Blueprint Genetics

FLEX Dystonia Panel Plus

THE ORIGINAL PANEL CONTENT HAS BEEN MODIFIED BY THE CUSTOMER.

REFERRING HEALTHCARE PROFESSIONAL

NAME   **HOSPITAL**

PATIENT

NAME	**DOB**	**AGE**	**GENDER**	**ORDER ID**
		1		

PRIMARY SAMPLE TYPE   **SAMPLE COLLECTION DATE**   **CUSTOMER SAMPLE ID**
Blood

SUMMARY OF RESULTS

PRIMARY FINDINGS

Negative for explaining the patient's phenotype.

ADDITIONAL FINDINGS

The patient is heterozygous for *GJB2* c.109G>A, p.(Val37Ile), which is pathogenic.
The patient is heterozygous for *MPZL2* c.463del, p.(Ala155Leufs*10), which is likely pathogenic.

Please see APPENDIX 2: Additional Findings for further details

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Dystonia Panel	41	655	126481	125861	187	99.51

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	16766	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Dystonia Flex Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ADCY5, ANO3, ATP1A2, ATP1A3, ATP2B2, BCAP31*, BSND, CACNA1B, CACNA1G, DCAF17, DIAPH3, DNAJC12, FA2H, FITM2, GCH1, GJB2, GJB6, GNAL, KCNMA1, KMT2B, MECR, MIPEP*, MPZL2, MT-ATP6, MT-ATP8, MT-CO1, MT-CO2, MT-CO3, MT-CYB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6, MT-RNR1, MT-RNR2, MT-TA, MT-TC, MT-TD, MT-TE,*

MT-TF, MT-TG, MT-TH, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TM, MT-TN, MT-TP, MT-TQ, MT-TR, MT-TS1, MT-TS2, MT-TT, MT-TV, MT-TW, MT-TY, NARS2, PDE10A, PDGFB, PDGFRB, PNKD, PRKRA, PRRT2, SCN1A, SCN2A, SGCE#, SLC2A1, SLC39A14#, SPR, TH, THAP1, TOR1A, UBTF and VAC14. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: SGCE (NM_001099401:10) and SLC39A14 (NM_001135154:9).

Genes added by the clinician: ATP1A2, ATP2B2, BSND, DIAPH3, GJB2, GJB6, MPZL2, NARS2, SCN1A and SCN2A

*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 22-month-old child with episodic focal neck dystonia, congenital right-sided sensorineural hearing loss and wide-based gait and frequent falls

Clinical reason for the custom panel: Movements disorders with sensorineural hearing loss.

CLINICAL REPORT

Sequence and Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) FLEX Dystonia 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 variants that were considered additional findings. Please see APPENDIX 2 for these results.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

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

Signature

Name

Title

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	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
GJB2	NM_004004.5	c.109G>A, p.(Val37Ile)	HET	missense_variant	AD,AR	**Pathogenic**
	ID rs72474224	**ASSEMBLY** GRCh37/hg19	**POS** 13:20763612	**REF/ALT** C/T		
	gnomAD AC/AN 2132/282164	**POLYPHEN** probably damaging	**SIFT** tolerated	**MUTTASTER** disease causing	**PHENOTYPE** Bart-Pumphrey syndrome, Deafness, Hystrix-like ichthyosis with deafness, Keratitis-icthyosis-deafness syndrome, Keratoderma, palmoplantar, with deafness, Vohwinkel syndrome	

| GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
MPZL2	NM_005797.3	c.463del, p.(Ala155Leufs*10)	HET	frameshift_variant	AR	**Likely pathogenic**
	ID	**ASSEMBLY** GRCh37/hg19	**POS** 11:118130889	**REF/ALT** GC/G		
	gnomAD AC/AN 8/282508	**POLYPHEN** N/A	**SIFT** N/A	**MUTTASTER** N/A	**PHENOTYPE** Sensorineural hearing loss	

NOTES REGARDING ADDITIONAL FINDINGS

The patient is heterozygous for a pathogenic variant *GJB2* c.109G>A, p.(Val37Ile). The *GJB2* c.109G>A, p.(Val37Ile) variant has been observed with a particularly high allele frequency in East Asian population (gnomAD: 8.2%, with 88/9434 individuals homozygous). It has been observed altogether in 2132 heterozygous and 99 homozygous individuals in the Genome Aggregation Database (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. The variant is predicted damaging by most of the *in silico* tools used. Despite of its high population frequency, the *GJB2* c.109G>A, p.(Val37Ile) variant is a well-known disease-causing variant with wide consensus on the pathogenicity. It has been detected in patients with autosomal recessive hearing loss in several studies (PMID: 10633133, 22106692, 12121355, 24654934, 23873582, 19043807, 26061099, 17036313, 22574200, 27153395, 17935238, 10830906). The variant has also been identified in clinical testing (ClinVar ID17023). The variant induces an inability to formation of homotypic gap junction channels, but is associated only with mild hearing loss (GeneReviews NBK1272). Recent comprehensive study of over 1000 patients with hearing loss found this variant in homozygous state in 13 patients and in compound heterozygous state in 14 patients (PMID: 26969326). Majority of these patients had mild-to-moderate hearing loss, but three patients (two compound heterozygous and one homozygous) were regarded as having severe-to-profound hearing loss.

