

## Anophthalmia and Microphthalmia Panel

### PANEL GENE LIST:

ALDH1A3, BCOR, BMP4, BMP7, COX7B, CRYBA4, FOXE3, GDF6, HCCS, MITF, NAA10, NDUFB11, OTX2, PAX6, PRSS56, RAX, SALL1, SHH, SIX6, SOX2, STRA6, TENM3, VSX2 (CHX10)

### CLINICAL FEATURES:

Anophthalmia is the complete absence of the globe, or bulb, of the eye and is the most severe structural eye malformation.<sup>1</sup> It may be termed as complete absence of tissue in the orbit (true anophthalmia), or the absence of ocular tissue in upon clinical examination (clinical anophthalmia).<sup>2</sup> Microphthalmia is a milder malformation of the eye in which the total axial length of the eye globe is at least two standard deviations below the mean for age.<sup>1</sup> In this condition the eyelids, conjunctiva, and lacrimal apparatus are normal. Simple or isolated microphthalmia refers to a small but otherwise structurally normal eye in the absence of other abnormalities.<sup>1</sup> When associated with other ocular abnormalities, the condition is referred to as complex microphthalmia. Additional abnormalities may include anterior segment dysgenesis, cataract, persistent hyperplastic primary vitreous, chorioretinal coloboma and/or retinal dysplasia, the most common being coloboma and cataracts.<sup>1</sup>

Anophthalmia and microphthalmia (A/M) conditions have been estimated to have a prevalence of 2-6 in 50,000 live births.<sup>4</sup> Both conditions occur more frequently bilaterally, except for isolated microphthalmia, which occurs more often unilaterally.<sup>1</sup> Approximately one half of A/M cases are syndromic involving abnormalities elsewhere such as craniofacial, skeletal, brain, genital, renal, and cardiac defects.<sup>4,5</sup> A syndromic diagnosis is identified in an estimated 20-45% of all cases. In addition, causative chromosome aberrations are found in 25-30% of individuals.<sup>3</sup> As a genetically heterogeneous condition, A/M may have dominant, recessive or X-linked inheritance.<sup>5</sup> The two most common single gene causes of A/M are due to variants in the SOX2 and OTX2 genes.

Variants in the SOX2 gene are the most common single-gene cause of A/M. Most individuals with variants in the SOX2 gene experience severe, bilateral A/M and have resulted from de novo mutations.<sup>5</sup> There is a spectrum of eye findings in individuals with SOX2 variants, which are inherited in an autosomal dominant manner. Common extra-ocular abnormalities associated with SOX2 variants include developmental delay, hearing loss, seizures, genitourinary tract malformations, brain abnormalities, myopathy, hypogonadotropic hypogonadism and trachea-esophageal fistula.<sup>5</sup> Genotype-phenotype correlations suggest that the most phenotypically severe correspond to complete loss of function variants.<sup>5</sup>

The OTX2 gene variants are the second most common single-gene cause of A/M. In addition to a range of ocular findings, OTX2 gene variants are associated with pituitary abnormalities, morphological and functional.<sup>5</sup> Pituitary findings have been identified in up to 30% of individuals.<sup>5</sup> Brain abnormalities, developmental delay, growth retardation, microcephaly, hypotonia, genital hypoplasia, cleft palate and hearing loss have also been reported.<sup>5</sup> As an autosomal dominant condition, approximately one third of affected individuals inherit the variant from a parent, however, the parent may be phenotypically unaffected as a result of incomplete penetrance or mosaicism.<sup>5</sup>

Additional genes associated with isolated or syndromic A/M include ALDH1A3, BCOR, BMP4, BMP7, COX7B, CRYBA4, FOXE3, GDF6, HCCS, MITF, NAA10, NDUFB11, PAX6, PRSS56, RAX, SALL1, SHH, SIX6, STRA6, TENM3, VSX2(CHX10).

The A/M panel may clarify a clinical diagnosis or identify a genetic diagnosis for A/M or an A/M-related disorder. If a genetic diagnosis is found, genetic testing and recurrence risk information would be available for at-risk family members. In addition, having an identified genetic diagnosis may or may not impact medical management or treatment of the condition.

## GENETICS:

Autosomal dominant, recessive, and x-linked inheritance.

## 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). 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. For the *FOXE3* gene, sequencing but not deletion/duplication analysis is performed. 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.

## TEST SENSITIVITY:

Molecular genetic testing, including sequence analysis, gene-targeted deletion/duplication analysis, and chromosome microarray analysis, can identify a genetic cause in 80% of individuals with bilateral anophthalmia or severe microphthalmia.<sup>3</sup> Overall, genetic testing can identify approximately 20% of all individuals with an ocular malformation in the anophthalmia, microphthalmia and coloboma spectrum.<sup>3</sup>

***ALDH1A3* gene:** Variants are estimated to be responsible for up to 10% of individuals with autosomal recessive A/M from consanguineous families.<sup>6</sup>

***BCOR* gene:** Variants resulting in XLR Lenz microphthalmia are rare, as they have been reported in few families.<sup>7</sup> *BCOR* is the only known gene associated with XLD orofacial/diaphragmatic syndrome, which can often cause microphthalmia and is lethal in males.<sup>7</sup> ***BMP4* gene:** Variants are rare, as they have only been reported in a small subset of individuals with microphthalmia.<sup>5,8,9</sup>

***BMP7* gene:** Variants are rare, as they have been reported in few individuals with A/M.<sup>26</sup> ***COX7B* gene:** Variants are rare, as they have only been reported in a small subset of families with microphthalmia with linear skin defects (MLS) syndrome.<sup>10</sup>

