Supporting Information

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SI Methods

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ThromboGenomics Working Group. This study is part of the ThromboGenomics working group of the Scientific and Standardization Committee of the International Society for Thrombosis and Haemostasis, which is committed to improving the diagnosis of rare inherited bleeding and platelet disorders by taking advantage of advances in next generation sequencing and DNA capture technologies. Its goal is to develop a publicly accessible reference database of genes and DNA sequence variants related to bleeding disorders and to establish a gene panel to test for the presence of such gene lesions. Descriptions of its projects and its curation and approval processes are described on its website (https://thrombogenomics.org.uk).

Mutagenesis, Cell Transfection, and αIIbβ3 Analysis. Variants αIIb P176H, P943A and β3 C547G were generated using the QuikChange XL Site-directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. The different cDNAs were all sequenced to confirm that the variants were introduced as predicted.

HEK293 cells were transfected with either normal human αIIb and β3 or variant cDNA's using Fugene 6 mammalian transfection reagent (Promega). Cells were harvested 48 h after transfection in Hepes-modified Tyrode's (HBMT) buffer containing 1 mM $MgCl_2$ and 2 mM $CaCl_2$ (10^5 cells/mL, 100 μL) and incubated with Alexafluor 488-conjugated mAb 10E5 (10 μg/mL), which binds to the cap region of the αIIb β-propeller domain (1), to assess αIIbβ3 expression. To assess αIIbβ3 ligand binding, cells were incubated for 30 min at 37 °C with 1 mM DTT and 200 μg/mL Alexa647-conjugated fibrinogen, with or without 10 mM EDTA. Cells were washed and resuspended in HBMT buffer containing 1 mM $MgCl_2$ and 2 mM $CaCl_2$ and analyzed by flow cytometry. Data are expressed as net normalized fibrinogen binding (Alexa647-conjugated fibrinogen geometric mean fluorescence intensity in the absence of EDTA minus Alexa647-conjugated fibrinogen geometric mean fluorescence intensity in the presence of EDTA, divided by αIIbβ3 expression as judged by the binding of mAb 10E5).

Transfected cells were also analyzed by immunoblotting for αIIb and β3 expression. Cells were washed in PBS and lysed by incubation for 60 min with lysis buffer consisting of 150 mM NaCl, 25 mM Tris/HCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride, and 10 μg/mL leupeptin, pH 7.4. After centrifugation, protein content was determined (BCA protein assay kit; Thermo Scientific) and 40–80 μg of total cell lysate was electrophoresed on 7.5% SDS/PAGE gels. Proteins were transferred to a nitrocellulose membrane (Millipore), blocked for 1 h with a proprietary blocking buffer (Odyssey), and incubated with the appropriate primary antibody at a dilution of 1:1,000 (vol/vol) in the same blocking buffer with 0.1% (vol/vol) Tween 20 (16 h, 4 °C). After washing, membranes were incubated at 4 °C with species-specific, infrared-labeled secondary antibodies (LI-COR). Protein bands were visualized using an infra-red detector (Odyssey).

1. Xiao T, Takagi J, Coller BS, Wang JH, Springer TA (2004) Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* 432(7013):59–67.

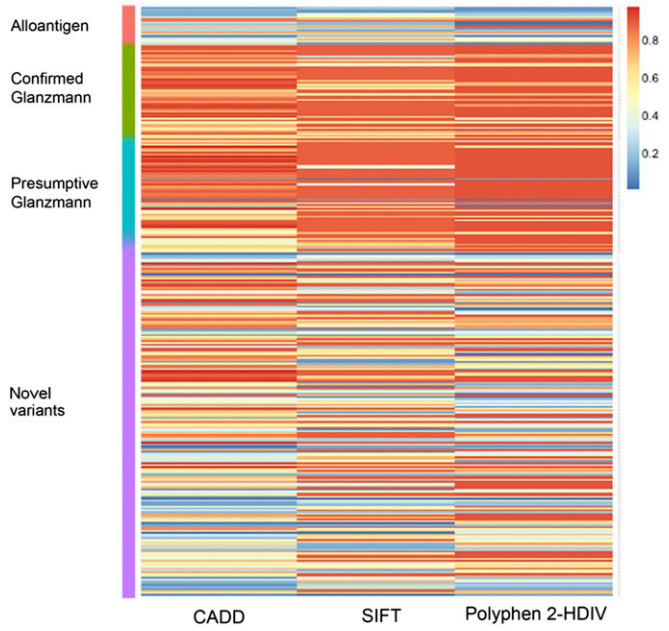


Fig. S1. Heat map showing the concordance among the algorithms for individual variants in each group.

