Published in final edited form as:

Birth Defects Res C Embryo Today. 2015 June ; 105(2): 96–113. doi:10.1002/bdrc.21097.

Conserved genetic pathways associated with microphthalmia, anophthalmia, and coloboma

Linda M. Reis¹ and Elena V. Semina^{1,2}

¹Department of Pediatrics and Children's Research Institute at the Medical College of Wisconsin, Milwaukee, Wisconsin, USA

²Departments of Ophthalmology and Cell Biology, Neurobiology, and Anatomy at the Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Abstract

The human eye is a complex organ whose development requires extraordinary coordination of developmental processes. The conservation of ocular developmental steps in vertebrates suggests possible common genetic mechanisms. Genetic diseases involving the eye represent a leading cause of blindness in children and adults. During the last decades, there has been an exponential increase in genetic studies of ocular disorders. In this review, we summarize current success in identification of genes responsible for microphthalmia, anophthalmia and coloboma (MAC) phenotypes, which are associated with early defects in embryonic eye development. Studies in animal models for the orthologous genes identified overlapping phenotypes for most factors confirming the conservation of their function in vertebrate development. These animal models allow for further investigation of the mechanisms of MAC, integration of various identified genes into common developmental pathways and, finally, provide an avenue for the development and testing of therapeutic interventions.

Overview of ocular development

The development of the vertebrate eye is a complex, evolutionarily conserved process that involves tissues from different embryonic origins (Adler and Canto-Soler, 2007; Chow and Lang, 2001; Graw, 2010). The optic vesicle is derived from the anterior part of the neural tube, the diencephalon, which evaginates bilaterally towards the overlying surface ectoderm (Figure 1). Morphogenesis of the lens begins as soon as the optic vesicle makes close contact with the surface ectoderm. The ectoderm thickens to form the lens placode, and then invaginates together with the optic vesicle to form the lens pit and optic cup, respectively. The bilayered optic cup becomes divided into the presumptive neural retina, retinal pigment epithelium, and optic stalk. The invagination occurs asymmetrically along the ventral aspect of the optic stalk, and is completed with the fusion along the dorsal axis and formation of the optic fissure, providing a path for developing retinal axons to exit, and blood vessels and extra-ocular cells to enter the optic cup. Concurrently, the lens pit deepens and finally separates from the ectoderm to form the lens vesicle: the cells at the posterior portion of the vesicle differentiate to form the primary lens fibers, whereas cells in the anterior part differentiate into the lens epithelium. Cell divisions occur in the epithelial region located anterior to the lens equator (germinative zone); the products of these cell divisions migrate

below the equator into the transitional zone, where they elongate and differentiate into fiber cells. The lens continually grows and maintains its distinct polarity throughout life (Lovicu and McAvoy, 2005). The anterior segment develops after separation of the lens vesicle from the head ectoderm. The embryonic origin of the anterior segment structures has been defined in avian (Johnston et al., 1979) and rodent (Gage et al., 2005) models, and involves neuroectoderm, primitive mesoderm, and neural crest cells. Neural crest is a transient embryonic structure in vertebrates that delaminates from the border of the neural plate; neural crest cells migrate throughout the embryo to multiple locations and differentiate into specialized tissues (Theveneau and Mayor, 2012). The ocular neural crest-derived cells migrate into the space between the lens rudiment and the ectoderm (Gage et al., 2005; Hausteiner, 1983) and make a major contribution to the anterior segment structures. Signaling from the lens was shown to play a role in the formation of the overlying cornea (Beebe and Coats, 2000; Coulombre and Coulombre, 1964; Genis-Galvez et al., 1967; Thut et al., 2001). At the same time, studies in mice demonstrated that formation of a corneal endothelium is required to prevent attachment of the lens to the corneal stroma, and therefore to allow the formation of the anterior segment as well as the lens (Reneker et al., 2000).

The steps described above are universally present in various vertebrate species, with some variation. For example, in zebrafish, surface ectoderm cells within the lens placode delaminate as a solid cluster of cells (lens mass), rather than a vesicle; the adult zebrafish lens is more spherical in shape, and its epithelial cell layer extends further toward the posterior suture than in mammals (Bibliowicz et al., 2011; Greiling and Clark, 2012). The conservation of ocular developmental processes in vertebrates suggests a possible common genetic program, even though variation should be expected and has been demonstrated for several genes (Fougerousse et al., 2000).

Developmental ocular disorders

Defects in proper induction, proliferation, migration, or differentiation of ocular tissues during embryonic development can lead to a spectrum of disorders. These conditions are highly debilitating and often lead to a partial or complete vision loss. Disruptions of earlier developmental steps predictably result in more severe structural anomalies involving multiple ocular tissues, while defects in differentiation of specialized tissues/cells produce more isolated ocular phenotypes. The first group of disorders includes the microphthalmia, anophthalmia, and coloboma (MAC) spectrum of disorders, characterized by a smaller or absent eye or a missing portion of the eye structure that can be limited to one or involve multiple tissues. Alterations of later steps of ocular development can result in congenital cataracts (lens defects), various conditions involving corneal opacification (corneal dystrophies, Peters anomaly), or other anterior segment defects (iris hypoplasia, aniridia, Axenfeld-Rieger anomaly), retinal dystrophies, optic nerve atrophies, and others. This review will focus on genetic mechanisms of MAC in humans and their conservation in different species.

The overall incidence of MAC has been estimated as 6–13 per 100,000 births, with the prevalence of anophthalmia being 0.6–4.2, microphthalmia 2–17 and coloboma 2–14 per 100,000 live births (Skalicky et al., 2013; Williamson and FitzPatrick, 2014). MAC

conditions are often associated with additional ocular and non-ocular anomalies, indicating the involvement of the responsible genes in multiple developmental processes. Approximately one-third of MAC cases are reported to be syndromic and include craniofacial, genital, skeletal, brain, renal, cardiac, and other abnormalities, with brain/neurological anomalies more commonly accompanying anophthalmia, and urological/genital defects being more prevalent in patients with coloboma or colobomatous microphthalmia (Skalicky et al., 2013; Williamson and FitzPatrick, 2014).

Conserved genetic pathways associated with MAC

Even though at least eighty-two genes are currently known to be associated with MAC (Table 1 and Supplemental Table 1), many cases remain unexplained, indicating that more factors are involved (Chassaing et al., 2013; Schilter et al., 2013). Among known genes involved in MAC with/without variable syndromic features, transcription factors represent the largest group (HMG-box domain-containing gene *SOX2*; homeodomain-containing factors *OTX2*, *PAX6*, *VSX2*, *PITX3*, *RAX*, and *SIX6*; paired domain gene *PAX6*; forkhead family member *FOXE3*; zinc-finger gene *SALL2*; and basic helix-loop-helix gene *ATHO7*), which is followed by TGF β /BMP signaling molecules (*BMP4*, *BMP7*, *GDF6*, *GDF3*), retinoic acid pathway genes (*ALDH1A3*, *STRA6*, *RARB*), and other genes with known or unknown functions (*SHH*, *ABCB6*, *MAB21L2*, *C12orf57*, *TENM3 (ODZ3)*, *PXDN*, *YAP1*, *HMGB3*, and *CRIM1*); these MAC genes will be the focus of this review. Genes mutated in many well characterized syndromes that involve MAC as a major feature, as well as ocular conditions that only occasionally include MAC, have also been identified, and include *PAX2*, *CHD7*, *HCCS*, *SMOC1*, *NDP*, *CRYBA4*, and other factors (Supplemental Table 1).

Transcription factors: *SOX2*, *OTX2*, *FOXE3*, *VSX2*, *RAX*, *PAX6*, *PITX3*, *SALL2*, *SIX6*, and *ATHO7*

Disruption of *SOX2* represents the most common genetic cause of MAC, explaining 20–40% of cases (Fantes et al., 2003; Gerth-Kahlert et al., 2013). In humans, heterozygous loss of *SOX2* function typically results in severe bilateral ocular defects, although unilateral cases are occasionally seen: 73% of reported cases had anophthalmia of at least one eye, and the majority of the remaining cases were affected with microphthalmia (Williamson and FitzPatrick, 2014). More isolated ocular anomalies, such as anterior segment dysgenesis, or coloboma, have occasionally been reported (Gerth-Kahlert et al., 2013; Mihelec et al., 2009; Wang et al., 2008). Patients with *SOX2* mutation or deletion usually, but not always, have systemic anomalies, including developmental delay/cognitive impairment, particularly affecting motor development, pituitary defects, short stature, structural brain anomalies, genitourinary defects, and gastrointestinal anomalies, including tracheoesophageal fistula (Schneider et al., 2009). The vast majority of mutations/deletions are *de novo* events; maternal mosaicism has occasionally been reported (Williamson and FitzPatrick, 2014). Expression of *Sox2* is seen early in development in the optic vesicle, and the presumptive lens ectoderm at the site of contact and expression continues in the lens vesicle (Furuta and Hogan, 1998). In *Sox2*-deficient mice (that show 40% of normal *Sox2* expression), variable anophthalmia, microphthalmia, and coloboma are present with abnormalities in development of ocular, as well as hypothalamic, tissue, evident as early as E10.5, thus showing strong

conservation in function between humans and mouse (Langer et al., 2012; Taranova et al., 2006).

OTX2 mutations, the next most common cause of MAC, present similarly to *SOX2*; 43% of reported cases exhibited anophthalmia of at least one eye with the majority of the remaining cases affected with microphthalmia (Ragge et al., 2005; Williamson and FitzPatrick, 2014). Milder ocular anomalies including coloboma, and retinal dystrophy have also been reported (Henderson et al., 2009; Wyatt et al., 2008). Affected individuals often have systemic anomalies, most commonly developmental delay/cognitive defects, abnormal pituitary structure or function, and short stature with occasional genital or structural brain anomalies also seen (Schilter et al., 2011). While most cases represent *de novo* mutations/deletions, up to 35% of mutations are inherited from a parent, who may or may not be affected (Schilter et al., 2011). Recently, *OTX2* has been shown to be involved in otocephaly-dysgnathia, characterized by mandibular hypoplasia or agnathia, malposition of the ears, and aglossia with ocular and brain anomalies in some patients, possibly through interaction with mutations at a second loci (Chassaing et al., 2012; Patat et al., 2013). *Otx2* expression is present throughout the optic vesicles at the early stage, but becomes restricted to the dorsal part upon contact with the surface ectoderm and then the outer layer, the presumptive pigment epithelium (Bovolenta et al., 1997). *Otx2*^{+/-} heterozygous mice show variable anophthalmia or microphthalmia, along with craniofacial anomalies including otocephaly in some animals, while *Otx2*^{-/-} knockout mice demonstrate absence of anterior head structures and early embryonic lethality (Matsuo et al., 1995); heterozygous male *Otx2* mutant mice also have decreased fertility, consistent with abnormal development of the hypothalamic-pituitary-gonadal axis, as seen in humans (Larder et al., 2013). Knockdown of *otx2* via morpholino in zebrafish resulted in mild microphthalmia and shortening of the pharyngeal skeleton, with more severe effects seen when combined with knockdown of other otocephaly genes (Chassaing et al., 2012).

Dominant mutations in *FOXE3*, typically causing read-through of the stop codon and resulting in an extension of the protein, are associated with anterior segment dysgenesis (ASD) and/or congenital cataract phenotypes (Reis and Semina, 2011). A second class of mutations resulting in a recessive phenotype of isolated bilateral primary aphakia, microphthalmia, and/or sclerocornea, has recently been identified (Valleix et al., 2006). Recessive mutations consist of missense, frameshift, and nonsense mutations; heterozygous carriers of recessive mutations are typically unaffected (Reis et al., 2010). *Foxe3* expression is seen in mouse embryos in the lens and presumptive midbrain (Blixt et al., 2000; Brownell et al., 2000); lens expression of *Foxe3* is initially uniform throughout the lens placode and lens vesicle, then becomes confined to the anterior lens epithelium during/after lens fiber differentiation (Blixt et al., 2000; Brownell et al., 2000). *Foxe3* deficiency in mice results in the *dysgenetic lens* phenotype characterized by a combination of microphthalmia, corneal opacity, small and irregular lenses, and lenticular-corneal adhesion, but without other systemic features (Blixt et al., 2007; Brownell et al., 2000); a proportion of heterozygous mice display corneal and lenticular anomalies resembling Peters anomaly (Ormestad et al., 2002). The Rinshoken cataract (*rcr*) recessive mouse phenotype characterized by microphthalmia and cataract was also found to be due to a deficiency of *Foxe3* resulting

from an upstream deletion (Wada et al., 2011). Knockdown of *foxe3* in zebrafish results in small eyes with small abnormal lenses as well as pupillary anomalies (Shi et al., 2006).

