CENTOGENE GUIDING PRECISION MEDICINE

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

Dr.
Address
Institution

Country

Order no.:
Order received: DD-MM-YYYY
Sample type / Sample collection date:
blood, CentoCard® / DD-MM-YYYY
Report date: DD-MM-YYYY
Report type: Final Report

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

Test(s) requested: CentoGenome® Solo

CLINICAL INFORMATION

Abnormal EKG; Abnormal T-wave; Cardiac arrest; Decreased body weight; Increased circulating lactate dehydrogenase concentration; Lower limb spasticity; Prolonged QT interval; Ventricular fibrillation
(Clinical information indicated above follows HPO nomenclature.)

Diagnosed condition(s): congenital prolonged QT syndrome.

Family history: Yes.
Consanguineous parents: No.
Rule out request(s): genetic cause of sudden cardiac death, Brugada syndrome.
Targeted gene request(s): *KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2. KCNJ5, CALM1, CALM2, CALM3.*

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the *CACNA1C* gene. **The result is consistent with a genetic diagnosis of autosomal dominant long QT syndrome type 8.**

Heterozygotes have a risk of 50% to transmit the variant to each offspring.

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

Coverage of the coding regions ±10bp at 10x nucleotide reading depth for the genes of specific interest (listed in the clinical information section): 100%

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 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***
CACNA1C	NM_199460.2:c.2573G>A	p.(Arg858His)	rs786205753	heterozygous	PolyPhen: - SIFT: - MutationTaster: Disease causing Conservation_nt: high	gnomAD: - ESP: - 1000 G: -	Missense Pathogenic

Variant annotation based on CentoCloud Bioinformatics pipeline. * Splicing predictions: Ada and RF scores. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G). *** Based on ACMG recommendations.

VARIANT INTERPRETATION

CACNA1C, c.2573G>A p.(Arg858His)

The *CACNA1C* variant c.2573G>A p.(Arg858His) causes an amino acid change from Arg to His at position 858 in exon(s) no. 19 (of 50). According to HGMD Professional 2024.1, this variant has previously been described as disease causing for Long QT syndrome (PMID:23174487, 29046645, 35932045). ClinVar lists this variant (Interpretation: Pathogenic/Likely pathogenic; Variation ID: 190653). It is classified as pathogenic based on CENTOGENE's implementation of the ACMG/AMP/ClinGen SVI guidelines.

Congenital long QT syndrome is electrocardiographically characterized by a prolonged QT interval and polymorphic ventricular arrhythmias (torsade de pointes). These cardiac arrhythmias may result in recurrent syncope, seizure, or sudden death (Jongbloed et al., 1999; PMID:10220144). Mode of Inheritance: Autosomal dominant (OMIM®: 618447).

SECONDARY FINDINGS

If consent is provided, we report secondary findings in line with ACMG recommendations (ACMG SF v3.3 list for reporting of secondary findings in clinical exome and genome sequencing; Genetics in Medicine, 2025; PMID: 40568962), i.e. relevant pathogenic and likely pathogenic variants in the ACMG-recommended genes.

We did not detect any relevant variants in the genes for which secondary findings are reported.

CARRIERSHIP FINDINGS

In this table, we list sequence variants previously ascertained, or evaluated and classified, by CENTOGENE as "pathogenic" or "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 or a complete representation of potentially relevant genetic variants in the patient. The complete gene list can be found at www.centogene.com/carriership-findings (please contact CENTOGENE 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.

In accordance with this disclaimer no relevant pathogenic or likely pathogenic variant was identified.

