SUMMARY OF RESULTS

NEGATIVE

No DNA variants that are expected to cause familial hypercholesterolemia were found in any of the four genes analyzed.

NOTES ABOUT YOUR RESULT

FH is the cause for only a small fraction of people with high cholesterol. There are other health and lifestyle factors that result in high cholesterol. Some people with FH may receive a negative test result because this test gives positive results for only 3 out of 4 people with FH.

RECOMMENDATIONS

It is recommended that you share this report with your healthcare provider. Information about the treatment and management of high cholesterol is available through the National Lipid Association (https://www.lipid.org/).

Genetic counseling is available to help understand what this means for you and family members and can be accessed through PWNHealth, the independent provider network that placed your order, by phone: (888) 494-7333 or email: gc@pwnhealth.com.

All DNA tests have limitations. Please see the following pages for the limitations of this specific test as well as other useful information.

GENES ANALYZED

The APOB, LDLR, LDLRAP1 and PCSK9 genes were analyzed and no DNA variants known to cause FH were found.

REVIEWED BY

_____________________________________________  ________________________________________
Ph.D. Name                                           Ph.D. Name
ABOUT FAMILIAL HYPERCHOLESTEROLEMIA (FH)

What is FH?

Hypercholesterolemia is a term that means having high levels of cholesterol in your blood. Cholesterol is a waxy substance that is found in all the cells of your body. Cholesterol can build up in blood vessels and block them; this is known as atherosclerosis. Having high blood cholesterol raises the risk for heart disease, the leading cause of death in the US, and for stroke, the fifth leading cause of death.

Sometimes, high cholesterol is caused by genetic variants found in a person's DNA. These variants may have been inherited from one or both of your parents and can be passed along to your children and grandchildren. Familial is a term that refers to a condition that is inherited or passed down through generations of a family. Therefore, the term Familial Hypercholesterolemia (FH) refers to high cholesterol that runs in a family.

Symptoms of high cholesterol are not always obvious. Many affected people are unaware that their cholesterol is too high.

Why get genetic testing for FH?

Genetic testing is important for people with high cholesterol because it might help you understand the cause of your high cholesterol. This would help you and your doctor set an appropriate treatment plan.

Genetic testing can also help identify family members who are at risk.

What is genetic testing?

Genetic testing is the process by which we isolate and test DNA from a patient's saliva sample. Within DNA, we look at a person's genes. Genes are instructions to tell our bodies how to build, maintain, and function throughout our lives. Genetic testing looks carefully at certain genes to determine if the instructions are not written properly (variants) and are causing certain medical conditions. This information can help us diagnose or give risks for developing a disorder such as FH.

When to suspect FH:

- If your cholesterol tests have been consistently high.
- If you have had a heart attack or if you have a family history of high cholesterol or early onset heart disease.
- If you have visible signs of FH such as swollen tendons or yellow “fatty” deposits in the skin.
**FH FACTS**

- Cells in your body need cholesterol in order to stay healthy. Too much cholesterol in your body can be harmful.

- There are two types of cholesterol: low-density lipoprotein (LDL) cholesterol, often called “bad” cholesterol and high-density lipoprotein (HDL) cholesterol, often called “good” cholesterol. Your body requires certain amounts of both types of cholesterol, but too much “bad” cholesterol or not enough “good” cholesterol can lead to atherosclerosis and increase the risk of heart disease and stroke. The following table shows LDL cholesterol levels that are considered high (Hopkins et al. 2011. PubMed ID: 21600530).

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>LDL Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>&gt;190</td>
</tr>
<tr>
<td>20 - 29</td>
<td>&gt;220</td>
</tr>
<tr>
<td>&gt;30</td>
<td>&gt;250</td>
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</tbody>
</table>

- Patients with FH often have one or two DNA variants in one of four important genes. Each of these genes has instructions for the body to make a certain kind of protein. Proteins made by the body are different than the protein we eat in our diet. Proteins are made using the instructions in DNA and are important to the structure, function and regulation of the body’s organs and tissues.

- The **LDLR** gene - the protein produced by this gene helps remove cholesterol from your blood. Variants in this gene are the most frequent cause of FH.

- The **APOB** gene - the protein produced by this gene helps transport cholesterol in your blood. Variants in this gene make it difficult for the **LDLR** protein to remove cholesterol from your blood.

- The **PCSK9** gene - the protein produced by this gene breaks down proteins used to remove cholesterol from your blood. Variants in this gene can result in not enough of the proteins needed for proper removal of cholesterol from your blood.

- The **LDLRAP1** gene – the protein produced by this gene also helps to remove cholesterol from your blood. Variants in this gene are a rare cause of FH.

