

The University of Chicago Genetic Services Laboratories



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Genetic Testing for Primary Microcephaly

Autosomal recessive primary microcephaly (MCPH):

- Congenital microcephaly (3 SD below the mean at birth or at least 4 SD below the mean at later ages)
- Mental retardation (MR), 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 weight and appearance except for the microcephaly

Brain imaging shows a mildly reduced number of gyri, and in some patients may also demonstrate agenesis of the corpus callosum or a few periventricular nodular heterotopia (numerous heterotopia suggest an alternative diagnosis). Prenatally, individuals have normal head size until approximately 20 weeks and decreased head size by 32 weeks, although this varies. The relative degree of microcephaly doesn't vary throughout life and doesn't vary within a family by more than 2 SD. MR is usually mild to moderate with no progressive decline or motor deficit [1].

Mutations in the *ASPM* [OMIM #605481] gene are the most common cause of MCPH [2]. Approximately 40% of patients (both consanguineous and non-consanguineous) with a strict diagnosis of MCPH have mutations in *ASPM*. However, very few patients (<10%) with a less restrictive phenotype have mutations in *ASPM* [3]. Thus, we expect a high detection rate for high-functioning MCPH, but a lower detection rate for low-functioning MCPH, especially if associated with other anomalies. 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. There is no correlation between the genotype and the degree of microcephaly or MR [3]. *Asp*, the *Drosophila* ortholog of *ASPM*, is necessary for the formation of the mitotic spindle in mitosis and meiosis [1].

Several other genes, including *CDK5RAP2*, *CENPJ*, *MCPH1*, *STIL*, and *CEP152* have been reported to cause MCPH in a small number of families.

- Homozygous mutations in *CDK5RAP2* [OMIM #608201] have been identified in three Pakistani families with MCPH [4,5]. *CDK5RAP2* is a centrosomal protein and may be involved in microtubule production during mitosis [1].
- Four Pakistani families with MCPH have been reported with homozygous mutations in *CENPJ* [OMIM #609279] [4,6]. *CENPJ* is a centrosomal protein and likely shares a very similar role with *CDK5RAP2* [1].
- Homozygous mutations in *MCPH1* [OMIM #607117] have been reported in multiple populations, including at least one Pakistani family and at least one Caucasian family [7-9]. *MCPH1* encodes the Microcephalin protein, which is believed to play a role in cell-cycle timing [1].
- Kumar, et al (2009) reported three Indian families with MCPH that were homozygous for mutations in *STIL* [OMIM #181590]. *STIL* is necessary for proper mitotic spindle organization [10].
- Homozygous or compound heterozygous mutations in the *CEP152* [OMIM #613529] gene were identified in 3 unrelated Canadian families with MCPH. *CEP152* is also a centrosomal protein [11].

Other Microcephaly Disorders:

[Autosomal recessive microcephaly, infantile-onset seizures, and developmental delay \(MCSZ\)](#) [OMIM #613402] and [microcephaly, cortical malformations, and MR](#) [OMIM #600176] are more severe disorders.

In patients with MCSZ, MR is usually severe to profound with variable behavioral problems and severe and intractable seizures. Mutations in the *PNKP* [OMIM #605610] gene have been described in seven families with MCSZ. Both homozygous, consanguineous patients and compound heterozygotes were reported. The *PNKP* protein is involved in DNA repair of both double and single-stranded breaks. Of the 14 patients described, none have reported a higher frequency of infections and no cancers have been reported up to the age of 21 years [12].

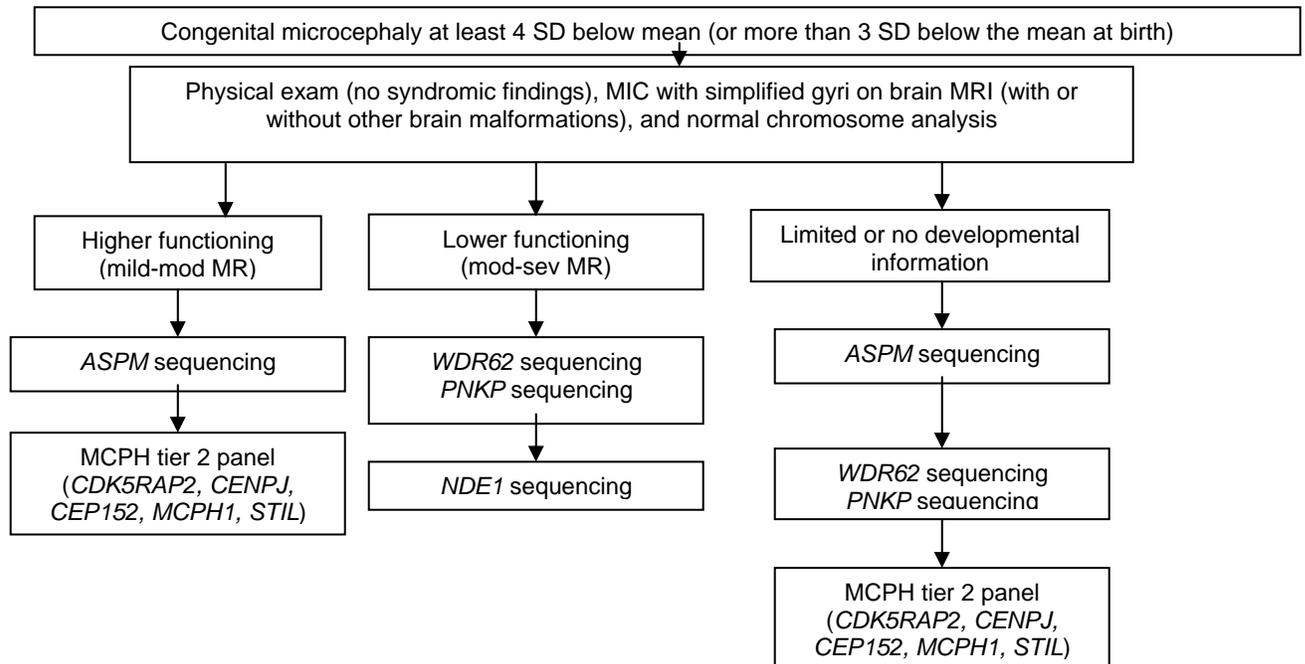
Patients with microcephaly, cortical malformations, and MR have moderate to severe MR and various brain malformations including callosal abnormalities, polymicrogyria, schizencephaly and subcortical nodular heterotopia. A subset of patients have seizures [13]. This form of MCPH is caused by mutations in the *WDR62* [OMIM#613583] gene. Homozygous missense and frameshift mutations were first reported in seven consanguineous families with primary microcephaly and simplified gyri. Like other MCPH genes, *WDR62* encodes a spindle pole protein that is expressed in neuronal precursor cells undergoing mitosis in the proliferative phase of neurogenesis [14].

