Syndromic Macrocephaly/Overgrowth Syndromes Panel
Sequence Analysis and Exon-Level Deletion/Duplication Testing of 11 Genes

Panel Gene List:
CUL4B, EZH2, GLI3, GPC3, MED12, NFIX*, NSD1, PHF6, PTCH1, PTEN**, UPF3B

* No sequencing or deletion/duplication testing of exon 1 of the NFIX gene
**The PTEN promoter region is not analyzed in this panel

Clinical Features:
Macrocephaly is defined as an occipitofrontal circumference (OFC) greater than the 98th percentile for age. Macrocephaly may occur for many reasons, including megalencephaly, hydrocephalus, cerebral edema, neoplasia, and structural anomalies. Syndromic forms of macrocephaly are often due to megalencephaly, which is defined as a brain weight/volume ratio greater than the 98th percentile for age due to hyperplasia of the central nervous system parenchyma.\(^{26}\) Individuals with these forms of syndromic macrocephaly may also exhibit somatic overgrowth. Other features commonly observed in individuals with syndromic macrocephaly include developmental delay, hypotonia, increased risk for neoplasia, dysmorphic features, and birth defects.\(^{26,27,28}\) In many cases, macrocephaly and/or overgrowth are identified in the neonatal period, although in other cases the onset may be postnatal and not noted until childhood. In some cases, growth parameters may normalize in adulthood.\(^{28}\)

Because of the significant clinical overlap and phenotypic heterogeneity of disorders causing syndromic macrocephaly, it can be difficult to make a clinical diagnosis, particularly in infancy. Additionally, variants in a single gene may be associated with a broad spectrum of clinical presentations (clinical heterogeneity). Therefore, a multi-gene panel is very useful in helping to establish the etiology of syndromic macrocephaly. A complete list of the disorders included on the Syndromic Macrocephaly/Overgrowth Syndromes Panel is available in the table on the last page of this information sheet.

Genetics:
The Syndromic Macrocephaly/Overgrowth Syndromes Panel at GeneDx includes sequencing and deletion/duplication analysis of 11 genes. Pathogenic variants in these genes are associated with X-linked or autosomal dominant disorders and typically have a loss-of-function effect. The variant spectrum includes missense, nonsense, splicing, and small insertion or deletion variants, as well as exonic deletions or duplications. Many of these genes encode proteins that play a role in cell growth and division, cell death, chromatin regulation, histone modification, cell migration, and angiogenesis (Pilia et al., 1996, \(^{29,30}\)).
**Test Methods:**
Using genomic DNA, coding exons and flanking splice junctions of the genes on this panel are enriched using a proprietary targeted capture method developed by GeneDx. Note that variants in exon 1 of the NFIX gene will not be detected. Additionally, the PTEN core promoter region is not analyzed in this panel; to our knowledge, promoter variants have not been associated with the macrocephaly/autism phenotype. The products are sequenced on an Illumina instrument using paired end reads. The sequence data is aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Sanger sequencing is used to compensate for low coverage and refractory amplifications. Concurrently, targeted array CGH analysis with exon-level resolution is performed to evaluate for a deletion or duplication of one or more exons for most of the genes included on the panel. The presence of any potentially disease-associated sequence variant(s) or copy number alteration(s) is confirmed by dideoxy DNA sequence analysis or quantitative PCR, respectively, or by other appropriate methods.

**Test Sensitivity:**
The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends on the patient’s clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the following table. The technical sensitivity of the sequencing test is estimated to be 98%. Deletions involving more than 20 bp and insertions involving more than 10 bp are not reliably detected by the sequencing methodology, and deletions or duplications of less than 500 bp are not reliably detected by array CGH. Note that small sections of a few genes have inherent sequence properties that result in suboptimal data and variants in those regions may not be reliably identified.

**Syndromic Macrocephaly/Overgrowth Syndromes Panel:**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Diagnostic Yield in Selected Population(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabezas syndrome</td>
<td>CUL4B</td>
<td>cullin-4B</td>
<td>XL</td>
<td>~3% of X-linked intellectual disability(^1,2)</td>
</tr>
<tr>
<td>Weaver syndrome</td>
<td>EZH2</td>
<td>histone-lysine N-methyltransferase EZH2</td>
<td>AD</td>
<td>5% patients with non-specific overgrowth(^3)</td>
</tr>
<tr>
<td>Griegl cephalopolysyndactyly syndrome (GCPS)</td>
<td>GLI3</td>
<td>transcriptional activator GLI3</td>
<td>AD</td>
<td>68% GCPS(^4,5) 91% PHS(^4,5)</td>
</tr>
<tr>
<td>Pallister-Hall syndrome (PHS)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Syndrome/Condition</th>
<th>Gene</th>
<th>Description</th>
<th>Inheritance</th>
<th>% Male with Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simpson-Golabi-Beahmel syndrome (SGBS)</td>
<td>GPC3</td>
<td>glypican-3</td>
<td>XL</td>
<td>56% males with SGBS(^*)(^7,^8)</td>
</tr>
<tr>
<td>FG syndrome</td>
<td>MED12</td>
<td>Mediator of RNA polymerase II transcription subunit 12</td>
<td>XL</td>
<td>13% males with clinical diagnosis of FG syndrome(^6)</td>
</tr>
<tr>
<td>Sotos syndrome-2</td>
<td>NFIX(^*)</td>
<td>Nuclear factor 1 X-type</td>
<td>AD</td>
<td>4% patients with Sotos-like features(^5)</td>
</tr>
<tr>
<td>Marshall-Smith syndrome</td>
<td>NSD1</td>
<td>nuclear receptor SET domain-containing protein</td>
<td>AD</td>
<td>90-93% of non-Japanese patients with Sotos syndrome(^11,^12) 63% Japanese patients with Sotos syndrome(^13)</td>
</tr>
<tr>
<td>Borjeson-Forssman-Lehmann syndrome (BFLS)</td>
<td>PHF6</td>
<td>PHD finger protein 6</td>
<td>XL</td>
<td>56% males with BFLS(^14)</td>
</tr>
<tr>
<td>Gorlin syndrome</td>
<td>PTCH</td>
<td>protein patched homolog 1</td>
<td>AD</td>
<td>72% of patients with Gorlin syndrome(^15,^16)</td>
</tr>
<tr>
<td>PTEN-related autism and macrocephaly</td>
<td>PTEN(^**)</td>
<td>phosphatase and tensin homolog</td>
<td>AD</td>
<td>2-17% of patients with autism spectrum disorders and macrocephaly(^17,^18,^19,^20) 81% of patients with CS(^21) 71% of patients with BRRS(^22,^23)</td>
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<tr>
<td>Cowden syndrome (CS)</td>
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<td>Bannayan-Riley-Ruvalcaba syndrome (BRRS)</td>
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<tr>
<td>Lujan syndrome</td>
<td>UPF3B</td>
<td>regulator of nonsense transcripts 3B</td>
<td>XL</td>
<td>~1% X-linked intellectual disability(^24,^25)</td>
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<tr>
<td>FG syndrome</td>
<td></td>
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<tr>
<td>Nonsyndromic X-linked intellectual disability</td>
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</tbody>
</table>

\(^*\) No sequencing or deletion/duplication testing of exon 1 of the NFIX gene

\(^**\) The PTEN promoter region is not analyzed in this panel

References: