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

Macrocephaly / Overgrowth Syndrome Panel (Malformations) Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL

PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
				CUSTOMER SAMPLE ID

PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE
DNA	

SUMMARY OF RESULTS

PRIMARY FINDINGS

The patient is heterozygous for *PTEN* c.445C>T, p.(Gln149*), which is pathogenic.

PRIMARY FINDINGS: SEQUENCE ALTERATIONS

GENE	TRANSCRIPT	NOMENCLATURE	GENOTYPE	CONSEQUENCE	INHERITANCE	CLASSIFICATION
PTEN	NM_000314.8	c.445C>T, p.(Gln149*)	HET	stop_gained	AD	**Pathogenic**

	ID	ASSEMBLY	POS	REF/ALT	
		GRCh37/hg19	10:89692961	C/T	

gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	PHENOTYPE
0/0	N/A	N/A	disease causing	Bannayan-Riley-Ruvalcaba syndrome, Cowden syndrome, Lhermitte-Duclos syndrome

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Macrocephaly / Overgrowth Syndrome Panel (Malformations)	48	929	190108	189740	189	99.81

TARGET REGION AND GENE LIST

The Blueprint Genetics Macrocephaly / Overgrowth Syndrome Panel Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *AKT1*, *AKT3*, *ASPA*, *ASXL2*, *BRWD3*, *CCND2*, *CDKN1C*, *CHD8*, *CUL4B*, *DHCR24*, *DIS3L2**, *DNMT3A*, *EED*, *EIF2B5*, *EZH2*, *GFAP*, *GLI3*, *GPC3*, *GPSM2*, *GRIA3*, *HEPACAM*, *HUWE1*, *KDM1A*, *KIAA0196*, *KIF7*, *KPTN*, *L1CAM*, *MED12*, *MLC1*, *MPDZ*, *NFIB*, *NFIX*, *NSD1*, *OFD1*, *PIGA**, *PIK3CA**, *PIK3R2*, *PTCH1*, *PTEN**, *RAB39B*, *RNF135*, *SETD2*, *SYN1*, *TMEM94*, *TSC1*, *TSC2*, *UPF3B* and *ZBTB20*. This panel targets protein coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants (listed in the SUMMARY OF THE TEST section). This panel should be used to detect single nucleotide variants and small insertions 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 detect balanced translocations or complex rearrangements, 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 sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 9-year-old child with macrocephaly and venous malformation of the right sole. FHx of an uncle with intracardial haemangioma (SUD). *PTEN*?

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Macrocephaly / Overgrowth Syndrome Panel identified a heterozygous nonsense variant *PTEN* c.445C>T, p.(Gln149*).

PTEN c.445C>T, p.(Gln149*)

This variant is absent 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 generates a premature stop codon in exon 5 (of a total of 9 exons) and is predicted to lead to a loss of normal protein function, either through protein truncation or nonsense-mediated mRNA decay. Loss of function is an established disease-mechanism in this gene (HGMD).

Previously, this variant has been identified in multiple patients with with features of Cowden syndrome/*PTEN* hamartoma tumor syndrome (PHTS) (PMID: 21194675, 22266152, 23335809, 27477328, 28262255, 29273943, 31336731). The variant has been submitted to ClinVar by other clinical testing laboratories (variation ID 404164).

PTEN

The *PTEN* gene (MIM *601728) on chromosome 10q23 encodes a ubiquitously expressed tumor suppressor dual-specificity phosphatase that antagonizes the PI3K signaling pathway through its lipid phosphatase activity and negatively regulates the MAPK pathway through its protein phosphatase activity (summarized in PMID: 18460397). It participates in cell division, normally acting to inhibit cell proliferation and tumor development. The diversity of phenotypes observed in *PTEN* mutation carriers and the numerous organs and cell types affected provide important evidence for the multiple actions of the PI3K/PTEN signaling network in the regulation of many cellular processes. Diseases associated with *PTEN* comprise autosomal dominant disorders (germline variants) with overlapping features such as macrocephaly/autism syndrome (MIM #605309) and the *PTEN* hamartoma tumor syndrome (PHTS) including Cowden syndrome (CS; MIM #158350), Bannayan-Riley-Ruvalcaba syndrome (BRRS; MIM #153480), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome (GeneReviews: NBK1488). Pathogenic variants in *PTEN* can also cause autism spectrum disorders (ASD; MIM #209850) with macrocephaly, congenital malformations, and/or increased risks for cancer.

