Blueprint Genetics

Macrocephaly / Overgrowth Syndrome Panel

Test code: MA1401

Is a 48 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical suspicion of syndromes resulting in early overgrowth or macrocephaly.

About Macrocephaly / Overgrowth Syndrome

Macrocephaly is a condition in which the head is abnormally large (circumference > +2.5 SD of normal for weight and gender). Many people with an unusually large head and large skull are healthy, however macrocephaly is also a feature of several syndromes. Macrocephaly may be due to megalencephaly (true enlargement of the brain) or due to other conditions such as hydrocephalus or cranial thickening and is a common reason for referral to the genetics clinic. Macrocephaly is associated with many genetic disorders and this panel can be used for their differential diagnostics. Syndromic and nonsyndromic forms of pathologic macrocephaly may be caused by congenital anatomic abnormalities or genetic conditions, but the disease may also be nongenetic and caused by environmental events. The genetic macrocephaly conditions cover a broad spectrum of gene disorders and their related proteins have diverse biological functions. As of yet it is not clear what precise biological pathways lead to generalized brain overgrowth, but several genes have been identified. Genetic types of macrocephaly include: 1) familial macrocephaly (benign asymptomatic), 2) autism disorder (multifactorial, non-syndromic type), 3) syndrome associations (multiple types) 3A) with cutaneous findings (*PTEN* hamartoma syndromes, neurofibromatosis, type 1 hemimegalencephaly), 3B) with overgrowth (Sotos, Weaver, Macrocephaly-Cutis Marmorata Telangiectasia Congenita, Simpson-Golabi-Behmel, Beckwith-Wiedemann Syndrome), 3C) neuro-cardio-facial-cutaneous syndromes (Noonan, Costello, Cardiofaciocutaneous, LEOPARD) with intellectual disability (Fragile X syndromes), 4) metabolic types with leukodystrophy (Alexander, Canavan, megalencephalic leukodystrophy, organic acidurias, glutaric aciduria, type 1, D-2-hydroxyglutaric aciduria) and 5) hydrocephalus (aqueductal stenosis types and multifactorial, non-obstructive types).

Availability

4 weeks

Gene Set Description

Genes in the Macrocephaly / Overgrowth Syndrome Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
AKT1	Proteus syndrome, Cowden syndrome	AD	5	6
AKT3	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome	AD	13	28
ASPA	Aspartoacylase deficiency (Canavan disease)	AR	54	102
ASXL2	Shashi-Pena syndrome	AD	8	6
BRWD3	Mental retardation	XL	9	17
CCND2	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome	AD	8	9
CDKN1C	Beckwith-Wiedemann syndrome, IMAGE syndrome	AD	35	81
CHD8	Autism	AD	41	66
CUL4B	Mental retardation, syndromic, Cabezas	XL	23	38
DHCR24	Desmosterolosis	AR	6	9

DIS3L2*	Perlman syndrome	AR	12	14
DNMT3A	Tatton-Brown-Rahman syndrome	AD	41	48
EED	Cohen-Gibson syndrome	AD	5	8
EIF2B5	Leukoencephalopathy with vanishing white matter, Ovarioleukodystrophy	AR	20	98
EZH2	Weaver syndrome	AD	29	41
GFAP	Alexander disease	AD	114	131
GLI3	Acrocallosal syndrome, Pallister-Hall syndrome, Grieg cephalopolysndactyly syndrome, Postaxial polydactyly type A, Preaxial polydactyly type 3, Preaxial polydactyly type 4	AD	70	235
GPC3	Simpson-Golabi-Behmel syndrome	XL	33	75
GPSM2	Deafness, Chudley-McCullough syndrome	AR	18	11
GRIA3	Mental retardation	XL	12	23
HEPACAM	Megalencephalic leukoencephalopathy with subcortical cysts, remitting	AD/AR	12	26
HUWE1	Mental retardation, syndromic, Turner	XL	37	54
KDM1A	Cleft palate, psychomotor retardation, and distinctive facial features	AD	5	17
KIAA0196	Spastic paraplegia, Ritscher-Schinzel syndrome (3C syndrome)	AD/AR	15	18
KIF7	Acrocallosal syndrome, Hydrolethalus syndrome, Al-Gazali-Bakalinova syndrome, Joubert syndrome	AR/Digenic	24	44
KPTN	Mental retardation, autosomal recessive 41	AR	5	5
L1CAM	Mental retardation, aphasia, shuffling gait, and adducted thumbs (MASA) syndrome, Hydrocephalus due to congenital stenosis of aqueduct of Sylvius, Spastic, CRASH syndrome, Corpus callosum, partial agenesis	XL	80	292
MED12	Ohdo syndrome, Mental retardation, with Marfanoid habitus, FG syndrome, Opitz-Kaveggia syndrome, Lujan-Fryns syndrome	XL	29	30
MLC1	Megalencephalic leukoencephalopathy with subcortical cysts	AR	39	108
MPDZ	Hydrocephalus, nonsyndromic, autosomal recessive 2	AR	14	24
NFIB	Macrocephaly	AD	17	2
NFIX	Marshall-Smithsyndrome, Sotos syndrome 2	AD	49	78
NSD1	Sotos syndrome, Weaver syndrome, Beckwith-Wiedemann syndrome	AD	329	517
OFD1	Simpson-Golabi-Behmel syndrome, Retinitis pigmentosa, Orofaciodigital syndrome, Joubert syndrome	XL	153	160
PIGA*	Multiple congenital anomalies-hypotonia-seizures syndrome	XL	24	27
PIK3CA*	Cowden syndrome, CLOVES	AD	85	56
PIK3R2	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 1	AD	8	8

