



Genetic Testing for Autosomal Recessive Primary Microcephaly

Clinical Features

Autosomal Recessive Primary Microcephaly (MCPH) is characterized by:

- Congenital microcephaly (3 SD below the mean at birth or at least 4 SD below the mean at later ages)
- Intellectual disability (ID), but no other neurological findings (febrile or other mild seizures do not exclude the diagnosis)
- Normal or mildly short stature that is less severe than the markedly small head circumference
- Normal facial appearance except for features of apparent microcephaly

Brain imaging in most cases of autosomal recessive primary microcephaly shows a reduced number of gyri and in some patients may also demonstrate agenesis of the corpus callosum. Microcephaly is typically congenital with a decreased head circumference apparent by 32 weeks of gestation, although variability exists. The relative degree of microcephaly doesn't vary throughout life and doesn't vary within a family by more than 2 SD. ID is usually mild to moderate with no progressive decline or motor deficit (1).

Molecular Genetics

Mutations in the *ASPM* [OMIM #605481] gene are the most common cause of autosomal recessive primary microcephaly (2). Approximately 40% of patients (both consanguineous and non-consanguineous) with a strict diagnosis of autosomal recessive primary microcephaly have mutations in *ASPM*. However, very few patients (<10%) with a less restrictive phenotype have mutations in *ASPM* (3). To date, over 85 mutations have been reported in the *ASPM* gene, spanning most of the 28 coding exons. Most *ASPM* mutations are predicted to result in a truncated protein.

Our Comprehensive Autosomal Recessive Microcephaly Panel includes sequence analysis of the 25 genes listed below, and deletion/duplication analysis of the 17 genes listed in bold.

Comprehensive Autosomal Recessive Primary Microcephaly Panel				
AGMO	CDK6	CEP152	MFSD2A	SLC25A19
ARFGEF2	CENPE	CEP63	NDE1	STAMPB
ASPM	CENPF	CIT	PHC1	STIL
CASC5	CENPJ	MCPH1	PNKP	WDR62
CDK5RAP2	CEP135	MED17	SASS6	ZNF335

Gene	Clinical Features and Molecular Pathology
<i>AGMO</i> [OMIM#613738]	Novel homozygous mutations in <i>AGMO</i> were identified in a consanguineous Saudi Arabian family with two children affected with primary microcephaly, developmental delay, short stature and intellectual disability (4).
<i>ARFGEF2</i> [OMIM#605371]	Missense and frameshift mutations were identified in two Turkish families with autosomal recessive periventricular heterotopia with microcephaly which is characterized by microcephaly, periventricular heterotopia, ID and recurrent infections (5).
<i>ASPM</i> [OMIM#]	Mutations in the <i>ASPM</i> gene are the most common cause of autosomal recessive primary microcephaly (2). Approximately 40% of patients with a strict diagnosis of MCPH have mutations in <i>ASPM</i> . However, fewer patients (<10%) with a less restrictive phenotype have mutations in <i>ASPM</i> (3).
<i>CASC5</i> [OMIM#609173]	Genin <i>et al.</i> (2012) identified the same <i>CASC5</i> frameshift mutation in the homozygous state in three separate consanguineous families with primary microcephaly (6). The <i>CASC5</i> protein is required for proper microtubule attachment to the chromosome centromere and for spindle-assembly checkpoint activation during mitosis (6).
<i>CDK5RAP2</i> [OMIM #608201]	Homozygous mutations in <i>CDK5RAP2</i> have been identified in three Pakistani families with autosomal recessive primary microcephaly (7, 8). <i>CDK5RAP2</i> is a centrosomal protein and may be involved in microtubule production during mitosis (1).
<i>CDK6</i> [OMIM#603368]	Homozygous missense mutations in <i>CDK6</i> were identified in a large Pakistani family with 10 individuals presenting with microcephaly (-4 SD to -6SD), sloping foreheads, and mild intellectual disability (9).
<i>CENPE</i>	Compound heterozygous mutations in <i>CENPE</i> have been described in one family with affected siblings

