CENTOGENE THE RARE DISEASE COMPANY

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type / Sample collection date:
blood, CentoCard® / xxx
Report date: xxx
Report type: Final Report

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: **male**, Your ref.: **xxx**

Additional report recipient(s): xxx

Test(s) requested: CentoXome® Trio

CLINICAL INFORMATION

Abnormal basal ganglia MRI signal intensity; Abnormal brainstem MRI signal intensity; Abnormal globus pallidus morphology; Abnormal thalamic MRI signal intensity; Abnormality of the cerebellar peduncle; Abnormality of the cerebral white matter; Abnormality of the medulla oblongata; Apnea; Bilateral tonic-clonic seizure; Encephalopathy; Generalized hypotonia; Muscular hypotonia; Seizure
(Clinical information indicated above follows HPO nomenclature.)

Age of onset: 1 month(s).

Family history: Unknown.
Consanguineous parents: No.

Please see reports of the parents: [Order: xxx, Name: xxx] and [Order: xxx, Name: xxx].

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homoplastic (100% of total reads) pathogenic variant was identified in the mitochondrial gene *MT-ND3*. **The obtained result is consistent with a genetic diagnosis of mitochondrial complex I deficiency (mitochondrial type 1).**

No further clinically relevant variants, including copy number variations, related to the described phenotype were identified by exome sequencing.

As secondary (incidental) finding, a heterozygous pathogenic variant was identified in the *SDHB* gene. **This is consistent with a genetic diagnosis of hereditary paraganglioma-pheochromocytoma (PGL/PCC) syndromes.**

RECOMMENDATIONS

- Clinical follow-up for symptoms associated to the secondary (incidental) finding is recommended.
- Genetic counselling is also recommended.

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RESULT SUMMARY

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
MT-ND3	NC_012920.1:m.10158T>C	NC_012920.1_ *MT-ND3*:p.Ser34Pro	rs199476117	homoplasmic (100% of the NGS reads)	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: - Conservation_aa: -	MITOMAP: 0/51673	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

MT-ND3, m.10158T>C NC_012920.1_*MT-ND3*:p.Ser34Pro

The variant detected in the *MT-ND3* gene, m.10158T>C, was identified in 100% of the reads (total number of reads 8992). The *MT-ND3* gene is involved in mitochondrial complex I (CI) function. The variant m.10158T>C has been identified in several patients with symptoms of Leigh disease (or subacute necrotizing encephalomyelopathy) and MELAS (Mitochondrial Encephalopathy, Lactic acidosis, and Stroke-like episodes) (GeneReviews - PMID: 20301352, MitoMAP: https://www.mitomap.org). McFarland et al. (2004 - PMID: 14705112) identified this variant as presumed de novo in three independent families affected with Leigh disease. Cells isolated from patients harboring this variant had reduced levels of fully assembled complex I, and cybrid cell lines generated with the m.10158T>C variant had a reduction in complex I activity concomitant with the degree of heteroplasmy in individual established lines (McFarland 2004). Additional Leigh disease patients carrying this variant have also been described (Bugiani et al., 2004 - PMID: 15576045). Mukai and Nagata (2017 - PMID: 28883258) identified the m.10158T>C ND3 variant in a patient diagnosed with MELAS. This variant was also detected in the mother of the patient in a heteroplasmic state. The detected variant is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in *MT-ND3* have been associated to Leigh syndrome and MELAS. Leigh syndrome is characterized by onset of symptoms typically between ages three and 12 months, often following a viral infection. Decompensation (often with elevated lactate levels in blood and/or cerebrospinal fluid) during an intercurrent illness is typically associated with psychomotor retardation or regression. Neurologic features include hypotonia, spasticity, movement disorders (including chorea), cerebellar ataxia, and peripheral neuropathy. Extraneurologic manifestations may include hypertrophic cardiomyopathy. About 50% of affected individuals die by age three years, most often as a result of respiratory or cardiac failure (PMID: 20301352). MELAS is a multisystem disorder with protean manifestations. The vast majority of affected individuals develop signs and symptoms of MELAS between ages two and 40 years. Common clinical manifestations include stroke-like episodes, encephalopathy with seizures and/or dementia, muscle weakness and exercise intolerance, normal early psychomotor development, recurrent headaches, recurrent vomiting, hearing impairment, peripheral neuropathy, learning disability, and short stature. During the stroke-like episodes neuroimaging shows increased T2-weighted signal areas that do not correspond to the classic vascular distribution (hence the term "stroke-like"). Lactic acidemia is very common and muscle biopsies typically show ragged red fibers (PMID: 20301411).

