ExomeNext

Ambry Genetics
A Konica Minolta Company

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

POSITIVE: Clinically Relevant Alteration(s) Detected

Clinically Relevant Findings			Notable Findings
Characterized genes	**Mitochondrial genome**	**Uncharacterized genes**	
1(1)*	**None**	**N/A**	None

* genes(alterations)

Indication for Testing

Macrocephaly, developmental delay, pulmonic valve stenosis, dysmorphic features.

Results and Interpretation

Gene (RefSeq ID)	Relevant Associated Syndrome	Mode of Inheritance	Characterized/ Uncharacterized Gene	Alteration	Genotype	Alteration Type	Alteration Classification	Clinical Correlation
PPP1CB (NM_002709)	Noonan-like syndrome with loose anagen hair	Autosomal dominant	Characterized	c.146C>G (p.P49R)	Heterozygous, *de novo*	Missense	Pathogenic Mutation	Positive

- **Overall, the evidence suggests that the identified *PPP1CB* alteration is the cause of the patient's clinical symptoms.**

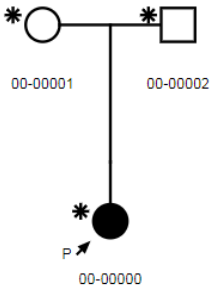
Notes

- 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 Sample Director, on 0/00/2018 at 0:00:00 PM

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 revealed an alteration with likely clinical relevance.

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

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	98.59	98.16
Mother	98.62	98.4
Father	98.68	98.3

PPP1CB Gene Details

Protein (Number of Amino Acids)	RefSeq ID	Location	Genomic Coordinates (GRCh37)	Genomic Size (bp)	Total Exons	Coding Exons
Protein phosphatase 1, catalytic subunit, beta isozyme (327 aa)	NM_002709	2p23	28974626-29025806	51181	8	8

The proband's clinical presentation is consistent with that of previously-reported patients with *PPP1CB* alterations:

The *PPP1CB* gene is located on chromosome 2p23.2 and encodes the protein phosphatase 1, catalytic subunit, beta isozyme protein (OMIM_600590). PPP1CB is one of three catalytic subunits of the protein phosphatase 1 (PP1) protein, which is involved in the regulation of multiple cellular functions including cell division, protein synthesis, carbohydrate metabolism, muscle contraction, and neuronal signaling (reviewed in Bollen, 2010; Heroes, 2013). PPP1CB is a component of the RAS/MAPK pathway (reviewed in Bertola, 2016). Pathogenic alterations in the *PPP1CB* gene are associated with Noonan-like syndrome with loose anagen hair, an autosomal dominant disorder which is typically caused by *de novo* alterations (OMIM_617506).

Noonan-like syndrome with loose anagen hair (NSLH) is characterized by developmental delay, intellectual disability, growth restriction, relative macrocephaly, dymorphic features, and unruly and/or slow-growing hair (reviewed in Bertola, 2016). Dysmorphic facial features reported are similar to those seen in Noonan syndrome, and include low-set, posteriorly rotated ears, prominent forehead/dolichocephaly, hypertelorism, epicanthus, and pectus anomalies (Gripp, 2016; Bertola, 2016; Ma, 2016). Other features reported in subsets of patients include ventriculomegaly, feeding difficulties (especially in the newborn period), irregular skin pigmentation, hypotonia, cryptorchidism and various eye anomalies. Cardiac involvement is commonly seen, but is highly variable between patients with reports of dilated aortic root, valve anomalies/insufficiency, patent ductus arteriosus, aortic arch anomalies, and right bundle branch block (Gripp, 2016; Bertola, 2016; Ma, 2016). Anxiety has also been reported in some patients (Hamdan, 2014; Gripp, 2016; Bertola, 2016). One patient to date has been reported with mild hearing loss, and a second patient was reported to have suspected hearing loss (Gripp, 2016).

A recurrent missense alteration c.146G>C (p.P49R) has been observed in more than half of patients reported to date (Gripp, 2016; Bertola, 2016; Ma, 2016).

Based on the available evidence, the clinical overlap of this gene with the patient's reported phenotype is positive. Clinical correlation is recommended.

PPP1CB c.146C>G (p.P49R)

The alteration results in an amino acid change:
The c.146C>G (p.P49R) alteration is located in coding exon 2 of the *PPP1CB* gene. This alteration results from a C to G substitution at nucleotide position 146, causing the proline (P) at amino acid position 49 to be replaced by an arginine (R).

The alteration has been observed in affected individuals:
The *de novo* c.146C>G (p.P49R) alteration has been reported in three individuals who presented with a phenotype resembling Noonan syndrome (Gripp, 2016). Clinical features included developmental delays, hypertelorism, relative macrocephaly with prominent forehead, low-set posteriorly rotated ears, various eye anomalies, slow growing and unruly hair, and some mild abnormalities on brain imaging. Cardiac anomalies were observed in two patients that included pulmonic valve stenosis and mitral valve thickening.

The alteration is not observed in population databases:
Based on data from the Genome Aggregation Database (gnomAD), the *PPP1CB* c.146C>G alteration was not observed, with coverage at this position.

The altered amino acid is conserved throughout evolution:
The p.P49 amino acid is conserved in available vertebrate species.

The alteration is predicted deleterious by *in silico* models:
The p.P49R alteration is predicted to be probably damaging by Polyphen and deleterious by SIFT *in silico* analyses.

