



\*Some, or all, of the gene is duplicated in the genome. Read more: <https://blueprintgenetics.com/pseudogene/>

#The gene has suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with a mapping quality score of MQ>20 reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (\*) or number sign (#).

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## STATEMENT

### CLINICAL HISTORY

Patient is a 28-year-old male who clinically looks to have amyoplasia of upper limbs. No involvement of lower limbs. Craniosynostoses, internal rotation of shoulders, extended elbows at birth. There is no family history of similar disease.

### CLINICAL REPORT

Sequence and Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) Arthrogryposes Panel did not detect any known disease-causing or rare variants that could explain the patient's phenotype as described to the laboratory at the time of interpretation.

The analysis detected a variant that was considered an additional finding. Please see APPENDIX 2 for this result.

STEP	DATE
Order date	Mar 25, 2022
Sample received	May 03, 2022
Sample in analysis	May 04, 2022
Reported	Jun 10, 2022

On Jun 10, 2022 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



Riitta Lindström, Ph.D.

Geneticist



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Lab Director, Chief Medical Officer



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Senior Geneticist

APPENDIX 2: ADDITIONAL FINDINGS

This table includes variants that:

1. are not thought to be the likely cause for, or sufficient to cause the patient’s phenotype
- a. a single variant (pathogenic, likely pathogenic or variant of uncertain significance) in a gene that causes an autosomal recessive or X-linked recessive disorder
2. are findings potentially relevant to the patient’s medical care
- a. risk variants identified in genes included on the panel

b. potentially disease-causing variants for an autosomal dominant disorder not related to patient’s current phenotype
3. indicate carrier status for pathogenic or likely pathogenic variants in a gene that causes an autosomal recessive or X-linked disorder not suspected in the patient

ADDITIONAL FINDINGS: SEQUENCE ALTERATIONS

GENE NEB	TRANSCRIPT NM_001271208.2	NOMENCLATURE c.20162T>C, p.(Leu6721Pro)	GENOTYPE HET	CONSEQUENCE missense_variant	INHERITANCE AR	CLASSIFICATION Variant of uncertain significance
ID rs111517514	ASSEMBLY GRCh37/hg19	POS 2:152404248	REF/ALT A/G			
gnomAD AC/AN 134/277716	POLYPHEN probably damaging	SIFT deleterious	MUTTASTER disease causing	PHENOTYPE Nemaline myopathy		

NOTES REGARDING ADDITIONAL FINDINGS

The patient is heterozygous for a variant of uncertain significance *NEB* c.20162T>C, p.(Leu6721Pro). The variant has been submitted to ClinVar by other clinical testing laboratories (variation ID [331437](#)). Disease caused by variants in this gene is inherited in an autosomal recessive manner. No second potentially disease-causing variant was detected in this gene. Therefore, this heterozygous variant is not expected to explain the patient’s reported phenotype.

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## APPENDIX 5: SUMMARY OF THE TEST

### PLUS ANALYSIS

**Laboratory process:** When required, the total genomic DNA was extracted from the biological sample using bead-based method. DNA quality and quantity were assessed using electrophoretic methods at Blueprint Genetics. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing. Sequencing library was prepared by ligating sequencing adapters to both ends of DNA fragments. Sequencing libraries were size-selected with bead-based method to ensure optimal template size and amplified by polymerase chain reaction (PCR). Regions of interest (exons and intronic targets) were targeted using hybridization-based target capture method. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primers and adapter-adapter dimers. Ready sequencing libraries that passed the quality control were sequenced using the Illumina's sequencing-by-synthesis method using paired-end sequencing (150 by 150 bases). Primary data analysis converting images into base calls and associated quality scores was carried out by the sequencing instrument using Illumina's proprietary software, generating CBCL files as the final output. These steps were performed at Blueprint Genetics.

