Blueprint Genetics Variant Classification Scheme for Dominant Monogenic Disorders

CLASSIFICATION	CATEGORY	CRITERIA
PATHOGENIC	1 Point Needed	1. Well-established disease-causing variant and wide consensus in the field on pathogenicity of the variant. (Typically significant family segregation has been established and several publications support pathogenicity) **– 1 Point** 2. In the setting of a novel disease in the family, de novo truncating variant absent from control populations in a gene where loss of gene function has been established as a mechanism of pathogenicity for patient´s disease (both paternity and maternity confirmed) **– 1 Point** 3. Loss-of-function variant seen in at least 2 patients (one of which can be the current patient) in a gene where loss of gene function has been established as a mechanism of pathogenicity for patient´s disease **– 1 Point**
	OR	**COMPULSORY A OR B:** A) Positive segregation with the disease (≥2 families) and at least 5 unrelated patients (one of which can be the current patient) with same variant and phenotype **– 2 Points** OR B) ≥ 5 cases (one of which can be the current patient) with same variant and phenotype reported **– 1 Point** **ADDITIONAL POINTS:** 1. Clear gene–phenotype association exists **– 1 Point** 2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations) **– 1 Point** 3. A missense or splice region variant predicted deleterious by majority of in silico tools applied and/or well-established paralogue mutation exists **– 1 Point** 4. An inframe deletion affecting conserved amino acid in a functional domain **– 1 Point** 5. De novo variant in the setting of a novel disease in the family (paternity and maternity unconfirmed) **– 1 Point** 6. Loss-of-function variant in a gene where loss-of gene function has been established as a mechanism of pathogenicity for the disease **– 1 Point** 7. Deficient protein function in appropriate functional assay(s), e.g. an animal model with equivalent mutation or splice site defect confirmed on mRNA level **– 1 Point** 8. Well-characterized other disease-causing variant at the same codon or same splice consensus site (+/-1, 2) **– 1 Point** 9. Other strong data supporting pathogenicity **– 1 Point**
	5 Points Needed	
LIKELY PATHOGENIC	2 Points Needed	1. Loss-of-function variant in a gene where loss of gene function has been established as a mechanism of pathogenicity for the disease **– 1 Point** 2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations) **– 1 Point**
	OR	1. Clear gene-phenotype association exist **– 1 Point** 2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations) **- 1 Point** 3. A missense or splice region variant predicted deleterious by majority of in silico tools applied **- 1 Point** 4. An inframe deletion affecting conserved aa in a functional domain **– 1 Point** 5. Variant has been identified in ≥2 individuals (one of which can be the current patient) with same disease manifestation **- 1 Point** 7. Evidence of a well-established paralogue mutation exists **– 1 Point** 6. De novo alteration in the setting of a novel disease in the family (paternity and maternity unconfirmed) **– 1 Point** 8. Deficient protein function in appropriate functional assay(s), e.g. an animal model with equivalent mutation or splice site defect confirmed on mRNA level **- 1 Point** 10. Well-characterized other disease-causing variant at the same codon or same splice consensus site (+/-1, 2) **- 1 Point** 11. Other strong data supporting pathogenicity **– 1 Point**
	4 Points Needed	
Variant of uncertain significance (VUS)	Variant has characteristics of being independent disease-causing mutation, however, insufficient or conflicting evidence exists.	
LIKELY BENIGN	1 Point Needed	1. Control population minor allele frequency in gnomAD or other publicly available database is considerable (MAF>0.001) - (disease prevalence must be taken into account) **– 1 Point**
	OR	1. MAF <0.001 in control populations but variant is detected in healthy controls with no disease association in a case-control study/studies **– 1 Point** 2. Homozygous variant in a gene with no association to the disease **– 1 Point** 3. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype **– 1 Point** 4. Majority of the in silico tools predict the substitution to be benign **- 1 Point** 5. Intact protein function observed in appropriate functional assay(s), e.g. splice region variant without abnormal splicing **- 1 Point** 6. Other data supporting benign classification **– 1 Point**
	2 Points Needed	
BENIGN	2 Points Needed	1. Does not segregate with the disease in family/ies with 2 or more affected individuals **- 1 Point** 2. Any additional criteria described below **– 1 Point**
	OR	1. Control population minor allele frequency is considerable (MAF>0.001) - (prevalence of the disease must be taken into account) **– 1 Point** 2. Homozygous variant in a gene with no association to the disease **- 1 Point** 3. Intact protein function observed in appropriate functional assay(s), e.g. splice region variant without abnormal splicing **- 1 Point** 4. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype **- 1 Point** 5. No disease association in small case-control study **- 1 Point** 6. Majority of the in silico tools predict the substitution to be benign **- 1 Point** 7. Other data supporting benign classification **- 1 Point**
	4 Points Needed	

Loss-of-function (LoF) variants: Variants considered deleterious (predicted out-of-frame consensus splice site (+/-1, 2), nonsense*, frameshift*, start lost•, gross deletion##, out-of-frame intragenic duplication variants).
*with cautious interpretation of the variants located in the last exon or in the last 50 base pairs of the penultimate exon as they might escape NMD.
•with cautious interpretation of the variants that have nearby inframe Methionine.
#with cautious interpretation of the variants affecting exons that are not present in all transcripts.

Non-truncating variants: missense and splice region variants, small inframe deletions/duplications

Disclaimers:

• Every case is examined by our team in the light of the literature, publicly available clinical databases and the BpG in-house mutation database. Exceptions to the scheme can be made in complex cases or in the setting of poorly described patient phenotype.
• This classification scheme is not designed for the interpretation of variants considered as genetic modifiers or alleles predisposing to a disease with low-risk. Several variants classified with this scheme as likely benign or benign could function as disease modifiers. Classification as disease modifier can be applied when adequate scientific evidence has been established for a variant.
• It is not optimal for interpretation of alterations confounded by incomplete penetrance, variable expressivity, recessive inheritance, oligogenic inheritance, or skewed X-inactivation.
• Final classifications are subject to review and approval by Blueprint Genetics clinical staff and may differ from those predicted by the scheme.