A pathogenic mutation was identified in the LDLR gene. Testing positive for a pathogenic mutation in the LDLR gene causes Familial Hypercholesterolemia (FH). FH is a hereditary disorder associated with very high levels of cholesterol at an early age, specifically a type of cholesterol called LDL-C.

Having too much LDL-C can cause it to build up in the arteries, which are the blood vessels that take blood from the heart to the rest of the body. As cholesterol and other substances build up in the arteries, they harden and narrow, restricting blood flow. This can lead to coronary heart disease (CHD), also known as coronary artery disease (CAD), which is the most common type of heart disease, and can lead to heart attack and stroke.

NOTES ABOUT YOUR RESULT

Speak with your healthcare provider to determine how your cholesterol treatment may be influenced by this result. Useful information is provided in the screening guidelines below.

DETAILS

<table>
<thead>
<tr>
<th>GENE</th>
<th>MUTATION</th>
<th>CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR</td>
<td>c.259T&gt;G (p.Trp87Gly)</td>
<td>Pathogenic</td>
</tr>
<tr>
<td></td>
<td>Alternate name(s): chr19:g.11213408T&gt;G</td>
<td></td>
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<tr>
<td></td>
<td>Transcript: ENST00000558518</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zygosity: Heterozygous</td>
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</tbody>
</table>

SUPPORTING EVIDENCE

LDLR c.1586+5G>A is an intronic variant in the splice donor region of intron 10. The variant is present in 0.0034% (4 / 117602) of individuals in the Exome Aggregation Consortium (ExAC) population database. Algorithms designed to predict impact on RNA splicing (MaxEntScan, SpliceSiteFinder-Like, NNSplice, HSF) consistently predict a significant impact on the intron 10 donor. Guanine is fully conserved in mammals. The variant has been reported in multiple unrelated individuals diagnosed with Familial Hypercholesterolemia (PMID 7635461, 10668928, 16250003, 17539906, 19446849, 25463123). Molecular studies of the impact of this variant to RNA splicing demonstrated two aberrant mRNAs due to either in-frame skipping of exon 10 or the activation of a cryptic splice site in intron 10 inserting 66 intronic base pairs (PMID 10668928). Although this variant is an intronic variant, the demonstrated impact to RNA splicing and the occurrence in individuals clinically diagnosed with hypercholesterolemia indicate the likelihood of pathogenicity. Consequently, this variant is classified as Likely Pathogenic.
ADDITIONAL GENES ANALYZED

The genes below were analyzed, and no pathogenic or likely pathogenic mutations associated with Familial Hypercholesterolemia were identified. Please see the test methodology and limitations section for additional information.

APOB, PCSK9

REVIEWED BY

Tom Sample, MD, Pathologist
Risk and Family Information

Risk among US individuals to develop coronary heart disease. Risk may vary based on age, diet, exercise, and other factors.

CORONARY HEART DISEASE

- FH + high cholesterol
- No FH + high cholesterol
- No FH + normal cholesterol

FAMILY

Consider sharing your results with relatives because:

- This mutation was most likely inherited from either your mother or your father. This would mean that one of your parents has the same mutation, and that your relatives on that side of the family may also have the same mutation. Fathers are just as likely to pass on a mutation as mothers.

- Each of your siblings has a 50% chance of having inherited this mutation. Brothers are just as likely to inherit it as sisters.

- Each of your children has a 50% chance of inheriting the same mutation. Men are just as likely as women to pass the mutation on to their children, and daughters and sons are equally likely to inherit it. Testing children for this mutation is recommended, as it impacts health and medical management in childhood.

- If genetic testing indicates that a relative does not have the mutation (tests negative), that relative’s children are not at risk to inherit this mutation. These mutations do not skip generations.

Know Your Screening Guidelines

Below is a summary of current screening guidelines from the International FH Foundation. These guidelines are for individuals who have Familial Hypercholesterolemia. Your healthcare provider may use these guidelines to help create a customized screening plan for you.

**CORONARY HEART DISEASE**  
2, 3, 4

- **Starting at age 8-10 or at diagnosis of FH:**
  - Speak to your provider to learn whether your cholesterol levels have already been checked and how often testing should be repeated.
  - Discuss ways to reduce your cholesterol with your provider. This may include certain medications as well as lifestyle modifications such as diet, exercise and quitting smoking.
  - Consider completing a baseline electrocardiogram, a test that checks the electrical activity of the heart.
- Women who are pregnant or are planning to become pregnant are recommended to speak with their healthcare provider about how to best manage their cholesterol before and during pregnancy.

**GENERAL HEART HEALTH RECOMMENDATIONS FOR ALL INDIVIDUALS** 5

- Don’t smoke and avoid second-hand smoke
- Treat high blood pressure if you have it
- Eat foods that are low in saturated fat, trans fat, sodium (salt) and added sugars
- Be physically active
- Reach and maintain a healthy weight
- Control your blood sugar if you have diabetes
- Get regular medical check-ups
- Take medicine as prescribed

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Common Questions

What does a positive result mean?
A positive result means that a mutation, or a genetic change, was identified in a specific gene that causes Familial Hypercholesterolemia. Your personal results contain more detailed risk information specific to the mutation identified in your genes, as well as information to share with your family members and healthcare provider.

