CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE • Am Strande 7 • 18055 Rostock • Germany

Order no.:
Order received: DD/MM/YYYY
Sample type / Sample collection date:
CentoCloud / no material / not available
Report date:
Report type: Final Report

Patient no.: , First Name: , Last Name:
DOB: **DD/MM/YYYY**, Sex: **female**, Your ref.:

Test(s) requested: CentoCloud Genome Solo

CLINICAL INFORMATION

Hypercholesterolemia; Hyperthyroidism; Oligohydramnios
(Clinical information indicated above follows HPO nomenclature.)

Family history: Unknown.
Consanguineous parents: Unknown.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *LDLR* gene. **The genetic diagnosis of autosomal dominant familial hypercholesterolemia-1 is confirmed.**

No further clinically relevant variants related to the described phenotype were detected.

RECOMMENDATIONS

- If possible, parental targeted testing is recommended as establishing the origin of the variant, inherited or *de novo*, is important for familial genetic counselling. Additionally, targeted testing for all affected and at-risk family members, if any, is recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant) is recommended.

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MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
LDLR	NM_000527.2:c.1135T>C	p.(Cys379Arg)	rs879254803	heterozygous	PolyPhen: - Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.000032 ESP: - 1000 G: 0.000032 CentoMD: -	Missense Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v94). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD (latest database available). *** based on ACMG recommendations.

VARIANT INTERPRETATION

LDLR, c.1135T>C p.(Cys379Arg)

The *LDLR* variant c.1135T>C p.(Cys379Arg) causes an amino acid change from Cys to Arg at position 379. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for hypercholesterolemia by Hobbs et al., 1992 (PMID: 1301956), Romano et al., 2011 (PMID: 21865347), Bertolini et al., 2013 (PMID: 23375686). ClinVar lists this variant (Interpretation: Pathogenic/Likely pathogenic; Variation ID: 251685). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Familial hypercholesterolemia is an autosomal dominant disorder 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). The disorder occurs in 2 clinical forms: homozygous and heterozygous (Hobbs et al., 1992; PMID:1301956). Mode of Inheritance: Autosomal dominant (OMIM®: 143890)

RESEARCH FINDINGS

Research variants (with potential relevance to the described phenotype) are variants in genes with no or only partial experimental evidence for their involvement in human disease.

The data was analyzed focusing on variants affecting protein function (nonsense, frameshift, conserved splice site and missense with high pathogenicity predictions) in genes with supporting evidence on zygosity, segregation or functional importance of the gene. Available literature or experimental data on expression and/or animal models were considered.

However, no such variants could be identified for the patient.

SECONDARY (INCIDENTAL) FINDINGS

If consent is provided, in line with ACMG recommendations for reporting of secondary (incidental) findings in clinical exome and genome sequencing (Genetics in Medicine, 2021; PMID: 34012068), we report secondary (incidental) findings, i.e., pathogenic variants (class 1) and likely pathogenic variants (class 2) in the recommended genes for the indicated phenotypes.

We did not detect any class 1 or 2 variants in the genes, for which secondary (incidental) findings are reported.

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CARRIERSHIP FINDINGS

In this table we list sequence variants previously ascertained or evaluated and classified in CENTOGENE as "pathogenic" and "likely pathogenic", in selected genes associated with recessive severe and early-onset Mendelian diseases. As only in-house classified variants are presented, it should not be considered a comprehensive list of variants in these genes and does not provide a complete list of potentially relevant genetic variants in the patient. The complete gene list can be found at www.centogene.com/carriership-findings (please contact CENTOGENE customer support if the gene list has been updated after this report was issued). Orthogonal validation was not performed for these variants. Therefore, if any variant is used for clinical management of the patient, confirmation by another method needs to be considered. Furthermore, the classification of these variants may change over time, however reclassification reports for these variants will not be issued. CENTOGENE is not liable for any missing variant in this list and/or any provided classification of the variants at a certain point of time. As the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or family and/or inform about reproductive risks, we recommend discussing these findings in the context of genetic counselling.

SEQUENCE VARIANTS

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BCHE	NM_000055.3:c.293A>G	p.(Asp98Gly)	rs1799807	heterozygous	PolyPhen: Possibly damaging Align-GVGD: C65 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.012 ESP: 0.014 1000 G: 0.0060 CentoMD: 0.015	Missense Pathogenic (class 1)

Variant annotation based on OTFA (using VEP v94). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function; splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD (latest database available). *** based on ACMG recommendations.

