THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Hypercholesterolemia

Familial hypercholesterolemia (FH), the most common inherited cardiovascular disease, is characterized by severly elevated low-density lipoprotein cholesterol (LDL-C). Elevated LDL-C levels lead to atherosclerotic plaque deposition and an increased risk for cardiovascular disease at an early age. Xanthomas, patches of yellowish cholesterol buildup, are also a common feature of FH and can occur around the eyelids and within tendons of the elbows, hands, knees, and feet. The most common cardiovascular disease is coronary artery disease (CAD), which may manifest as angina and myocardial infarction. If left untreated, men are at a 50% risk and women at a 30% risk for a coronary event by age 50 and 60 years, respectively. The most common cause of FH are heterozygous mutations in the *APOB*, *LDLR*, and *PCSK9* genes. Additionally, biallelic mutations in these genes can also cause a more severe form of FH with most individuals experiencing severe CAD by their mid-20s (1,2). Rarely, elevated high-density lipoprotein cholesterol (HDL-C) levels can be elevated in individuals with mutations in *STAP1*, these patients are also at increased risk for CAD (3).

Our Hypercholesterolemia Panel includes analysis of all 10 genes listed below.

Hypercholesterolemia Panel Genes			
ABCG5	**LDLR**	**LIPC**	**STAP1**
ABCG8	**LDLRAP1**	**PCSK9**	
APOB	**LIPA**	**SCARB1**	

Gene	Clinical Features	Details
ABCG5	Sitosterolemia	Sitosterolemia (aka phytosterolemia) is an autosomal recessive condition due to unobstructed intestinal absorption of cholesterol and cholesterol-like molecules derived from plants (i.e. – sitosterol). Patients have very high levels of plant sterols in plasma and develop tendon and tuberous xanthomas (knee, heel, elbow) in childhood, premature atherosclerosis and coronary artery disease [OMIM# 210250].
ABCG8		
APOB	Hypercholesterolemia, Hypobetalipoproteinemia Abetalipoproteinemia	Mutations in *APOB* have been associated with autosomal dominant/recessive FH and hypobetalipoproteinemia. Individuals with dominant FH have LDL-C levels >190 mg/dL >130 mg/dL and total cholesterol levels >310 mg/dL and >230 mg/dL in untreated adults and children, respectively, while individuals with recessive FH have extremely high LDL-C levels (>500 mg/dL in untreated adults) and severe coronary artery disease in their mid-20s. An estimated 1-5% of patients with familial hypercholesterolemia have mutations in *APOB* (1). Loss of function mutations in the *APOB* gene have been associated with autosomal recessive hypobetalipoproteinemia or abetalipoproteinemia, characterized by hypocholesterolemia and defective absorption of lipid-soluble vitamins leading to retinal degeneration, neuropathy, and coagulopathy [OMIM# 615558].
LDLR	Familial Hypercholesterolemia	Heterozygous mutations in *LDLR* are the most common cause of familial hypercholesterolemia, accounting for 60-80% of patients. Loss of function mutations lead to reduced numbers or activity of LDLR molecules resulting in reduced clearance of LDL cholesterol from the plasma. Patients have also been described with biallelic mutations in *LDLR*. These patients typically have a more severe clinical picture with earlier presentation, usually in the first 2 decades of life (1).
LDLRAP1	Familial Hypercholesterolemia	Biallelic mutations in the *LDLRAP1* gene, which encodes the LDL receptor adaptor protein 1 required for LDLR internalization, have been associated with hypercholesterolemia. Patients with mutations in *LDLRAP1* typically have a robust response to statin treatment. Differentiation of *LDLRAP1* patients from individuals with biallelic *LDLR* mutations can be performed by testing parents. Parents who carry *LDLRAP1* mutations will have normal cholesterol levels while parents of individuals with biallelic *LDLR* mutations will have elevated cholesterol levels (5).
LIPA	Cholesteryl ester storage disease, Wolman disease	Mutations in *LIPA* cause two distinct autosomal recessive phenotypes. Wolman disease is associated with infantile onset presenting with vomiting, steatorrhea, abdominal distention, hepatomegaly and splenomegaly, caused by infiltration of macrophages with build-up of cholesterol esters and triglycerides [OMIM# 278000]. Cholesteryl ester storage disease is associated with a later onset phenotype with a wide range of phenotypes from a milder form of Wolman disease

		to hypercholesterolemia [OMIM# 278000]. Typically, Wolman disease is associated with null mutations while cholesteryl ester storage disease is associated with hypomorphic mutations. (6)
LIPC	Hepatic lipase deficiency	Hepatic lipase deficiency is an autosomal recessive disorder characterized by abnormally triglyceride-rich LDL and HDL as well as beta-migrating very low density lipoproteins [OMIM# 614025].
PCSK9	Familial Hypercholesterolemia 3	Depending on the mutation type, mutations in *PCSK9* can lead to either hypercholesterolemia or hypocholesterolemia. Loss-of-function mutations cause hypocholesterolemia, while gain-of-function (overexpression or hyperactivity) mutations result in rapid LDLR internalization and degradation and reduced numbers of LDLR molecules and are associated with familial hypercholesterolemia [OMIM# 603776] and account for ~1-3% of all patients with familial hypercholesterolemia (1).
SCARB1	Hypercholesterolemia	Recently, Zanoni *et al.* identified a loss of function mutation in *SCARB1*, p.Pro376Leu, in both the heterozygous and homozygous state in individuals with elevated high HDL cholesterol levels. These individuals were at an increased risk for coronary heart disease (7).
STAP1	Hypercholesterolemia	Fouchier *et al.* recently described heterozygous mutations in the *STAP1* gene in five families with hypercholesterolemia. Compared to patients with *LDLR* mutations, individuals with *STAP1* mutations showed lower (but still significantly elevated) LDL levels (2).

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Hypercholesterolemia Panel (10 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406 81407
Turn-around time:	8 weeks

Results:
Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Youngblom *et al.* Familial Hypercholesterolemia. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018., 2014 Jan 2.
2. Ramasamy I, Update on the molecular biology of dyslipidemias. Clin Chim Acta. 2016. 454:143-185.
3. Fouchier *et al.* Mutations in *STAP1* are associated with autosomal dominant hypercholesterolemia. Circ Res. 2014. 115(6):552-555.
4. Bird TD, Alzheimer Disease Overview. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018., 1998 Oct 23.
5. Fellin R, *et al.* The history of Autosomal Recessive Hypercholesterolemia (ARH): From clinical observations to gene identification. Gene. 2015. 555(1):23-32.
6. Hoffman EP, *et al.* Lysosomal Acid Lipase Deficiency. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018., 2015 Jul 30.
7. Zanoni P, *et al.* Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. Science. 2016. 351(6278):1166-1171.