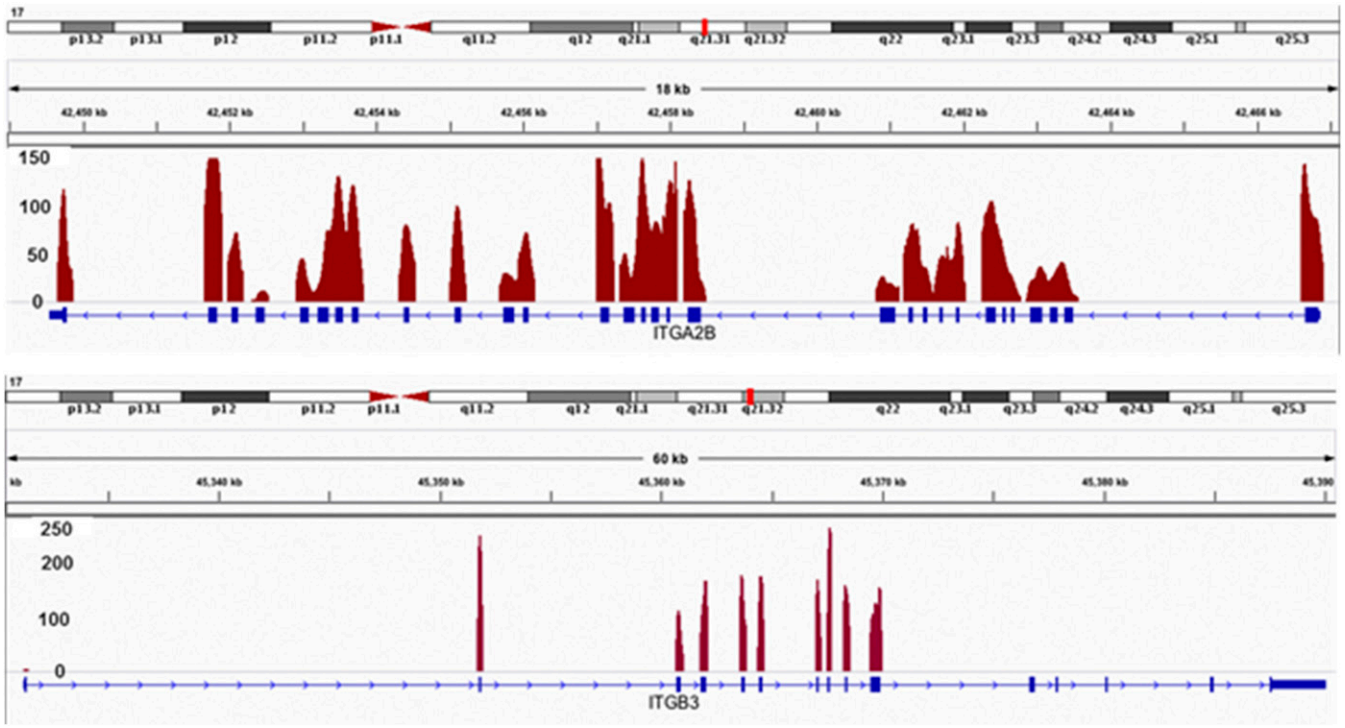
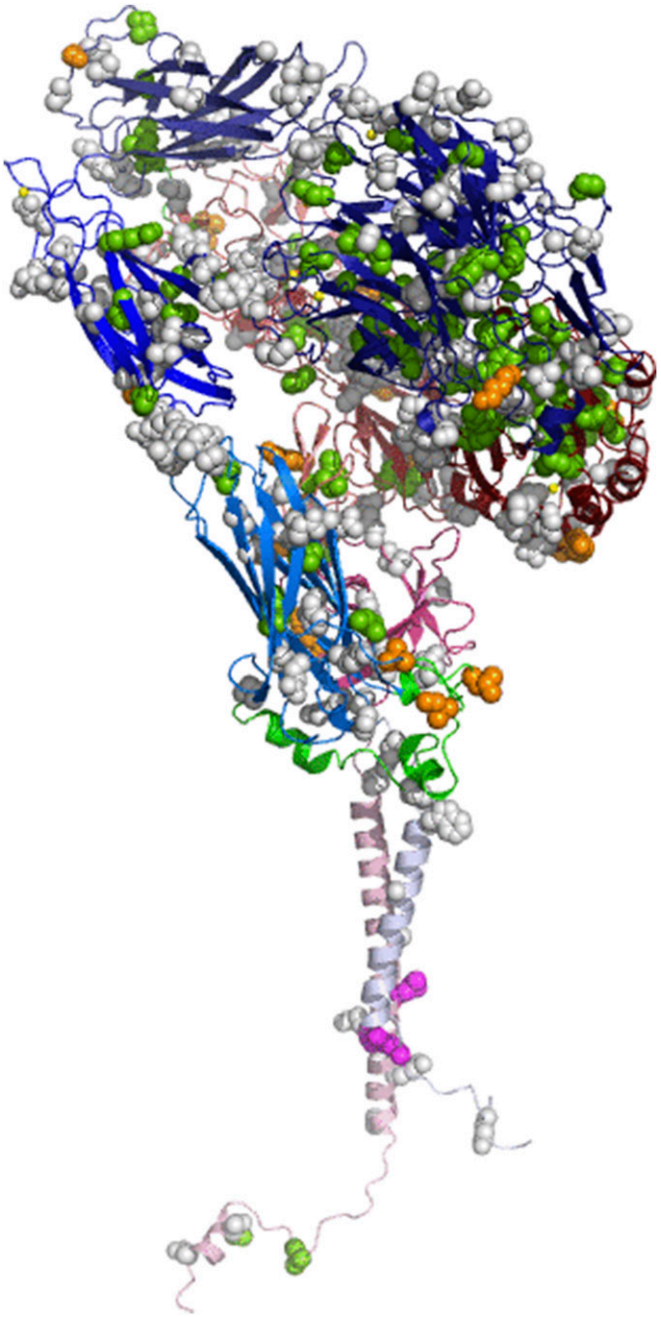


Fig. S2. The average coverage of the ESP data over the genes of interest. Not all exons have equal coverage; this is particularly true for *ITGB3* where the first and the last four exons are not covered. For *ITA2B* a minimum coverage of 20× is achieved over most exons with the exception of exon 27.



Movie S1. Model of αIIbβ3 with Glanzmann thrombasthenia mutations (green spheres), macro/anisothrombocytopenia mutations (magenta spheres), alloantigens (orange spheres), and novel missense variants (gray spheres).

Movie S1

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)