Cowden syndrome is a multiple hamartoma syndrome with a high risk for benign and malignant tumors of the thyroid, breast, and endometrium. People with Cowden syndrome have also found to have an increased risk for colorectal cancer, kidney cancer, and melanoma. Nearly everyone with Cowden syndrome obtain hamartomas. These growths are typically detected on the skin and mucous membranes (often occurring in the lining of the mouth and nose), but they can also be found in the intestine and elsewhere in the body as well. Affected individuals usually have macrocephaly, trichilemmomas, and papillomatous papules, presenting by the late 20s and over 90% of the patients shows some symptoms of Cowden syndrome before fourth decade (GeneReviews: NBK1488). Macrocephaly defined as having a head circumference two or more standard deviations above the mean is one of the most consistent features of CS, seen in over 90% of patients (PMID: 27471403). The lifetime risk of developing breast cancer is 85%, with an average age of diagnosis between 38 and 46 years, but there may be increased risk for breast cancer also in males (PMID: 11238682). The lifetime risk for thyroid cancer (usually follicular, rarely papillary, but never medullary thyroid cancer) is approximately 35%. The risk for endometrial cancer may approach 28%. CS is a rare condition, affecting approximately one in 200,000 individuals worldwide, although this number

may be an underestimate (PMID: 27471403). Bannayan-Riley-Ruvalcaba is a congenital disorder characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glans penis (GeneReviews: NBK1488). Proteus syndrome is a complex, genetically heterogeneous highly variable disorder involving congenital malformations and hamartomatous overgrowth of multiple tissues; it can be caused by either germline or somatic *PTEN* variants. Proteus-like syndrome is undefined but refers to individuals with significant clinical features of PS who do not meet the diagnostic criteria for PS (GeneReviews: NBK1488).

The majority of Cowden syndrome cases are simplex (GeneReviews: NBK1488). Perhaps 10%-50% of individuals with CS have an affected parent. If the genetic status of the parents is unknown but they have no clinical signs of CS/BRRS and are in their thirties, it is unlikely either parent is heterozygous for a *PTEN* pathogenic variant and the risk to sibs is therefore minimal, since the penetrance of PHTS is close to 99% by the thirties in individuals with a *PTEN* pathogenic variant (GeneReviews: NBK1488).

Macrocephaly/autism syndrome (MIM #605309) caused by heterozygous mutations in *PTEN* is an autosomal dominant disorder characterized by increased head circumference, abnormal facial features, and delayed psychomotor development resulting in autistic behavior or intellectual disability (PMID: 17286265). Also polymicrogyria associates with pathogenic variants in *PTEN* (GeneReviews NBK1329). Some patients may have a primary immunodeficiency disorder with recurrent infections associated with variably abnormal T- and B-cell function (PMID: 27426521). It is recognized that 5-10% of children with macrocephaly and ASD and/or intellectual disability (ID) have a heterozygous pathogenic mutation in *PTEN*. However, the clinical features and course in children with a pathogenic *PTEN* mutation are unclear. A recent retrospective study collected data from 47 patients with *PTEN* mutations from 38 eligible families and reported macrocephaly (average head circumference of +5.7 SD) with developmental delay, ID and/or ASD to be the most common presenting signs/symptoms (66%) (PMID: 28526761). Other clinical features in this study included dermatological findings (66%), gastrointestinal (GI) symptoms (34%), ASD diagnosis (50%), abnormal brain imaging (53% of those examined) and abnormal thyroid imaging (26%).

