CENTOGENE THE RARE DISEASE COMPANY

CENTOGENE AG Am Strande 7 • 18055 Rostock • Germany

XXX

Order no.: xxx
Order received: xxx
Sample type: blood, filter card
Sample collection date: xxx
Report type: Final Report
Report date: xxx

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

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

CLINICAL INFORMATION*

Abnormality of cardiovascular system morphology, Atrioventricular canal defect, C1-C2 vertebral abnormality, Cleft palate, Webbed neck
*: Clinical information indicated above follows HPO nomenclature.

NO GENETIC DIAGNOSIS

INTERPRETATION

Regarding the phenotype of the patient no clinically relevant variant has been detected. Also no research variant with potential relevance for the phenotype has been identified.

RECOMMENDATIONS

- Proceeding to whole genome sequencing with an additional 15-18% clarification rate compared to whole exome sequencing is recommended.
- Genetic counseling is also recommended.

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RESULT SUMMARY

By whole exome sequencing, we did not detect any variant clinically relevant to the described phenotype of the patient. We paid specific attention to genes known to be associated with Noonan – CFC syndrome or Klippel-Feil syndrome. 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 90.25% was achieved (>20x), with 5581 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.

Genes associated with Noonan – CFC syndrome or Klippel-Feil syndrome:
BRAF, CBL, GDF3, GDF6, HRAS, KAT6B, KRAS, LZTR1, MAP2K1, MAP2K2, MEOX1, MYO18B, NF1, NRAS, PTPN11, RAF1, RASA2, RIT1, SHOC2, SOS1, SOS2, SPRED1

RESEARCH VARIANTS

Research variants with potential relevance to the described phenotype are detected in genes with no or partial experimental evidence on their involvement in human disease. Whole exome 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.

ANALYSIS STATISTICS WES

AVERAGE COVERAGE (X)	% TARGET BP COVERED					
	0X	≥ 1X	≥ 5X	≥ 10X	≥ 20X	≥ 50X
115.14	0.32	99.68	99.17	98.15	94.33	73.64

METHODS

RNA capture baits against approximately 60 Mb of the Human Exome (targeting >99% of regions in CCDS, RefSeq and Gencode databases) is used to enrich regions of interest from fragmented genomic DNA with Agilent's SureSelect Human All Exon V6 kit. The generated library is sequenced on an Illumina platform to obtain an average coverage depth of ~100x. Typically, ~97% of the targeted bases are covered >10x. 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.
Centogene has established stringent quality criteria and validation processes for variants detected by NGS. Lower quality single nucleotide or deletion insertion variants are thus being confirmed by Sanger. As a result of this we warrant specificity of >99.9% for all reported variants.

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.
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.

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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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