Human Gene Mutation Database (HGMD) variants associated with the BPD genes were included in the design.[6] For TG.V2, 9,280 variants were included (HGMDPro 2015.2) and for TG.V3, 11,442 variants were included (HGMDPro 2016.4).

A set of known variants was used to prioritise the variants identified in patient samples (see below). All variants in HGMD_PRO_2017.2 were used to perform the prioritisation analysis of all TG.V2 samples. HGMD_PRO_2017.4 variants were used for the prioritisation of TG.V3 samples alongside a curated list of variants from disease specific databases. These included; 2,498 variants, with a gnomAD minor allele frequency (MAF) <0.25, from the F7, F8, F9 and VWF gene EAHAD databases (http://www.eahad-db.org/, accessed February 2017); 438 MYH9, GP1BA, GP1BB, GP9, and WAS variants with a MAF <0.01 from the LOVD databases[7] (accessed May 2017); 203 ITGA2B and ITGB3 variants from the Glanzmann Thrombasthenia Database (https://glanzmann.mcw.edu/, accessed May 2017). In addition, all variants previously reported by ThromboGenomics were used for prioritisation.

Library preparation, enrichment and sequencing

DNA samples were processed as previously described with minor modifications.[1] In short, samples were processed in batches with 500ng of each sample fragmented using a Covaris E220 (Covaris Inc., Woburn, MA, USA). Samples were processed using the ROCHE KAPA HTP Library Preparation kit (Roche Diagnostics Ltd., Burgess Hill, UK). DNA libraries were captured using ROCHE NimbleGen SeqCap ThromboGenomics capture baits (ROCHE NimbleGen, Inc. Madison, WI USA). Final libraries were quantified, samples pooled and sequenced using an Illumina Hiseq 4000 sequencer, 150 base pair (bp) paired-end (PE) run.

Initially for TG.V2, 48 samples were multiplexed per sequencing reaction. In an effort to increase sample throughput and reduce costs, methods were adjusted to multiplex 96 samples. At the same time, modifications were made to increase the DNA library fragment size with the aim of increasing read coverage in poorly performing regions. DNA was fragmented to obtain an average insert size of 220 bp for TG.V2 and 350 bp for TG.V3.

Variant calling

Single nucleotide variants (SNVs) and short insertions or deletions (INDELs) were called using GATK 3.3 using GRCh37.[8] HaplotypeCaller in a single sample mode and filtered using the following VariantFiltration expressions "MQ< 40.0 || QD < 2.0 || FS > 100.0" for SNVs and "FS > 200.0 || QD < 2.0 || ReadPosRankSum < -20.0" for INDELs. Variants were merged into multi-sample VCF files. SNVs and INDELs were annotated with their predicted impact against Ensembl 75, presence in the human gene using SnpEff 4.0.[9]

Relatedness and ancestry estimation

A panel of 10,000 SNVs were incorporated into the design of TG.V3 to estimate the degree of relatedness between individuals and to categorise an individual's ancestry into European, African, East Asian, South Asian or Other. These variants were selected from a larger panel of SNVs recommended by ROCHE for this purpose (personal communication, Todd Richmond, Roche Sequencing Solutions). Principal component analysis of samples from the 1000 Genomes Project with known ethnicity were used to generate a reference data set. Patient samples were then compared to this reference to estimate ancestry.

Relatedness was estimated using the 10,000 common SNVs using the PC-Relate function from GENESIS R package. For each sample pair, a relatedness score was calculated, ranging from 0 to 1.

Copy Number Variation

CVNs were called using a custom pipeline based on the ExomeDepth R-package (version 1.1.10).[10] ExomeDepth makes a copy number gain or loss call in a specified genomic interval by comparing the read depths in a sample and an optimised reference set of other samples. Our customisation reduces false negative and positive calls by specifically defining a set of ten unrelated reference samples. To detect small CNVs within large exons, genomic intervals of no more than 500bp were used to calculate read depth. Modifications were also made to the ExomeDepth read counting method to avoid inflation caused by reads overlapping two adjacent genomic intervals. CNVs observed in more than 10% of samples within a batch were filtered out as technical artefacts or common CNVs.

Region of interest

The region of interest (ROI) for variant prioritisation was defined as:

- all coding regions for each curated gene transcript
- +/- 15 bps into the introns
- 5' and 3' UTRs sequences
- the position of all known variants at the time of the panel design

Variant prioritisation

For each patient sample, variants identified were prioritised to provide a list of potentially pathogenic variants for interpretation by the multi-disciplinary team (MDT).

Variants were prioritised if:

- predicted to have a moderate or a high impact effect according to SnpEff
- within the snRNA gene RNU4ATAC
- located at the same nucleotide position as a known variant with a gnomAD MAF <0.025
  or
- novel with a gnomAD MAF <0.001

Variants were not prioritised if they had >3 alternate alleles (to guard against sequencing errors in repetitive regions) or if observed in the HTS samples with a frequency >=10% (to remove systematic artifacts).

Problematic regions

A number of regions are not well covered by aligned sequence generated using the HTS test and bioinformatic pipeline. A region in the UTRs of the ORAI1 gene has poor sequencing coverage, although only 6bp have a read depth less than 20x (GRCh37:Chr12:122064774-122064779).

Exon 26 of the VWF gene has poor aligned sequence coverage due to a homologous region within VWFP1, an unprocessed pseudogene on chromosome 22 (<20x aligned reads at GRCh37:Chr12:6131938-6132010). Raw sequencing reads that align to this region are manually inspected in patients with an indication of von Willebrand Disease to identify any possible variants within this exon. Any potential pathogenic variants are confirmed using PCR primers specific to the VWF gene sequence before issuing a report.

Supplemental References:

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Supplemental Tables and Figures

Supplemental Table 3 - excel sheet

Genes and disorders included in the ThromboGenomics HTS test.
TIER1 genes sequenced using the ThromboGenomics HTS panel including disease category, HGNC-approved gene symbol, gene name, disorder tested, mode of inheritance, reporting transcript, LRG accession and version of test.
AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.
*Gene without sufficient evidence for TIER1 status as of July 2018.

Supplemental Table 6 - excel sheet

Variants reported in index patients using the ThromboGenomics HTS test.
Single nucleotide variants and small insertion-deletions are in a separate tab to the CNVs.