There are currently over 700 variants in *PTEN* annotated as disease-causing (DM) in the HGMD Professional variant database (version 2022.2), including missense and truncating variants (nonsense, frameshift, variants affecting splicing), small inframe deletions and insertions, gross deletions and insertions as well as complex rearrangements. In addition to being mutated in the germline in PHTS, somatic loss-of-function *PTEN* mutations are seen in a wide range of sporadic human tumors.

Mutation nomenclature is based on GenBank accession NM_000314.8 (*PTEN*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

PTEN c.445C>T, p.(Gln149*) is classified as pathogenic, based on currently available evidence supporting its disease-causing role (established association between the gene and the patient's phenotype, the variant's absence in control populations, previous identification of the variant in multiple individuals with the same phenotype, and variant type (nonsense)). Disease caused by *PTEN* variants is inherited in an autosomal dominant manner. Any offspring of the patient are at 50% risk of inheriting the variant and of being affected. *PTEN*-related disease may be caused by a de novo variant. Genetic counseling and family member testing are recommended.

| 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.
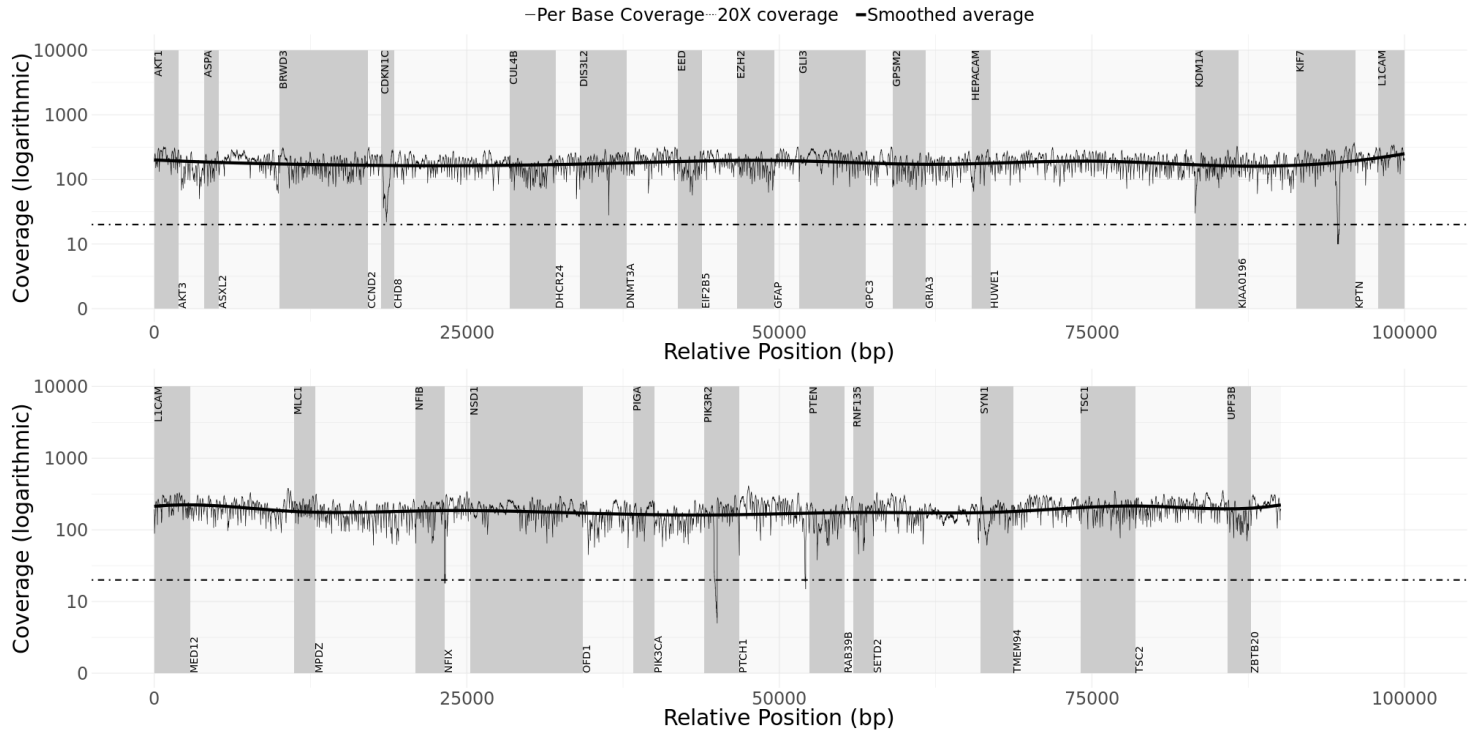
APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: When required, the total genomic DNA was extracted from the biological sample using bead-based method. Quantity of DNA was assessed using fluorometric method. After assessment of DNA quantity, 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 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. Inquiries regarding potential changes to the classification of the variant is strongly recommended prior to making any future clinical decisions. 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.

