Blueprint Genetics

*Sample report as of July 1st, 2022. Regional differences may apply. For complete and up-to-date test methodology description, please see your report in Nucleus online portal. Accreditation and certification information available at **blueprintgenetics.com/certifications***

Spinal Muscular Atrophy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** Age **GENDER** **ORDER ID**

PRIMARY SAMPLE TYPE **CUSTOMER SAMPLE ID**

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient patient is homozygous for a deletion of exon 7 in the *SMN1* gene, this variant is classified as pathogenic.

PRIMARY FINDINGS: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
SMN1	COPY NUMBER LOSS	0	HOM	Partial gene	UCSC	**Pathogenic**
	OMIM	**PHENOTYPE** Spinal muscular atrophy			**COMMENT** -	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Spinal Muscular Atrophy Panel	30	442	83350	83215	269	99.84

TARGET REGION AND GENE LIST

The Blueprint Genetics Spinal Muscular Atrophy Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: AARS, ASAH1, ATP7A, BICD2, BSCL2, CHCHD10, DCTN1, DNAJB2, DYNC1H1, EXOSC3, EXOSC8, FBXO38, GARS, HEXA, HSPB1, HSPB3, HSPB8, IGHMBP2, LAS1L, PLEKHG5, REEP1, SCO2, SLC5A7, SMN1#*, SMN2#*, TBCE, TRPV4, UBA1, VAPB and VRK1. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELs) and copy number variations defined as single exon or larger deletions and

duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

*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 mapping quality score (MQ>20) reads.
The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#).

STATEMENT

CLINICAL HISTORY

Patient is a 29-year-old individual with congenital myopathy: muscle biopsy (age 9) revealed congenital myopathy with type I fiber predominance (type II affected; no inflammatory myopathy, no abnormal storage or inclusion material). Difficulty with gross motor skills since 2-year-old, especially traversing stairs or long distances (needs to take them slowly with rests). Previously, "Focused Neuromuscular Disease NGS Gene Sequencing Panel and Deletion/Duplication Analysis" was negative (performed in another laboratory). The analysis of Comprehensive Muscular Dystrophy / Myopathy Panel performed in BpG resulted negative (BpG ID 117823).

CLINICAL REPORT

Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) Spinal Muscular Atrophy Panel identified a homozygous deletion on chromosome 5 including at least exon 7 of the *SMN1* gene, which is considered compatible to whole gene deletion. There is limited sensitivity to detect the exact break points of the deletion due to the complexity of the *SMN1* gene region. In addition, >=3 copies of *SMN2* were detected.

SMN1

The survival of motor neuron 1 (*SMN1*) gene (MIM *600354) shares more than 99% nucleotide identity with the *SMN2* gene (MIM *601627), both genes encode a 294-amino acid RNA-binding protein, SMN, that is required for efficient assembly of small nuclear ribonucleoprotein (snRNP) complexes. These two genes, both containing nine exons, can be distinguished only by eight nucleotides (5 intronic, 3 exonic, 1 each located in exons 6, 7, and 8) (PMID: 9950358). *SMN1* produces a full-length transcript, whereas *SMN2* produces predominantly an alternatively spliced transcript lacking exon 7, which results into a less stable SMN protein (PMID: 10339583, 10607836). *SMN1* and *SMN2* are located close to each other at the complex SMN region on chromosome 5q12.2-q13.3 where repetitive sequences, pseudogenes, transposable elements, deletions and inverted duplications are not unusual (PMID: 9950358). Homozygous loss of *SMN1,* caused by deletion or point mutation, causes spinal muscular atrophy (SMA; OMIM #253300); absence of exon 7 of *SMN1* has been identified in about 95% of patients with SMA (PMID: 9950358). Absence of *SMN1* is partially compensated for by *SMN2*, which produces enough SMN protein to allow for relatively normal development in cell types other than motor neurons (MIM *600354). However, *SMN2* cannot fully compensate for loss of *SMN1* because a majority of *SMN2* transcripts are truncated and less stable than *SMN1* transcripts (PMID: 7813012, 17307868).