Supplemental Table 1

Summary of the subjects from the ThromboGenomics, PANE and VIBB collections sequenced using the HTS test

	Cohort size					**2390 Index patients (prioritised variants)**		**Diagnostic yield**
	Index cases	Hemophilia carrier query[$]	Affected relatives	Non affected relatives[&]	Non affected individuals[#]	TG.V2	TG.V3	% patients
TG	1,602	18	98*	33*	-	1136 patients (4.75 variants)	466 patients (4.70 variants)	52.2%
PANE	193	-	-	-	19	193 patients (3.28 variants)	-	4.6%
VIBB	594	-	7 (mother child pairs)	-	4	-	594 patients (4.21 variants)	10.4%

TG: ThromboGenomics
TG.V2: ThromboGenomics version 2
TG.V3: ThromboGenomics version 3

* 225 subjects are related, in 97 families.
[$] Hemophilia A and B carrier query referred with no index case
[&] Non-affected relatives - includes four hemophilia carrier queries
[#] Non-affected subjects - participants in the study without appended HPO codes.

Supplemental Table 2

Key laboratory tests performed in the PANE and VIBB study subjects.

	PANE	**VIBB**
Coagulation	aPTT, PT, TT, Fibrinogen, Factors II, V, VII, VIII, IX, X, XI, XII and XIII activity	aPTT, PT, TT, Fibrinogen, Factors VIII, IX and XIII activity
vWF	VWF:Ag, VWF:Rco	VWF:Ag, VWF:Rco, VWF:CB
Platelet function	PFA, LTA with AA, TRAP, Collagen, Epinephrine, Ristocetin, ADP	PFA, LTA with AA, TRAP, Collagen, Epinephrine, Ristocetin, ADP. Platelet receptor phenotyping (GPIIb, GPIIIa, GPIX, GPIbalpha and GPIV)
Fibrinolysis	tPA, PAI, α2-antiplasmin activity, Plasminogen activity	tPA, PAI, Tpa-PAI complex, α2-antiplasmin activity

Supplemental Table 4

Variant interpretation guidelines used by ThromboGenomics multi-disciplinary team for study participants sequenced using TG.V2.

Variant classification	**Criteria**
Pathogenic	Variant that has been described in at least 3 unrelated index patients that have been reported in: - HGMD as disease mutation (DM) - F7/F8/F9/VWF EAHAD variant database (http://eahad-db.org) - ThromboGenomics BPD variant dataset
Likely Pathogenic	Variant that has been described in <3 unrelated index patients but has been reported in: - HGMD as DM - F7/F8/F9/VWF EAHAD variant database - ThromboGenomics BPD variant dataset OR Variant that is absent from control datasets (1000G, ExAC and UK10K sequencing data) and present in a gene that strongly matches the clinical and laboratory phenotype for the patient. OR Variant that is absent from control datasets (1000G, ExAC and UK10K sequencing data) and predicted to cause a loss of function
Variant of uncertain clinical significance (VUS)	Variant that has a low minor allele frequency (MAF) or is absent from control datasets (1000G, gnomAD and UK10K sequencing data) and present in a gene that matches the clinical and laboratory phenotype for the patient

Supplemental Table 5

Summary of differences between the ThromboGenomics TG.V2 and TG.V3 HTS tests, analysis methods and variant interpretation.

	TG.V2	**TG.V3**
Panel content	19 Coagulation genes 8 Thrombotic genes 53 Platelet genes HGMDPro 2015.2 variants	21 Coagulation genes 9 Thrombotic genes 66 Platelet genes HGMDPro 2016.4 variants 10,000 SNVs
Region of Interest (ROI)	0.222 Mb	0.275 Mb
Methods	DNA fragmentation – 220 bp Multiplex – 48 samples	DNA fragmentation – 350 bp Multiplex – 96 samples
Variants used in prioritisation analysis	HGMD_PRO_2017.2	HGMD_PRO_2017.4 Curated known variants
Variant interpretation	In house ThromboGenomics MDT criteria (see Supplemental Table 4)	ACMG guidelines*

***** Richards S, Aziz N, Bale S, et al. 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(5):405-424.

Supplemental Table 7

Details of index patients with oligogenic findings. VUS – Variants of Uncertain Significance.

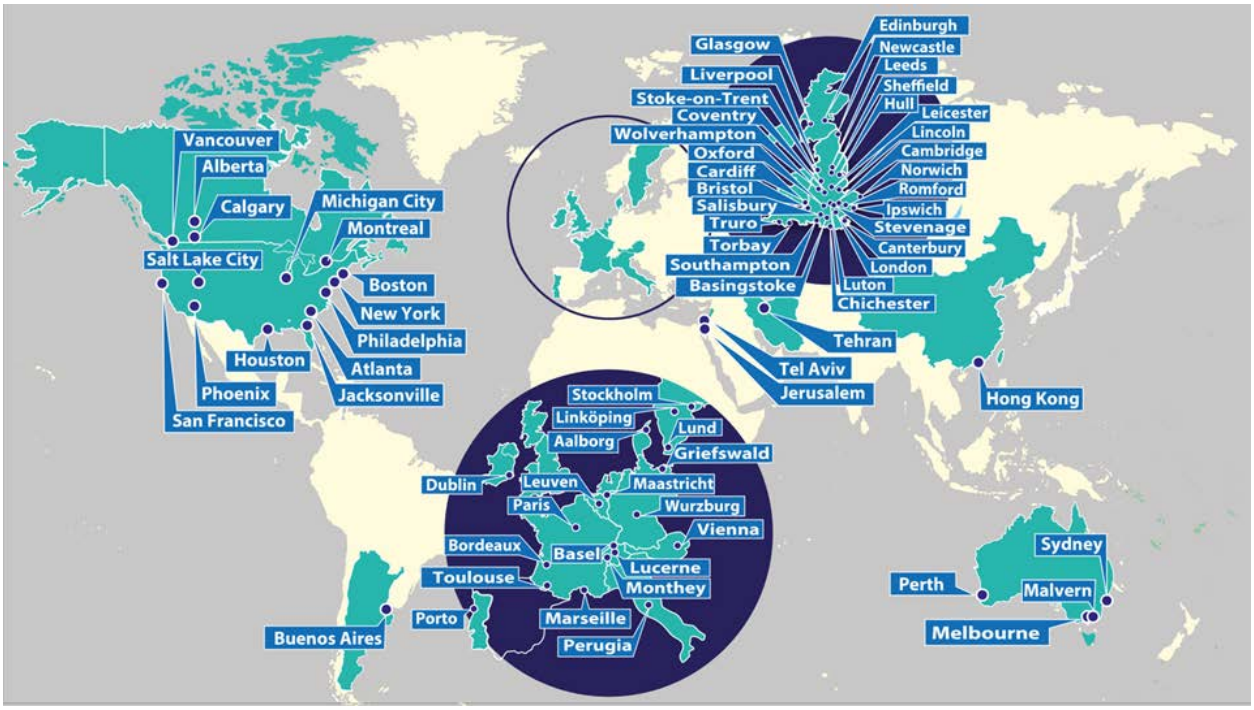
Disease class	**Index patient**	**Gene 1**	**Gene 2**	**Gene 3**
		Variant (heterozygous/homozygous) – Variant classification		
Thrombotic	**1**	F5 (het)- Pathogenic	PROS1 (het)- Likely Pathogenic	
	2	F5 (het)- Pathogenic	PROS1 (het)- VUS	
	3	SERPIND1 (het)- VUS	FGB (het)- VUS	F5 (het)- Pathogenic
	4	SERPINC1 (het)- Likely Pathogenic	F5 (het)- Pathogenic	
	5	F2 (het)- Likely Pathogenic	PROS1 (het)- Likely Pathogenic	
	6	SERPINC1 (het)- Pathogenic	F5 (het)- Pathogenic	
	7	PROS1 (het)- Likely Pathogenic	PROC (het)- Likely Pathogenic	F2 (het)- Pathogenic
	8	F2 (het)- Pathogenic	PROS1 (het:variant 1)- Likely Pathogenic PROS1 (het:variant 2)- Likely Pathogenic	
	9	F2 (het)- Pathogenic	PROS1 (het)- Pathogenic	
	10	PROC (het)- Likely Pathogenic	PROS1 (het)- Pathogenic	
	11	PROC (het)- Likely Pathogenic PROC (het)- Likely Pathogenic	SERPINC1 (het)- VUS	
Coagulation	**1**	F8 (homo) - Pathogenic	F9 (homo)- VUS	
	2	F11 (het:variant 1)- Likely Pathogenic F11 (het:variant 2) - Likely Pathogenic	F8 (het)- VUS	

