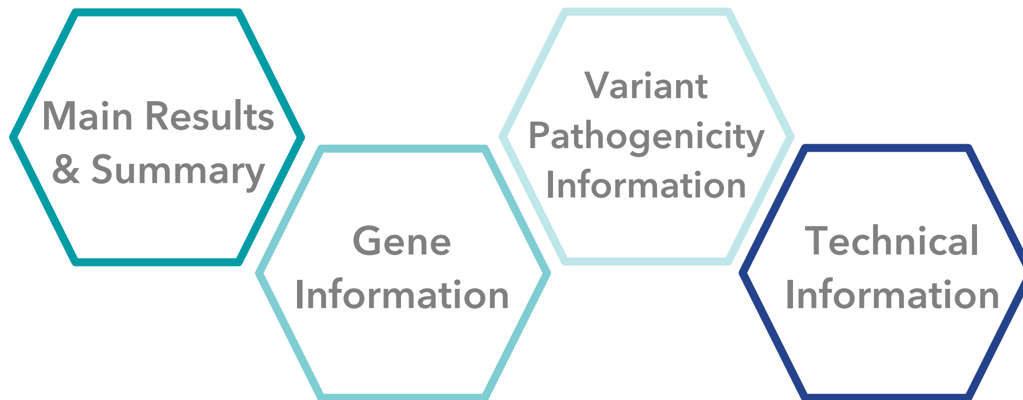
Reading a Genetic Test Report

Common components of a genetic testing report:



MAIN RESULTS AND SUMMARY

On the first page of the report, you will typically find basic information about the patient and test, such as:

- ✓ **Patient information**: name, date of birth, medical record #
- ✓ Ordering provider
- ✓ **Indication** for test performed
- ✓ **Type of test** performed - examples include: single gene analysis, multi-gene panel, familial variant test, karyotype, microarray, exome or genome sequencing

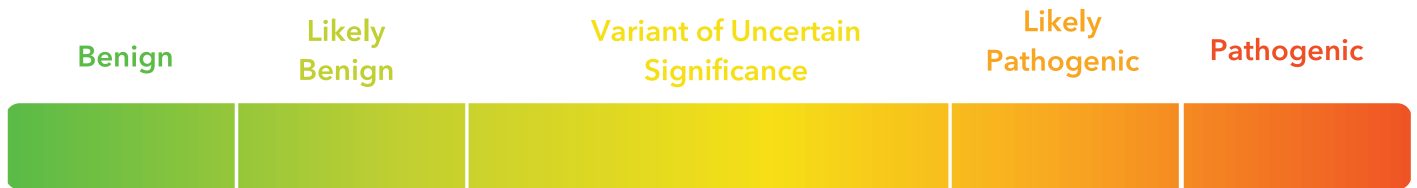
  TIP: Not all testing methodologies can detect every type of genetic variant. Please see Table 1 for more information about these tests and what they can and cannot detect

Result Summary:

- ✓ **Positive**: the analysis has detected a genetic variant that is interpreted as likely disease causing, or implies that the patient is a carrier of a recessive condition
- ✓ **Negative:** no gene variants were identified on analysis, further genetic testing may be indicated and/or current testing cannot identify a genetic etiology
- ✓ **Inconclusive:** one or more genetic variants were identified on testing that we do not know the impact of. It may be that the variant itself is a Variant of Uncertain Significance (VUS), or that the variant(s) are not able to fully explain the patient's phenotype

  TIP: Over time, our understanding of VUS results may change - clinicians can reach out to the lab to ask for updates to variant classifications over time

Variant Pathogenicity:



- ✓ **Benign/Likely Benign**: these variants typically will not appear on genetic testing reports.
- ✓ **Variant of Uncertain Significance:** the clinical importance of this variant is uncertain, there may be little or no evidence that this variant causes disease or there may be conflicting evidence
- ✓ **Likely Pathogenic:** the variant is probably disease causing
- ✓ **Pathogenic:** the variant has strong evidence to be disease causing

Variant Nomenclature:

