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SAMPLE REPORT

Ordered By	Contact ID:1332838 Org ID:8141	Patient Name: **8680, Test15**	
Physician:	Rhodarmer, Jake, MA	Accession #: **00-135418**	Specimen #:
	Ph:123-123-1234 Fx:123-123-1223	AP2 Order #: 614287	Specimen: Blood EDTA (Purple top)
Client:	MOCKORG44 (10829) 123 Somewhere Lane Suite 4 Heaven NV 78872 US	Birthdate: 09/08/9999	Age: y m
		Gender: U	
		MRN #: N/A	Collected: N/A
		Indication: Internal Testing	Received: 11/20/2018
		Ethnicity: N/A	

FHNext: *Analyses of 5 Genes Associated with Familial Hypercholesterolemia*

RESULTS

APOB **Pathogenic Mutation: p.R3527Q**

SUMMARY

POSITIVE: Pathogenic Mutation Detected

INTERPRETATION

- This individual is heterozygous for the **p.R3527Q** pathogenic mutation in the *APOB* gene.
- This result is consistent with a diagnosis of familial hypercholesterolemia (FH).
- The expression and severity of disease for this individual cannot be predicted.
- Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No additional pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected. Genes Analyzed (5 total): ***APOB, LDLR, LDLRAP1*** **and** ***PCSK9*** **(sequencing and deletion/duplication);** ***SLCO1B1*** **(sequencing only).**

COMMENT: For *SLCO1B1*, only the status of the c.521T>C alteration is analyzed and reported. The c.521T>C (rs4149056T>C or p.V174A) single nucleotide polymorphism (SNP) in the *SLCO1B1* gene was detected in heterozygous state in this individual. The presence of one normal function T allele and one decreased function C allele (11-36% of individuals) confers intermediate myopathy risk; a lower dose of simvastatin or use of an alternative statin and routine creatine kinase (CK) surveillance are recommended (Ramsey LB et al. *Clin Pharmacol Ther*. 2014 Oct;96(4):423-8).

APOB **Additional Information**

The **p.R3527Q** pathogenic mutation (also known as c.10580G>A), located in coding exon 26 of the *APOB* gene, results from a G to A substitution at nucleotide position 10580. The arginine at codon 3527 is replaced by glutamine, an amino acid with highly similar properties. This alteration (also reported as p.R3500Q) was identified in seven unrelated probands with familial hypercholesterolemia (FH) and found to segregate with disease in two families (Soria et al. *Proc Natl Acad Sci USA*. 1989; 86(2):587-91). This pathogenic mutation has been reported to be responsible for 2-6% of Western European FH cases and has been reported as an Amish founder mutation (Heath KE et al. *Atherosclerosis.* 1999;143(1):41-54; Lombardi et al. *Clin Genet*. 2000;57(2):116-24; Chmara M et al. *J Appl Genet.* 2010;51(1):95-106; Shen H et al. *Arch Intern Med.* 2010;170(20):1850-5). In addition, this alteration has been reported to result in defective low-density lipoprotein receptor binding (Boren J et al. *J Clin Invest.* 1998;101(5):1084-93). Two alterations at the same codon, p.R3527L and p.R3527W (reported as p.R3500L and p.R3500W), have also been associated with FH (Gaffney D et al. *Arterioscler. Thromb. Vasc. Biol.* 1995;15:1025-9; Fouchier SW et al. *Hum. Mutat.* 2005;26:550-6). Based on the supporting evidence, p.R3527Q is interpreted as a disease-causing mutation.

APOB (NM_000384.2) encodes the apolipoprotein B glycoprotein (both apoB-100 and apoB-48 form), which plays a central role in the human lipoprotein metabolism. The apoB-100 protein is the ligand for receptor-mediated endocytosis of low density lipoprotein and affects cholesterol absorption in plasma. The *APOB* gene is located at 2p24.1 and contains 29 coding exons. Mutations in the *APOB* gene cause autosomal dominant familial ligand defective apoB-100 (FDB) and familial hypobetalipoproteinemia (FHBL). FDB is characterized by hypercholesterolemia,

tendon xanthomas, and premature coronary artery disease. FHBL presents with acanthocytosis, deficiencies in fat soluble vitamins, atypical retinitis pigmentosa and neuromuscular abnormalities (Whitfield AJ et al. *Clin Chem*. 2004;50(10):1725-32).

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.

- FHNext (Product Code 8680)

ASSAY INFORMATION

General Information: Familial hypercholesterolemia (FH) is typically an autosomal dominant disease characterized by extremely high levels of plasma LDL (low density lipoprotein) cholesterol in the body, causing atherosclerotic plaque formation in the arteries and therefore a significantly increased risk for premature coronary artery disease (CHD) and myocardial infarction. Rarely, FH may be also inherited in an autosomal recessive manner (such as the more severe form associated with biallelic pathogenic variants in the *LDLRAP1* gene). Abnormally functioning LDL-receptors cause deposition of cholesterol in different parts of the body, including xanthelasma (skin), xanthomas (tendons), and coronary arteries (atherosclerosis). Historically, it has been estimated that 1 in 500 individuals has one *LDLR* mutation ("heterozygous" or "HeFH" – autosomal dominant form assumed) worldwide, while recent studies have suggested a higher prevalence of 1 in 200 individuals. It has been estimated that 1 in 160,000 individuals has two *LDLR* mutations ("homozygous" or "HoFH" – autosomal dominant form assumed) worldwide. Those with HeFH usually have a 2- to 3- fold elevation in plasma LDL-cholesterol and develop symptoms such as tendinous xanthomas, corneal arcus, and premature coronary artery disease. Those with HoFH also present planar xanthomas, with plasma LDL cholesterol increased 6- to 8-fold; death from myocardial infarctions during the first two decades of life is common. Due to a founder effect, FH is much more common in some population groups such as French Canadians, Afrikaners, Lebanese, Finns, and Ashkenazi Jews. Proper diet, exercise, and certain medications can help treat FH. Those with HeFH usually respond well with a combination of diet change and drugs (statins), while in some cases, additional therapies (LDL apheresis, MTP inhibitor, apoB antisense inhibitor, PCSK9 inhibitor) or liver transplantation may be recommended for those with HoFH or patients with FH who are intolerant of statins. A proactive diagnosis, in combination with selective treatments, can help decrease the incidence and progression of FH effects. 1-5% of those treated with statins experience myalgia; this can be related to a specific pharmacogenetic marker. The c.521T>C SNP pharmacogenetic marker in *SLCO1B1* confers an intermediate (in the heterozygous form) to high (in the homozygous form) simvastatin-induced myopathy risk; a lower dose of simvastatin or use of an alternative statin and routine creatine kinase (CK) surveillance are recommended in these cases. Data also support using genetic testing to offer cascade testing to family members, as it increases the detection of FH when compared to using serum cholesterol levels alone for diagnosis.

Methodology: The FHNext test is an analysis of 5 genes associated with familial hypercholesterolemia. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using a standardized kit and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes, and is followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100x depth of coverage and 40% het ratio thresholds are verified by Sanger sequencing (Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647). Gross deletion/duplication analysis is performed for all genes (excluding *SLCO1B1*) using a custom pipeline based on read-depth from NGS data followed by a confirmatory orthogonal method, as needed. Exon-level resolution may not be achieved for every gene. Sequence analysis is based on the following NCBI reference sequences: *APOB* NM_000384.2, *LDLR* NM_000527.4, *LDLRAP1*NM_015627.2, *PCSK9* NM_174936.3, and *SLCO1B1* NM_006446.4.

Analytical Range: The FHNext test targets detection of DNA sequence mutations in 4 genes (*APOB, LDLR, LDLRAP1,* and *PCSK9*) by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. For *SLCO1B1*, only the status of the rs4149056T>C (c.521T>C) alteration is analyzed and reported with respect to risk with simvastatin exposure. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of all genes (excluding *SLCO1B1*).