Coagulation	**3**	F2 (het)- Likely Pathogenic	FGB (het)- Likely Pathogenic	F9 (het)- Pathogenic
	4	VWF (het)- Likely Pathogenic	F11 (het)- Likely Pathogenic	
	5	F2 (het)- Likely Pathogenic	F7 (het)- Likely Pathogenic	
	6	VWF (het:variant1)– VUS VWF (het:variant2)– VUS	F8 (het)- VUS	
	7	VWF (het)- VUS	F8 (het)- Pathogenic	
	8	F11 (het)- VLP Likely Pathogenic	F10 (het)- VUS	F7 (het)- VUS
	9	F11 (het)- Pathogenic	VWF (het)- Likely Pathogenic	
	10	F8 (homo)- Likely Pathogenic	VWF (het)- VUS	
	11	F5 (het)- Likely Pathogenic	F10 (het)- VUS	
	12	F8 (homo)- VUS	F11 (het)- Likely Pathogenic	
	13	F13A1 (het:variant 1) - Likely Pathogenic F13A1 (het:variant 2) - Likely Pathogenic	F7 (het)- VUS	
Coagulation and Platelet function	**1**	VWF (het)- Likely Pathogenic	P2RY12 (het)- Likely Pathogenic	
	2	VWF (het)- Likely Pathogenic	GP1BB (het)- VUS	
Coagulation and Platelet count	**1**	VWF (het)- Pathogenic	GATA1 (het)- VUS	
	2	F11 (het)- VUS	TUBB1 (het)- VUS	MYH9 (het)- VUS
Bleeding	**1**	SERPINF2 (het)- VUS	THBD (het)- VUS	

Supplemental Figures

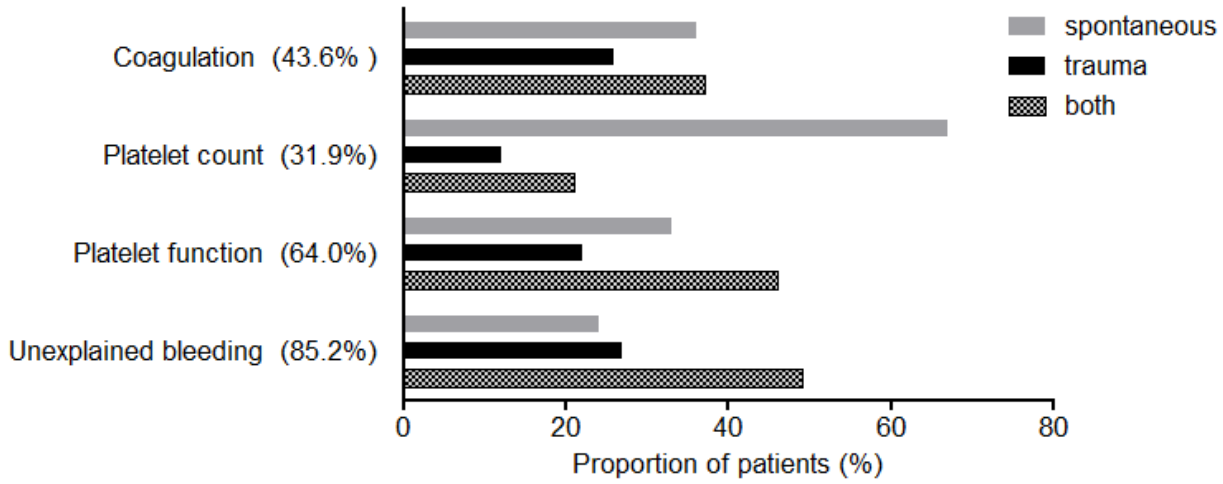
Supplemental Figure 1

A global map with the location of 72 UK and 46 non-UK hospitals which referred samples to ThromboGenomics.



