Blueprint Genetics Variant Classification Scheme for Recessive 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 **- 1 Point** **OR** 2. ≥2 cases (one of which can be the current patient) with the same phenotype and the same loss-of-function variant: in one patient observed in homozygous or compound heterozygous state (confirmed in trans) and in one patient observed together with another disease-causing variant in the same gene (phase not determined) **OR** ≥3 cases (one of which can be the current patient) with the same phenotype and the same loss-of-function variant observed together with another disease-causing variant in the same gene (phase not determined) **AND** Variant frequency in control populations is consistent with an autosomal recessive disorder **AND** loss of gene function is a well-established disease mechanism **- 1 Point**
	OR	
	4 Points Needed	**COMPULSORY A OR B:** **A)** ≥3 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant: • In at least two patients the variant is observed in homozygous or compound heterozygous state with a disease-causing variant (confirmed in *trans*) • One patient may be included where the variant is observed either in homozygous state or together with another disease-causing variant in the same gene (phase not determined) **- 1 Point** **OR** **B)** ≥5 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant: • In at least four patients the variant is observed together with a well-established variant in the same gene (phase not determined) • One patient may be included where the variant is observed either in homozygous state or together with another disease-causing variant in the same gene (phase not determined) **- 1 Point** **ADDITIONAL POINTS:** 1. Clear gene-phenotype association exists **- 1 Point** 2. Variant frequency in control populations is consistent with an autosomal recessive disorder **- 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 amino acid in a functional domain **– 1 Point** 5. *De novo* variant is observed together with pathogenic or likely pathogenic variant in the same gene **- 1 Point** 6. Deficient protein function in appropriate functional assay(s) **- 1 Point** 7. Well-characterized other disease-causing variant at the same codon or same consensus splice site (+/-1, 2) **- 1 Point** 8. Other strong data supporting pathogenicity **- 1 Point**
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** AND 2. Variant frequency in control populations is consistent with an autosomal recessive disorder **- 1 Point**
	OR	
	4 Points Needed	1. Clear gene-phenotype association exists - **1 Point** 2. Variant frequency in control populations is consistent with an autosomal recessive disorder **- 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 amino acid in a functional domain **– 1 Point** 5. ≥2 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant: in one patient observed in homozygous or compound heterozygous state (confirmed in trans) and in one patient observed either in homozygous state or together with a pathogenic or likely pathogenic variant in the same gene (phase not determined) OR 6. Variant has been reported in trans with a pathogenic or likely pathogenic variant in the same gene or in the current patient it occurs in trans with a pathogenic or likely pathogenic variant in the same gene (Note: This requires testing of parents to determine phase or close proximity of the variants, when NGS data can be used to determine the phase) **- 1 Point** OR ≥3 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant together with a pathogenic variant in the same gene (phase not determined) **- 1 Point** 7. *De novo* variant is observed together with pathogenic or likely pathogenic variant in the same gene **- 1 Point** 8. Deficient protein function in appropriate functional assay(s) **- 1 Point** 9. Well-characterized other disease-causing variant at the same codon or same consensus splice site **– 1 Point** 10. Other strong data supporting pathogenicity **- 1 Point**
Variant of uncertain significance (VUS)	Variant has characteristics of being a disease-causing variant, however, insufficient or conflicting evidence exists.	
LIKELY BENIGN	1 Point Needed	1. The allele frequency of the variant or the number of individuals homozygous for the variant in gnomAD or other publicly available database is greater than expected for the disorder (the prevalence of the disease in the population and the fraction explained by the specific gene must be taken into consideration) **– 1 Point**
BENIGN	1 Point Needed	1. The allele frequency of the variant in gnomAD or other publicly available database is more than 5% **– 1 Point**

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, dominant 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.