ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: [https://www.clinicalgenome.org/affiliation/50040](https://www.clinicalgenome.org/affiliation/50040)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease (MONDO ID)</th>
<th>Clinically significant transcript</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ITGA2B</em></td>
<td>Glanzmann thrombasthenia (MONDO: 0010119)</td>
<td>NM_000419.4</td>
</tr>
<tr>
<td><em>ITGB3</em></td>
<td>Glanzmann thrombasthenia (MONDO: 0010119)</td>
<td>NM_000212.2</td>
</tr>
</tbody>
</table>

**Pathogenic Criteria**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Original Criteria Description</th>
<th>Specification(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Strong Criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVS1</td>
<td>Null variant in a gene where LOF is a known mechanism of disease</td>
<td>Use decision tree as per SVI WG with specified “regions critical to protein function”.</td>
</tr>
</tbody>
</table>
| PS2/PM6_VeryStrong | *De novo* in a patient with disease and no family history | Use proposed SVI point recommendations.  
- Only applicable when proband has a known pathogenic or likely pathogenic variant with the *de novo* variant. |
| PM3_VeryStrong | For recessive disorders, detected in trans with a pathogenic variant | Use proposed SVI point recommendations. Both variants must be classified using *ITGA2B/ITGB3 Rule Specifications*. |
| **Strong Criteria** | | |
| PS1 | Same amino acid change as a previously established pathogenic variant regardless of nucleotide change | Use with no specification. |

**Related publication(s):**

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<table>
<thead>
<tr>
<th>Moderate Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PM1</strong></td>
</tr>
<tr>
<td><strong>PM3</strong></td>
</tr>
<tr>
<td><strong>PM4</strong></td>
</tr>
</tbody>
</table>
### ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *ITGA2B, ITGB3*

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<table>
<thead>
<tr>
<th>PM5</th>
<th>Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before</th>
<th>Use with no specification.</th>
</tr>
</thead>
</table>
| PS2_Moderate/PM6 | De novo in a patient with disease and no family history | Use proposed SVI point recommendations.  
- Only applicable when proband has a known pathogenic or likely pathogenic variant with the de novo variant |
| PS3_Moderate | Well-established in vitro or in vivo, functional studies supportive of a damaging effect on the gene or gene product | In a model organism or heterologous cell line, significantly reduced surface protein expression (5-25%). |
| PP1_Moderate | Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease | Segregation in proband plus 2 affected relatives.  
*Affected relatives must have both variants identified in proband. |
| PP4_Moderate | Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology | Proband with clinical diagnosis of GT based on the presence of mucocutaneous bleeding and appropriate lab abnormalities. Full sequencing of both genes is required at this strength. |

### Supporting Criteria

<table>
<thead>
<tr>
<th>PM2_Supporting</th>
<th>Absent in population databases (or at extremely low frequency if recessive)</th>
<th>Prevalence &lt;1/10,000 (&lt;0.0001) alleles in gnomAD.</th>
</tr>
</thead>
</table>
| PP1 | Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease | Segregation in proband plus 1 affected relative.  
*Affected relatives must have both variants identified in proband. |
| PP2 | Missense variant in a gene that has a low rate of benign missense | This rule does not apply because benign missense variants are not rare. |

**Related publication(s):**

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ClinGen_Platelet ACMG Specifications_v1
ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

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<table>
<thead>
<tr>
<th>Rule</th>
<th>Description</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP3</td>
<td>Multiple lines of computational evidence support a deleterious effect on the gene or gene product</td>
<td>REVEL score of $&gt;0.7$ -OR- $&gt;2$ independent in silico missense predictors predict a damaging impact</td>
</tr>
<tr>
<td>PP4</td>
<td>Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology</td>
<td>Proband with clinical diagnosis of GT based on the presence of mucocutaneous bleeding and appropriate lab abnormalities.</td>
</tr>
<tr>
<td>PP5</td>
<td>Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.</td>
<td>As per SVI recommendation, do not use this rule.</td>
</tr>
<tr>
<td>PS2/PM6_Supporting</td>
<td><em>De novo</em> in a patient with disease and no family history</td>
<td>Use proposed SVI point recommendations. - Only applicable when proband has a known pathogenic or likely pathogenic variant with the <em>de novo</em> variant</td>
</tr>
<tr>
<td>PM3_Supporting</td>
<td>For recessive disorders, detected in trans with a pathogenic variant</td>
<td>Use proposed SVI point recommendations. Both variants must be classified using <em>ITGA2B/ITGB3</em> Rule Specifications.</td>
</tr>
<tr>
<td>PM5_Supporting</td>
<td>Novel missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before</td>
<td>Use at adjusted strength.</td>
</tr>
</tbody>
</table>

**Related publication(s):**

Please check [https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria](https://www.clinicalgenome.org/affiliation/50040/docs/assertion-criteria) for the most recent version.

