PROGENE LAB
For Medical And Genetic Tests
معمل بروجين للتحاليل الطبية

# Single Gene Analysis by NGS

| | |
|---|---|
| **Patient Name:** ********** | **اسم المريض:**********|
| **Sample type:** Blood sample | **DOB:** ********** |
| **Patient phone No.:** ********** | **Sex:** Male |
| **Referring Doctor:** ********** | **City, Country:** ********** |
| **Report type:** *LDLR* Gene Analysis by NGS | **Order received:** ********** |
| **Report date:** ********** | |
| **Reason of referral:** Suspicion of genetic etiology based on clinical features | |

**Clinical Information:** A 16-year-old male child born to consanguineous parents complaining of hypercholesterolemia and nodular swellings at the joints.

## Summary of The Results

**Positive result**

A variant was detected that may account for the patient's phenotype for clinical correlation and differential diagnosis

**A homozygous variant in *LDLR* gene causing Hypercholesterolemia, Familial, 1; FHCL1, with autosomal recessive mode of inheritance**



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## Interpretation of the test results:

### Primary findings:

| Gene | Variant Coordinates | Zygosity/Protein effect | Heredity | MAF | Classification |
|---|---|---|---|---|---|
| *LDLR* | NC_000019.9:g.11240282A>G<br>NM_000527.5:c.2483A>G<br>NP_000518.1:p.(Tyr828Cys) | Homozygous/ Missense | AR | 0.00 | **Pathogenic** |

Low Density Lipoprotein Receptor gene (LDLR; OMIM* 606945) was regionalized to 19p13.1-p13.3[1]. The low-density lipoprotein receptor is a cell surface receptor that plays an important role in cholesterol homeostasis. The low-density lipoprotein receptor (LDLR) is a ubiquitous transmembrane glycoprotein of 839 amino acids that mediates the transport of LDL into cells, via receptor-mediated endocytosis[2]. Monoallelic and biallelic variants of LDLR gene have been reported to cause Hypercholesterolemia, Familial, 1 (FHCL1; OMIM# 143890).

Hypercholesterolemia, Familial, 1 is characterized by elevation of serum cholesterol bound to low density lipoprotein (LDL), which promotes deposition of cholesterol in the skin (xanthelasma), tendons (xanthomas), and coronary arteries (atherosclerosis)[3]. Patients with homozygous familial hypercholesterolemia have extremely high serum cholesterol levels and may develop advanced atherosclerotic plaque before 10 years of age. The plaque formation can occur at unusual sites, including the ascending aorta and around the coronary ostia. These atheromata can interfere with aortic valve function and cause patients to present with angina, myocardial infarction, and even sudden death[4].

The homozygous stop gain variant in exon-17 of the *LDLR* gene in our proband, **NM_000527.5:c.2483A>G, NP_000518.1:p.(Tyr828Cys)** is evaluated as **Pathogenic**, according to the American College of Medical Genetics (ACMG) criteria for the classification of pathogenic variants[5]. The variant has been described previously as disease causing in several studies, such as **Ranheim et al. 2006** and **Thormaehlen et al. 2015**[6,7]. Furthermore, the variant is predicted as deleterious by the predictor tools, location is highly conserved among different species and are extremely rare in population databases (MAF 0.000000684 in gnomAD).



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## Information for Table Interpretation:

Human Genome Variation Society (HGVS) recommendations were used to describe sequence variants (http://www.hgvs.org).

Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria according to ACMG guidelines[5], variants were assigned to one of five interpretation categories (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign) and using computational pathogenicity calculators. All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance (VUS).

## Recommendation:

- We recommend family segregation and the confirmation of the detected homozygous variant in *LDLR* with Sanger sequencing.
- Genetic counseling has to be offered to the family.

## Core disease genes list:

Whole exome of the patient was sequenced. Analysis was based on gene panels according to the clinical data. In this case, > 99.9% of the targeted regions were covered by a minimum of 85 high-quality sequencing reads per base. The evaluation of variants is dependent on available clinical information at the time of analysis. This prediction can be complemented with additional in-silico predictions in individual cases. Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

## Methods:

Sequencing: The coding and flanking intronic regions were enriched using in solution hybridization technology and were sequenced using the Illumina HiSeq /Nova Seq system. NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high quality reads using an internally developed method based on sequencing coverage depth. Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage.

## Bioinformatics and quality control:

The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner



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(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 Freebayes. Variant data was annotated with public variant databases (VcfAnno, VEP). The sequencing depth and coverage for the tested sample was calculated based on the alignments. 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 as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. 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, which processes aligned sequence reads. 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.

## Analytic validation:

This laboratory-developed test has been independently validated. The sensitivity of this test is expected to be in the same range as the validated next generation sequencing assay used to generate the data (sensitivity for SNVs 99.9%, indels 11-50 bps 99.1%, one-exon deletions 100% and 1-9 exon duplications 75%, specificity >99.9% for most variant types). It does not detect very low-level mosaicism as a variant.

## Test restrictions and limitations:

Next generation sequencing (NGS) approaches are now routinely adopted to accurately detect single nucleotide variants (SNVs) and have emerged as a technology with the capability to detect accurately both SNVs and CNVs in a single assay, but CNV analysis via NGS is not yet routinely adopted in diagnosis. CNV calls from NGS data depend on high depth and uniformity of coverage across target sites, and currently available bioinformatics tools are still not sensitive enough to reliably pick up all CNVs.

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.

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered. Specific genetic events like copy number variants, translocations and repeat expansions may not be reliably detected with Exome Sequencing.



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In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected. Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g., Multiplex Ligation Dependent Probe Amplification (MLPA). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

## Disclaimer:

DNA studies don't constitute a definitive test for the selected condition(s) in all individuals. It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems. This test should be one of many aspects used by the healthcare provider to help with a diagnosis and treatment plan, but it shouldn't be a sole diagnostic criterion. This test is used for clinical purposes. It should not be regarded as investigational or for research. Any preparation and processing of a sample from patient material provided by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic testing itself is based on the highest and most current scientific and analytical standards.

## References:

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*With kind regards*

**Clinical Laboratory Geneticists Staff:**

**Prof. Dr. Marwa Elsharkawy**

**Associate Prof. Noha Abd Elhalim**

**Signature**

*Prof. Dr. Mohamed Abd Elmonem*



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