CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type: DNA
Sample collection date: xxx
Report type: Final Report
Report date: xxx

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

Test(s) requested: Whole Genome Sequencing (CentoGenome®)

CLINICAL INFORMATION

The patient is presenting since the age of 6 years with a clinical picture of myopathy, progressing and mainly in lower limbs. EMG suggestive of myopathy. Muscle biopsy resulted normal. Normal level of CPK. Parents are consanguineous and healthy. They have two other sons who are healthy and history of an abortion.

NO GENETIC DIAGNOSIS

INTERPRETATION

No clinically relevant variant to the described phenotype has been detected.

No variants with potential relevance for the patient's phenotype have been detected in research genes.

RECOMMENDATIONS

- Genetic counselling is recommended.

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RESULT SUMMARY

By whole exome sequencing, we have not detected any variant clinically relevant to the described phenotype of the patient. Please see below for the list of specifically analyzed genes. We did not detect any relevant variant in these genes. However, pathogenic variants cannot be completely excluded since not all exons were fully covered due to limitations of the method. For these genes, an overall coverage of 98.72% was achieved (>20x), with 3274 missing base pairs (coding region including +/- 2bp). Note that whole exome sequencing for diagnostic purposes does not provide full coverage for all genes and cannot detect large deletions/duplications. If needed, it is possible to test for every single gene that might likely explain the given phenotype.

Myopathy-related genes:
ACTA1, BIN1, CCDC78, CFL2, CNTN1, DNM2, FHL1, KBTBD13, MAMLD1, MTM1, MTMR14, MYF6, MYH7, NEB, RYR1, SELENON, TNNT1, TPM2, TPM3, ABHD5, ACADVL, AGL, CPT2, ENO3, ETFA, ETFB, ETFDH, GAA, GBE1, GYG1, GYS1, LDHA, LPIN1, PFKM, PGAM2, PGK1, PGM1, PHKA1, PNPLA2, PRKAG2, PYGM, SLC22A5, SLC25A20, TAZ, B3GALNT2, B4GAT1, DAG1, FKRP, FKTN, GMPPB, ISPD, LARGE, POMGNT1, POMGNT2, POMK, POMT1, POMT2, TMEM5, BAG3, CRYAB, DES, DNAJB6, FHL1, FLNC, LDB3, MYOT, ACAD9, ACADM, ACADVL, AGL, AMPD1, CPT2, ETFA, ETFB, GAA, GYS1, HADHA, HADHB, LPIN1, OPA1, OPA3, PFKM, PGAM2, PGM1, PHKA1, POLG, POLG2, PYGM, RRM2B, SUCLA2, TK2, TWNK, TYMP, ACTA1, CFL2, KBTBD13, KLHL40, KLHL41, LMOD3, NEB, TNNT1, TPM2, TPM3, ATP2A1, CACNA1S, CAV3, CLCN1, HINT1, HSPG2, KCNA1, KCNE3, SCN4A.

RESEARCH VARIANTS

Research variants with potential relevance to the described phenotype: Research variants, which were detected in genes with no or partial experimental evidence on their involvement in human disease, were analyzed.
Whole genome sequencing 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/functional importance of the gene. Available literature or experimental data on expression/animal models were considered.
We also searched for regions of homozygosity and evaluated the genes and variants with respect to a possible so far undescribed involvement in human diseases corresponding to your patient.
However, no such variants could be identified for the patient.

INCIDENTAL FINDINGS

Incidental findings which we list according to the ACMG guidelines are not provided here due to the lack of consent.

ANALYSIS STATISTICS WGS

AVERAGE COVERAGE (X)	% TARGET BP COVERED					
	0X	≥ 1X	≥ 5X	≥ 10X	≥ 20X	≥ 50X
36.0034	0.29975	99.7002	99.4413	98.8964	93.7397	5.50792

Additionally, other types of clinical relevant variants can be identified (e.g. risk factors, modifiers).

METHODS

Genomic DNA was fragmented by sonication and Illumina adapters were ligated to generated fragments for subsequent sequencing on the HiSeqX platform (Illumina) to yield an average coverage depth of ~30X. An end to end in-house bioinformatics pipeline including base calling, primary filtering of low quality reads and probable artefacts, and annotation of variants was applied. CNV calling is based on HAS pipeline. All disease causing variants reported in HGMD®, in ClinVar or in CentoMD® in addition to all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. Evaluation is focused on coding exons along with flanking +/-20 intronic bases, however extended to the complete gene region for candidate genes or in search for a second previously described variant in AR inheritance pattern. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized in classes 1 - 5. All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. CNVs of unknown significance are not reported. Reported CNVs are confirmed with another method such as MLPA and qPCR.
Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet our internal QC criteria (based on extensive validation processes) are not validated by Sanger.

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LIMITATIONS

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered.
Due to limited read length and other contributing technical limitations, repeat expansions (i.e. in the Huntington gene, the SCA-genes, the myotonic dystrophy repeat region, and other similar regions) cannot be assessed with the applied method. Of note, CNV calls from Whole Genome Sequencing have a limited accuracy and sensitivity, and structural changes below 2 kb at a genome-wide level are not called by our pipeline.

ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. 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.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

To also 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 (dmqc@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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