**ClinGen_Platelet ACMG Specifications_v1**
### Benign Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Original Criteria Description</th>
<th>Specification(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stand Alone Criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA1</td>
<td>Allele frequency is greater than expected for disorder</td>
<td>Frequency cutoff of 0.24% (&gt;0.0024 at 99.99% CI w/subpopulation w/min of 5 alleles).</td>
</tr>
<tr>
<td><strong>Strong Criteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS1</td>
<td>Allele frequency is greater than expected for the disorder</td>
<td>Frequency cutoff of 0.158% (&gt;0.00158 at 99.99% CI w/subpopulation w/min of 5 alleles)</td>
</tr>
<tr>
<td>BS2</td>
<td>Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age</td>
<td>&gt;1 homozygote who is unaffected proven with at least aggregometry.</td>
</tr>
</tbody>
</table>
| BS3 | Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing | - Must demonstrate normal aggregometry in a transgenic mouse model.  
-OR-  
- In a heterologous cell line, must demonstrate BOTH normal expression and normal protein function. |
| BS4 | Lack of segregation in affected members of a family | Variant not detected in an affected family member. |
| **Supporting Criteria** | | |
| BP1 | Missense variant in a gene for which primarily truncating variants are known to cause disease | Rule does not apply as truncating variants do not predominate and missense variants are a known cause of disease. |
| BP2 | Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed *in cis* with a pathogenic variant in any inheritance pattern. | Use as written for recessive variants (i.e. - variant must be observed *in cis* with a pathogenic variant). |

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**ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1**

This version specified for the following genes: *ITGA2B, ITGB3*

**Expert Panel Page:** https://www.clinicalgenome.org/affiliation/50040

**Date Approved:** June 12, 2020
BP3: In-frame deletions/insertions in a repetitive region without a known function
   Use with no specification.

BP4: Multiple lines of computational evidence suggest no impact on gene/gene product
   REVEL score of < 0.25

BP5: Variant found in a case with an alternate molecular basis for disease
   Do not use this rule as an individual can be a carrier of an unrelated pathogenic variant for a recessive disorder.

BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation
   As per SVI recommendation, do not use this rule.

BP7: A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.
   Use with no specification.

RULES FOR COMBINING PATHOGENIC CRITERIA – Unchanged at this time

PATHOGENIC
   1. 1 Very Strong AND
      a. ≥1 Strong OR
      b. ≥2 Moderate OR
      c. 1 Moderate and 1 Supporting OR
      d. ≥2 Supporting
   2. ≥2 Strong OR
   3. 1 Strong AND
      a. ≥3 Moderate OR
      b. 2 Moderate AND ≥2 Supporting OR
      c. 1 Moderate AND ≥4 Supporting

LIKELY PATHOGENIC
   1. 1 Very Strong AND 1 Moderate OR
   2. 1 Strong AND 1-2 Moderate OR
   3. 1 Strong AND ≥2 Supporting OR
   4. ≥3 Moderate OR
   5. 2 Moderate AND ≥2 Supporting OR

Related publication(s):

Date Approved: June 12, 2020

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ClinGen_Platelet ACMG Specifications_v1
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Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

6. 1 Moderate AND ≥4 Supporting
7. 1 Very strong AND 1 Supporting

RULES FOR COMBINING BENIGN CRITERIA - Unchanged at this time

Benign
1. 1 Stand-Alone OR
2. ≥2 Strong

Likely Benign
1. 1 Strong and 1 Supporting OR
2. ≥2 Supporting

Introduction to Glanzmann thrombasthenia (GT)

Glanzmann thrombasthenia (GT) is an inherited platelet disorder, in which platelets fail to aggregate to physiologic stimuli due to quantitative or qualitative defects in integrins αIIb or β3 (GPIIb/IIIa). Of note, the classical/standard definition of GT was not based on genotype, and is primarily a phenotypic diagnosis based on clinical presentation and platelet aggregation, and does not consider protein defects other than integrins αIIb or β3.