What is a pathogenic mutation?
A pathogenic mutation is a variant in the DNA sequence of a gene that affects its ability to function. A pathogenic mutation is also referred to as a mutation in this report.

Who will see these test results?
Your results are available to you and the healthcare provider who ordered your test, as well as any additional providers you designated. Your results will not be sent by Color to your insurance company, employer, or any other healthcare provider without your explicit request.

Should I share my results with my healthcare provider?
Color recommends you share your results with your healthcare provider. Sharing your results allows your provider to guide you to appropriate resources and discuss tailored options for screening and monitoring of cholesterol levels.

Are there any protections against discrimination based on these results?
In 2008, a federal law called the Genetic Information Non-Discrimination Act (GINA) was passed. Under the terms of GINA, medical insurance companies and employers are prohibited from discriminating against individuals on the basis of genetic information. GINA defines genetic information as including not only genetic test results, but also family history, and the fact that genetic testing occurred. The terms of GINA specify that insurance companies cannot raise rates, cancel a plan, or determine eligibility because of genetic testing. Employers also are prohibited from making hiring, firing, or promotion decisions based on genetic testing. The terms of GINA carry exceptions. For example, an exception might include employers with fewer than 15 employees and those with military insurance. Additionally, GINA does not extend to life, disability, or long-term insurance companies. Some states may have protections regarding discrimination from these types of insurance. Individuals may consider purchasing these policies prior to undergoing genetic testing.

CORONARY HEART DISEASE

If there is no one in my family who has high cholesterol, do I still have an increased risk?
Yes. Even if others in your family have cholesterol levels in the normal range, mutations that cause Familial Hypercholesterolemia increase cholesterol levels, which can increase the risk of heart attack and stroke. We encourage you to speak with your healthcare provider and to schedule an appointment with a board-certified genetic counselor at Color.

How can I reduce my risk of developing coronary heart disease?
You and your healthcare provider can use this information to make a personalized screening and management plan. Following your plan may lower your cholesterol levels and your chance of developing coronary heart disease. For more detailed information about some of the options
that your healthcare provider could discuss with you, see the screening and management guidelines provided in your results.

**ABOUT FAMILY**

**How did I get this mutation?**
Both men and women can have and pass on mutations in the \textit{LDLR} gene. You may have inherited the mutation from either your mother or your father. Based on this genetic analysis alone, it is not possible to determine how you inherited this mutation. In very rare instances, a mutation could originate with you and would not be present in your mother or father. However, the great majority of \textit{LDLR} mutations are passed from generation to generation. Please keep in mind that parents do not choose to pass a specific gene mutation to their children. Your risk of heart disease is not affected by whether a mutation was passed to you from your father or your mother.

**Should I talk with my relatives about my result?**
You are encouraged to share these results with your relatives. It is normal to feel some anxiety about this. Knowing this information may help your relatives understand their own future risk of developing heart disease, which may help them prevent a heart attack or stroke. However, keep in mind that not everyone wants to know their genetic status and genetic testing is a personal decision. Talking about genetic test results and their impact on the family is an ongoing discussion rather than a one-time conversation.

**Who specifically in my family should also get tested?**
Familial Hypercholesterolemia is a dominant condition. This means that each of your children has a 50% chance of having inherited the same mutation from you. This mutation was likely inherited from one of your parents, though in some instances, a mutation could originate with you and would not be present in your mother or father. If they choose to undergo testing, this can determine whether or not your siblings and extended relatives are also at risk to have this \textit{LDLR} mutation. It is typically recommended that an individual undergo genetic testing at the age at which it will impact their medical care (see Screening and Management guidelines). Your children, parents, and siblings may wish to discuss genetic testing with their own providers to determine if they have the same \textit{LDLR} mutation in order to begin early screening and management to reduce cholesterol levels. Schedule a time to speak with a genetic counselor at Color for more detailed recommendations regarding testing your family members.

**What is the impact on my children if my partner also has a gene mutation associated with Familial Hypercholesterolemia?**
Mutations that cause FH are not common (one out of every 250 people in the general population).\textsuperscript{1} However, if a child inherits two mutations that cause FH (one from each parent), they have a much more severe form of the condition called Homozygous Familial Hypercholesterolemia (HoFH). If you have children, or are planning to have children, it is generally recommended that your partner consider genetic testing to understand your children’s chance of having HoFH. We recommend discussing this possibility with a genetic counselor at Color for more information.
Methodology
This test is designed to assess clinically relevant mutations in genes associated with Familial Hypercholesterolemia. Genomic DNA is extracted from a saliva or peripheral blood sample using standard methods. For the genes analyzed, Next Generation Sequencing libraries compatible with the Illumina platform are generated and enriched via a custom designed Agilent SureSelect bait library. DNA fragments enriched from these genes are retrieved and analyzed using 2x150 paired-end sequencing with an Illumina NextSeq 500 instrument. After alignment to reference genome GRCh37.p12 (hg19), low quality and duplicate reads are removed and variants are identified using the GATK Haplotypecaller. This test detects single nucleotide substitutions (SNV), small insertions and deletions (indels) in the DNA coding sequences, nearby flanking regions (+/- 20bp) and known splice regions in the genes targeted by the Color panel. In addition, copy number variations (CNVs), large insertions and inversions overlapping coding exons, are reported. The Color test has >20X coverage for 100% of regions in our reportable range. The median coverage across our samples is >250X and our minimum acceptance criteria for depth is: >99% at >50X and 100% at 20X. Any exceptions to this are noted in the Limitations section.

