Blueprint Genetics

Sample report as of June 14th, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at ***blueprintgenetics.com/certifications***

Hyperlipidemia Panel (Endocrinology) Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *LDLR* c.1646G>A, p.(Gly549Asp), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
LDLR	NM_000527.5	c.1646G>A, p.(Gly549Asp)	HET	missense_variant	AD,AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs28941776	GRCh37/hg19	19:11226829	G/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	6/251466	probably damaging	deleterious	disease causing	Hypercholesterolemia	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Hyperlipidemia Panel (Endocrinology)	20	253	65609	65609	300	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Hyperlipidemia Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABCA1*, *ABCG5*, *ABCG8*, *ALMS1**, *APOA1*, *APOA5*, *APOB*, *APOC2*, *APOC3*, *APOE*, *CREB3L3*, *CYP27A1*, *GPD1*, *GPIHBP1*, *LDLR*, *LDLRAP1*, *LIPA*, *LMF1*, *LPL* and *PCSK9*. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This panel should be used to detect single nucleotide variants and small insertions deletions (INDELs) and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion

disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not detect balanced translocations or complex rearrangements, and it may not detect low-level mosaicism.

*Some, or all, of the gene is duplicated in the genome. Read more: https://blueprintgenetics.com/pseudogene/
The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 23-year-old adult with hypercholesterolemia, untreated LDL chol > 6. Family history: High chol and CAD.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Hyperlipidemia Panel identified a heterozygous missense variant *LDLR* c.1646G>A, p.(Gly549Asp).

LDLR c.1646G>A, p.(Gly549Asp)

There are 6 individuals heterozygous for this variant in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease.

The *LDLR* c.1646G>A, p.(Gly549Asp) variant (also reported as p.Gly528Asp, Palermo-1 or Genoa) results in the substitution of glycine (Gly) by aspartic acid (Asp) at protein position 549. The affected residue is highly conserved among species, suggesting that substitutions at this position may not be tolerated. There is a small physicochemical difference between glycine and aspartic acid, therefore this is considered a conservative substitution (Grantham score = 94). The variant is predicted damaging by *in silico* tools PolyPhen, SIFT and MutationTaster.

We have identified this variant in two patients with high LDL and total cholesterol (BpG unpublished observations). The variant located in the EGF precursor homology domain has been reported in patients with familial hypercholesterolemia (FH) (PMID 19837725, 15241806, 11810272, 2088165, 23375686) and also in patients with myocardial infarction (PMID 25487149) and in a patient with chylomicronemia syndrome (PMID 28391899). Pirillo et al. reported 107 FH affected individuals with heterozygous p.(Gly549Asp) variant and a patient with p.(Gly549Asp) variant with a variant in the *PCSK9* gene (PMID 28965616). Traeger-Synodinos reported 34 children, age between 2 months to 16 years, heterozygous for the p.(Gly549Asp) variant (PMID 9544850). The variant has also been reported in the clinical variation databases in several FH affected individuals ClinVar 3698, the Dutch FH database and the UMD-LDLR database. Functional studies have shown the variant to reduce LDLR activity, in stimulated T-lymphocytes and EBV-transformed B-lymphocytes and to inhibit LDL transport and reduce LDL uptake in cells (PMID 21865347, 25647241). In addition, another variant p.(Gly549Val) or p.(Gly528Val) affecting the same amino acid residue has been reported in association with FH (PMID 1301940, ClinVar 251955).

LDLR

The *LDLR* gene (MIM *606945) encodes low density lipoprotein receptor, a cell surface protein involved in receptor-mediated endocytosis of specific ligands. Low density lipoprotein (LDL) is normally bound at the cell membrane and taken into the cell, eventually reaching the lysosomes where the protein is degraded and the cholesterol made available for repression of microsomal enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting step in cholesterol synthesis.

Heterozygous pathogenic variants in *LDLR* cause familial hypercholesterolemia (FH, OMIM *143890), whereas biallelic variants cause more severe FH. FH is a relatively common (prevalence 1:200-500) hereditary metabolic disorder (MIM: #143890). Autosomal dominant FH is genetically heterogeneous and can be caused by defects in at least three different genes (*LDLR*, *APOB* and *PCSK9*), all of which encode proteins involved in the hepatic clearance of LDL cholesterol mediated by the LDL receptor

(LDLR). Heterozygous FH (HeFH) is attributed to a heterozygous disease causing variant in one of these three genes. Persons with untreated FH are at an approximately 20-fold increased risk of coronary heart disease (CHD). Untreated men are at a 50% risk of fatal or non-fatal coronary event by the age of 50; untreated women are at a 30% risk by the age of 60.