***CRYBA4* gene:** Variants identified in individuals with microphthalmia are rare.<sup>8,11</sup>

***FOXE3* gene:** Variants account for approximately 1% of all individuals with A/M.<sup>2</sup> However, *FOXE3* variants were identified in 15% (4/26) individuals affected with bilateral microphthalmia, all of which were in consanguineous families.<sup>12</sup>

***GDF6* gene:** Variants have been reported in approximately 2% of A/M cases.<sup>13</sup> ***HCCS* gene:** Variants are rare, as they have been reported in few individuals who have microphthalmia with linear skin defects (MLS) syndrome.<sup>14</sup>

***MITF* gene:** Variants are rare, as they have only been reported in a single family.<sup>15</sup>

***NAA10* gene:** Variants are rare, as they have only been reported in a single family.<sup>16</sup>

***NDUFB11* gene:** Variants are rare, as they have been reported in few individuals who have MLS syndrome.<sup>17</sup>

**OTX2 gene:** Variants have been reported in approximately 2 to 8% of all A/M cases.<sup>5</sup> **PAX6 gene:** Variants identified in individuals with microphthalmia are generally rare, accounting for less than 1% of individuals with microphthalmia.<sup>2,4</sup> However, PAX6 is a leading cause of aniridia.<sup>5</sup>

**PRSS56 gene:** Variants are rare, as they have been reported in few individuals who have microphthalmia.<sup>18</sup>

**RAX gene:** Variants have been identified in approximately 3% of individuals with A/M.<sup>2</sup> **SALL1 gene:** Variants are responsible for Townes-Brocks syndrome, which has been associated with microphthalmia in rare cases.<sup>19</sup>

**SHH gene:** Variants identified in individuals with microphthalmia are rare.<sup>5,20</sup> **SIX6 gene:** Variants are rare, as they have been identified in few individuals with microphthalmia.<sup>21,22</sup>

**SOX2 gene:** Variants are the most commonly identified, accounting for 10-20% of A/M cases.<sup>8</sup>

**STRA6 gene:** Variants account for less than 2% of individuals with microphthalmia.<sup>5,23</sup> **TENM3 gene:** Variants are rare, as they have been identified in few individuals with microphthalmia.<sup>24</sup>

**VSX2 (CHX10) gene:** Variants account for approximately 2% microphthalmia cases and have only been identified in consanguineous families to date.<sup>8,25</sup>

| Gene           | Protein   | Inheritance | Disease Associations   |
|----------------|---|-------------|--|
| <b>ALDH1A3</b> | Aldehyde dehydrogenase 1 family, member A3      | AR          | Anophthalmia/microphthalmia  |
| <b>BCOR</b>    | BCL6 corepressor                                | XLD         | Lenz microphthalmia, oculofaciocardiodental syndrome                     |
| <b>BMP4</b>    | Bone morphogenetic protein 4                    | AD          | Microphthalmia, orofacial cleft  |
| <b>BMP7</b>    | Bone morphogenetic protein 7                    | AD          | Anophthalmia/Microphthalmia  |
| <b>COX7B</b>   | Cytochrome C oxidase subunit 7B                 | AD          | Microphthalmia with linear skin defects, SHORT syndrome, orofacial cleft |
| <b>CRYBA4</b>  | Crystallin, beta-A4                             | AD          | Microphthalmia, cataract   |
| <b>FOXE3</b>   | Forkhead box E3                                 | AR          | Microphthalmia, anterior segment dysgenesis                              |
| <b>GDF6</b>    | Growth differentiation factor 6                 | AD          | Microphthalmia, coloboma   |
| <b>HCCS</b>    | Hepatocellular carcinoma                        | XLD         | Microphthalmia with linear skin defects                                  |
| <b>MITF</b>    | Microphthalmia-associated transcription factor  | AD          | Wardenburg syndrome, Tietz albinism-deafness syndrome                    |
| <b>NAA10</b>   | N-alpha-acetyltransferase 10, catalytic subunit | XL          | Lenz microphthalmia syndrome, NAT deficiency, Ogden syndrome             |
| <b>NDUFB11</b> | NADH dehydrogenase 1-beta subcomplex, 11        | XLD         | Microphthalmia with linear skin defects                                  |
| <b>OTX2</b>    | Orthodenticle, homolog of drosophila, 2         | AD          | Microphthalmia, pituitary hormone deficiency, retinal dystrophy          |

|                     |  |    |   |
|---------------------|--|----|---|
| <b>PAX6</b>         | Paired box gene 6                                | AD | Aniridia, microphthalmia, cataracts                 |
| <b>PRSS56</b>       | Protease, serine, 56                             | AR | Microphthalmia, nanophthalmia                       |
| <b>RAX</b>          | Retina and anterior neural fold homeobox gene    | AR | Microphthalmia                                      |
| <b>SALL1</b>        | Sal-like 1                                       | AD | Townes-Brock syndrome                               |
| <b>SHH</b>          | Sonic hedgehog                                   | AD | Microphthalmia, Holoprosencephaly                   |
| <b>SIX6</b>         | Sine oculous homeoboxof 6, homolog of drosophila | AR | Microphthalmia, optic disc abnormalities            |
| <b>SOX2</b>         | SRY-box 2  | AD | Anophthalmia/microphthalmia, optic nerve hypoplasia |
| <b>STRA6</b>        | Stimulated by retinoic acid 6                    | AR | Anophthalmia/microphthalmia, Matthew-Wood syndrome  |
| <b>TENM3</b>        | Teneurin transmembrane protein 3                 | AR | Microphthalmia                                      |
| <b>VSX2 (CHX10)</b> | Visual systems homeobox 2                        | AR | Microphthalmia                                      |

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