Blueprint Genetics

Microcephaly and Pontocerebellar Hypoplasia Panel

Test code: MA0701

Is a 78 gene panel that includes assessment of non-coding variants.

Is ideal for patients with a clinical suspicion of microcephaly or pontocerebellar hypoplasias.

About Microcephaly and Pontocerebellar Hypoplasia

Microcephaly is a neurodevelopmental disorder. It is usually defined as a head circumference (HC) more than two (or three) standard deviations below the mean for age and sex and serves as an important neurological indication or warning sign, however uniformity in its definition is lacking. Microcephaly may be congenital or develop in the first few years of life. In general, life expectancy for individuals with microcephaly is reduced and the prognosis for normal brain function is poor. It may stem from a wide variety of conditions that cause abnormal growth of the brain, or from syndromes associated with chromosomal abnormalities. A homozygous mutation in one of the microcephalin genes (*MCPH1, ASPM, WDR62*) causes primary microcephaly. Najm type X-linked intellectual deficit (point mutations and deletions in the *CASK* gene) is a rare cerebellar dysgenesis syndrome associated with microcephaly in most cases. Examples of monogenic syndromes associated with microcephaly are Seckel syndrome spectrum disorders. Nonsyndromic pontocerebellar hypoplasias (PCH) are a rare heterogeneous group of diseases characterized by hypoplasia and atrophy and/or early neurodegeneration of the cerebellum and pons. PCH patients of all subtypes present with progressive microencephaly, delayed or absence of cognitive and voluntary motor development, intellectual deficit, spasticity, chorea/dyskinesia, swallowing difficulties and seizures. The majority of PCH cases are caused by mutations in tRNA splicing endonuclease (TSEN genes). Approximately half the cases of PCH subtype 1 are due to mutations in the *EXOSC3* gene. Other subtypes include mutations in for example *TSEN2* and *TSEN54* genes. Diagnosis is made based on clinical symptoms and neuroradiological findings (MRI) and can be confirmed by molecular genetic analyses. Nonsyndromic pontocerebellar hypoplasias (PCH) are generally inherited in an autosomal recessive pattern. Isolated microcephaly is known to have autosomal dominant, autosomal recessive and X-linked inheritance.

Availability

4 weeks

Gene Set Description

Genes in the Microcephaly and Pontocerebellar Hypoplasia Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
AKT3	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome	AD	13	28
AMPD2	Pontocerebellar hypoplasia type 9, Spastic paraplegia 63	AR	14	18
ASPM	Microcephaly	AR	176	212
ASXL1	Bohring-Opitz syndrome	AD	41	39
ASXL3	Bainbridge-Ropers syndrome	AD	45	49
ATR	Cutaneous telangiectasia and cancer syndrome, Seckel syndrome	AD/AR	10	33
CASK	Mental retardation and microcephaly with pontine and cerebellar hypoplasia, FG syndrome, Mental retardation	XL	87	112
CCDC47	Microcephaly, Malformations	AR		1
CDK5RAP2	Microcephaly	AR	19	21

CENPF	Ciliary dyskinesia -Lethal Ciliopathy	AR	13	8
CENPJ	Seckel syndrome, Microcephaly	AR	34	9
CEP152#	Seckel syndrome, Microcephaly	AR	20	20
CEP63	Seckel syndrome	AR	7	2
CSNK2A1		AD	14	20
DONSON	Microcephaly, short stature, and limb abnormalities (MISSLA), Microcephaly-Micromelia syndrome	AR	10	19
DYNC1H1	Spinal muscular atrophy, Charcot-Marie-Tooth disease, Mental retardation	AD	60	71
DYRK1A	Mental retardation	AD	94	77
EFTUD2	Mandibulofacial dysostosis with microcephaly, Esophageal atresia, syndromic	AD	45	99
EXOSC3	Pontocerebellar hypoplasia	AR	11	19
GFM1	Combined oxidative phosphorylation deficiency	AR	19	19
GPT2	Mental retardation, autosomal recessive 49, Microcephaly, Spastic paraplegia	AR	5	7
KANSL1*	Koolen-de Vries syndrome	AD	61	64
KATNB1	Lissencephaly 6, with microcephaly	AR	6	10
KIF11	Microcephaly	AD	39	69
LIG4	Severe combined immunodeficiency with sensitivity to ionizing radiation, LIG4 syndrome	AR	18	36
MBD5	Mental retardation	AD	62	90
MCPH1	Microcephaly	AR	23	32
MED17	Microcephaly, postnatal progressive, with seizures and brain atrophy	AR	4	4
MFSD2A	Microcephaly 15, primary, autosomal recessive	AR	4	4
MIPEP	Combined oxidative phosphorylation deficiency 31	AR	5	8
MRE11A	Ataxia-telangiectasia-like disorder-1	AR	57	56
MYCN	Feingold syndrome	AD	27	41
MYO18B	Klippel-Feil syndrome 4, autosomal recessive, with myopathy and facial dysmorphism	AR	2	4
NCAPD3	Microcephaly	AR	3	5
NDE1	Microhydranencephaly, Lissencephaly	AR	13	18
NHEJ1	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation	AR	15	16

OPHN1	Mental retardation, with cerebellar hypoplasia and distinctive facial appearance	XL	28	42
PAFAH1B1	Lissencephaly, Subcortical laminar heterotopia	AD	121	169
PCDH12	Microcephaly	AR	1	6
PCLO	Pontocerebellar hypoplasia	AR	1	2
PCNT	Microcephalic osteodysplastic primordial dwarfism	AR	49	88
PHGDH	Neu-Laxova syndrome 1	AR	13	23
PLK4	Microcephaly and chorioretinopathy, autosomal recessive 2	AR	3	6
PNKP	Epileptic encephalopathy, early infantile, Ataxia-oculomotor	AR	34	23
POMT1	Muscular dystrophy-dystroglycanopathy	AR	47	96
PQBP1	Renpenning syndrome	XL	14	18
QARS	Microcephaly, progressive, seizures, and cerebral and cerebellar atrophy	AR	14	10
RARS2#	Pontocerebellar hypoplasia	AR	23	37
RTTN	Microcephaly, short stature, and polymicrogyria with or without seizures	AR	16	16
SEPSECS	Pontocerebellar hypoplasia, type 2D	AR	10	15
SLC1A4	Spastic tetraplegia, thin corpus callosum, and progressive microcephaly	AR	4	8
SMARCA2	Nicolaides-Baraitser syndrome	AD	41	73
SMARCE1	Coffin-Siris syndrome	AD	14	12
SOX11	Mental retardation, autosomal dominant 27	AD	11	14
STAG2	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder	XL	6	14
STAMBP	Microcephaly-capillary malformation syndrome	AR	15	19
STIL	Microcephaly	AR	13	17
TBC1D20	Warburg micro syndrome 4	AR	6	6
TBC1D23	Pontocerebellar hypoplasia, type 11		5	9
THOC6	Microcephaly	AR	8	9
TMTC3	Lissencephaly 8		6	10
TOE1	Pontocerebellar hypoplasia type 7		11	12
TOP3A			8	
TRMT10A	Microcephaly, short stature, and impaired glucose metabolism 1	AR	2	7
TSEN2	Pontocerebellar hypoplasia	AR	8	5
TSEN54	Pontocerebellar hypoplasia	AR	23	21

TUBB	Congenital symmetric circumferential skin creases 1, Cortical dysplasia, complex, with other brain malformations 6	AD	11	7
TUBB2B*,#	Polymicrogyria, asymmetric	AD	21	30
TUBGCP4	Microcephaly and chorioretinopathy, autosomal recessive 3	AR	7	6
TUBGCP6	Microcephaly and chorioretinopathy, autosomal recessive 1	AR	16	7
UBE3B	Blepharophimosis-Ptosis-Intellectual-Disability syndrome (Kaufman oculocerebrofacial syndrome)	AR	14	24
VARS	Early-onset progressive encephalopathy with brain atrophy and thin corpus callosum (PEBAT), Encephalopathy, progressive	AR	12	6
VRK1	Pontocerebellar hypoplasia	AR	9	9
WDR62	Microcephaly	AR	33	48
WDR73	Galloway-Mowat syndrome	AR	9	12
XRCC4	Short stature, microcephaly, and endocrine dysfunction	AR	9	10
ZNF148	Global developmental delay, absent or hypoplastic corpus callosum, and dysmorphic facies (GDACCF)		5	4
ZNF335	Microcephaly 10, primary, autosomal recessive	AR	8	12

*

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

#

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

ASPM	Chr1:197097820	c.2761-25A>G	NM_018136.4	rs199422149
CDK5RAP2	Chr9:123182253	c.4005-15A>G	NM_018249.5	rs387906274
CEP152	Chr15:49059406	c.2148-17G>A	NM_001194998.1	rs751691427
DONSON	Chr21:34955994	c.786-22A>G	NM_017613.3	rs1135401960
EXOSC3	Chr9:37782146	c.475-12A>G	NM_016042.3	rs370087266
NCAPD3	Chr11:134086816	c.382+14A>G	NM_015261.2	
PNKP	Chr19:50364799	c.1387-33_1386+49delCCTCCTCCCCTGACCCC	NM_007254.3	rs752902474
POMT1	Chr9:134379574	c.-30-2A>G	NM_007171.3	
RARS2	Chr6:88244587	c.613-3927C>T	NM_020320.3	
RTTN	Chr18:67727297	c.4748-19T>A	NM_173630.3	
RTTN	Chr18:67815044	c.2309+1093G>A	NM_173630.3	
STAMBP	Chr2:74077998	c.1005+358A>G	NM_006463.4	
XRCC4	Chr5:82400728	c.-10-1G>T	NM_022406.2	rs869320678

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

The following exons are not included in the panel as they are not sufficiently covered with high quality sequence reads: *MCPH1* (NM_001322042:14), *TSEN2* (NM_001321278:12). Genes with suboptimal coverage in our assay are marked with number sign (#) and 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. Gene is considered to have suboptimal coverage when >90% of the gene's target nucleotides are not covered at >20x with mapping quality score (MQ>20) reads. 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) *

81175, 81404, 81405 X2, 81406 X2, 81407 X2, 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

- Cornelia de Lange –oireyhtymä
- Cortical Foundation
- Foundation for Children with Microcephaly
- GeneReviews - *CASK*-Related Disorders
- GeneReviews - *EXOSC3* -Related Pontocerebellar Hypoplasia
- GeneReviews - *TSEN54* -Related Pontocerebellar Hypoplasia
- GeneReviews - CASK-Related Disorders
- GeneReviews - Primary Autosomal Recessive Microcephalies and Seckel Syndrome Spectrum Disorders
- GeneReviews - Seckel Syndrome
- GeneReviews - Smith-Lemli-Opitz Syndrome
- Microcephaly Support Group UK
- NORD - Cornelia de Lange Syndrome
- NORD - Pontocerebellar Hypoplasia
- NORD - Seckel Syndrome
- NORD - Smith-Lemli-Opitz Syndrome
- Smith Lemli Opitz Foundation