[OMIM# 117143]	with primary microcephaly, short stature, and severe developmental delays (10). Brain imaging showed simplified gyral pattern, partial agenesis of the corpus callosum and cerebellar hypoplasia.
<i>CENPF</i> [OMIM# 600236]	Truncating mutations in <i>CENPF</i> have been reported on one family with an affected child with autosomal recessive primary microcephaly (11).
<i>CENPJ</i> [OMIM #609279]	Four Pakistani families with autosomal recessive primary microcephaly have been reported with homozygous mutations in <i>CENPJ</i> (8, 12). <i>CENPJ</i> is a centrosomal protein and likely shares a very similar role with <i>CDK5RAP2</i> (1).
<i>CEP135</i> [OMIM#614673]	Hussain <i>et al.</i> identified a homozygous frameshift mutation in a consanguineous family with two siblings affected by primary microcephaly (13). Reducing <i>CEP135</i> amounts in cells via RNA interference caused a disorganization of interphase and mitotic spindles, leading to the hypothesis that the <i>CEP135</i> protein has a role in maintaining the structure and organization of the centrosome and microtubules (13).
<i>CEP152</i> [OMIM #613529]	Homozygous or compound heterozygous mutations in the <i>CEP152</i> gene were identified in 3 unrelated Canadian families with autosomal recessive primary microcephaly. <i>CEP152</i> is also a centrosomal protein (14).
<i>CEP63</i> [OMIM#614728]	A homozygous nonsense mutation was identified in <i>CEP63</i> in a consanguineous family of Pakistani descent with three members with primary microcephaly and, to a lesser extent, proportionate short stature [21]. The <i>CEP63</i> protein forms a complex with <i>CEP152</i> , and helps to maintain normal centrosome numbers within cells (15).
<i>CIT</i> [OMIM# 605629]	Homozygous missense mutations in the <i>CIT</i> gene have been identified in three consanguineous families affected with autosomal recessive primary microcephaly, and in three unrelated families with multiple affected children affected by severe microlissencephaly (16, 17)
<i>MCPH1</i> [OMIM #607117]	Homozygous mutations in <i>MCPH1</i> associated with autosomal recessive primary microcephaly have been reported in multiple populations, including at least one Pakistani family and at least one Caucasian family (18-20). <i>MCPH1</i> encodes the Microcephalin protein, which is believed to play a role in cell-cycle timing (1).
<i>MED17</i> [OMIM#603810]	A homozygous missense mutation was identified in 5 infants from 4 Jewish families with postnatal progressive microcephaly and severe developmental retardation associated with cerebral and cerebellar atrophy (21).
<i>MFSD2A</i> [OMIM# 614397]	Homozygous mutations in <i>MFSD2A</i> have been identified in at least three consanguineous families with autosomal recessive primary microcephaly (22).
<i>NDE1</i> [OMIM#609449]	Mutations in <i>NDE1</i> have been reported in children with severe congenital MIC, with simplified gyri, and profound ID. Homozygous mutations have been reported in one Turkish, two Saudi and two Pakistani consanguineous families. <i>NDE1</i> is highly expressed in the developing human and mouse cerebral cortex, particularly at the centrosome, and has a role in mitotic spindle assembly during early neurogenesis. Deficiency of <i>NDE1</i> therefore appears to cause failure of neurogenesis and a deficiency of cortical lamination (23).
<i>PHC1</i> [OMIM#602978]	A homozygous missense <i>PHC1</i> mutation was identified in a consanguineous Saudi family in which 2 of 6 children were affected with microcephaly (-4.3 SD and -5.8 SD) and short stature (-2.3 SD and -3.6 SD) with an IQ of 80 recorded in the older child (24).
<i>PNKP</i> [OMIM #605610]	Mutations in the <i>PNKP</i> gene have been described in seven families with autosomal recessive Microcephaly, infantile-onset seizures, and developmental delay (MCSZ). Both homozygous and compound heterozygous mutations have been reported. The <i>PNKP</i> protein is involved in DNA repair of both double and single-stranded breaks. In patients with MCSZ, ID is usually severe to profound with variable behavioral problems and severe and intractable seizures (25).
<i>SASS6</i> [OMIM# 616402]	One family with autosomal recessive primary microcephaly and a homozygous missense mutation in <i>SASS6</i> has been reported (26).
<i>SLC25A19</i> [OMIM#606521]	Amish lethal microcephaly is characterized by the presence of microcephaly and a tenfold increase in the levels of urinary organic acid 2-ketoglutarate. To date, all affected individuals within the Old Order Amish population (in which the prevalence of this condition in this population is approximately 1 in 500 births) are homozygous for a founder mutation in <i>SLC25A19</i> (27). The <i>SLC25A19</i> gene encodes a mitochondrial thiamine pyrophosphate carrier.
<i>STAMBIP</i> [OMIM#606247]	McDonnell <i>et al.</i> identified mutations in <i>STAMBIP</i> in a cohort of patients with Microcephaly-Capillary malformation (MIC-CAP) syndrome. MIC-CAP is characterized by small scattered capillary malformations, congenital microcephaly, early-onset intractable epilepsy, profound global developmental delay, spastic quadriplegia, hypoplastic distal phalanges and poor growth (28).
<i>STIL</i> [OMIM #181590]	Kumar, <i>et al</i> (2009) reported three Indian families with autosomal recessive primary microcephaly that were homozygous for mutations in <i>STIL</i> . <i>STIL</i> is necessary for proper mitotic spindle organization (29).
<i>WDR62</i> [OMIM#613583]	Mutations in <i>WDR62</i> have been reported in a subset of patients with microcephaly, cortical malformations, and moderate to severe ID. Besides Microcephaly, these patients had various brain malformations including callosal abnormalities, polymicrogyria, schizencephaly and subcortical nodular heterotopia. A subset has seizures (30). Homozygous missense and frameshift mutations were first reported in seven consanguineous families. Like other autosomal recessive primary microcephaly genes, <i>WDR62</i> encodes a spindle pole protein that is expressed in neuronal precursor cells undergoing mitosis in the proliferative phase of neurogenesis (31).
<i>ZNF335</i> [OMIM#610827]	Yang <i>et al.</i> (2012) identified a homozygous mutation in <i>ZNF335</i> in a large consanguineous Arab-Israeli family with severe primary microcephaly (32). <i>ZNF335</i> interacts with a chromatin remodeling complex which regulates the expression of genes in a range of different pathways (32).

