color

Hereditary High Cholesterol Test

Results to date: August 10, 2017

Executive Summary

Familial Hypercholesterolemia

- The majority of people with Familial Hypercholesterolemia (FH) have severely elevated LDL-C levels, which significantly increases their risk for cardiovascular events. Their clinical risk is even higher compared to people with similar LDL-C levels without FH.[1]
- In addition, a subset of FH carriers do not present with increased LDL-C levels, but are still at increased cardiovascular risk compared to people who do not carry an FH mutation.[2]
- Untreated men with FH have a 50% risk of a coronary event by age 50 years, and untreated women have a 30% risk by age 60 years.[3] Thus, lifetime risk of CAD in FH mutation carriers is increased 22-fold compared to the population average.[2]
- The estimated prevalence of FH is approximately 1 in 250 individuals worldwide.[4,5] Approximately 90% of individuals with FH have not been diagnosed yet.[6]

Color's Hereditary High Cholesterol Test

- Color has developed a high-sensitivity and low-cost assay to sequence the three genes most strongly associated with FH: *LDLR*, *APOB* and *PCSK9*.
- In a blinded study, Color correctly identified 71 reportable FH mutations with 100% accuracy. No false positives nor false negatives were identified.

Introduction

Familial Hypercholesterolemia (FH) is an inherited disorder associated with a severe elevation of Low-Density-Lipoprotein-Cholesterol (LDL-C) in blood, which significantly increases the risk for developing cardiovascular disease. FH is estimated to affect at least 1 in ~250 individuals worldwide.[2,4,5] Individuals with FH have high LDL-C levels from birth, which leads to atherosclerosis in the coronary arteries at an early age. This greatly increases the risk of premature coronary artery disease (CAD), also known as coronary heart disease (CHD), which may present as angina pectoris or myocardial infarction. If FH is not identified and intensively treated at an early age, individuals with a FH mutation have a 22-fold increased lifetime risk of CAD compared with the general population.[2] It is important to note that this risk is much higher than those with elevated LDL-C levels without a FH mutation, who have only a 6-fold increased lifetime risk.[2] FH causes an estimated 5% of annual myocardial infarction in adults under the age of 60 in the United States.[3] Due to the severity of FH and its distinction from other forms of high cholesterol, in 2016 it was included in the updated ICD-10 codes, which will facilitate improved clinical management of this condition.[7]

Genetics

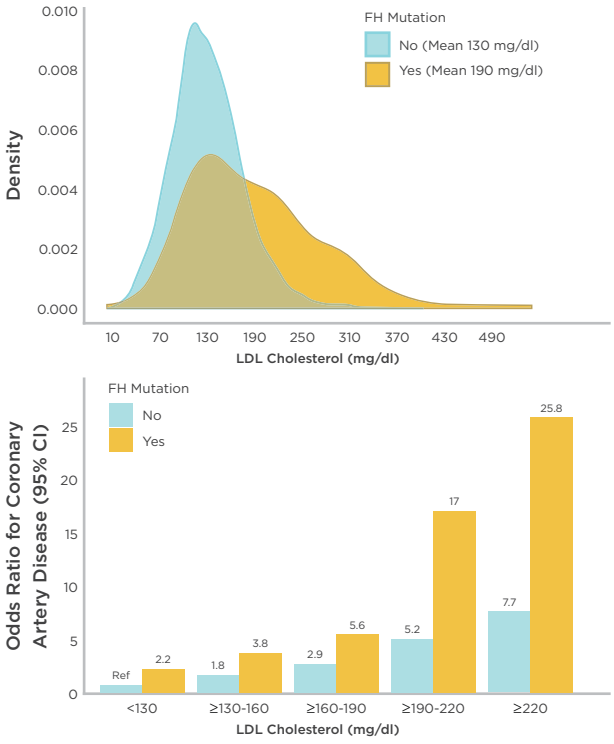
A loss-of-function mutation in a single copy of either the *LDLR* or *APOB* gene, or a gain-of-function mutation in a single copy of the *PCSK9* gene are the most common genetic alterations that can cause FH. The estimated contributions of each gene to overall FH cases is listed in Table 1.

Table 1: Percent of FH Cases Attributed to Pathogenic Variants in Each Gene.[8]

Gene	Percent of FH Cases
LDLR	60-80%
APOB	1-5%
PCSK9	0-3%
Unknown Cause	20-40%*

* Possible causes of FH in this group include polygenic forms of FH or mutations in genes not currently identified.