VSX2 (previously known as *CHX10*) is typically associated with recessive bilateral microphthalmia (often accompanied by cataract, iris coloboma, or other ocular anomalies), or anophthalmia, but one patient with normal eye size, lens subluxation, cone-rod dysfunction, and high myopia, has also been reported (Bar-Yosef et al., 2004; Ferda Percin et al., 2000; Khan et al., 2015). The *VSX2* mutant phenotype is typically limited to the eye, but few extraocular anomalies, including learning difficulties, are also reported (Iseri et al., 2010). To date, mutations were identified in 14 MAC families; in all but one pedigree, homozygous mutations were identified in consanguineous families (Chassaing et al., 2013; Reis et al., 2011). The majority of heterozygous parents are phenotypically normal, but macular scarring was noted in one mother with reduced vision and ERG demonstrated abnormalities of the rod system in both her and her spouse (Iseri et al., 2010). *Vsx2* expression has been identified within the early developing retina (retinal progenitor cells), and is restricted to the inner nuclear layer of the retina (specifically noted in the bipolar cells and Müller glial cells) during later stages of development and in the adult eye in human, mouse, and zebrafish (Barabino et al., 1997; Ferda Percin et al., 2000; Liu et al., 1994; Passini et al., 1997; Vitorino et al., 2009). A nonsense mutation in *Vsx2* was found to underlie the recessive ocular retardation J (*orJ*) phenotype characterized by microphthalmia, a thin hypoplastic retina, and optic nerve aplasia, without any extraocular anomalies (Burmeister et al., 1996). Knockdown of *vsx2* via morpholino in zebrafish resulted in microphthalmia with reduced numbers of Müller glial cells and PKC-positive bipolar cells in the retina (Vitorino et al., 2009). Additional animal models were generated by knock-in of specific missense mutations in the homeodomain or other regions in mice: mutant mice exhibited a similar phenotype to the *orJ* mutant, with variable microphthalmia, thinning of the retina, and smaller lens (Zou and Levine, 2012).

Homozygous or compound heterozygous mutations in the homeobox gene *RAX*, also known as *RX*, cause bilateral anophthalmia or occasionally microphthalmia; mutations consisting of nonsense, splicing, truncating, and missense mutations in the homeodomain have been reported in eight families (Abouzeid et al., 2012; Chassaing et al., 2013; Voronina et al., 2004). In most cases, the phenotype is isolated, but occasionally cognitive impairment or cerebral malformations have been reported. While all heterozygous carrier parents of these children have been unaffected, heterozygous missense mutations earlier in the gene have been reported in three patients with unilateral MAC and cerebral malformations in two; testing of the parents was not reported, and even though none of the mutations were initially detected in controls, since then all three variants have been reported at a low frequency in general population (ExAC), rendering their significance currently uncertain (Gonzalez-Rodriguez et al., 2010; London et al., 2009). In *Xenopus*, the two *Rx* genes, *Xrx1/2*, are expressed early in the ventral forebrain, the optic cups, and pineal gland (Mathers et al., 1997). In mouse, *MRx* expression is similarly seen in the anterior neural plate (E8.5), the ventral forebrain (E9.0–E10.5), the optic sulci/developing eye (E9.0–E10.5), the neuroretina (E15.5), and the hypothalamus and posterior pituitary (E10.5–E12.5) (Mathers et al., 1997). Similarly, the three zebrafish *rx* (*rx1*, *rx2*, and *rx3*) genes are expressed in the anterior neural plate and presumptive optic vesicles, with later restriction to the developing retina and

ventral forebrain (Loosli et al., 2003; Mathers et al., 1997). In *Xenopus*, a homozygous nonsense mutation in *rax* (*Xrx1*) resulted in an anophthalmic phenotype, which could be rescued by injection of wild-type *rax* mRNA (Fish et al., 2014). At the same time, overexpression of *Xrx1* in *Xenopus* led to development of ectopic retinal pigment epithelium outside the eye, duplicated retinal layers within the eye, and neural hyperplasia or duplication of the neural tube (Mathers et al., 1997). Mice homozygous for a null *Rx* allele fail to form optic vesicles, and show variable brain anomalies, ranging from absence of the ventral forebrain to lacking the entire forebrain, abnormal midbrain structures, and perinatal lethality; mice heterozygous for the *Rx* null allele are phenotypically normal (Mathers et al., 1997; Voronina et al., 2005). A hypomorphic *Rx* allele associated with anophthalmia and hypothalamic abnormalities, but normal viability in homozygous animals, has also been identified (Tucker et al., 2001). Similarly, in zebrafish, loss of *rx3* causes the anophthalmic zebrafish *chokh* (*chk*) phenotype (Kennedy et al., 2004; Loosli et al., 2003). In medaka fish, an intronic insertion in *Rx3* was identified as the cause of the recessive *eyeless* mutant, with defective optic vesicle evagination (Loosli et al., 2001).

Heterozygous *PAX6* mutations typically cause isolated aniridia, but several individuals with missense (5) or nonsense (1) *PAX6* mutations and microphthalmia (accompanied by anterior segment anomalies) have been reported (Azuma et al., 1999; Azuma et al., 2003; Dansault et al., 2007; Hanson et al., 1994; Henderson et al., 2007; Vincent et al., 2003; Xiao et al., 2012). Disruptions of two copies of *PAX6* by compound heterozygous mutations have been identified in several cases and resulted in anophthalmia (Glaser et al., 1994; Schmidt-Sidor et al., 2009; Solomon et al., 2009). In animal models, *Pax6* is first detected in the anterior neural plate, and then continues to be strongly present in both the lens field and the optic vesicle; normal *Pax6* expression is also critical for the developing cornea, and it is maintained throughout life in the ocular tissues (Davis et al., 2003; Hever et al., 2006). Multiple mouse *Pax6* mutations have been reported, resulting in *small eye* and other ocular phenotypes (Hanson, 2003). The rat small eye mutant, *Sey*, characterized by the absence of eyes and nose and abnormal brain development in homozygotes, is also caused by a mutation in the *Pax6* gene (Matsuo et al., 1993); heterozygous rats demonstrated corneal abnormalities which resemble the keratopathy observed in humans with aniridia (Ramaesh et al., 2003). In zebrafish, homozygous *pax6b* mutants displayed thick cornea, iris hypoplasia, shallow anterior chamber, and small lens (Takamiya et al., 2015).

While heterozygous mutations in *PITX3* result in ASD and/or congenital cataract with normal eye size (Semina et al., 1998), a homozygous dominant mutation in *PITX3*, c.650delG, p.(Gly217Alafs*92), was found to be associated with bilateral microphthalmia (along with corneal opacity) and severe developmental delay/neurological deficits in two siblings whose parents were both affected with congenital cataract (Bidinost et al., 2006). In addition, a recessive mutation associated with severe microphthalmia and sclerocornea has been reported in *PITX3*: a homozygous c.640_656del, p.(Ala214Argfs*42), mutation, the inverse of a 17-bp duplication commonly seen in ASD/cataract, was seen in an affected child while her carrier parents were normal (Aldahmesh et al., 2011). *Pitx3* expression is seen in mouse embryos in the developing lens, midbrain, and craniofacial and skeletal muscle (Semina et al., 1998). Lens expression of *Pitx3* is initially seen in the lens vesicle; during later steps of lens development, *Pitx3* was seen in both the lens epithelium and

differentiating fiber cells, but expression was strongest in the lens epithelium and equator region (Ho et al., 2009; Medina-Martinez, 2010; Semina et al., 1997). The *aphakia* (*ak*) and *eyeless* (*eyl*) mouse mutants are recessive phenotypes caused by *Pitx3* deficiency; both lines are characterized by microphthalmia with arrested lens development, and corneal opacity and lenticular-corneal adhesions, along with lack of dopamine neurons in the substantia nigra and behavioral changes (Rieger et al., 2001; Rosemann et al., 2010; Semina et al., 2000; Smidt et al., 2004). An additional recessive mouse mutant with a nonsense mutation in *Pitx3* resulting in microphthalmia/aphakia was recently identified (Wada et al., 2014). Knockdown of *pitx3* via Morpholino in zebrafish resulted in small eyes with lens degeneration and retinal defects, misshapen heads, and jaw abnormalities (Shi et al., 2006; Sorokina et al., 2011). Recently, a recessive *PITX3* mutation was identified in a large vertebrate model, Texel sheep, affected with microphthalmia and lens vesicle abnormalities (Becker et al., 2010; van der Linde-Sipman et al., 2003).

In a single family affected with a variable nonsyndromic coloboma phenotype, a homozygous nonsense mutation in *SALL2* was identified in three affected siblings (Kelberman et al., 2014). Within the mouse eye, expression of *Sall2* is seen in the lens placode and optic cup at E11.5, during the time of optic fissure closure (Bohm et al., 2008; Kelberman et al., 2014). Similarly, studies of human embryonic and fetal eye tissues identified *SALL2* expression throughout the retina, developing lens vesicle, and periocular mesenchyme at 5 weeks of development (when optic fissure closure begins), with restriction to the inner neuroblastic layer of the retina by 8 weeks when closure is complete; subsequent expression of *SALL2* was identified in the developing cornea, retina, and lens (Kelberman et al., 2014). *Sall2*^{-/-} knockout resulted in variable neural tube defects in some genetic backgrounds (Bohm et al., 2008); histological analysis of the eyes of *Sall2* null mutant mice identified a penetrant bilateral colobomatous phenotype (Kelberman et al., 2014).

Both recessive and dominant mutations in *SIX6* have been reported in MAC conditions. A heterozygous missense mutation in *SIX6* was seen in a proband with bilateral microphthalmia and cataract (Gallardo et al., 2004). The mutation is not seen in the general population (ExAC; exac.broadinstitute.org) but was inherited from the unaffected father, so the significance of this finding is unclear. Another group screened a large cohort of MAC patients for mutations in *SIX6*; three missense mutations were identified but were present at a similar frequency in controls and interpreted as polymorphisms (Aijaz et al., 2004). More recently, a homozygous frameshift mutation in *SIX6* was identified in two siblings affected with bilateral microphthalmia with anterior and posterior segment dysgenesis; both carrier parents were unaffected (Aldahmesh et al., 2013). *Six6/six6* expression within the eye has been studied in mouse, chick, and *Xenopus*, with expression initially seen in the optic vesicle and optic stalk with subsequent expansion to the neural retina and lens, and finally restriction to the retina at later stages of development (Kumar, 2009). Analysis in human embryonic tissues identified strong expression of *SIX6* in the eye beginning at 12–14 weeks gestation, with specific expression noted in the developing layered retinal ganglion and inner nuclear layer cells (Gallardo et al., 1999). *Six6*^{-/-} mice demonstrated hypoplastic pituitary gland and variable degrees of retinal hypoplasia, with optic nerve aplasia in some animals (Li et al., 2002).

In two families with nonsyndromic severe disruption of ocular development, including microphthalmia, severe vitreo-retinal dysplasia, cataract, corneal opacity, and hypoplastic optic nerves, homozygous mutations in *ATH7* were identified (Khan et al., 2012). An overlapping phenotype, which did not specify microphthalmia, was reported with deletion of an enhancer upstream of *ATH7* (Ghiasvand et al., 2011). *Math5* (*Atoh7*) $-/-$ mice have normal eye size but hypoplastic/absent optic nerves and chiasmata; histological analysis identified thinning of the inner plexiform and nuclear layers of the retina, fewer cells in the ganglion cell layer, abnormal cell layering, absence of the central artery and vein, and ectopic blood vessels within the posterior chamber (Brown et al., 2001; Wang et al., 2001). Similarly, homozygous disruption of *ath5* was found to be the cause of the *lakritz* (*lak*) mutant in zebrafish characterized by blindness caused by a loss of retinal ganglion cells in the ganglion cell layer (Kay et al., 2001).

TGF β /BMP signaling molecules: BMP4, BMP7, GDF6, GDF3

Although deletions of *BMP4* frequently occur along with deletion of *OTX2* in MAC patients, several cases of specific *BMP4* removal, as well as heterozygous loss-of-function mutations, have also been reported in MAC and other ocular conditions (Bakrania et al., 2008; Martinez-Fernandez et al., 2014; Reis et al., 2011). In patients with deletion of *BMP4* (not including *OTX2*), ocular findings include microphthalmia, anterior segment anomalies, or normal eyes in some cases. Anophthalmia is seen in multiple patients with intragenic mutations. Non-ocular anomalies are typically observed, but vary widely with polydactyly, short stature, cognitive impairment, structural brain anomalies, macrocephaly, diaphragmatic hernia, and dental anomalies being reported in more than one family (Reis et al., 2011; Martinez-Fernandez et al., 2014). In the developing mouse eye, *Bmp4* expression is first detected in the distal optic vesicle and overlying surface ectoderm, with later restriction to the dorsal margin of the optic cup (Furuta and Hogan, 1998) and at late stages, expression can be seen in the iris, ciliary body, and retinal pigment epithelium (Chang et al., 2001). In human embryos, *BMP4* was shown to be expressed in the optic vesicle at early stages, with later restriction to the developing lens; outside the eye, *BMP4* was detected in the developing brain and hand plate (Bakrania et al., 2008). Homozygous null *Bmp4* allele in mice results in death by the time of gastrulation in most embryos, with a failure of lens induction noted in animals that survive to 10.5 dpc (Furuta and Hogan, 1998). Mice with heterozygous null *Bmp4* mutations showed highly variable anomalies that differed with genetic background, including anophthalmia/microphthalmia, kidney defects, craniofacial anomalies, and polydactyly in one study, and anterior segment anomalies with increased intraocular pressure along with abnormalities of the posterior segment in another (Chang et al., 2001; Dunn et al., 1997).

