CENTOGENE • Am Strande 7 • 18055 Rostock • Germany

Dr. (Physician)
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: CentoGenome MOx 1.0 Solo

CLINICAL INFORMATION

Infantile onset; Pancytopenia, Hepatosplenomegaly, recurrent infections
(Clinical information indicated above follows HPO nomenclature.)

Family history: Yes.
Sister: Affected
Consanguineous parents: Yes.

Clinician suspects: Gaucher disease.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A homozygous pathogenic variant was identified in the *GBA* gene. Additionally, the concentration of the biomarker lyso-Gb1 is massively increased. The enzymatic activity of beta-glucocerebrosidase is pathologically reduced. **The genetic diagnosis of autosomal recessive Gaucher disease is confirmed.**

As a secondary finding, a heterozygous pathogenic variant was identified in the *BRCA1* gene. **This finding is consistent with the increased genetic susceptibility to *BRCA1*-related malignancies.**

RECOMMENDATIONS

- If possible, parental targeted testing is recommended to confirm the homozygosity of the identified *GBA* variant in place of a compound heterozygosity with a large deletion. Additionally, targeted testing for affected family members, if any, and familial cascade carrier testing is recommended.
- If possible, parental targeted testing is recommended as establishing the origin of the *BRCA1* 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.

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

MAIN FINDINGS

BIOCHEMICAL TESTING				
NAME OF GENE/ENZYME/BIOMARKER	RESULT	REFERENCE	INTERPRETATION	METHOD
beta-glucocerebrosidase	**< 1 (LOQ) µmol/L/h** LOQ=limit of quantification	≥ 4,1 µmol/L/h	pathologic	fluorimetry
lyso-Gb1	**435,0 ng/ml**	≤ 6,8 ng/ml	pathologic	liquid chromatography mass spectrometry

Updated reference March 2019: value obtained as mean + 2 standard deviations of the lyso-Gb1 concentration in healthy controls. Lyso-Gb1 (glucosylsphingosine) is a well validated and highly reliable marker in Gaucher disease, reflecting the severity and progress of the disease (Rolfs et al, PLoS One. 2013). The sensitivity of the marker for the initial diagnosis of untreated Gaucher disease for a concentration of > 10 ng/ml is > 99.9%. The method for measuring the concentration of lyso-Gb1 in DBS, EDTA-blood, plasma, or serum is CE IVD labeled and only offered from CENTOGENE/Germany.

Fluorimetric assay is an analytical method with a sensitivity of nearly 100% and specificity of 96%. In other words, it is not as specific as enzyme testing in leukocyte preparations. Therefore, there is always an independent confirmation test, e.g., genetic testing or specific biomarker analysis mandatory.

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
GBA	NM_000157.3:c.1448T>C	p.(Leu483Pro)	N/A	homozygous	PolyPhen: Possibly damaging Align-GVGD: C0 SIFT: Deleterious MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: 0.0012 ESP: - 1000 G: 0.010 CentoMD: 0.011	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
GBA, c.1448T>C p.(Leu483Pro)

The *GBA* variant c.1448T>C p.(Leu483Pro) causes an amino acid change from Leu to Pro at position 483. According to HGMD Professional 2021.3, this variant has been frequently described as disease causing for Gaucher disease, firstly by Tsuji et al., 1987 (PMID: 2880291), Grace et al., 1994 (PMID: 8294487), Zimran et al., 1994 (PMID: 8160756). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Pathogenic variants in the *GBA* gene are associated with autosomal recessive inherited Gaucher disease (GD), resulting from a deficiency or reduced activity of the lysosomal enzyme beta-glucocerebrosidase (GCase) with consecutive accumulation of glucosylceramide (GlcCer) primarily in mononuclear cells (macrophages), forming characteristic “Gaucher cells” infiltrating primarily the bone marrow, spleen, and liver. Very rarely, GD can also be caused by a deficiency in the GCase activator, saposin C (OMIM®: 610539) (Vacaro, 2010; PMID: 20484222). Three major clinical forms have been identified with type 1 GD (non-neuronopathic, OMIM®: 230800) being the most common (90%). Typical symptoms are organomegaly (liver and spleen), anemia, bleeding tendency and increased risk of infections due to cytopenia as well as bone involvement with consecutive bone pain, growth retardation, pathological fractures, and premature osteoarthritis (Stirnemann, 2017; PMID: 28218669). Type 2 GD (acute neuronopathic, OMIM®: 230900) is characterized by early and severe neurological impairment (ophthalmoplegia, psychomotor retardation, epilepsy) starting in infants aged 3–6 months old and by systemic involvement with hepatosplenomegaly. Bone disease is not present in type 2 GD. Death occurs before the third year of life due to aspiration or prolonged apnea (Mignot, 2006; PMID: 16485335).