- ✓ **Transcript**: Typically starts with NM_ followed by a set of numbers (eg NM_000399.3). One gene can have many transcripts (unique combination of exons and introns that make up an mRNA). Since the same variant can have different nomenclature depending on the transcript it is reported on, it is important to refer to a variant by both the transcript and c. (or p.).
- ✓ **Coding (c.):** the specific nucleotide change on the DNA reference sequence
- ✓ **Protein (p.):** the amino acid change at the protein level

Zygosity:

- ✓ **Heterozygous**: Only one copy or allele of the patient's gene has this variant
- ✓ **Homozygous**: Both copies of the patient's alleles for this gene contain the variant (please note it is possible that a variant may appear homozygous when it is actually heterozygous if a deletion is found on the other copy)
- ✓ **Hemizygous**: For variants found the sole copy of a gene. In most cases, this refers to variants on the X-chromosome, where males are considered hemizygous if one copy of the variant is found (as they typically do not have a second X-chromosome)
- ✓ **Compound Heterozygous**: When two variants are found in the same gene and are shown to be in trans (see below).
- ✓ **Cis:** two variants in the same gene are found, however they are on the same copy/ allele of the gene, and may not be sufficient to explain the disease
- ✓ **Trans:** two variants in the same gene are found, they are on different copies of the gene, therefore both alleles are affected

GENE INFORMATION

Summary of the gene's role in disease and related phenotype(s)

The report should include the following information about the gene that is reported:

- ✓ The **gene name**
- ✓ **Disease** that this gene has been associated with and what body system(s) this gene has an impact on
- ✓ Relevant **publications** that support an association
- ✓ **Inheritance pattern** of the related conditions:

Recessive: Both copies of the gene must be affected in order for an individual to have the condition. Therefore the patient must have a homozygous variant or two compound heterozygous variants in trans. If only one copy is affected, this individual is a carrier (see below)

Carrier status: If a patient is listed as a carrier for a recessive condition, it typically has an associated reproductive risk but typically does NOT implicate disease risk for the patient themselves i.e. if a patient is a carrier for Pompe disease, we do not expect that they will have clinical features related to that condition, but if they reproduce with another carrier their offspring has a 25% risk of having the condition

Dominant: Only one copy of the gene needs to be affected in order for the individual to have the condition, or an increased risk for the condition.

X-linked Dominant: Males and females will both present with the condition if the variant is in the heterozygous or hemizygous state

X-linked Recessive: Males will present with the condition if the variant is in the hemizygous state. Females need both copies of the X chromosome to be affected. In some cases, females may still have symptoms, but a milder form of the condition.

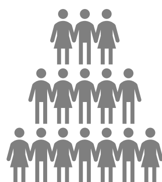
Please note that the following may impact the patient's presentation:

Penetrance: usually represented as a percent – the proportion of people with a particular gene change who will have signs/symptoms of a condition. In some cases (like hereditary cardiovascular disease or cancers), there will be "reduced penetrance". E.g., if someone with a gene change had a 75% chance of having an arrhythmia
Expressivity: patients with the same gene change may have different presentations. E.g., one family member with a BRCA1/2 gene change may have breast cancer, while another family member may have ovarian cancer.

VARIANT PATHOGENICITY INFORMATION

The lab report should include information (such as a variant evidence summary) which describes the evidence used to determine the pathogenicity of this variant. For more information on variant interpretation and how to do your own variant assessment, please see the *Variant Curation Evidence Handout*

In particular, take a look for the following information that may be included in the variant blurb:



Population Database

Literature and Publications

In Silico Predictors

Functional Studies

Population Database

- ✓ Low frequency in population databases (like gnomAD) is considered a supporting piece of evidence towards the variant pathogenicity
- ✓ Higher frequency in population databases implicates lower likelihood that that the variant is pathogenic, and higher likelihood it is part of normal human variation

Literature and Publications

- ✓ If the variant is reported in the literature in affected individuals, particularly in the case of rare variants and rare disorders, that is typically considered to be evidence supporting that the variant is disease-causing. The more affected individuals reported with the same variant and the same phenotype, the more compelling the evidence is.

