CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

Patient	XXX, XX
ID #	Male (*DD.MM.YYYY)
Sample receipt	xxx
Material	EDTA blood
Report date	xxx
Report-ID	R#

Genetic Report – XXX, XX (*DD.MM.YYYY)

Order ACMG *in silico* panel

Result: Report with Significant Findings

- **Detection of a likely pathogenic variant in gene *MYH7*, which is associated with hypertrophic cardiomyopathy.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants within the ACMG *in silico* panel.

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
MYH7	**c.1750G>A; p.Gly584Ser** **chr14:23896932 C>T (hg19)**	**het.**	**AD, AR**	**< 0.01**	**likely pathogenic**

Information for the interpretation of this table can be found in section *Additional Information*.

Recommendation

We recommend further clinical evaluation and management according to the current guidelines for *MYH7*-associated hypertrophic cardiomyopathy (Cirino and Ho, updated 2021, PMID: 20301725, GeneReviews).

Testing of asymptomatic family members regarding the variant c.1750G>A; p.Gly584Ser identified in gene *MYH7* may only be performed following genetic counseling.

If you wish to perform further genetic analyses, please do not hesitate to contact us.

Genetic Relevance

Your patient is heterozygous for a likely pathogenic variant in gene *MYH7*. This may be of relevance for family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring.

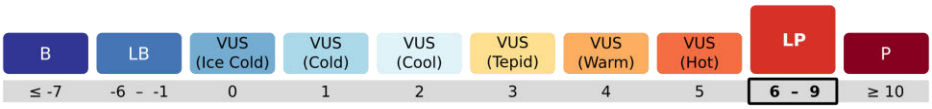
Clinical Information and Variant Interpretation

MYH7, NM_000257.4

OMIM / Reference	Phenotype	Heredity
613426	Dilated cardiomyopathy 1S (CMD1S)	AD
192600	Hypertrophic cardiomyopathy 1 (CMH1)	AD
160500	Laing distal myopathy (MPD1)	AD
608358	Myosin storage myopathy, autosomal dominant (MSMA)	AD
255160	Myosin storage myopathy, autosomal recessive (MSMB)	AR
181430	Scapuloperoneal syndrome, myopathic type (SPMM)	AD
613426	Left ventricular noncompaction cardiomyopathy 5 (LVNC5)	AD

MYH7 encodes the beta heavy chain subunit of myosin. It is expressed in embryonic cardiac tissue and adult skeletal muscle tissues rich in slow-twitch type I muscle fibers and is crucial for muscle contraction. Patients with pathogenic variants in *MYH7* present with varying symptoms, however, they are most commonly associated with autosomal dominant hypertrophic and dilated cardiomyopathy (Das et al., 2014; PMID: 24113344). The majority of the reported pathogenic aberrations in *MYH7* are missense variants, that lead to a form of cardiomyopathy. Myopathy occurs much less frequently (see HGMD Professional, OMIM).

MYH7, c.1750G>A; p.Gly584Ser (het.), ClinVar ID: 42862

ACMG/ACGS Criterion	Points	Description
PS4 (moderate)	+2	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Bourfiss et al., 2022, PMID: 36264615; Erdmann et al., 2003, PMID: 12974739; Van Hout et al., 2020, PMID: 33087929
PM1	+2	The variant is located within a critical region of the gene *MYH7*.
PM2	+2	This variant is listed in the gnomAD global population dataset with very low frequency.
PM5	+2	The variant results in the change of an amino acid residue, for which a different amino acid change (p.Gly584Arg) has already been described as pathogenic. Watkins et al., 1992, PMID: 1552912
ACMG/ACGS Classification: likely pathogenic	+8	

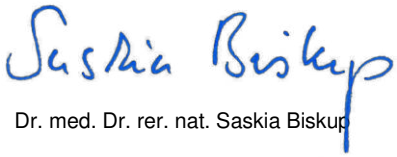
Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,



Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

Additional Information

Requested Regions

ACTA2, ***ACTC1***, ***ACVRL1***, ***APC***, ***APOB***, ***ATP7B***, ***BAG3***, ***BMPR1A***, ***BRCA1***, ***BRCA2***, ***BTD***, ***CACNA1S***, ***CASQ2***, ***COL3A1***, ***DES***, ***DSC2***, ***DSG2***, ***DSP***, ***ENG***, ***FBN1***, ***FLNC***, ***GAA***, ***GLA***, ***HFE***, ***HNF1A***, ***KCNH2***, ***KCNQ1***, ***LDLR***, ***LMNA***, ***MAX***, ***MEN1***, ***MLH1***, ***MSH2***, ***MSH6***, ***MUTYH***, ***MYBPC3***, ***MYH11***, ***MYH7***, ***MYL2***, ***MYL3***, ***NF2***, ***OTC***, ***PALB2***, ***PCSK9***, ***PKP2***, ***PMS2***, ***PRKAG2***, ***PTEN***, ***RB1***, ***RBM20***, ***RET***, ***RPE65***, ***RYR1***, ***RYR2***, ***SCN5A***, ***SDHAF2***, ***SDHB***, ***SDHC***, ***SDHD***, ***SMAD3***, ***SMAD4***, ***STK11***, ***TGFBR1***, ***TGFBR2***, ***TMEM127***, ***TMEM43***, ***TNNC1***, ***TNNI3***, ***TNNT2***, ***TP53***, ***TPM1***, ***TRDN***, ***TSC1***, ***TSC2***, ***TTN***, ***TTR***, ***VHL***, ***WT1*** (ACMG genes (SF v3.1))

Known pathogenic or expected pathogenic variants in the 78 genes associated with childhood and adult onset disorders described by the American College of Medical Genetics and Genomics (ACMG) are reported according to current recommendations (Miller et al., 2022, PMID: 35802134).

General Remarks

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Information for the interpretation of the tables

Heredity: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

MAF: The ***minor allele frequency*** describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot*, *warm*, *tepid*, *cool*, *cold*, and *ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

Methods

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the

Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. CNV calling was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

Computational Analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database. Regions with low sequence coverage are not typically resequenced for ACMG reports. A negative ACMG *in silico* panel report cannot be used to exclude the possibility of disease. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

In this case, 97.88% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. The medical report contains all variants not classified as uncertain, benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).