

Published in final edited form as:

*Mol Genet Metab.* 2011 December ; 104(4): 448–456. doi:10.1016/j.ymgme.2011.09.029.

## Eye Development Genes and Known Syndromes

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### Abstract

Anophthalmia and microphthalmia (A/M) are significant eye defects because they can have profound effects on visual acuity. A/M is associated with non-ocular abnormalities in an estimated 33–95% of cases and around 25% of patients have an underlying genetic syndrome that is diagnosable. Syndrome recognition is important for targeted molecular genetic testing, prognosis and for counseling regarding recurrence risks. This review provides clinical and molecular information for several of the commonest syndromes associated with A/M: Anophthalmia-Esophageal-Genital syndrome, caused by *SOX2* mutations, Anophthalmia and pituitary abnormalities caused by *OTX2* mutations, Matthew-Wood syndrome caused by *STRA6* mutations, Oculocardiofaciodental syndrome and Lenz microphthalmia caused by *BCOR* mutations, Microphthalmia Linear Skin pigmentation syndrome caused by *HCCS* mutations, Anophthalmia, pituitary abnormalities, polysyndactyly caused by *BMP4* mutations and Waardenburg anophthalmia caused by mutations in *SMO1*. In addition, we briefly discuss the ocular and extraocular phenotypes associated with several other important eye developmental genes, including *GDF6*, *VSX2*, *RAX*, *SHH*, *SIX6* and *PAX6*.

### Keywords

Anophthalmia/microphthalmia; *SOX2*; Anophthalmia-Esophageal-Genital (AEG) syndrome; *OTX2*; Matthew-Wood syndrome; *STRA6*; Oculofaciocardiodental syndrome; *BCOR*; Microphthalmia-Linear Skin Streaks; *HCCS*; *BMP4*

### Introduction

Anophthalmia and/or microphthalmia (A/M) can be defined as an absence or reduced size of the globe in the orbit [1, 2]. A/M is rare, with an estimated incidence of 1 to 3.2 cases per 10,000 live births [3, 4]. More than 50% (published range 33–95%) of individuals with A/M have extraocular findings, most commonly involving the limbs, musculoskeletal system and the craniofacial region with anomalies of the face, ear and neck [4, 5]. Causative chromosome aberrations are found in an estimated 25–30% of patients with A/M and a syndrome diagnosis is forthcoming in an estimated 20–45% of individuals [4, 5, 6, 7, 8]. The genetic syndromes associated with A/M often have characteristic and recognizable

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phenotypes and an awareness of their clinical features is essential, as the correct diagnosis can inform appropriate management decisions and genetic testing.

In this review, we provide a brief synopsis of the physical findings present in the commonest and most distinctive syndromes associated with A/M. We focus on seven eye genes associated with A/M that have well-defined phenotypes – *SOX2*, *OTX2*, *STRA6*, *BCOR*, *HCCS*, *BMP4* and *SMOCL1*. We briefly comment on the ocular and systemic phenotypes that have been associated with several other genes that are known to cause A/M in humans – *GDF6*, *VSX2*, *RAX*, *SHH*, *SIX6* and *PAX6*. Mutations in many of the A/M causative genes result in pleiotropic phenotypes with variable expressivity and incomplete penetrance. We then discuss possible relationships between the clinical findings that result from gene mutations in interacting genes and networks.

### 1.1 Anophthalmia-Esophageal-Genital (AEG) syndrome and *SOX2* mutations

An estimated 4–20% of cases of A/M are caused by mutations or deletions in *SOX2* and there are more than 60 case reports in the medical literature [6, 9, 10, 11, 12]. Although *SOX2* gene mutations or deletions are still relatively rare, with an estimated frequency of 1 in 250,000 births, they constitute the most common known genetic cause of A/M to date.

The majority of patients with *SOX2* mutations/deletions suffer from severe, bilateral A/M [6]. However, a wide spectrum of eye findings that are individually rare have been reported, ranging from unilateral A/M to anterior segment dysgenesis with iris hypoplasia, cataracts and colobomas, papillary defects with hypermetropia and retinal dystrophy, colobomas and retinal detachments [6, 11, 12, 13, 14, 15]. The variability in humans mirrors the wide range of ocular phenotypes that result from altered *Sox2* gene dosage in the mouse [13, 16]. Some individuals with *SOX2* mutations have had no ocular phenotype [17] and one pair of monozygous twins with the recurrent intragenic *SOX2* deletion (c.70del20, resulting in p.Asn24fs88X) had discordant ocular phenotypes [18].

*SOX2* mutations and deletions have been connected to a wide variety of extra-ocular findings. Neurological features that are relatively consistent in type but highly variable in severity usually dominate the clinical presentation. Brain abnormalities (29/62; 47%) have included mesial temporal malformations, grey matter heterotopias, hamartomas of the tuber cinereum, cavum septum pellucidum, non-specific periventricular white matter abnormalities and agenesis of the corpus callosum [6]. Developmental delays are frequent and in one review, 28 of 47 (60%) patients from whom information was available had global delays and 7/47 (15%) had motor delays, although 12/47 (26%) individuals were reported to be developmentally normal [6]. 11/62 (18%) individuals have had seizures [6]. Other neurological abnormalities have included aberrant motor planning and dysdiadochokinesis, athetosis, bradykinesia, oromotor dyspraxia, disordered muscle tone with evidence of mild basal ganglia dysfunction and a wide-based gait [10].