Clinical phenotype: moderate-severe mucocutaneous bleeding; platelets fail to aggregate to physiologic stimuli, but agglutinate to ristocetin; vast majority with normal platelet number and size. Historically, patients grouped as Type I (<5% αIIbβ3) type II (5-15% [or 5-25%] αIIbβ3) and type III or variant GT with normal levels of dysfunctional αIIbβ3.

Inheritance: primarily autosomal recessive; compound heterozygotes predominate outside ethnic groups.

Genetic epidemiology/Incidence: GT is a rare disease (defined in U.S. as less than 1 in 200,000). Some sources estimate an incidence of 1 in a million worldwide (https://ghr.nlm.nih.gov/condition/glanzmann-thrombasthenia#statistics), but in reality, there are no reliable estimates of the worldwide incidence.

Most GT families have private mutations, although a few mutations re-occur in non-related families suggesting mutational hotspots. The incidence is often reported as higher in certain ethnic groups (Iraqi Jews, Palestinian Arabs and French gypsies of the Manouche tribe) where consanguinity is more likely.
GT molecular defects:

Almost all reported causative variants have been in either ITGA2B or ITGB3. The ITGA2B gene is 17 kb and has 30 exons; the ITGB3 gene is 59 kb and has 15 exons

- Missense variants (most common cause of GT) in any part of gene; retention and degradation in ER/Golgi; some associated with altered exon splicing
- Nonsense variants (premature stop codons)
- Small indels that alter frame and introduce premature stop codons
- mRNA splicing defects
- Large deletions, inversions and duplications are rare
- Other rare mechanisms
- Generally, mutations in ITGA2B and ITGB3 produce indistinguishable phenotypes
- Variant GTs are more likely to be caused by genetic changes in ITGB3
- Variants in ITGB3 extracellular Cys residues can cause gain-of-function changes
- A GT-like phenotype has been described in several patients with mutations in FERMT3 (kindlin-3) and RASGRP2 (CalDAG-GEFI), genes regulating integrin activation

ACMG Classification Rule Specifications for Glanzmann thrombasthenia (GT)

SUMMARY OF CLASSIFICATION CRITERIA
(Rules in black required no specification. Rules in grey are not applicable to GT. GT specified rules are in red)

PATHOGENIC

Very Strong
PV51 – Null variant in gene with established LOF as disease mechanism

Strong
PS1 - Same amino acid change from different DNA change
PS2 – De novo with paternity and maternity confirmed
PS3 – Well-established in vitro or in vivo, functional studies supportive of a damaging effect
PS4 - Prevalence of variant in affected individuals is significantly increased over controls.

Moderate
PM1 – Present in functional region without benign variation
PM2 – Rarity/Absence in control populations
PM3 - Detected in trans with a pathogenic or likely pathogenic variant
PM4 – Protein length changes
PM5 – Missense change at same codon as another pathogenic missense variant
PM6 – Assumed de novo with specifications as recommended by SVI WG

Supporting

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This version specified for the following genes: ITGA2B, ITGB3

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

PP1 – Cosegregation with disease in multiple affected family members
PP2 - Missense variant in a gene with low rate of benign missense variation
PP3 –Multiple lines of computational evidence supporting a deleterious effect
PP4 – Phenotype and family history specific for disease with single genetic etiology
PP5 - Reputable source reports as pathogenic

BENIGN

**Stand-Alone**

BA1 – Allele frequency cutoff >0.24% (99.99% CI w/subpopulation w/min of 5 alleles)

**Strong**

BS1 – Allele frequency cutoff 0.158% (99.99% CI w/subpopulation w/min of 5 alleles)
BS2 - Observed in homozygous state in healthy individuals
BS3 – Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing
BS4 - Non-segregation in affected relatives

**Supporting**

BP1 - Missense variant in gene where only LOF causes disease
BP2 – Bi-allelic variants for a fully penetrant disorder
BP3 - In-frame deletions/insertions in a repetitive region without a known function
BP4 - Multiple lines of computational evidence support no impact
BP5 - Variant found in a case with an alternate molecular basis for disease
BP6 - Reputable source reports as benign
BP7 - Synonymous variant for which splicing prediction algorithms predict no impact