Supplemental Figure 2

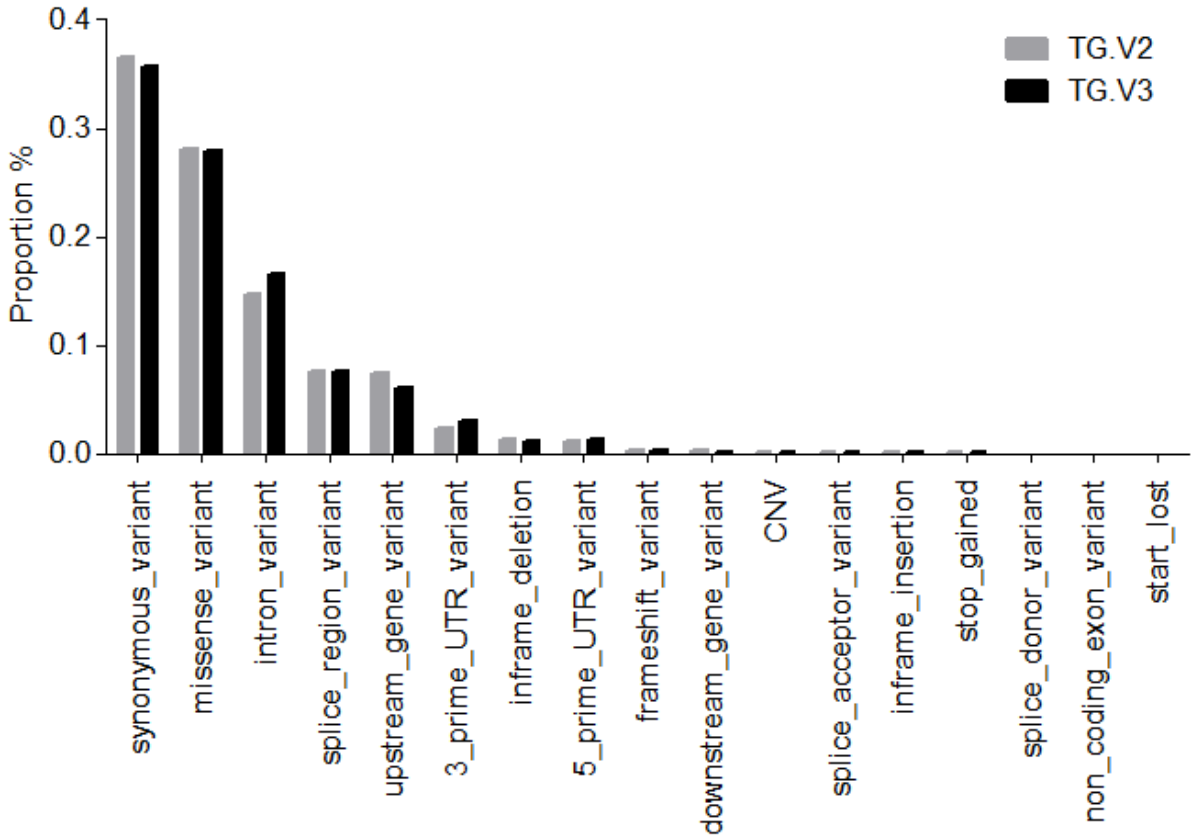
Distribution of bleeding symptoms present in the different disease classes.
The figure represents information from all patients with assigned HPO codes for 'clinically significant bleeding events' that were present in at least 10 patients. The proportion of patients per type of bleeding is on the horizontal axis. The HPO term for menorrhagia was excluded when selecting patients for this analysis. Between brackets are the proportion of patients within each disease class with bleeding phenotypes used in this analysis.



Supplemental Figure 3

The average proportion of variants and predicted effects identified in patients sequenced using TG.V2 or TG.V3.

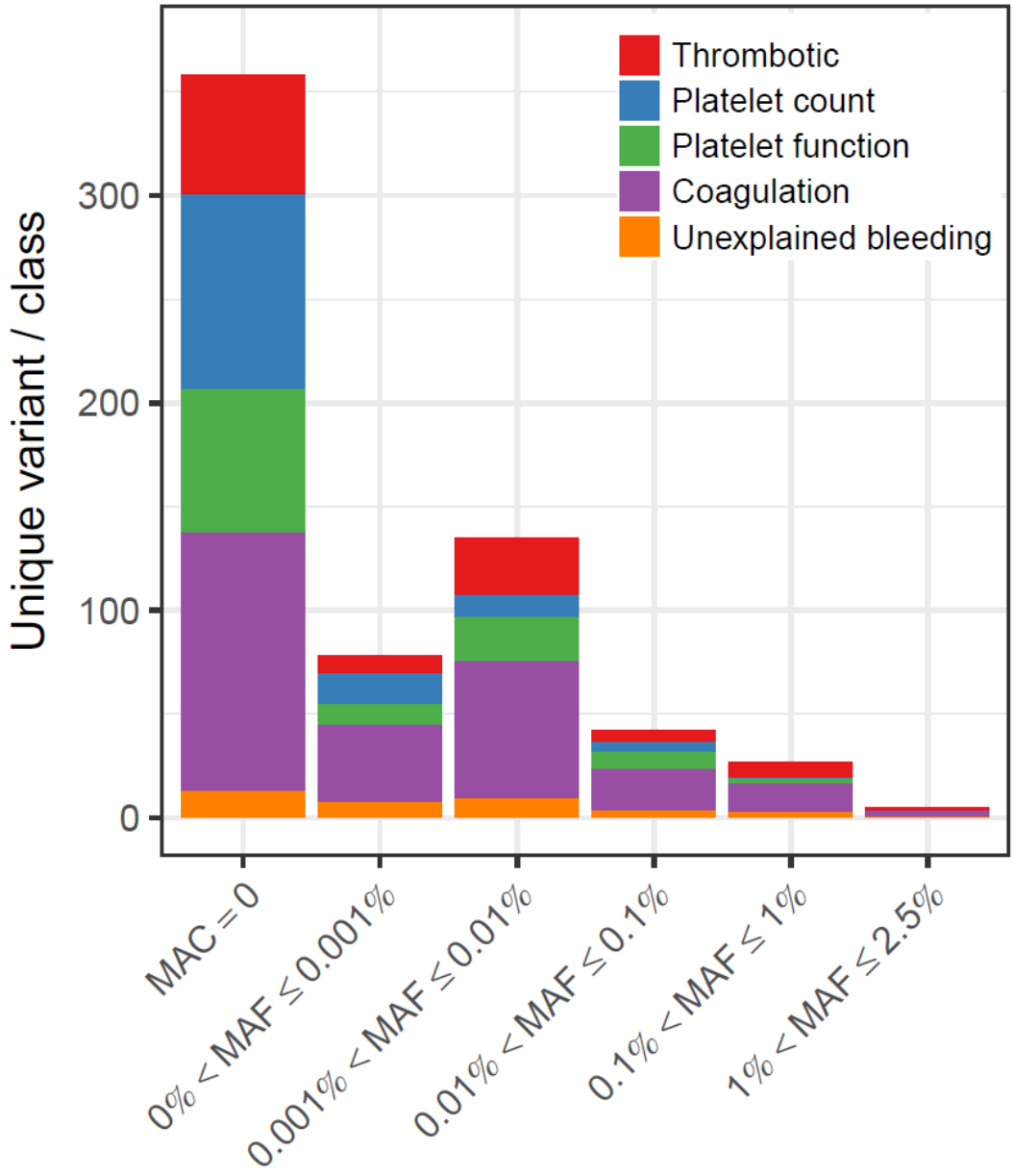
Only variants within the region of interest (ROI) were included. The average number of variants within the ROI for each patient was 156.3 and 202.4 for TG.V2 and TG.V3 respectively.



