Blueprint Genetics

Sample report as of June 14th, 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***

Leukodystrophy and Leukoencephalopathy Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

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

CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE**

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *POLR3B* c.1568T>A, p.(Val523Glu), which is pathogenic.

The patient is heterozygous for a deletion c.(846+1_847-1)_(966+1_967-1)del, which encompasses exon 11 of *POLR3B*. This alteration is classified as likely pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
POLR3B	NM_018082.6	c.1568T>A, p.(Val523Glu)	HET	missense_variant	AR	**Pathogenic**
	ID	**ASSEMBLY**	**POS**	**REF/ALT**		
	rs138249161	GRCh37/hg19	12:106826199	T/A		
	gnomAD AC/AN	**POLYPHEN**	**SIFT**	**MUTTASTER**	**PHENOTYPE**	
	82/282478	benign	deleterious	disease causing	Leukodystrophy hypomyelinating	

PRIMARY FINDINGS: COPY NUMBER ABERRATIONS

GENE	EVENT	COPY NUMBER	GENOTYPE	IMPACT	LINKS	CLASSIFICATION
POLR3B	COPY NUMBER LOSS	1	HET	POLR3B:Partial gene	UCSC	**Likely pathogenic**
	OMIM	**PHENOTYPE**			**COMMENT**	
		Leukodystrophy hypomyelinating			-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Leukodystrophy and Leukoencephalopathy Panel	81	986	179645	179123	170	99.71

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 1000X	MEDIAN COVERAGE	PERCENT > 1000X
Mitochondrial genome	37	-	15358	15358	9597	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Leukodystrophy and Leukoencephalopathy Flex Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *ABCD1**, *ADAR*, *AIFM1*, *AIMP1*, *ALDH3A2*, *AP4B1*, *AP4E1*, *AP4M1*, *AP4S1#**, *APOPT1*, *ARSA*, *ASPA*, *CLCN2*, *COA7*, *COL4A1*, *COX15*, *COX6B1*, *CSF1R*, *CTC1*, *CYP27A1*, *D2HGDH*, *DARS*, *DARS2*, *DEGS1#*, *EARS2*, *EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4*, *EIF2B5*, *EPRS*, *FA2H*, *FAM126A*, *FDX1L*, *FOLR1*, *FOXRED1*, *GALC*, *GFAP*, *GFM1*, *GJC2*, *HEPACAM*, *HIBCH*, *HSPD1**, *HTRA1*, *IBA57*, *L2HGDH*, *LMNB1*, *LYRM7*, *MARS2*, *MLC1*, *MRPL44*, *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*, *MTFMT*, *NDUFAF5*, *NFU1*, *NKX6-2*, *NOTCH3*, *NT5C2*, *NUBPL*, *PLP1*, *POLR1C#*, *POLR3A*, *POLR3B*, *PSAP*, *PYCR2*, *RARS*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *RNASET2*, *RNF216**, *SAMHD1*, *SCO1*, *SDHAF1*, *SERAC1*, *SLC1A4*, *SNORD118*, *SOX10*, *SUMF1*, *TREX1*, *TTC19*, *TUBB4A** and *ZFYVE26*. The following exons are not included in the panel as they are not covered with sufficient high quality sequence reads: AP4S1 (NM_001254727:6), DEGS1 (NM_001321541:3) and POLR1C (NM_001318876:9).

*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 (#).

STATEMENT

CLINICAL HISTORY

Patient is a 35-year-old adult with delayed puberty, hypodontia, ataxia, cerebellar leukodystrophy, hypogonadotropic hypogonadism; hypomyelination. There is no family history of similar disease.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) FLEX Leukodystrophy and Leukoencephalopathy Panel identified a heterozygous missense variant *POLR3B* c.1568T>A, p.(Val523Glu).

***POLR3B* c.1568T>A, p.(Val523Glu)**

There are 82 individuals heterozygous for this variant in gnomAD, a large reference population database (n>120,000 exomes and >15,000 genomes) which aims to exclude individuals with severe pediatric disease. The variant is predicted to be deleterious by most *in silico* tools utilized. The variant is a recurrent pathogenic variant. It has been reported in homozygous or compound heterozygous state with another disease causing *POLR3B* variant in several individuals with hypomyelinating

leukodystrophy-8 with hypodontia and hypogonadotropic hypogonadism (PMID: 22036172, 23355746, 24190003, 25339210, 26204956, 28589944). Based on electron microscopy structure analysis, the p.(Val523Glu) variant was predicted to affect local structure and impair proper function of pol III (PMID: 22036172). The variant has been submitted to ClinVar by other clinical testing laboratories (variation ID 31166).