Figure 1: LDL Cholesterol Values According to FH Mutation Status. Adapted from Khera et al. 2016.[2]



Having a pathogenic mutation in one copy of either the *LDLR*, *APOB*, or *PCSK9* gene causes FH. When untreated, children with FH usually have LDL-C levels >160 mg/dL, and adults usually have levels >190 mg/dL.[2] The range of LDL-C levels in people who do and do not carry FH mutations, and the risk of CAD by LDL-C level, are shown in Figure 1. It is important to note that while not all individuals with FH will have elevated LDL-C, they are still at increased risk of CAD.[2] Because FH is a hereditary disease, if an individual has FH, roughly half of their immediate relatives are likely to also have FH and an increased risk of CAD, and are recommended to undergo genetic testing to determine their mutation status. Education of family members is important, as many may be unaware that they have had high cholesterol and are at increased risk for premature CAD.

Underdiagnosis of FH

Studies across different populations have estimated that FH affects between 1 in 220 to 1 in 300 individuals worldwide.[2,4,5] It is estimated that although approximately 1.5 million people in the US have FH, over 90% have not been properly diagnosed, representing a missed opportunity to prevent CAD-related morbidity and mortality.[6] The diagnosis of FH is often missed due to misconceptions and lack of awareness about FH by both healthcare providers and the general public. For example, it is a commonly held belief that FH can be unambiguously diagnosed based on cholesterol levels alone, or that a person with FH will always have physical findings such as corneal arcus or xanthoma, when in fact this is not the case.[3]

Diagnosis of FH is crucial to inform appropriate treatment and prevent premature CAD, and the use of genetic testing in conjunction with clinical criteria increases correct identification of people with FH.[2,9,10] One study found that nearly a quarter of FH mutation carriers would not have been diagnosed based on clinical criteria alone.[9] The highest rates of diagnosis have been achieved in countries that use cascade screening strategies, with over 70% of affected individuals diagnosed in The Netherlands.[6] Children as young as 2 can be screened if there is a strong family history of high cholesterol, which allows for earlier intervention of healthy diet and lifestyle changes, and statins considered by age 8.[8]

Prevention and Treatment

When diagnosed, FH is usually managed at specialty clinics, such as lipidology, endocrinology, or cardiovascular clinics. In addition to diet and lifestyle changes, individuals with FH are recommended to begin treatment with high-dose statins starting as early as age 8-10 years to achieve desired LDL-C levels. Additional increases in statin dose, and/or other medications, may be recommended to achieve healthy levels of LDL-C. Treatment is typically needed for life.[10] All individuals with FH are considered high risk for CAD based upon several professional guidelines (NCEP, NICE, NLA, and AHA/ACC), and standard risk calculators for CAD are not applicable to those with FH. Importantly, with early detection and optimal treatment, an affected individual's risk of CAD is similar to that of the general population.[3]

Methodology of The Color Hereditary High Cholesterol Test

The Color Hereditary High Cholesterol Test uses the same, proven technology employed in the Color Hereditary Cancer Test. The Color laboratory, certified by CLIA (05D2081492) and accredited by CAP (8975161), has developed a systematic process of automated laboratory protocols and tailored bioinformatics analysis to achieve reliable next-generation sequencing (NGS) results. This process is based on laboratory products from industry leaders such as Agilent, Illumina and Hamilton. Specifically, it includes library preparation using Kapa Biosystems HyperPlus reagents, target enrichment by Agilent's SureSelect method (v1.7), and sequencing by Illumina's NextSeq 500 (paired-end 150bp, High Output kit). At several points along the process, automated quality control checks have been incorporated to ensure sample identification, high quality of DNA isolation, library preparation, target capture, and sequencing. In addition, each sequencing test contains two fully characterized positive controls and a no-template control. The bioinformatics pipeline employs well-established algorithms such as BWA-MEM, SAMtools, Picard and GATK. Copy number variants (CNVs), insertions and inversions are detected using CNVkit, LUMPY, and internally developed algorithms for read depth analysis and split-read alignment detection. The coverage requirements for reporting are 20X for each base of the reportable range. Median coverage

typically ranges between 200-300X. 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 ABMGG board certified medical geneticist or pathologist. Variants classified as pathogenic or likely pathogenic are confirmed by a secondary technology (Sanger sequencing, aCGH or MLPA) per our confirmation policy.

The Color Hereditary High Cholesterol Test analyzes the DNA coding sequences, nearby flanking regions (+/- 20bp), and known splice regions of three genes in which pathogenic variants are known to cause Familial Hypercholesterolemia: *APOB, LDLR, PCSK9*.