Supplemental Figure 4

Minor allele frequency or allele count (gnomAD) for the reported autosomal SNVs and indels for each disease class.

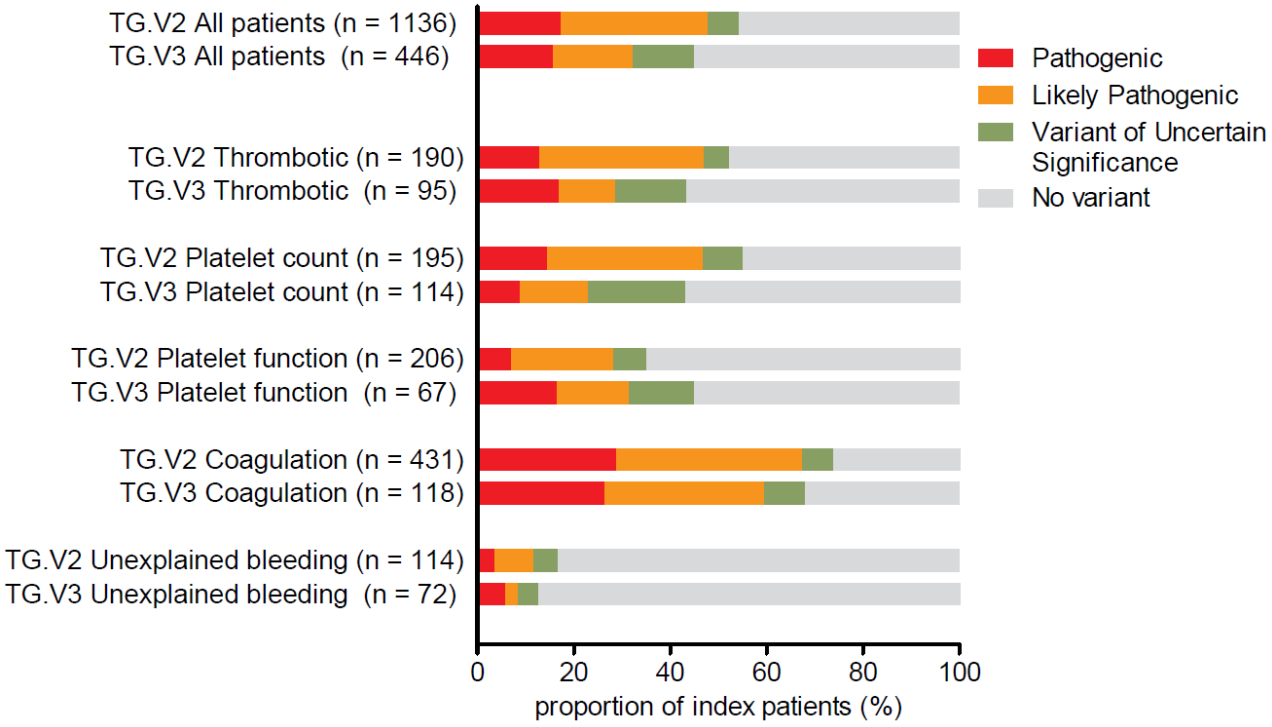
MAC: Minor Allele Count. MAF: Minor Allele Frequency. The first bin in the plot (MAC=0) corresponds to variants not observed in gnomAD.



Supplemental Figure 5

Diagnostic yield and pathogenicity of variants reported using the TG.V2 and TG.V3 test.

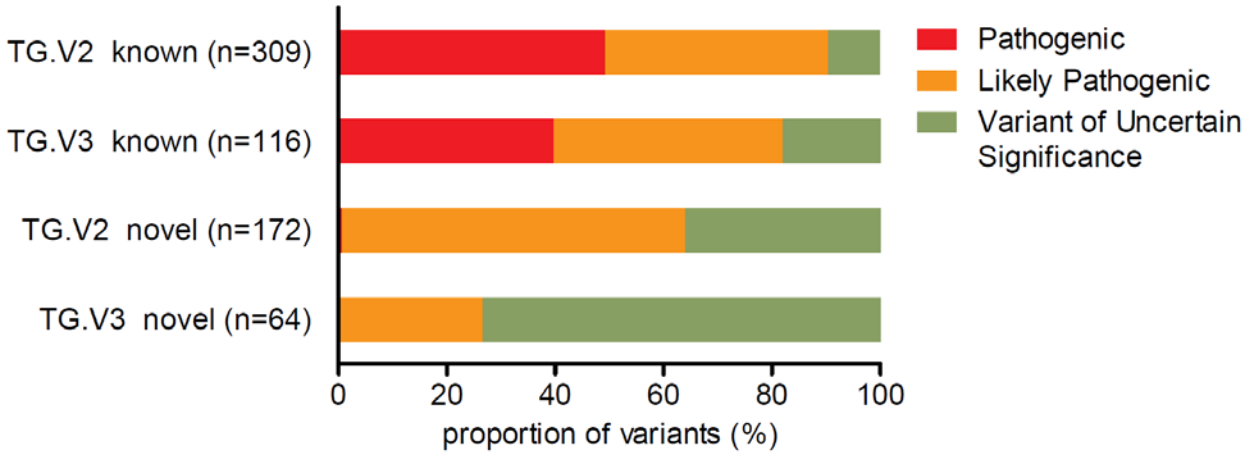
Only index patients from the ThromboGenomics collection were used for this analysis as both the TG.V2 and TG.V3 platforms were used for to sequence samples from these patients (TG.V2; 1,136 patients and TG.V3; 466 patients).



