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Genetics of syndromic ocular coloboma: CHARGE and COACH syndromes

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Abstract

Optic fissure closure defects result in uveal coloboma, a potentially blinding condition affecting between 0.5 and 2.6 per 10,000 births that may cause up to 10% of childhood blindness. Uveal coloboma is on a phenotypic continuum with microphthalmia (small eye) and anophthalmia (primordial/no ocular tissue), the so-called MAC spectrum. This review gives a brief overview of the developmental biology behind coloboma and its clinical presentation/spectrum. Special attention will be given to two prominent, syndromic forms of coloboma, namely, CHARGE (Coloboma, Heart defect, Atresia choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies/deafness) and COACH (Cerebellar vermis hypoplasia, Oligophrenia, Ataxia, Coloboma, and Hepatic fibrosis) syndromes. Approaches employed to identify genes involved in optic fissure closure in animal models and recent advances in live imaging of zebrafish eye development are also discussed.

Introduction

The eye develops starting the third week of human gestation as an evagination of the diencephalic neuroepithelium to form the optic vesicle, which subsequently invaginates to form the optic cup. During evagination, the optic vesicle comes in close proximity to the surface ectoderm, which subsequently thickens, forming the lens placode. As the optic vesicle invaginates to give rise to a bilayered optic cup (innermost neural retina and outermost retinal pigment epithelium, RPE), the lens placode also invaginates in a coordinated morphogenetic process to form the primordial crystalline lens, the lens vesicle (Figure 1). Optic vesicle invagination, however, is asymmetric, such that a ventral opening, the optic fissure, forms around the fifth week of human gestation (O'Rahilly 1983). For the eye to develop normally, the two edges of the fissure must approximate and fuse. If the optic fissure margins fail to fuse, uveal coloboma, a potentially blinding congenital malformation, ensues.

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Clinically, coloboma presents as lens, iris, neural retina/RPE/choroid and/or optic nerve defects in the inferior (and often slightly nasal) quadrant (Figure 2A, B). The visual impact is largely determined by whether the defect affects macular development. Coloboma may occur in normal-sized eyes but it is also frequently accompanied by small eyes (microphthalmia), or even rudimentary eye tissue or clinically absent (anophthalmia) eyes (i.e., the microphthalmia anophthalmia-coloboma disease or MAC spectrum). In the broadest sense, any eye with an antero-posterior axial length two standard deviations shorter than the age-appropriate mean is microphthalmic, even if this may not be clinically obvious to inspection. Mild forms of optic fissure closure defects include peaking of the pupil towards the inferonasal quadrant and minor RPE defects seen on fundoscopy (Figure 2C, D). Variable expressivity and incomplete penetrance often exist within families where multiple affected individuals are present and the phenotype may be asymmetrically severe within an individual. Both observations suggest that genetic and/or environmental as well as stochastic processes influence the phenotype.

The term “coloboma” is often used in the nomenclature of other eye defects such as the “eyelid coloboma” of Treacher-Collins syndrome and the “macular coloboma” in severe forms of Leber congenital amaurosis; the “morning glory” anomaly is sometimes erroneously referred to as “optic nerve coloboma.” Each of these conditions is characterized by missing and/or dysplastic tissue in an ocular or adnexal structure. However, they all arise from processes quite distinct from optic fissure closure and should not be confused with uveal coloboma. For the purposes of this article, the terms “uveal coloboma” and “coloboma” can be considered synonymous.

Coloboma presents with considerable genetic heterogeneity; it is associated with many chromosomal abnormalities and is likely influenced by environmental factors that are reviewed in detail in the literature (Chang et al., 2006). Mutations in several developmentally-regulated genes encoding transcription factors, cell-cell adhesion proteins, growth factors, cytoskeletal proteins and enzymes have been reported in patients with uveal coloboma. We performed pathway analysis with human MAC associated genes using Enrichr website (https://amp.pharm.mssm.edu/Enrichr/) to identify signaling pathways that significantly contribute towards the human MAC phenotype. To test the accuracy of our analysis we checked the “Disease/Drug” tab and the top entry in the ClinVar option was “anophthalmia microphthalmia syndrome” with an adjusted *P*-value of 3.856e-24, the top entry in the “Cell Types” tab was retina (*P*=0.000003883) according to the Human Gene Atlas option (Figure 3). Top ten pathways based on the adjusted *P*-value in the KEGG 2019 option according to our analysis are show in Figure 3. Some usual suspects like TGF-β signaling, adherens junctions and Hedgehog signaling were observed, providing credence to our analysis. The most significant and rather unexpected was the Hippo signaling pathway, which is involved in the regulation of organ size (Harvey et al., 2003; Wu et al., 2003). Some recent studies have shown the association of Hippo signaling genes like *FAT1*, *RERE* and *YAP1* with human MAC phenotype (Lahrouchi et al., 2019; Fregeau et al., 2016; Williamson et al., 2014).