Endocrine abnormalities were found in 8/62 (13%) patients with *SOX2* mutations [6]. Pituitary hypoplasia in those with *SOX2* mutations can cause profound gonadotropin deficiency with hypogonadotropic hypogonadism despite relative sparing of growth hormone [6, 13, 19]. 7/62 (11%) individuals had intra-uterine growth retardation and postnatal growth retardation [6]. Dysmorphic features were subtle in many cases and included dolichocephaly, facial asymmetry, a tall forehead, short and narrow palpebral fissures with deep-set or widely spaced eyes, dysplastic ears that were small and cupped with prominent anti-helices and fleshy ear lobes, midface hypoplasia, crowded teeth with widely spaced or prominent central incisors and micro-retrognathia or a prominent chin [6, 20]. Other findings described in patients with *SOX2* mutations, but that may be incidental, were pyloric stenosis, mild pectus excavatum, hyperextensible joints, fifth finger

clinodactyly, 2–3 toe syndactyly and a sacral dimple [6, 10, 20]. Sensorineural hearing loss of moderate severity has also been reported [13], but was relatively rare (4/62; 6%) [6]. Cardiovascular defects were uncommon (2/62; 3%) [6].

The association of A/M with tracheo-esophageal fistula (TEF) and genital abnormalities has been termed Anophthalmia-Esophageal-Genital (AEG) syndrome [6, 10, 20]. TEF and/or esophageal atresia are relatively unusual malformations in ocular disease, but were described in 15/62 (24%) individuals with *SOX2* mutations [6, 10, 20]. Genitourinary tract malformations (22/62; 35%) have comprised cryptorchidism, micropenis and hypospadias in males and renal hypoplasia, horseshoe kidney or duplex kidney in both sexes [6, 11]. Various vertebral and rib malformations (1/62; 2%) such as rib fusion, 11 pairs of ribs, hemivertebrae and butterfly vertebrae have also been noted with the AEG phenotype [6, 17].

*SOX2* is a transcription factor with one coding exon that produces a 317 amino acid protein containing a high mobility group (HMG) DNA binding domain, a C-terminal transactivation domain and a transactivator complex or partner factor interaction domain. Sox2 interacts with Pax6 and Otx2 to effect gene regulation, activating  $\delta$ -crystallin for lens formation from the former interaction and co-regulating *Rax* expression from the latter [21, 22]. *Sox2* expression in mice occurred within the optic vesicle and stalk, neural retina, the lens and placodal area of the surface ectoderm; in humans expression was also seen in the neural retina, optic stalk and lens [12, 13]. The *SOX2* mutational spectrum has included whole gene deletions and intragenic deletions, nonsense and frameshift alterations (more common than missense substitutions and more likely to be deleterious) and missense mutations affecting the DNA-binding or transactivation domains [11]. There was one recurrent, 20 bp deletion (c.70del20, resulting in p.Asn24fs88X), which was found in an estimated 18–30% of all patients with A/M and that resulted in a severe phenotype that has included AEG syndrome [6, 12]. Two similar deletions of 17 bp and 23 bp have occurred in the same region, possibly caused by a GGCGGC repeat sequence flanking the region and mispairing [10, 17]. Recurrent mutations were otherwise rare [9, 11, 12]. There has been a single frameshift mutation that has predicted protein elongation (c.943del2) [9].

Although the vast majority of *SOX2* mutations have arisen as *de novo* events, *SOX2* mutations can be inherited in an autosomal dominant pattern with intrafamilial variability [19]. Maternal mosaicism has been described [6, 13, 17, 23, 24], as has paternal transmission, with an absent or milder phenotype in the father [13, 14]. The pathogenesis of *SOX2* mutations has been attributed to loss of gene function, based on the phenotypic similarity between cases with premature protein truncation and deletion cases, but dominant negative effects from the mutations are also possible. A phenotype-genotype correlation has been suggested because severe mutations that result in complete loss of function were more likely to cause A/M (47/50; 94%) compared to missense mutations, which were more likely to be associated with a non-microphthalmic phenotype (11/21; 52%) or a less severe ocular phenotype with fewer developmental or systemic abnormalities [6].

## 1.2 Pituitary Abnormalities and *OTX2* mutations

Mutations in *OTX2* have been responsible for an estimated 2–3% to 8% of A/M and there are more than 30 examples in the medical literature [8, 25, 26]. A/M has been consistently associated with *OTX2* mutations, although transmitting parents can have normal eyes [26]. In addition to A/M, a range of ocular abnormalities, including anterior segment defects (4/37; 11%), Leber's congenital amaurosis, hypoplasia/aplasia of the optic nerve (13/37; 35%) and hypoplasia of the optic chiasm and dysplastic optic globes [26, 27, 28], have been observed.

Mutations in *OTX2* have been strongly associated with pituitary abnormalities, either morphological (examples include hypoplasia of the anterior portion of the gland or the entire gland and an ectopic posterior pituitary) or functional (examples include growth hormone deficiency or combined hormonal deficiencies). Pituitary findings have been present in 4/21 (19%) to 11/37 (30%) of cases in different studies [26, 27, 29] and these frequencies may be underestimates because of incomplete investigations in some individuals. The pituitary defects have shown variation in people with the same mutation and have been reported in the absence of A/M [29]. Brain abnormalities, including Chiari malformations, have been present in 7/37 (19%) of those with *OTX2* mutations [26, 27]. Developmental delays ranging from mild to severe were found in 17/37 (46%) and attention deficit hyperactivity disorder (ADHD) and autistic features have been described [26].

Other relatively common findings in individuals with *OTX2* mutations were failure to thrive with growth retardation (12/37; 32%), microcephaly (7/37; 19%), feeding difficulties (5/37; 14%) and hypotonia (2/37; 5%) [26]. Genital hypoplasia, with small labia majora in females and cryptorchidism and a small penis in males, has been reported [27]. Cleft palate (2/21; 10%), seizures and sensorineural hearing loss were rare [26, 28, 30]. Sacral dimple, an anteriorly placed anus and Wolf-Parkinson-White syndrome likely represent coincidental findings [26].