However, mostly because of its population frequency this variant has been previously classified as a benign polymorphism (PMID: 12792423, 9529365) or hypomorphic allele (PMID: 24645897). However, in 2016 a comprehensive publication evaluating the data using American College of Medical Genetics and Genomics (ACMG) Guidelines-based variant classification scheme concluded that this variant is likely pathogenic and overrode the preliminary classification because of the extensive supporting literature (PMID: 27153395). The ClinGen Hearing Loss Variant Curation Expert Panel recently classified the p.(Val37Ile) variant as pathogenic based on ACMG Guidelines modified for hearing loss (PMID: 30311386, 31160754). It was concluded that the *GJB2* c.109G>A, p.(Val37Ile) variant is one of the most common pathogenic variants in hearing loss, and is considered as a low penetrant variant based on the ClinGen Expert Panel's judgement (PMID: 30311386, 31160754). Genetic counseling is recommended.

The patient is also heterozygous for a likely pathogenic variant *MPZL2* c.463del,p.(Ala155Leufs*10). Disease caused by *MPZL2* variants is inherited in an autosomal recessive manner, and no second rare or potentially disease-causing variant in *MPZL2* was detected in the patient. Therefore, this heterozygous variant is not expected to be related to the patient's clinical presentation. However, as the patient is a carrier of a likely pathogenic variant for recessive disease, genetic counseling is recommended.