Genetic diversity may help explain, in part, the phenotypic complexity of the disease and suggests that multiple developmental mechanisms (e.g., cell autonomous, non-cell

autonomous) may be at work to produce a similar human disease presentation. Because developmentally- regulated genes are expressed in discrete anatomical locations at defined times, genetic diversity may also explain specific phenotypic patterns. For example, mutations in the paired box 2 (*PAX2)* transcription factor gene in Papillorenal Syndrome tend to result in colobomas most often affecting the optic nerve, as this gene is expressed prominently in the optic stalk, the precursor of the optic nerve (Sanyanusin et al., 1995; Pichaud and Desplan, 2002).

MAC may be isolated (non-syndromic) or associated with other systemic abnormalities (syndromic). Nomenclature classifications have been made in the Online Mendelian Inheritance in Man (OMIM) based, in part, on this distinction, as well as on the gene(s) associated with the phenotype (Table 1). The occurrence of coloboma with a myriad of syndromic conditions suggests that the process of optic fissure closure shares the same developmental pathways at play in the development of other organ systems. Using the example of *PAX2* in Papillorenal Syndrome again, both the kidney and the eye rely on this transcription factor during development and *PAX2* mutations result in congenital ocular and renal defects. The explanations provided by these patterns of expression, however, are not absolute. For example, genes associated with syndromic forms of coloboma may also present with isolated microphthalmia and/or coloboma and some syndromic forms of MAC have circumscribed phenotypes despite a much wider pattern of mutant gene expression.

Excellent, detailed reviews on non-syndromic coloboma have been published (Williamson and FitzPatrick, 2014; Reis and Semina, 2015) and this review only briefly discusses the genetics of non-syndromic coloboma. Most of our attention will be on the etiology and pathogenesis of syndromic coloboma, particularly in CHARGE and COACH syndromes, in which coloboma is highly prevalent.

Genetics of non-syndromic uveal coloboma: isolated microphthalmia (MCOP) and isolated microphthalmia with coloboma (MCOPCB)

Non-syndromic forms of coloboma can present in dominant, recessive, or X-linked patterns, although, most often, coloboma occurs sporadically, and the precise inheritance pattern is difficult to discern. A comprehensive list of genes associated to date with non-syndromic coloboma is presented in Table 2 and we refer the interested readers to the in-depth review of the genetics of non-syndromic coloboma by Williamson and FitzPatrick (2014). Most of the genes associated with non-syndromic coloboma tend to be eye-specific transcription factors that are involved in developmental processes.

Genetics of syndromic forms of coloboma: syndromic microphthalmia (MCOPS) loci, CHARGE, and COACH

The genes associated with syndromic forms of coloboma tend to be widely expressed and generally have pleiotropic effects. A list of all the syndromes involving coloboma is presented in Table 3. The prevalence varies among syndromes, with CHARGE and COACH being strongly associated with coloboma; coloboma, in fact, is one of their diagnostic

criteria. However, genotyped-confirmed cases of CHARGE and COACH in the absence of coloboma are also observed.

Syndromic coloboma associated with transcription factor mutations

Transcription factors known to play a key role in early eye development (e.g. SOX2, OTX2, and MITF) are strongly associated with syndromic MAC phenotypes. These are among the earliest eye field transcription factors and play multiple roles in several aspects of eye development. *SOX2* (OMIM *184429) is one of the most frequently identified genes associated with bilateral anophthalmia and severe microphthalmia. The majority of known cases of *SOX2* are *de novo* mutations (Fantes et al., 2003), but familial, autosomal dominant transmission has also been observed (Chassaing et al., 2007; Williamson and FitzPatrick, 2014). Germline mosaicism is reported in five cases where the *SOX2* mutations were maternally transmitted (Chassaing et al., 2007, Faivre et al., 2006, Schneider et al., 2008, Schneider et al., 2009, Stark et al., 2011) and germline transmission of a *SOX2* loss-of-function allele has been reported only for one family (Gerth-Kahlert et al., 2013). Mosaicism is important to look for clinically, as it potentially affects genetic counseling, changing a rare, *de novo* mutation event unlikely to recur into a recurrence risk of up to 50% with each pregnancy. Numerous systemic abnormalities including pituitary/hypothalamus dysfunction (Kelberman et al., 2006), intellectual disability (Zenteno et al., 2005), esophageal atresia (Williamson et al., 2006; Zenteno et al., 2006), microcephaly (Faivre et al., 2006) and (possibly) dental abnormalities (Numakura et al., 2010) have been reported.