Three heterozygous mutations in *BMP7* were reported in MAC probands with variable phenotypes; two had syndromic anophthalmia (unilateral or bilateral) and the other was affected with unilateral coloboma and mild learning difficulties (Wyatt et al., 2010). All three mutations were inherited from an unaffected mother, but they are rarely or never seen in the general population (ExAC; exac.broadinstitute.org), so the significance of these mutations is unclear; no additional cases of *BMP7* mutations in MAC have been reported. In the mouse eye, *Bmp7* is expressed in the neuroepithelium of the optic vesicle and the

overlying surface ectoderm at early stages, with later expression at the junction between the neuroepithelium and pigmented retinal epithelium, throughout the outer pigmented retinal layer, in the mesenchymal cells adjacent to the optic cup, and lens and sheath cells surrounding the optic nerve (Dudley et al., 1995). Within the developing human eye, *BMP7* expression was noted in the optic stalk, optic cup, and lens vesicle at CS13 and in the developing retina and lens at CS16, 17, and 19, with strong expression noted at the fusional edges of the optic fissure (Wyatt et al., 2010). Mice with a homozygous *Bmp7* null allele are universally affected with bilateral anophthalmia or microphthalmia and severe renal dysplasia, along with variable hind-limb polydactyly and axial skeletal abnormalities, and typically die within the first day of life (Dudley et al., 1995). Characterization of another *Bmp7* mouse model identified anophthalmia, dental defects, cleft palate, and other craniofacial defects (Zouvelou et al., 2009).

Multiple groups have reported dominant mutations in *GDF6* in patients with MAC conditions; ocular phenotypes ranged from bilateral anophthalmia to unilateral coloboma and extraocular anomalies, and included cleft lip/palate, polydactyly, and absent ossicles (Asai-Coakwell et al., 2007; Chassaing et al., 2013; Williamson and FitzPatrick, 2014). The contribution of this gene to MAC is not fully unclear, as many of the identified mutations are inherited from an unaffected parent and/or present in control populations (Chassaing et al., 2013; Williamson and FitzPatrick, 2014). Heterozygous mutations in *GDF6* with similarly incomplete penetrance have also been reported in non-MAC skeletal conditions, including Klippel-Feil syndrome (Asai-Coakwell et al., 2009; Tassabehji et al., 2008), as well as Leber congenital amaurosis (recessive mutations also reported for LCA) (Asai-Coakwell et al., 2013). In mouse, *Gdf6* expression was noted in various skeletal regions affected in Klippel-Feil syndrome, and the *Gdf6*^{−/−} knockout had skeletal defects resembling this condition; ocular findings were not specified (Tassabehji et al., 2008); ocular findings were characterized in *Gdf6*^{+/-} mice and showed optic disc excavation, marked asymmetry, optic disc cupping, and a low incidence of microphthalmia (Asai-Coakwell et al., 2009). Knockdown of *Gdf6a* in *Xenopus laevis* via morpholino resulted in reduction in eye size with a disorganized retina in 22–65% of embryos in one study, and anophthalmia, microphthalmia, and/or coloboma in 14% of embryos in another (Hanel and Hensey, 2006; Tassabehji et al., 2008). Expression of *gdf6a* in the zebrafish eye is seen in the dorso-temporal region of the developing retina at 18–24 hpf (Asai-Coakwell et al., 2007). Knockdown of *gdf6a* via morpholino in zebrafish demonstrated variable ocular defects, including small eye with disorganized retina and hypoplastic optic nerve, ventral coloboma and persistent dorsal-retinal groove, along with various non-ocular defects; increasing the morpholino dose led to development of an anophthalmia phenotype in one study (Asai-Coakwell et al., 2007) (Asai-Coakwell et al., 2009). Genetic mutations in *gdf6a* (also known as *radar*) in zebrafish, *dark half*^{s327}, and *out*^{m233}, are characterized by a severe reduction in eye size (small retina and lens) and increased cell death/apoptosis in both tissues; partial rescue was achieved with injection of wild-type *gdf6a* (den Hollander et al., 2010; Gosse and Baier, 2009).

Heterozygous missense mutations in a related gene, *GDF3*, have also been identified in seven families with variable ocular (microphthalmia and/or coloboma) and/or skeletal (vertebral fusion, scoliosis, rudimentary 12th rib) phenotypes (Ye et al., 2010). Incomplete

penetrance was confirmed in one family and an additional mutation in *GDF6* (see above) was identified in one proband, but was not present in the more mildly affected mother with the same *GDF3* mutation. Since the time of publication, all of the missense variants were reported in significant numbers (25–246 alleles) in the general population with homozygous individuals reported for two of the variants (ExAC.), suggesting that these may not be causative alleles. No ocular defects were reported in *Gdf3*^{−/−} mice (Andersson et al., 2008), but morpholino knockdown of *dvr1*, an ortholog of *gdf1/3*, resulted in coloboma, reduced eye size, tail curvature, and reduced tail length in some embryos (Ye et al., 2010).

Retinoic acid pathway: *ALDH1A3*, *STRA6*, *RARB*

ALDH1A3, also known as *RALDH3*, one of three retinaldehyde dehydrogenases responsible for the oxidation of retinaldehyde to retinoic acid (RA), was identified by multiple groups as a cause of recessive MAC; bilateral anophthalmia or microphthalmia was the most frequent ocular phenotype, although colobomatous microphthalmia as well as coloboma with normal eye size were also reported, and the phenotype was typically isolated (Abouzeid et al., 2014; Fares-Taie et al., 2013). In the mouse, *Raldh3* is expressed in multiple tissues during embryonic development, with a pattern distinct from *Raldh1* or *Raldh2* (Niederreither et al., 2002). Within the developing eye, generation of retinoic acid in the surface ectoderm over the eye field by *Raldh3* was shown to begin by at E8.75, with later activity confined to the dorsal retinal pigment epithelium (E9.5); after optic cup formation, *Raldh3* expression is seen in the ventral neural retina (from E10.5) until birth (Duester, 2009). Importantly, *Raldh3* is the only source of retinoic acid in the ventral neural retina from E11.5 to 13.5, while the choroid fissure is formed from the ventral folds of the optic cup (Duester, 2009). Embryos with knockout of *Raldh3* demonstrated shortening of the ventral retina, excessive thickening of the central neural retina, loss of the vitreous body, and retrolenticular membrane between the retina and lens, suggesting that *Raldh3* is required to complete closure of the choroid fissure, as well as choanal atresia leading to perinatal lethality; *Raldh3*^{−/−} embryos showed a significant decrease in retinoic acid activity in the eye, with complete loss of RA in the ventral retina and olfactory pit at E10.5, and the phenotype was rescued by maternal treatment with RA (Dupe et al., 2003; Molotkov et al., 2006). In zebrafish, knockdown of *aldh1a3* via morpholino resulted in microphthalmia with delayed closure of the optic fissure and coloboma in some larvae; the phenotype was rescued with injection of wild-type, but not mutant, *ALDH1A3* mRNA (Yahyavi et al., 2013).

Homozygous or compound heterozygous mutations in *STRA6* cause PDAC (pulmonary aplasia/dysgenesis/hypoplasia, diaphragmatic eventration/hernia, anophthalmia/microphthalmia, and cardiac defect) syndrome (Chitayat et al., 2007; Pasutto et al., 2007). Bilateral anophthalmia or severe microphthalmia are typical for this condition; additional features include abnormalities of the kidneys, uterus, and spleen, cognitive impairment, cryptorchidism, inguinal hernia, and intrauterine growth retardation and/or short stature and early lethality in many (Chassaing et al., 2009). Milder PDAC phenotypes have been reported, often associated with missense mutations, including a family with isolated, bilateral colobomatous microphthalmia (Casey et al., 2011). While the phenotype is usually recessive, occasionally an isolated microphthalmia/coloboma has been reported in relatives with a heterozygous truncating mutation (Golzio et al., 2007; Ng et al., 2013); since the

majority of heterozygous relatives are unaffected, the significance of these findings is unclear. *Strab* is widely expressed in the developing mouse embryo: it is observed in multiple regions of the eye starting at 9.5-dpc, as well as the nervous system, face, ear, muscle, skeletal system, gut derivatives, and urinary and genital systems (Bouillet et al., 1997). Despite this wide expression pattern and in contrast to the severe human phenotype, *Strab*-null mice are relatively normal and display a mild ocular phenotype of persistent and hyperplastic primary vitreous; retinoid levels and RA-dependent signaling were normal in all tissues of the *Strab*-null mice (Berry et al., 2013). Pharmacological inhibition of retinoic acid synthesis in zebrafish, intended to mimic the effects of *strab* loss of function mutations, resulted in variable microphthalmia, coloboma of the retinal pigment epithelium, and heart defects (Casey et al., 2011).

Finally, mutations in the retinoic acid receptor *RARB* have been associated with both dominant and recessive anophthalmia and microphthalmia. Compound heterozygous nonsense or frameshift mutations in *RARB* were first identified in siblings with a PDAC phenotype resembling that associated with mutations in *STRA6* (Srour et al., 2013). Additional screening of patients with a broader spectrum of anomalies identified *de novo* heterozygous missense mutations affecting a single residue (Arg387) in three additional families; the probands in these families were similarly affected with anophthalmia or microphthalmia (bilateral in all but one), diaphragmatic hernia, lung dysplasia in two, and cognitive impairment in the single child who survived the neonatal period (Srour et al., 2013). Mice with *Rarb* deficiency were described to exhibit lung hypoplasia (Massaro et al., 2000).

Genes with other or unknown function

Holoprosencephaly (HPE), a severe disruption of forebrain development, is frequently associated with craniofacial and ocular anomalies, including microphthalmia or coloboma in some cases, and is highly variable in phenotypic presentation (Dubourg et al., 2004). Disruption of *SHH*, a gene associated with HPE, has also been identified in three families with MAC phenotypes without HPE: a family with nonsyndromic dominant coloboma, a mother with cognitive impairment and colobomatous microphthalmia (whose son was affected with HPE), and a proband with colobomatous microphthalmia, microcephaly, developmental delays, short stature, and mild dysmorphic features (Dubourg et al., 2004; Ginocchio et al., 2008; Schimmenti et al., 2003). Similarly, a 5-Mb deletion 14q13.1-q21.1 including the HPE8 locus, a region associated with HPE, was identified in a proband with bilateral colobomatous microphthalmia and cognitive impairment without HPE (Piccione et al., 2012). During optic cup formation in chick, *Shh* is expressed in the forebrain region connected to the ventral optic stalk (stage 13–18); at later stages, expression is detected in the inner portion of the central retina (stage 24) (Zhang and Yang, 2001). Specific misexpression or blocked expression of *Shh* in the developing chick eye caused disruption of ventral ocular tissues (Zhang and Yang, 2001). In zebrafish, homozygous sonic-you (*syu*^{−/−}) mutants display extensive retinal cell death before embryonic mortality, while heterozygous *syu*^{+/-} retinas were found to undergo progressive cone photoreceptor loss (Stenkamp et al., 2002; Wang and Gregory-Evans, 2015).

Two different heterozygous missense mutations in *ABCB6*, a member of the ABC transporter family, were identified in four families affected with nonsyndromic coloboma and microphthalmia in three (Wang et al., 2012). Study of human cell lines and tissues identified the strongest expression for *ABCB6* in retinal and RPE cells (Wang et al., 2012). In zebrafish, *abcb6* was expressed in the developing CNS tissue and eyes beginning at early stages of development (24-hpf), with continued ocular expression around the lens at later time points (Wang et al., 2012). Knockdown of *abcb6* via morpholino resulted in highly penetrant eye defects, including iris coloboma in the lower papillary margin; this phenotype could be efficiently rescued by injection of wild-type but not mutant *ABCB6* (Wang et al., 2012).

MAB21L2, a gene with unknown function, was recently reported as a dominant cause of bilateral anophthalmia, colobomatous microphthalmia, or coloboma in five families with cognitive impairment or skeletal dysplasia identified in some individuals; all heterozygous mutations affected one of two residues within N-terminal domain (Deml et al., 2015; Rainger et al., 2014). In siblings from another family, a homozygous missense mutation in a C-terminal region of the protein was associated with isolated bilateral retinal coloboma with microphthalmia in one eye (Rainger et al., 2014). Study of *Mab21l2* in mouse identified strong expression in the developing eye, pharyngeal arches, and limb bud (Rainger et al., 2014); within the eye, *Mab21l2* was specifically seen in the optic vesicle at E8.5–9.5 and in the neural retina, optic nerve, and RPE at E12 (Saito et al., 2012; Yamada et al., 2004). Zebrafish *mab21l2* is similarly expressed in the developing eye, brain, pharyngeal arches, and fins; within the eye, expression was seen in the presumptive eye field at 18-hpf, in the retina and lens at 24-hpf, and in the ciliary marginal zone (CMZ), inner nuclear, and ganglion cell layers of the retina as well as around the optic fissure in 48–72-hpf embryos (Deml et al., 2015). Mice with a homozygous knockout allele had improper invagination of the optic vesicle, resulting in rudimentary retina and aphakia along with ventral body wall defects, improper formation of the heart and liver, and embryonic lethality (Saito et al., 2012; Yamada et al., 2004). Morpholino mediated knockdown of *mab21l2* in zebrafish resulted in microphthalmia and incomplete retinal development (Kennedy et al., 2004). Zebrafish with homozygous frameshift mutations demonstrated microphthalmia with small or absent lenses, coloboma in 76%, shortened body/curved tail in 23%, and 100% embryonic lethality; zebrafish with a homozygous in frame deletion of the same region involved in dominant human mutations were affected with a more severe coloboma phenotype, but generally normal eye size and lens as well as reduced viability; both mutants demonstrated retinal invagination defects (Deml et al., 2015). The mutant zebrafish phenotype was able to be rescued with wild-type, but not mutant, *MAB21L2* mRNA (Deml et al., 2015).

