ExomeNext® Report

Secondary Findings Report

FINAL REPORT - 2/20/2023

Ambry Genetics

PATIENT

Legal Name: Last, First

Chosen Name
Sex at Birth: F
Gender

Accession #: 22-000000
Birthdate: 01/01/1980
MRN #: 000000
Indication: Diagnostic

TEST INFORMATION

Specimen #: 44-55-66
Specimen: Blood EDTA
AP2 Order #: 00000
Collected: 12/30/2022
Received: 12/31/2022
Test Started: 12/31/2022

ORDERING PROVIDER

Ordering Provider:
Sample Doctor, MD

Client:
Sample Facility

ADDITIONAL RECIPIENTS

Sample GC, CGC

NEGATIVE: No Pathogenic or Likely Pathogenic Variants Detected

RESULTS

- This individual was not found to have pathogenic or likely pathogenic variants in the ACMG recommended minimum list of genes (Miller 2022). Alterations included in secondary findings reports are previously described alterations reported within peer-reviewed publications and/or pathogenic or likely pathogenic variants based on ACMG guidelines (Richards, 2015). Only pathogenic and likely pathogenic alterations are reported.
- A negative secondary findings result does not rule out the possibility that the individual carries a pathogenic or likely pathogenic alteration in a gene on the ACMG list. Not all exons in the genome are sequenced and certain genomic regions may have low coverage.
- Please note that copy number variants are not analyzed for secondary findings, therefore any large deletions/duplications in the tested genes will not be reported.

RECOMMENDATIONS

- Clinical correlation is recommended, and genetic testing results should be interpreted in the context of the patient's clinical and family history.
- Genetic counseling is recommended and can assist with additional testing, evaluation, medical management, appropriate testing of family members, and/or family planning.

Notes

- Alteration(s) related to this individual's phenotype are reported within the primary report (when applicable), and are not included in secondary findings reports.
- Reclassification reports will be issued for positive secondary findings that are downgraded to a variant of uncertain significance or lower after the time of the report. Results are not systematically reanalyzed for additional secondary findings after the time of the report.

ORDER SUMMARY

The following products were included in the test order for this individual. Please Note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- ExomeNext -Trio (Product Code 9995)
- ExomeNext ACMG Secondary Findings (Product Code 9920)

Electronically Signed By

All content hereafter is supplemental information to the preceding report.

ACMG RECOMMENDED SECONDARY FINDINGS LIST

Genes and associated phenotypes as recommended in Miller, 2023

*ACTA2, ACTC1, ACVRL1, APC, APOB, ATP7B** , *BAG3, BMPR1A, BRCA1, BRCA2, BTD**, *CACNA1S* , *CALM1* , *CALM2* , *CALM3* , *CASQ2**,*COL3A1, DES, DSC2, DSG2, DSP, ENG, FBN1, FLNC* , *GAA**, *GLA, HFE** (homozygous c.845G>A p.C282Y only), *HNF1A, KCNH2, KCNQ1, LDLR, LMNA, MAX, MEN1, MLH1, MSH2, MSH6, MUTYH**, *MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PALB2, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RBM20, RET, RPE65**, *RYR1* , *RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM127, TMEM43, TNNC1, TNNI3, TNNT2, TP53, TPM1, TRDN**, *TSC1, TSC2, TTN*^, *TTR, VHL*, and *WT1*.

* only reported when homozygous or 2 pathogenic or likely pathogenic variants present
^ only pathogenic or likely pathogenic frameshift and nonsense variants, and variants known to impact the splicing of TTN exons with high PSI sequencing analysis only

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. 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. Secondary findings analysis is a separate analysis of the exome data which is not performed during the primary data analysis.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's whole blood. Short Tandem Repeat markers are used for relationship study of proband and related family members. 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 nonsense variants, small insertions and deletions, canonical splice site alterations or non-synonymous variants. 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), NHLBI Exome Sequencing Project (ESP), 1000 genomes, HapMap data, and online search engines (e.g., PubMed). Variants are then filtered based on Mendelian inheritance model filtering. Alterations to be reported for secondary findings include previously described pathogenic alterations reported within peer-reviewed publications or pathogenic/likely pathogenic (VLP) alterations classified using criteria obtained from Ambry's General Variant Classification Scheme (https://www.ambrygen.com/science/variant-classification). Variants with reduced penetrance may not reported.

Literature support is evaluated for evidence. 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 validated coverage and alternate read ratio established confidence thresholds

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, 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.

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

Result Reports: Pathogenic mutations or VLPs identified within the ACMG SF gene list are reported unless opted out (Kalia, 2016; Miller D, 2021). Expanded childhood onset secondary findings are available for prenatal exome testing orders.

Disclaimer: This test was developed by 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 relegated 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, trinucleotide repeat sequences, mutations involved or tri-allelic inheritance, 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 mutation in the undetectable region. 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.

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations,

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