XXX

**Order no.:** xxx
**Order received:** xxx
**Sample type:** blood, filter card
**Sample collection date:** xxx
**Report type:** Final Report
**Report date:** xxx

Patient no.: **xxx**, First Name: **xxx**, Last Name: **xxx**
DOB: **xxx**, Sex: **male**, Your ref.: **xxx**

**Test(s) requested: Clinical Exome Sequencing (CentoDX™)**

## CLINICAL INFORMATION

The patient is presenting since birth with dark blue sclerae and recurrent fractures. Suspicion of osteogenesis imperfecta. Parents are not related. No affected siblings reported.

**POSITIVE RESULT**
**Pathogenic variant identified**

## INTERPRETATION

A heterozygous pathogenic variant was identified in the COL1A1 gene. **The genetic diagnosis of autosomal dominant COL1A1-related osteogenesis imperfecta is confirmed.**

## RECOMMENDATIONS

- Parental carrier testing is requested to establish whether the detect variant is inherited or de novo.
- Genetic counselling is recommended.

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

## RESULT SUMMARY

| GENE | VARIANT COORDINATES | ZYGOSITY | IN SILICO PARAMETERS* | ALLELE FREQUENCIES** | TYPE AND CLASSIFICATION*** |
|---|---|---|---|---|---|
| *COL1A1* | Chr17(GRCh37):g.48267264C>A<br>NM_000088.3:c.2569G>T<br>p.(Gly857Cys)<br>Exon 37 | Het | PolyPhen: Probably damaging<br>Align-GVGD: C65<br>SIFT: Deleterious<br>MutationTaster: Disease causing<br>Conservation: nt high/aa high | gnomAD: -<br>ESP: -<br>1000 G: -<br>CentoMD: - | Missense<br>Pathogenic<br>(class 1) |

Variant description based on Alamut Batch (latest database available). * AlignGVD: C0: least likely to interfere with function, C65: most likely to interfere with function, splice prediction tools: SSF, MaxEnt, HSF. ** Genome Aggregation Database (gnomAD), Exome Sequencing Project (ESP), 1000Genome project (1000G) and CentoMD® (latest database available). *** based on ACMG recommendations

## VARIANT INTERPRETATION

### COL1A1, c.2569G>T p.(Gly857Cys)

The COL1A1 variant c.2569G>T p.(Gly857Cys) causes an amino acid change from Gly to Cys at position 857. This variant has been confirmed by Sanger sequencing. According to HGMD Professional 2017.3, this variant has previously been described as disease causing for Osteogenesis imperfecta III/IV by Marini et al., 2007 (PMID: 17078022) and Bardai et al., 2016 (PMID: 27509835). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below).

COL1A1-related osteogenesis imperfecta (OI) is characterized by fractures with minimal or absent trauma, variable dentinogenesis imperfecta (DI), and, in adult years, hearing loss. The clinical features of COL1A1-related OI represent a continuum ranging from perinatal lethality to individuals with severe skeletal deformities, mobility impairments, and very short stature to nearly asymptomatic individuals with a mild predisposition to fractures, normal dentition, normal stature, and normal life span. Fractures can occur in any bone, but are most common in the extremities. DI is characterized by grey or brown teeth that may appear translucent and wear down and break easily. COL1A1-related OI has been classified into four types (I, II, III, and IV) based on clinical presentation and radiographic findings. This classification system can be helpful in providing information about prognosis and management for a given individual. The four OI types are now referred to as follows: OI type I: classic non-deforming OI with blue sclerae; OI type II: perinatally lethal OI; OI type III: progressively deforming OI; OI type IV: common variable OI with normal sclerae. COL1A1-related OI is inherited in an autosomal dominant manner. The proportion of cases caused by a de novo COL1A1 mutation varies by the severity of disease: approximately 60% of cases of classic non-deforming OI with blue sclerae or common variable OI with normal sclerae, virtually 100% of perinatally lethal OI, and close to 100% of progressively deforming OI are de novo (Steiner, et al., 2013 - PMID: 20301472).

## INCIDENTAL FINDINGS

We did not detect any class 1 or 2 variants in the genes for which incidental findings are reported based on the ACMG guidelines.

## ANALYSIS STATISTICS for the offered genes

| AVERAGE COVERAGE (X) | % TARGET BP COVERED | | | | | |
|---|---|---|---|---|---|---|
| | 0X | ≥ 1X | ≥ 5X | ≥ 10X | ≥ 20X | ≥ 50X |
| 137.69 | 0.11 | 99.89 | 99.70 | 99.40 | 98.17 | 89.52 |

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

## 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

Genomic DNA is enzymatically fragmented and regions of interest are selectively enriched using capture probes targeted against coding regions of ~6700 genes with known clinical significance. Libraries are generated with Illumina compatible adaptors and sequenced on an Illumina platform.
Evaluation is focused on coding exons along with flanking +/-10 intronic bases within the captured region. Due to limitations of the method, the target region is not covered 100%. Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling is performed using validated in-house software. Relevant variants reported in HGMD®, in ClinVar or in CentoMD® as well as all variants with minor allele frequency (MAF) of less than 1% in gnomAD database are considered. All pertinent inheritance patterns are considered. In addition, provided family history and clinical information are used to evaluate eventually identified variants. All identified variants are evaluated with respect to their pathogenicity and causality, and these are categorized in classes 1 - 5 (see above). All variants related to the phenotype of the patient, except benign or likely benign variants, are reported. Any relevant variant(s) identified by NGS is(are) Sanger sequenced to exclude NGS artefacts before being reported.
Exon 37 of the *COL1A1* gene was analyzed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon-intron splice junctions. The reference sequence is: *COL1A1*: NM_000088.3.

## LIMITATIONS

Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. If results obtained do not match the clinical findings, additional testing should be considered.
Due to limited read length and other contributing technical limitations, repeat expansions (i.e. in the Huntington gene, the SCA-genes, the myotonic dystrophy repeat region, and other similar regions) cannot be assessed with the applied method. Of note, CNV calls from Whole Genome Sequencing have a limited accuracy and sensitivity, and structural changes below 2 kb at a genome-wide level are not called by our pipeline.

## ADDITIONAL INFORMATION

This test was developed and its performance validated by CENTOGENE AG. 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.

In line with ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Genetics in Medicine, 2016), we report incidental findings, i.e. pathogenic variants (class 1) and likely pathogenic variants (class 2) only in the recommended genes for the recommended phenotypes.

To also 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 (dmqc@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.

## COPYRIGHT NOTICE



Chief Scientific Officer
Human Geneticist

Clinical Scientist

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