CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

XXX

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

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

Test(s) requested: CentoScreen™ panel

CLINICAL INFORMATION

The proband is unaffected. Family history: a child with SMA (deceased); consanguinity.

CARRIER STATUS
Pathogenic variant identified

INTERPRETATION

We detected a heterozygous deletion encompassing exon 7 of the SMN1 gene. Deletion of SMN1 is associated with autosomal recessive spinal muscular atrophy (SMA).

Should the proband's partner also be a carrier of a clinically relevant variant in the SMN1 gene, the risk for their offspring of being affected is 25%. Please note that the recurrence risks deviate slightly from the norm for autosomal recessive inheritance because about 2% of affected individuals have a de novo SMN1 variant on one allele (PMID: 20301526).

We did not detect any pathogenic or likely pathogenic variant by sequencing of the CentoScreen™ panel genes, including CYP21A2.

We did not detect any copy number variation (CNV) in the panel genes (see limitations) by analyzing the NGS data.

We did not detect an expanded allele in the FMR1 gene by repeat expansion analysis. We detected a normal heterozygous allele – please, see the table below. CGG repeats in this patient were found to be interrupted by AGG repeats. In stable normal alleles these AGG triplets are thought to anchor the region during replication and prevent strand slippage (Monaghan et al., 2013 - PMID: 23765048). FMR1-related disorders are inherited in an X-linked dominant manner. Alleles in the normal range have no meiotic or mitotic instability and are transmitted without any increase or decrease in repeat number (Saul and Tarleton, 2012 - PMID: 20301558).

RECOMMENDATIONS

- For a better assessment of the risk to have another child with SMA, deletion/duplication and if negative, sequencing of the SMN1 gene needs to be performed for the proband's partner.
- Genetic counselling is recommended.

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RESULT SUMMARY

GENE (TRANSCRIPT, METHOD)	OUTCOME
SMN1 (NM_000344.3; dPCR)	heterozygous deletion encompassing exon 7

VARIANT INTERPRETATION

We detected a deletion encompassing exon 7 of the SMN1 gene. Similar deletions as well as deletions of the entire SMN1 gene have been described as disease-causing for spinal muscular atrophy by several authors (HGMD Professional 2017.4). The deletion is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the SMN1 gene are associated with spinal muscular atrophy types 1 to 4, which are inherited in an autosomal recessive manner. Spinal muscular atrophy (SMA) is characterized by progressive muscle weakness resulting from degeneration and loss of the anterior horn cells (i.e., lower motor neurons) in the spinal cord and the brain stem nuclei (Prior and Finanger, 2016 - PMID: 20301526).

ANALYSIS STATISTICS

An overall coverage of 99.97% was achieved (coding region including +/- 10bp).

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

METHODS

Custom RNA capture baits against 331 panel genes (covering >99% of regions in CCDS and sequence variants including known deep intronic pathogenic variants, splicing, regulatory, any known mutation in CentoMD® 4.0 or HGMD® Professional 2017.3) are used to enrich regions of interest from fragmented genomic DNA. The generated library is sequenced on an Illumina HiSeq 4000 platform. Typically, over 99% of the targeted bases are covered >20x. An end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low quality reads and probable artefacts, and subsequent annotation of variants, is applied. All disease causing variants reported in HGMD®, in ClinVar or in CentoMD® as well as 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 and all identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized into classes 1 - 5 (please see https://www.centogene.com/genetic-testing/reporting-at-centogene.html). Only Class 1 and Class 2 variants along with few selected risk factor variants are reported. 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.
An in-house developed pipeline was used to determine copy number variations (CNV) within the panel genes (see limitations). The approach utilizes CNV calling based on coverage information of a particular target region when compared to a normalized coverage of control reference data set. Each target region was analyzed as a 100-300bp non-overlapping genomic window. To eliminate variations or noise due to genomic complexity, regions of confidence are selectively probed to eliminate false positives. Fold change values were calculated for each target window against normalized mean of all reference samples. Only regions showing ratio off-set of >2 SD from all genes in the sample were considered as a candidate for CNV. All CNVs were confirmed by an orthogonal method (MLPA or qPCR) before reporting. This approach offers a sensitivity of ~90% for detecting CNVs.
Four locus-specific oligonucleotides were used (according to Keen-Kim et al., 2005) that hybridize upstream and downstream of either the CYP21A2 gene or its pseudogene CYP21A1P. The four primers were combined with each other to obtain 4 different amplicons corresponding to: the normal copy of the gene, pseudogene, gene/pseudogene rearrangement product and the deletion/conversion product. The product of the normal copy of the gene was analyzed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence of the CYP21A2 gene is: NM_000500.7.
The copy number of SMN1 exon 7 was quantified using digital droplet PCR. Quantification is based on the assumption that molecules are distributed uniformly in partitions and have a Poisson distribution. The reference sequence is NM_000344.3.

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GENE	PHENOTYPE (OMIM®)	INHRT.	RESULTS OF REPEAT EXPANSION ANALYSIS	NORMAL	MUTABLE NORMAL (INTERMEDIATE)	PREMUTATION	PATHOGENIC WITH FULL PENETRANCE	REFERENCE
FMR1	FMR1-related (309550)	X-linked	**Allele 1: 29±1 repeats** **Allele 2: 30±1 repeats**	<45 CGG repeats	45-54 CGG repeats	55-200 CGG repeats	>200 CGG repeats	Saul et al., 2012 (PMID: 20301558)

The FMR1 gene was analysed using the AmplideXTM FMR1 PCR Kit to screen the trinucleotide repeat region in the promoter. The reference sequence of the FMR1 gene is: NM_002024.5.

LIMITATIONS

CentoScreen® is a screening test designed to assess the risk for the proband's offspring to be affected with an autosomal recessive or X-linked recessive disorder. It is not intended to establish a genetic diagnosis for the proband – unaffected or affected. However, the test result may include information about a medical condition of the proband that requires medical follow-up. Please note that a negative result for this panel does not rule out the possibility of a genetic condition in the proband, the proband's partner and/or their offspring. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical or family history, additional testing should be considered.
CentoScreen® panel focuses on 331 genes (list available at www.centogene.com) related to frequently occurring disorders within the population. Pathogenic or likely pathogenic variants outside the panel genes will not be detected. Variants of uncertain significance within the targeted region are not reported. Please note that they may become better understood and reclassified over time.
Copy number variations (CNVs) assessment with NGS is limited to 34 genes (ABCC6, ALDH3A2, COL4A5, CTNS, DBT, DMD, EDA, F8, FANCA, FKTN, GAA, GALC, GBE1, GJB6, GLDC, HBA1, HBA2, HBB, HEXB, HPRT1, HPS3, HSD17B4, IDS, MCOLN1, NEB, OTC, PAH, PCCA, PCDH15, PDHA1, RAPSN, SGCB, STS and XPC) within the Panel. Any CNVs lying outside the coding regions of these genes will not be reported.
Specific genetic events like translocations and repeat expansions may not be reliably detected with Next Generation Sequencing. Recombination of GBA with its pseudogene and inversion of Intron 1 and Intron 22 within F8 gene is not directly assessed and hence may be missed. Analysis of variants lying within repetitive regions of NEB and TTN may have limitations when only sequenced with NGS. In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected.
Repeat Expansion testing may not be able to detect exact number of repeats beyond 200. Mosaic expansions may be missed.
Deletions other than exon 7 in SMN1 are not covered within the assay.

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.
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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