Homozygous and compound heterozygous mutations in *C12orf57*, a gene with unknown function, have been reported in seven families and result in a variable ocular phenotype of coloboma, colobomatous microphthalmia, or other visual impairment in 50% of reported patients, along with severe cognitive impairment with absent speech, intractable seizures, and abnormal development of the corpus callosum (Akizu et al., 2013; Platzer et al., 2014; Zahrani et al., 2013). *C12orf57* was shown to be expressed in many adult mouse tissues, including the eye and brain, as well as in tissue from an E10.5 embryo (Zahrani et al., 2013).

Similarly, RT-PCR of various human tissues demonstrated broad expression of *C12orf57* with enrichment noted for the fetal brain (Akizu et al., 2013).

A homozygous frameshift mutation in *TENM3*, also known as *ODZ3*, was identified in siblings affected with isolated nonsyndromic bilateral colobomatous microphthalmia; the parents were third cousins (Aldahmesh et al., 2012). No additional cases have been reported since this publication. Mouse *Odz3* was shown to be widely expressed in the developing nervous system and eye, along with sites in the craniofacial region, gut and urogenital system, dermis, and limbs (Ben-Zur et al., 2000). In the eye, high levels of expression of *Odz3/Tenm3* were seen in the optic recess of the diencephalon, the optic stalk, lens, and the corneal ectoderm (Ben-Zur et al., 2000; Leamey et al., 2007). In the zebrafish retina, *tenm3* is expressed in the retinal ganglion cells, amacrine cells, and tectal neurons from 2- to 5-dpf (Antinucci et al., 2013). Knockout of *Tenm3* in mice resulted in mice with structurally normal eyes but impaired binocular vision, due to abnormal mapping of ipsilateral retinal projections (Leamey et al., 2007). Similarly, knockdown of *tenm3* via morpholino resulted in morphologically normal embryos with abnormal visual function caused by abnormal retinal ganglion cell development and function (Antinucci et al., 2013).

In two families with recessive microphthalmia and anterior segment dysgenesis (and developmental delay in one family), WES analysis identified compound heterozygous mutations in *PXDN*, a peroxidase previously associated with cataract-microcornea or glaucoma and corneal opacity (Choi et al., 2015). In the developing mouse eye, *Pxdn* is expressed faintly in the lens epithelium and primary fiber cells at the time of lens vesicle formation (E11.5), with stronger expression in the epithelium cells and inner limiting membrane of the lens, along with the ocular mesenchymal cells and vitreous at later stages (E13.5). At 17.5, *Pxdn* expression is seen throughout the lens and in the inner neuroblast layer (Yan et al., 2014). Mice with a homozygous nonsense mutation in *Pxdn* (*KTA048*) are affected with severe anterior segment dysgenesis, microphthalmia, early-onset glaucoma, and progressive retinal dysgenesis; mild ocular anomalies in the mutant mice are first seen at E13.5 with the phenotype worsening as development progresses (Yan et al., 2014).

Recently, whole genome/exome sequence (WGS/WES) analysis has been utilized as an efficient method for the identification of new genes involved in MAC conditions, as well as expanding the phenotypic spectrum for known genes not previously associated with MAC (Deml et al., 2014; Deml et al., 2015). In addition to the genes discussed above, WES has resulted in numerous single reports of genes involved in MAC conditions, with limited functional data/animal models. Heterozygous mutations in *YAPI*, a member of the HIPPO-pathway-induced growth response, were identified in two multigenerational families affected with coloboma and variable hearing loss, cognitive impairment, hematuria, and cleft lip/palate in one family (Williamson et al., 2014). In a single pedigree affected with colobomatous microphthalmia, microcephaly, cognitive impairment, and short stature, a hemizygous mutation in *HMGB3* was found in the proband whose unaffected mother was a heterozygous carrier (Scott et al., 2014). In another family with non-syndromic colobomatous microphthalmia with microcornea syndrome, a heterozygous 22-kb deletion, including the final three exons of *CRIMI* was identified; loss of *Crim1* expression in the eye resulted in overlapping ocular anomalies (Beleggia et al., 2015).

Concluding remarks

Studies presented above of human MAC genes in animal models allowed for examination of expression of various ocular markers and investigation into the affected cellular processes. These experiments allowed integration of some of the identified factors into common pathways. For example, expression of *Pax6* was found to be disrupted in many animal models, including *Sox2* mutant mice (Langer et al., 2012), *gdf6a* morphant zebrafish (Hanel and Hensey, 2006; Tassabehji et al., 2008), *Xrx1* mutant xenopus (Fish et al., 2014), chick eyes with misexpression of *Shh* (Zhang and Yang, 2001) and *Pxdn* mutant mice (Yan et al., 2014); at the same time, expression of *Pax6* was unaffected in *Bmp4* and *Sall2* mutant mice (Furuta and Hogan, 1998; Kelberman et al., 2014). The effects of *RAX* deficiency has been extensively studied in zebrafish *rx3* mutants and downregulation of several homeodomain transcription factors and mediators of retinoid-signaling, including *vsx2* and *mab21l2*, was noted while Wnt signaling was found to be upregulated (Kennedy et al., 2004; Yin et al., 2014). In the *Pitx3* mutant lens, expression of *Foxe3*, as well as *Prox1*, *Mip* and four crystalline genes was downregulated (Wada et al., 2014).

Defects in the control of cellular proliferation have been linked to MAC phenotypes by several studies. The absence of *pax2* was shown to result in up-regulated proliferation throughout the eye, most likely due to the loss of fadd activity that was found to induce cell death through a caspase-dependent mechanism (Viringipurampeer et al., 2012). The rate of apoptosis was elevated in developing eyes of *gdf6a*^{-/-} zebrafish embryos (French et al., 2013). Additionally, the role of an alternative form of programmed cell death, necroptosis (a programmed form of necrotic cell death), in ocular development and disease has been demonstrated in animal models. Viringipurampeer and coauthors (2012) demonstrated that downstream effector of pax2.1 in zebrafish, fadd, is involved in repression of necroptotic cell death as fadd-deficient embryos exhibited an activation of RIP1/RIP3-mediated necroptosis in the developing eye, and especially in the cells lining the margins of the optic fissure. Most remarkably, the authors demonstrated that the inhibition of cell death occurring through the necroptosis pathway is sufficient to enable optic fissure closure in *pax2*-deficient embryos. Another recent article from the same group demonstrated that developmental defects associated with *Pax6*-deficient (and therefore possibly other dosage-sensitive genes) can be reversed through nonsense suppression therapeutics (Gregory-Evans et al., 2014; Wang and Gregory-Evans, 2015). Further studies in animal models will allow for additional integration of various identified genes into common developmental pathways, and provide an avenue for the development and testing of novel therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Abouzeid H, Favez T, Schmid A, Agosti C, Youssef M, Marzouk I, El Shakankiry N, Bayoumi N, Munier FL, Schorderet DF. Mutations in ALDH1A3 represent a frequent cause of microphthalmia/anophthalmia in consanguineous families. Hum Mutat. 2014; 35:949–953. [PubMed: 24777706]

- Abouzeid H, Youssef MA, Bayoumi N, ElShakankiri N, Marzouk I, Hauser P, Schorderet DF. RAX and anophthalmia in humans: evidence of brain anomalies. *Mol Vis.* 2012; 18:1449–1456. [PubMed: 22736936]
- Adler R, Canto-Soler MV. Molecular mechanisms of optic vesicle development: complexities, ambiguities and controversies. *Dev Biol.* 2007; 305:1–13. [PubMed: 17335797]
- Aijaz S, Clark BJ, Williamson K, van Heyningen V, Morrison D, Fitzpatrick D, Collin R, Ragge N, Christoforou A, Brown A, Hanson I. Absence of SIX6 mutations in microphthalmia, anophthalmia, and coloboma. *Invest Ophthalmol Vis Sci.* 2004; 45:3871–3876. [PubMed: 15505031]
- Akizu N, Shembesh NM, Ben-Omran T, Bastaki L, Al-Tawari A, Zaki MS, Koul R, Spencer E, Rosti RO, Scott E, Nickerson E, Gabriel S, da Gente G, Li J, Deardorff MA, Conlin LK, Horton MA, Zackai EH, Sherr EH, Gleeson JG. Whole-exome sequencing identifies mutated c12orf57 in recessive corpus callosum hypoplasia. *Am J Hum Genet.* 2013; 92:392–400. [PubMed: 23453666]
- Aldahmesh MA, Khan AO, Hijazi H, Alkuraya FS. Homozygous truncation of SIX6 causes complex microphthalmia in humans. *Clin Genet.* 2013; 84:198–199. [PubMed: 23167593]
- Aldahmesh MA, Khan AO, Mohamed J, Alkuraya FS. Novel recessive BFSP2 and PITX3 mutations: Insights into mutational mechanisms from consanguineous populations. *Genet Med.* 2011
- Aldahmesh MA, Mohammed JY, Al-Hazaa S, Alkuraya FS. Homozygous null mutation in ODZ3 causes microphthalmia in humans. *Genet Med.* 2012
- Andersson O, Korach-Andre M, Reissmann E, Ibanez CF, Bertolino P. Growth/differentiation factor 3 signals through ALK7 and regulates accumulation of adipose tissue and diet-induced obesity. *Proc Natl Acad Sci U S A.* 2008; 105:7252–7256. [PubMed: 18480259]
- Antinucci P, Nikolaou N, Meyer MP, Hindges R. Teneurin-3 specifies morphological and functional connectivity of retinal ganglion cells in the vertebrate visual system. *Cell Rep.* 2013; 5:582–592. [PubMed: 24183672]
- Asai-Coakwell M, French CR, Berry KM, Ye M, Koss R, Somerville M, Mueller R, van Heyningen V, Waskiewicz AJ, Lehmann OJ. GDF6, a novel locus for a spectrum of ocular developmental anomalies. *Am J Hum Genet.* 2007; 80:306–315. [PubMed: 17236135]
- Asai-Coakwell M, French CR, Ye M, Garcha K, Bigot K, Perera AG, Staehling-Hampton K, Mema SC, Chanda B, Mushegian A, Bamforth S, Doschak MR, Li G, Dobbs MB, Giampietro PF, Brooks BP, Vijayalakshmi P, Sauve Y, Abitbol M, Sundaresan P, van Heyningen V, Pourquie O, Underhill TM, Waskiewicz AJ, Lehmann OJ. Incomplete penetrance and phenotypic variability characterize Gdf6-attributable oculo-skeletal phenotypes. *Hum Mol Genet.* 2009; 18:1110–1121. [PubMed: 19129173]
- Asai-Coakwell M, March L, Dai XH, Duval M, Lopez I, French CR, Famulski J, De Baere E, Francis PJ, Sundaresan P, Sauve Y, Koenekoop RK, Berry FB, Allison WT, Waskiewicz AJ, Lehmann OJ. Contribution of growth differentiation factor 6-dependent cell survival to early-onset retinal dystrophies. *Hum Mol Genet.* 2013; 22:1432–1442. [PubMed: 23307924]
- Azuma N, Yamaguchi Y, Handa H, Hayakawa M, Kanai A, Yamada M. Missense mutation in the alternative splice region of the PAX6 gene in eye anomalies. *Am J Hum Genet.* 1999; 65:656–663. [PubMed: 10441571]
- Azuma N, Yamaguchi Y, Handa H, Tadokoro K, Asaka A, Kawase E, Yamada M. Mutations of the PAX6 gene detected in patients with a variety of optic-nerve malformations. *Am J Hum Genet.* 2003; 72:1565–1570. [PubMed: 12721955]
- Bakrania P, Efthymiou M, Klein JC, Salt A, Bunyan DJ, Wyatt A, Ponting CP, Martin A, Williams S, Lindley V, Gilmore J, Restori M, Robson AG, Neveu MM, Holder GE, Collin JR, Robinson DO, Farndon P, Johansen-Berg H, Gerrelli D, Ragge NK. Mutations in BMP4 cause eye, brain, and digit developmental anomalies: overlap between the BMP4 and hedgehog signaling pathways. *Am J Hum Genet.* 2008; 82:304–319. [PubMed: 18252212]
- Barabino SM, Spada F, Cotelli F, Boncinelli E. Inactivation of the zebrafish homologue of Chx10 by antisense oligonucleotides causes eye malformations similar to the ocular retardation phenotype. *Mech Dev.* 1997; 63:133–143. [PubMed: 9203137]
- Bar-Yosef U, Abuellaish I, Harel T, Hendler N, Ofir R, Birk OS. CHX10 mutations cause non-syndromic microphthalmia/anophthalmia in Arab and Jewish kindreds. *Hum Genet.* 2004; 115:302–309. [PubMed: 15257456]