Del/Dup (CNV) analysis using the Blueprint Genetics (BpG) FLEX Leukodystrophy and Leukoencephalopathy Panel identified a heterozygous deletion c.(846+1_847-1)_(966+1_967-1)del encompassing exon 11 of *POLR3B*. This deletion is estimated to cover the genomic region 12:106799574-106799814 and is approximately 240 base pairs in size. However, the exact breakpoints of the deletion cannot be determined using the present method, and therefore its exact size and genomic position are unknown. Deletions encompassing the *POLR3B* gene have been reported in 8 individuals in the Exome Aggregation Consortium (ExAC) control cohorts and in 2 individuals in the Genome Aggregation Database control cohorts (gnomAD SVs v2.1, ExAC data available in the gnomAD browser). To the best of our knowledge, this variant has not been reported in the medical literature or on disease-related variation databases.

Due to the large genomic distance between these variants, we cannot determine if they are on the same parental chromosome (*in cis*) or on different parental chromosomes (*in trans*).

POLR3B

POLR3B encodes the second largest subunit of RNA polymerase III (RNA Pol III). RNA Pol III is involved in the transcription of small noncoding RNAs such as 5S ribosomal RNA, U6 small nuclear RNA, short interspersed nuclear elements (SINES), and all transfer RNAs. Variants in *POLR3B* have been identified in individuals with autosomal recessive hypomyelinating leukoencephalopathy (PMID: 22036171,25339210, 27512013, 28589944). Hypomyelinating leukodystrophy is characterized by varying combinations of four clinical findings: neurologic dysfunction that is typically predominated by motor dysfunction, abnormal dentition, endocrine abnormalities such as short stature, and ocular abnormality that is usually myopia (Genereviews #NBK99167). This disorder has also been referred to as 4H syndrome (hypomyelination, hypodontia, hypogonadotrophic hypogonadism), ADDH (ataxia, delayed dentition, and hypomeylination), TACH (tremor-ataxia with central hypomyelination), LO (leukodystrophy with oligodontia), and HCAHC (hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum). Variants in *POLR3B* account for approximately 49% of cases with *POLR3*-related leukodystrophy. Individuals with variants in *POLR3B* typically have earlier disease onset and an increased frequency of cerebellar atrophy seen on MRI than those with *POLR3A* variants. Individuals with *POLR3B* variants also tend to have a slower disease course.

Recently, Djordjevic et al. (2021) has described six unrelated individuals with *de novo* missense variants in *POLR3B* and a clinical presentation substantially different from POLR3-related leukodystrophy. These individuals had afferent ataxia, spasticity, variable intellectual disability and epilepsy, and predominantly demyelinating sensory motor peripheral neuropathy (PMID: 33417887). In addition, a de novo missense variant of *POLR3B* is reported to cause demyelinating Charcot-Marie-Tooth disease in a Chinese patient (PMID: 34666706).

There are currently 77 variants in *POLR3B* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2022.1), including both missense variants and truncating variants (nonsense, frameshift, variants affecting splicing, gross deletions).

Mutation nomenclature is based on GenBank accession NM_018082.6 (*POLR3B*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

POLR3B c.1568T>A, p.(Val523Glu) is classified as pathogenic, based on currently available evidence supporting its disease-causing role. Deletion *POLR3B* c.(846+1_847-1)_(966+1_967-1)del, affecting exon 11, is classified as likely pathogenic, based on the established association between the gene and the patient's phenotype, the variant's absence in control populations, and variant type (gross deletion). Disease caused by these variants is expected to be inherited in an autosomal recessive manner. Testing of parental/offspring samples is needed to determine whether the variants occur in cis (on the same allele) or in trans (on different alleles). Compound heterozygosity of the variants (in trans) would explain the patient's clinical presentation. If both of these variants are parentally inherited, any siblings of the patient will have a 25% chance of being compound heterozygous and thus affected, a 50% chance of being an unaffected carrier, and a 25% chance of being an unaffected non-carrier. Genetic counseling and family member testing are recommended.

The original panel content has been modified by the customer.

CONFIRMATION

Deletion in *POLR3B* has been confirmed by digital PCR.