Supplemental Figure 6

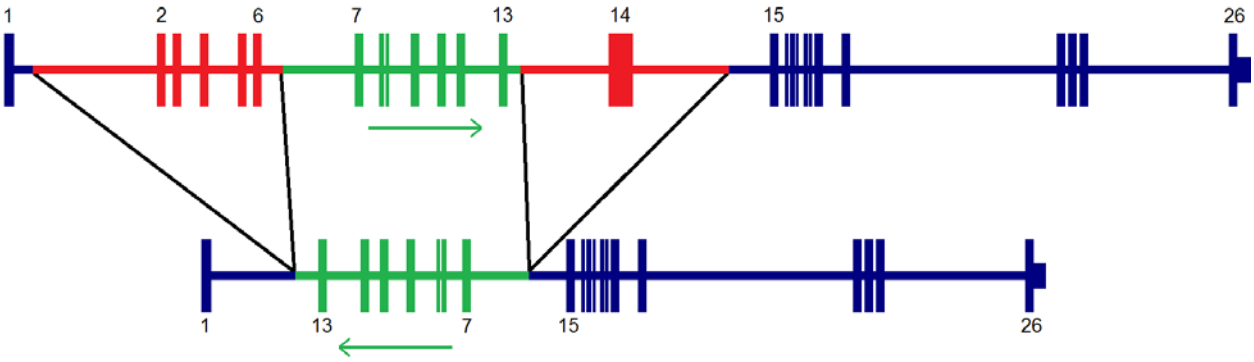
The pathogenicity of novel and known missense variants reported for TG.V2 and TG.V3 in patients from the ThromboGenomics collection.

ACMG guidelines were followed for TG.V3 patients. Analysis included 661 missense variants reported in index patients using TG.V2 (481 variants) and TG.V3 (180 variants).



Supplemental Figure 7

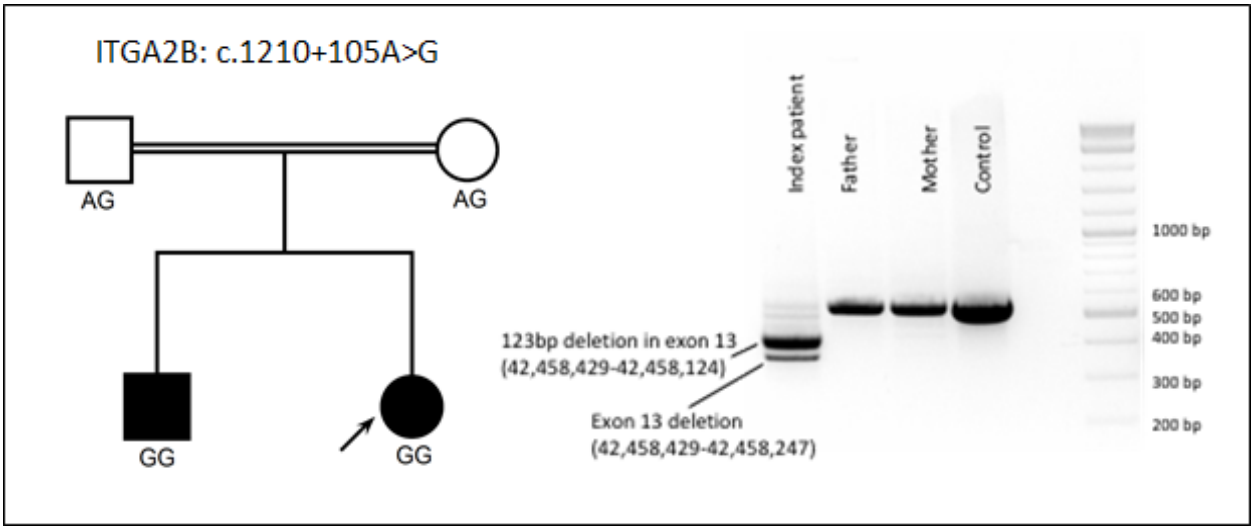
Hemizygous complex CNV associated with severe hemophilia A identified using the ThromboGenomics HTS test. Deletion of intron 1 to intron 6 (red) and intron 13 to intron 14 (red), flanking an inversion of intron 6 to intron 13 (green).



Supplemental Figure 8

Pedigree of proband with Glanzmann Thrombasthenia caused by a homozygous deep intronic variant.

Electrophoresis gel results of PCR amplicons of platelet cDNA reveals that aberrant splicing of the *ITGA2B* mRNA is associated with the deep intronic variant (c.1210+105A>G). Sanger sequencing of the aberrant splice products revealed two mRNAs, one with a deletion of all of exon 13 coding sequence and the second with a 123 bp deletion in the exon 13 coding sequence.



Supplemental Table 3

Category	Gene symbol (HGNC)	Gene name	Associated disorder	Mode of inheritance	Transcript	LRG	TG versions
Thrombotic	ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif 13	Thrombotic thrombocytopenic purpura	AR	NM_139025	LRG_544	V3
Thrombotic	HRG	histidine rich glycoprotein	Histidine-rich glycoprotein deficiency	AD	NM_000412	LRG_601	V1; V2; V3
Thrombotic	PLAT	plasminogen activator, tissue type	Tissue Plasminogen Activator deficiency	AD	NM_000930	LRG_570	V1; V2; V3
Thrombotic	PLG	plasminogen	Plasminogen deficiency	AR	NM_000301	LRG_571	V1; V2; V3
Thrombotic	PROC	protein C, inactivator of coagulation factors Va and VIIIa	Protein C deficiency	AR; AD	NM_000312	LRG_599	V1; V2; V3
Thrombotic	PROS1	protein S	Protein S deficiency	AR; AD	NM_000313	LRG_572	V1; V2; V3
Thrombotic	SERPINC1	serpin family C member 1	Antithrombin deficiency	AR; AD	NM_000488	LRG_577	V1; V2; V3
Thrombotic	SERPIND1	serpin family D member 1	Heparin cofactor 2 deficiency	AD	NM_000185	LRG_594	V1; V2; V3
Thrombotic	THBD	thrombomodulin	Thrombomodulin deficiency	AD	NM_000361	LRG_168	V1; V2; V3
Coagulation	F10	coagulation factor X	Factor X deficiency	AR	NM_000504	LRG_548	V1; V2; V3
Coagulation	F11	coagulation factor XI	Factor XI deficiency	AR; AD	NM_000128	LRG_583	V1; V2; V3
Coagulation	F12	coagulation factor XII	Factor XII deficiency	AR; AD	NM_000505	LRG_145	V3
Coagulation	F13A1	coagulation factor XIII A chain	Factor XIII deficiency	AR	NM_000129	LRG_549	V1; V2; V3
Coagulation	F13B	coagulation factor XIII B chain	Factor XIII deficiency	AR	NM_001994	LRG_550	V1; V2; V3
Coagulation	F2	coagulation factor II, thrombin	Prothrombin deficiency	AR; AD	NM_000506	LRG_551	V1; V2; V3
Coagulation	F5	coagulation factor V	Factor V deficiency	AR; AD	NM_000130	LRG_553	V1; V2; V3
Coagulation	F7	coagulation factor VII	Factor VII deficiency	AR	NM_000131	LRG_554	V1; V2; V3
Coagulation	F8	coagulation factor VIII	Haemophilia A	XLR	NM_000132	LRG_555	V1; V2; V3
Coagulation	F9	coagulation factor IX	Haemophilia B	XLR	NM_000133	LRG_556	V1; V2; V3
Coagulation	FGA	fibrinogen alpha chain	Fibrinogen deficiency	AR; AD	NM_000508	LRG_557	V1; V2; V3
Coagulation	FGB	fibrinogen beta chain	Fibrinogen deficiency	AR; AD	NM_005141	LRG_558	V1; V2; V3
Coagulation	FGG	fibrinogen gamma chain	Fibrinogen deficiency	AR; AD	NM_021870	LRG_585	V1; V2; V3
Coagulation	GGCX	gamma-glutamyl carboxylase	Multiple coagulation factor deficiency	AR	NM_000821	LRG_592	V1; V2; V3