Spinal muscular atrophies are autosomal recessive disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy (OMIM #253300). Five types of SMA are recognized based on the age of onset, the maximum muscular activity achieved, and survivorship (GeneReviews): type 0 (SMA0), congenital SMA; type I (SMA1), severe infantile acute SMA / Werdnig-Hoffman disease; type II (SMA2, OMIM #253550), or infantile chronic SMA; type III (SMA3, OMIM #253400), juvenile SMA, or Wohlfart-Kugelberg-Welander disease; and type IV (SMA4, OMIM #271150), or adult-onset SMA. All SMA types are caused by recessive mutations in *SMN1*. SMA1 has age of onset under 6 months and the life

span of the patients is most often under 2 years. Milder SMA phenotypes are usually associated with a gene conversion of *SMN1* sequences into *SMN2* sequences, or more than 2 copies of *SMN2* (PMID: 14705979, 16508748, 11839954).

SMA disease incidence per 100,000 live births is estimated 4-10 in Europe and USA, and the carrier frequency is estimated 1/50-90 (GeneReviews). There have been no approved drug treatments for SMA until recently when a novel drug Nusinersen became available (PMID: 27939059).

CONCLUSION

Considering the current literature and well-established role of *SMN1* deletion as a disease causing variant, we classify it as pathogenic. Disease caused by *SMN1* variants is inherited in an autosomal recessive manner. The patient is homozygous for the variant, which is in line with autosomal recessive inheritance. The recurrence risks deviate slightly from the norm for autosomal recessive inheritance because about 2% of affected individuals have a *de novo SMN1* variant on one allele; in these instances, only one parent is a carrier of an *SMN1* variant, and thus the sibs are not at increased risk for SMA. If both parents are found to be carriers of this mutation, each sibling of an affected individual has a 25% chance of being a homozygous carrier of the mutation and thus being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Genetic counseling and family member testing are recommended.

STEP	DATE
Order date	
Sample received	
Reported	

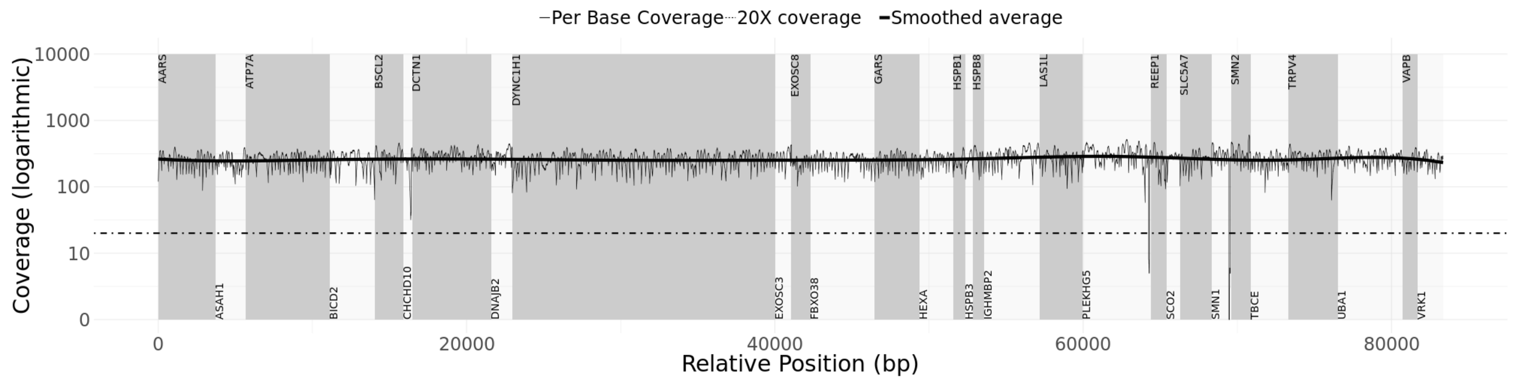
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

Name

Title

Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.



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. 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.
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.
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.
Variant classification: Our variant classification follows the Blueprint Genetics 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.
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.
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.
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 ±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 and digital PCR confirmation).

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000052.5(ATP7A):c.2916+2480T>G, NM_000052.5(ATP7A):c.3294+763C>G, NM_001122955.3(BSCL2):c.405-11A>G, NM_016042.3(EXOSC3):c.475-12A>G, NM_000520.4(HEXA):c.1146+18A>G, NM_001540.3(HSPB1):c.-217T>C, NM_002180.2(IGHMBP2):c.1235+894C>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.