CENTOGENE
THE RARE DISEASE COMPANY

**CENTOGENE GmbH** • Am Strande 7 • 18055 Rostock • Germany

Dr. Saeed Al Tala
Armed Forces Hospital Southern Region
CMLSO
**Saudi Arabia**

**Order no.:** 63135312
**Order received:** 25 Aug. 2023
**Sample type / Sample collection date:** blood, CentoCard® / 21 Aug. 2023
**Report date:** 14 Sep. 2023
**Report type:** Final report

Patient no: **1835027**, First Name: **Malak Fayez Abdulrahman**, Last Name: **Alshehri**
DOB: **02 May 2023**, Sex: **female**, Your ref.: **3580244737/022300147608**

**Test(s) requested: CentoGenome® MOx 1.0 Solo**

## CLINICAL INFORMATION

Abnormal foot morphology; Abnormal morphology of ulna; Abnormal skeletal morphology; Diarrhea; Long hallux; Metatarsus adductus; Microcephaly; Seizure; Ulnar deviation of the hand or of fingers of the hand
(Clinical information indicated above follows HPO nomenclature.)
Diagnosed Condition(s): ulnar inward deviation.
Family history: Unknown.
Consanguineous parents: Unknown.
Clinician suspects: Omenn syndrome.

NEGATIVE RESULT

## INTERPRETATION

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

## RECOMMENDATIONS

- Reevaluation of the sequence dataset is recommended every 12 months or if there are phenotypic changes.
- Genetic counselling is recommended.

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



CAP ACCREDITED ISO 15189 CAP ACCREDITED

## 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 FINDINGS

If consent is provided, in line with ACMG recommendations (ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing; Genetics in Medicine, 2023; PMID: 37347242) we report secondary findings, i.e. relevant pathogenic and likely pathogenic variants in the recommended genes for the indicated phenotypes in this publication.

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 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.

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

| SEQUENCE VARIANTS | | | | | | | |
|---|---|---|---|---|---|---|---|
| GENE | VARIANT COORDINATES | AMINO ACID CHANGE | SNP IDENTIFIER | ZYGOSITY | IN SILICO PARAMETERS* | ALLELE FREQUENCIES ** | TYPE AND CLASSIFICATION *** |
| FECH | NM_001012515.2:c.333-48T>C | p.(?) | rs2272783 | Heterozygous | PolyPhen: - Align-GVDG: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A | gnomAD: 0.11 ESP: 0.037 1000 G: 0.14 CentoMD: 0.064 | Effect unknown 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).

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

| SEQUENCE VARIANTS | | | | | | | |
|---|---|---|---|---|---|---|---|
| GENE | VARIANT COORDINATES | AMINO ACID CHANGE | SNP IDENTIFIER | ZYGOSITY | IN SILICO PARAMETERS* | ALLELE FREQUENCIES ** | TYPE AND CLASSIFICATION *** |
| FECH | NM_001012515.2:c.333-48T>C | p.(?) | rs2272783 | Heterozygous | PolyPhen: - Align-GVDG: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A | gnomAD: 0.11 ESP: 0.037 1000 G: 0.14 CentoMD: 0.064 | Effect unknown 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).

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

## 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).

## ANALYSIS STATISTICS

**CentoGenome® MOx 1.0 Solo**

| | | |
|---|---|---|
| Targeted nucleotides covered | ≥ 10x | 99.03% |

**> Contact Details**

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

## 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.

## COPYRIGHT NOTICE



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

**Prof Dr Peter Bauer, MD**
Chief Medical and Genomic Officer
Human Geneticist

**Dr Uros Hladnik, MD**
Human Geneticist

**Dr Moneeb Othman, PhD**
Clinical Scientist

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