Coagulation	KLKB1	kallikrein B1	Fletcher factor (prekallikrein) deficiency	AR	NM_000892		V1
Coagulation	KNG1	kininogen 1	Kininogen Deficiency	AR	NM_000893	LRG_598	V1; V3
Coagulation	LMAN1	lectin, mannose binding 1	Combined factor V and VIII deficiency	AR	NM_005570	LRG_595	V1; V2; V3
Coagulation	MCFD2	multiple coagulation factor deficiency 2	Combined factor V and VIII deficiency	AR	NM_139279	LRG_566	V1; V2; V3
Coagulation	SERPINE1	serpin family E member 1	Plasminogen activator Inhibitor 1 deficiency	AR; AD	NM_000602	LRG_597	V1; V2; V3
Coagulation	SERPINF2	serpin family F member 2	Alpha 2 antiplasmin deficiency	AR	NM_000934	LRG_885	V2; V3
Coagulation	VKORC1	vitamin K epoxide reductase complex subunit 1	Multiple coagulation factor deficiency type 2	AR; AD	NM_024006	LRG_582	V1; V2; V3
Coagulation	VWF	von Willebrand factor	von Willebrand disease	AR; AD	NM_000552	LRG_587	V1; V2; V3
Platelet	ABCG5	ATP binding cassette subfamily G member 5	Sitosterolemia and macrothrombocytopenia	AR	NM_022436	LRG_1181	V3
Platelet	ABCG8	ATP binding cassette subfamily G member 8	Sitosterolemia and macrothrombocytopenia	AR	NM_022437	LRG_1182	V3
Platelet	ACTN1	actinin alpha 1	Macrothrombocytopenia	AD	NM_001102	LRG_886	V2; V3
Platelet	ANKRD26	ankyrin repeat domain 26	Autosomal dominant thrombocytopenia 2	AD	NM_014915	LRG_605	V1; V2; V3
Platelet	ANO6	anoctamin 6	Scott syndrome	AR	NM_001025356	LRG_877	V2; V3
Platelet	AP3B1	adaptor related protein complex 3 beta 1 subunit	Hermansky-Pudlak syndrome	AR	NM_003664	LRG_170	V1; V2; V3
Platelet	AP3D1	adaptor related protein complex 3 delta 1 subunit	Hermansky-Pudlak syndrome	AR	NM_001261826	LRG_1207	V3
Platelet	ARPC1B	actin related protein 2/3 complex subunit 1B	Thrombocytopenia and immune deficiency	AR	NM_005720	LRG_1188	V3
Platelet	BLOC1S3	biogenesis of lysosomal organelles complex 1 subunit 3	Hermansky-Pudlak syndrome	AR	NM_212550	LRG_546	V1; V2; V3
Platelet	BLOC1S6	biogenesis of lysosomal organelles complex 1 subunit 6	Hermansky-Pudlak syndrome	AR	NM_012388	LRG_883	V2; V3
Platelet	CHST14	carbohydrate sulfotransferase 14	Ehlers-Danlos syndrome, musculocontractural type	AR	NM_130468	LRG_600	V2; V3
Platelet	COL1A1	collagen type I alpha 1 chain	Ehlers-Danlos syndrome, classic type	AD	NM_000088	LRG_1	V3
Platelet	COL3A1	collagen type V alpha 1 chain	Ehlers-Danlos syndrome, vascular type	AD	NM_000090	LRG_3	V3
Platelet	COL5A1	collagen type V alpha 2 chain	Ehlers-Danlos syndrome, classic type	AD	NM_000093	LRG_737	V3
Platelet	COL5A2	collagen type III alpha 1 chain	Ehlers-Danlos syndrome, classic type	AD	NM_000393	LRG_738	V3
Platelet	CYCS	cytochrome c, somatic	Autosomal dominant thrombocytopenia 4	AD	NM_018947	LRG_876	V1; V2; V3