- Becker D, Tetens J, Brunner A, Burstel D, Ganter M, Kijas J, International Sheep Genomics Consortium. Drogemuller C. Microphthalmia in Texel sheep is associated with a missense mutation in the paired-like homeodomain 3 (PITX3) gene. *PLoS One*. 2010; 5:e8689. [PubMed: 20084168]
- Beebe DC, Coats JM. The lens organizes the anterior segment: specification of neural crest cell differentiation in the avian eye. *Dev Biol*. 2000; 220:424–431. [PubMed: 10753528]
- Beleggia F, Li Y, Fan J, Elcioglu NH, Toker E, Wieland T, Maumenee IH, Akarsu NA, Meitinger T, Strom T, Lang R, Wollnik B. CRIM1 haploinsufficiency causes defects in eye development in human and mouse. *Hum Mol Genet*. 2015
- Ben-Zur T, Feige E, Motro B, Wides R. The mammalian Odz gene family: homologs of a *Drosophila* pair-rule gene with expression implying distinct yet overlapping developmental roles. *Dev Biol*. 2000; 217:107–120. [PubMed: 10625539]
- Berry DC, Jacobs H, Marwarha G, Gely-Pernot A, O'Byrne SM, DeSantis D, Klopfenstein M, Feret B, Dennefeld C, Blanner WS, Croniger CM, Mark M, Noy N, Ghyselinck NB. The STRA6 receptor is essential for retinol-binding protein-induced insulin resistance but not for maintaining vitamin A homeostasis in tissues other than the eye. *J Biol Chem*. 2013; 288:24528–24539. [PubMed: 23839944]
- Bibliowicz J, Tittle RK, Gross JM. Toward a better understanding of human eye disease insights from the zebrafish, *Danio rerio*. *Prog Mol Biol Transl Sci*. 2011; 100:287–330. [PubMed: 21377629]
- Bidinost C, Matsumoto M, Chung D, Salem N, Zhang K, Stockton DW, Khoury A, Megarbane A, Bejjani BA, Traboulsi EI. Heterozygous and homozygous mutations in PITX3 in a large Lebanese family with posterior polar cataracts and neurodevelopmental abnormalities. *Invest Ophthalmol Vis Sci*. 2006; 47:1274–1280. [PubMed: 16565358]
- Blixt A, Landgren H, Johansson BR, Carlsson P. Foxe3 is required for morphogenesis and differentiation of the anterior segment of the eye and is sensitive to Pax6 gene dosage. *Dev Biol*. 2007; 302:218–229. [PubMed: 17064680]
- Blixt A, Mahlapuu M, Aitola M, Peltö-Huikko M, Enerback S, Carlsson P. A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. *Genes Dev*. 2000; 14:245–254. [PubMed: 10652278]
- Bohm J, Buck A, Borozdin W, Mannan AU, Matysiak-Scholze U, Adham I, Schulz-Schaeffer W, Floss T, Wurst W, Kohlhaase J, Barrionuevo F. Sall1, sall2, and sall4 are required for neural tube closure in mice. *Am J Pathol*. 2008; 173:1455–1463. [PubMed: 18818376]
- Bouillet P, Sapin V, Chazaud C, Messaddeq N, Decimo D, Dolle P, Chambon P. Developmental expression pattern of Stra6, a retinoic acid-responsive gene encoding a new type of membrane protein. *Mech Dev*. 1997; 63:173–186. [PubMed: 9203140]
- Bovolenta P, Mallamaci A, Briata P, Corte G, Boncinelli E. Implication of OTX2 in pigment epithelium determination and neural retina differentiation. *J Neurosci*. 1997; 17:4243–4252. [PubMed: 9151741]
- Brown NL, Patel S, Brzezinski J, Glaser T. Math5 is required for retinal ganglion cell and optic nerve formation. *Development*. 2001; 128:2497–2508. [PubMed: 11493566]
- Brownell I, Dirksen M, Jamrich M. Forkhead Foxe3 maps to the dysgenetic lens locus and is critical in lens development and differentiation. *Genesis*. 2000; 27:81–93. [PubMed: 10890982]
- Burmeister M, Novak J, Liang MY, Basu S, Ploder L, Hawes NL, Vidgen D, Hoover F, Goldman D, Kalnins VI, Roderick TH, Taylor BA, Hankin MH, McInnes RR. Ocular retardation mouse caused by Chx10 homeobox null allele: impaired retinal progenitor proliferation and bipolar cell differentiation. *Nat Genet*. 1996; 12:376–384. [PubMed: 8630490]
- Casey J, Kawaguchi R, Morrissey M, Sun H, McGettigan P, Nielsen JE, Conroy J, Regan R, Kenny E, Cormican P, Morris DW, Tormey P, Ni Chroinin M, Kennedy BN, Lynch S, Green A, Ennis S. First implication of STRA6 mutations in isolated anophthalmia, microphthalmia, and coloboma: A new dimension to the STRA6 phenotype. *Hum Mutat*. 2011
- Chang B, Smith RS, Peters M, Savinova OV, Hawes NL, Zabaleta A, Nusinowitz S, Martin JE, Davisson ML, Cepko CL, Hogan BL, John SW. Haploinsufficient Bmp4 ocular phenotypes include anterior segment dysgenesis with elevated intraocular pressure. *BMC Genet*. 2001; 2:18. [PubMed: 11722794]

- Chassaing N, Causse A, Vigouroux A, Delahaye A, Alessandri JL, Boespflug-Tanguy O, Boute-Benejean O, Dollfus H, Duban-Bedu B, Gilbert-Dussardier B, Giuliano F, Gonzales M, Holder-Espinasse M, Isidor B, Jacquemont ML, Lacombe D, Martin-Coignard D, Mathieu-Dramard M, Odent S, Picone O, Pinson L, Quelin C, Sigaudy S, Toutain A, Thauvin-Robinet C, Kaplan J, Calvas P. Molecular findings and clinical data in a cohort of 150 patients with anophthalmia/microphthalmia. *Clin Genet*. 2013
- Chassaing N, Golzio C, Odent S, Lequeux L, Vigouroux A, Martinovic-Bouriel J, Tiziano FD, Masini L, Piro F, Maragliano G, Delezoide AL, Attie-Bitach T, Manouvrier-Hanu S, Etchevers HC, Calvas P. Phenotypic spectrum of STRA6 mutations: from Matthew-Wood syndrome to non-lethal anophthalmia. *Hum Mutat*. 2009; 30:E673–81. [PubMed: 19309693]
- Chassaing N, Sorrentino S, Davis EE, Martin-Coignard D, Iacovelli A, Paznekas W, Webb BD, Faye-Petersen O, Encha-Razavi F, Lequeux L, Vigouroux A, Yesilyurt A, Boyadjiev SA, Kayserili H, Loget P, Carles D, Sergi C, Puvabanditsin S, Chen CP, Etchevers HC, Katsanis N, Mercer CL, Calvas P, Jabs EW. OTX2 mutations contribute to the otocephaly-dysgnathia complex. *J Med Genet*. 2012; 49:373–379. [PubMed: 22577225]
- Chitayat D, Sroka H, Keating S, Colby RS, Ryan G, Toi A, Blaser S, Viero S, Devisme L, Boute-Benejean O, Manouvrier-Hanu S, Mortier G, Loeys B, Rauch A, Bitoun P. The PDAC syndrome (pulmonary hypoplasia/agenesis, diaphragmatic hernia/eventration, anophthalmia/microphthalmia, and cardiac defect) (Spear syndrome, Matthew-Wood syndrome): report of eight cases including a living child and further evidence for autosomal recessive inheritance. *Am J Med Genet A*. 2007; 143A:1268–1281. [PubMed: 17506106]
- Choi A, Lao R, Ling-Fung Tang P, Wan E, Mayer W, Bardakjian T, Shaw GM, Kwok PY, Schneider A, Slavotinek A. Novel mutations in PXDN cause microphthalmia and anterior segment dysgenesis. *Eur J Hum Genet*. 2015; 23:337–341. [PubMed: 24939590]
- Chow RL, Lang RA. Early eye development in vertebrates. *Annu Rev Cell Dev Biol*. 2001; 17:255–296. [PubMed: 11687490]
- Coulombre AJ, Coulombre JL. Lens Development. I. Role of the Lens in Eye Growth. *J Exp Zool*. 1964; 156:39–47. [PubMed: 14189921]
- Dansault A, David G, Schwartz C, Jaliffa C, Vieira V, de la Houssaye G, Bigot K, Catin F, Tattu L, Chopin C, Halimi P, Roche O, Van Regemorter N, Munier F, Schorderet D, Dufier JL, Marsac C, Ricquier D, Menasche M, Penfornis A, Abitbol M. Three new PAX6 mutations including one causing an unusual ophthalmic phenotype associated with neurodevelopmental abnormalities. *Mol Vis*. 2007; 13:511–523. [PubMed: 17417613]
- Davis J, Duncan MK, Robison WG Jr, Piatigorsky J. Requirement for Pax6 in corneal morphogenesis: a role in adhesion. *J Cell Sci*. 2003; 116:2157–2167. [PubMed: 12692153]
- Deml B, Kariminejad A, Borujerdi RH, Muheisen S, Reis LM, Semina EV. Mutations in MAB21L2 result in ocular coloboma, microcornea and cataracts. *PLoS Genet*. 2015; 11:e1005002. [PubMed: 25719200]
- Deml B, Reis LM, Maheshwari M, Griffis C, Bick D, Semina EV. Whole exome analysis identifies dominant COL4A1 mutations in patients with complex ocular phenotypes involving microphthalmia. *Clin Genet*. 2014; 86:475–481. [PubMed: 24628545]
- den Hollander AI, Biyanwila J, Kovach P, Bardakjian T, Traboulsi EI, Ragge NK, Schneider A, Malicki J. Genetic defects of GDF6 in the zebrafish out of sight mutant and in human eye developmental anomalies. *BMC Genet*. 2010; 11 102-2156-11-102.
- Dubourg C, Lazaro L, Pasquier L, Bendavid C, Blayau M, Le Duff F, Durou MR, Odent S, David V. Molecular screening of SHH, ZIC2, SIX3, and TGIF genes in patients with features of holoprosencephaly spectrum: Mutation review and genotype-phenotype correlations. *Hum Mutat*. 2004; 24:43–51. [PubMed: 15221788]
- Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev*. 1995; 9:2795–2807. [PubMed: 7590254]
- Duester G. Keeping an eye on retinoic acid signaling during eye development. *Chem Biol Interact*. 2009; 178:178–181. [PubMed: 18831967]

- Dunn NR, Winnier GE, Hargett LK, Schrick JJ, Fogo AB, Hogan BL. Haploinsufficient phenotypes in Bmp4 heterozygous null mice and modification by mutations in Gli3 and Alx4. *Dev Biol.* 1997; 188:235–247. [PubMed: 9268572]
- Dupe V, Matt N, Garnier JM, Chambon P, Mark M, Ghyselinck NB. A newborn lethal defect due to inactivation of retinaldehyde dehydrogenase type 3 is prevented by maternal retinoic acid treatment. *Proc Natl Acad Sci U S A.* 2003; 100:14036–14041. [PubMed: 14623956]
- ExAC. Exome Aggregation Consortium, Cambridge, MA. 2015.
- Fantes J, Ragge NK, Lynch SA, McGill NI, Collin JR, Howard-Peebles PN, Hayward C, Vivian AJ, Williamson K, van Heyningen V, FitzPatrick DR. Mutations in SOX2 cause anophthalmia. *Nat Genet.* 2003; 33:461–463. [PubMed: 12612584]
- Fares-Taie L, Gerber S, Chassaing N, Clayton-Smith J, Hanein S, Silva E, Serey M, Serre V, Gerard X, Baumann C, Plessis G, Demeer B, Bretillon L, Bole C, Nitschke P, Munnich A, Lyonnet S, Calvas P, Kaplan J, Ragge N, Rozet JM. ALDH1A3 mutations cause recessive anophthalmia and microphthalmia. *Am J Hum Genet.* 2013; 92:265–270. [PubMed: 23312594]
- Ferda Percin E, Ploder LA, Yu JJ, Arici K, Horsford DJ, Rutherford A, Bapat B, Cox DW, Duncan AM, Kalnins VI, Kocak-Altintas A, Sowden JC, Traboulsi E, Sarfarazi M, McInnes RR. Human microphthalmia associated with mutations in the retinal homeobox gene CHX10. *Nat Genet.* 2000; 25:397–401. [PubMed: 10932181]
- Fish MB, Nakayama T, Fisher M, Hirsch N, Cox A, Reeder R, Carruthers S, Hall A, Stemple DL, Grainger RM. *Xenopus* mutant reveals necessity of rax for specifying the eye field which otherwise forms tissue with telencephalic and diencephalic character. *Dev Biol.* 2014; 395:317–330. [PubMed: 25224223]
- Fougerousse F, Bullen P, Herasse M, Lindsay S, Richard I, Wilson D, Suel L, Durand M, Robson S, Abitbol M, Beckmann JS, Strachan T. Human-mouse differences in the embryonic expression patterns of developmental control genes and disease genes. *Hum Mol Genet.* 2000; 9:165–173. [PubMed: 10607827]
- French CR, Stach TR, March LD, Lehmann OJ, Waskiewicz AJ. Apoptotic and proliferative defects characterize ocular development in a microphthalmic BMP model. *Invest Ophthalmol Vis Sci.* 2013; 54:4636–4647. [PubMed: 23737474]
- Furuta Y, Hogan BL. BMP4 is essential for lens induction in the mouse embryo. *Genes Dev.* 1998; 12:3764–3775. [PubMed: 9851982]
- Gage PJ, Rhoades W, Prucka SK, Hjalt T. Fate maps of neural crest and mesoderm in the mammalian eye. *Invest Ophthalmol Vis Sci.* 2005; 46:4200–4208. [PubMed: 16249499]
- Gallardo ME, Lopez-Rios J, Feraud-Espinosa I, Granadino B, Sanz R, Ramos C, Ayuso C, Seller MJ, Brunner HG, Bovolenta P, Rodriguez de Cordoba S. Genomic cloning and characterization of the human homeobox gene SIX6 reveals a cluster of SIX genes in chromosome 14 and associates SIX6 hemizygosity with bilateral anophthalmia and pituitary anomalies. *Genomics.* 1999; 61:82–91. [PubMed: 10512683]
- Gallardo ME, Rodriguez De Cordoba S, Schneider AS, Dwyer MA, Ayuso C, Bovolenta P. Analysis of the developmental SIX6 homeobox gene in patients with anophthalmia/microphthalmia. *Am J Med Genet A.* 2004; 129A:92–94. [PubMed: 15266624]
- Genis-Galvez JM, Santos-Gutierrez L, Rios-Gonzalez A. Causal factors in corneal development: an experimental analysis in the chick embryo. *Exp Eye Res.* 1967; 6:48–56. [PubMed: 6019480]
- Gerth-Kahlert C, Williamson K, Ansari M, Rainger JK, Hingst V, Zimmermann T, Tech S, Guthoff RF, van Heyningen V, Fitzpatrick DR. Clinical and mutation analysis of 51 probands with anophthalmia and/or severe microphthalmia from a single center. *Mol Genet Genomic Med.* 2013; 1:15–31. [PubMed: 24498598]
- Ghiasvand NM, Rudolph DD, Mashayekhi M, Brzezinski JA 4th, Goldman D, Glaser T. Deletion of a remote enhancer near ATOH7 disrupts retinal neurogenesis, causing NCRNA disease. *Nat Neurosci.* 2011; 14:578–586. [PubMed: 21441919]
- Ginocchio VM, De Brasi D, Genesio R, Ciccone R, Gimelli S, Fimiani F, de Berardinis T, Nitsch L, Banfi S, Magli A, Della Casa R. Sonic Hedgehog deletion and distal trisomy 3p in a patient with microphthalmia and microcephaly, lacking cerebral anomalies typical of holoprosencephaly. *Eur J Med Genet.* 2008; 51:658–665. [PubMed: 18762283]

