	Evidence Type		Case Information	Suggested Points/Case		Points Given	Max Score
				Default	Range		
Case-Level Data[1]	Variant Evidence	Autosomal Dominant OR X-Linked Disorder[2]	Variant is *de novo*[3]	2	0-3		12
			Proband with predicted or proven null variant[4]	1.5	0-2		10
			Proband with other variant type with some evidence of gene impact[5]	0.5	0-1.5		7
		Autosomal Recessive Disorder	Two variants in *trans* and at least one *de novo*[3] or a predicted/proven null variant[4]	2	0-3		12
			Two variants (not predicted/proven null) with some evidence of gene impact[5] in *trans*	1	0-1.5		
	Segregation Evidence		Evidence of segregation in one or more families[6]	Sequencing Method	0-3		3

Total LOD Score	Candidate Gene Sequencing	Exome/ Genome or all genes sequenced in linkage region
2-2.99	0.5	1
3-4.99	1	2
≥5	1.5	3

	Case-Control Study Type[8]	Case-Control Quality Criteria	Suggested Points/Study	Points Given	Max Score
Case-Control Data[7]	Single Variant Analysis[8a]	• Variant Detection Methodology[9a] • Power[9b] • Bias and Confounding Factors[9c] • Statistical Significance[9d]	0-6		12
	Aggregate Variant Analysis[8b]		0-6		
			TOTAL ALLOWABLE POINTS for Genetic Evidence		12

General Notes

- All variants under consideration should be rare enough in the general population to be consistent with disease.
- Cohorts/cases should not be double counted. For example, individual cases included as part of case-control studies should not be given points from both the "Case Level Data" and "Case-Control Data" categories.
- Case-Level Data includes studies describing individuals or families with variation in the gene of interest.
- Case-Control studies are those in which statistical analysis is used to evaluate variation in cases compared to controls.

Case-Level Data

1. Each case may be given points for A) variant evidence (in the context of the appropriate mode of inheritance) and B) segregation evidence, if applicable (see footnote 5 and Figure S1 for more details on segregation evidence).
2. In X-linked disorders, affected probands will often be hemizygous males (in the case of truly "recessive" disorders) and/or heterozygous females (in the case of "dominant" disorders). Recognizing that there can be rare cases of females affected by X-linked recessive disorders (due to chromosomal aneuploidy, skewed X inactivation, or homozygosity for a sequence variant) evaluators must be aware of the nuances of interpretation of individual cases and X-linked pedigrees. Points can be assigned at the discretion of the expert reviewer taking into account the available evidence. Furthermore, there are known cases of female carriers of X-linked recessive conditions manifesting symptoms that are milder or later in onset compared to males, and scoring of genetic evidence in these examples should be subject to expert review with regard to the assigned gene/disease/inheritance combination.

3. Points should be adjusted depending on statistical expectation of *de novo* variation in the gene in question.

4. As described in the 2015 ACMG/AMP sequence variant interpretation guidelines[1], null variants (typically nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletions) are considered "very strong evidence for pathogenicity" in genes for which loss of function is a known disease mechanism. Disease mechanism can be assumed loss of function (LOF) if the gene is LOF constrained. LOF constraint scores must be interpreted in the context of the disease in question – genes associated with severe, pediatric-onset disorders are more likely to show constraint than adult-onset conditions where overall fitness is not impacted. More points can be assigned if the gene is known to be highly constrained for LOF variants; fewer points can be assigned if there is alternative splicing, or the LOF variant is near the C terminus and/or NMD is not predicted (stop codon downstream of the last 50 bp of the penultimate exon).

5. For variants not considered to be "null" (typically missense), at least some impact to gene function must be demonstrated for the case to count. Impact based on predictions only would score less than the default 0.5 points and impact based on functional validation can score 0.5 or above (up to 1.5/case) depending on the validation quality and biological representativeness of the functional assay.