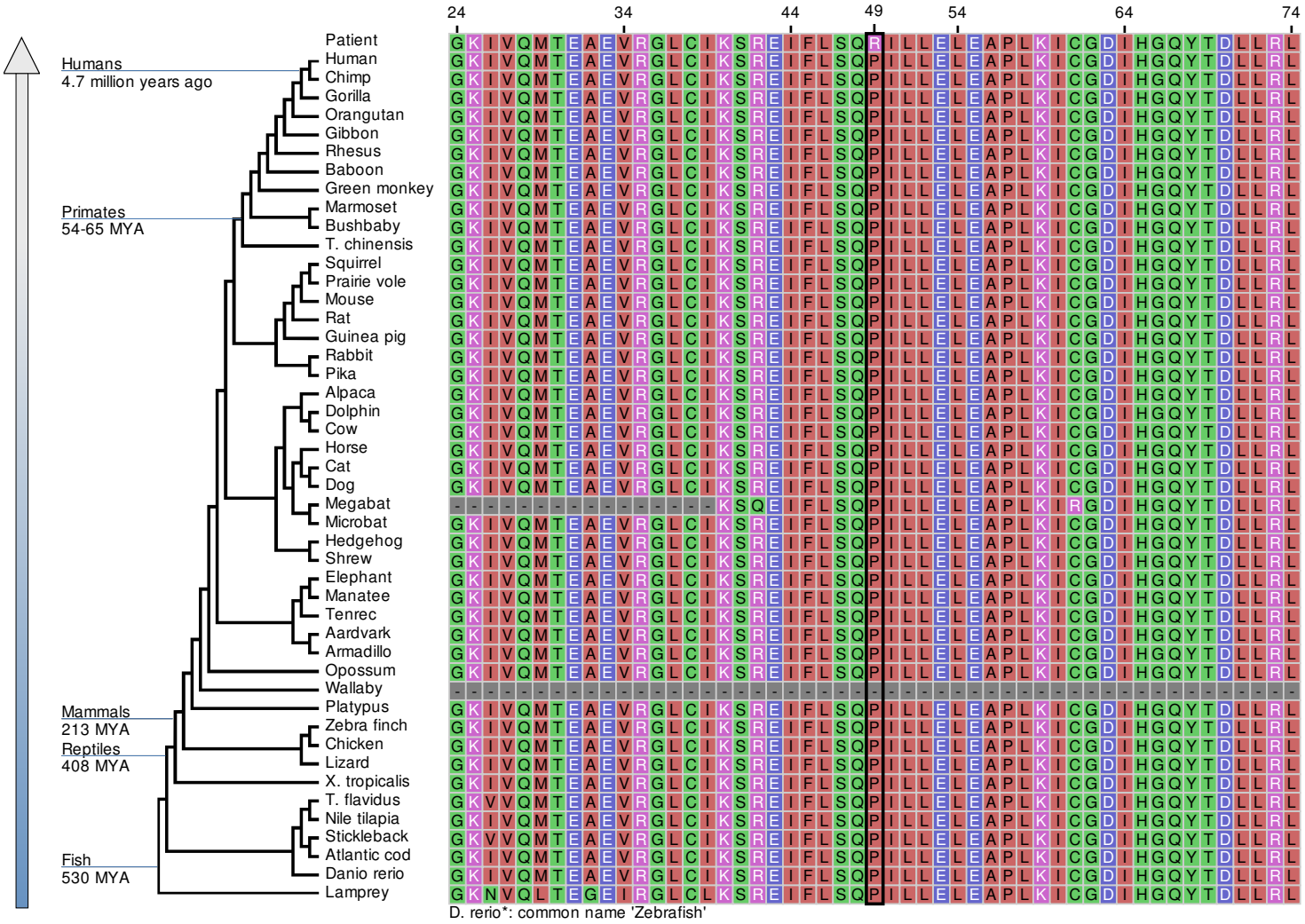
The alteration is *de novo* in the proband herein:
Co-segregation analysis of the c.146C>G (p.P49R) alteration in this family revealed that the unaffected mother and father do not carry this alteration, indicating a likely *de novo* mutation occurrence. (Note that the possibility for germline mosaicism cannot be ruled out.)

Gene (RefSeq ID)	Alteration	Exon	Proband (14-108227)	Mother (14-108261)	Father (14-108262)	Conclusion
PPP1CB^ (NM_002709)	c.146C>G (p.P49R)	CDS 2	Heterozygous	Negative	Negative	*De novo*

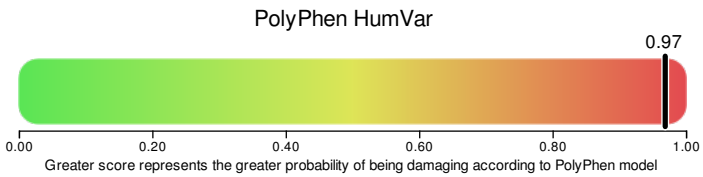
^Alteration(s) confirmed by automated fluorescence dideoxy sequencing (aka "Sanger") sequencing.

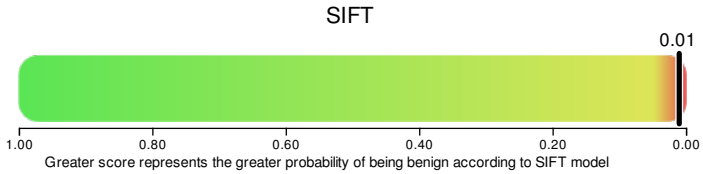
Based on the available evidence, the *PPP1CB* c.146C>G (p.P49R) alteration is classified as pathogenic.

PPP1CB c.146C>G (p.P49R)



Trait	Pro (P)	Arg (R)
Amino Acid Name	Proline	Arginine
Polarity/Charge	non-polar	positively charged
pH	neutral	basic
Residue Weight	97	156
Hydrophobicity Score	-1.6	-4.5
Hydrophilicity Score	0	3
Secondary Structure Propensity	strong α breaker / strong β breaker	α indifferent / β indifferent





Notable Findings (alterations of unlikely clinical relevance)

None identified.

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.

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 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.