STEP	DATE
Order date	
Sample received	
Sample in analysis	
Reported	

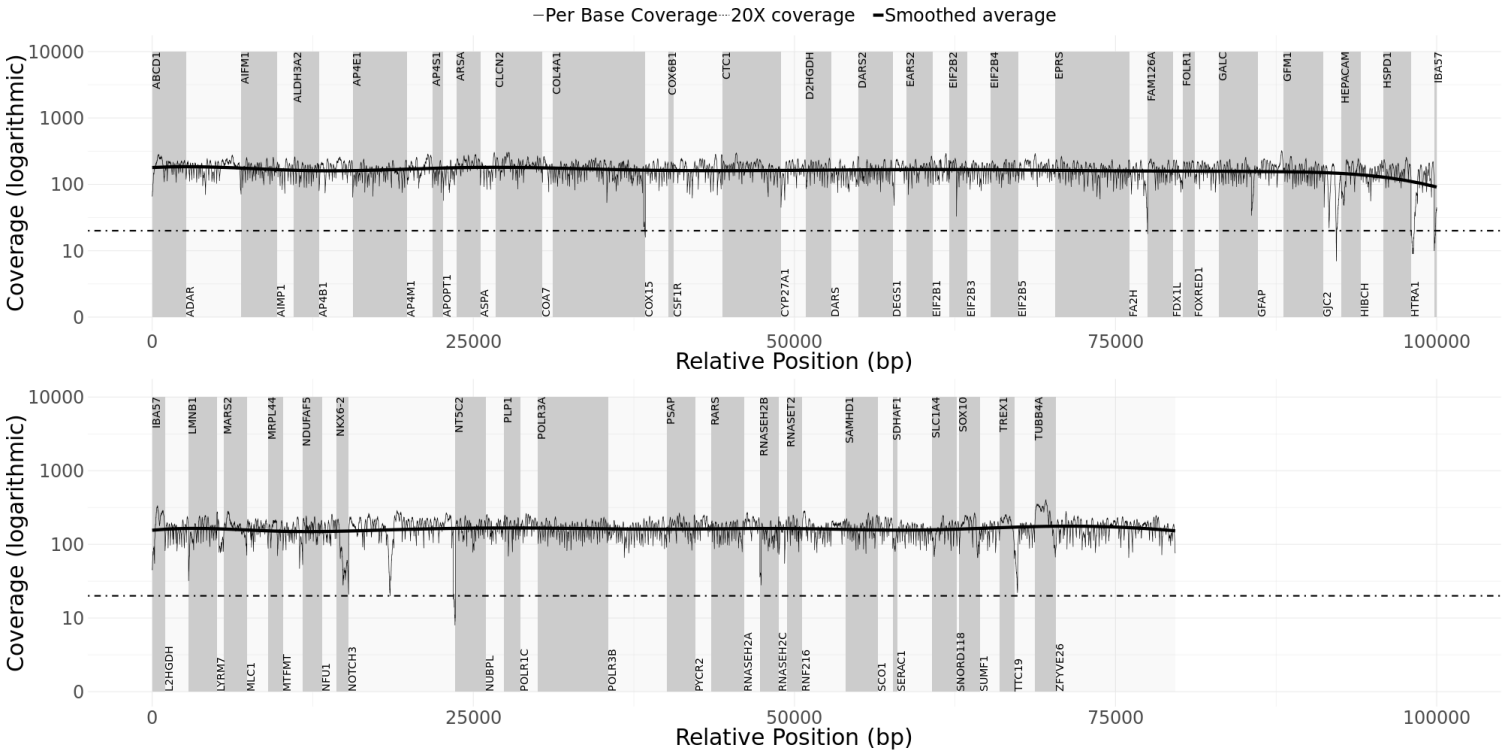
This statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

