STEP	DATE
Reported	Sep 26, 2018

On Sep 26, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:

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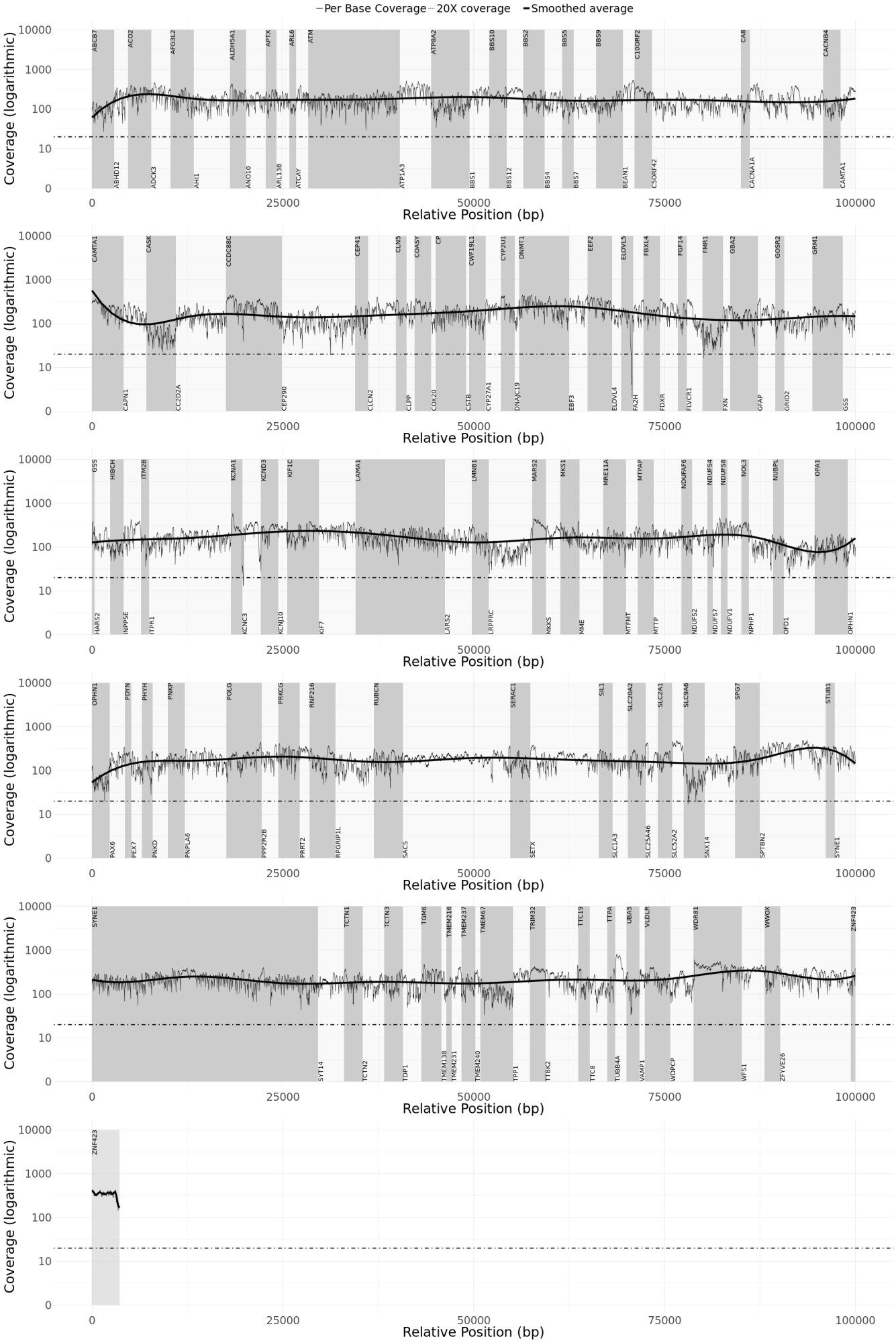
Per Base Coverage
20X coverage
Smoothed average
Coverage (logarithmic)
Relative Position (bp)
ABCB7 ACO2 AFG3L2 ALDH5A1 APTX ARL6 ATM ATP8A2 BBS10 BBS2 BBS5 BBS9 C10ORF2 CA8 CACNB4
ABHD12 ADCK3 AHI1 ANO10 ARL13B ATCAY ATP1A3 BBS1 BBS12 BBS4 BBS7 BEAN1 C5ORF42 CACNA1A CAMTA1
CAMTA1 CASK CCDC88C CEP41 CLN5 COASY CP CWF19L1 CYP2U1 DNMT1 EEF2 ELOVL5 FBXL4 FGF14 FMR1 GBA2 GOSR2 GRM1
CAPN1 CC2D2A CEP290 CLCN2 CLPP COX20 CSTB CYP27A1 DNAJC19 EBF3 ELOVL4 FA2H FDXR FLVCR1 FXN GFAP GRID2 GSS
GSS HIBCH ITM2B KCNA1 KCND3 KIF1C LAMA1 LMNB1 MARS2 MKS1 MRE11A MTPAP NDUFAF6 NDUFS4 NDUFS8 NOL3 NUBPL OPA1
HARS2 INPP5E ITPR1 KCNC3 KCNJ10 KIF7 LARS2 LRPPRC MKKS MME MTFMT MTTP NDUFS2 NDUFS7 NDUFV1 NPHP1 OFD1 OPHN1
OPHN1 PDYN PHYH PNKP POLG PRKCG RNF216 RUBCN SERAC1 SIL1 SLC20A2 SLC2A1 SLC9A6 SPG7 STUB1
PAX6 PEX7 PNKD PNPLA6 PPP2R2B PRRT2 RPGRIP1L SACS SETX SLC1A3 SLC25A46 SLC52A2 SNX14 SPTBN2 SYNE1
SYNE1 TCTN1 TCTN3 TGM6 TMEM216 TMEM237 TMEM67 TRIM32 TTC19 TTPA UBA5 VLDLR WDR81 WWOX ZNF423
SYT14 TCTN2 TDP1 TMEM138 TMEM231 TMEM240 TPP1 TTBK2 TTC8 TUBB4A VAMP1 WDPCP WFS1 ZFYVE26
ZNF423

APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column 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 and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.
Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean 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). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. 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 as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. 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, which processes aligned sequence reads. 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: 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. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon 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 1000 Genomes Project, gnomAD, ClinVar and HGMD. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen, MutationTaster were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as 1000 Genomes Project, Database of Genomic Variants, ExAC, DECIPHER. The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.
Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.
Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). 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.
Regulation and accreditations: This test has not been cleared or approved by the FDA. 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.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000051.3(ATM):c.-174A>G
NM_000051.3(ATM):c.-31+595G>A
NM_000051.3(ATM):c.-30-1G>T
NM_000051.3(ATM):c.1236-404C>T
NM_000051.3(ATM):c.2639-384A>G
NM_000051.3(ATM):c.2839-579_2839-576delAAGT
NM_000051.3(ATM):c.3403-12T>A
NM_000051.3(ATM):c.3994-159A>G
NM_000051.3(ATM):c.5763-1050A>G
NM_024649.4(BBS1):c.951+58C>T
NM_024649.4(BBS1):c.1110+329C>T
NM_033028.4(BBS4):c.77-216delA
NM_152384.2(BBS5):c.619-27T>G
NM_001127221.1(CACNA1A):c.5404-13G>A
NM_025114.3(CEP290):c.6012-12T>A
NM_025114.3(CEP290):c.2991+1655A>G
NM_000178.2(GSS):c.-9+5G>A
NM_002241.4(KCNJ10):c.-1+1G>T
NM_000253.2(MTTP):c.619-5_619-2delTTTA

NM_000253.2(MTTP):c.1237-28A>G
NM_152416.3(NDUFAF6):c.298-768T>C
NM_024407.4(NDUFS7):c.17-1167C>G
NM_025152.2(NUBPL):c.815-27T>C
NM_003611.2(OFD1):c.935+706A>G
NM_003611.2(OFD1):c.1130-22_1130-19delAATT
NM_130837.2(OPA1):c.610+360G>A
NM_130837.2(OPA1):c.610+364G>A
NM_130837.2(OPA1):c.2179-40G>C
NM_000280.4(PAX6):c.*125537G>T
NM_000280.4(PAX6):c.524-41T>G
NM_000280.4(PAX6):c.-52+1G>A
NM_000280.4(PAX6):c.-128-1G>T
NM_000280.4(PAX6):c.-129+2T>A
NM_000288.3(PEX7):c.-45C>T
NM_007254.3(PNKP):c.1387-33_1386+49delCCTCCTCCCCTGACCCC
NM_022464.4(SIL1):c.1030-18G>A
NM_006516.2(SLC2A1):c.680-11G>A
NM_182961.3(SYNE1):c.15918-12A>G
NM_001077416.2(TMEM231):c.824-11T>C
NM_000391.3(TPP1):c.887-18A>G
NM_006005.3(WFS1):c.-43G>T

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant
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
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.