- Glaser T, Jepeal L, Edwards JG, Young SR, Favor J, Maas RL. PAX6 gene dosage effect in a family with congenital cataracts, aniridia, anophthalmia and central nervous system defects. *Nat Genet.* 1994; 7:463–471. [PubMed: 7951315]
- Golzio C, Martinovic-Bouriel J, Thomas S, Mougou-Zrelli S, Grattagliano-Bessieres B, Bonniere M, Delahaye S, Munnich A, Encha-Razavi F, Lyonnet S, Vekemans M, Attie-Bitach T, Etchevers HC. Matthew-Wood syndrome is caused by truncating mutations in the retinol-binding protein receptor gene STRA6. *Am J Hum Genet.* 2007; 80:1179–1187. [PubMed: 17503335]
- Gonzalez-Rodriguez J, Pelcastre EL, Tovilla-Canales JL, Garcia-Ortiz JE, Amato-Almanza M, Villanueva-Mendoza C, Espinosa-Mattar Z, Zenteno JC. Mutational screening of CHX10, GDF6, OTX2, RAX and SOX2 genes in 50 unrelated microphthalmia-anophthalmia-coloboma (MAC) spectrum cases. *Br J Ophthalmol.* 2010; 94:1100–1104. [PubMed: 20494911]
- Gosse NJ, Baier H. An essential role for Radar (Gdf6a) in inducing dorsal fate in the zebrafish retina. *Proc Natl Acad Sci U S A.* 2009; 106:2236–2241. [PubMed: 19164594]
- Graw J. Eye development. *Curr Top Dev Biol.* 2010; 90:343–386. [PubMed: 20691855]
- Gregory-Evans CY, Wang X, Wasan KM, Zhao J, Metcalfe AL, Gregory-Evans K. Postnatal manipulation of Pax6 dosage reverses congenital tissue malformation defects. *J Clin Invest.* 2014; 124:111–116. [PubMed: 24355924]
- Greiling TM, Clark JI. New insights into the mechanism of lens development using zebra fish. *Int Rev Cell Mol Biol.* 2012; 296:1–61. [PubMed: 22559937]
- Hanel ML, Hensey C. Eye and neural defects associated with loss of GDF6. *BMC Dev Biol.* 2006; 6:43. [PubMed: 17010201]
- Hanson IM. PAX6 and congenital eye malformations. *Pediatr Res.* 2003; 54:791–796. [PubMed: 14561779]
- Hanson IM, Fletcher JM, Jordan T, Brown A, Taylor D, Adams RJ, Punnett HH, van Heyningen V. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. *Nat Genet.* 1994; 6:168–173. [PubMed: 8162071]
- Haustein J. On the ultrastructure of the developing and adult mouse corneal stroma. *Anat Embryol (Berl).* 1983; 168:291–305. [PubMed: 6660567]
- Henderson RA, Williamson K, Cumming S, Clarke MP, Lynch SA, Hanson IM, FitzPatrick DR, Sisodiya S, van Heyningen V. Inherited PAX6, NF1 and OTX2 mutations in a child with microphthalmia and aniridia. *Eur J Hum Genet.* 2007; 15:898–901. [PubMed: 17406642]
- Henderson RH, Williamson KA, Kennedy JS, Webster AR, Holder GE, Robson AG, FitzPatrick DR, van Heyningen V, Moore AT. A rare de novo nonsense mutation in OTX2 causes early onset retinal dystrophy and pituitary dysfunction. *Mol Vis.* 2009; 15:2442–2447. [PubMed: 19956411]
- Hever AM, Williamson KA, van Heyningen V. Developmental malformations of the eye: the role of PAX6, SOX2 and OTX2. *Clin Genet.* 2006; 69:459–470. [PubMed: 16712695]
- Ho HY, Chang KH, Nichols J, Li M. Homeodomain protein Pitx3 maintains the mitotic activity of lens epithelial cells. *Mech Dev.* 2009; 126:18–29. [PubMed: 19007884]
- Iseri SU, Wyatt AW, Nurnberg G, Kluck C, Nurnberg P, Holder GE, Blair E, Salt A, Ragge NK. Use of genome-wide SNP homozygosity mapping in small pedigrees to identify new mutations in VSX2 causing recessive microphthalmia and a semidominant inner retinal dystrophy. *Hum Genet.* 2010; 128:51–60. [PubMed: 20414678]
- Johnston MC, Noden DM, Hazelton RD, Coulombre JL, Coulombre AJ. Origins of avian ocular and periocular tissues. *Exp Eye Res.* 1979; 29:27–43. [PubMed: 510425]
- Kay JN, Finger-Baier KC, Roeser T, Staub W, Baier H. Retinal ganglion cell genesis requires lakritz, a Zebrafish atonal Homolog. *Neuron.* 2001; 30:725–736. [PubMed: 11430806]
- Kelberman D, Islam L, Lakowski J, Bacchelli C, Chanudet E, Lescai F, Patel A, Stupka E, Buck A, Wolf S, Beales PL, Jacques TS, Bitner-Glindzicz M, Liasis A, Lehmann OJ, Kohlhaase J, Nischal KK, Sowden JC. Mutation of SALL2 causes recessive ocular coloboma in humans and mice. *Hum Mol Genet.* 2014; 23:2511–2526. [PubMed: 24412933]
- Kennedy BN, Stearns GW, Smyth VA, Ramamurthy V, van Eeden F, Ankoudinova I, Raible D, Hurley JB, Brouckhoff SE. Zebrafish rx3 and mab21l2 are required during eye morphogenesis. *Dev Biol.* 2004; 270:336–349. [PubMed: 15183718]

- Khan AO, Aldahmesh MA, Noor J, Salem A, Alkuraya FS. Lens Subluxation and Retinal Dysfunction in a Girl with Homozygous VSX2 Mutation. *Ophthalmic Genet.* 2015; 36:8–13. [PubMed: 24001013]
- Khan K, Logan CV, McKibbin M, Sheridan E, Elcioglu NH, Yenice O, Parry DA, Fernandez-Fuentes N, Abdelhamed ZI, Al-Maskari A, Poulter JA, Mohamed MD, Carr IM, Morgan JE, Jafri H, Raashid Y, Taylor GR, Johnson CA, Inglehearn CF, Toomes C, Ali M. Next generation sequencing identifies mutations in Atonal homolog 7 (ATOH7) in families with global eye developmental defects. *Hum Mol Genet.* 2012; 21:776–783. [PubMed: 22068589]
- Kumar JP. The sine oculis homeobox (SIX) family of transcription factors as regulators of development and disease. *Cell Mol Life Sci.* 2009; 66:565–583. [PubMed: 18989625]
- Langer L, Taranova O, Sulik K, Pevny L. SOX2 hypomorphism disrupts development of the prechordal floor and optic cup. *Mech Dev.* 2012; 129:1–12. [PubMed: 22522080]
- Larder R, Kimura I, Meadows J, Clark DD, Mayo S, Mellon PL. Gene dosage of Otx2 is important for fertility in male mice. *Mol Cell Endocrinol.* 2013; 377:16–22. [PubMed: 23811236]
- Leamey CA, Merlin S, Lattouf P, Sawatari A, Zhou X, Demel N, Glendinning KA, Ohashi T, Sur M, Fassler R. Ten_m3 regulates eye-specific patterning in the mammalian visual pathway and is required for binocular vision. *PLoS Biol.* 2007; 5:e241. [PubMed: 17803360]
- Li X, Perissi V, Liu F, Rose DW, Rosenfeld MG. Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science.* 2002; 297:1180–1183. [PubMed: 12130660]
- Liu IS, Chen JD, Ploder L, Vidgen D, van der Kooy D, Kalnins VI, McInnes RR. Developmental expression of a novel murine homeobox gene (Chx10): evidence for roles in determination of the neuroretina and inner nuclear layer. *Neuron.* 1994; 13:377–393. [PubMed: 7914735]
- London NJ, Kessler P, Williams B, Pauer GJ, Hagstrom SA, Traboulsi EI. Sequence alterations in RX in patients with microphthalmia, anophthalmia, and coloboma. *Mol Vis.* 2009; 15:162–167. [PubMed: 19158959]
- Loosli F, Staub W, Finger-Baier KC, Ober EA, Verkade H, Wittbrodt J, Baier H. Loss of eyes in zebrafish caused by mutation of chokh/rx3. *EMBO Rep.* 2003; 4:894–899. [PubMed: 12947416]
- Loosli F, Winkler S, Burgdorf C, Wurmbach E, Ansorge W, Henrich T, Grabher C, Arendt D, Carl M, Krone A, Grzebisz E, Wittbrodt J. Medaka eyeless is the key factor linking retinal determination and eye growth. *Development.* 2001; 128:4035–4044. [PubMed: 11641226]
- Lovicu FJ, McAvoy JW. Growth factor regulation of lens development. *Dev Biol.* 2005; 280:1–14. [PubMed: 15766743]
- Martinez-Fernandez ML, Bermejo-Sanchez E, Fernandez B, MacDonald A, Fernandez-Toral J, Martinez-Frias ML. Haploinsufficiency of BMP4 gene may be the underlying cause of Frias syndrome. *Am J Med Genet A.* 2014; 164A:338–345. [PubMed: 24311462]
- Massaro GD, Massaro D, Chan WY, Clerch LB, Ghyselinck N, Chambon P, Chandraratna RA. Retinoic acid receptor-beta: an endogenous inhibitor of the perinatal formation of pulmonary alveoli. *Physiol Genomics.* 2000; 4:51–57. [PubMed: 11074013]
- Mathers PH, Grinberg A, Mahon KA, Jamrich M. The Rx homeobox gene is essential for vertebrate eye development. *Nature.* 1997; 387:603–607. [PubMed: 9177348]
- Matsuo I, Kuratani S, Kimura C, Takeda N, Aizawa S. Mouse Otx2 functions in the formation and patterning of rostral head. *Genes Dev.* 1995; 9:2646–2658. [PubMed: 7590242]
- Matsuo T, Osumi-Yamashita N, Noji S, Ohuchi H, Koyama E, Myokai F, Matsuo N, Taniguchi S, Doi H, Iseki S. A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. *Nat Genet.* 1993; 3:299–304. [PubMed: 7981749]
- Medina-Martinez O. Focus on Molecules: Pitx3. *Exp Eye Res.* 2010
- Mihelec M, Abraham P, Gibson K, Krowka R, Susman R, Storen R, Chen Y, Donald J, Tam PP, Grigg JR, Flaherty M, Gole GA, Jamieson RV. Novel SOX2 partner-factor domain mutation in a four-generation family. *Eur J Hum Genet.* 2009; 17:1417–1422. [PubMed: 19471311]
- Molotkov A, Molotkova N, Duester G. Retinoic acid guides eye morphogenetic movements via paracrine signaling but is unnecessary for retinal dorsoventral patterning. *Development.* 2006; 133:1901–1910. [PubMed: 16611695]

