Dr. (Physician)
Institution
Address
Country

Order no.:
Order received: DD-MM-YYYY
Sample type / Sample collection date:
blood, CentoCard® / DD-MM-YYYY
Report date: DD-MM-YYYY
Report type: Final Report

Patient no.:, First Name: , Last Name:
DOB: **DD-MM-YYYY**, Sex: **male**, Your ref.:

Test(s) requested: CentoCancer® (sequencing and NGS-based CNV analyses)

CLINICAL INFORMATION

Prostate cancer
(Clinical information indicated above follows HPO nomenclature.)

Family history: No.
Consanguineous parents: No.

POSITIVE RESULT
Pathogenic variant identified

INTERPRETATION

A heterozygous pathogenic variant was identified in the MLH1 gene. **This result is compatible with the genetic diagnosis of autosomal dominant Lynch syndrome.**

Heterozygotes have a 50% risk of transmitting the variant to each offspring. In case of transmission the offspring will have an increased genetic risk of developing malignancies associated with this gene.

No further clinically relevant variants were detected.

RECOMMENDATIONS

- Oncology evaluation and follow-up for an optimal management of the cancer risk are recommended.
- Targeted testing for affected family members is recommended. For adult at-risk relatives, genetic counselling should be offered, and predictive testing is available.
- Genetic counselling is recommended

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

MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
MLH1	NM_000249.2:c.2195_2198dup	p.(His733Glnfs*14)	rs267607899	heterozygous	PolyPhen: N/A 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 CentoCloud Bioinformatics pipeline. * 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

MLH1, c.2195_2198dup p.(His733Glnfs*14)

The *MLH1* variant c.2195_2198dup p.(His733Glnfs*14) creates a shift in the reading frame starting at codon 733 in exon(s) no. 19 (of 19). According to HGMD Professional 2023.3, this variant has previously been described as disease causing for Lynch syndrome (PMID:8646682, 12658575, 15217520, 15849733, 19459153). ClinVar lists this variant (Interpretation: Pathogenic; Variation ID: 90088). It is classified as pathogenic according to the recommendations of CENTOGENE and ACMG/AMP ClinGen SVI general recommendations (please, see additional information below).

Lynch syndrome, also known as Hereditary Nonpolyposis Colon Cancer (HNPCC), is characterized by an increased risk for colorectal cancer (CRC) and cancers of the endometrium, ovary, stomach, small bowel, urinary tract, biliary tract, brain (usually glioblastoma), skin (sebaceous adenomas, sebaceous carcinomas and keratoacanthomas), pancreas, and prostate. Cancer risks and age of onset vary depending on the associated gene. Several other cancer types have been reported to occur in individuals with Lynch syndrome (e.g., breast, sarcomas, adrenocortical carcinoma). However, the data are not sufficient to demonstrate that the risk of developing these cancers is increased in individuals with Lynch syndrome (PMID: 20301390). Mode of inheritance: autosomal dominant (OMIM®: 120435)

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

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Genomic DNA is enzymatically fragmented, and regions of interest are enriched using DNA capture probes. The final indexed libraries are sequenced on an Illumina platform. For the CentoCancer®, the coding regions of the panel genes, 10 bp of flanking intronic sequences, and known pathogenic/likely pathogenic variants within these genes included in the enrichment design (coding and non-coding), are targeted for analysis. The panel gene list can be obtained in the appendix of this report. Data analysis, including alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37), variant calling and annotation is performed using validated in-house software. All identified variants are evaluated with respect to their pathogenicity and disease causality, and are categorized into five classes (pathogenic, likely pathogenic, variant of uncertain significance [VUS], likely benign, and benign) according to ACMG/AMP guidelines for classification of variants in addition to ClinGen recommendations. All potentially clinically relevant variants that may explain or contribute to the phenotype are reported. VUSs are usually not reported in the following cases: the described phenotype is already explained by detected pathogenic or likely pathogenic variants, the detected VUSs are not considered to be related to the described phenotype, there is a lack of clinical information, and/or the individual is asymptomatic or unaffected. CENTOGENE has established stringent quality criteria and validation processes for variants detected by NGS. Variants with low sequencing quality and/or unclear zygosity are confirmed by orthogonal methods. Consequently, a specificity of >

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99.9% for all reported variants is warranted. The copy number variation (CNV) detection software has a sensitivity of more than 95%.

ANALYSIS STATISTICS

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Targeted nucleotides covered	≥ 20x	99.80%

LIMITATIONS

CentoCancer®
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 genetic data or patient information is inaccurate and/or incomplete. If the obtained genetic results are not compatible with the clinical findings, additional testing should be considered.
More complex genetic events such as inversions, translocations, and repeat expansions, are not analyzed in this test. In addition, due to technology limitations, certain regions may be poorly covered, or not covered at all. In these regions and others encompassing repetitive, high-homology (such as pseudogene homology), and GC-rich sequences, relevant variants can be missed. Extremely low-coverage calls (homo/hemizygous or heterozygous calls with less than three or four reads, respectively) are expected to be artifacts based on our extensive validations and are consequently not considered during the analysis. The CNV detection sensitivity is decreased for repetitive and homologous regions such as pseudogenes, as well as for events spanning two or less exons. It is expected that lower quality samples (prenatal, product of conception, blood from patients with hematologic disorders, and highly degraded DNA) may generate lower quality NGS data; in these cases, CNV analysis may not be possible to perform. Potential aberrant splicing is assessed with splice prediction tools. Intronic variants that are beyond 10 nucleotides from exon-intron boundaries are not considered for aberrant splicing analysis, with the exception of known pathogenic splicing variants evidenced by external sources.

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

Chief Medical and Genomic Officer Human Geneticist	Human Geneticist	Clinical Scientist

> Contact Details
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Fax: +49 (0)381 80113 401
customer.support@centogene.com
www.centogene.com

APPENDIX

CentoCancer®

APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, DIS3L2, EPCAM, FANCC, FH, FLCN, GALNT12, HOXB13, KIT, MC1R, MEN1, MET, MITF, MLH1, MLH3, MRE11, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, POT1, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RECQL, RET, RNF43, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL, WT1, XRCC2, XRCC3

> Contact Details
Tel.: +49 (0)381 80113 416
Fax: +49 (0)381 80113 401
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