PTCH1	Basal cell nevus syndrome	AD	193	522
PTEN*	Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos syndrome, Cowden syndrome	AD	435	638
RAB39B	Waisman parkinsonism-mental retardation syndrome, Mental retardation	XL	6	17
RNF135	Macrocephaly, macrosomia, facial dysmorphism syndrome	AD	6	6
SETD2	Luscan-Lumish syndrome	AD	10	17
SYN1	Epilepsy, with variable learning disabilities and behavior disorders	XL	12	8
TMEM94	Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart (NEDBEH)	AR		3
TSC1	Lymphangioleiomyomatosis, Tuberous sclerosis	AD	177	372
TSC2	Lymphangioleiomyomatosis, Tuberous sclerosis	AD	396	1195
UPF3B	Mental retardation, syndromic	XL	9	21
ZBTB20	Primrose syndrome	AD	17	23

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Some, or all, of the gene is duplicated in the genome. Read more.

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The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#). Due to possible limitations these genes may not be available as single gene tests.

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number
CDKN1C	Chr11:2905209	c.*5+20G>T	NM_000076.2	rs760540648
EIF2B5	Chr3:183855941	c.685-13C>G	NM_003907.2	
L1CAM	ChrX:153128846	c.3531-12G>A	NM_000425.4	
L1CAM	ChrX:153131293	c.2432-19A>C	NM_000425.4	

L1CAM	ChrX:153133652	c.1704-75G>T	NM_000425.4	
L1CAM	ChrX:153133926	c.1547-14delC	NM_000425.4	
L1CAM	ChrX:153136500	c.523+12C>T	NM_000425.4	
MLC1	Chr22:50502853	c.895-226T>G	NM_015166.3	
MLC1	Chr22:50523373	c.-42C>T	NM_015166.3	rs771159578
OFD1	ChrX:13768358	c.935+706A>G	NM_003611.2	rs730880283
OFD1	ChrX:13773245	c.1130-22_1130-19delAATT	NM_003611.2	rs312262865
OFD1	ChrX:13773249	c.1130-20_1130-16delTTGGT	NM_003611.2	
PTCH1	Chr9:98226337	c.2561-2057A>G	NM_000264.3	
PTEN	Chr10:89622883–89623482			
PTEN	Chr10:89622988	c.-1239A>G	NM_000314.6	
PTEN	Chr10:89623049	c.-1178C>T	NM_000314.6	
PTEN	Chr10:89623056	c.-1171C>T	NM_000314.6	rs587779981
PTEN	Chr10:89623116	c.-1111A>G	NM_000314.6	
PTEN	Chr10:89623226	c.-1001T>C	NM_000314.4	
PTEN	Chr10:89623296	c.-931G>A	NM_000314.4	rs587781959
PTEN	Chr10:89623306	c.-921G>T	NM_000314.4	
PTEN	Chr10:89623331	c.-896T>C	NM_000314.4	
PTEN	Chr10:89623365	c.-862G>T	NM_000314.4	rs587776675
PTEN	Chr10:89623373	c.-854C>G	NM_000314.4	
PTEN	Chr10:89623392	c.-835C>T	NM_000314.4	rs587779994
PTEN	Chr10:89623428	c.-799G>C	NM_000314.4	rs587779992
PTEN	Chr10:89623462	c.-765G>A	NM_000314.4	
PTEN	Chr10:89690791	c.210-8dupT	NM_000314.4	
PTEN	Chr10:89692749	c.254-21G>C	NM_000314.4	
PTEN	Chr10:89725294	c.*65T>A	NM_000314.4	
PTEN	Chr10:89725304	c.*75_*92delTAATGGCAATAGGACATTinsCTATGGCAATAGGACATTG	NM_000314.4	
TSC1	Chr9:135800306	c.363+668G>A	NM_000368.4	
TSC2	Chr16:2098067	c.-30+1G>C	NM_000548.3	rs587778004
TSC2	Chr16:2106052	c.600-145C>T	NM_000548.3	
TSC2	Chr16:2107460	c.848+281C>T	NM_000548.3	rs45517132
TSC2	Chr16:2110656	c.976-15G>A	NM_000548.3	rs45517150
TSC2	Chr16:2127477	c.2838-122G>A	NM_000548.3	
TSC2	Chr16:2138031	c.5069-18A>G	NM_000548.3	rs45484794

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

Genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

Test Performance

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection

performance table for details regarding our ability to detect different types of alterations (Table).

