Macrocephaly and Overgrowth Syndromes: Sequencing and Beckwith-Wiedemann Syndrome Methylation Panel

**Test Code:** MM260  
**Turnaround time:** 6 weeks  
**CPT Codes:** 81321 x1, 81401 x1, 81403 x1, 81404 x1, 81406 x1

**Condition Description**

Macrocephaly is defined as a head circumference, which is 2 standard deviations larger than the average when matched for age and sex. It refers to an abnormally large head inclusive of the scalp, cranial bone, and intracranial contents. Macrocephaly can arise due to a true enlargement of the brain (megalencephaly) or other conditions such as hydrocephalus and be either syndromic or non-syndromic. The genetic subtypes of macrocephaly include familial forms of macrocephaly, autism, syndromic associations such as PTEN hamartoma syndrome, Noonan syndrome, Sotos syndrome, and metabolic disorders such as glutaric aciduria type 1 and D-2-hydroxyglutaric aciduria.

Reference:

**Genes**

AKT1, AKT2, AKT3, CDKN1C, CUL4B, DNMT3A, EZH2, GLI3, GNAQ, GPC3, H19, LIT1, MED12, MET10, NFI, NSD1, PHF6, PIK3CA, PIK3R2, PTCH1, PTEN, RNF135, UPP3B

**Indications**

This test is indicated for:
- Patients with a clinical diagnosis of macrocephaly or other overgrowth syndrome.

**Methodology**

**Next Generation Sequencing:** In-solution hybridization of all coding exons is performed on the patient's genomic DNA. Although some deep intronic regions may also be analyzed, this assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements, and does not detect single or multi-exon deletions or duplications. Direct sequencing of the captured regions is performed using next generation sequencing. The patient's gene sequences are then compared to a standard reference sequence. Potentially causative variants and areas of low coverage are Sanger-sequenced. Sequence variations are classified as pathogenic, likely pathogenic, benign, likely benign, or variants of unknown significance. Variants of unknown significance may require further studies of the patient and/or family members.

**Beckwith-Wiedemann Methylation:** Methylation-specific MLPA (MS-MLPA) is used to test for BWS. One advantage of MS-MLPA is that in addition to detecting DNA methylation abnormalities (epimutations), similar to Southern blot and quantitative methylation sensitive PCR, it also detects deletions and duplications (CNVs) of the 11p15 region. CNVs are estimated to be present in ~10% of patients with BWS. The presence of a CNV can increase the recurrence risk from that of the general population up to a 50% risk. Both methylation and CNVs will be reported from this analysis.

**Detection**

**Next Generation Sequencing:** Clinical Sensitivity: Unknown. Pathogenic variants in the promoter region, some pathogenic variants in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions/duplications will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's clinical/biochemical phenotype.

Analytical Sensitivity: ~99%.

**Beckwith-Wiedemann Methylation:** Hypomethylation of DMR2 (*Lit1* gene) is expected to detect up to 60-70% of individuals with BWS. Hypermethylation of DMR1 (*H19* gene) is expected to detect an additional 2-13% of individuals with BWS. Therefore, the total detection rate for both DMR1 and DMR2 methylation analysis is estimated to be 62-83%.

**Reference Range**

**Beckwith-Wiedemann Methylation:** For DMR1 (*H19* gene), an increase in DNA methylation of greater than two standard deviations above the mean of normal is consistent with BWS. For DMR2 (*Lit1* gene), a decrease in DNA methylation of greater than two standard deviations below the mean of normal is consistent with BWS.

**Specimen Requirements**

Submit only 1 of the following specimen types

**Type:** Whole Blood

Specimen Requirements:
In EDTA (purple top) tube:
Infants (Children (>2 years)): 3-5 ml
Older Children & Adults: 5-10 ml.

Specimen Collection and Shipping: Ship sample at room temperature with overnight delivery.

**Type: Isolated DNA**

Specimen Requirements:

In microtainer: 60 ug

Isolation using the Qiagen\textsuperscript{TM} Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping: Refrigerate until time of shipment in 100 ng/ul of TE buffer. Ship sample at room temperature with overnight delivery.

**Related Tests**

- Macrocephaly: Deletion/Duplication Panel