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SECONDARY (INCIDENTAL) FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
SDHB	M_003000.2:c.72+1G>T		rs587782703	heterozygous	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: Disease-causing Conservation_nt: moderate Conservation_aa: N/A	gnomAD:- ESP:- 1000 G:0.0000084 CentoMD:-	SplicingPathogenic (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

SDHB, c.72+1G>T

The *SDHB* variant c.72+1G>T is predicted to disrupt the highly conserved donor splice site of exon 1. According to HGMD® Professional 2019.1, this variant has previously been described as disease-causing for Phaeochromocytoma by Benn et al., 2006 (PMID: 16317055), Schrader et al., 2016 (PMID: 26556299), and Whitworth et al., 2018 (PMID: 29909963). ClinVar lists this variant as pathogenic (clinical testing/research, Variation ID: 142764). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *SDHB* gene have been associated with autosomal dominant hereditary paraganglioma-pheochromocytoma (PGL/PCC) syndromes. These are characterized by paragangliomas (tumors that arise from neuroendocrine tissues distributed along the paravertebral axis from the base of the skull to the pelvis) and pheochromocytomas (paragangliomas that are confined to the adrenal medulla). Sympathetic paragangliomas cause catecholamine excess; parasympathetic paragangliomas are most often non-secretory. Extra-adrenal parasympathetic paragangliomas are located predominantly in the skull base and neck (referred to as head and neck PGL [HNPGL]) and sometimes in the upper mediastinum; approximately 95% of such tumors are non-secretory. In contrast, sympathetic extra-adrenal paragangliomas are generally confined to the lower mediastinum, abdomen, and pelvis, and are typically secretory. Pheochromocytomas, which arise from the adrenal medulla, typically lead to catecholamine excess. Symptoms of PGL/PCC result from either mass effects or catecholamine hypersecretion (e.g., sustained, or paroxysmal elevations in blood pressure, headache, episodic profuse sweating, forceful palpitations, pallor, and apprehension or anxiety). The risk for developing metastatic disease is greater for extra-adrenal sympathetic paragangliomas than for pheochromocytomas (GeneReviews®; PMID: 20301715).

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TABULAR LIST OF ADDITIONAL PATHOGENIC AND LIKELY PATHOGENIC VARIANTS

To provide the most comprehensive and relevant genetic information, we list selected variants found in genes associated with **severe and early-onset disease.** The gene selection is based on OMIM® phenotypes and CENTOGENE internal data. We classified these variants at the time of reporting as "pathogenic" and "likely pathogenic" (see our mutation database CentoMD® for further information). Variants not included and classified in the current release of CentoMD®, and low-quality variants that usually represent technical artifacts, are not included. The complete gene list can be found at https://www.centogene.com/diagnostics/medical-reporting/p-lp-gene-reporting.html (please contact CENTOGENE customer support if the gene list has been updated after this report was issued).
The listed variants may not directly answer the diagnostic request, at least not with the clinical information provided to CENTOGENE or current scientific understanding of relevant genetic disease mechanisms. However, these variants may help to close a potential diagnostic gap regarding the current clinical picture and are therefore provided here for a full diagnostic overview. In case this additional information is used in the further differential diagnosis process, orthogonal validation of relevant variants might be necessary.
Beyond the requested test, variants in genes related to late-onset diseases with unclear (considerably reduced) penetrance and/or cancer-related genes with onset in adulthood are not included in this list. This table does therefore not provide a complete list of potentially relevant genetic variants in the patient. Furthermore, the classification of these variants may change over time. 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.
Insofar, as the identified variants may indicate (additional) genetic risks or diagnoses in the patient and/or his family and/or inform about reproductive risks, we strongly recommend following applicable local guidelines with regard to informing the patient about such findings. Particularly, if the patient decided not to be informed about "secondary (incidental) findings" (to avoid any misunderstanding the list given here is not covering "secondary (incidental) findings" according to ACMG), it should be clarified with the patient whether he/she wants to be informed about these additional variants.

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)		heterozygous	PolyPhen: Align-GVGD: SIFT: MutationTaster: Conservation_nt: moderate Conservation_aa: weak	gnomAD: ESP: 1000 G: CentoMD:	MissensePathogenic (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).

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METHODS

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 CentoMD® are evaluated. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic nucleotides of genes with a 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 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. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of > 99.9% for all reported variants is warranted. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. The copy number variation (CNV) detection software has a sensitivity of more than 95% for all homozygous/hemizygous and mitochondrial deletions, as well as heterozygous deletions/duplications and homozygous/hemizygous duplications spanning at least three consecutive exons. For the uniparental disomy (UPD) screening, a specific algorithm is used to assess the well-known clinically relevant chromosomal regions (6q24, 7, 11p15.5, 14q32, 15q11q13, 20q13 and 20).

ANALYSIS STATISTICS

CentoXome® Trio

Targeted nucleotides covered	≥ 20x	98.73%

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 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 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. Complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. The UPD 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, 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. Heterozygous CNVs spanning less than three exons cannot reliably be detected, are therefore excluded from routine analysis, and will only be inspected and reported upon medical or technical indication. 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. Synonymous variants and intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis. However, pathogenic splicing variants evidenced by external sources are considered in the analysis.

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.

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.

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.

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DISCLAIMER

Any preparation and processing of a sample from patient material provided to CENTOGENE by a physician, clinical institute or a laboratory (by a "Partner") and the requested genetic and/or biochemical testing itself is based on the highest and most current scientific and analytical standards. However, in very few cases genetic or biochemical tests may not show the correct result, e.g. because of the quality of the material provided by a Partner to CENTOGENE or in cases where any test provided by CENTOGENE fails for unforeseeable or unknown reasons that cannot be influenced by CENTOGENE in advance. In such cases, CENTOGENE shall not be responsible and/or liable for the incomplete, potentially misleading or even wrong result of any testing if such issue could not be recognized by CENTOGENE in advance.

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