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Type 3 GD (chronic neuronopathic, OMIM®: 231000) exhibits the visceral manifestations described in GD1, combined with varying neurological signs including oculomotor paresis, progressive myoclonus epilepsy, cerebellar ataxia or spasticity (Tylki-Szymanska, 2010; PMID: 20084461). As additional rare subtypes are sometimes described a) fetal GD ("collodion-baby phenotype", "perinatal-lethal phenotype"; OMIM®: 608013) as the most severe manifestation of type 2 GD with hydrops fetalis, hepatosplenomegaly, ichthyosis, arthrogryposis, facial dysmorphia and death often occurring in utero or soon after birth (Mignot, 2003; PMID: 12838552) and b) type 3C GD (OMIM®: 231005) with cardiac involvement (valve calcification), corneal involvement and hydrocephaly, often associated with a homozygous D409H genotype (Cindik, 2010; PMID: 19816973). Enzyme replacement (GD type 1-3) and substrate reduction therapies (GD type 1) are available for treatment.

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.

SEQUENCE VARIANTS

GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
BRCA1	NM_007300.3:c.5566C>T	p.(Arg1856*)	rs41293465	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: Disease causing Conservation_nt: weak Conservation_aa: N/A	gnomAD: 0.000012 ESP: - 1000 G: 0.000012 CentoMD: 0.000018	Nonsense 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

BRCA1, c.5566C>T p.(Arg1856*)

The *BRCA1* variant c.5566C>T p.(Arg1856*) creates a premature stop codon. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for Breast cancer by Serova et al., 1996 (PMID: 8554067), Cunningham et al., 2014 (PMID: 24504028), Pruss et al., 2014 (PMID: 25085752). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 55601). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).

Familial breast-ovarian cancer type 1, also known as hereditary breast and ovarian cancer syndrome (HBOC) is an autosomal dominant disorder. It is characterized with an increased life time risk for breast cancer (46%-87%), ovarian cancer (39%-63%), prostate cancer (9%), and pancreatic cancer (1%-3%), and possibly also melanoma. Breast cancer is one of the most common forms of cancer, accounting for about 25% of all cancers in women. It is 100 times more common in women than in men, although men tend to have poorer outcomes due to delays in diagnosis. About 5 to 10% of all breast cancers are inherited, and most of them are associated with *BRCA1* and *BRCA2* genes. *BRCA1/BRCA2* germline mutations might also have implications in cancer therapy which should be discussed with the oncologist/gynecologist. Mode of inheritance: autosomal dominant (OMIM®: 604370)

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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***
GJB2	NM_004004.5:c.35del	p.(Gly12Valfs*2)	rs80338939	heterozygous	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: 0.0062 ESP: 0.0074 1000 G: 0.0024 CentoMD: 0.0051	Frameshift 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

CentoGenome MOx 1.0 Solo

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 variations (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. Variants are categorized into five classes (pathogenic, likely pathogenic, VUS, likely benign, and benign) according to ACMG guidelines for classification of variants in addition to ClinGen recommendations. All relevant variants related to the phenotype of the patient are reported. Likely benign and benign variants are not reported. For CentoGenome® MOx, if biochemical analysis is applicable, this is performed upon detection of relevant variants by sequencing in specific genes. This 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. 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. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods. 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. Any detected CNV is only confirmed by an orthogonal method and reported when considered relevant to the phenotype. Screening of uniparental disomy (UPD) 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).

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Targeted nucleotides covered	≥ 10x	98.79%

LIMITATIONS

CentoGenome MOx 1.0 Solo
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. 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. 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 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 UPD detection is a screening method, and therefore false-positive and false-negative results may occur.

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

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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Signature

Chief Genomic Officer	Senior Medical Director	Clinical Scientist

Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com