Recently, mutations in *NDE1* [OMIM#609449] were reported in children with severe congenital MIC, with brains smaller than 10 SD below the mean, with simplified gyri, and profound developmental handicap with normal body growth. Homozygous mutations have been reported in one Turkish, two Saudi and two Pakistani consanguineous families. *NDE1* 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 NDE1 therefore appears to cause failure of neurogenesis and a deficiency of cortical lamination [15,16].

Clinical Work-up and Counseling:

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



Epidemiology:

MCPH occurs in approximately 1 in 10,000 individuals in Pakistan and an estimated 1 in 1,000,000 in the Caucasian population [1]. It is more common in consanguineous populations. *ASPM* mutations have been found in all ethnic groups studied [3]. *PNKP* and *WDR62* mutations have been reported in Palestinian, Turkish, and Mixed European ethnicities [11-13], whereas *NDE1* mutations have been found in Pakistani, Turkish, and Middle Eastern ethnicities [15-16].

Additional Resources:

Foundation for Children with Microcephaly

Phone: 602-487-6445

email: jenni@childrenwithmicro.org

www.childrenwithmicro.org

Test methods:

We offer mutation analysis of all 28 coding exons and intron/exon boundaries of *ASPM* by direct sequencing of amplification products in both the forward and reverse directions. We also offer deletion/duplication analysis of the *ASPM* gene by oligonucleotide array-CGH to identify copy number changes involving one or more exons.

We also offer mutation analysis of all 16 coding exons and intron/exon boundaries of *PNKP*, all 30 coding exons and intron/exon boundaries of *WDR62*, and all 8 coding exons and intron/exon boundaries of *NDE1* by direct sequencing of amplification products in both the forward and reverse directions.

Physicians may also choose to order our MCPH Tier 2 Panel. This panel includes full gene sequencing and deletion/duplication analysis for the *CDK5RAP2*, *CENPJ*, *MCPH1*, and *STIL* genes, along with full gene sequencing of *CEP152*. As *CEP152* has been recently added to this panel, it will be offered individually for patients that have had the panel done previously. Sequencing for the other genes cannot be ordered individually.

Deletion/duplication analysis of the *ASPM*, *CDK5RAP2*, *CENPJ*, *MCPH1* and *STIL* genes by oligonucleotide array-CGH identifies copy number changes involving one or more exons. Partial exonic copy number changes and rearrangements of less than 400 bp for *ASPM*, *CDK5RAP2*, *MCPH1*, and *STIL*, and of less than 2kb for *CENPJ* may not be detected by this methodology.

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) or his coordinator, Hailly Butler (hailly.butler@seattlechildrens.org) 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. Dobyans and stored anonymously in a microcephaly database.

ASPM sequencing analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$2400
CPT codes: 83891, 83898 x9, 83904 x9, 83912
Turn-around time: 6 - 8 weeks

Note: We cannot bill insurance for ASPM sequencing.

ASPM deletion/duplication analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$1000
CPT codes: 83891, 88386 x2
Turn-around time: 4 - 6 weeks

MCPH Tier 2 Panel (CDK5RAP2, CENPJ, MCPH1, and STIL sequence and deletion/duplication analysis, and CEP152 sequence analysis)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$5530
CPT codes: 83891, 83898 x17, 83904 x16, 88386 x3
Turn-around time: 8 - 10 weeks

Note: We cannot bill insurance for MCPH Tier 2 Panel.

CEP152 sequence analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$2400
CPT codes: 83891, 83898 x9, 83904 x9, 83912
Turn-around time: 4 - 6 weeks

PNKP sequencing analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$1675
CPT codes: 83891, 83898 x4, 83904 x7, 83912
Turn-around time: 4 - 6 weeks

WDR62 sequencing analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$2400
CPT codes: 83891, 83898 x9, 83904 x9, 83912
Turn-around time: 4 - 6 weeks

NDE1 sequencing analysis

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$1000
CPT codes: 83891, 83898 x2, 83904 x4, 83912
Turn-around time: 4 - 6 weeks

Deletion/Duplication analysis for two or more genes (by array-CGH)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: \$1545
CPT codes: 83891, 83894, 88386 x3
Turn-around time: 4 - 6 weeks

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