*OTX2* is a member of the family of homeobox-containing transcription factors related to the *D. Melanogaster* gene orthodenticle (*otd*) [31]. Located at chromosome 14q22.23, *OTX2* has 3 exons and encodes two isoforms, both with homeodomains, DNA-binding motifs and N- and C-terminal transactivation domains [28, 29, 32]. Expression occurs in the brain, including the pituitary gland and hypothalamus, eyes, ears and nose. The shorter, 289 amino acid isoform is predominantly expressed in comparison to the longer, 297 amino acid isoform [29]. Homozygous *Otx2* 'knock-out' mice die prior to birth with significant brain malformations [28]. Heterozygous mutant mice show a range of malformations, including A/M, anencephaly, ethmocephaly, holoprosencephaly, short nose and micrognathia/agnathia [33].

*OTX2* mutations are inherited in an autosomal dominant pattern. The mutational spectrum has included nonsense alterations, insertions, intragenic deletions, whole gene deletions and missense mutations [26, 28]. Most mutations predicted premature protein truncation and loss-of-function, with the exception of p.Asn225Ser, which has been shown to have a dominant negative effect on target gene expression [26, 34]. There was substantial intrafamilial variability and the same mutation can result in normal eye development or severe A/M [25, 26, 34]. One third of affected individuals inherit their mutation from a parent who can be phenotypically normal due to mosaicism or incomplete penetrance, despite frameshift or nonsense mutations [30]. A genotype-phenotype correlation was found by some authors, who have suggested that the pituitary abnormalities occur more commonly in patients with mutations in the second half of the gene that result in retention of the homeodomain and an SGQFTP motif, which may confer dominant negative effects [26]. However, other studies have not confirmed this relationship [28].

### 1.3 Matthew-Wood syndrome/PDAC syndrome and *STRA6* mutations

There are more than 30 patients reported in the literature with Stimulated by retinoic acid gene 6 homolog (*STRA6*) gene mutations that result in a severe, but variable phenotype named both Matthew-Wood syndrome and Pulmonary hypoplasia/agenesis, Diaphragmatic hernia/eventration, Anophthalmia/microphthalmia and Cardiac defects (PDAC) syndrome [35, 36, 37, 38, 39]. Anophthalmia or severe microphthalmia have been invariable in those with two *STRA6* mutations (20/20; 100%). Almost all patients with mutations in *STRA6* have had at least one other major phenotypic component of PDAC, with pulmonary

hypoplasia or pulmonary agenesis in 12/21 (57%), congenital diaphragmatic hernia and/or diaphragmatic eventration in 10/21 (48%) and cardiac defects, particularly involving the pulmonary arteries or the common aortopulmonary trunk in 14/21 (67%) [40]. One more mildly affected patient has been described who was a compound heterozygote for two mutations [40].

Craniofacial dysmorphism was frequent in PDAC syndrome and was characterized by distinct and 'bushy' eyebrows [35, 39, 40], hypoplastic nipples and hypoplastic toenails [40]. Other common findings were renal anomalies (6/21; 29%) including pelvic kidney, horseshoe kidney, renal hypoplasia and malrotation of the kidney, and genital malformations with a bicornuate or hypoplastic uterus and cryptorchidism [38, 39]. Gastrointestinal malformations such as duodenal stenosis, an annular pancreas or absent pancreas, intestinal malrotation, an accessory spleen or a hypoplastic spleen have also been observed [35, 38, 39]. Less frequent findings were pre- and postnatal growth retardation, thymic hypoplasia, subglottic laryngeal stenosis, cleft palate, pulmonary capillary dysplasia, a thin or absent corpus callosum, small optic nerves, arrhinencephaly and Dandy-Walker malformation [38, 40].

Individuals with *STRA6* mutations generally have poor survival, and the majority of cases were reported in childhood, with the exception of one child aged 9 years of age and one adult [35, 36, 38, 40]. Mental retardation was common in those who survive the neonatal period, but a developmental profile within the expected range was described in a blind, 30-month old female with p.Asp560His and p.Arg655His mutations [35, 40].

The *STRA6* gene is located at chromosome 15q24.1 and has 20 exons that produce a 667 amino acid protein [35, 36]. *STRA6* was widely expressed and was present in the optic vesicle and developing eye, optic nerve meninges, lung, endodermal gut derivatives, limbs and somites [41, 42]. The protein functions as a receptor that mediates the cellular uptake of retinol binding protein that bears circulating vitamin A [41]. The subsequent intracellular metabolism of retinol to retinoic acid regulates the expression of multiple developmental target genes [43]. It is possible that some of the phenotypic variability observed with *STRA6* mutations can be explained by differences in vitamin A metabolism [38].

The inheritance pattern of *STRA6* mutations is autosomal recessive and the mutational spectrum has included three whole-gene deletions, nonsense and frameshift mutations and missense alterations that result in loss of function [40]. A phenotype genotype correlation has not been established [38]. PDAC syndrome is likely to be genetically heterogeneous [35, 36], but further genes have not been forthcoming. *STRA6* has also been sequenced in 18 patients with isolated A/M [37]. One child who was 6 years and 9 months of age and who had bilateral microphthalmia and a duplicated renal collecting system had two mutations: p.Gln592X and p.Gly217Glu [37]. *STRA6* was sequenced in a patient with Pulmonary and pulmonary artery hypoplasia, a<sub>g</sub>onadism, o<sub>m</sub>phalocele, d<sub>i</sub>aphragmatic defect and d<sub>e</sub>xtrocardia (PAGOD syndrome) [42], but no mutations were identified.