Related publication(s): Date Approved: June 12, 2020

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**VERY STRONG EVIDENCE OF PATHOGENICITY**

PVS1  Null variant in a gene where LOF is a known mechanism of disease. Null variants to include truncating variants, canonical splice sites, exon gross deletions, intragenic exon tandem duplications, and the initiation codon

- Use ClinGen SVI approved decision tree below:

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**ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1**

This version specified for the following genes: ITGA2B, ITGB3

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

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**Related publication(s):**

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This version specified for the following genes: ITGA2B, ITGB3

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- In-frame exons: ITGA2B = 7, 8, 10, 12, 13, 15, 18, 21, 23, 25-30; ITGB3 = 6-9, 13, 15
- Regions “critical to protein function” have been specified to include:
  - Extracellular domains involved in ligand binding:
    - ITGB3 β1 domain, including amino acids 135-197 & 237-240
    - ITGA2B β Propeller domain (amino acids 32-482)
  - The transmembrane domains of ITGA2B (amino acids 994-1019) and ITGB3 (amino acids 719-741)
  - The cytoplasmic domain of ITGB3 (amino acids 742-788)

STRONG EVIDENCE OF PATHOGENICITY

PS1  Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

  Example: Val->Leu caused by either G>C or G>T in the same codon.

  Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

PS2  De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.

  - Use only when proband has an additional pathogenic or likely pathogenic variant with the de novo variant. Additionally, the P/LP variant must have been classified as such by the Platelet VC EP.
  - Use ClinGen SVI’s proposed point recommendation table to determine the appropriate evidence code weight (see below). To be scored at the highest level with “Phenotype highly specific for gene” the proband must meet the PP4 criteria.

Related publication(s): Date Approved: June 12, 2020

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ClinGen_Platelet ACMG Specifications_v1
**ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1**

This version specified for the following genes: *ITGA2B, ITGB3*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

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<table>
<thead>
<tr>
<th>Table 1. Points awarded per de novo occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotypic consistency</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Phenotype highly specific for gene</td>
</tr>
<tr>
<td>Phenotype consistent with gene but not highly specific</td>
</tr>
<tr>
<td>Phenotype consistent with gene but not highly specific and high genetic heterogeneity*</td>
</tr>
<tr>
<td>Phenotype not consistent with gene</td>
</tr>
</tbody>
</table>

*Maximum allowable value of 1 may contribute to overall score

<table>
<thead>
<tr>
<th>Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for de novo occurrence(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supporting</strong> (PS2_Supporting or PM6_Supporting)</td>
</tr>
<tr>
<td>0.5</td>
</tr>
</tbody>
</table>

**PS3**

**Well-established in vitro or in vivo, functional studies supportive of a damaging effect on the gene or gene product**

- **PS3**: In a transgenic animal model, must demonstrate minimal to no function (flow cytometry JON/A, aggregation, or alternate assay analyzing integrin function)

- **OR**

  In a model organism or heterologous cell line, EITHER (A) when expression is normal or reduced (>5% expression compared to WT), disruption of protein function must be demonstrated by significantly reduced binding to fibrinogen or ligand mimetic antibodies (e.g. PAC-1) OR (B) Absent surface protein expression (<5% expression compared to WT) shown by at least 1 of 2 of the following assays: flow cytometry or Western blotting.

- **PS3_Moderate**: In a model organism or heterologous cell line, significantly reduced surface protein expression (5-25% expression compared to WT) on at least 1 of 2 of the following assays: flow cytometry or Western blotting.

**PS4**

**The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.**

- This rule does not apply to GT due to the rarity of this disorder and lack of appropriate studies. Additionally, most pathogenic variants are private (Nurden et al. Hum Mutat 2015; PMID: 25728920).

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**MODERATE EVIDENCE OF PATHOGENICITY**

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PM1  Located in a mutational critical and well-established functional domain/splice site without benign variation
- This rule does not apply to GT due to the fact that these genes are thought to be highly polymorphic (PMID: 25827233); and there are no known hot spots.

