color

Hereditary Heart Health Genetic Test

Results to date April 2, 2018

Executive Summary

Hereditary cardiovascular disorders can be asymptomatic until a person experiences a major event, leading to poor outcomes. Color developed the Hereditary Heart Health Genetic Test using next-generation sequencing (NGS) to identify pathogenic variants in 30 genes associated with four hereditary cardiovascular disorders (arrhythmias, arteriopathies, cardiomyopathies, and familial hypercholesterolemia) for which preventative measures or treatments exist. The test can detect single nucleotide variants (SNVs), small insertions and deletions (indels), and copy number variants (CNVs). A validation study of variants in these genes showed 100% accuracy for all variant types across disorders and specimen types.

Introduction

Cardiovascular disease is the leading cause of death for women and men in the US, accounting for one-third of deaths worldwide.[1] Major risk factors include high blood pressure, elevated LDL cholesterol, and smoking. In addition, cardiovascular disease can be caused by hereditary disorders that increase the risk of serious events such as cardiac arrest, heart failure, sudden cardiac death, and stroke. The prevalence of individuals with hereditary cardiovascular disease is estimated to be between 0.01% and 0.5% in the general population.[2,3] Many individuals with hereditary cardiovascular disorders may be asymptomatic, which makes early diagnosis and treatment difficult and puts individuals at risk for adverse cardiovascular events.

Color's Hereditary Heart Health Genetic Test is designed to detect pathogenic variants in 30 genes associated with four major categories of cardiovascular disorders: arrhythmias, arteriopathies, cardiomyopathies, and familial hypercholesterolemia. These cardiovascular genes met expert consensus for having clinical validity and utility and are associated with cardiovascular disorders that have preventive measures and/or treatments available.[4]

Arrhythmias

Arrhythmias are disorders of the heart that cause the heart's sinus rhythm to be abnormal. These abnormalities may present as a change in the heartbeat's speed, such as tachycardia (when the heart beats faster than normal while at rest) or bradycardia (when the heart beats slower than normal), or a change in the normal pattern. Although many cases of arrhythmia are caused by non-genetic factors, pathogenic variants can lead to disruptions in the normal conduction pathway or the function of the sinoatrial node (the heart pacemaker) which can also cause arrhythmias. This test analyzes genes associated with four types of hereditary arrhythmias:

- Long QT syndrome (LQTS): Pathogenic variants in the *KCNH2, KCNQ1,* and *SCN5A* genes account for approximately 75% of clinically diagnosed cases of hereditary LQTS.[5–7]
- Short QT syndrome (SQTS): Pathogenic variants in the *KCNH2* and *KCNQ1* genes account for almost 20% of cases of hereditary SQTS.[8]
- Brugada syndrome: Pathogenic variants in the *SCN5A* gene account for approximately 15-30% of clinically diagnosed cases of Brugada syndrome.[9]
- Catecholaminergic polymorphic ventricular tachycardia (CPVT): Pathogenic variants in the *RYR2* gene account for approximately 50-55% of clinically diagnosed cases of hereditary CPVT.[10–12]

Arteriopathies

Arteriopathies are disorders of the arteries, most of which affect the aorta (aortopathies). Disorders of the aorta include thoracic and abdominal aortic aneurysms and dissections. Individuals with arteriopathy are often unaware of their condition, due to only 5% of individuals with arteriopathy experiencing early symptoms. Sudden cardiac death is the most common adverse event associated with arthropathies due to the asymptomatic presentation of the disorder.[13] While arteriopathies may be caused by non-genetic factors, thoracic arteriopathies have a strong genetic component. This test analyzes genes associated with four types of hereditary arteriopathies:

- Marfan syndrome: Pathogenic variants in the *FBN1* gene cause Marfan syndrome. Approximately 25% of individuals have a *de novo* pathogenic variant and are the first in their family to be diagnosed with Marfan syndrome.[14]
- Loeys-Dietz syndrome (LDS): Pathogenic variants in the *SMAD3, TGFBR1,* and *TGFBR2* genes account for the majority of clinically diagnosed cases of LDS. Approximately 75% of individuals have a de novo pathogenic variant.[15]
- Vascular Ehlers-Danlos syndrome (vEDS or type IV): Pathogenic variants in the COL3A1 gene cause vEDS. Approximately 50% of individuals have a de novo pathogenic variant.[16]
- Familial thoracic aortic aneurysm and dissection (FTAAD): Pathogenic variants in the *MYH11* and *ACTA2* genes account for approximately 20% of clinically diagnosed cases of FTAAD while pathogenic variants in the *COL3A1, FBN1, SMAD3, TGFBR1,* and *TGFBR2* genes account for approximately 15% of these cases.[17]