In Silico Predictors

- ✓ Have computational tools predicted that this variant causes disease?
- ✓ Particularly in the case of missense variants, pathogenic in silico predictions, in isolation, are not highly weighted when determining pathogenicity

Functional Studies

- ✓ Are there in vitro or in vivo functional studies which provide evidence of pathogenicity? If so, this is typically evidence towards pathogenicity.

Recommendation for family testing

Some laboratories recommend family testing to confirm presence or absence in affected or unaffected family members. Please see the *Assessing a VUS Handout* for more information about

when it is appropriate to test family members. **TIP**: Some labs offer free family testing (sometimes called VUS resolution) if they are close to reclassifying VUS towards LB or LP.

TECHNICAL INFORMATION

At the end of the report, the lab should include a methodology section, which gives you information about the test that was run and any limitations of the testing that may exist. This section may also indicate with types of findings might not be reported (for example, carrier status, variants in genes that aren't felt to be relevant to the patient's reported clinical phenotype, copy number variants, etc.)

Genes Analyzed

If it is an older report, newer genes of interest related to the disease in question may not have been analyzed and more updated genetic testing can be considered. Look for a list of genes which were included in a panel. For genome or exome sequencing, contact the laboratory directly to see if the gene that you are interested would have been covered by the test.

Methodology

Test Method	Description	Detection Limitations
Gene panels (Sequencing **+ / -**deletion / duplication)	Single gene or multi-gene panels typically include sequencing of gene(s), however. Attention should be given to whether or not deletion/duplication analysis was performed, as all variant types may not be covered Can identify variants like: ✓ Single nucleotide variants (SNVs): missense, nonsense, frameshift ✓ Splice site variants	DOES NOT identify: χ Variants in genes not included on the panel χ Some Copy number variants (CNVs) - dependent on size of the variant χ Sometimes intronic variants
Family variant testing	✓ Typically only test the variant identified in family member(s) or sequence of whole gene of interest	DOES NOT identify: χ Variants or genes outside of the specific ones tested

Test	Benefits	Limitations
Exome sequencing **+ / -** mitochondrial analysis	✓ Sequencing of coding regions of genes (exons) ✓ Phenotype - driven analysis ✓ Re-analysis is typically offered and recommended if new phenotype information is gained ✓ May be ran as a “duo” or “trio" (biological parents may be included)	DOES NOT identify: χ Large copy number variants (depending on lab methods) χ Repeat expansion disorders χ Variants in genes not related to the indication for testing (secondary findings may be included in some cases) χ Some genes with homology, repetitive regions or pseudogenes (check with the lab about your particular gene)
Genome sequencing	✓ Sequencing of coding and some non-coding regions of gene ✓ Assay has more uniform coverage across the genome ✓ Potential to detect certain repeat expansion disorders and mitochondrial related disorders (check with lab)	DOES NOT identify: χ Some repeat expansion disorders (check with individual labs) χ Some genes with homology, repetitive regions or pseudogenes (check with the lab about your particular gene)
Karyotype	✓ Analyzes full chromosome differences - extra or missing chromosomes, balanced/imbalance chromosome translocation or rearrangements	DOES NOT identify: χ Genetic changes <5-10MB in size
Chromosomal microarray	✓ Can detect chromosomal microdeletions and duplications >1kb in size	DOES NOT identify: χ Balanced rearrangements or small single gene exonic deletions/duplications χ Low levels of mosaicism
Repeat expansion analysis	✓ Can detect repeat expansion disorders (i.e. Fragile X, SCAs, HD, C9orf72, and some neuromuscular conditions ✓ Some labs can report exact repeat number while others may not, depend on the lab technology	DOES NOT identify: χ All other variant types including SNV, CNVs

Example Report:

Test ordered

ExomeNext®

SAMPLE REPORT

Main results and Summary

Variant nomenclature

PATIENT
Name: **Patient, Sample**
Accession #: 00-300896
DOB: 01/01/1901
Sex at Birth: Male
MRN: N/A
Indication: Diagnostic