- Ng WY, Pasutto F, Bardakjian TM, Wilson MJ, Watson G, Schneider A, Mackey DA, Grigg JR, Zenker M, Jamieson RV. A puzzle over several decades: eye anomalies with FRAS1 and STRA6 mutations in the same family. *Clin Genet*. 2013; 83:162–168. [PubMed: 22283518]
- Niederreither K, Fraulob V, Garnier JM, Chambon P, Dolle P. Differential expression of retinoic acid-synthesizing (RALDH) enzymes during fetal development and organ differentiation in the mouse. *Mech Dev*. 2002; 110:165–171. [PubMed: 11744377]
- Ormestad M, Blixt A, Churchill A, Martinsson T, Enerback S, Carlsson P. Foxe3 haploinsufficiency in mice: a model for Peters' anomaly. *Invest Ophthalmol Vis Sci*. 2002; 43:1350–1357. [PubMed: 11980846]
- Passini MA, Levine EM, Canger AK, Raymond PA, Schechter N. Vsx-1 and Vsx-2: differential expression of two paired-like homeobox genes during zebrafish and goldfish retinogenesis. *J Comp Neurol*. 1997; 388:495–505. [PubMed: 9368856]
- Pasutto F, Sticht H, Hammersen G, Gillessen-Kaesbach G, Fitzpatrick DR, Nurnberg G, Brasch F, Schirmer-Zimmermann H, Tolmie JL, Chitayat D, Houge G, Fernandez-Martinez L, Keating S, Mortier G, Hennekam RC, von der Wense A, Slavotinek A, Meinecke P, Bitoun P, Becker C, Nurnberg P, Reis A, Rauch A. Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, and mental retardation. *Am J Hum Genet*. 2007; 80:550–560. [PubMed: 17273977]
- Patat O, van Ravenswaaij-Arts CM, Tantau J, Corsten-Janssen N, van Tintelen JP, Dijkhuizen T, Kaplan J, Chassaing N. Otocephaly-Dysgnathia Complex: Description of Four Cases and Confirmation of the Role of OTX2. *Mol Syndromol*. 2013; 4:302–305. [PubMed: 24167467]
- Piccione M, Serra G, Consiglio V, Di Fiore A, Cavani S, Grasso M, Malacarne M, Pierluigi M, Viaggi C, Corsello G. 14q13.1–21.1 deletion encompassing the HPE8 locus in an adolescent with intellectual disability and bilateral microphthalmia, but without holoprosencephaly. *Am J Med Genet A*. 2012; 158A:1427–1433. [PubMed: 22581785]
- Platzer K, Huning I, Obieglo C, Schwarzmayer T, Gabriel R, Strom TM, Gillessen-Kaesbach G, Kaiser FJ. Exome sequencing identifies compound heterozygous mutations in C12orf57 in two siblings with severe intellectual disability, hypoplasia of the corpus callosum, chorioretinal coloboma, and intractable seizures. *Am J Med Genet A*. 2014; 164A:1976–1980. [PubMed: 24798461]
- Ragge NK, Brown AG, Poloschek CM, Lorenz B, Henderson RA, Clarke MP, Russell-Eggitt I, Fielder A, Gerrelli D, Martinez-Barbera JP, Ruddell P, Hurst J, Collin JR, Salt A, Cooper ST, Thompson PJ, Sisodiya SM, Williamson KA, Fitzpatrick DR, van Heyningen V, Hanson IM. Heterozygous mutations of OTX2 cause severe ocular malformations. *Am J Hum Genet*. 2005; 76:1008–1022. [PubMed: 15846561]
- Rainger J, Pehlivan D, Johansson S, Bengani H, Sanchez-Pulido L, Williamson KA, Ture M, Barker H, Rosendahl K, Spranger J, Horn D, Meynert A, Floyd JA, Prescott T, Anderson CA, Rainger JK, Karaca E, Gonzaga-Jauregui C, Jhangiani S, Muzny DM, Seawright A, Soares DC, Kharbada M, Murday V, Finch A, UK10K, Baylor-Hopkins Center for Mendelian Genomics, Gibbs RA, van Heyningen V, Taylor MS, Yakut T, Knappskog PM, Hurles ME, Ponting CP, Lupski JR, Houge G, FitzPatrick DR. Monoallelic and biallelic mutations in MAB21L2 cause a spectrum of major eye malformations. *Am J Hum Genet*. 2014; 94:915–923. [PubMed: 24906020]
- Ramaesh T, Collinson JM, Ramaesh K, Kaufman MH, West JD, Dhillon B. Corneal abnormalities in Pax6+/- small eye mice mimic human aniridia-related keratopathy. *Invest Ophthalmol Vis Sci*. 2003; 44:1871–1878. [PubMed: 12714618]
- Reis LM, Khan A, Kariminejad A, Ebadi F, Tyler RC, Semina EV. VSX2 mutations in autosomal recessive microphthalmia. *Mol Vis*. 2011; 17:2527–2532. [PubMed: 21976963]
- Reis LM, Khan A, Kariminejad A, Ebadi F, Tyler RC, Semina EV. VSX2 mutations in autosomal recessive microphthalmia. *Mol Vis*. 2011; 17:2527–2532. [PubMed: 21976963]
- Reis LM, Semina EV. Genetics of anterior segment dysgenesis disorders. *Curr Opin Ophthalmol*. 2011; 22:314–324. [PubMed: 21730847]
- Reis LM, Tyler RC, Schilter KF, Abdul-Rahman O, Innis JW, Kozel BA, Schneider AS, Bardakjian TM, Lose EJ, Martin DM, Broeckel U, Semina EV. BMP4 loss-of-function mutations in developmental eye disorders including SHORT syndrome. *Hum Genet*. 2011; 130:495–504. [PubMed: 21340693]

- Reis LM, Tyler RC, Schneider A, Bardakjian T, Stoler JM, Melancon SB, Semina EV. FOXE3 plays a significant role in autosomal recessive microphthalmia. *Am J Med Genet A*. 2010; 152A:582–590. [PubMed: 20140963]
- Reneker LW, Silversides DW, Xu L, Overbeek PA. Formation of corneal endothelium is essential for anterior segment development - a transgenic mouse model of anterior segment dysgenesis. *Development*. 2000; 127:533–542. [PubMed: 10631174]
- Rieger DK, Reichenberger E, McLean W, Sidow A, Olsen BR. A double-deletion mutation in the Pitx3 gene causes arrested lens development in aphakia mice. *Genomics*. 2001; 72:61–72. [PubMed: 11247667]
- Rosemann M, Ivashkevich A, Favor J, Dalke C, Holter SM, Becker L, Racz I, Bolle I, Klempt M, Rathkolb B, Kalaydjiev S, Adler T, Aguilar A, Hans W, Horsch M, Rozman J, Calzada-Wack J, Kunder S, Naton B, Gailus-Durner V, Fuchs H, Schulz H, Beckers J, Busch DH, Burbach JP, Smidt MP, Quintanilla-Martinez L, Esposito I, Klopstock T, Klingenspor M, Ollert M, Wolf E, Wurst W, Zimmer A, de Angelis MH, Atkinson M, Heinzmann U, Graw J. Microphthalmia, parkinsonism, and enhanced nociception in Pitx3 (416insG) mice. *Mamm Genome*. 2010; 21:13–27. [PubMed: 20033184]
- Saito Y, Kojima T, Takahashi N. Mab2112 is essential for embryonic heart and liver development. *PLoS One*. 2012; 7:e32991. [PubMed: 22412967]
- Schilter KF, Reis LM, Schneider A, Bardakjian TM, Abdul-Rahman O, Kozel BA, Zimmerman HH, Broeckel U, Semina EV. Whole-genome copy number variation analysis in anophthalmia and microphthalmia. *Clin Genet*. 2013; 84:473–481. [PubMed: 23701296]
- Schilter KF, Schneider A, Bardakjian T, Soucy JF, Tyler RC, Reis LM, Semina EV. OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clin Genet*. 2011; 79:158–168. [PubMed: 20486942]
- Schimmenti LA, de la Cruz J, Lewis RA, Karkera JD, Manligas GS, Roessler E, Muenke M. Novel mutation in sonic hedgehog in non-syndromic colobomatous microphthalmia. *Am J Med Genet A*. 2003; 116A:215–221. [PubMed: 12503095]
- Schmidt-Sidor B, Szymanska K, Williamson K, van Heyningen V, Roszkowski T, Wierzb-Bobrowicz T, Zaremba J. Malformations of the brain in two fetuses with a compound heterozygosity for two PAX6 mutations. *Folia Neuropathol*. 2009; 47:372–382. [PubMed: 20054790]
- Schneider A, Bardakjian T, Reis LM, Tyler RC, Semina EV. Novel SOX2 mutations and genotype-phenotype correlation in anophthalmia and microphthalmia. *Am J Med Genet A*. 2009; 149A:2706–2715. [PubMed: 19921648]
- Scott AF, Mohr DW, Kasch LM, Barton JA, Pittiglio R, Ingersoll R, Craig B, Marosy BA, Doheny KF, Bromley WC, Roderick TH, Chassaing N, Calvas P, Prabhu SS, Jabs EW. Identification of an HMGB3 frameshift mutation in a family with an X-linked colobomatous microphthalmia syndrome using whole-genome and X-exome sequencing. *JAMA Ophthalmol*. 2014; 132:1215–1220. [PubMed: 24993872]
- Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. *Nat Genet*. 1998; 19:167–170. [PubMed: 9620774]
- Semina EV, Murray JC, Reiter R, Hrstka RF, Graw J. Deletion in the promoter region and altered expression of Pitx3 homeobox gene in aphakia mice. *Hum Mol Genet*. 2000; 9:1575–1585. [PubMed: 10861284]
- Semina EV, Reiter RS, Murray JC. Isolation of a new homeobox gene belonging to the Pitx/Rieg family: expression during lens development and mapping to the aphakia region on mouse chromosome 19. *Hum Mol Genet*. 1997; 6:2109–2116. [PubMed: 9328475]
- Shi X, Luo Y, Howley S, Dzialo A, Foley S, Hyde DR, Vihtelic TS. Zebrafish foxe3: roles in ocular lens morphogenesis through interaction with pitx3. *Mech Dev*. 2006; 123:761–782. [PubMed: 16963235]
- Skalicky SE, White AJ, Grigg JR, Martin F, Smith J, Jones M, Donaldson C, Smith JE, Flaherty M, Jamieson RV. Microphthalmia, anophthalmia, and coloboma and associated ocular and systemic features: understanding the spectrum. *JAMA Ophthalmol*. 2013; 131:1517–1524. [PubMed: 24177921]

- Smidt MP, Smits SM, Burbach JP. Homeobox gene Pitx3 and its role in the development of dopamine neurons of the substantia nigra. *Cell Tissue Res*. 2004; 318:35–43. [PubMed: 15300495]
- Solomon BD, Pineda-Alvarez DE, Balog JZ, Hadley D, Gropman AL, Nandagopal R, Han JC, Hahn JS, Blain D, Brooks B, Muenke M. Compound heterozygosity for mutations in PAX6 in a patient with complex brain anomaly, neonatal diabetes mellitus, and microphthalmia. *Am J Med Genet A*. 2009; 149A:2543–2546. [PubMed: 19876904]
- Sorokina EA, Muheisen S, Mlodik N, Semina EV. MIP/Aquaporin 0 represents a direct transcriptional target of PITX3 in the developing lens. *PLoS One*. 2011; 6:e21122. [PubMed: 21698120]
- Srour M, Chitayat D, Caron V, Chassaing N, Bitoun P, Patry L, Cordier MP, Capo-Chichi JM, Francannet C, Calvas P, Ragge N, Dobrzeniecka S, Hamdan FF, Rouleau GA, Tremblay A, Michaud JL. Recessive and dominant mutations in retinoic acid receptor beta in cases with microphthalmia and diaphragmatic hernia. *Am J Hum Genet*. 2013; 93:765–772. [PubMed: 24075189]
- Stenkamp DL, Frey RA, Mallory DE, Shupe EE. Embryonic retinal gene expression in sonic-you mutant zebrafish. *Dev Dyn*. 2002; 225:344–350. [PubMed: 12412019]
- Takamiya M, Weger BD, Schindler S, Beil T, Yang L, Armant O, Ferg M, Schlunck G, Reinhard T, Dickmeis T, Rastegar S, Strahle U. Molecular Description of Eye Defects in the Zebrafish Pax6b Mutant, sunrise, Reveals a Pax6b-Dependent Genetic Network in the Developing Anterior Chamber. *PLoS One*. 2015; 10:e0117645. [PubMed: 25692557]
- Taranova OV, Magness ST, Fagan BM, Wu Y, Surzenko N, Hutton SR, Pevny LH. SOX2 is a dose-dependent regulator of retinal neural progenitor competence. *Genes Dev*. 2006; 20:1187–1202. [PubMed: 16651659]
- Tassabehji M, Fang ZM, Hilton EN, McGaughan J, Zhao Z, de Bock CE, Howard E, Malass M, Donnai D, Diwan A, Manson FD, Murrell D, Clarke RA. Mutations in GDF6 are associated with vertebral segmentation defects in Klippel-Feil syndrome. *Hum Mutat*. 2008; 29:1017–1027. [PubMed: 18425797]
- Theveneau E, Mayor R. Neural crest delamination and migration: from epithelium-to-mesenchyme transition to collective cell migration. *Dev Biol*. 2012; 366:34–54. [PubMed: 22261150]
- Thut CJ, Rountree RB, Hwa M, Kingsley DM. A large-scale in situ screen provides molecular evidence for the induction of eye anterior segment structures by the developing lens. *Dev Biol*. 2001; 231:63–76. [PubMed: 11180952]
- Tucker P, Laemle L, Munson A, Kanekar S, Oliver ER, Brown N, Schlecht H, Vetter M, Glaser T. The eyeless mouse mutation (ey1) removes an alternative start codon from the Rx/rax homeobox gene. *Genesis*. 2001; 31:43–53. [PubMed: 11668677]
- Valleix S, Niel F, Nedelec B, Algros MP, Schwartz C, Delbosc B, Delpech M, Kantelip B. Homozygous nonsense mutation in the FOXE3 gene as a cause of congenital primary aphakia in humans. *Am J Hum Genet*. 2006; 79:358–364. [PubMed: 16826526]
- van der Linde-Sipman JS, van den Ingh TS, Vellema P. Morphology and morphogenesis of hereditary microphthalmia in Texel sheep. *J Comp Pathol*. 2003; 128:269–275. [PubMed: 12834610]
- Vincent MC, Pujo AL, Olivier D, Calvas P. Screening for PAX6 gene mutations is consistent with haploinsufficiency as the main mechanism leading to various ocular defects. *Eur J Hum Genet*. 2003; 11:163–169. [PubMed: 12634864]
- Viringipurampeer IA, Ferreira T, DeMaria S, Yoon JJ, Shan X, Moosajee M, Gregory-Evans K, Ngai J, Gregory-Evans CY. Pax2 regulates a fadd-dependent molecular switch that drives tissue fusion during eye development. *Hum Mol Genet*. 2012; 21:2357–2369. [PubMed: 22357656]
- Vitorino M, Jusuf PR, Maurus D, Kimura Y, Higashijima S, Harris WA. Vsx2 in the zebrafish retina: restricted lineages through derepression. *Neural Dev*. 2009; 4:14. [PubMed: 19344499]
- Voronina VA, Kozhemyakina EA, O'Kernick CM, Kahn ND, Wenger SL, Linberg JV, Schneider AS, Mathers PH. Mutations in the human RAX homeobox gene in a patient with anophthalmia and sclerocornea. *Hum Mol Genet*. 2004; 13:315–322. [PubMed: 14662654]
- Voronina VA, Kozlov S, Mathers PH, Lewandoski M. Conditional alleles for activation and inactivation of the mouse Rx homeobox gene. *Genesis*. 2005; 41:160–164. [PubMed: 15789424]
- Wada K, Maeda YY, Watanabe K, Oshio T, Ueda T, Takahashi G, Yokohama M, Saito J, Seki Y, Takahama S, Ishii R, Shitara H, Taya C, Yonekawa H, Kikkawa Y. A deletion in a cis element of

