Ambry Genetics®

Ambry's Exome Reporting Categories

REPORTING CATEGORIES SPECIFIC TO OUR EXOME SEQUENCING:

Alteration Pathogenicity + **Gene Overlap** = **Overall Results Category**

CHARACTERIZED GENETIC ETIOLOGIES	NOVEL GENETIC ETIOLOGIES
Positive: Clinically relevant alteration(s) detected	**Uncertain, Candidate:** Alteration(s) of potential clinical relevance detected
Likely Positive: Alteration(s) with likely clinical relevance detected	**Uncertain, Suspected Candidate:** Alteration(s) of potential clinical relevance detected
Uncertain: Alteration(s) of uncertain clinical relevance detected	**Negative:** No alterations with potential clinical relevance detected
Negative: No clinically relevant alterations detected	

Final overall conclusion incorporates the classification of the alteration and the strength of overlap between the phenotype observed in the patient of interest and previously reported patients with alterations in the same gene (gene overlap).

ALTERATION CLASSIFICATION	GENE OVERLAP	FINAL RESULT
Pathogenic	Positive	POSITIVE
Pathogenic	Likely Positive	LIKELY POSITIVE
Pathogenic	Uncertain	UNCERTAIN
Likely Pathogenic	Positive	LIKELY POSITIVE
Likely Pathogenic	Likely Positive	LIKELY POSITIVE
Likely Pathogenic	Uncertain	UNCERTAIN
Uncertain	Positive	UNCERTAIN
Uncertain	Likely Positive	UNCERTAIN
Uncertain	Uncertain	UNCERTAIN

To view the Ambry reporting categories for alterations submitted to ClinVar, refer to the following fields in ClinVar:

AMBRY CLASSIFICATION	CLINVAR DATA FIELD
Alteration Classification	Clinical Significance
Overall Results Category	Comment of Clinical Significance

NOTE: the overall conclusion considers all reported genes/alterations

For further details see Farwell KD, *et al.* Genet Med. 2015 Jul;17(7):578-86.

CATEGORIZATION OF POST-FILTERED ALTERATIONS FOR DIAGNOSTIC EXOME SEQUENCING (DES)

GENE OVERLAP	ALTERATION CLASSIFICATION	ZYGOSITY AND GENE INHERITANCE	CATEGORIZATION
Positive / Likely Positive	MUT/VLP	Consistent	Pos/Likely Pos Candidate
		Inconsistent	Uncertain Candidate*/Notable
	VUS	Consistent	Uncertain Candidate
		Inconsistent	Notable
	VLB/Poly	Consistent/ Inconsistent	Maybe Notable as a modifier ˆ
Likely Positive, limited features#	MUT/VLP	Consistent	Likely Pos Candidate, Partial
		Inconsistent	Notable
	VUS	Consistent	Uncertain Candidate, Partial
		Inconsistent	Not Reported
Uncertain	MUT/VLP	Consistent	Uncertain Candidate
		Inconsistent	Notable
	VUS	Consistent	Uncertain Candidate
		Inconsistent	Not Reported
None	MUT/VLP/VUS	Consistent	Not Reported (may be reported as secondary finding)
		Inconsistent	Not Reported (may be reported as secondary finding)

*For one mutant allele detected in an AR gene with very strong gene overlap and for a condition with little locus heterogeneity.
ˆ with a MUT/VLP/VUS candidate in the same gene.
When the gene is associated with specific and isolated features (e.g. hearing loss, muscular dystrophy) that are only a minor part of the clinical concerns of the patient.

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Ambry's Variant Classification Categories

All alterations, across all report types, follow our variant classification schema as follows:

- Pathogenic Mutation: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk family members and appropriate changes in medical management (*i.e.* high risk surveillance) for pathogenic mutation carriers recommended. A pathogenic mutation is always included in results reports.
- Variant, Likely Pathogenic (VLP): alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk family members and appropriate changes in medical management (*i.e.* high risk surveillance) for VLP carriers recommended. A VLP is always included in results reports.
- Variant, Unknown Significance (VUS): alterations with limited and/or conflicting evidence regarding pathogenicity. Targeted testing of informative family members to collect cosegregation data via our Family Studies Program recommended. Medical management based on personal and family clinical histories, not VUS carrier status. A VUS is always included in results reports.
- Variant, Likely Benign (VLB): alterations with strong evidence against pathogenicity. Targeted testing of at-risk family members not recommended. Medical management based on personal and family clinical histories. A VLB is not routinely included in results reports.
- Benign: alterations with very strong evidence against pathogenicity. Targeted testing of at-risk family members not recommended. Medical management based on personal and family clinical histories. Benign alterations are not routinely included in results reports.

For further details see ambrygen.com/variant-classification and LaDuca H, *et al.* Genet Med. 2014 Nov;16(11):830-7.