TEST INFORMATION
Portal Order #: 0000000
Family #: 0000000
Specimen #: N/A
Specimen type: Blood EDTA
Collection date: 01/01/2023
Received date: 01/01/2023
Test Started: 01/01/2023
Final Report: 02/20/2023

MEDICAL PROFESSIONAL
Sample Doctor
Sample Facility

ADDITIONAL RECIPIENTS
Sample GC

POSITIVE: Clinically Relevant Alteration(s) Detected

Reportable Findings		
Single gene alterations	Contiguous gene deletions/duplications	Mitochondrial genome
1(1)*	None	Not Ordered

* genes(alterations)

Indication for Testing

Autism, developmental regression, seizures

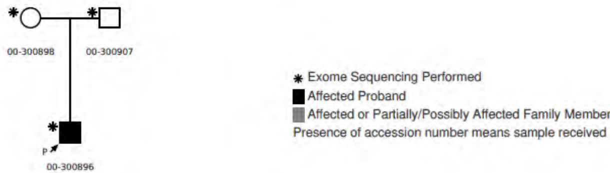
Results

Gene (RefSeq ID)	Characterized/ Uncharacterized Gene	Relevant Associated Syndrome	Mode of Inheritance	Genotype	Alteration	Alteration Classification
CHD2 (NM_001271)	Characterized	*CHD2*-related developmental and epileptic encephalopathy	Autosomal dominant	Heterozygous, *de novo*	c.443+1G>A	Pathogenic Mutation

Interpretation

- **Overall, the evidence suggests that the identified *CHD2* alteration is the cause of the patient's clinical symptoms.** Clinical correlation is recommended.

Family Pedigree



Analyses Performed

i) Full exome sequencing, bioinformatics, filtering and manual review based on autosomal and X-linked dominant and recessive and Y-linked inheritance models of the proband, mother, and father was performed. Medical review of characterized genetic etiologies revealed an alteration with likely clinical relevance.

ii) Because a characterized finding was identified, medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings was not performed.

*Uncharacterized genes are not currently established to underlie Mendelian genetic conditions. An uncharacterized gene will be classified as a "candidate" or "suspected candidate" when sufficient evidence, based on Ambry's comprehensive, rule-based scoring criteria, is available (Farwell Hagman, 2017) (http://www.ambrygen.com/candidate-gene-reporting).

Raw Data

A table with additional variant filtering details can be found with the raw data filtered variant list (if requested). This list includes clinically irrelevant characterized genes and uncharacterized genes which could not be ruled out (if analyzed); these alterations are not systematically confirmed via Sanger sequencing. The filtered variant list can be requested via this form (www.ambrygen.com/file/material/view/1262/Raw_Sequence_Data_Consent_0619_final.pdf).

Metrics and Coverage

The values below represent metrics from the family's exome sequencing. Complete coverage data for this proband can be e-mailed or made available for download through AmbryPort by request.

	Depth of coverage	
Relationship	% Bases ≥ 10x	% Bases ≥ 20x
Proband	98.5	98.2

MRN #: N/A Accession #: 00-300896

CHD2 Gene Details

Gene Symbol	RefSeq ID	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons	Number of Amino Acids
CHD2	NM_001271	chr15:93443551-93571237	127687	39	38	1828 aa

The *CHD2* gene is located on chromosome 15q26.1 and encodes the chromodomain-helicase-DNA-binding protein 2. Pathogenic alterations in this gene are known to cause *CHD2*-related developmental and epileptic encephalopathy, which is an autosomal dominant condition that generally occurs *de novo*. *CHD2*-related developmental and epileptic encephalopathy is characterized by early-onset refractory seizures and cognitive slowing or regression associated with frequent ongoing epileptiform activity, developmental delay, intellectual disability, and autism spectrum disorders. Seizure onset is typically between ages six months and four years, and seizure types include drop attacks, myoclonus, and rapid onset of multiple seizure types associated with generalized spike-wave on EEG, atonic-myoclonic-absence seizures, and clinical photosensitivity (Wilson, 2021). Loss of function has been reported as the mechanism of disease for *CHD2*-related developmental and epileptic encephalopathy.