#### 1.4 Oculofaciocardiodental (OFCD) syndrome, Lenz microphthalmia and *BCOR* mutations

Oculofaciocardiodental (OFCD) syndrome and Lenz microphthalmia syndrome are both caused by mutations in the BCL-6 corepressor gene (*BCOR*) [44] and hence are often considered together. There have been more than 60 reported patients with OFCD syndrome [45, 46, 47, 48, 49, 50]. As the name suggests, Ocular, Facial, Cardiac and Dental manifestations were the cardinal findings and the syndrome has been among the more easily recognizable eye disorders with A/M.

Congenital cataracts are the predominant ocular finding (100%) in OFCD syndrome, but microphthalmia and/or microcornea 28/34 (82%) are also frequent; ptosis, iris synechiae, lens dislocation, retinal detachment and optic nerve dysplasia have been noted [50]. The eye findings may be unilateral [51]. The facial anomalies are recognizable, with a long and narrow face in 26/34 (76%), a high nasal bridge and midface hypoplasia and a broad or septate nasal tip in 25/26 (96%) [45, 50]. Cardiac anomalies, seen in 20/27 (74%), comprised atrial/ventricular septal defects (17/20; 85%) and less commonly, double-outlet right ventricle, aortic stenosis, mitral and tricuspid insufficiencies, Pentalogy of Fallot, dextrocardia and patent ductus arteriosus [50]. Dental findings included canine radiiculomegaly or enlarged dental roots (20/22; 91%), root dilacerations, oligodontia and retained deciduous teeth [50]. In 22 patients for whom information was available for both primary and secondary dentition, dental abnormalities were present in all individuals [50]. Soft tissue syndactyly of the second and third toes (16/28; 57%), hammer-type flexion deformities of the toes (16/28; 54%) and radioulnar synostosis (7/28; 25%) have been common musculoskeletal features [50]. Cleft palate, a high-arched palate or a bifid uvula have been reported in 8/26 (31%) [50]. Developmental problems were seen in 6/34 (18%) of patients, but were generally mild [50]. Hearing loss was present in 5/34 (15%) [50]. Laterality defects involving the heart and viscera have also been linked to *BCOR* mutations [49]. The clinical findings of OFCD are only apparent in females; phenotypic variability can also occur due to somatic mosaicism and X chromosome inactivation [50].

In contrast to OFCD, Lenz microphthalmia was first described in males with a more complex phenotype comprising A/M with colobomas, microcephaly and structural brain abnormalities, mental retardation, palatal defects, congenital heart disease including atrial septal defects, anomalies of the fingers and clavicles, unilateral renal aplasia and cryptorchidism [44, 50]. This phenotype has been associated with a specific missense mutation in *BCOR*, c.254C>T, predicting p.Pro85Leu, in two apparently unrelated families. No further *BCOR* mutations have been found in individuals with Lenz microphthalmia, suggesting that genetic heterogeneity exists and that *BCOR* is not the predominant gene [46, 50].

*BCOR* is located at chromosome Xp11.4 and nonsense and frameshift mutations (most common; resulting in premature termination of the protein with deletion of the C-terminal domain), splicing mutations and gene deletions have been described [44, 46, 47]. The frameshift mutations were predicted to cause nonsense mediated decay, and thus loss of gene function is likely to be the pathogenic mechanism. The expression pattern of the murine gene in tooth primordium, eye, neural tube and the branchial arches has correlated well with the tissues that are affected in OFCD and Lenz microphthalmia [52]. *BCOR* acts as a co-repressor of BCL-6, itself a transcriptional repressor, by altering chromatin modification [53]. *BCOR* was sequenced in 96 patients with apparently isolated microphthalmia, coloboma and/or mental retardation [50]. A single frameshift mutation was identified in an affected female with bilateral cataracts and unilateral microphthalmia who was subsequently revealed to have had numerous teeth removed in her teenage years and 2–3 toe syndactyly, suggesting that she was, in fact, affected with OFCD.

### 1.5 Microphthalmia with Linear Skin defects (MLS) Syndrome and *HCCS* mutations

There are more than 50 case reports of Microphthalmia with Linear Skin defects (MLS; also known as Microphthalmia, Dermal Aplasia and Sclerocornea [MIDAS]) in the medical literature [54, 55, 56, 57]. The two major diagnostic criteria for MLS are unilateral or bilateral A/M (92%) [57] and congenital defects of the skin (89%) [57, 58]. The skin lesions were commonly present on the scalp, face and neck and have been described as a linear and patchy erythroderma, ‘weeping’ or jagged; however, they can heal with minimal residual

scarring during the first few months or later in childhood [55, 57, 59]. The skin lesions were rarely present without eye defects [57].

Minor features of MLS were wide-ranging, with significant intrafamilial variability [55]. Eye defects most frequently involved the anterior segment (sclerocornea/microcornea and orbital cysts), but there were less commonly observed ocular manifestations that were diverse, such as corneal leukoma, iridocorneal adhesions, congenital glaucoma with total/peripheral anterior synechiae, aniridia and cataracts [55, 57, 59, 60]. Central nervous system involvement has included anencephaly, agenesis of the corpus callosum, hydrocephalus, mental retardation, infantile seizures and developmental delays, but there was little data on incidence of these findings [57]. Congenital heart defects, such as hypertrophic and oncocytic cardiomyopathy [61], atrial and ventricular septal defects and arrhythmias with supraventricular tachycardia should also be considered part of the phenotype [57]. Short stature, diaphragmatic hernia, nail dystrophy, preauricular pits and hearing loss, genitourinary malformations, anterior or imperforate anus, bicornuate uterus, ambiguous genitalia and penile hypospadias in males with a 46,XX karyotype have been noted [55, 56, 57, 58]. The clinical features are likely to have been influenced by the presence of differently sized Xp22 deletions in affected individuals.