Variants are classified according to the standards and guidelines for sequence variant interpretation of the American College of Medical Genetics and Genomics (ACMG). Variant classification categories include pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. All variants are evaluated by a board certified medical geneticist or pathologist. Identified likely benign and benign variants are not reported. The presence of a VUS is always reported, and the details are available upon request. All VUS and likely pathogenic variants are reviewed bi-annually for updates in the scientific literature. As part of the Color service, we will attempt to recontact the provider and/or the person that was tested if any reported variant’s classification changes.

Clinically actionable variants (i.e. likely pathogenic and pathogenic) are confirmed using an alternative technology (Sanger sequencing, aCGH or MLPA) in compliance with Color’s internal protocols and relevant ACMG guidelines. For SNVs and indels, a confidence model (color.com/variantconfidence) is used to identify high and low confidence variants. Low confidence variants, and structural variants (CNVs, insertions and inversions) are confirmed using an alternative technology. High confidence, clinically actionable variants that have been independently confirmed at least three times will not be submitted for further secondary confirmation. High confidence VUSs are reported without secondary confirmation.

This test was developed and its performance characteristics determined by Color Genomics, a clinical laboratory accredited by the College of American Pathologists (CAP) and certified under the Clinical Laboratory Improvement Amendments (CLIA) to perform high-complexity testing (CAP #8975161 - CLIA #05D2081492). This test has not been cleared or approved by the United States Food and Drug Administration (FDA). The 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.

Genes
APOB*, LDLR**, PCSK9*

* Please see the Limitations section for more information.
** The promoter region is analyzed for CNVs. In addition, a small region of the promoter (LDLR, Chr19:11200025-11200130) is also analyzed for the presence of clinically relevant SNVs and indels.

** Limitations

This test aims to detect all clinically relevant variants within the genes analyzed (defined above). The majority of these genes are assessed for variants within all coding exons (+/- 20bp in the nearby flanking regions). For PCSK9 gene exon 8, only the first 7bp of intron 8 are analyzed. In APOB, exon 1 is not analyzed. For the LDLR promoter region, the detection of deletions, duplications, and complex structural rearrangements may be limited.

This test is not designed to detect chromosomal aneuploidy or complex rearrangements such as translocations. It also does not reliably detect mosaicism. The sensitivity to detect deletions and duplications in the range of 40-250bp, as well as those which deletion/duplication do not overlap more than 250bp of contiguous coding sequence, may be reduced. The presence of a large insertion may interfere with the chemistry used to target the genes of interest, which could decrease the detection sensitivity. In addition, the sequence and identity of a large insertion may not be completely resolved. Inversions including at least one coding exon will be detected only if the breakpoints are covered by the Color test. The sensitivity to detect variants in the vicinity of homopolymer regions may be reduced.

Color only reports findings within the genes that are on the panel. It is important to understand that there may be variants in those genes that current technology is not able to detect. Additionally, there may be genes associated with Familial Hypercholesterolemia whose clinical association has not yet been definitively established. The test may therefore not detect all variants associated with Familial Hypercholesterolemia. Additionally, in the unlikely event a variant is detected that is associated with a disorder other than Familial Hypercholesterolemia, this information will not be included in the report. Genetic counseling and/or physician consultation may be warranted to ensure complete understanding of your test results.

In very rare cases, such as circulating hematolymphoid neoplasm, allogeneic bone marrow transplant, or recent blood transfusion (within 7 days of testing), the results of germline DNA analysis may be complicated by somatic and/or donor mutations. DNA quality may be affected if a participant has received chemotherapy within the last 120 days.

** Disclaimers

Color implements several safeguards to avoid technical errors, such as 2-dimensional barcoding and barcode scanning at several steps throughout the sequencing process. Color is not responsible for errors in specimen collection, transportation, and activation or other errors made prior to receipt at our laboratory. Due to the complexity of genetic testing, diagnostic errors, although rare, may occur due to sample mix-up, DNA contamination, or other laboratory operational errors. In addition, poor sample DNA quality and certain characteristics inherent to specific regions of an individual’s genomic DNA may limit the accuracy of results in those regions.

In the absence of an identified pathogenic or likely pathogenic mutation, standard risk models may be employed to determine potential risk of Familial Hypercholesterolemia and guidelines displayed on this report. All risk estimation is approximate, sometimes cannot be specifically
calculated, and is based on previously analyzed cohorts. Additionally, risk estimation may be incorrect if inaccurate personal or family history is provided. An elevated risk for Familial Hypercholesterolemia is not a diagnosis and does not guarantee that a person will develop the disease.