

## Syndromic Macrocephaly/Overgrowth Syndromes Panel Sequence Analysis and Exon-Level Deletion/Duplication Testing of 29 Genes

**Panel Gene List:** AKT3, BRWD3, CCND2, CHD8, CUL4B, DNMT3A, EZH2, GLI3, GPC3, HEPACAM, HERC1, MED12, MTOR, NFIA, NFIX, NSD1, OFD1, PHF6, PIK3CA, PIK3R2, PPP2R5D, PTCH1, PTEN\*, RAB39B, RNF135, SETD2, SNX14, TBC1D7, UPF3B

### **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.<sup>58</sup> 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.<sup>58,59,60</sup> 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.<sup>60</sup>

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.

### **Inheritance Pattern/Genetics:**

The Syndromic Macrocephaly/Overgrowth Syndromes Panel at GeneDx includes sequencing and deletion/duplication analysis of 30 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. <sup>61,62</sup>

## Test Methods:

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). For PTEN, approximately nucleotides c.-700 through c.-1300 in the promoter region are also captured. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size.

| Gene   | Inheritance | Disease Associations                               | Diagnostic Yield in Selected Population(s)             |
|--------|-------------|--|--|
| AKT3   | AD          | MPPH2  | ~30% of MPPH syndrome <sup>1</sup>                     |
| BRWD3  | XL          | X-linked intellectual disability with macrocephaly | Rare <sup>2,3</sup>                                    |
| CCND2  | AD          | MPPH3  | ~30% of MPPH syndrome <sup>1</sup>                     |
| CHD8   | AD          | Neurodevelopmental and autism spectrum disorders   | Rare <sup>4,5</sup>                                    |
| CUL4B  | XL          | Cabezas syndrome                                   | ~3% of X-linked intellectual disability <sup>6,7</sup> |
| DNMT3A | AD          | Tatton-Brown-Rahman syndrome                       | Rare <sup>4,8,9</sup>                                  |
| EZH2   | AD          | Weaver syndrome                                    | 5% patients with non-specific overgrowth <sup>10</sup> |

|         |       |  |   |
|---------|-------|--|---|
| GLI3    | AD    | Grieg cephalopolysyndactyly syndrome (GCPS)<br>Pallister-Hall syndrome (PHS)                             | 68% GCPS <sup>11,12</sup><br>91% PHS <sup>11,12</sup>   |
| GPC3    | XL    | Simpson-Golabi-Behmel syndrome (SGBS)  | 56% males with SGBS <sup>13,14,15</sup>   |
| HEPACAM | AD/AR | Megalencephalic leukoencephalopathy with subcortical cysts   | Rare <sup>16,17</sup>   |
| HERC1   | AR    | Macrocephaly, dysmorphic facies, and psychomotor retardation   | Rare <sup>18,19,20,21</sup>   |
| MED12   | XL    | FG syndrome<br>Lujan syndrome  | 13% males with clinical diagnosis of FG syndrome <sup>24</sup>  |
| MTOR    | AD    | Smith-Kingsmore syndrome   | Rare <sup>4,25</sup>  |
| NFIA    | AD    | Brain malformations with or without urinary tract defects  | Rare <sup>26,27</sup>   |
| NFIX    | AD    | Sotos syndrome-2<br>Marshall-Smith syndrome  | 4% patients with Sotos-like features <sup>28</sup>  |
| NSD1    | AD    | Sotos syndrome   | 90-93% of non-Japanese patients with Sotos syndrome <sup>29,30</sup><br>63% Japanese patients with Sotos syndrome <sup>31</sup>   |
| OFD1    | XL    | Joubert syndrome<br>Orofaciodigital syndrome   | Rare in JSRD <sup>32,33,34</sup>  |
| PHF6    | XL    | Borjeson-Forsman-Lehmann syndrome (BFLS)   | 56% males with BFLS <sup>35</sup>   |
| PIK3CA  | AD    | megalencephaly-capillary malformation (MCAP) syndrome, CLOVE syndrome, and fibroadipose hyperplasia      | ~40% of megalencephaly-capillary malformation (MCAP) <sup>36</sup>  |
| PIK3R2  | AD    | MPPH1  | ~40% of MPPH syndrome <sup>1</sup>  |
| PPP2R5D | AD    | autosomal dominant intellectual disability   | Rare <sup>37</sup>  |
| PTCH1   | AD    | Gorlin syndrome  | 72% of patients with Gorlin syndrome <sup>38,39</sup>   |
| PTEN    | AD    | PTEN-related autism and macrocephaly<br>Cowden syndrome (CS)<br>Bannayan-Riley-Ruvalcaba syndrome (BRRS) | 2-17% of patients with autism spectrum disorders and macrocephaly <sup>40,41,42,43</sup><br>81% of patients with CS <sup>44</sup><br>71% of patients with BRRS <sup>45,46</sup> |
| RAB39B  | XL    | X-linked intellectual disability   | Rare <sup>47,48</sup>   |
| RNF135  | AD    | Macrocephaly, macrosomia, facial dysmorphism syndrome  | Rare <sup>49</sup>  |
| SETD2   | AD    | Luscan-Lumish syndrome   | Rare <sup>50,51</sup>   |

|        |    |  |   |
|--------|----|--|---|
| SNX14  | AR | autosomal recessive spinocerebellar ataxia-20                                  | Rare <sup>52,53</sup>                                 |
| TBC1D7 | AR | Macrocephaly/megalencephaly syndrome   | Rare <sup>54,55</sup>                                 |
| UPF3B  | XL | Lujan syndrome<br>FG syndrome<br>Nonsyndromic X-linked intellectual disability | ~1% X-linked intellectual disability <sup>56,57</sup> |

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