Inheritance:

Recurrence risk for parents of an affected individual with a confirmed mutation causing MCPH is 25%. Empiric studies have shown that non-consanguineous couples having one child with MCPH and normal chromosomes and neuroimaging have a 20% risk of recurrence [17].

Additional Resources:

Foundation for Children with Microcephaly

Phone: 877-476-5503

Email: jenni@childrenwithmicro.org

www.childrenwithmicro.org

Test methods:

Our Comprehensive Autosomal Recessive Primary Microcephaly Panel includes sequence analysis of 25 genes and deletion/duplication analysis of 17 genes. Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Deletion/duplication analysis of *ASPM* and the panel genes is performed by oligonucleotide array-CGH. Deletion/duplication analysis for individual genes is also available. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Dr. William Dobyns at the Seattle Children's Research Institute is available to review MRI scans and give recommendations regarding genetic testing. Please contact Dr. Dobyns (wbd@uw.edu) to arrange this, if desired.

Please send a completed Microcephaly Clinical Checklist with each sample. This information will be used to aid in interpretation of the test result. The clinical data form, along with the test result, will be shared with Dr. Dobyns and stored anonymously in a microcephaly database.

ASPM sequence analysis

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81407
Turn-around time:	4 weeks

ASPM deletion/duplication analysis

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81406
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Comprehensive Autosomal Recessive Primary Microcephaly Panel (includes sequencing analysis of 25 genes including ASPM, and deletion/duplication analysis of 17 genes)

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	\$3000
CPT codes:	81406, 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this panel.

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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