Cardiomyopathies

Cardiomyopathies are disorders of the heart that cause the myocardium to be abnormal, which leads to mechanical and/or electrical dysfunction. Cardiomyopathies may present as heart failure, syncope, arrhythmia, thromboembolic disease, or sudden cardiac death in individuals of all ages. Cardiomyopathies may either be hereditary or secondary to generalized systemic disorders such as diabetes, long-term hypertension, thyroid disease, sarcoidosis, and exposure to various medications, drugs, and infections. This test analyzes genes associated with five types of hereditary cardiomyopathies and Fabry disease:

- Dilated cardiomyopathy (DCM): Pathogenic variants in the *ACTC1, DSC2, DSG2, DSP, LMNA, MYBPC3, MYH7, PKP2, TNNI3, TNNT2,* and *TPM1* genes account for approximately 25% of clinically diagnosed cases of hereditary DCM.[18]
- Hypertrophic cardiomyopathy (HCM): Pathogenic variants in the *MYBPC3* and *MYH7* genes are responsible for approximately 80% of clinically diagnosed cases of hereditary HCM.[19] Pathogenic variants in other genes including *ACTC1, MYL2, MYL3, PRKAG2, TNNI3, TNNT2,* and *TPM1* account for a smaller percentage of these cases.
- Restrictive cardiomyopathy (RCM): Pathogenic variants in the *ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI3, TNNT2,* and *TPM1* genes have been associated with hereditary RCM.
- Arrhythmogenic cardiomyopathy (ACM): Pathogenic variants in the *DSC2, DSG2, DSP, LMNA, PKP2* and *TMEM43* genes account for the majority of clinically diagnosed cases of hereditary ACM.[20]
- Left ventricular noncompaction cardiomyopathy (LVNC): Pathogenic variants in the *MYBPC3* and *MYH7* genes are responsible for the majority of clinically diagnosed cases of hereditary LVNC.[21,22] Pathogenic variants in other genes including *ACTC1, LMNA, MYBPC3, MYH7, TNNT2,* and *TPM1* are also associated with hereditary LVNC.
- Fabry disease: Pathogenic variants in the *GLA* gene cause Fabry disease, which is an X-linked hereditary disorder. The classic form of Fabry disease occurs in males with little or no α-Gal A enzyme activity. Common cardiovascular symptoms associated with Fabry disease include mitral insufficiency, left ventricular hypertrophy (which appears similar to HCM), arrhythmia, and conduction abnormalities, and heart failure.[23,24]

Familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a hereditary disorder associated with a severe elevation of low-density lipoprotein cholesterol (LDL-C) in the blood and a significantly increased risk of cardiovascular disease. 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 which may present as angina pectoris or myocardial infarction. FH causes an estimated 5% of annual myocardial infarction in adults under the age of 60 in the US.[25] It is estimated that 1 in 250 individuals in the general population have FH.[26–28] Pathogenic variants in the *APOB, LDLR,* and *PCSK9* genes are responsible for 60-80% of clinically diagnosed cases of FH.[29]

Methods

Laboratory Procedures

The Color Hereditary Heart Health Genetic Test uses the same technology (described below) utilized in the Color Hereditary Cancer Genetic Test and the Color Hereditary High Cholesterol Genetic Test. Laboratory procedures were performed at the Color laboratory (Burlingame, CA) under CLIA (Clinical Laboratory Improvements Amendments, #05D2081492) and CAP (College of American Pathologists, #8975161) regulatory guidance. DNA was extracted from blood or saliva samples and purified using the Perkin Elmer Chemagic DNA Extraction Kit (Perkin Elmer, Waltham, MA) automated on the Hamilton STAR (Hamilton, Reno, NV) and the Chemagic Liquid Handler (Perkin Elmer, Waltham, MA). The quality and quantity of the extracted DNA were assessed by UV spectroscopy (BioTek, Winooski, VT). High molecular weight genomic DNA was enzymatically fragmented and prepared using the Kapa HyperPlus Library Preparation Kit (Kapa Biosciences, Cape Town, South Africa) automated on the Hamilton Star liquid handler. Target enrichment was performed with an automated (Hamilton Star, Reno, NV) hybrid capture procedure using SureSelect XT probes (Agilent, Santa Clara, CA). Sequencing was performed with the NextSeq 500/550 instrument (Illumina, San Diego, CA) for 150 bp paired-end sequencing.