The MLS causative gene, Holocytochrome c-type synthase (*HCCS*), is located at chromosome Xp22.2 and has 7 exons, of which 6 encode a 268 amino acid protein. *HCCS* is an enzyme located in the inner mitochondrial membrane that adds heme to apocytochrome c and c<sub>1</sub>, resulting in mature holocytochrome c [55]. Deficiency or overexpression of the murine *Hccs* protein is associated with lethality, developmental abnormalities and defective apoptosis in mice [62]. Defects in oxidative phosphorylation and the mitochondrial respiratory chain can be anticipated with *Hccs* deficiency, which triggers cell death by necrosis [55]. Chromosome deletions or intragenic deletions are commonest in the mutational spectrum and segmental monosomy for *HCCS* was found in 77% of those with MLS [63], but missense mutations and nonsense mutation have also been described [55]. Inheritance is X-linked dominant and the syndrome has been reported to be lethal in males [57].

Sequencing of *HCCS* in 27 females with isolated A/M showed heterozygosity for the missense mutation c.475G>A, predicting p.Glu159Iys, in a female with bilateral microphthalmia and sclerocornea [64]. This sequence alteration did not complement respiratory deficiency of the CYC3- *S. cerevisiae* mutant strain and was not found in 460 control chromosomes; in addition, a skewed X-inactivation pattern was identified in the affected individual, suggesting that the alteration was pathogenic [64]. This report suggests that MLS can occur without skin findings and/or in patients with isolated A/M and other ocular defects – further descriptions of those with only ocular findings were a female with a deletion encompassing part of *HCCS* presenting with a left opaque cornea, congenital glaucoma and a white anterior cataract [55] and a girl with bilateral sclerocornea and partial aniridia in the left eye resulting from an X/Y translocation and a heterozygous deletion of the MLS critical region [59].

### 1.6 Pituitary defects, brain malformations, digital anomalies and *BMP4* mutations

The involvement of Bone Morphogenetic Protein 4 (*BMP4*) in the pathogenesis of A/M was first suggested from two patients with *de novo* chromosome deletions including *BMP4* and *OTX2*, karyotypes 46,XX,del(14)(q22.3q23.2) and 46,XY,del(14)(q22.2q23.1) [65, 66]. Both patients had bilateral A/M, partial agenesis of the corpus callosum, cerebellar hypoplasia and developmental delays [65, 66]. One patient had an abnormal pituitary gland. The subsequent report of an individual with congenital glaucoma and sclerocornea, brain anomalies and developmental delays who had a *BMP4* deletion that did not include *OTX2*

implicated *BMP4* in the pathogenesis of A/M [67] and several mutations in *BMP4* have since been described [66, 68, 69]. The ocular findings in individuals with *BMP4* mutations have included Axenfeld-Rieger spectrum and anterior segment dysgenesis, coloboma and retinal dystrophy in addition to the A/M, glaucoma and sclerocornea described above.

*BMP4* mutations have caused pleiotropic phenotypes and there was variable expressivity and incomplete penetrance [66, 69]. Findings in more than one affected individual have included short stature and weight less than the 3<sup>rd</sup> centile, developmental delays, macrocephaly, hydrocephalus and bilateral postaxial polydactyly [66, 67, 69, 70]. Missense mutations have been found in individuals with cleft lip and palate [70] and renal malformations [71]. A patient with SHORT syndrome (short stature, hyperextensibility of joints/inguinal hernia, ocular depression, Rieger anomaly and delayed dental eruption) had a deletion involving *BMP4* and at least 12 other genes [69]. A further example of the phenotypic complexity can be found with the c.226del2 mutation that predicts p.Ser76fs104X, which was associated with A/M, retinal dystrophy, polydactyly and syndactyly, diffuse brain atrophy with widened sulci and partial agenesis of the corpus callosum [69].

The *BMP4* gene is located at chromosome 14q22.23 and contains 3 coding exons in a total of 4 exons. The protein is 408 amino acids long and contains a TGF- $\beta$ 1 propeptide and a TGF- $\beta$  domain that induces intracellular signaling through the SMAD family [66]. *Bmp4* cooperates with *Pax6* and *Bmp7* in lens placode formation and *Bmp4* +/- heterozygous null mice have shown a variety of eye defects, including A/M, anterior segment dysgenesis with failure of lens induction and retinal and optic nerve aplasia [66, 72]. Inheritance is autosomal dominant and the mutational spectrum has included deletions, nonsense and missense mutations, although some of the missense mutations may prove to be rare polymorphisms [69].

### 1.7 Waardenburg anophthalmia syndrome and *SMOC1* mutations

Waardenburg anophthalmia syndrome [WAS; OMIM 206920; also known as ophthalmomelic syndrome] was first reported in two families – one with two sisters who had unilateral anophthalmia, synostosis of the fourth and fifth metacarpals and postaxial oligodactyly of the toes with soft tissue syndactyly [73]. Variable expressivity was evident, as one of the affected sisters had coloboma in the contralateral eye and a brother from the same family presented with only limb manifestations [73]. In the second, unrelated family, an affected female had bilateral anophthalmia, brachydactyly of the fingers, postaxial oligodactyly with four toes on each foot and significant learning disabilities [73]. More than 30 affected individuals have since been reported and the phenotypic features have been summarized as unilateral or bilateral anophthalmia (32/35; 91.4%), postaxial oligodactyly of lower limbs (29/35; 83%), synostosis of the fourth and fifth digits of the hands (20/35; 57%) and learning disabilities (13/35; 37%) [74]. Other common features were orofacial clefts (4/35) and perinatal or postnatal death (10/35); less frequent features comprised absent or hypoplastic optic nerves, horseshoe kidneys, cryptorchidism and hypospadias, contractures of the fingers and elbows, bowed tibiae, coxa valga, talipes equino varus and cutaneous syndactyly of the toes [74]. The syndrome was initially linked to chromosome 10p11.23 in three unrelated families [75], but no causative gene was identified.