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).

The sample was analyzed using CE marked Blueprint Genetics CES Platform and/or Blueprint Genetics WES Platform in vitro diagnostic medical device manufactured by Blueprint Genetics Oy. For more information please see Accreditations and Certifications.

PERFORMING SITE:

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

- DNA extraction and QC
- Next-generation sequencing
- Bioinformatic analysis
- Confirmation of sequence alterations
- Confirmation of copy number variants
- Interpretation

NON-CODING VARIANTS COVERED BY THE PANEL:

chr10:g.89622883-89623482, NM_000314.6(*PTEN*):c.-1239A>G, NM_000314.6(*PTEN*):c.-1178C>T, NM_000314.6(*PTEN*):c.-1171C>T, NM_000314.6(*PTEN*):c.-1111A>G, NM_000314.4(*PTEN*):c.-1001T>C, NM_000314.4(*PTEN*):c.-931G>A, NM_000314.4(*PTEN*):c.-921G>T, NM_000314.4(*PTEN*):c.-896T>C, NM_000314.4(*PTEN*):c.-862G>T, NM_000314.4(*PTEN*):c.-854C>G, NM_000314.4(*PTEN*):c.-835C>T, NM_000314.4(*PTEN*):c.-799G>C, NM_000314.4(*PTEN*):c.-765G>A, NM_000314.4(*PTEN*):c.210-8dupT, NM_000314.4(*PTEN*):c.254-21G>C, NM_000314.4(*PTEN*):c.*65T>A, NM_000314.4(*PTEN*):c.*75_*92delTAATGGCAATAGGACATTinsCTATGGCAATAGGACATTG, NM_000076.2(*CDKN1C*):c.*5+20G>T, NM_000548.3(*TSC2*):c.-30+1G>C, NM_000548.3(*TSC2*):c.600-145C>T, NM_000548.3(*TSC2*):c.848+281C>T, NM_000548.3(*TSC2*):c.976-15G>A, NM_000548.3(*TSC2*):c.2838-122G>A, NM_000548.3(*TSC2*):c.5069-18A>G, NM_015166.3(*MLC1*):c.895-226T>G, NM_015166.3(*MLC1*):c.-42C>T, NM_003907.2(*EIF2B5*):c.685-13C>G, NM_000264.3(*PTCH1*):c.2561-2057A>G, NM_000368.4(*TSC1*):c.363+668G>A,

NM_003611.2(*OFD1*):c.935+706A>G, NM_003611.2(*OFD1*):c.1130-22_1130-19delAATT, NM_003611.2(*OFD1*):c.1130-20_1130-16delTTGGT, NM_000425.4(*L1CAM*):c.3531-12G>A, NM_000425.4(*L1CAM*):c.2432-19A>C, NM_000425.4(*L1CAM*):c.1704-75G>T, NM_000425.4(*L1CAM*):c.1547-14delC, NM_000425.4(*L1CAM*):c.523+12C>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.