CENTOGENE
THE RARE DISEASE COMPANY

CENTOGENE GmbH • Am Strande 7 • 18055 Rostock • Germany

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: **female**, Your ref.:

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

CLINICAL INFORMATION

Atrial septal defect; Cardiomyopathy; Left atrial enlargement; Mitral regurgitation; Noncompaction cardiomyopathy; Ventricular septal defect
(Clinical information indicated above follows HPO nomenclature.)

Family history: No.
Consanguineous parents: Yes.

POSITIVE RESULT
Likely pathogenic variant identified

INTERPRETATION

A heterozygous likely pathogenic variant was identified in the *MYBPC3* gene. **This result is consistent with the genetic diagnosis of autosomal dominant familial hypertrophic cardiomyopathy type 4.**

With each pregnancy there is a 50% risk that the variant will be transmitted to the offspring.

No further clinically relevant variants were detected.

RECOMMENDATIONS

- If possible, parental targeted testing is recommended as establishing the origin of the variant, inherited or *de novo*, is important for familial genetic counselling. Additionally, targeted testing for all affected and at-risk family members, if any, is recommended.
- Genetic counselling, including reproductive counselling (discussing prenatal and preimplantation diagnoses, if relevant), is recommended.

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MAIN FINDINGS

SEQUENCE VARIANTS							
GENE	VARIANT COORDINATES	AMINO ACID CHANGE	SNP IDENTIFIER	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
MYBPC3	NM_000256.3:c.3713T>C	p.(Leu1238Pro)	rs730880702	homozygous	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: - MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high	gnomAD: - ESP: - 1000 G: - CentoMD: -	Missense Likely Pathogenic (class 2)

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

MYBPC3, c.3713T>C p.(Leu1238Pro)

The *MYBPC3* variant c.3713T>C p.(Leu1238Pro) causes an amino acid change from Leu to Pro at position 1238. According to HGMD Professional 2021.3, this variant has previously been described as disease causing for Cardiomyopathy, hypertrophic by Choi et al., 2010 (PMID: 20641121), Walsh et al., 2017 (PMID: 27532257). ClinVar lists this variant (Interpretation: Likely pathogenic; Variation ID: 181136). 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 *MYBPC3* gene are associated with familial hypertrophic cardiomyopathy type 4 (OMIM®: 115197) with an autosomal dominant pattern of inheritance. *MYBPC3* has also been shown to be associated with an autosomal recessive pattern of inheritance, with earlier and more severe presentation of hypertrophic cardiomyopathy phenotypes and represents a semi-dominant condition.
Familial hypertrophic cardiomyopathy type 4 (HCM) is typically defined by the presence of unexplained left ventricular hypertrophy (LVH). The clinical manifestations of HCM range from asymptomatic LVH to progressive heart failure to sudden cardiac death. The disease may show reduced penetrance and variable expressivity, thus varying from individual to individual even within the same family (PMID: 20301725). Mode of Inheritance: Autosomal dominant (OMIM®: 115197).

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 CentoCardio®, 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) as well as the mitochondrial genome, 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

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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 > 99.9% for all reported variants is warranted. Mitochondrial variants are reported for heteroplasmy levels of 15% or higher. The copy number variation (CNV) detection software has a sensitivity of more than 95%.

ANALYSIS STATISTICS

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Targeted nucleotides covered	≥ 20x	99.70%

LIMITATIONS

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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. Mitochondrial variants with heteroplasmy levels below 15% may not be detected. 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 and/or mitochondrial genome 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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APPENDIX

CentoCardio®

ABCC9, ACTA1, ACTA2, ACTC1, ACTN2, ACVR2B, ACVRL1, ADAMTS10, ADAMTS19, AGL, AKAP9, ALPK3, ANK2, ANKRD1, ANKS6, ARHGAP31, ATM, B3GAT3, BAG3, BCOR, BMPR2, BRAF, CACNA1C, CACNA2D1, CACNB2, CALM1, CALM2, CALM3, CASQ2, CAV3, CAVIN4, CBL, CCDC103, CCDC39, CCDC40, CDH2, CFAP298, CFAP300, CFAP53, CHD7, CITED2, CLDN16, CLDN19, CNNM2, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A1, COL5A2, COX15, CPT2, CREBBP, CRELD1, CRYAB, CSRP3, CTNNA3, DES, DLL4, DMD, DNAAF1, DNAAF11, DNAAF2, DNAAF3, DNAAF4, DNAAF5, DNAAF6, DNAH11, DNAH5, DNAH9, DNAI1, DNAI2, DNAJC19, DNAL1, DOLK, DPP6, DSC2, DSG2, DSP, DTNA, EFEMP2, EGF, EHMT1, ELAC2, ELN, EMD, ENG, EOGT, EP300, EVC, EVC2, EYA4, FBN1, FBN2, FHL1, FKRP, FKTN, FLNA, FLNC, FOXC1, FOXF1, FOXH1, FOXJ1, FXYD2, GAA, GAS8, GATA4, GATA5, GATA6, GDF1, GDF2, GJA1, GJA5, GLA, GNB5, GPC3, GPD1L, HADHA, HAND1, HCCS, HCN4, HFE, HRAS, HTRA1, ILK, JAG1, JPH2, JUP, KANSL1, KCNA1, KCNA5, KCND3, KCNE1, KCNE2, KCNE3, KCNE5, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNK3, KCNQ1, KDM6A, KLF10, KMT2D, KRAS, LAMA2, LAMA4, LAMP2, LDB3, LDLR, LDLRAP1, LEFTY2, LMNA, LRRC56, LZTR1, MAP2K1, MAP2K2, MED12, MED13L, MEIS2, MFAP5, MGP, MIB1, MMP15, MMP21, MMP3, MRAS, MT-ATP6, MT-ATP8, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-RNR1, MT-RNR2, MT-TA, MT-TC, MT-TD, MT-TE, MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYL4, MYLK, MYLK2, MYO6, MYOM1, MYOZ2, MYPN, NEBL, NEXN, NF1, NIPBL, NKX2-5, NKX2-6, NME8, NODAL, NOTCH1, NOTCH2, NOTCH3, NPPA, NR2F2, NRAS, NSD1, ODAD1, ODAD2, ODAD3, ODAD4, PDLIM3, PKD1L1, PKD2, PKP2, PLN, PPP1CB, PRDM16, PRKAG2, PRKAR1A, PRKG1, PSEN1, PSEN2, PTPN11, RAF1, RANGRF, RARB, RASA1, RBM10, RBM20, RIT1, ROBO4, RYR1, RYR2, SALL1, SALL4, SCN10A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SCO2, SDHA, SELENON, SEMA3A, SGCD, SGCG, SHOC2, SKI, SLC12A3, SLC22A5, SLC25A4, SLC2A10, SLMAP, SMAD3, SMAD4, SMAD6, SMC3, SNTA1, SOS1, SOS2, SOX2, SPAG1, STRA6, SYNE1, SYNE2, TAB2, TAFAZZIN, TBX1, TBX20, TBX3, TBX5, TCAP, TECRL, TFAP2B, TGFB2, TGFB3, TGFBR1, TGFBR2, TLL1, TMEM43, TNNC1, TNNI3, TNNI3K, TNNT2, TPM1, TRDN, TREX1, TRIM63, TRPM4, TRPM6, TTC12, TTN, TTR, VCL, ZEB2, ZFPM2, ZIC3, ZMYND10, TTC25, TAZ, LRRC6, PIH1D3, CCDC114, ARMC4, CCDC151

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