Platelet	DIAPH1	diaphanous related formin 1	Macrothrombocytopenia and sensorineural hearing loss	AD	NM_001079812	LRG_1117	V2; V3
Platelet	DTNBP1	dystrobrevin binding protein 1	Hermansky-Pudlak syndrome	AR	NM_032122	LRG_588	V1; V2; V3
Platelet	ETV6	ETS variant 6	Thrombocytopenia and susceptibility to cancer	AD	NM_001987	LRG_609	V2; V3
Platelet	FERMT3	fermitin family member 3	Leukocyte integrin adhesion deficiency, type III	AR	NM_178443	LRG_180	V2; V3
Platelet	FLI1	Fli-1 proto-oncogene, ETS transcription factor	Paris-Trousseau thrombocytopenia and Jacobson syndrome	AD	NM_002017	LRG_646	V1; V2; V3
Platelet	FLNA	filamin A	Macrothrombocytopenia	XLR	NM_001110556		V1; V2; V3
Platelet	FYB1	FYN binding protein 1	Thrombocytopenia 3	AR	NM_001465		V3
Platelet	GATA1	GATA binding protein 1	X-linked thrombocytopenia with dyserythropoiesis	XLR	NM_002049	LRG_559	V1; V2; V3
Platelet	GFI1B	growth factor independent 1B transcriptional repressor	Platelet-type bleeding disorder 17	AD	NM_004188	LRG_879	V2; V3
Platelet	GNE	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	Myopathy associated with thrombocytopenia	AR	NM_005476	LRG_1197	V2; V3
Platelet	GP1BA	glycoprotein Ib platelet alpha subunit	Bernard-Soulier syndrome; AD macrothrombocytopenia; Platelet-type von Willebrand disease	AR; AD	NM_000173	LRG_480	V1; V2; V3
Platelet	GP1BB	glycoprotein Ib platelet beta subunit	Bernard-Soulier syndrome; AD macrothrombocytopenia	AR; AD	NM_000407	LRG_478	V1; V2; V3
Platelet	GP6	glycoprotein VI platelet	Bleeding diathesis due to glycoprotein VI deficiency	AR	NM_016363	LRG_560	V1; V2; V3
Platelet	GP9	glycoprotein IX platelet	Bernard-Soulier syndrome	AR	NM_000174	LRG_477	V1; V2; V3
Platelet	HOXA11	homeobox A11	Amegakaryocytic thrombocytopenia with radioulnar synostosis	AD	NM_005523	LRG_561	V1; V2; V3
Platelet	HPS1	HPS1, biogenesis of lysosomal organelles complex 3 subunit 1	Hermansky-Pudlak syndrome	AR	NM_000195	LRG_562	V1; V2; V3
Platelet	HPS3	HPS3, biogenesis of lysosomal organelles complex 2 subunit 1	Hermansky-Pudlak syndrome	AR	NM_032383	LRG_563	V1; V2; V3
Platelet	HPS4	HPS4, biogenesis of lysosomal organelles complex 3 subunit 2	Hermansky-Pudlak syndrome	AR	NM_022081	LRG_590	V1; V2; V3
Platelet	HPS5	HPS5, biogenesis of lysosomal organelles complex 2 subunit 2	Hermansky-Pudlak syndrome	AR	NM_181507	LRG_586	V1; V2; V3
Platelet	HPS6	HPS6, biogenesis of lysosomal organelles complex 2 subunit 3	Hermansky-Pudlak syndrome	AR	NM_024747	LRG_564	V1; V2; V3
Platelet	ITGA2B	integrin subunit alpha 2b	Glanzmann thrombasthenia; Platelet-type bleeding disorder 16	AR; AD	NM_000419	LRG_479	V1; V2; V3
Platelet	ITGB3	integrin subunit beta 3	Glanzmann thrombasthenia; Platelet-type bleeding disorder 16	AR; AD	NM_000212	LRG_481	V1; V2; V3
Platelet	KDSR	3-ketodihydrosphingosine reductase	Thrombocytopenia and erythrokeraderma	AR	NM_002035	LRG_1196	V3

Platelet	LYST	lysosomal trafficking regulator	Chediak-Higashi syndrome (CHS)	AR	NM_000081	LRG_143	V1; V2; V3
Platelet	MECOM	MDS1 and EVI1 complex locus	Amegakaryocytic thrombocytopenia with radioulnar synostosis 2	AD	NM_001105078	LRG_1118	V3
Platelet	MPIG6B	megakaryocyte and platelet inhibitory receptor G6b	Thrombocytopenia, anemia and myelofibrosis	AR	NM_138272	LRG_1003	V3
Platelet	MPL	MPL proto-oncogene, thrombopoietin receptor	Congenital amegakaryocytic thrombocytopenia (CAMT)	AR	NM_005373	LRG_510	V1; V2; V3
Platelet	MYH9	myosin heavy chain 9	May-Hegglin and other MYH9 disorders	AD	NM_002473	LRG_567	V1; V2; V3
Platelet	NBEA	neurobeachin	Dense granule abnormality	AD	NM_015678	LRG_602	V1; V2; V3
Platelet	NBEAL2	neurobeachin like 2	Gray platelet syndrome	AR	NM_015175	LRG_568	V1; V2; V3
Platelet	ORAI1	ORAI calcium release-activated calcium modulator 1	Stormorken syndrome	AD	NM_032790	LRG_93	V2; V3 *
Platelet	P2RY12	purinergic receptor P2Y12	ADP receptor defect	AR	NM_022788	LRG_569	V1; V2; V3
Platelet	PLA2G4A	phospholipase A2 group IVA	Deficiency of phospholipase A2, group IV A	AR	NM_024420	LRG_596	V1; V2; V3
Platelet	PLAU	plasminogen activator, urokinase	Quebec platelet disorder	AD	NM_002658	LRG_593	V1; V2; V3
Platelet	PTPN11	protein tyrosine phosphatase, non-receptor type 11	Noonan Syndrome	AD	NM_002834	LRG_614	V3
Platelet	RASGRP2	RAS guanyl releasing protein 2	Platelet-type bleeding disorder 18	AR	NM_153819	LRG_100	V2; V3
Platelet	RBM8A	RNA binding motif protein 8A	Thrombocytopenia-absent radius syndrome (TAR)	AR	NM_005105	LRG_574	V1; V2; V3
Platelet	RNU4ATAC	RNA, U4atac small nuclear (U12-dependent splicing)	Roifman Syndrome	AR	NR_023343	LRG_1202	V3
Platelet	RUNX1	runt related transcription factor 1	Familial platelet disorder with predisposition to AML	AD	NM_001754	LRG_482	V1; V2; V3
Platelet	SLFN14	schlafen family member 14	Platelet-type bleeding disorder 20	AD	NM_001129820	LRG_1114	V3
Platelet	STIM1	stromal interaction molecule 1	Stormorken syndrome	AD	NM_003156	LRG_164	V2; V3
Platelet	STXBP2	syntaxin binding protein 2	Familial hemophagocytic lymphohistiocytosis type 5	AR	NM_006949	LRG_165	V2; V3
Platelet	TBXA2R	thromboxane A2 receptor	Thromboxane A2 receptor defect	AR; AD	NM_001060	LRG_578	V1; V2; V3
Platelet	TBXAS1	thromboxane A synthase 1	Ghosal syndrome	AR	NM_030984	LRG_579	V1; V2; V3
Platelet	THPO	thrombopoietin	Thrombocytopenia and thrombocythemia 1	AD	NM_000460	LRG_580	V1; V2; V3
Platelet	TPM4	tropomyosin 4	Macrothrombocytopenia	AD	NM_001145160		V3 *
Platelet	TUBB1	tubulin beta 1 class VI	Macrothrombocytopenia, beta-tubulin 1 related	AD	NM_030773	LRG_581	V1; V2; V3

Platelet	VIPAS39	VPS33B interacting protein, apical-basolateral polarity regulator, spe-39 homolog	ARC syndrome (Arthrogryposis, renal dysfunction, and cholestasis 1)	AR	NM_001193315	LRG_1019	V2; V3
Platelet	VPS33B	VPS33B, late endosome and lysosome associated	ARC syndrome (Arthrogryposis, renal dysfunction, and cholestasis 2)	AR	NM_018668	LRG_884	V2; V3
Platelet	WAS	Wiskott-Aldrich syndrome	Wiskott-Aldrich syndrome	XLR	NM_000377	LRG_125	V1; V2; V3