Signature

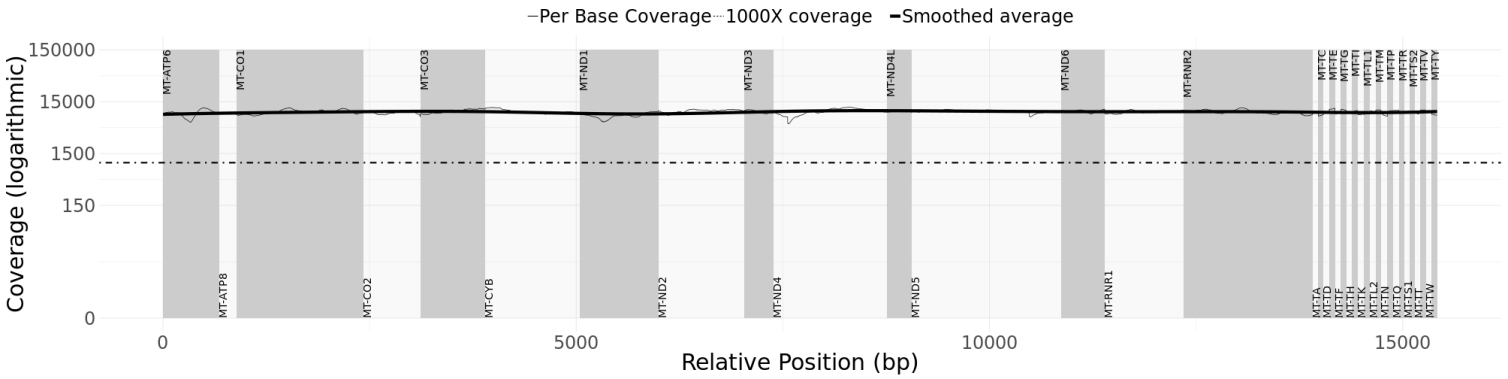
Name

Title

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 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
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 ±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: MD, PhD, CLIA: 99D2092375

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_018122.4(*DARS2*):c.228-22T>C, NM_018122.4(*DARS2*):c.228-22T>A, NM_018122.4(*DARS2*):c.228-21_228-20delTTinsC, NM_018122.4(*DARS2*):c.228-21_228-20delTTinsCC, NM_018122.4(*DARS2*):c.228-16C>A, NM_018122.4(*DARS2*):c.228-16C>G, NM_018122.4(*DARS2*):c.228-15C>G, NM_018122.4(*DARS2*):c.228-15C>A, NM_018122.4(*DARS2*):c.228-12C>A, NM_018122.4(*DARS2*):c.228-11C>G, NM_020435.3(*GJC2*):c.-170A>G, NM_020435.3(*GJC2*):c.-167A>G, NM_020435.3(*GJC2*):c.-20+1G>C, NM_001042465.1(*PSAP*):c.778-26C>A, NM_007055.3(*POLR3A*):c.*18C>T, NM_007055.3(*POLR3A*):c.3337-11T>C, NM_007055.3(*POLR3A*):c.1909+22G>A, NM_007055.3(*POLR3A*):c.1909+18G>A, NM_018082.5(*POLR3B*):c.967-15A>G, NM_018082.5(*POLR3B*):c.1857-12A>G, NM_024570.3(*RNASEH2B*):c.65-13G>A, NM_024570.3(*RNASEH2B*):c.511-13G>A, NM_001845.4(*COL4A1*):c.*35C>A, NM_001845.4(*COL4A1*):c.*32G>A/T, NM_001845.4(*COL4A1*):c.*31G>T, NM_024884.2(*L2HGDH*):c.906+354G>A, NM_000153.3(*GALC*):c.*12G>A, NM_000153.3(*GALC*):c.-66G>C, NM_000153.3(*GALC*):c.-67T>G, NM_001201402.1(*GALC*):c.-74T>A, NM_001201402.1(*GALC*):c.-128C>T, chr17:g.8076761-8076761, chr17:g.8076761-8076761, chr17:g.8076762-8076762, NM_017775.3(*TTC19*):c.-42G>T, NM_001031806.1(*ALDH3A2*):c.681-14T>A/G, NM_001031806.1(*ALDH3A2*):c.681-14T>A, NM_001031806.1(*ALDH3A2*):c.681-14T>G, NM_000435.2(*NOTCH3*):c.341-26_341-24delAAC,

NM_152783.3(*D2HGDH*):c.293-23A>G, NM_024120.4(*NDUFAF5*):c.223-907A>C, NM_006941.3(*SOX10*):c.-84-2A>T, NM_006941.3(*SOX10*):c.-31954C>T, NM_006941.3(*SOX10*):c.-32520C>G, NM_015166.3(*MLC1*):c.895-226T>G, NM_015166.3(*MLC1*):c.-42C>T, NM_000487.5(*ARSA*):c.1108-12C>G, NM_000487.5(*ARSA*):c.1108-20A>G, NM_003907.2(*EIF2B5*):c.685-13C>G, NM_005211.3(*CSF1R*):c.1859-119G>A, NM_032861.3(*SERAC1*):c.92-165C>T, NM_032861.3(*SERAC1*):c.92-239G>C, NM_000533.3(*PLP1*):c.4+78_4+85delGGGGGTTC, NM_000533.3(*PLP1*):c.453+28_453+46delTAACAAGGGGTGGGGGAAA, NM_000533.3(*PLP1*):c.454-322G>A, NM_000533.3(*PLP1*):c.454-314T>A/G, NM_000533.3(*PLP1*):c.454-314T>A, NM_000533.3(*PLP1*):c.454-314T>G, NM_004208.3(*AIFM1*):c.697-44T>G, NM_004208.3(*AIFM1*):c.-123G>C

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.