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

Dr. Jane Doe
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| | |
|---|---|
| **Patient** | XXX, XX |
| ID # | Male (*DD.MM.YYYY) |
| **Sample receipt** | xxx |
| **Material** | Dried blood |
| **Report date** | xxx |
| **Report-ID** | R# |

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

**Indication** Short limb dwarfism, skeletal dysplasia, history of intrauterine growth restriction, antenatal short stature, short nose, low-set ears, proptosis, brachydactyly, inguinal hernia

**Order** ExomeFocus

## Result: Report with Significant Findings

- **Detection of a homozygous likely pathogenic variant in gene *CHST3*, which is consistent with spondyloepiphyseal dysplasia with congenital joint dislocations in your patient.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants ≥50 kb which are likely to be causative for your patient's disease.

| Gene | Variant | Zygosity | Heredity | MAF (%) | Classification |
|---|---|---|---|---|---|
| ***CHST3*** | **c.460C>T; p.Gln154*** chr10:73767249 C>T (hg19) | **homo.** | **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, management and surveillance according to the current guidelines for *CHST3*-related skeletal dysplasia (Superti-Furga & Unger, updated 2019, PMID: 21882400, GeneReviews).

Carrier testing of both parents regarding the identified variant in gene *CHST3* in your patient may be performed in order to ascertain their carrier status, and to determine the risk of reoccurrence for further offspring of the parents of your patient.

## Genetic Relevance

Your patient is homozygous for a likely pathogenic variant in gene *CHST3*. This may be of relevance for future family planning and at-risk family members.

One altered *CHST3* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

Our sequencing data indicated that both gene copies of *CHST3* are present. The data did not show evidence for a deletion of one allele, which would mimic homozygosity.

## Clinical Information and Variant Interpretation

### *CHST3*, NM_004273.5

| OMIM / Reference | Phenotype | Heredity |
|---|---|---|
| 143095 | Spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD) | AR |

The ***CHST3*** gene encodes chondroitin 6-O-sulfotransferase 1 or C6ST-1. This enzyme has an important role in the development and maintenance of the skeleton. In particular, it is essential for the normal development of cartilage. Biallelic pathogenic variants in this gene result in spondyloepiphyseal dysplasia with congenital joint dislocations (SEDCJD). The features of this skeletal dysplasia are prenatal onset short stature, multiple joint dislocations, club feet, and progressive kyphosis and occasionally scoliosis. Cardiac abnormalities and hearing impairment may be present, although vision and intellectual development are not affected (GeneReviews "CHST3-Related Skeletal Dysplasia," Superti-Furga & Unger, updated 2019, PMID: 21882400).

### *CHST3*, c.460C>T; p.Gln154* (homo.)

| ACMG/ACGS Criterion | Points | Description |
|---|---|---|
| **PVS1** (moderate) | +2 | The variant likely results in a loss (or truncation) of the protein, which is a known pathomechanism for *CHST3*-associated disease. |
| **PM2** | +2 | This variant is listed in the gnomAD global population dataset with very low frequency. |
| **PM3** (supporting) | +1 | The variant has been detected *in trans* with a pathogenic variant and/or in a homozygous state. |
| **PP4** | +1 | The associated disease is consistent with specific symptoms in the patient. |
| **ACMG/ACGS Classification:** likely pathogenic | +6 | B (≤ -7) \| LB (-6 - -1) \| VUS (Ice Cold) (0) \| VUS (Cold) (1) \| VUS (Cool) (2) \| VUS (Tepid) (3) \| VUS (Warm) (4) \| VUS (Hot) (5) \| **LP (6 - 9)** \| P (≥ 10) |

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

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## Additional Information

**Scope of analysis**

The whole exome of the individual above was targeted and sequenced. Analysis was restricted to large copy number variations (CNVs) (≥50 kb in size) and a set of automatically selected potentially high impact single nucleotide variants (SNVs) in disease causing genes. This gene set is additionally screened for the occurrence of CNVs <50 kb in size.

Reported variants are limited to pathogenic, likely pathogenic, and variants of uncertain significance (ACGS temperature scale = hot)) associated with the clinical phenotype of the patient, according to current scientific understanding.

**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). CNVs below a size of 50 kb were only evaluated for a precomputed set of genes of potential relevance. 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

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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. Candidate CNV calls are evaluated manually.

**Variants identified through exome analysis were automatically ranked and evaluated with reference to the indicated phenotype. Single heterozygous variants in genes associated with autosomal recessive inheritance may not have been reported.**

In this case, 97.75% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. *In silico* predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

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).

**Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.**

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