Assays have been validated for various sample types including EDTA-blood, isolated DNA (excluding from formalin fixed paraffin embedded tissue), saliva and dry blood spots (filter cards). These sample types were selected in order to maximize the likelihood for high-quality DNA yield. The diagnostic yield varies depending on the assay used, referring healthcare professional, hospital and country. Plus analysis increases the likelihood of finding a genetic diagnosis for your patient, as large deletions and duplications cannot be detected using sequence analysis alone. Blueprint Genetics' Plus Analysis is a combination of both sequencing and deletion/duplication (copy number variant (CNV)) analysis.

The performance metrics listed below are from an initial validation performed at our main laboratory in Finland. The performance metrics of our laboratory in Seattle, WA, are equivalent.

Performance of Blueprint Genetics high-quality, clinical grade NGS sequencing assay for panels.

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	99.2% (7,745/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Microdeletion/-duplication sdrs (large CNVs, n=37))		
Size range (0.1-47 Mb)	100% (25/25)	

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth	143X
Nucleotides with >20x sequencing coverage (%)	99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

	Sensitivity %	Specificity %
ANALYTIC VALIDATION (NA samples; n=4)		

Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2026 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.3% (12/13)	99.98%
Heteroplasmic (<5%)	88.9% (48/54)	99.93%
Insertions and deletions by sequence analysis n=40 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	99,997%
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		
Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%

Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding and regulatory variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the 'Test limitations" section above. If the test includes the mitochondrial genome the target region gene list contains the mitochondrial genes. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases including, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, *in silico* variant prediction tools such as SIFT, PolyPhen,MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, ordering providers have access to the details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with suboptimal coverage (<20X for nuclear genes and <1000X for mtDNA) if applicable. This reflects our mission to build fully transparent diagnostics where ordering providers can easily visualize the crucial details of the analysis process.

Clinical Interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the corner stone of clinical interpretation and resulting patient management decisions. Our classifications follow the ACMG guideline 2015.

The final step in the analysis is orthogonal confirmation. Sequence and copy number variants classified as pathogenic, likely pathogenic and variants of uncertain significance (VUS) are confirmed using bi-directional Sanger sequencing or by orthogonal methods such as qPCR/ddPCR when they do not meet our stringent NGS quality metrics for a true positive call.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information

(genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene's variation in population cohorts and detailed information about related phenotypes. We also provide links to the references, abstracts and variant databases used to help ordering providers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification. We do not recommend using variants of uncertain significance (VUS) for family member risk stratification or patient management. Genetic counseling is recommended.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Our internal database and our understanding of variants and related phenotypes increases with every case analyzed. Our laboratory is therefore well-positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

CPT code(s) *

81236, 81321, 81323, 81405 x3, 81406 x4, 81407 x3, 81479

* The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.

ICD Codes

Refer to the most current version of ICD-10-CM manual for a complete list of ICD-10 codes.

Sample Requirements

- Blood (min. 1ml) in an EDTA tube
- Extracted DNA, min. 2 µg in TE buffer or equivalent
- Saliva (Please see Sample Requirements for accepted saliva kits)

Label the sample tube with your patient's name, date of birth and the date of sample collection.

We do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue. In addition, if the patient is affected with a hematological malignancy, DNA extracted from a non-hematological source (e.g. skin fibroblasts) is strongly recommended.

Please note that, in rare cases, mitochondrial genome (mtDNA) variants may not be detectable in blood or saliva in which case DNA extracted from post-mitotic tissue such as skeletal muscle may be a better option.

Read more about our sample requirements here.

For Patients

Other

- *PTEN* Hamartoma Tumor Syndrome Foundation
- Child Growth Foundation

- Child Growth Foundation - Sotos Syndrome
- GeneReviews - *PIK3CA*-Related Segmental Overgrowth
- GeneReviews - *PTEN* Hamartoma Tumor Syndrome
- GeneReviews - Megalencephalic Leukoencephalopathy with Subcortical Cysts
- GeneReviews - PIK3CA-Related Segmental Overgrowth
- GeneReviews - Sotos Syndrome
- M-CM Network
- NORD - Beckwith-Wiedemann Syndrome
- NORD - BeckwithSotos Syndrome
- NORD - Cutis Marmorata Telangiectatica Congenita
- NORD - Megalencephaly-Capillary Malformation
- NORD - Sotos Syndrome
- Sotos Syndrome Support Association