6. LOD scores reported by the authors of a peer-reviewed journal article may be used to assign segregation points as outlined in the scoring matrix above. If a LOD score is not provided by the authors, one may be estimated for informative families with rare, highly penetrant disorders in which phenocopies are expected to be rare or absent. Below are guidelines for calculating estimated LOD scores in the appropriate scenarios:
   - Curators may estimate a LOD score using the simplified formula(s) in Figure S1 if the following conditions are met:
     - The disorder is rare and highly penetrant.
     - Phenocopies are rare or absent.
     - For **dominant or X-linked disorders**, the estimated LOD score should be calculated using ONLY **families with 4 or more segregations present** (Figure S1A). The affected individuals may be within the same generation, or across multiple generations.
     - For **recessive disorders**, the estimated LOD score should be calculated using **ONLY families with at least three affected individuals** in the pedigree (Figure S1B). Genotypes must be specified for all affected and unaffected individuals counted.
     - Families included in the calculation must not demonstrate any non-explainable non-segregations (for example, a genotype$^-$/phenotype$^+$ individual in a family affected by a disorder with no known phenocopies). Families with non-explainable non-segregations should not be used in LOD score calculations.
   - If any of the previous conditions are not met, do not use the formula(s) in Figure S1 to estimate a LOD score.
   - To be conservative in our simplified LOD score estimations, for autosomal dominant or X-linked disorders only affected individuals (genotype+/phenotype+ individuals) or obligate carriers (regardless of phenotype) should be included in calculations.
   - Expert reviewers may choose to specify the most appropriate way to approach segregation scoring within their disease domain, including enacting more formal, rigorous LOD score calculations.

Case-Control Data

7. Each case-control study should be independently assessed to evaluate the quality of the study design. Consensus with a clinical domain expert group is highly recommended.

8. Case-control studies are classified based on how variation in cases and controls is evaluated: single variant analysis or aggregate variant analysis. Studies presenting both types of analyses may be counted in either category at the discretion of the curator/expert, but the same variants should not be counted in both categories.
   a. *Single variant analysis* studies are those in which individual variants are evaluated for statistical enrichment in cases compared to controls. More than one variant may be analyzed, but the variants should be independently assessed with appropriate statistical correction for multiple testing.
   b. *Aggregate variant analysis* studies are those in which the statistical enrichment of two or more variants as an aggregate is assessed in cases compared to controls. This comparison could be accomplished by genotyping specific variants or by sequencing the entire gene.

9. Points for case-control studies may be assigned at the discretion of expert opinion based on the overall quality of each study. The following should be considered when evaluating case-control study quality:
   a. *Variant Detection Methodology*: Cases and controls should ideally be analyzed using methods with equivalent analytical performance (e.g. equivalent genotype methods, sufficient and equivalent depth and quality of sequencing coverage, correction for batch effects).
   b. *Power*: The study should analyze a sufficient number of cases and controls given the prevalence of the disease, the allele frequency, and the expected effect size in question to provide appropriate statistical power to detect an association.
   c. *Bias and Confounding factors:* The manner in which cases and controls were selected for participation and the degree of case-control matching may impact the outcome of the study. The following are some factors that should be considered:
      i. Are there systematic differences between individuals selected for study and individuals not selected for study?
      ii. Are the cases and controls matched by demographic information (e.g., age, ethnicity, location of recruitment, etc.)?
      iii. Are the cases and controls matched for genetic ancestry, if not did investigators account for genetic ancestry in the analysis?
      iv. Have the cases and controls been equivalently evaluated for presence or absence of a phenotype, and/or family history of disease?
   d. *Statistical Significance* – The level of statistical significance should be weighed carefully. When an odds ratio is presented, its magnitude should be consistent with a monogenic disease etiology. When p-values or 95% confidence intervals (CI) are presented, the strength of the statistical association can be weighed in the final points assigned. Factors, such as multiple testing, that might impact that interpretation of uncorrected p-values and CIs should be considered when assigning points.