ExomeNext

Ambry Genetics
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PATIENT
Name: **Patient, Sample**
Accession #: 00-00000
DOB: 01/01/1901
Gender: Female
MRN: N/A
Indication: Diagnostic

TEST INFORMATION
Portal Order #:
Family #: 0000
Specimen #: N/A
Specimen type: Blood EDTA
Collection date: 00/00/2018
Final Report: 00/00/2018

PHYSICIAN
Sample Doctor, MD

ADDITIONAL RECIPIENTS
Sample GC
Phone: 000-000-0000
Fax: 000-000-0000

FACILITY
Sample Facility
12345 Wonderful Lane
Somewhere, NY 99999-9999
Phone: 000-000-0000
Fax: 000-000-0000

UNCERTAIN: Suspected Candidate: Alteration(s) of potential clinical relevance detected

Clinically Relevant Findings			Notable Findings
Characterized genes	**Mitochondrial genome**	**Uncharacterized genes**	
None	**None**	**1(1)***	1(1)^

* genes(alterations)

^ Notable findings are alterations of unlikely clinical relevance, but which were unable to be ruled out. See details in the notable findings section of the supplemental report.

Indication for Testing

Epilepsy, brain malformations, spastic quadriplegic cerebral palsy, intellectual disability

Results and Interpretation

Gene (RefSeq ID)	Characterized/ Uncharacterized Gene	Alteration	Genotype	Alteration Type	Alteration Classification
MAST1 (NM_014975)	Uncharacterized	c.1577T>C (p.L526P)	Heterozygous, *de novo*	Missense	Variant of Uncertain Significance

- **Overall, the evidence suggests it is uncertain if the identified *MAST1* alteration is the cause of the patient's clinical symptoms.**
- Notable findings were detected in the following gene(s): *TRIT1*. See the supplemental report for additional information.

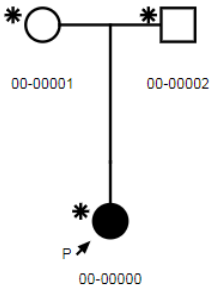
Notes

- Secondary findings were issued in a separate report.
- Genetic counseling is a recommended option for all patients undergoing genetic testing.
- Any tests on hold, previously reported, and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) have not been included in this report. For additional information, please contact Ambry Genetics.

Electronically Signed By {This value is automatically generated when clickin "Save & Sign"}

All content hereafter is supplemental information to the preceding report.

Family Pedigree



✱ Exome Sequencing Performed
Affected
Partially or Possibly Affected
Presence of accession number means sample received

Analyses Performed

i) Full exome sequencing, bioinformatics, filtering and manual review based on autosomal and X-linked dominant and recessive inheritance models of the proband, mother, and father was performed. Medical review of characterized genetic etiologies did not reveal any alterations with likely clinical relevance.

ii) Medical review of uncharacterized genes* and gene-disease relationships for potential candidate gene findings revealed alterations with likely clinical relevance.

iii) Sequencing of mitochondrial DNA (mtDNA) followed by screening and analysis of 68 known pathogenic alterations related to the proband's clinical phenotype did not reveal any alterations with likely clinical relevance.

*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.

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	98.48	97.86
Mother	98.53	98.28
Father	98.59	97.89

MAST1 Gene Details

Protein (Number of Amino Acids)	RefSeq ID	Location	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons
Microtubule associated serine/threonine kinase 1 (1570 aa)	NM_014975	19p13.13	12949259-12985766	36508	26	26

Lines of evidence to evaluate the involvement of *MAST1* in the patient's clinical phenotype:

The *MAST1* gene currently has limited evidence for involvement in human Mendelian disease based on the ClinGen clinical validity assessment criteria (aka “uncharacterized”) (Rehm, 2015). The functional consequences of presumably deleterious alterations within uncharacterized genes are typically unknown, including whether mutation mechanism is gain-of-function or dominant negative versus loss-of-function or whether a phenotype is produced in a dominant or recessive manner. While evidence may support the involvement with a patient's phenotype, not all alterations in uncharacterized genes can be definitively stated as disease-causing until further functional studies and multiple case reports have proven their clinical significance.

As part of an ongoing process to better understand the clinical significance of the reported candidate gene finding in your patient and to characterize novel candidate disease genes, Ambry participates in data sharing and follow-up research collaborations with physicians, clinics, and researchers. Please let us know if you would like us to enroll your patient in a research collaboration if one is/becomes available by e-mailing cde@ambrygen.com.

Supportive evidence:

• **Gene function:**
The *MAST1* gene encodes the microtubule-associated serine/threonine kinase 1 (MAST1) protein, characterized by the presence of an N-terminal kinase domain followed by a postsynaptic density protein-95/discs large/zona occludens-1 (PDZ) scaffolding domain involved in protein-protein interactions (reviewed in Garland, 2008). MAST1 is also known as syntrophin-associated serine/threonine kinase (SAST) due to its interaction with the syntrophin protein at neuronal postsynaptic densities in the central nervous system (Lumeng, 1999). An essential role for *MAST1* in the regulation of brain size and nervous system development has been suggested (Shen, 2018).