**Bioinformatics and quality control:** Base called raw sequencing data was transformed into FASTQ format using Illumina's software (bcl2fastq). Sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon) for nDNA. Variant data for was annotated using a collection of tools (VcfAnno and VEP) with a variety of public variant databases including but not limited to gnomAD, ClinVar and HGMD. The median sequencing depth and coverage across the target regions for the tested sample were calculated based on MQ0 aligned reads. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures including assessments for contamination and sample mix-up. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content. Bioinformatics and quality control processes were performed by Blueprint Genetics.

**Interpretation:** The clinical interpretation team assessed the pathogenicity of the identified variants by evaluating the information in the patient requisition, reviewing the relevant scientific literature and manually inspecting the sequencing data if needed. All available evidence of the identified variants was compared to classification criteria. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines. Likely benign and benign variants were not reported. The interpretation was performed at Blueprint Genetics.

**Variant classification:** Our variant classification follows the Blueprint Genetics [Variant Classification Schemes](#) modified from the [ACMG guideline 2015](#). Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. The classification and interpretation of the variant(s) identified reflect the current state of Blueprint Genetics' understanding at the time of this report. Variant classification and interpretation are subject to professional judgment, and may change for a variety of reasons, including but not limited to, updates in classification guidelines and availability of additional scientific and clinical information. This test result should be used in conjunction with the health care provider's clinical evaluation. Inquiry regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decision. For questions regarding variant classification updates, please contact us at [support@blueprintgenetics.com](mailto:support@blueprintgenetics.com)

**Databases:** The pathogenicity potential of the identified variants were assessed by considering the predicted consequence of the change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited to, the [gnomAD](#), [ClinVar](#), HGMD Professional and Alamut Visual. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as [Database of Genomic Variants](#) and [DECIPHER](#). For interpretation of mtDNA variants specific databases including e.g. Mitomap, HmtVar and 1000G were used.

**Confirmation of sequence alterations:** Sequence variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) were confirmed using bi-directional Sanger sequencing when they did not meet our stringent

NGS quality metrics for a true positive call. In addition, prenatal case with diagnostic findings were confirmed. The confirmation of sequence alterations was performed at Blueprint Genetics.

**Confirmation of copy number variants:** CNVs (Deletions/Duplications) were confirmed using a digital PCR assay if they covered less than 10 exons (heterozygous), less than 3 exons (homo/hemizygous) or were not confirmed at least three times previously at our laboratory. Furthermore, CNVs of any size were not confirmed when the breakpoints of the call could be determined. The confirmation of copy number variants was performed at Blueprint Genetics.

**Analytic validation:** The detection performance of this panel is expected to be in the same range as our high-quality, clinical grade NGS sequencing assay used to generate the panel data (nuclear DNA: sensitivity for SNVs 99.89%, indels 1-50 bps 99.2%, one-exon deletion 100% and five exons CNV 98.7%, and specificity >99.9% for most variant types). It does not detect very low level mosaicism as a variant with minor allele fraction of 14.6% can be detected in 90% of the cases. Detection performance for mtDNA variants (analytic and clinical validation): sensitivity for SNVs and INDELs 100.0% (10-100% heteroplasmy level), 94.7% (5-10% heteroplasmy level), 87.3% (<5% heteroplasmy level) and for gross deletions 100.0%. Specificity is >99.9% for all.

**Test restrictions:** A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

**Technical limitations:** This test does not detect the following: complex inversions, gene conversions, balanced translocations, repeat expansion disorders unless specifically mentioned, non-coding variants deeper than  $\pm 20$  base pairs from exon-intron boundary unless otherwise indicated (please see the list of non-coding variants covered by the test). Additionally, this test may not reliably detect the following: low level mosaicism, stretches of mononucleotide repeats, indels larger than 50bp, single exon deletions or duplications, and variants within pseudogene regions/duplicated segments. The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics. Laboratory error is also possible. Please see the Analytic validation above.

**Regulation and accreditations:** This test was developed and its performance characteristics determined by Blueprint Genetics (see Analytic validation). It has not been cleared or approved by the US Food and Drug Administration. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation (excluding mtDNA testing).