Foxe3 causes cataracts and microphthalmia in rct mice. *Mamm Genome*. 2011; 22:693–702. [PubMed: 22002806]

Wada K, Matsushima Y, Tada T, Hasegawa S, Obara Y, Yoshizawa Y, Takahashi G, Hiai H, Shimanuki M, Suzuki S, Saitou J, Yamamoto N, Ichikawa M, Watanabe K, Kikkawa Y. Expression of Truncated PITX3 in the Developing Lens Leads to Microphthalmia and Aphakia in Mice. *PLoS One*. 2014; 9:e111432. [PubMed: 25347445]

Wang L, He F, Bu J, Zhen Y, Liu X, Du W, Dong J, Cooney JD, Dubey SK, Shi Y, Gong B, Li J, McBride PF, Jia Y, Lu F, Soltis KA, Lin Y, Namburi P, Liang C, Sundaresan P, Paw BH, Li W, Li DY, Phillips JD, Yang Z. ABCB6 mutations cause ocular coloboma. *Am J Hum Genet*. 2012; 90:40–48. [PubMed: 22226084]

Wang P, Liang X, Yi J, Zhang Q. Novel SOX2 mutation associated with ocular coloboma in a Chinese family. *Arch Ophthalmol*. 2008; 126:709–713. [PubMed: 18474784]

Wang SW, Kim BS, Ding K, Wang H, Sun D, Johnson RL, Klein WH, Gan L. Requirement for math5 in the development of retinal ganglion cells. *Genes Dev*. 2001; 15:24–29. [PubMed: 11156601]

Wang X, Gregory-Evans CY. Nonsense suppression therapies in ocular genetic diseases. *Cell Mol Life Sci*. 2015

Williamson KA, FitzPatrick DR. The genetic architecture of microphthalmia, anophthalmia and coloboma. *Eur J Med Genet*. 2014

Williamson KA, Rainger J, Floyd JA, Ansari M, Meynert A, Aldridge KV, Rainger JK, Anderson CA, Moore AT, Hurler ME, Clarke A, van Heyningen V, Verloes A, Taylor MS, Wilkie AO, UK10K Consortium, Fitzpatrick DR. Heterozygous loss-of-function mutations in YAP1 cause both isolated and syndromic optic fissure closure defects. *Am J Hum Genet*. 2014; 94:295–302. [PubMed: 24462371]

Wyatt A, Bakrania P, Bunyan DJ, Osborne RJ, Crolla JA, Salt A, Ayuso C, Newbury-Ecob R, Abou-Rayyah Y, Collin JR, Robinson D, Ragge N. Novel heterozygous OTX2 mutations and whole gene deletions in anophthalmia, microphthalmia and coloboma. *Hum Mutat*. 2008; 29:E278–83. [PubMed: 18781617]

Wyatt AW, Osborne RJ, Stewart H, Ragge NK. Bone morphogenetic protein 7 (BMP7) mutations are associated with variable ocular, brain, ear, palate, and skeletal anomalies. *Hum Mutat*. 2010; 31:781–787. [PubMed: 20506283]

Xiao X, Li S, Zhang Q. Microphthalmia, late onset keratitis, and iris coloboma/aniridia in a family with a novel PAX6 mutation. *Ophthalmic Genet*. 2012; 33:119–121. [PubMed: 22171686]

Yahyavi M, Abouzeid H, Gawdat G, de Preux AS, Xiao T, Bardakjian T, Schneider A, Choi A, Jorgenson E, Baier H, El Sada M, Schorderet DF, Slavotinek AM. ALDH1A3 loss of function causes bilateral anophthalmia/microphthalmia and hypoplasia of the optic nerve and optic chiasm. *Hum Mol Genet*. 2013; 22:3250–3258. [PubMed: 23591992]

Yamada R, Mizutani-Koseki Y, Koseki H, Takahashi N. Requirement for Mab21l2 during development of murine retina and ventral body wall. *Dev Biol*. 2004; 274:295–307. [PubMed: 15385160]

Yan X, Sabrautzki S, Horsch M, Fuchs H, Gailus-Durner V, Beckers J, Hrabe de Angelis M, Graw J. Peroxidase is essential for eye development in the mouse. *Hum Mol Genet*. 2014; 23:5597–5614. [PubMed: 24895407]

Ye M, Berry-Wynne KM, Asai-Coakwell M, Sundaresan P, Footz T, French CR, Abitbol M, Fleisch VC, Corbett N, Allison WT, Drummond G, Walter MA, Underhill TM, Waskiewicz AJ, Lehmann OJ. Mutation of the bone morphogenetic protein GDF3 causes ocular and skeletal anomalies. *Hum Mol Genet*. 2010; 19:287–298. [PubMed: 19864492]

Yin J, Morrissey ME, Shine L, Kennedy C, Higgins DG, Kennedy BN. Genes and signaling networks regulated during zebrafish optic vesicle morphogenesis. *BMC Genomics*. 2014; 15:825–2164–15–825.

Zahrani F, Aldahmesh MA, Alshammari MJ, Al-Hazaa SA, Alkuraya FS. Mutations in c12orf57 cause a syndromic form of colobomatous microphthalmia. *Am J Hum Genet*. 2013; 92:387–391. [PubMed: 23453665]

Zhang XM, Yang XJ. Temporal and spatial effects of Sonic hedgehog signaling in chick eye morphogenesis. *Dev Biol*. 2001; 233:271–290. [PubMed: 11336495]

- Zou C, Levine EM. Vsx2 controls eye organogenesis and retinal progenitor identity via homeodomain and non-homeodomain residues required for high affinity DNA binding. *PLoS Genet.* 2012; 8:e1002924. [PubMed: 23028343]
- Zouvelou V, Luder HU, Mitsiadis TA, Graf D. Deletion of BMP7 affects the development of bones, teeth, and other ectodermal appendages of the orofacial complex. *J Exp Zool B Mol Dev Evol.* 2009; 312B:361–374. [PubMed: 19127565]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Schematic drawing of vertebrate ocular development. (A) Optic vesicle formation as an evagination from the diencephalon. (B) Following close contact with the surface ectoderm, the optic vesicle becomes divided into presumptive RPE, neural retina, and optic stalk, while the surface ectoderm is induced to form the lens placode. (C, D) The optic vesicle and lens placode co-invaginate, giving rise to the bilayered optic cup and the lens vesicle, respectively. (E) The lens differentiates to form the primary lens fibers (posterior) and the lens epithelium (anterior); the neural retina and the pigment epithelium continue to develop; the optic stalk gives rise to the optic nerve; the surface ectoderm adjacent to the lens gives rise to the corneal epithelium. Abbreviations: C: Cornea; L: lens; LP: lens placode; LV: lens vesicle; MS: mesenchyme; NR: neural retina; ON: optic nerve; OS: optic stalk; OV: optic vesicle; RPE: retinal pigment epithelium; S: sclera; SE: surface ectoderm. **Modified from Adler and Canto-Soler with permission** (Adler and Canto-Soler, 2007).

Figure 2.

Images of MAC patients with different genetic mutations. Images of patients affected with *SOX2* (A–C), *FOXE3* (D) and *BMP4* (E–G) mutations. Of note, some patients have prosthetic eyes in place. **Images A–B, E–G are reproduced with permission from** (Reis et al., 2011; Schneider et al., 2009).

Table 1

Summary of human genes associated with isolated or variable syndromic MAC.

Gene	MIM	Locus	Mode	MAC phenotype	B/U	Syndromic	Animal model ¹
Transcription factors							
<i>SOX2</i>	184429	3q26.33	AD	A, M, CM, C	B/U	Y/N	M
<i>OTX2</i>	600037	14q22.3	AD	A, M, CM, C	B/U	Y/N	M, ZF(MO)
<i>FOXE3</i>	601094	1p32	AR	M, CM	B	N	M, ZF
<i>VSX2</i>	142993	14q24.3	AR	A, M, CM	B	N/Y	M, ZF(MO)
<i>RAX</i>	601881	18q21.32	AR	A, M	B	N/Y	M, ZF, XP, MK
			AD	A, M, C	U	N/Y	
<i>PAX6</i>	607108	11p13	AD	M, C	B/U	N	M, ZF, R
			AR ²	A, M	B	Y	
<i>PTX3</i>	602669	10q25	AR/AR ²	M	B	Y/N	M, ZF, SH
<i>SALL2</i> [*]	602219	14q11.2	AR	C	B	N	M
<i>SIX6</i> [*]	606326	14q23.1	AD/AR	M	B	N	M
<i>ATOH7</i>	609875	10q21.3	AR	M	B	N	M, ZF
TGFB/BMP signaling molecules							
<i>BMP4</i>	112262	14q22.2	AD	A, M	B/U	Y	M
<i>BMP7</i>	112267	20q13.31	AD	A, C	B/U	Y/N	M
<i>GDF6</i>	601147	8q22.1	AD	A, M, CM, C	B/U	Y/N	M, XP(MO), ZF
<i>GDF3</i>	606522	12p13.31	AD	M, CM, C	B/U	Y/N	ZF(MO)
Retinoic Acid Pathway							
<i>ALDH1A3</i>	600463	15q26.3	AR	A, M, CM, C	B	N/Y	M, ZF(MO)
<i>STRA6</i>	610745	15q24.1	AR	A, CM	B	Y/N	M
<i>RARB</i>	180220	3p24.2	AD	A, M	B/U	Y	M
Genes with other or unknown function							
<i>SHH</i>	600725	7q36	AD	M, CM, C	B/U	N/Y	ZF, CH
<i>ABCB6</i>	605452	2q35	AD	C, CM	NS	N	ZF(MO)
<i>MAB21L2</i>	604357	4q31.3	AD	A, CM, C	B	Y/N	M, ZF

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Gene	MIM	Locus	Mode	MAC phenotype	B/U	Syndromic	Animal model ¹
			AR	C, CM	B	N	
<i>CL2orf57</i>	615140	12p13.31	AR	CM, C	B	Y	–
<i>TENM3*</i> (<i>ODZ3</i>)	610083	4q35.1	AR	CM	B	N	M, Z(MO)
<i>PXDN</i>	605158	2p25.3	AR	M	B	N	M
<i>YAPI</i>	606608	11q22.1-q22.2	AD	C	B	Y/N	–
<i>HMGCB3*</i>	300193	Xq28	XR	CM	B	Y	XP(MO)
<i>CRIMI*</i>	606189	2p22.3-p22.2	AD	CM	B	N	M

AD, Autosomal Dominant; AR, Autosomal Recessive; XR, X-linked Recessive; U, Unilateral; B, Bilateral; NS- not specified; A, Anophthalmia; M, Microphthalmia; CM, Colobomatous microphthalmia; C, Coloboma; Y, Syndromic cases only; N, No syndromic cases reported; Y/N, Usually syndromic but isolated cases also reported; N/Y, Usually non-syndromic, but extra-ocular features occasionally reported.

¹ Animal models displaying ocular anomalies (MAC for most, please see text) are listed: M, Mouse; ZF, Zebrafish; (MO), Morpholino only; XP, Xenopus; MK, Medaka; SH, Sheep; R, Rat; Chicken, CH.

² Autosomal-recessive inheritance of MAC due to compound heterozygous or homozygous mutations inherited from heterozygous parents affected with a less severe ocular phenotype

* Only one affected family has been reported