Recently, three groups used autozygosity/homozygosity mapping to narrow the locus for WAS to a 1–3 Mb interval at chromosome 14q24.2 [74, 76, 77]. All three researchers found mutations in the secreted protein acidic and rich in cysteine (SPARC)-related modular calcium binding 1 (*SMOC1*) gene that were predicted to cause loss of function [74, 76, 77]. Mutations have so far been identified in 12 families, with evidence of genetic heterogeneity. The mutational spectrum has included nonsense, single base-pair deletions, missense and



splice site mutations and the mutations were spread across the gene [74, 76]. Inheritance was autosomal recessive.

Studies in mice have shown that *Smoc1* is expressed in the forebrain, midbrain, hindbrain, pharyngeal arches, rostral neural tube, forelimb anlage, frontonasal region and somites at E9.5 [74, 76]. Later in development, expression was also visualized in the dorsal hindlimbs, medial region of the dorsal and ventral forelimbs, the optic stalk, ventral optic nerve and the site of closure of the optic cup [74, 76]. Mice with a targeted, pre-conditional mutation in *Smoc1* containing a LacZ reporter allele (*Smoc*<sup>tm1a/tm1a</sup> mice) were created and had 10% of the wildtype level of *Smoc1* mRNA [74]. These mice had highly penetrant oligodactyly of the hindlimbs, iris and retinal colobomas, small body size, cleft palate and a high perinatal mortality that accurately recapitulated the human phenotype [74]. Homozygous null *Smoc1*<sup>TP/TP</sup> mutant mice also shared delayed growth, small eyes with aplasia or hypoplasia of the optic nerves, atrophy of the anteroventral part of the retina and extension of the retinal pigment epithelium to the optic nerve [76]. Examination of the hindlimbs of these mutant mice showed strain dependent synostosis between the fourth and fifth metatarsals, syndactyly and pes valgus, bowed tibiae and hypoplastic fibulae and soft tissue syndactyly in the forelimbs [76]. Expression in *Danio rerio* included the choroid fissure, brain and pharyngeal arches [77] and targeting of the *Danio rerio* *SMOC1* orthologue with antisense morpholinos caused microphthalmia, coloboma with delayed closure of the choroid fissure, defects of the forebrain and possibly the pharyngeal arches [77].

The SMOC1 protein is secreted and contains a follistatin-like domain, two thyroglobulin type I (Tg1) domains and an EF-hand calcium-binding domain [74, 76, 77]. The orthologue of *SMOC1* in *Xenopus* has been shown to function as a BMP antagonist [78], although the molecular mechanism for this is unknown.

### 1.8 A/M and *GDF6* mutations

Mutations in Growth and differentiation factor 6 (*GDF6*) have primarily been linked to Klippel-Feil syndrome (KFS), a condition defined by congenital fusion of the cervical spine vertebrae, a low posterior hairline and a short neck with limited mobility [79]. However, *GDF6* mutations have also been associated with a broad range of phenotypes, including A/M and coloboma [80, 81, 82]. A chromosome deletion that encompassed *GDF6* and 30 other genes was identified in a patient with bilateral chorioretinal colobomas, developmental delays, an atrial septal defect and soft-tissue syndactyly of the toes [80]. Further screening of *GDF6* in 489 individuals with eye disease revealed four missense mutations in patients with microphthalmia and coloboma and extraocular features that included KFS, hemivertebrae and malformed ribs, post-axial polydactyly and spondylocostal dysostosis [81]. More recently, sequencing in 50 patients with ocular malformations identified *GDF6* mutations in four (8%) patients, with three patients carrying the recurrent p.Ala249Glu sequence alteration [76]. *Gdf6* is expressed in the palate and one of these individuals also had cleft lip and palate, although this finding could be coincidental [82].

*GDF6* is a member of the Growth and differentiation factor family, a subgroup of the bone morphogenic protein (BMP) family that functions by signaling through the Smad pathway [80, 83]. In homozygous null mice, variable and asymmetric eye defects were observed, including excavation of the optic disc (8/12), marked asymmetry (6/12) and microphthalmia (1/12) [81]. *Gdf6* is expressed in the retina and gene knockdown experiments using morpholinos to reduce expression of the orthologous genes in *Danio rerio* and *Xenopus* have produced larvae with small eyes, absent optic lobes and disordered retinal layering [80, 83, 84]. Mutations have included deletions and missense mutations and two missense mutations, p.Ala249Glu and p.Lys424Arg, were shown to reduce the amount of secreted, mature GDF6 and result in a protein that was less effective in activating *SOX9* reporter gene activity

compared to wildtype GDF6 [81]. Incomplete penetrance was seen with three of the variants and inheritance was autosomal dominant [81].

### 1.9 A/M and *VSX2*

Mutations in the Visual system homeobox 2 gene (*VSX2*, previously known as *CHX10*) have been described in several families with non-syndromic microphthalmia [85, 86, 87]. Although other eye abnormalities including cataracts, iris abnormalities, retinal dystrophy and colobomas have been found in those with mutations, to date there are no extraocular findings [2].