## PERFORMING SITE:

BLUEPRINT GENETICS OY, KEILARANTA 16 A-B, 02150 ESPOO, FINLAND Laboratory Director: JUHA KOSKENVUO, MD, PhD, CLIA: 99D2092375

## NON-CODING VARIANTS COVERED BY THE PANEL:

NM\_020451.2(*SELENON*):c.\*1107T>C  
 NM\_000157.3(*GBA*):c.1225-14\_1225-11delTGTCinsAGT  
 NM\_000157.3(*GBA*):c.589-12C>G  
 NM\_000157.3(*GBA*):c.-150A>G  
 NM\_170707.3(*LMNA*):c.513+45T>G  
 NM\_170707.3(*LMNA*):c.937-11C>G  
 NM\_170707.3(*LMNA*):c.1608+14G>A  
 NM\_170707.3(*LMNA*):c.1609-12T>G  
 NM\_000124.3(*ERCC6*):c.2599-26A>G  
 NM\_005055.4(*RAPSN*):c.193-15C>A  
 NM\_005055.4(*RAPSN*):c.-199C>G  
 NM\_005055.4(*RAPSN*):c.-210A>G  
 NM\_000123.3(*ERCC5*):c.881-26T>G  
 NM\_003239.2(*TGFB3*):c.\*495C>T  
 NM\_003239.2(*TGFB3*):c.-30G>A  
 NM\_018668.3(*VPS33B*):c.499-11G>A  
 chr16:g.8891573-8891573  
 NM\_000303.2(*PMM2*):c.179-25A>G



NM\_000303.2(PMM2):c.640-15479C>T  
 NM\_000303.2(PMM2):c.640-23A>G  
 NM\_000080.3(CHRNE):c.501-16G>A  
 NM\_000080.3(CHRNE):c.-94G>A  
 NM\_000080.3(CHRNE):c.-95G>A  
 NM\_000080.3(CHRNE):c.-96C>T  
 NM\_001271208.1(NEB):c.24220-151C>A  
 NM\_001271208.1(NEB):c.19429-381\_19429-379delTTTinsA  
 NM\_001849.3(COL6A2):c.1117-35\_1118dupAAAAGACGTGAGGCTGATTCTGCAAACCCCTTCCAGGG  
 NM\_001849.3(COL6A2):c.1459-63G>A  
 NM\_000158.3(GBE1):c.2053-3358\_2053-3350delGTGTGGTGGinsTGTTTTTTACATGACAGGT  
 NM\_001999.3(FBN2):c.3974-24A>C  
 NM\_001999.3(FBN2):c.3974-26T>G  
 NM\_001999.3(FBN2):c.3725-15A>G  
 NM\_020320.3(RARS2):c.613-3927C>T  
 NM\_016042.3(EXOSC3):c.475-12A>G  
 NM\_006731.2(FKTN):c.648-1243G>T  
 NM\_000252.2(MTM1):c.137-19\_137-16delACTT  
 NM\_000252.2(MTM1):c.137-11T>A  
 NM\_000252.2(MTM1):c.232-26\_232-23delGACT  
 NM\_000252.2(MTM1):c.529-909A>G  
 NM\_000252.2(MTM1):c.868-13T>A

## GLOSSARY OF USED ABBREVIATIONS:

**AD** = autosomal dominant

**AF** = allele fraction (proportion of reads with mutated DNA / all reads)

**AR** = autosomal recessive

**CNV** = Copy Number Variation e.g. one exon or multiexon deletion or duplication

**gnomAD** = genome Aggregation Database (reference population database; >138,600 individuals)

**gnomAD AC/AN** = allele count/allele number in the genome Aggregation Database (gnomAD)

**HEM** = hemizygous

**HET** = heterozygous

**HOM** = homozygous

**ID** = rsID in dbSNP

**MT** = Mitochondria

**MutationTaster** = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.

**Nomenclature** = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

**OMIM** = Online Mendelian Inheritance in Man®

**PolyPhen** = *in silico* prediction tool used to evaluate the significance of amino acid changes.

**POS** = genomic position of the variant in the format of chromosome:position

**SIFT** = *in silico* prediction tool used to evaluate the significance of amino acid changes.