PM2_Supporting  Absent or rare in population databases (gnomAD: http://gnomad.broadinstitute.org).
- This evidence code is available when a variant is present in fewer than 1 in 10,000 alleles in the ExAC or gnomAD population cohorts.
- This cutoff was recommended based on work from Buitrago, et al showing that none of the established GT pathogenic variants were identified in ~32,000 alleles studies; suggesting that pathogenic variants have allele frequencies less than 0.01% in studied populations (PMID: 25827233)

PM3  Variant detected in trans with a pathogenic variant
- Both variants must be classified using ITGA2B/ITGB3 rule specifications.
- Use ClinGen SVI’s proposed point recommendation table to determine the appropriate evidence code weight:

<table>
<thead>
<tr>
<th>Classification/Zygosity of other variant†</th>
<th>Points per Proband</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Confirmed in trans</td>
</tr>
<tr>
<td>Pathogenic or Likely pathogenic variant</td>
<td>1.0</td>
</tr>
<tr>
<td>Homozygous occurrence (max point 1.0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Uncertain significance variant on other allele (max point 0.5)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

†All variants should be sufficiently rare (meet PM2 specification)

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for PM3

<table>
<thead>
<tr>
<th>PM3_Supporting</th>
<th>PM3</th>
<th>PM3_Strong</th>
<th>PM3_VeryStrong</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

PM4  Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants
- This rule can be applied as originally intended by the ACMG/AMP guidelines.

PM5  Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
- The strength of this rule may be adjusted based on the classification of the previously observed variant.

Related publication(s):  
Date Approved: June 12, 2020

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ClinGen_Platelet ACMG_Specifications_v1
ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: ITGA2B, ITGB3

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

- PM5: use as written
- PM5_Supporting: Novel missense change at an amino acid residue where a different missense change determined to be likely pathogenic has been seen before

PM6 Assumed de novo, but without confirmation of paternity and maternity.
- Use only when proband has a pathogenic or likely pathogenic variant in trans with the de novo variant.
- Use ClinGen SVI’s proposed point recommendation table to determine the appropriate evidence code weight (see PS2). To be scored at the highest level with “Phenotype highly specific for gene” the patient must meet the PP4 criteria.

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease (evidence code dependent on number of meiosis and families reported)
- At each strength level the affected relative(s) should have both variants identified in the proband.
  
  **Supporting:** Segregation in proband plus 1 affected relative when phase is confirmed in trans
  
  **Moderate:** Segregations in proband plus 2 affected relatives when phase is confirmed in trans
  
  **Strong:** Segregations in proband plus >2 affected relatives when phase is confirmed in trans

PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
- This rule does not apply to GT due to the fact that these genes are thought to be highly polymorphic (PMID: 25827233)

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product.

For missense variants:
  
  REVEL score of $\geq 0.7$ is required for use of this rule code.

Buitrago et al. (PNAS 2015) reported that three commonly used computational algorithms (Polyphen, SIFT, and CADD) to predict Pathogenicity had a 69–98% sensitivity in detecting GT pathogenic variants. (PMID: 25827233).

For splicing variants:
  
  $>2$ independent in silico missense predictors predict a damaging impact

We have used Human Splicing Finder and Maximum Entropy Scan with the following parameters: (1) the threshold for a position to be considered a splice site is $>65$ for HSF and $>3$ for MaxEntScan, (2) a broken splice site is defined as a position that has shifted below the threshold with a difference in score of $<-10\%$ for HSF or $<-30\%$ for MaxEntScan, and (3) a new

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ClinGen_Platelet ACMG_Specifications_v1
splice site is defined as a position that has shifted above the threshold where the difference in score is >10% for HSF or >30% for MaxEntScan.

PP4 Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology
- Proband must meet diagnostic criteria for GT, which includes phenotypic and laboratory findings.
  ● PP4. Moderate & PP4 Bleeding phenotype: mucocutaneous bleeding including epistaxis, petechiae, easy bruising, oral bleeding, gastrointestinal bleeding and menorrhagia (proband must have at least one bleeding symptom)
  ● PP4. Moderate Laboratory phenotype:
    - Full sequencing of the ITGA2B and ITGB3 genes is required to use this code at a moderate weight.
    - Abnormal lab values/patterns:
      (1) must demonstrate minimal to no aggregation with all, at least 2, tested agonists (except ristocetin, which must be normal)

      -AND-

      (2) EITHER (A) reduced (<25%) or absent surface protein expression (demonstrated by flow cytometry or Western blotting with patient cells) OR (B) functional flow cytometry of patient cells to demonstrate the inability of platelet binding to activated GPIIa/IIia.