### 1.10 A/M and *RAX*

In screening 75 A/M individuals for mutations, an individual with A/M and sclerocornea had a nonsense mutation, p.Glu147X and a missense mutation, p.Arg192Glu 83 in *RAX* [88]. Both of these mutations were located within the DNA-binding homeodomain of the *RAX* gene, and biochemical studies showed that both alterations were functionally significant [88]. Mutations in *RAX* have since been identified in other A/M patients and in patients with colobomas [89, 90]. Systemic findings have been absent with the exception of two patients who were heterozygotes - p.Thr50Pro was seen in a 11 year old child with unilateral microphthalmia, septum pellucidum agenesis, cortical atrophy and optic nerve atrophy and p.Arg110Gly was present in a 2 year old with anophthalmia, hydrocephalus and congenital hip dislocation [82]. However, these sequence alterations have not been shown to be pathogenic and thus it was not proven that these extraocular findings were related to altered *RAX*.

### 1.11 A/M and *SIX6*

*SIX6* was a candidate gene for A/M because of three independent reports of interstitial deletions at chromosome 14q22.3-q23, one of which included *SIX6*, in patients with bilateral anophthalmia and pituitary anomalies [91]. However, *SIX6* mutations seem to be uncommon or not causative for A/M and one study of 173 patients was negative for significant sequence alterations [92, 93].

### 1.12 A/M and *SHH*

Mutations in *SHH* were rare as a cause of isolated A/M and/or coloboma [94], although A/M can occur with holoprosencephaly caused by mutations in this gene [95]. One three-generation pedigree has also been published, in which a novel 24 bp deletion in *SHH* segregated with iris and uveoretinal colobomas [96].

### 1.13 A/M and *PAX6*

Mutations in *PAX6* have long been known to cause aniridia [97, 98], but *PAX6* mutations have been associated with a wider spectrum of eye and brain defects. Missense mutations in the paired domain can result in Peter's anomaly, corectopia with nystagmus, macular and foveal hypoplasia. Microphthalmia is rare [99]. Anophthalmia has been described in mice with loss of both copies of the gene [99]. Brain abnormalities, in particular agenesis of the corpus callosum, absence or hypoplasia of the anterior commissure and absence of the pineal gland, auditory processing defects and anosmia have been described [100, 101, 102]. The clinical features seen with *PAX6* mutations relate well to the expression of the murine gene, which includes the developing lens, the surface ectoderm of the head, the pit of the optic vesicle, prosencephalon, telencephalon, dicephalon and olfactory bulb [99].

## Discussion

We have described the phenotypes that arise from mutations in seven developmental eye genes associated with extra-ocular features – *SOX2*, *OTX2*, *STRA6*, *BCOR*, *HCCS*, *BMP4* and *SMO1*. We briefly mention the physical findings associated with six other A/M genes that show less phenotypic pleiotropy with regard to extraocular findings – *GDF6*, *VSX2*, *RAX*, *SIX6*, *SHH* and *PAX6*. The diversity of genes emphasizes the need for numerous intact genes and networks to achieve normal eye formation. The finding of non-penetrance associated with mutations in several of the genes also emphasizes the likely importance of modifier genes, epigenetic factors and environmental effects in determining the clinical presentation.

At present, there is too little clinical and molecular data to make definite correlations between the clinical findings caused by mutated genes that act in interrelated networks. There can be variation in ocular and extraocular expression for the genes that do interact and it is likely that different tissues have varying sensitivities to aberrant gene dosage. The spectrum of extraocular features associated with *OTX2* mutations (brain malformations, pituitary abnormalities and short stature, feeding difficulties and developmental delays) [25, 26, 28, 30] overlaps with the clinical features found with *SOX2* mutations [6] and a relationship between the phenotypes caused by mutations in *SOX2* and *OTX2* has been described (Table 1) [26]. The two proteins have been shown to interact through the HMG domain of Sox2 and the homeodomain of Otx2 [22, 99]. However, the lack of gastrointestinal abnormalities seen with *SOX2* mutations, such as EA/TEF, in patients with *OTX2* mutations, can provide clinical distinction. Both *Sox2* and *Otx2* coregulate *Rax*; as *Rax* is expressed in the developing pineal gland, ventral hypothalamus, cells lining the third ventricle and the posterior pituitary gland in addition to the eye [103], it is not perhaps surprising that brain malformations have been seen in patients with *Rax* sequence alterations, although not proven to be caused by them. *Pax6* and *Sox2* are mutually induced by each other and both may influence *Otx2* expression [22]; all three genes have been associated with cerebral malformations. Finally, mutations in both *GDF6* and *BMP4* ultimately act to perturb SMAD signaling [81] and both have caused skeletal abnormalities, including polydactyly and syndactyly, and orofacial clefting, although there still remains little information for both phenotypes. The partial overlap between Waardenburg anophthalmia and the phenotypes caused by disruption of *BMP4* indicate a possible functional relationship between *SMO1* and *BMP4* [76].

As the syndromes associated with A/M become better delineated, the requirement for a thorough evaluation of dysmorphic features, growth and development and other malformations in a patient with A/M is mandatory. Cranial imaging may be useful, including computerized tomography (CT) or magnetic resonance imaging (MRI) of the brain that includes the pituitary gland and the orbits and optic nerves [2]. Pituitary hormone testing and an audiological evaluation should be considered. Array comparative genomic hybridization and karyotyping should be undertaken in cases that have complex phenotypes in which cytogenetic aberrations should be considered [2]. Finally, a family history is vital and parental eye examinations are often important, especially for consideration of recurrence risks. These examinations and investigations, together with knowledge of the likely phenotypes associated with different A/M genes, can guide targeted genetic testing.

## Acknowledgments

Anne Slavotinek was funded by an R21 grant EY19999 from the Eunice Kennedy Shriver Institute for Child Health and Development whilst writing this review.