Gene information

CHD2 c.443+1G>A

Alteration description:
The c.443+1G>A intronic variant results from a G to A substitution one nucleotide after exon 5 (coding exon 4) of the *CHD2* gene. Alterations that disrupt the canonical splice site are expected to cause aberrant splicing, resulting in an abnormal protein or a transcript that is subject to nonsense-mediated mRNA decay.

Affected individuals:
This variant has been determined to be the result of a *de novo* mutation in an individual with *CHD2*-related developmental and epileptic encephalopathy (Yang, 2020).

Population frequency:
This variant was not reported in population-based cohorts in the Genome Aggregation Database (gnomAD).

Family inheritance:

Gene (RefSeq ID)	Alteration	Exon	Proband (00-300896)	Mother (00-300898)	Father (00-300897)	Inheritance
CHD2# (NM_001271)	c.443+1G>A	Intron 5	Heterozygous	Negative	Negative	*De novo*♦

#Alteration(s) detected via exome sequencing with Q-score and read depth above established confidence thresholds. Confirmation by automated fluorescence dideoxy sequencing (aka "Sanger") sequencing not performed.
♦Note that the possibility of germline mosaicism cannot be ruled out.

Based on the available evidence, the *CHD2* c.443+1G>A alteration is classified as pathogenic.

Variant Pathogenicity Information

MRN #: N/A Accession #: 00-300896

ExomeNext® Assay Information

General Information: Ambry's ExomeNext® is a cost-effective, comprehensive, integrated exome sequencing assay designed to increase the diagnostic yield for genetic disorders that have eluded definitive delineation using traditional diagnostic approaches. The exome represents all the exons, which are the regions in the human genome that are translated into proteins. It is estimated that the protein-coding regions of the human genome contain about 85% of the disease-causing mutations. Whole-exome sequencing has been successfully applied to identify both inherited and de novo mutations in a diverse variety of autosomal dominant, recessive, and X-linked disorders. In addition to the primary analysis, which is performed with the purpose of uncovering the underlying genetic cause for a given clinical presentation, the clinical diagnostic exome may also be utilized to provide secondary findings, which are pathogenic or likely pathogenic alterations in genes that lead to diseases unrelated to the patient's present clinical presentation or reason for referral.

Result Reports: A primary clinical report will only be generated for the proband regardless of number of family members submitted. However, it may be possible to infer information about family member's results based on the proband's report. Pathogenic mutation(s) likely to factor into the patient's current clinical condition are always reported. Since new scientific information becomes available on a regular basis, this could alter the interpretation of previously reported results. In the event of a change in interpretation, an unsolicited reclassification/amended report may be issued to the ordering clinician. Pathogenic mutations or likely pathogenic variants identified within the ACMG secondary findings gene list are reported separately unless opted out (Kalia, 2016; Miller, 2023). Expanded childhood onset secondary findings are available in a separate report for prenatal exome testing orders.