  ● PP4 Laboratory phenotype:
    - Abnormal lab values/patterns:
      (1) must demonstrate minimal to no aggregation with all, at least 2, tested agonists (except ristocetin, which must be normal)
ClinGen Platelet Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: ITGA2B, ITGB3

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50040

PP5  Reputable source reports as pathogenic
- Do not use this rule code

**STAND-ALONE EVIDENCE OF BENIGN IMPACT**

**BA1**  Allele frequency is greater than expected for disorder
- Use a minor allele frequency cutoff of $\geq 0.0024$ or 0.24% (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

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The prevalence of GT worldwide is not well defined. Therefore, we took the conservative approach of using the reported prevalence of 1 in 200,000 in the French Manouche population, which is the highest reported prevalence for this calculation and assumed 100% penetrance. Additionally, the calculator was set to 1 for gene and allele heterogeneity to produce a conservative number.

### STRONG EVIDENCE OF BENIGN IMPACT

**BS1**  
**Allele frequency is greater than expected for disorder**  
- Use a minor allele frequency cutoff of >0.00158 but <0.0024 (99.99% CI, sub-population must have a minimum of 5 alleles present in the sub-population) based on the Whiffen-Ware calculator.

To set the strong MAF cutoff, we use the rationale discussed above and accounted for the two known genes associated with GT; whereas the above calculation assumed only one causative gene.

**BS2**  
**Observed in homozygous state in healthy individuals**  
- This evidence code is available when the variant is identified in ≥1 homozygotes who are unaffected (proven with at least aggregometry).
- It is important to note that in order to use this rule one must have phenotypic information about an individual who is homozygous for a particular variant. This means one could not use population data (i.e. gnomAD or ExAC) as evidence. This code would more likely be used in the setting of gene panel testing, where an individual with a different phenotype was found to be homozygous for a variant in one of those two genes.

**BS3**  
**Well-established *in vitro or in vivo* functional studies show no damaging effect on protein function or Splicing**  
- Must demonstrate normal aggregometry in a transgenic mouse model.
  - OR -
- In a heterologous cell line, must demonstrate BOTH normal expression (>75%) and normal protein function, based on binding to fibrinogen or ligand mimetic antibodies (e.g. PAC-1).

**BS4**  
**Lack of segregation in affected members of a family**  
- The variant is not detected in an affected family member, with a confirmed bleeding phenotype and appropriate lab values as described above.

### SUPPORTING EVIDENCE OF BENIGN IMPACT

**BP1**  
**Missense variant in a gene for which primarily truncating variants are known to cause disease**
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- This rule code does not apply to these genes, as truncating variants account for only a portion of disease causing variants.

BP2  **Observed in cis with a pathogenic variant**
- This rule can be applied as originally intended by the ACMG/AMP guidelines.

BP3  **In-frame deletions/insertions in a repetitive region without a known function**
- This rule can be applied as originally intended by the ACMG/AMP guidelines in the ITG3B gene, which has three repetitive microsatellite regions. There are no known repetitive regions in the ITGA2B gene.

BP4  **Multiple lines of computational evidence suggest no impact on gene/gene product**
- Use this code for missense variants with a REVEL score of < 0.25.
  - This recommendation was based on evaluating the REVEL scores of variants assessed as benign by this VCEP using our rule specifications with the exception of this rule code. All benign and likely benign variants fell under this threshold.
  - We are not recommending the use of other missense *in silico* model predictors at this time due to a previous concern about their validity for these genes as demonstrated by Buitrago et al. (PNAS 2015). They found that the three computational algorithms they used were less reliable predicting non-pathogenicity of previously known benign variants. (PMID: 25827233)

BP5  **Variant found in a case with an alternate molecular basis for disease**
- This rule code is not recommended for use at this time.
- Diminished platelet expression and function of αIIb β3 integrin is very specific for GT. Therefore, pathogenic variants in other genes (such as FERMT2 and RASGRP2) are subtypes of GT in which there is only diminished function of αIIb β3 integrin may have similar laboratory features to other platelet disorders. In these cases, pathogenic variants in other platelet genes may be causative.

BP6  **Reputable source reports as benign**
- Do not use this rule code

BP7  **A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.**
- This rule can be applied as originally intended by the ACMG/AMP guidelines.

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**Related publication(s):**

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