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

Dr. Jane Doe
Paul-Ehrlich-Str. 23
72076 TÜBINGEN
GERMANY

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

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

Order ACMG *in silico* panel for minors

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a likely pathogenic variant in gene *TTN*, which is considered to be a risk factor for dilatative cardiomyopathy.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants within the ACMG *in silico* panel for minors.

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
TTN	**c.95656delG; p.Glu31886Asn*fs**3**	**het.**	**AD, AR**	**< 0.01**	**likely pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene variant at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows the **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene variant at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
mitochondrial: gene encoded in the mitochondrial DNA. Information on degree of heteroplasmy (heteropl. or homopl.), frequency and read count (variant/total reads).
MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).
Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: pathogenic, likely pathogenic, and uncertain significance. **All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION

***TTN*, c.95656delG; p.Glu31886Asn*fs**3 (het.), NM_133378.4, rs760768093**

OMIM / Reference	Phenotype	Heredity
600334	Tibial muscular dystrophy, tardive	AD
603689	Hereditary myopathy with early respiratory failure	AD
604145	**Dilated cardiomyopathy 1G**	**AD**
613765	Familial hypertrophic cardiomyopathy 9	AD
608807	Muscular dystrophy, limb-girdle, 2J	AR
611705	Myopathy, early-onset, with fatal cardiomyopathy (Salih myopathy)	AR
ORPHA293910, PMID: 21810661, PMID: 25157032	Familial isolated arrhythmogenic ventricular dysplasia/cardiomyopathy	AR?, AD
PMID: 24315344	Restrictive cardiomyopathy	AD?
PMID: 27625337, PMID: 29029073	Non-compaction cardiomyopathy (NCCM/LVNC)	AD?
PMID: 23975875	Centronuclear myopathy	AR
PMID: 31660661, PMID: 29575618, PMID: 28040389	Arthrogryposis multiplex congenita and myopathy	AR

The gene ***TTN*** encodes a giant, elastic protein which is composed of multiple domains and forms part of the sarcomere within the striated muscles. Titin functions as a molecular spring which is responsible for the passive elasticity of muscle.

Pathogenic variants in gene *TTN* are associated with a heterogeneous group of cardiac and muscle disorders. The highly variable phenotypes associated with *TTN* can be explained due to multiple TTN-transcripts, created by alternative splicing. The resulting isoforms have shown to be tissue specific and also specific for different developmental and physiological states (Savarese et al., 2018, PMID: 29598826).

The variant **c.95656delG; p.Glu31886Asn*fs**3** (also known as c.103360delG (NM_001267550.2) in the literature) in gene *TTN* has been identified in your patient in a heterozygous state. The detected variant creates a shift in the reading frame, which either results in a truncated protein or in nonsense-mediated decay of the mRNA transcript. The variant thus probably results in a loss of the protein function, which is a known pathomechanism for *TTN* associated dilatative cardiomyopathy. This variant is located in one of the constitutive *TTN*-exons of the M-band which are expressed in the cardiac isoforms of titin (e.g. N2B, N2A) and where only a few loss-of-function variants have been identified in controls (Roberts et al., 2015, PMID: 25589632). Roberts et al. (2015, PMID: 25589632) as well as Schafer et al. (2017, PMID: 27869827) could show that loss-of-function variants in the M-band were significantly enriched in patient-cohorts with dilatative cardiomyopathy as compared to healthy controls, even though loss-of-function variants in the A-band of titin are typically associated with dilatative cardiomyopathy (Herman et al., 2012, PMID: 22335739). This variant is present within the gnomAD global population dataset at a maximum population frequency of < 0.01%. It has been reported as pathogenic in patient-cohorts for recessive titinopathies (Perić et al., 2017, PMID: 28295036; Savarese et al., 2020, PMID: 32778822; Božović et al., 2021, PMID: 34106991), left ventricular noncompaction cardiomyopathy (Schultze-Berndt et al., 2021, PMID: 34540771) and as a possible founder mutation in the Serbian population (Perić et al., 2017, PMID: 28295036). This variant is also listed as

pathogenic or likely pathogenic for recessive as well as dominant titinopathies (incl. dilatative cardiomyopathy) in ClinVar (Variation ID: 374145).

We consider the identified variant c.95656delG; p.Glu31886Asn*fs3 to be likely pathogenic and a likely risk factor for dilatative cardiomyopathy.**

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. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

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

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

RECOMMENDATION

We recommend further clinical evaluation according to the current guidelines for dilatative cardiomyopathy (Hershberger and Jordan, updated 2022, PMID: 20301486, GeneReviews).

Testing of asymptomatic family members regarding the variant c.95656delG; p.Glu31886Asn*fs**3 identified in gene *TTN* may only be performed following genetic counseling.

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. Following the identification of the molecular cause of a genetic disease, genetic counseling has to be offered.

Medical report written by: xxx

Proofread by: xxx

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup Dr. med. Friedmar Kreuz, M.A.	xxx
Consultant for Human Genetics	Diagnostics

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).

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 NovaSeq6000 system.

NGS based CNV-Calling: (only applicable for nuclear encoded genes) Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. 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 can not 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, 98.1% 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).

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