- These genes do not change over the course of a person’s lifetime. Therefore, a test for a specific gene or genes only needs to be taken once.
It’s possible that more genes or variants that cause FH will be discovered in the future. Therefore, a negative test result now does not guarantee that you do not have FH.

Cholesterol comes from two sources: your body naturally makes cholesterol, and it is also in the foods we eat.

High cholesterol occurs in men and women, children and adults, and in overweight and lean people.

Being physically active, eating a healthy diet, avoiding tobacco products, and maintaining a healthy weight can all help control cholesterol levels in most people. Most people with FH also need to take medications that help lower cholesterol. In some cases, additional treatments to help lower cholesterol may be required.

RISK AND INHERITANCE INFORMATION

RISK

Approximately one in every six adults in the United States has high cholesterol (CDC: High serum total cholesterol—an indicator for monitoring cholesterol lowering efforts; U.S. adults, 2005–2006. www.cdc.gov/nchs/data/databriefs/db02.pdf)

A person with a variant in the LDLR, APOB, PCSK9, or LDLRAP1 genes has a 1 in 2 (50%) chance of passing the variant to each of their children. People with two variants in these genes have a 3 in 4 (75%) chance of passing one of the causative variants to their children.
SCREENING GUIDELINES

It is generally recommended that adults at average risk of developing heart disease should have their cholesterol tested every 5 years (www.mayoclinic.org).

Screening may be more frequent in patients with specific risk factors including having a family history of FH, being overweight, using tobacco, not being physically active, and being a man over 45 or a woman older than 55.

WHO IS THIS FH TEST FOR?

- Must be over age 18.
- Cannot have had a bone marrow transplant.
- Must have high cholesterol and know your cholesterol levels.
- Testing is particularly important for anyone with high cholesterol and a parent, sibling or child who also has high cholesterol or with premature heart disease.

COMMON QUESTIONS

WHAT IS GENETIC TESTING?

Genetic testing is the process by which we isolate and examine DNA from a patient’s saliva sample. Within DNA, we look at a person’s genes.

WHAT ARE GENES?

Genes are instructions to tell our bodies how to build, maintain, and function throughout our lives. Genetic testing allows us to look at certain genes to determine if the instructions are not written properly. This information can help us diagnose a disorder or give risks for developing a disorder such as FH.

WHAT ARE DNA VARIANTS?

When we do a genetic test, we compare a person’s genes to average genes from many other people. Differences between the person’s genes and the average genes are called variants. All people have many DNA variants. DNA variants usually have little or no impact on a person’s health. Occasionally, DNA variants may cause disease.

WHAT ARE PROTEINS?

Proteins made by the body are different than the protein we eat in our diet. Proteins are made using the instructions in DNA and are important to the structure, function and regulation of the body’s organs and tissues.
WHAT DOES FAMILIAL MEAN?
Familial is a term that refers to a condition that is inherited, or passed down through generations in a family, through their DNA.

WHAT ARE REASONS TO TAKE THIS GENETIC TEST?
People take this test for a variety of reasons. Some want to know their genetic risk for FH. Others want to help their family members understand their risk.

WHAT ABOUT GENETIC DISCRIMINATION?
The Federal Genetic Information Non-Discrimination Act, also known as GINA, is a law set in place to protect you from employers and health insurers using your genetic information against you. It should be noted the law does not protect against long-term, life, and disability insurers from asking for and using your genetic information in making coverage decisions. Visit www.ginahelp.org for more information.

WHERE IS MY TEST PERFORMED?
All Seq2Know tests are performed at an accredited DNA testing laboratory, PreventionGenetics. PreventionGenetics was founded in 2004 and is located in Marshfield, WI.

WHAT IF I HAVE QUESTIONS?
If you have questions about the testing or how to interpret the results, please contact PWNHealth, the independent provider network that placed your order, by phone: 888-494-7333 or email: gc@pwnhealth.com.

If you have questions about kits, sample collection or testing status contact PreventionGenetics client services: 715-387-0484, option 0.

WHO HAS ACCESS TO MY RESULTS?
PreventionGenetics and our Independent Physician Network, PWNHealth, will have access to your results. We will not release any identifiable patient data to anyone other than the ordering doctor without your documented consent. Your results will not be sent to your insurance company, employer or others without your written consent.

ARE MY RESULTS KEPT CONFIDENTIAL?
Yes. We take confidentiality and patient privacy very seriously. We follow confidentiality laws related to protected health information and are a government certified laboratory.

DOES PREVENTIONGENETICS SHARE MY INFORMATION OR TEST RESULTS WITH ANY RESEARCHERS OR OTHERS?
We will not release any identifiable patient data to anyone other than the contracted ordering doctor at the independent physician network without documented patient consent.
WHAT IS AN INDEPENDENT PHYSICIAN NETWORK?
An independent physician network is a group of qualified healthcare providers who are not directly associated with the testing laboratory.