Result Reports: In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation:** alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP):** alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carries typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS):** alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based on personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

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

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
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8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. PolyPhen [Internet]: Adzhubei IA, et al. *Nat Methods.* 2010;7(4):248-249. genetics.bwh.harvard.edu/pph2.
13. SIFT [Internet]: Ng PC & Henikoff S. *Hum Genet.* 2006;7:61-80. http://sift.jcvi.org.
14. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
15. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
16. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. 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 forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of 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 **FHNext** test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels, and gross deletions/duplications. These tests are not intended to analyze the following types of mutations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. 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 group. The **FHNext** test is designed and validated to be capable of detecting >99% of described mutations in the genes represented on this test (analytical sensitivity). The clinical sensitivity of the **FHNext** test may vary widely according to the specific clinical and family history. Familial hypercholesterolemia is a complex clinical disorder. Mutations in other genes or the regions not analyzed by **FHNext** test can also give rise to similar clinical conditions. 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 maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, low-level mosaicism, presence of pre-malignant or malignant cells in the sample, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data.

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Understanding Your Positive Familial Hypercholesterolemia (FH) Genetic Test Result

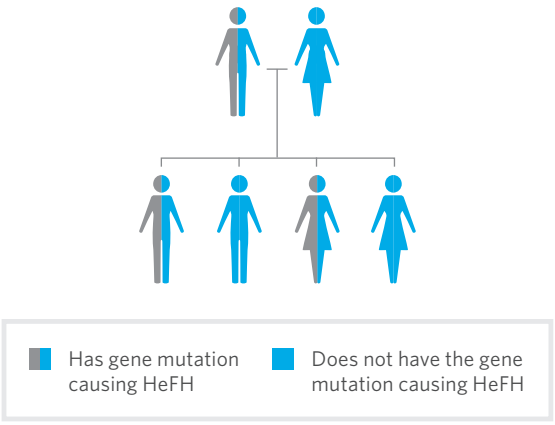
INFORMATION FOR PATIENTS WITH **ONE PATHOGENIC MUTATION** OR **VARIANT THAT IS LIKELY PATHOGENIC**

Result	**POSITIVE**	Your testing shows that you have a pathogenic (disease-causing) mutation or a variant that is likely disease-causing in a gene that causes FH. Both of these should be treated as the same type of positive result.
Diagnosis	**FH**	People with one mutation typically have HeFH, and those with two mutations have HoFH. However, if your cholesterol levels are consistent with HoFH and only one mutation was found, you could actually have HoFH (and the second mutation was unable to be found from the testing).
Gene	**DEFINITION**	Everyone has two copies of each gene. We get one copy of each gene from each of our parents.
Heterozygous Familial Hypercholesterolemia	**HEFH**	One mutation (change in the gene, like spelling mistakes) in one copy of any of the genes in this test can cause HeFH. Adults with untreated HeFH often have total serum cholesterol levels >310mg/dL. Children or adolescents with untreated HeFH often have total serum cholesterol levels >230mg/dL.
Homozygous Familial Hypercholesterolemia	**HOFH**	Two mutations (changes in the gene, like spelling mistakes) in any of the genes in this test can cause HoFH. People with untreated HoFH often have total serum cholesterol levels >500mg/dL.
Management Options	**FOR PATIENTS WITH FH**	People with HeFH usually respond well to a combination of diet change and medications (e.g. statins and PCSK9 inhibitors). Lipoprotein apheresis treatment, and sometimes even surgery like a liver transplant, might be needed for patients with HoFH. Talk to your doctor about treatment that may be right for you.
Screening Options	**FAMILY MEMBERS**	Careful monitoring of cholesterol levels is important for all close relatives of patients with FH. Talk to your doctor about which options may be right for you and/or your family.
Next Steps	**DISCUSS**	Please share this with family members so they can talk with their doctors and learn more. They can now be tested for this same mutation, if they choose to.
Reach Out	**RESOURCES**	• National Society of Genetic Counselors nsgc.org • Canadian Association of Genetic Counsellors cagc-accg.ca • The FH Foundation thefhfoundation.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.org

HeFH in the Family

Your close family members (like your parents, brothers, sisters, children) have a 50/50 chance of having the mutation that you carry, and other family members (like your aunts, uncles, cousins) may also have it. Your relatives can now be tested for this same mutation, if they wish.

Please talk with your doctor or genetic counselor about this. The field of genetics is continuously changing, so updates related to your result, medical recommendations, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or taken as medical advice.



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