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METHODS

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Genomic DNA is enzymatically fragmented and tagged with Illumina compatible adapter sequences. The libraries are paired-end sequenced on an Illumina platform to yield an average coverage depth of ~ 30x. A bioinformatics pipeline based on the DRAGEN pipeline from Illumina, as well as CENTOGENE's in-house pipeline is applied. The sequencing reads are aligned to the Genome Reference Consortium Human Build 37 (GRCh37/hg19), as well as the revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA (NC_012920). Sequence variants (SNVs/indels) and copy number variants (CNVs) are called using DRAGEN, Manta and in-house algorithms. Variants with a minor allele frequency (MAF) of less than 1% in gnomAD database, or disease-causing variants reported in HGMD®, in ClinVar or in CENTOGENE's in-house Biodatabank are evaluated. Although the evaluation is focused on coding exons and flanking intronic regions, the complete gene is interrogated for candidate variants with plausible association to the phenotype. All potential modes of inheritance are considered. In addition, the provided clinical information and family history are used to evaluate identified variants with respect to their pathogenicity and disease causality. Sequence variants are categorized into five classes (pathogenic, likely pathogenic, variant of uncertain significance [VUS], likely benign, and benign) according to ACMG/AMP guidelines for classification of variants in addition to ClinGen recommendations. CNVs are categorized into five classes (pathogenic, likely pathogenic, variant of uncertain significance [VUS], likely benign, and benign) according to ACMG/ClinGen guidelines for classification of CNVs. Additionally, other types of clinically relevant variants can be identified (e.g. risk factors, modifiers). All relevant variants related to the phenotype of the patient are reported. Likely benign and benign variants are not reported. CNVs of unknown significance with no apparent relation to the patient's phenotype are not reported. Mitochondrial variants with a heteroplasmy level of 15% or higher are reported. For detection of SNVs and indels in the regions targeted for downstream analysis a sensitivity of 99.9%, a specificity of 99.9%, and an accuracy of 99.9% is achieved. CNV detection software has a sensitivity of more than 95%. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Reportable variants detected by NGS that do not reach internal quality and validation standards (including but not limited to, variants with unclear zygosity including mosaic calls, CNVs encompassing complex genomic regions, CNVs below 500kb) are confirmed by orthogonal methods. CNVs involving clinically significant imprinted regions are further investigated by MS-MLPA analysis. Consequently, a specificity of > 99.9% for all reported variants is warranted. Screening of repeat expansions is performed by the ExpansionHunter algorithm for the following genes: *AR, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, CACNA1A, CNBP, CSTB, C9orf72, DMPK, FMR1, FXN, HTT, JPH3, NOP56, PABPN1, PHOX2B, PPP2R2B, PRNP* and *TBP*. The technical results of repeat expansion screenings will be correlated with the clinical information provided. Any repeat expansion called and considered relevant to the phenotype will be confirmed by an orthogonal method. *GBA1* screening is performed using Gauchian algorithm to detect recombination events affecting the region encompassing exons 9-11 (NM_000157.3), a region which has the highest homology to *GBAP1*. Any detected recombination event is reported only when considered relevant to the phenotype. Spinal muscular atrophy (SMA) screening is performed using SMN Caller algorithm to detect the copy number of the *SMN1* gene. Screening of uniparental isodisomy (isoUPD) is performed using an in-house algorithm for Mendelian inheritance errors (MIE) to detect runs of homozygosity (ROH) for the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

ANALYSIS STATISTICS

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Targeted nucleotides covered	≥ 10x	99.71%

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the provided patient information or genetic data is inaccurate or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.

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Only variants in genes potentially related to the proband's provided clinical information are reported. Genes with mapping issues in the genome assembly used, 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 not mentioned in the methods section, such as inversions and translocations, 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. 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. The CNV detection sensitivity is decreased for repetitive regions, homologous regions such as pseudogenes, and for events spanning 2 or less exons. Due to methodological limitations, the sensitivity for detecting mosaic copy number variants (CNVs) is reduced. Furthermore, in cases where mosaic CNVs are detected, high allelic fractions may not be correctly recognized as mosaic. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. It is expected that lower quality samples (e.g. 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, mitochondrial genome analysis, and/or additional integrated screening analyses in this test may not be possible to perform. The repeat expansion algorithm used is not designed to handle complex

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loci that harbor multiple repeats. Repeats are only genotyped if the coverage at the locus is at least 10x. The Gauchian algorithm can only detect non-recombinant-like variants from a set of 111 known *GBA1* variants and can detect recombination events affecting exons 9-11 only. Therefore, recombinations affecting other regions are not in the scope of this screening. Silent carriers may be missed with the SMN Caller algorithm. The isoUPD detection is a screening method, and therefore false-positive and false-negative results may occur.

ADDITIONAL INFORMATION

This test was developed and validated by CENTOGENE. It is intended for clinical purposes. All test results are reviewed, interpreted, and reported by our scientific and medical experts.

To exclude mistaken identity in sample collection, several guidelines recommend testing a second sample independently obtained from the consultand.

As variant classification can change over time, please feel free to contact CENTOGENE in the future to inquire about classification changes of reported variants.

DISCLAIMER

The preparation and processing of patient samples provided to CENTOGENE by a physician, clinical institution, or laboratory (by a “Partner”), as well as the genetic and/or biochemical testing requested, are performed according to the highest and most current scientific and analytical standards. However, in rare cases, genetic or biochemical tests may yield incorrect results—for example, due to the quality of the sample provided by the Partner, or due to unforeseeable or unknown factors beyond CENTOGENE’s control. In such cases, CENTOGENE shall not be held responsible or liable for incomplete, potentially misleading, or incorrect results if the issue could not have been identified in advance.

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