• **Expression profile:**
Two splice isoforms of MAST1/SAST have been reported, SAST170 (molecular weight of ~170 kDa) with 1570 amino acids and SAST124 (molecular weight of ~124 kDa) with 1117 amino acids, which are both predominantly expressed in the mammalian brain (Lumeng, 1999; Yano, 2003). Additionally, SAST is expressed within the vascular endothelium close to neuronal nuclei throughout the cortex and cerebellum, in developing spermatid acrosomes, and in cell bodies and axons of motor neurons (Lumeng, 1999).

• **Previously-reported patients:**
Using exome sequencing, Shen et al. (2018) reported *de novo* missense alterations in the *MAST1* gene in three unrelated patients and an unrelated patient identified previously by de Ligt et al. (2012) and Gilissen et al. (2014). Clinical features in all patients included global developmental delay, moderate to severe intellectual disability (ID), delayed to absent speech, and microcephaly (Shen, 2018). Brain MRI revealed hypoplasia or partial agenesis of the corpus callosum, cerebellar vermis and pontocerebellar hypoplasia, and arachnoid cysts (Shen, 2018). All three patients identified by Shen et al. (2018) had constipation and other variable gastrointestinal issues including vomiting and feeding difficulties. Two had sleep disturbances, while the third had obstructive sleep apnea. One patient also had seizures, scoliosis, strabismus, hydronephrosis, and ureter pelvic junction obstruction. *De novo* missense alterations in *MAST1* were also reported in one patient with diplegic cerebral palsy (McMichael, 2015) and in another with moderate ID, speech delay, facial dysmorphism, autism, seizures, and dystonia (Bowling, 2017).

• **Overlapping microdeletions/CNV:**
The *MAST1* gene is considered to be one of the candidate genes responsible for the clinical findings in the 19p13.13 microdeletion/microduplication syndrome (Auvin, 2009; Dolan, 2010). Patients with 19p13.13 microdeletions present with macrocephaly with frontal bossing, variable ophthalmologic abnormalities including strabismus, nystagmus, eso/exotropia, and optic nerve hypoplasia, and gastrointestinal issues including abdominal pain and vomiting. Additional features in these patients included global developmental delay, hypotonia, and seizures (Auvin, 2009; Dolan, 2010). Patients with 19p13.13 microduplications had microcephaly, but shared most of the other features of the 19p13.13 microdeletion patients (Dolan, 2010).

• **Protein family, co-localization, or interaction:**
MAST family kinases bind to the phosphatase and tensin homolog (PTEN) protein via their PDZ domains and mediate its phosphorylation, increasing its stability (Valiente, 2005). Mutations in *PTEN* are associated with *PTEN* hamartoma tumor syndrome (PHTS) which is characterized by a wide spectrum of clinical features including skin, neurologic, and gastrointestinal manifestations (reviewed in Tan, 2011). Neurological features of PHTS include macrocephaly, autism, global developmental delay, and cognitive impairment (Butler, 2005; Buxbaum, 2007; Herman, 2007; Varga, 2009).

• **Mutational mechanism:**
Notably, the ExAC database reports significantly lower-than-expected numbers of both truncating (probability of LoF intolerance, pLI=1) and missense alterations (z score=7.64) in the *MAST1* gene, indicating that this gene is highly intolerant of any kind of variation.

MAST1 c.1577T>C (p.L526P)

The alteration results in an amino acid change:
The c.1577T>C (p.L526P) alteration is located in coding exon 14 of the *MAST1* gene. This alteration results from a T to C substitution at nucleotide position 1577, causing the leucine (L) at amino acid position 526 to be replaced by a proline (P).

The alteration is not observed in population databases:
Based on data from the Genome Aggregation Database (gnomAD), the *MAST1* c.1577T>C alteration was not observed, with coverage at this position.

The altered amino acid is conserved throughout evolution:
The p.L526 amino acid is well conserved in available higher vertebrate species.

The alteration is predicted deleterious by *in silico* models:
The p.L526P alteration is predicted to be probably damaging by Polyphen and deleterious by SIFT *in silico* analyses.

The amino acid is located in a functionally important protein domain:
The p.L526 amino acid is located in the protein kinase domain of the MAST1 protein, which mediates the phosphorylation of target proteins (Yano, 2003).