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIONS)

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign

Additionally, other types of clinically relevant variants can be identified (e.g., risk factors, modifiers).

METHODS

An in-house bioinformatics pipeline, including read alignment to GRCh37/hg19 genome assembly and revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920), variant calling, annotation, and comprehensive variant filtering is applied. Copy number variation (CNV) calling is based on the DRAGEN pipeline from Illumina. All variants with minor allele frequency (MAF) of less than 1% in gnomAD database, and disease-causing variants reported in HGMD®, in ClinVar or in CentoMD® are evaluated. Although the evaluation is focused on coding exons and flanking intronic regions, the complete gene region is interrogated for candidate variants with plausible association to the phenotype. All potential patterns for mode of inheritance are considered. In addition, provided family history and clinical information are used to evaluate identified variants with respect to their pathogenicity and disease causality. Variants are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) along ACMG guidelines for classification of variants. All relevant variants related to the phenotype of the patient are reported. CNVs of unknown significance are not reported. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods only when a DNA sample has been provided. In such cases, a specificity of > 99.9% for all reported variants is warranted.

ANALYSIS STATISTICS

CentoCloud® Genome Solo

Targeted nucleotides covered	≥ 10x	99.02%

LIMITATIO

NS

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LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Misinterpretation of results may occur if the provided genetic data or patient information is inaccurate and/or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.
The genes with mapping issues in GRCh37/hg19 genome assembly, the non-protein-coding disease-associated genes, and genomic regions that are hard to sequence by current technology and are without evidenced relevance for monogenic disorders, are excluded from this analysis. More complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. In addition, due to technology limitations, certain regions may be poorly covered, or not covered at all. In these regions and others encompassing repetitive, high-homology (such as pseudogene homology), and GC-rich sequences, relevant variants can be missed. Extremely low-coverage calls are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis. The CNV detection sensitivity is decreased for repetitive and homologous regions, such as pseudogenes. It is expected that lower quality samples (prenatal, product of conception, blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis may not be possible to perform. Mitochondrial variants with heteroplasmy levels below 15% may not be detected (for the products of conception considering the sample quality and possible maternal contamination, mitochondrial genome is excluded from the analysis). Potential aberrant splicing is assessed with splice prediction tools. Deep intronic variants without strong prediction of aberrant splicing may not be reported, with the exception of known pathogenic splicing variants evidenced by external sources.

ADDITIONAL INFORMATION

This test was developed, and its performance was validated, by CENTOGENE. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. This test has been developed for clinical purposes. All test results are reviewed, interpreted, and reported by our scientific and medical experts.

To exclude mistaken identity in your clinic, several guidelines recommend testing a second sample that is independently obtained from the proband. Please note that any further analysis will result in additional costs.

The classification of variants can change over the time. Please feel free to contact CENTOGENE (customer.support@centogene.com) in the future to determine if there have been any changes in classification of any reported variants.

DISCLAIMER

The CentoCloud® from CENTOGENE provides state-of-the-art bioinformatic services applying a validated diagnostic pipeline to filter variants from the sequencing raw data provided by our customers. Although regularly and extensively tested and monitored, the CentoCloud® system – like any software product including bioinformatic analyses and particularly as third-party software is included - might contain unforeseeable errors which may lead to misalignment, incorrect base calls, and mis-annotation of variants and thus may have an impact on the accuracy of the medical interpretation of results.
Although CENTOGENE monitors and reviews the received sequencing raw data and its quality insofar as technically possible, it is not responsible for any errors resulting from the preparation and sequencing of a sample by its customers (including, but not limited to, sample identification and/or sample swap). Such errors are then already contained within the raw data and will lead to an erroneous result.
CENTOGENE shall only be responsible for providing a fully annotated variant list in the range and format as agreed with its customers. Any filtering criteria may be subject to further modification at the partner's site in accordance with clinical information or family information. The selection of highlighted variants by CENTOGENE does not preclude any other variant within the variant list that should be considered for medical interpretation.
Any clinical interpretation and conclusion of variants highlighted in this report is the responsibility of the customer. Test results should always be interpreted in the context of clinical findings, family history, and any other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Please note, rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing of samples through CENTOGENE's laboratory should be considered, this also applies for a confirmative analysis of any positive result.

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Chief Genomic Officer Human Geneticist	Human Geneticist	Clinical Scientist

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