SCHEME FOR AUTOSOMAL DOMINANT AND X-LINKED MENDELIAN DISEASES

CLASS	AMBRY CLASSIFICATION	CATEGORY	CRITERIA	EXCEPTIONS (NEW BASELINE CLASS)
5	Pathogenic	A 1 Needed	• Confirmed *de novo* alteration in the setting of a new disease (appropriate phenotype) in the family	• Confirmed *de novo* alteration in a novel gene with possible disease implications (4) • Likely *de novo* alteration (i.e. paternity not confirmed) with known disease association (4) • Confirmed *de novo* alteration in the setting of a discordant phenotype (3)
			• Alterations resulting in premature truncation (e.g.reading frame shift, nonsense)	• Truncation in close proximity to 3' terminus (3/4 gene specific) • LOF has not been established as mechanism of pathogenicity (e.g. MYH7) (3)
			• Other ACMG-defined mutation (i.e. initiation codon or gross deletion)	• In-frame gross deletion of a single exon not in a known protein functional domain (3), Initiation codon that is not well conserved or possible alternate start (3/4), LOF has not been established as a mechanism of pathogenicity (3)
			• Strong segregation with disease (LOD >3 = >10 meioses)	
			• Functionally-validated splicing mutation	• In-frame skipping a single exon not in a known protein functional domain (4) LOF has not been established as a mechanism of pathogenicity (3)
		B 4 Needed	• Significant disease association in appropriately sized case-control study(ies)	
			• Detected in individual satisfying established diagnostic critera for classic disease without a clear mutation	
			• Last nucleotide of exon	• When poorly conserved or *in silico* doesn't predict significant effect
			• Good segregation with disease (LOD 1.5-3 = 5-9 meioses)	
			• Deficient protein function in appropriate functional assay(s)	
			• Well-characterized tmutation at same position	• Different disease causing mechanism, i.e. if other mutation affects splicing, and this particular variant is predicted to affect protein, but not slicing or nonsense vs. missense • When well characterized mutation is a proline
			• Other strong data supporting pathogenic classification	
4	Likely Pathogenic	1 Needed	• Alterations at the canonical donor/acceptor sites (+/- 1, 2) without other strong (B-level) evidence supporting pathogenicity	• LOF has not been established as a mechanism of pathogenicity (3)
		C 4 Needed	• Rarity in general population databases (dbSNP, ESP, 1000 Genomes, ExAC)	• Dependent on disease penetrance and inheritance pattern.
			• *in silico* models in agreement (deleterious) and/or completely conserved position in appropriate species	• *in silico* splicing predictions not used as independent line of evidence for last nucleotide of exon.
			• Moderate segregation with disease (at least 3 informative meioses) for rare diseases.	
			• Other data supporting pathogenic classification	
		3 of B		
		2 of B and at least 1 of C		
		1 of B and at least 3 of C		
3	VUS	Insufficient or Conflicting Evidence		
		Gross Duplications without Strong Evidence for Pathogenic or Benign		
2	Likely Benign	D 1 Needed	• Intact protein function observed in appropriate functional assay(s)	
			• Intronic alteration with no splicing impact by RT-PCR analysis or other splicing assay	
			• Other strong data supporting benign classification	
		E 2 Needed	• Co-occurence with mutations in same gene (phase unknown)	• Genes without a defined, severe biallelic phenotype (3) When always linked to a the same mutation (can't rule out synergenic effect)
			• Co-occurence with mutations in other high penetrant genes that clearly explains a proband's phenotype	
			• Subpopulation frequency in support of benign classification	
			• *in silico* models in agreement (benign)	
			• Does not segregate with disease in family study (genes with incomplete penetrance)	
			• No disease association in small case-control study	
			• Other data supporting benign classification	
1	Benign	F 1 Needed	• General population or subpopulation frequency is too high to be a pathogenic mutation based on disease/syndrome prevalence and penetrance	
			• Does not segregate with disease in family study (genes with complete penetrance)	
			• Internal frequency is too high to be a pathogenic mutation based on disease/ syndrome prevalence and penetrance	
			• Seen *in trans* with a mutation or in homozygous state in individual without severe disease for that gene	• Genes without a defined, severe biallelic phenotype (3)
			• No disease association in appropriately sized case-control study(ies)	
		1 of D and at least 2 of E		
		2 or more of D		
		>3 of E w/o conflicting data		
		>4 of E w/conflicting data		

The variant classification scheme is not intended for the interpretation of alterations considered epigenetic factors including genetic modifiers, multifactorial disease, or low-risk disease association alleles and may be limited in the interpretation of alterations confounded by incomplete penetrance, variable expressivity, phenocopies, triallelic or oligogenic inheritance, or skewed X-inactivation.

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