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.
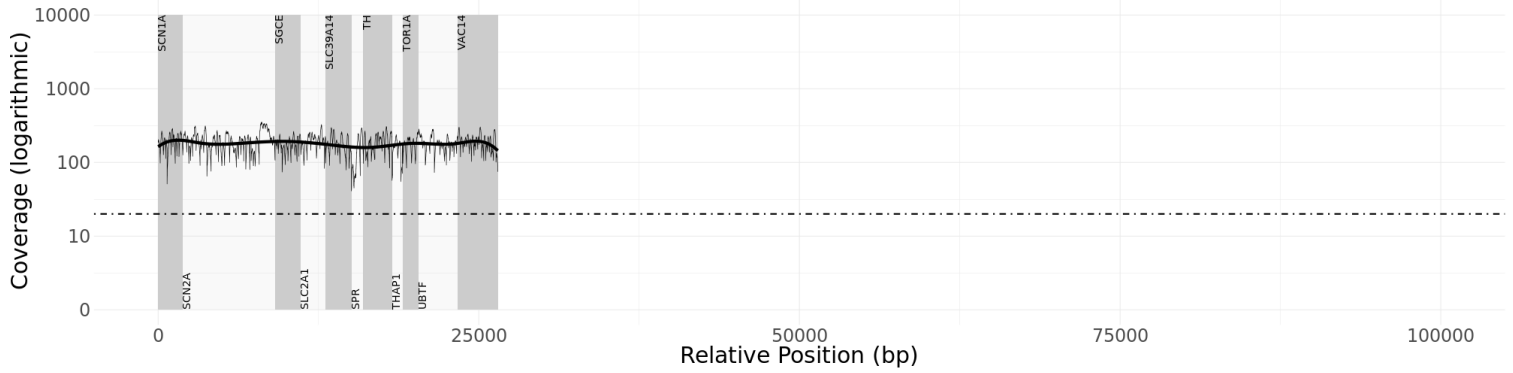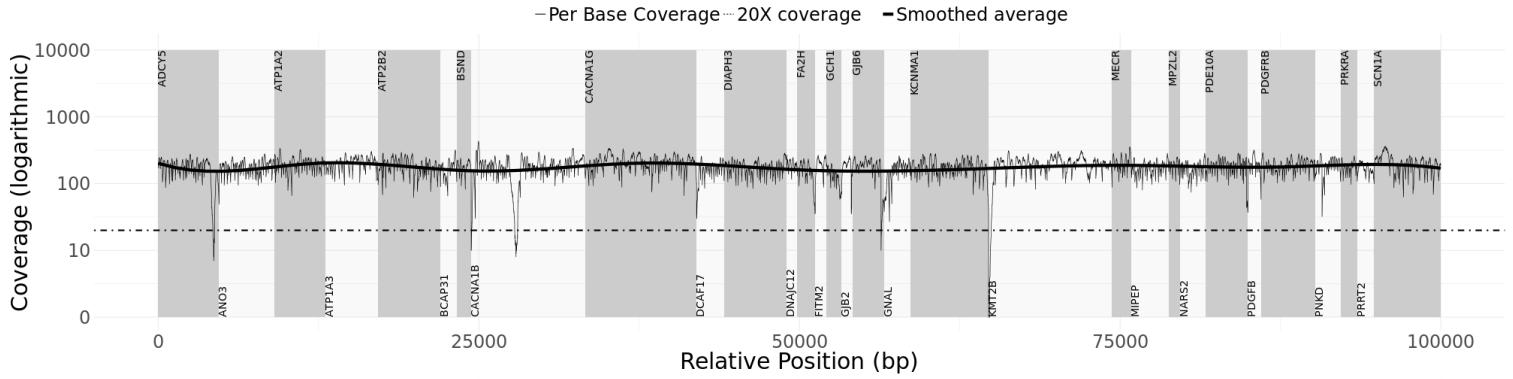
Readability of the coverage plot may be hindered by faxing. A high quality coverage plot can be found with the full report on nucleus.blueprintgenetics.com.
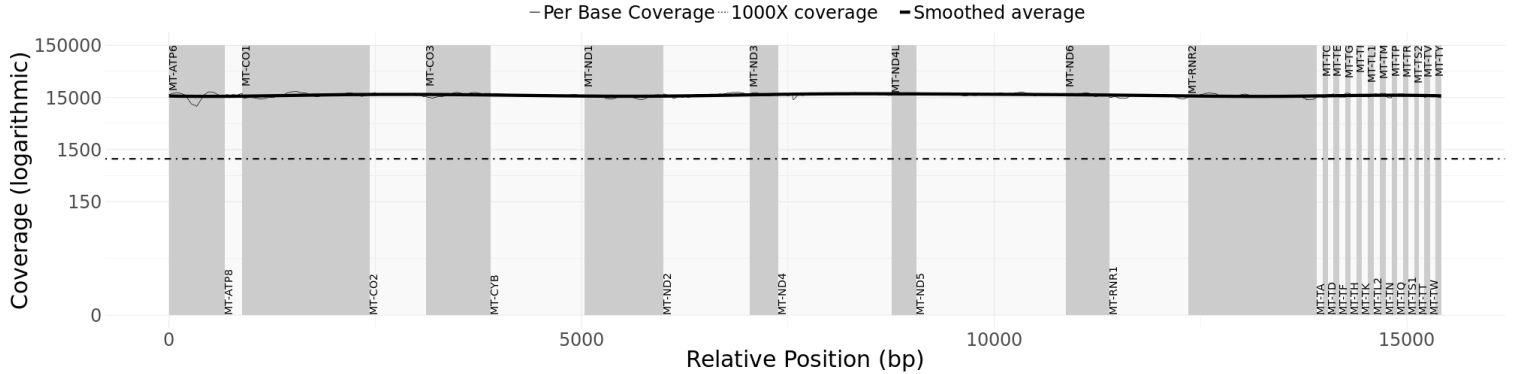
APPENDIX 5: SUMMARY OF THE TEST

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_025000.3(DCAF17):c.322-14delC
NM_001042517.1(DIAPH3):c.-172G>A
NM_001042517.1(DIAPH3):c.-173C>T
NM_000161.2(GCH1):c.-22C>T
NM_004004.5(GJB2):c.-22-2A>C
NM_004004.5(GJB2):c.-23+2T>A
NM_004004.5(GJB2):c.-23+1G>A
NM_004004.5(GJB2):c.-23G>T
NM_004004.5(GJB2):c.-259C>T
NM_004004.5(GJB2):c.-260C>T
NM_001256443.1(PRRT2):c.*345G>A
NM_006920.4(SCN1A):c.4820-14T>G
NM_006920.4(SCN1A):c.4306-14T>G
NM_006920.4(SCN1A):c.964+14T>G
NM_006920.4(SCN1A):c.474-13T>A
NM_006516.2(SLC2A1):c.680-11G>A
NM_006516.2(SLC2A1):c.-107G>A
NM_003124.4(SPR):c.-13G>A
NM_199292.2(TH):c.1198-24T>A
NM_199292.2(TH):c.738-34G>C
NM_199292.2(TH):c.-69T>A
NM_199292.2(TH):c.-70G>A
NM_199292.2(TH):c.-71C>T
NM_018105.2(THAP1):c.*157T>C
NM_018105.2(THAP1):c.-220C>T

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.