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### Highlights

Carefully describing the phenotype can aid identification of the correct gene

Many of the genes that cause anophthalmia/microphthalmia interact

Predicting phenotypes caused by interacting genes is complicated

Mutations in causative genes can be non-penetrant or vary in expressivity

Modifier genes, epigenetic factors and environment can influence clinical features

**Table 1**  
Clinical Features Found with Mutations in a Selection of Anophthalmia/Microphthalmia Genes

|  | SOX2 | OTX2              | PAX6 | RAX                   | BMP4 | SMOC1             | GDF6               |
|--|------|-------------------|------|-----------------------|------|-------------------|--------------------|
| <b>Ocular findings</b>                       |      |                   |      |                       |      |                   |                    |
| A/M <sup>a</sup>                             | +    | <b>29/37; 78%</b> | +    | <b>IU<sup>b</sup></b> | +    | <b>32/35; 91%</b> | +/-; <b>0.8-8%</b> |
| Coloboma                                     | +    | +                 | +    | +                     | +    | +                 | +                  |
| ASD <sup>c</sup>                             | +    | +                 | +    | +                     | +    | +                 | +                  |
| Aniridia                                     | +    | +                 | +    | +                     | +    | +                 | +                  |
| Cataracts                                    | +    | +                 | +    | +                     | +    | +                 | +                  |
| Congenital glaucoma                          | +    | +                 | +    | +                     | +    | +                 | +                  |
| Retinal dystrophy                            | +    | +                 | +    | +                     | +    | +                 | +                  |
| Optic nerve atrophy/hypoplasia/dysplasia     | +    | +                 | +    | +                     | +    | +                 | +                  |
| <b>CNS<sup>d</sup> – Structural defects</b>  | +    | +                 | +    | +                     | +    | +                 | +                  |
| Heterotopias                                 | +    | +                 | +    | +                     | +    | +                 | +                  |
| ACC <sup>e</sup> /hypoplasia corpus callosum | +    | +                 | +    | +                     | +    | +                 | +                  |
| Mesial temporal malformation                 | +    | +                 | +    | +                     | +    | +                 | +                  |
| Abnormal white matter                        | +    | +                 | +    | +                     | +    | +                 | +                  |
| Arnold-Chiari malformation                   | +    | +                 | +    | +                     | +    | +                 | +                  |
| Anterior commissure defect                   | +    | +                 | +    | +                     | +    | +                 | +                  |
| Olfactory bulb hypoplasia                    | +    | +                 | +    | +                     | +    | +                 | +                  |
| Absent pineal gland                          | +    | +                 | +    | +                     | +    | +                 | +                  |
| Hydrocephalus/ventricular dilatation         | +    | +                 | +    | +                     | +    | +                 | +                  |
| <b>CNS</b>                                   |      |                   |      |                       |      |                   |                    |
| Developmental differences                    | +    | +                 | +    | +                     | +    | +                 | +                  |
| - global delays                              | +    | +                 | +    | +                     | +    | +                 | +                  |
| - motor delays                               | +    | +                 | +    | +                     | +    | +                 | +                  |
| Seizures                                     | +    | +                 | +    | +                     | +    | +                 | +                  |
| Hypotonia                                    | +    | +                 | +    | +                     | +    | +                 | +                  |
| <b>Endocrine</b>                             |      |                   |      |                       |      |                   |                    |
|  | +    | +                 | +    | +                     | +    | +                 | +                  |

|  | SOX2                 | OTX2                 | PAX6 | RAX | BMP4                 | SMOC1                | GDF6 |
|--|----------------------|----------------------|------|-----|----------------------|----------------------|------|
| Pituitary gland/Hypopituitarism          | +:IU                 | +: <b>19–30%</b>     |      |     |                      |                      | +:IU |
| <u>Growth retardation</u>                | +: 7/62; 11%         | +: <b>12/37; 32%</b> |      |     | +: <b>4/9; 44%</b>   |                      |      |
| Microcephaly                             | +: 7/37; 19%         |                      |      |     |                      |                      |      |
| Feeding difficulties                     | +: 5/37; 14%         |                      |      |     |                      |                      |      |
| <u>Dysmorphic findings</u>               | +:IU                 | +:IU                 |      |     | +: <b>6/9; 66%</b>   |                      |      |
| Cardiac defects                          | +: 2/62; 3%          |                      |      |     |                      |                      |      |
| <u>Genitourinary tract malformations</u> | +: <b>22/62; 35%</b> | +:IU                 |      |     |                      | +:IU                 | +:IU |
| <u>Other</u>                             |                      |                      |      |     |                      |                      |      |
| Tracheo-esophageal fistula               | +: <b>15/62; 24%</b> |                      |      |     |                      |                      | +:IU |
| Vertebral/rib malformations              | +: 1/62; 2%          |                      |      |     |                      |                      |      |
| Hearing loss                             | +: 4/62; 6%          | +:IU                 |      |     | +: 1/8; 12.5%        | +: 4/35; 11%         | +:IU |
| Cleft lip/palate                         |                      | +: 2/21; 10%         |      |     | +: <b>3/8; 37.5%</b> | +: <b>20/35; 57%</b> | +:IU |
| Polydactyly/syndactyly                   |                      |                      |      |     |                      | +: <b>29/35; 83%</b> |      |
| Oligodactyly                             |                      |                      |      |     |                      |                      |      |

References: SOX2 = [6]; OTX2 = [26] and [27]; PAX6 = [99], [100] and [101]; RAX = [88], [89] and [90]; BMP4 = [69]; SMOC1 = [74] and [76]; GDF6 = [86], [87], [88] and [90]. A/M<sup>a</sup> = anophthalmia/microphthalmia; IU<sup>b</sup> = incidence unknown; ASD<sup>c</sup> = Anterior Segment Dysgenesis; CNS<sup>d</sup> = Central Nervous System; ACC<sup>e</sup> = agenesis of corpus callosum.