UNDERSTANDING MY RESULTS

WILL MY RESULTS CHANGE OR WILL I NEED TO RETAKE THE TEST IN THE FUTURE?
No. Your DNA does not change. You are born with your genes. However, our knowledge and ability to understand genetic variants do change. For this test, we are only reporting genetic changes that are currently known or expected to cause FH. Knowledge about the genetic causes of FH is likely to improve over time.

WITH WHOM SHOULD I SHARE MY RESULTS?
PreventionGenetics recommends that you share your results with your healthcare provider. Your provider may recommend additional screening and/or lifestyle changes. We also recommend that you share results with your relatives. We do not recommend that you share your results with anyone else.

WHAT DOES THIS MEAN FOR MY FAMILY MEMBERS?
Your results could impact risks for blood relatives, including children, siblings, parents, and other family members.

WHAT DOES THE INDEPENDENT PHYSICIAN NETWORK DO?
PWN Health, the independent physician network, will review your personal and family history information. They will approve your testing request.
TEST METHODOLOGY AND LIMITATIONS

GENETICS
Several genes are reported to be associated with FH, however most patients are found to harbor one DNA variant in one of the following genes: LDLR, APOB, or PCSK9. Data from several studies indicate that variants in LDLR, APOB, and PCSK9 are found in approximately 37-82%, 0-7%, and 0-3% of FH cases, respectively (Varret et al. Clin Genet 73:1, 2008). Over 2300 pathogenic variants have been reported in the LDLR gene alone. Patients with more than one pathogenic variant in the LDLR, APOB, or PCSK9 genes are rare and generally have a severe form of FH. Two pathogenic variants in the LDLRAP1 gene, one inherited from each parent are required to cause FH. A single pathogenic variant in LDLRAP1 does not cause high cholesterol.

TESTING STRATEGY
This test provides full coverage of all coding regions of the genes tested. See Testing Methods for more details.

INDICATIONS FOR TEST
This test is recommended for patients with high levels of LDL.

TEST METHODS
We use a combination of Next Generation Sequencing (NGS) and Sanger sequencing technologies to cover the full coding regions of the listed genes plus ~20 bases of non-coding DNA flanking each exon. As required, genomic DNA is extracted from the patient specimen. For NGS, patient DNA corresponding to these regions is captured using an optimized set of DNA hybridization probes. Captured DNA is sequenced using Illumina’s Reversible Dye Terminator (RDT) platform (Illumina, San Diego, CA, USA). Minimum NGS coverage is ≥20x for all exons and +/-10bp of intronic flank and ≥10x from 11-20bp of intronic flank. All regions with coverage that does not meet this threshold are backfilled with Sanger sequencing. All pathogenic and likely pathogenic NGS variant calls are confirmed by Sanger sequencing.

For Sanger sequencing, Polymerase Chain Reaction (PCR) is used to amplify targeted regions. After purification of the PCR products, cycle sequencing is carried out using the ABI Big Dye Terminator v.3.1 kit. PCR products are resolved by electrophoresis on an ABI 3730xl capillary sequencer. In nearly all cases, cycle sequencing is performed separately in both the forward and reverse directions.

Human Genome Variation Society (HGVS) recommendations are used to describe sequence variants (http://www.hgvs.org). Rare variants and undocumented variants are nearly always classified as likely benign if there is no indication that they alter protein sequence or disrupt splicing.
LIMITATIONS

This test is designed to detect only specific types of DNA variants; this test may not detect all types of variants known to cause FH including large deletions or duplications of the genes tested.

We only report DNA variants that are currently known or expected to cause FH.

Interpretation of the test results is limited by the information that is available currently. Better interpretation should be possible in the future as more data and knowledge about human genetics and this specific disorder are accumulated.

Due to the testing methods used, we cannot be certain that we were able to test the genes inherited from both parents.

We sequence all coding regions for each gene. Unless specifically indicated, test reports contain no information about other portions of the genes.

In most cases, we are unable to determine whether variants were inherited from either the mother or the father without testing them also.

We only test DNA from the specimen provided, in this case usually saliva. It is unlikely, but possible that other tissues in the patient’s body contain DNA that is different from the DNA in the specimen provided.

DNA variants that are present in less than 50% of the patient’s cells may not be detected.

We cannot be certain that the reference sequences are correct. Genome build hg19, GRCh37 (Feb2009) is used as our reference.

We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the specimen arrives at PreventionGenetics.

Genetic counseling to help to explain test results to the patients and to discuss reproductive options is recommended.

FDA NOTES

These results should be used in the context of available clinical findings and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by PreventionGenetics. US Food and Drug Administration (FDA) does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.