

Prenatal Testing for *SOX2*, *OTX2* and *VSX2* Variants: Anophthalmia/Microphthalmia

CLINICAL FEATURES

Several developmental eye disorders have a known genetic basis, including microphthalmia and anophthalmia. Anophthalmia is the complete absence of the globe, or bulb, of the eye and hence, the most severe structural eye malformation. A milder form is microphthalmia, where the total axial length of the eye globe is at least two standard deviations below the mean for age. Simple microphthalmos refers to a structurally normal eye with short total axial length. In each of these conditions, the eyelids, conjunctiva and lacrimal apparatus are normal. In complex microphthalmia, additional abnormalities are present and may include anterior segment dysgenesis, cataract, persistent hyperplastic primary vitreous, chorioretinal coloboma and/or retinal dysplasia. Anophthalmia/microphthalmia has been observed in association with various genetic syndromes and approximately 25% of individuals with anophthalmia/microphthalmia have identifiable chromosomal abnormalities.¹ Pathogenic variants in the *SOX2*, *OTX2* and *VSX2* genes leading to haploinsufficiency may be associated with anophthalmia and microphthalmia. *SOX2* variants are also known to be associated with hearing loss, developmental delay, esophageal atresia, genitourinary abnormalities, myopathy, and spastic diplegia. *OTX2* variants have been reported in patients with anophthalmia/microphthalmia associated with brain malformations and pituitary insufficiency. *VSX2* variants are usually associated with isolated ocular finding without other systemic malformations.

Anophthalmia/microphthalmia can be detected by the evaluation of the inter- and intra-orbital distances during the 2nd trimester. It can be isolated or associated with other malformations. *SOX2*-related eye abnormalities are usually bilateral, severe, and detectable by prenatal ultrasound examination.^{1,7} Prenatal molecular analysis for the anophthalmia/microphthalmia panel should be considered even in the absence of known family history. Ultrasound examination may be normal in affected fetuses; therefore, pregnancies at risk to inherit a specific known familial pathogenic variant can be offered targeted molecular testing regardless of ultrasound findings, if desired.

INHERITANCE PATTERN/GENETICS

Isolated anophthalmia/microphthalmia is genetically heterogeneous and may be inherited as autosomal dominant, recessive or X-linked trait.

TEST METHODS

Using genomic DNA, bi-directional DNA sequence of the coding region of *SOX2* (single large exon), *VSX2* (exons 1-5) and *OTX2* (exons 3-5) is obtained and analyzed. Concurrently, deletion/duplication testing by targeted array CGH analysis with exon-level resolution (ExonArrayDx) will be performed to evaluate for a deletion or duplication of one or more exons of the *SOX2* and *OTX2* genes. For known familial variants, the relevant portion of the *SOX2*, *OTX2* and/or *VSX2* gene will be analyzed in duplicate.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination will be performed. Therefore, in all prenatal cases a maternal sample should accompany the fetal sample.

The majority of *OTX2* variants are nonsense and frameshift variants leading to haploinsufficiency. Nonsense and frameshift variants, as well as whole gene deletions, have been observed in the *SOX2* gene.⁵ *VSX2* variants are mainly missense and a partial gene deletion including the entire exon 3 has been also reported.^{3,6} Interstitial deletions involving both *SOX2* have been observed in patients with bilateral anophthalmia. Interstitial deletions at

14q22-q23 including the OTX2 gene have been reported in two cases with bilateral anophthalmia and pituitary abnormality.

TEST SENSITIVITY

Heterozygous missense and protein-truncating variants in the SOX2 gene have been observed in 11-20% of individuals with anophthalmia/microphthalmia.^{2,4} SOX2 whole gene deletions have been observed in approximately 10% (5/52) of individuals with severe microphthalmia/anophthalmia.⁵ OTX2 variants and deletions account for 2-3% of anophthalmia/microphthalmia cases. VSX2 variants account for approximately 2% (2/117) of the patients with non-syndromic microphthalmia.⁶ Sequence and deletion/duplication analysis as performed by GeneDx is expected to identify all reported variants in the SOX2, VSX2 and OTX2 genes. The sensitivity of SOX2, OTX2 and VSX2 testing in pregnancies with ultrasound anomalies suggestive of anophthalmia/microphthalmia is currently unknown.

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