CENTOGENE THE RARE DISEASE COMPANY

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BioGene
Clinical Genetics
Cairo
Egypt

Order no.: 63001065
Order received: 10 Jul. 2022
Sample type / Sample collection date: blood, EDTA / 25 May 2022
Report date: 19 Aug. 2022
Report type: Final Report

Patient no.: **1699028**, First Name: **Seliem**, Last Name: **Mohamed Atef**
DOB: **12 Dec. 2020**, Sex: **male**, Your ref.: **NHS-Cairo**

Test(s) requested: sequence analysis of *SMN1* gene (OMIM®: 600354)

CLINICAL INFORMATION

Delayed ability to roll over; Fasciculations; Hand tremor; Hyporeflexia; Hypotonia; Limb muscle weakness; Motor delay
(Clinical information indicated above follows HPO nomenclature.)

Previous CENTOGENE testing, results negative: CentoXome Solo (Order 62984961). Previous CENTOGENE testing, results positive: SMN1, SMN2 genes (deletion/duplication) (1 copy detected of SMN1, 3 copies detected of SMN2, Order 62978555).

Family history: Unknown.
Consanguineous parents: No.

Please note the exon numbering for the *SMN*1 gene that is used throughout MLPA analysis (Product: P021-B1 SMA) product description is based on the classic exon numbering as used in most scientific literature: exons 1, 2a, 2b, 3-8. In contrast, the exon numbering that is used in this sanger analysis, NM_000344.3 reference sequence (LRG_676t1), counts the exons 1-9. **Thus, exon 7 in MLPA analysis corresponds to exon 8 in sanger sequencing analysis.**

POTENTIALLY RELEVANT RESULT

INTERPRETATION

A pathogenic variant was identified in the *SMN1* gene or *SMN2* gene by sanger sequencing. The variant appears as a hemizygous *SMN1* variant by gene reference sequence. By MLPA analysis, a single copy of the *SMN1* gene was identified and three copies of the *SMN2* gene (please refer to our previous report for MLPA analysis for *SMN1/SMN2* genes, Order ID: 62978555 on 20 May 2022). **Considering also the patient's phenotype, this finding is consistent with a genetic diagnosis of autosomal recessive *SMN1*-associated spinal muscular atrophy**. Further testing is necessary to definitely confirm if the variant is localized in the *SMN1* or in the *SMN2* gene.

RECOMMENDATIONS

- Parental targeted testing is recommended to confirm the phase (*cis* or *trans*) of the reported variants (SNV and one copy loss of exon 7, in MLPA analysis, of *SMN1*). If in *trans*, this will support the SMA diagnosis. Additionally, targeted testing for affected family members, if any, and familial cascade carrier testing are recommended. Segregation of the SNV with the disease in the family would further support its localization in *SMN1*.
- Proceeding to an allele-specific mRNA study (AS-RT-PCR) (PMID: 32169315) to distinguish if the

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detected variant localizes on *SMN1* or *SMN2* could be considered. Please be informed that we do not offer this specific test.
- Genetic counselling is recommended.

RESULT SUMMARY

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
SMN1/SMN2	NM_000344.3:c.735dup	p.(Pro246Thrfs*10)	N/A	Hemizygous (?)	PolyPhen: - Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	gnomAD: - ESP: - 1000 G: - CentoMD: -	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.

VARIANT INTERPRETATION
SMN1/SMN2, c.735dup p.(Pro246Thrfs*10)

The *SMN1/SMN2* variant c.735dup p.(Pro246Thrfs*10) creates a shift in the reading frame starting at codon 246. The new reading frame ends in a stop codon 9 positions downstream. According to HGMD Professional 2022.1, this variant has previously been described as disease causing for Spinal muscular atrophy by Sharifi et al., 2021 (PMID: 33481221). It is classified as pathogenic (class 1) according to the recommendations of CENTOGENE and ACMG (please, see additional information below).
The variant was detected by sanger sequencing. Initially, long range PCR using a primer which binds specifically to *SMN1* gene (based on RefSeq sequence) was used, subsequently the product was used for amplification and sequencing analysis. However, *SMN1* and *SMN2* share more than 99% nucleotide identity and the SMN region (chr5q13) is highly variable leading to frequent deletions, duplications and gene conversions (PMID: 20301526, 9950358). Due to this and to limitations of the methods used, it is not possible to discriminate with certainty whether the variant localizes in the *SMN1* or *SMN2* gene.

Pathogenic variants (predominantly homozygous deletions) in the *SMN1* gene are associated with autosomal recessive inherited spinal muscular atrophy types 1-4 (5q-SMA; OMIM®: 253300, 253500, 253400, 271150) resulting in insufficient levels of survival of motor neuron (SMN) protein in motor neurons with consecutive degeneration of alfa-motor neurons in the anterior horn of the spinal cord or in some cases, motor nuclei in the brain stem. The SMA-types differ by means of symptom onset and rate of progression. Type 1 (syn.: Werdnig-Hoffmann disease) is the most severe form, having onset in utero, muscular paresis and hypotonia (“Floppy baby”), progressive muscle wasting and feeding difficulties. Affected children have normal intelligence but are unable to sit unaided and die from respiratory failure or aspiration before the age of 2 years. Type 2 is of intermediate severity. Patients are able to sit unsupported, but cannot stand or walk unaided. Survival depends on the degree of respiratory muscle involvement but is usually greater than 4 years. Type 3 (syn.: Kugelberg-Welander disease) has an onset after the age of two and patients are able to walk unaided (shuffling gait, frequent falls) having difficulty with stairs. Slow deterioration results in scoliosis and wheel chair dependence. Longevity can extend well into middle adult life. Type 4 onset in patients older than 30 years showing mild to moderate muscle weakness in upper arms and legs with minor breathing problems. An additional phenotype (type 0) describes disease courses with prenatal death. The severity of the SMA phenotype is modified by the copy number of SMN2 gene, producing some amount of full-length SMN proteins. The less SMN2 copies are present the more severe is the phenotype of SMA (Srivastava, 2019; PMID: 31271088). Two modern therapies are available with SMN1 gene replacement (recombinant AAV9 vector encoding full-length SMN1 protein) and SMN2 splicing modification (antisense oligonucleotide approach with increase in SMN protein synthesis), respectively. (OMIM®: 253300).

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 clinical relevant variants can be identified (e.g. risk factors, modifiers).

METHODS

The SMN1 gene was analysed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence is / sequences are: SMN1: NM_000344.3.

LIMITATIONS

The genetic results are interpreted in the context of the provided clinical findings, family history, and other laboratory data. Misinterpretation of results may occur, if the provided information is inaccurate and/or incomplete. If the obtained genetic results do not concur with the clinical findings, additional testing should be considered.
Allele drop-out cannot be excluded by the used method; polymorphic/normal genomic variation in the patient sample may interfere with variant detection.
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 will be reported.

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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