Multi-gene Panel

The Color Hereditary Heart Health Genetic Test analyzes 30 genes: *ACTA2, ACTC1, APOB, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MYBPC3, MYH7, MYH11, MYL2, MYL3, PCSK9, PKP2, PRKAG2, RYR2, SCN5A, SMAD3, TGFBR1, TGFBR2, TMEM43, TNNI3, TNNT2,* and *TPM1.* Coding regions for all of these genes are analyzed in most cases. The following regions are not analyzed: *KCNH2* exons 4 and 14, *KCNQ1* exon 1, *MYBPC3* exon 11, *PRKAG2* exon 5, and *TGFBR1* exon 1. For *APOB* analysis is limited to codon 3527.

Bioinformatics

Sequence reads were aligned against the human genome reference GRCh37.p12 with the Burrows-Wheeler Aligner version 0.7.15, and duplicate and low-quality reads were removed. Single-nucleotide variants (SNV) and small insertions and deletions of 2 to 50 bps (indel) were called by the HaplotypeCaller module of GATK3.4, DeepVariant 0.6.0, and Scalpel 0.5.3. Variants in homopolymer regions were called by an internally developed algorithm using SAMtools version 1.8. Copy number variants of >50 bps (CNV) were detected using dedicated algorithms based on read depth (CNVkit version 0.9.5, optimized in-house), paired reads, and split reads (LUMPY version 0.2.13, in-house developed algorithms). On pipeline completion, the sequencing run quality was checked. A no template control and two positive controls containing a set of known variants (NA12878 and NA19240) were concurrently run in every batch of samples. The coverage requirements for reporting were ≥20 unique reads (20x) for each base. Median coverage typically ranged between 200x and 300x.

Test Accuracy

Previous Validation

The Color Hereditary Heart Health Genetic Test uses the same assay that was previously validated, as reported in the Color Hereditary Cancer Genetic Test white paper (available at color.com). There, we reported 100% accuracy, sensitivity, specificity, positive predictive value (PPV), and repeatability and 99.98% reproducibility.

Sample Selection

To specifically validate the accuracy for genes included in the Color Hereditary Heart Health Genetic Test, a total of 41 independent and blinded samples were compared to previously characterized results. The validation set consisted of DNA derived from saliva (n = 4), peripheral blood (n = 25), and cell lines (n = 12) that contain variants in genes associated with hereditary cardiovascular disorders as detailed in Table 1. Three variant types were assessed: SNVs, indels, and CNVs. DNA from blood and saliva samples were sent to an independent CLIA-certified laboratory for confirmation.

The description of the specific variants, variant types, and specimen types assessed are detailed in the appendix (Table 2). Variants in the three genes associated with FH were previously assessed, as described in the Hereditary High Cholesterol Test white paper (available at color.com).

Table 1. Variant types included in validation

	VARIANT TYPE			
Disorder Area	**SNV**	**Indel**	**CNV**	**TOTAL**
Arrhythmias	4	2	2	8
Arteriopathies	5	1	1	7
Cardiomyopathies	22	12	2	36
TOTAL	**31**	**15**	**5**	**51**

Accuracy Results

The Color Hereditary Heart Health Genetic Test showed 100% concordance across genes, variant types, and specimen types assessed in all samples. In this analysis, 51 variants were identified, with no false positive or false negative calls in any of the 41 samples.

Conclusions

Color's Hereditary Heart Health Genetic Test detects variants in 30 genes associated with hereditary cardiovascular disorders. Using NGS technology, the test accurately identified variants across variant types, sample types (DNA collected from saliva or peripheral blood), and cardiovascular disease areas.

