CENTOGENE GUIDING PRECISION MEDICINE

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

Dr.
Institution
Address

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: **male**, Your ref.: **-**

Test(s) requested: CentoXome® MOx 1.0 Solo

CLINICAL INFORMATION

Atonic seizure; Delayed speech and language development; EEG with generalized epileptiform discharges; Encephalopathy; Epileptic encephalopathy; Gait imbalance; Hydronephrosis; Hypsarrhythmia; Motor delay; Seizure
(Clinical information indicated above follows HPO nomenclature.)

Family history: No.
Consanguineous parents: No.

POSITIVE RESULT
Pathogenic variants identified

INTERPRETATION

Two heterozygous pathogenic variants were identified in the *TPP1* gene. According to the NGS data (BAM alignment files) the trans phase of the variants is confirmed. Additionally, the activity of the enzyme tripeptidyl peptidase was pathologically decreased. **The genetic diagnosis of autosomal recessive neuronal ceroid lipofuscinosistype 2 is confirmed.**

No further variants clinically relevant to the described phenotype were identified

RECOMMENDATIONS

- Parental carrier testing is recommended. Additionally, targeted testing for affected family members, if any, and familial cascade carrier testing are 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***
TPP1	NM_000391.3:c.509-1G>C	p.(?)	rs56144125	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: high	gnomAD: 0.00040 ESP: - 1000 G: -	Splicing Pathogenic
TPP1	NM_000391.3:c.622C>T	p.(Arg208*)	rs119455955	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: moderate	gnomAD: 0.00048 ESP: - 1000 G: -	Nonsense 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.

BIOCHEMICAL TESTING§				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
tripeptidyl peptidase 1	1,9 µmol/L/h	≥ 26,3 µmol/L/h	pathologic	fluorimetry

§ For biochemical testing, one normal control was included in each assay; the control result was in the normal range based on the reference mentioned above.

VARIANT INTERPRETATION

TPP1, c.509-1G>C p.(?)

The *TPP1* variant c.509-1G>C affects the canonical donor splice-site nucleotide and skipping of the exon is predicted. According to HGMD Professional 2024.2, this variant has previously been described as disease causing for Neuronal ceroid lipofuscinosis, late infantile (PMID:9295267, 32631363, 30977854). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 2644). It is classified as pathogenic based on CENTOGENE's implementation of the ACMG/AMP/ClinGen SVI guidelines.

TPP1, c.622C>T p.(Arg208*)

The *TPP1* variant c.622C>T p.(Arg208*) creates a premature stop codon in exon(s) no. 6 (of 13). According to HGMD Professional 2024.2, this variant has previously been described as disease causing for Neuronal ceroid lipofuscinosis, late infantile (PMID:9295267, 38374194, 30977854). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 2643). It is classified as pathogenic based on CENTOGENE's implementation of the ACMG/AMP/ClinGen SVI guidelines.

Pathogenic variants in the *TPP1* gene are associated with autosomal recessive neuronal ceroid lipofuscinosistype 2 (CLN2). The neuronal ceroid lipofuscinoses (NCL; CLN) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by the intracellular accumulation of autofluorescent lipopigment storage material in different patterns ultrastructurally. The clinical course includes progressive dementia, seizures, and progressive visual failure. The lipopigment pattern seen most often in CLN2 consists of 'curvilinear' profiles (PMID: 15965709). (OMIM®: 204500)

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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.

SEQUENCE VARIANTS

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BTD	NM_001281723.2:c.1336G>C	p.(Asp446His)	rs13078881	heterozygous	PolyPhen: Probably damaging SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: moderate	gnomAD: 0.032 ESP: - 1000 G: -	Missense Pathogenic
CLCN1	NM_000083.2:c.2680C>T	p.(Arg894*)	rs55960271	heterozygous	PolyPhen: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: weak	gnomAD: 0.0056 ESP: - 1000 G: -	Nonsense 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.

METHODS

CentoXome® MOx 1.0 Solo
Genomic DNA is enzymatically fragmented, and target regions are enriched using DNA capture probes. These regions include approximately 41 Mb of the human coding exome (targeting > 98% of the coding RefSeq from the human genome build GRCh37/hg19), as well as the mitochondrial genome. The generated library is sequenced on an Illumina platform to obtain at least 20x coverage depth for > 98% of the targeted bases. 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. 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 CENTOGENE's in-house Biodatabank are evaluated. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM® information). 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. 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. Copy number variants (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. For CentoXome® MOx, if applicable, biochemical analysis is performed upon detection of relevant variants by sequencing. This

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enhances the diagnosis of metabolic disorders, optimizes variant classification, and helps to ascertain the eventual contribution to the phenotype; the list of enzyme-activity assays and biomarkers can be obtained at www.centogene.com/mox. 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. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. CNV detection software has a sensitivity of more than 95%. 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).

tripeptidyl peptidase activity testing
Biochemical testing for tripeptidyl-peptidase is performed using fluorimetry. This is an analytical screening method, with a sensitivity of approximately 100% and specificity of above 96%.

ANALYSIS STATISTICS

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Targeted nucleotides covered	≥ 20x	98.74%

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. The genes with mapping issues in GRCh37/hg19 genome assembly, the non-protein-coding disease-associated genes, and approximately 0.2 Mb of genomic regions that are hard to sequence by current enrichment 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. The isoUPD detection is a screening method, and therefore false-positive and false-negative results may occur. 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 (homo/hemizygous or heterozygous calls with less than three or four reads, respectively) 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, as well as for events spanning two or less exons. Considering that exome sequencing focuses on coding regions, CNV size estimates may be imprecise particularly when breakpoints fall in intronic or intergenic areas. 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 (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 and/or mitochondrial genome analysis may not be possible to perform. Potential aberrant splicing is assessed with splice prediction tools. Intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis, with the exception of known pathogenic splicing variants evidenced by external sources.

tripeptidyl-peptidase activity testing
The biochemical 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 genetic data or patient information is inaccurate and/or incomplete. If the obtained biochemical results are not compatible with the clinical findings, additional testing should be considered. Using fluorimetry, enzyme activity assays with dried blood spot (DBS) are not as specific as with leukocyte preparation. Therefore, these assays when not combined with further analyses, such as genetic or specific biomarker testing, may lead to inconclusive or incorrect interpretations.

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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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