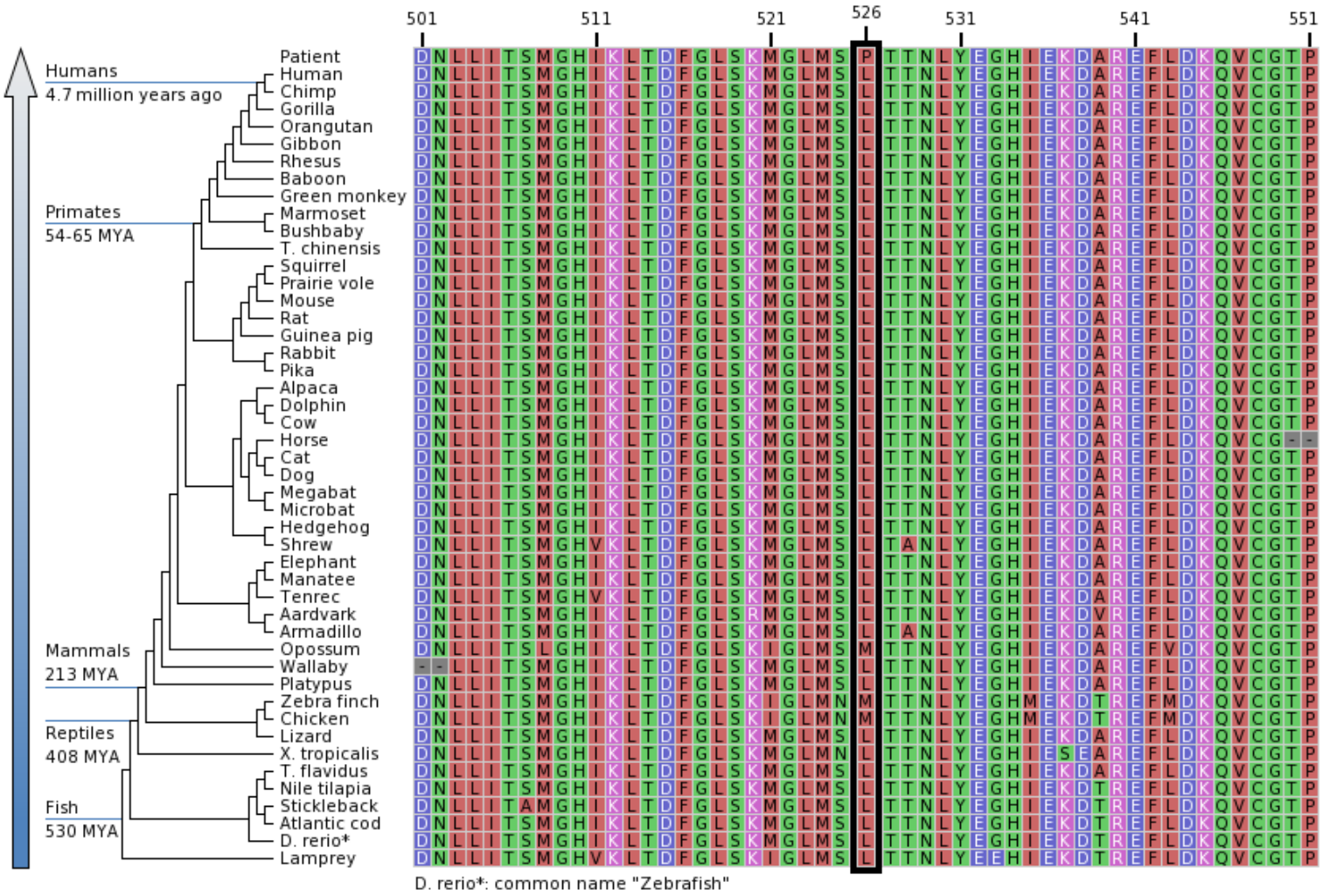
The alteration is *de novo* in the proband herein:
Co-segregation analysis of the c.1577T>C (p.L526P) alteration in this family revealed that the unaffected mother and father do not carry this alteration, indicating a likely *de novo* mutation occurrence. (Note that the possibility for germline mosaicism cannot be ruled out.)

Gene (RefSeq ID)	Alteration	Exon	Proband (18-077878)	Mother (18-078267)	Father (18-086264)	Conclusion
MAST1† (NM_014975)	c.1577T>C (p.L526P)	CDS 14	Heterozygous	Negative	Negative	*De novo*

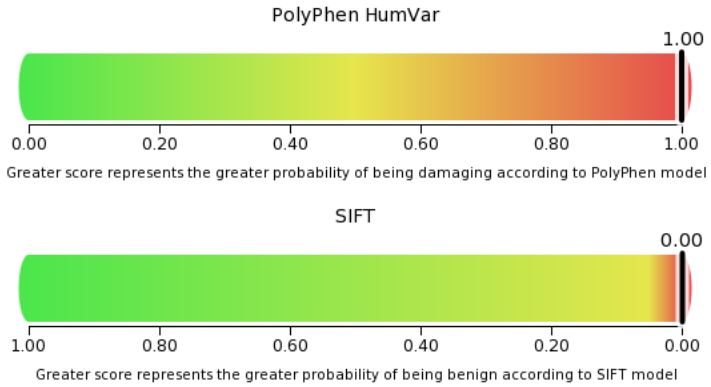
†Alteration(s) detected via exome sequencing with Q-score and read depth above established confidence thresholds. Confirmation by automated fluorescence dideoxy sequencing (aka "Sanger") sequencing not performed.