In contrast, homozygous FH (HoFH) results from either biallelic pathogenic variants in one of the known FH-associated genes or a heterozygous pathogenic variant in each of two different genes. HoFH is much rarer than HeFH (prevalence 1:160,000 to 1:1,000,000). Cholesterol levels and prognosis associated with HoFH are generally dependent on whether the causative variant wholly disrupts receptor function (receptor negative) or leads to a reduction in function (receptor defective). Cholesterol levels are higher in receptor-negative HoFH (22.9±3.5 mmol/l) vs. receptor-defective HoFH patients (16.9±2.4 mmol/l) (PMID: 16343504) and response to statins is poorer in receptor-negative patients (LDL-C decrease 15% vs. 26%) (PMID: 11772418). Most individuals with HoFH experience severe CHD by their mid-20s. The rate of either death or coronary bypass surgery by the teenage years is high. Severe aortic stenosis is also common.

FH is treated actively with diet/lifestyle changes and pharmacotherapy to lower lipid levels. Pharmacotherapy should initially be statin-based, followed by addition of other drugs such as ezetimibe, niacin, bile acid sequestrants and fibrates if the targeted LDL-C level is not achieved. Children and adults with HoFH usually require LDL apheresis. Liver transplantation is also being used in rare circumstances at some centers. In some countries new targeted drugs, lomitapide and mipomersen, are available for HoFH treatment (Familial Hypercholesterolemia - GeneReviews).

There are currently more than 2330 variants in *LDLR* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2022.1), including all types of variants.

Mutation nomenclature is based on GenBank accession NM_000527.5 (*LDLR*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

LDLR c.1646G>A, p.(Gly549Asp) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Disease caused by *LDLR* c.1646G>A, p.(Gly549Asp) is expected to be inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

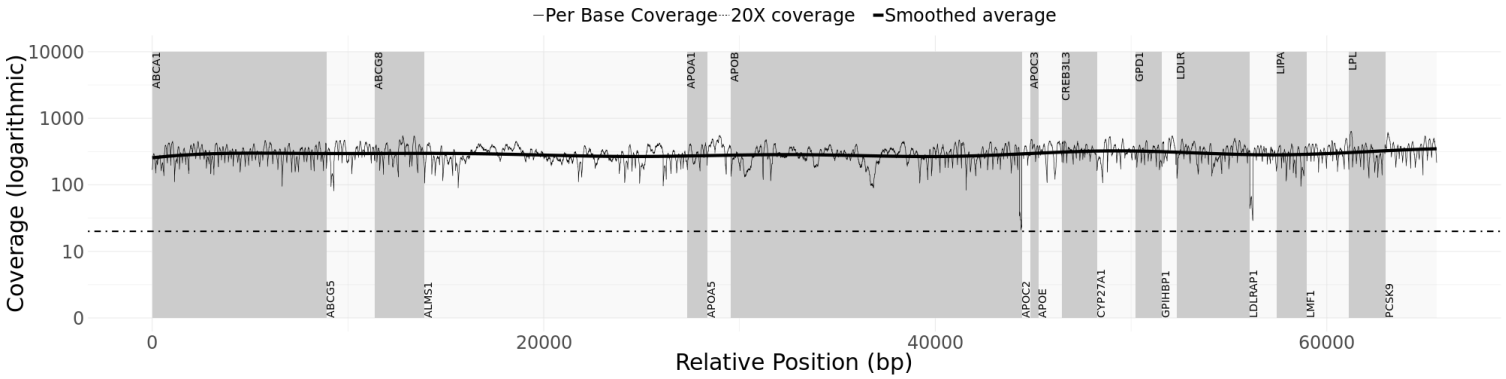
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. Quantity of DNA was assessed using fluorometric method. After assessment of DNA quantity, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output.
Bioinformatics and quality control: Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.
Interpretation: The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported.
Variant classification: Our variant classification follows the Blueprint Genetics Variant Classification Schemes modified from the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiries regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decisions. For questions regarding variant classification updates, please contact us at support@blueprintgenetics.com
Databases: The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the gnomAD, ClinVar, HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as Database of Genomic Variants and DECIPHER. For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.

Confirmation of sequence alterations: Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed.
Confirmation of copy number variants: CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined.
Analytic validation: The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.
Test restrictions: A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.
Technical limitations: This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.
Regulation and accreditations: This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing).

The sample was analyzed using CE marked Blueprint Genetics CES Platform and/or Blueprint Genetics WES Platform in vitro diagnostic medical device manufactured by Blueprint Genetics Oy. For more information please see Accreditations and Certifications.

PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: MD, PhD, CLIA: 99D2092375

- DNA extraction and QC
- Next-generation sequencing
- Bioinformatic analysis
- Confirmation of sequence alterations
- Confirmation of copy number variants
- Interpretation

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_015627.2(*LDLRAP1*):c.-17_-12dupGGCGGC, NM_015627.2(*LDLRAP1*):c.748-608G>A, NM_174936.3(*PCSK9*):c.-331C>A, NM_000040.1(*APOC3*):c.-13-2A>C, NM_000039.1(*APOA1*):c.-21+22G>A, NM_000039.1(*APOA1*):c.-65A>C, chr19:g.11199939-11199939, NM_000527.4(*LDLR*):c.-267A>G, NM_000527.4(*LDLR*):c.-228G>C, chr19:g.11200000-11200000, NM_000527.4(*LDLR*):c.-206C>T, chr19:g.11200031-11200031, chr19:g.11200032-11200032, chr19:g.11200032-11200032,

NM_000527.4(*LDLR*):c.-191C>A, NM_000527.4(*LDLR*):c.-188C>T, NM_000527.4(*LDLR*):c.-185_-183delCTT, NM_000527.4(*LDLR*):c.-172G>A, NM_000527.4(*LDLR*):c.-168A>G, NM_000527.4(*LDLR*):c.-163T>C, NM_000527.4(*LDLR*):c.-161A>C, NM_000527.4(*LDLR*):c.-156C>T, NM_000527.4(*LDLR*):c.-155_-154delACinsTTCTGCAAACTCCT, NM_000527.4(*LDLR*):c.-155_-150delACCCCA, NM_000527.4(*LDLR*):c.-155_-154delACinsTTCTGCAAACTCCT, NM_000527.4(*LDLR*):c.-155_-150delACCCCAinsTT, NM_000527.4(*LDLR*):c.-154C>T, NM_000527.4(*LDLR*):c.-153C>T, NM_000527.4(*LDLR*):c.-152C>T, NM_000527.4(*LDLR*):c.-151C>G, NM_000527.4(*LDLR*):c.-150A>G, NM_000527.4(*LDLR*):c.-149C>A, NM_000527.4(*LDLR*):c.-146C>A, NM_000527.4(*LDLR*):c.-142C>G/T, NM_000527.4(*LDLR*):c.-139_-130delCTCCCCCTGC, NM_000527.4(*LDLR*):c.-140C>A/G/T, NM_000527.4(*LDLR*):c.-139C>A/G, NM_000527.4(*LDLR*):c.-138delT, NM_000527.4(*LDLR*):c.-138T>C, NM_000527.4(*LDLR*):c.-137C>T, NM_000527.4(*LDLR*):c.-136C>G/T, NM_000527.4(*LDLR*):c.-136C>G, NM_000527.4(*LDLR*):c.-136C>T, NM_000527.4(*LDLR*):c.-135C>G, NM_000527.4(*LDLR*):c.-134C>T, NM_000527.4(*LDLR*):c.-124dupA, NM_000527.4(*LDLR*):c.-120C>T, NM_000527.4(*LDLR*):c.-101T>C, NM_000527.4(*LDLR*):c.-99A>G, NM_000527.4(*LDLR*):c.-98C>T, NM_000527.4(*LDLR*):c.-23A>C, NM_000527.4(*LDLR*):c.-22delC, NM_000527.4(*LDLR*):c.-14C>A, NM_000527.4(*LDLR*):c.940+14delC, NM_000527.4(*LDLR*):c.941-13T>A, NM_000527.4(*LDLR*):c.1359-31_1359-23delGCGCTGATGinsCGGCT, NM_000527.4(*LDLR*):c.1359-25A>G, NM_000527.4(*LDLR*):c.1845+11C>G, NM_000527.4(*LDLR*):c.1845+15C>A, NM_000527.4(*LDLR*):c.2140+86C>G, NM_000527.4(*LDLR*):c.2140+103G>T, NM_000527.4(*LDLR*):c.*43G>A, NM_000237.2(*LPL*):c.-241G>C, NM_000237.2(*LPL*):c.-227T>C, NM_005502.3(*ABCA1*):c.6205-39delT, NM_005502.3(*ABCA1*):c.4465-34A>G, NM_005502.3(*ABCA1*):c.4176-11T>G, NM_005502.3(*ABCA1*):c.1195-27G>A, NM_005502.3(*ABCA1*):c.-93+2dupT

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
AF = allele fraction (proportion of reads with mutated DNA / all reads)
AR = autosomal recessive
CNV = Copy Number Variation e.g. one exon or multiexon deletion or duplication
gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)
gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)
HEM = hemizygous
HET = heterozygous
HOM = homozygous
ID = rsID in dbSNP
MT = Mitochondria
MutationTaster = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.
Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level
OMIM = Online Mendelian Inheritance in Man®
PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.
POS = genomic position of the variant in the format of chromosome:position
SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.