Test Limitations: This test was developed and its performance characteristics were determined by Ambry Genetics. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be referred to a genetic counselor, medical geneticist, or physician skilled in evaluating the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The following types of mutations are detectable: nucleotide substitutions, small deletions, small insertions, small indels, and gross deletions and duplications. The overall coverage of each gene varies and each individual may have slightly different coverage yield. Accurate exon-level gross deletion and duplication detection by exome sequencing is dependent on several factors such as inherent sequence properties of the targeted regions, including shared homology and exon size, depth-of-coverage, efficiency of capture, and degree of read depth variation in the selected reference samples. Therefore, the specificity and sensitivity of gross deletion and duplication detection by exome sequencing may be reduced. Exome sequencing is not intended to analyze the following types of mutations: gross rearrangements, deep intronic variations, long repeat sequences, portions of genes with highly homologous pseudogenes, trinucleotide repeat sequences, mutations involved in tri-allelic inheritance, certain mitochondrial genome mutations, epigenetic effects, oligogenic inheritance, and X-linked recessive mutations in females who manifest disease due to skewed X-inactivation and other unknown abnormalities. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutations in an undetectable region. Ambry's ExomeNext® detection rate is 30% for positive or likely positive relevant findings identified in established disease-gene associations, and an additional 7% for candidate gene findings (Farwell, 2015; Farwell Hagman, 2017). Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from rare genetic variants that may interfere with analysis, or from other sources.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Samples are prepared using the IDT xGen Exome Research Panel V1.0 (IDT). Each DNA sample is sheared, adaptor ligated, PCR-amplified and incubated with the exome baits. Captured DNA is eluted and PCR amplified. Final quantified libraries are seeded onto an Illumina flow cell and sequenced using paired-end, 150 cycle chemistry on the Illumina NovaSeq, NextSeq or HiSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools using genome assembly GRCh 37/hg19. Data is annotated with the Ambry Variant Analyzer tool (AVA), including: nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, and predicted functional impact. Data analysis is focused on small insertions and deletions, canonical splice site alterations, and non-synonymous alterations. Gross deletion/duplication analysis is assessed for proband only for all genes within the targeted exome using a custom pipeline based on coverage and/or breakpoint analysis from NGS data and is followed by a confirmatory orthogonal method, as needed. The following sites are used to search for previously described gene mutations and polymorphisms: the Human Gene Mutation Database (HGMD), the Single Nucleotide Polymorphism database (dbSNP), ExAC, ESP, 1000 genomes, and online search engines (e.g., PubMed). Variants are then filtered further based on applicable inheritance models. Co-segregation studies are performed if family members are available. All relevant findings undergo manual review by molecular geneticists using integrated genomics software (IGV) and/or undergo confirmation either by automated fluorescence dideoxy (aka "Sanger") sequencing or via trio exome sequencing with coverage and alternate read ratios above established confidence thresholds. Additionally, gross deletions/duplications are confirmed by SNP Microarray (Affymetrix® CytoScan™ HD Array), in-house targeted array, MLPA, or Sanger sequencing. Co-segregation results may be confounded by many factors which cannot be completely ruled out including haploinsufficiency, reduced penetrance, age-of-onset, and/or variable expressivity. Relevant findings are evaluated from among the genes in Ambry's internal, dynamic gene database which classifies genes as characterized or uncharacterized Mendelian disease genes based on clinical validity (Smith, 2017). Characterized genes are those currently known to underlie at least one Mendelian genetic condition. Uncharacterized genes are those not currently known or with insufficient evidence to be associated with a Mendelian genetic condition. Characterized genes are analyzed first. If no positive findings are identified, reflex analysis of uncharacterized genes occurs for potential identification of a candidate gene finding. The analysis of candidate gene findings is only performed when an informative trio is received for testing and focuses on de novo, autosomal recessive, or X-linked inherited alterations. Each alteration remaining after inheritance model filtering is analyzed and/or reviewed by board certified molecular geneticists to identify the most likely causative alteration(s). Interpretation is based on the clinical, family, and test information provided by the referring provider and the current knowledge of genes and alterations at the time of reporting. Screening and analysis of known mtDNA mutations related to the proband's clinical phenotype is included if ordered. Amplification of the entire mitochondrial genome is carried out by long distance PCR and sequencing of mitochondrial DNA (mtDNA) is performed separately on Illumina MiSeq.

Methodology and Limitations

If you have any questions or feedback on this handout, would like to suggest changes or inquire about additional training, please contact the Variant Curation Task Force at variantcurationrequests@mgb.org.

Thank you to the following people for helping in the development of this handout:
Lauren Briere, MS, CGC, Helen Chen, MS, CGC, Emma Henricks, MS, CGC, Anna Nagy, MS, CGC, Chelsea Stevens, MS, CGC, the Variant Curation Task Force members