# Microcephaly Xpanded Panel
## A targeted test for genetic causes of microcephaly using a trio approach

**Clinical Features:**

Microcephaly is defined as a small cranium with an occipito-frontal head circumference (OFC) of more than two standard deviations (SD) below the mean for age, sex, and ethnicity.[1] Microcephaly can be congenital (primary microcephaly) or acquired postnatally (secondary microcephaly). Either type can be caused by environmental or genetic factors.[2] Individuals with primary microcephaly have inadequate brain growth during pregnancy, and are born with a significantly small head size (OFC of < 3 SDs). They may also have non-progressive intellectual disabilities, febrile or other mild seizures, mild short stature, and a narrow sloping forehead due to the reduced cranial size.[3] Brain imaging typically reveals a normal brain structure with reduction in size, particularly of the cerebral cortex. The brain size in cases of secondary microcephaly has the expected size at birth but subsequently fails to grow normally.[4] Common causes of secondary microcephaly include environmental insults, such as infections or a brain injury. In addition, secondary microcephaly is observed in some metabolic disorders or genetic syndromes, such as Rett syndrome and Angelman syndrome, in which a progressive reduction in head circumference is seen in infancy or early childhood.[5] Congenital and postnatal microcephaly could present as an isolated finding in an individual, be associated with other brain malformations such as cerebellar hypoplasia, or as part of an underlying syndrome.

The cause of microcephaly can be difficult to discern as there are many genes contributing to microcephaly, either as an isolated finding in an individual or as part of an underlying syndrome. It is often necessary to perform testing of multiple genes (concurrently or as reflex tests) to identify the underlying genetic cause in an individual. Moreover, new genes associated with microcephaly are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels updated. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify which variants are de novo or inherited.

The Microcephaly Xpanded Panel uses a trio approach that includes concurrent analysis of the affected proband and both parents, which increases the likelihood of identifying a definitive genetic explanation for the cause of microcephaly in an individual. Depending on the family structure, family history, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the Microcephaly Xpanded Panel. The Microcephaly Xpanded Panel is based on whole exome capture, Next

Generation sequencing (NGS), and targeted analysis of a comprehensive list of approximately 800 genes currently associated with microcephaly. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with microcephaly.

**Inheritance Pattern/Genetics:**

The etiology of microcephaly is complex, including multiple genetic, epigenetic and environmental factors. Approximately 15-50% of individuals with microcephaly have been reported to have an underlying genetic etiology.[1] The general incidence of microcephaly at birth varies from 1.3 to 150 per 100,000 live births, depending on the population and the applied SD threshold to define microcephaly.[2] Microcephaly can be caused by chromosomal abnormalities, inborn errors of metabolism, single gene disorders, trauma and infection. The inheritance patterns can be autosomal dominant, recessive or x-linked. One type of isolated congenital microcephaly that has a genetic etiology is primary autosomal recessive microcephaly (MCPH), which is a rare and heterogeneous disorder, with an incidence in consanguineous populations of approximately one in 10,000 and less in non-consanguineous populations.[2] To date, there are twelve subtypes of MCPH and between 37-54% of patients with a strictly characterized MCPH diagnosis have a pathogenic variant in the ASPM gene, associated with MCPH5.[3,6] Syndromic forms of microcephaly are associated with underlying chromosomal aberrations, contiguous gene deletions, and single gene disorders that are inherited in an autosomal dominant, recessive, or X-linked manner.[5] Several of these syndromes have postnatal onset of microcephaly, including Rett syndrome and Ataxia telangectasia with intellectual disability, whereas other syndromes, such as Cornelia de Lange, Smith-Lemli Opitz, and Seckel, present with congenital microcephaly.

**Test Methods:**

Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome were captured using a proprietary system developed by GeneDx and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19 and analyzed for sequence variants in targeted genes using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified in the proband and relative samples, if submitted. Sequence and copy number alterations are reported according to the Human Genome Variation Society (HGVS) and International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. A list of additional variants not included in the report is available upon request.

Please note that while the Microcephaly Xpanded Panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The

Microcephaly Xpanded Panel gene list includes approximately 800 genes. The list was developed by searching for genes associated with microcephaly in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. Additionally, genes were added to the list using GeneDx data from clinical whole exome sequencing done on patients reported to have microcephaly. The gene list is systematically updated at least quarterly. The current gene list is available on our website (http://www.genedx.com/test-catalog/genetic-testing-for-neurological-disorders/).

**Result Reporting:**
The Microcephaly Xpanded Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, extra fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with microcephaly in the published or emerging literature. Pathogenic/likely pathogenic variants in genes responsible for the phenotype of the patient will be reported; however, because this is a phenotype-driven test of a large number of genes, variants of uncertain significance (VUS) are not routinely reported, only at our discretion. Variants that are considered to be benign or likely benign will not be reported. As the Microcephaly Xpanded Panel includes approximately 800 genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing.

In rare instances, this test may reveal a pathogenic variant that is not directly related to the test indication. For example, pathogenic variants in genes associated with an increased risk for cancer, cardiac abnormalities, or metabolic defects could be identified. In the event that an incidental finding is identified, this information will be disclosed to the ordering health care provider if it is likely to impact medical care. The absence of reportable incidental findings for any particular gene does not rule out the possibility of pathogenic variants in that gene.

**Test Sensitivity:**
The clinical sensitivity of the Microcephaly Xpanded panel test depends in part on the patient's clinical phenotype. Currently, patients with a clinical indication that includes microcephaly have a 33.5% positive rate by whole exome sequencing (WES). When parents are sequenced and analyzed concurrently (trio-based testing), the positive rate is 35% and for cases where parents are unavailable, the positive rate is significantly lower at 25%.[7]

The average coverage of all genes on the panel is greater than 98% at 10X (with a depth of 10 or more reads), and approximately 88% of the genes on the panel have an average coverage of greater than 99.0% coverage at 10X . Only a few genes with a high clinical sensitivity for microcephaly have an average coverage of less than 90% coverage at 10X. Note that these numbers represent the average coverage for the genes on the panel, derived by combining data from a large number of patients. The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

Some types of genetic disorders, such as those due to nucleotide repeat expansion/contraction, abnormal DNA methylation, and other mechanisms may not be detectable with this test. Additionally, small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be reliably detected. For example, the polyalanine repeat expansions in ARX and abnormal methylation of UBE3A causing Angelman syndrome would not be detectable by this Microcephaly Xpanded Panel.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the Microcephaly Xpanded Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the Microcephaly Xpanded Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

**References:**

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