Based on the available evidence, the clinical significance of the *MAST1* c.1577T>C (p.L526P) alteration is uncertain.

MAST1 c.1577T>C (p.L526P)



Trait	Leu (L)	Pro (P)
Amino Acid Name	Leucine	Proline
Polarity/Charge	non-polar	non-polar
pH	neutral	neutral
Residue Weight	113	97
Hydrophobicity Score	3.8	-1.6
Hydrophilicity Score	-1.8	0
Secondary Structure Propensity	strong α former / β former	strong α breaker / strong β breaker



Notable Findings (alterations of unlikely clinical relevance)

Total "notable findings" detected: 1 notable gene (1 unique alteration).

Notable Findings are alterations of unlikely clinical relevance, but which were unable to be ruled out. These may include (1) alterations in characterized genes with minimal or inconsistent clinical overlap, (2) a single heterozygous alteration in a recessive gene with limited or inconsistent clinical overlap, or (3) alterations in characterized or uncharacterized genes which failed to co-segregate with disease in the current family. These alterations are not systematically confirmed via Sanger sequencing.

Gene (RefSeq ID): *TRIT1* (NM_017646)

Alteration(s)	Genotype	Alteration Type	Alteration Classification	MAF[1]	Database Records[2]
c.334delC (p.R112Efs*36)	Heterozygous, paternal	Frameshift	Pathogenic Mutation	0.07%	N/A

Relevant Associated Syndrome (Inheritance)[3]: Combined oxidative phosphorylation deficiency 35 (AR)

Rationale: The alteration is unlikely to explain the patient's phenotype due to minimal phenotypic overlap and because the patient has a single heterozygous alteration in a gene generally associated with an autosomal recessive syndrome. All coding exons of this gene were covered 100% at ≥20x. Overlapping clinical features with this patient include developmental delay, hypotonia, and epilepsy; however, this patient is not reported to have microcephaly.

Clinical Correlation: Uncertain (carrier only)

[1]MAF = minor allele frequency. The number displayed reflects the highest ethnicity-specific MAF in gnomAD.
[2]dbSNP and/or HGMD alteration record.
[3]Relevant syndrome data collected from HGMD, OMIM, the medical literature, and/or internal Ambry data.
AD= autosomal dominant, AR= autosomal recessive, XL= X-linked, XLR= X-linked recessive, XLD= X-linked dominant, YL= Y-linked, CX= complex inheritance (including multiple modes of inheritance).
Recessive Diseases: Cannot rule out undetectable biallelic alteration or potential gain-of-function, haploinsufficiency, or dominant-negative effect.

Report References

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Resources Used for Bioinformatics, Medical Review Filtering, and Reporting

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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 virtually 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.

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 re-classification/amended report may be issued to the ordering clinician.

Expected (Normal) Value: Diagnostic: 0, 1, or more mutation(s) detected.

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 and small indels. Exome sequencing is not intended to analyze the following types of mutations: gross deletions/duplications, 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 K, *et al.*, Genet Med., 2014 and Farwell Hagman K, *et al.*, Genet Med., 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 SeqCap EZ VCRome 2.0 (Roche NimbleGen) or 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, 100 or 150 cycle chemistry on the Illumina HiSeq or NextSeq. Initial data processing, base calling, alignments and variant calls are generated by various bioinformatics tools. 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. 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. 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 E, *et al.*, Hum Mutat, 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 uncharacterzied 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.

Analysis of Alterations: The following lines of evidence are used to assess the pathogenic nature of an alteration: presence in affected and healthy populations, co-segregation information, functional studies, alteration type, conservation, *in silico* predictions, and presence in a functional protein domain. The absence of a particular line of evidence implies that no information was found or it does not apply for that alteration type (eg. *in silico* for truncating alterations).

Analytical range: Approximately 75% of the bases are expected to have quality scores of Q30 or higher, which translates to an expected base-calling error rate of 1:1000, or an expected base-calling accuracy of 99.9%. Additionally, 90% and 95% of the exome will be covered at ≥20x and ≥10x respectively under current run conditions, generally sufficient for high quality heterozygous and homozygous variant calling for germline variants. For any given individual ~ 10% of the targeted exome is not sequenced well enough to make a confident call. Each individual may have slightly different coverage yield distributions within the exome. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. The mean depth of coverage for targeted mitochondrial bases is greater than 1,000X.