CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

Dr. Name
Example hospital

Example city
Example country

Order no.: xxxxxxx
Order received: xxxxxx
Sample type: xxxxxx
Sample collection date: xxxxxx
Report type: xxxxxxx
Report date: xxxxxxxx

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

Test(s) requested: Whole Exome Sequencing (CentoXome GOLD®)

The requested test is clearly pointed out

CLINICAL INFORMATION*:

Patient presents abnormality of the urinary system, renal tubular dysfunction, m
hypokalemia, dehydration

*: *Clinical information indicated above follows HPO nomenclature*

This paragraph describes the patient's clinical symptomatology following the HPO nomenclature. More Information concerning HPO nomenclature to be found here: http://human-phenotype-ontology.github.io/

POSITIVE RESULT
Pathogenic or likely pathogenic variant(s) identified

The main assertion is indicated here

INTERPRETATION

A homozygous likely pathogenic variant was identified in SLC12A1 gene. This result is cons
genetic diagnosis of Bartter syndrome, inherited in autosomal recessive manner.

The final genetic diagnosis is provided in the Interpretation (blue highlighted) section

RECOMMENDATIONS

- Parental carrier testing is recommended to confirm homozygosity of the detected variant.
- Genetic counseling is recommended.

In this section, specific recommendations are given

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

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Patient no.: 1245194
Order no.: 62424957

RESULT SUMMARY:

The clinically relevant variants are represented in the table format for clear visualization

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCI	
SLC12A1	Chr15(GRCh37):g.48580645dup NM_000338.2:c.2805dup p.(Trp936Metfs*5) Exon23	Hom	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A	gnomAD: - ESP: - 1000 G: - CentoMD: -	Frameshift Likely pathogenic (class 2)

Variant description based on Alamut Batch (latest database available). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

VARIANT INTERPRETATION

For every clinically relevant variant indicated in the result summary table a detailed variant description and related disease characterization are indicated

SLC12A1; c.2805dup, p.(Trp936Metfs*5)

The SLC12A1 variant c.2805dup p.(Trp936Metfs*5) creates a shift in the reading frame starting at codon 936. The new reading frame ends in a stop codon 4 positions downstream. This variant has been confirmed by Sanger sequencing. It is classified as likely pathogenic (class 2) according to the recommendations of Centogene and ACMG (please, see additional information below).

Pathogenic variants in the SLC12A1 gene are associated with Bartter syndrome type 1, which is an autosomal recessive disorder. Bartter syndrome refers to a group of disorders resulting from impaired salt reabsorption in the thick ascending loop of Henle with pronounced salt wasting, hypokalemic metabolic alkalosis, and hypercalciuria. Patients with antenatal forms of Bartter syndrome typically present with premature birth associated with polyhydramnios and low birth weight and may develop life-threatening dehydration in the neonatal period. Patients with classic Bartter syndrome present later in life and may be sporadically asymptomatic or mildly symptomatic (PMID: 9326936 and 22282380).

ANALYSIS STATISTICS WES

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AVERAGE COVERAGE (X)	% TARGET BP COVERED					
	0X	≥ 1X	≥ 5X	≥ 10X	≥ 20X	≥ 50X
89.65	0.13	99.87	99.30	97.83	92.08	64.72

CENTOGENE VARIANT CLASSIFICATION (BASED ON ACMG RECOMMENDATIO

Classification of germline genetic variants according to ACMG guidelines

Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign

METHODS

This paragraph describes the methodology used by Centogene for the requested test

RNA capture baits against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCD is used to enrich regions of interest from fragmented genomic DNA with Agilent's SureSelect Human All sequenced on an Illumina platform to obtain an average coverage depth of ~100x. Typically, ~97% of t
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. 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 into classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported.
Variants of relevance identified by NGS are continuously and individually in-house validated for quality aspects; those variants which meet

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our internal QC criteria (based on extensive validation processes) are not validated by Sanger.
Exon 23 of the SLC12A1 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: SLC12A1: NM_000338.2, NM_001184832.1.

LIMITATIONS

This paragraph describes the limitations of the requested test

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only v
related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or pos
of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match
testing should be considered.
Specific genetic events like copy number variants, translocations and repeat expansions may not be reliably detected with Exome Sequencing. 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.

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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Signatures:

Chief Medical Director

Clinical Scientist

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