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Appendix

Table 2. Validation set for accuracy

Disease Area	Gene	cHGVS	pHGVS	Variant Type	Specimen Type
Arrhythmias	*KCNH2*	Deletion of exons 12-14		CNV	Cell Line
	KCNQ1	c.1781G>A	p.Arg594Gln	SNV	Blood
	KCNQ1	c.826delT	p.Ser276Profs*13	Indel	Blood
	RYR2	Deletion of exon 3		CNV	Blood
	SCN5A	c.5038G>A	p.Ala1680Thr	SNV	Blood
	SCN5A	c.1336G>A	p.Glu446Lys	SNV	Blood
	SCN5A	c.5350G>A	p.Glu1784Lys	SNV	Blood
	SCN5A	c.1936delC	p.Gln646Argfs*5	Indel	Blood
Arteriopathies	*ACTA2*	c.353G>A	p.Arg118Gln	SNV	Blood
	COL3A1	c.766delA	p.Ile256Tyrfs*7	Indel	Cell Line
	FBN1	c.1468+5G>A		SNV	Blood
	FBN1	c.4364T>G	p.Ile1455Ser	SNV	Saliva
	FBN1	Deletion of exons 43-44		CNV	Cell Line
	TGFBR1	c.1460G>A	p.Arg487Gln	SNV	Blood
	TGFBR2	c.1583G>A	p.Arg528His	SNV	Blood
Cardiomyopathies	*DSC2*	c.96delC	p.Cys32*	Indel	Blood
	DSG2	c.1773_1774delTG	p.Cys591*	Indel	Blood
	DSP	c.1146delT	p.Phe382Leufs*11	Indel	Blood
	DSP	Duplication of exon 24		CNV	Blood
	PKP2	c.1307_1315delinsATTTAGTT	p.Leu436Hisfs*11	Indel	Saliva
	PKP2	c.2197_2202delinsG	p.His733Alafs*8	Indel	Blood
	ACTC1	c.301G>A	p.Glu101Lys	SNV	Blood
	GLA	c.658C>T	p.Arg220*	SNV	Cell Line

Appendix cont'd

Disease Area	Gene	cHGVS	pHGVS	Variant Type	Specimen Type
Cardiomyopathies cont'd	*GLA*	c.644A>G	p.Asn215Ser	SNV	Cell Line
	GLA	c.485G>A	p.Trp162*	SNV	Cell Line
	GLA	c.1212_1214delAAG	p.Arg404del	SNV	Cell Line
	LMNA	c.1824C>T	p.Gly608=	SNV	Cell Line
	LMNA	c.1411C>T	p.Arg471Cys	SNV	Cell Line
	LMNA	c.1579C>T	p.Arg527Cys	SNV	Cell Line
	LMNA	c.104T>C	p.Leu35Pro	SNV	Cell Line
	MYBPC3	c.1504C>T	p.Arg502Trp	SNV	Blood, Cell Line
	MYBPC3	c.3628-41_3628-17del		Indel	Blood, Cell Line
	MYBPC3	c.2373_2374insG	p.Trp792Valfs*41	Indel	Cell Line
	MYH7	c.1988G>A	p.Arg663His	SNV	Saliva, Cell Line
	MYH7	c.3578G>A	p.Arg1193His	SNV	Blood
	MYH7	c.1357C>T	p.Arg453Cys	SNV	Cell Line
	MYH7	c.1750G>C	p.Gly584Arg	SNV	Cell Line
	MYH7	Whole gene duplication		CNV	Cell Line
	MYL2	c.173G>A	p.Arg58Gln	SNV	Blood
	MYL3	c.281G>A	p.Arg94His	SNV	Blood
	PRKAG2	c.905G>A	p.Arg302Gln	SNV	Blood
	TNNI3	c.532_534delAAG	p.Lys178del	Indel	Saliva, Cell Line
	TNNI3	c.575G>A	p.Arg192His	SNV	Cell Line
	TNNT2	c.629_631delAGA	p.Lys210del	Indel	Blood
	TNNT2	c.487_489delGAG	p.Glu163del	Indel	Cell Line
	TPM1	c.574G>A	p.Glu192Lys	SNV	Blood, Cell Line