Blinded Study Design

A set of samples known to contain single nucleotide variants (SNVs) and copy number variants (CNVs) in the three FH genes were tested using the Color Hereditary High Cholesterol Test. The cohort consisted of 126 anonymized DNA specimens provided by Steve Humphries, Ph.D. and Michael Sheridan, Ph.D. of StoreGene, Cardiovascular Genetics at University College London, United Kingdom. Color did not have information regarding the status or genetic makeup of the samples. Results were submitted to our collaborators to be compared against their results. This allowed Color to test the accuracy of its assay in the absence of any a priori knowledge of genetic variants.

Results

Within the 126 samples examined, 71 reportable (VUS/LP/P) variants were identified in 51 samples. We confirmed that in those 51 samples, previous clinical testing had identified a variant in at least one of the genes, and the other 75 specimens had previously tested negative for pathogenic variants. Identified variants are listed in Table 2 and Table 3 by type and classification. Locations of variants identified across diverse domains of *LDLR* are represented in Figure 2.

In this study, 71 expected variants were identified by the Color Hereditary High Cholesterol Test, for a 100% concordance. Similar to what has been previously reported,[10] we observed a diverse set of variants in clinically defined FH patients, including SNVs, indels, and CNVs, a majority of which were identified in the *LDLR* gene. Of note, 2 specimens were identified as having pathogenic CNVs that had not been identified by the reference genetic analysis. In addition, in 2 specimens a second pathogenic mutation was identified which was not previously detected by the reference analysis due to ending the diagnostic odyssey after finding the first mutation. These new discoveries were examined through Sanger sequencing of the DNA samples held in the UK, and all of Color's initial findings were confirmed. The identification of these individuals as compound heterozygotes is consistent with their extremely elevated LDL-C levels, and has clear utility for effective testing in family members. These clinically relevant and useful results demonstrate the effectiveness of panel testing for FH.

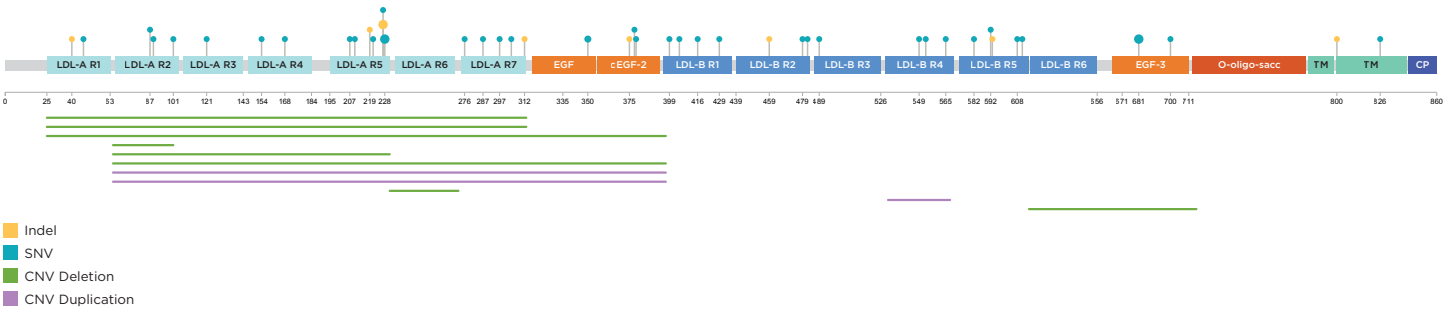
Table 2: Reportable variants observed in clinical samples (n=126) in blinded study, by variant type and previous detection status.

Variant Class	SNV	Indel	CNV	False Negatives	False Positives	Total
Known Mutations	47	10	10	0	0	67
Newly Discovered Mutations	2	0	2	0	0	4
Total	49	10	12	0	0	71

Table 3: Reportable variants observed in clinical samples (n=126) in blinded study, by variant type and gene.

Variant type / gene	% of Total reportable variants
SNV	69%
CNV	17%
Indel / MNV	14%
LDLR	82%
APOB	17%
PCSK9	1%

Figure 2: Spectrum of variants observed in *LDLR*. Functional domains indicated as colored boxes.



Conclusions

Familial Hypercholesterolemia is a relatively common and life-threatening disorder whose diagnosis and treatment has been hampered by lack of awareness. Genetic testing can identify those with FH, and while not all people with FH display visible symptoms or elevated LDL-C levels, they are still at higher risk for CAD. Color has developed a next generation sequencing-based Hereditary High Cholesterol Test for FH. Our blinded validation study of 126 previously tested samples showed 100% accuracy.

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