Mutations in *OTX2* (OMIM *600037) were first reported by Ragge et al. (2005a) in a cohort of patients with microphthalmia/clinical anophthalmia. Additional ocular (retinal degeneration, microcornea, cataract, optic nerve hypoplasia, coloboma) and non-ocular (developmental delay, structural brain abnormalities, hypotonia, seizures, pituitary dysfunction) abnormalities were also noted in this report and largely confirmed in subsequent studies (Dateki et al, 2008; Tajima et al., 2009; Chassaing et al., 2012). Mutations can appear *de novo* or are transmitted in an autosomal dominant fashion. Unlike *SOX2*, there is no evident transmission bias between paternal vs. maternal alleles. Non-penetrance and variable expressivity have been reported for non-synonymous or clear loss-of-function alleles. Two confirmed and one suspected case of gonadal mosaicism have been reported concomitant with *OTX2* mutation (Ragge et al., 2005a, Wyatt et al., 2008).

Autosomal dominant mutations in *PAX2* (OMIM *167409) cause Papillorenal Syndrome and are strongly associated with optic nerve coloboma/congenital excavation of the optic nerve and renal dysfunction (Sanyanusin et al., 1995). Microphthalmia is not a common presentation with the Papillorenal Syndrome. *De novo* mutations and familial cases are approximately equally reported. Non-penetrance and highly variable expressivity can be present within the same family as well as maternal and paternal germline mosaicism (Amiel et al., 2000; Cheong et al., 2007).

We recently reported two cases of *MITF* (OMIM *156845) compound heterozygosity (George et al., 2016) resulting in colobomatous microphthalmia, macrocephaly, severe albinism, sensorineural deafness and osteopetrosis (COMMAD syndrome). Both probands with a strong phenotypic overlap were born of non-consanguineous unions and parents were

diagnosed with classic Waardenburg syndrome, type 2a (WS2A) due to heterozygous *MITF* mutations. Each parent in both families had a unique variant described in the literature to be present in all the known *MITF* isoforms. In both probands, there was complete lack of melanin pigment in the hair, skin, and eyes, severe colobomatous microphthalmia, profound congenital sensorineural hearing loss, and osteopetrosis.

Syndromic coloboma associated with secreted growth factors

Genes of the transforming growth factor beta (TGFβ) superfamily signaling pathway play important roles in many aspects of eye development. For example, bone morphogenetic protein (BMP) ligands are involved in the formation of the retina, lens, iris, and ciliary body (for review see Wang et al., 2014). Furthermore, growth differentiation factors (GDFs) are involved in determining the dorso-ventral symmetry of the optic cup (Yang, 2004). Dominant mutations in genes encoding growth factors from these two families are known to cause coloboma. Heterozygous mutations in *BMP4* (OMIM*112262, Reis et al., 2011) and *BMP7* (OMIM*112267, Wyatt et al., 2010) are associated with bilateral anophthalmia, microphthalmia and/or coloboma. MAC phenotypes ranging from anophthalmia (bilateral or unilateral) to uveal coloboma are associated with incompletely penetrant autosomal dominant *GDF3* and *GDF6* mutations (Asai-Coakwell et al, 2007; Asai-Coakwell et al., 2009; Ye et al., 2010).

Colobomatous microphthalmia in CHARGE syndrome due to CHD7 mutation

CHARGE syndrome (OMIM 214800) is a rare genetic condition arising during early fetal development that affects multiple organ systems. Diagnosis is classically clinically based, relying on the presence of major and minor criteria (Table 4) (Blake et al., 1998; Verloes, 2005; Hale et al., 2016). CHARGE syndrome has an estimated prevalence of approximately 1:10,000 births worldwide and presents with considerable clinical variability (Issekutz et al., 2005; Sanlaville and Verloes, 2007). The term CHARGE itself is an acronym first coined in 1982 for the association of Coloboma, Heart defect, Atresia choanae, Retarded growth and development, Genital hypoplasia, and Ear anomalies/deafness (Pagon et al., 1981). Coloboma is present in 75–81% of patients; conversely, approximately 15–30% of patients diagnosed with microphthalmia/coloboma present with CHARGE syndrome (Traboulsi, 1999). Additional consistent clinical observations in CHARGE syndrome patients include semicircular canal hypoplasia, external ear abnormalities, and cranial nerve dysfunction (over 90% of the patients); choanal atresis (i.e., blockage of the posterior nasal apertures found between the nasal cavity and the throat), resulting from failure of recanalization of the nasal fossae during fetal development (38–55%); congenital heart defects (76–77%); genital hypoplasia (62–81%); cleft lip and/or palate (33–48%) and tracheoesophageal anomalies (19–29%) (Bergman et al., 2011; Zentner et al., 2010b).

In 2004, Vissers and colleagues identified an overlapping 2.3Mb *de novo* microdeletion on chromosome 8q12 by using comparative genomic hybridization (CGH) in patients with CHARGE syndrome (Vissers et al., 2004). This region includes the Chromodomain Helicase DNA-binding 7 (*CHD7*) gene, which encodes a 2997 amino acid helicase-domain-containing protein, localizing to both the nucleoplasm and nucleolus (Schnetz et al., 2009). Point mutations were further identified in *CHD7* in unrelated CHARGE patients,

establishing this gene as causative for the syndrome (Jongmans et al., 2006; Lalani et al., 2006). Although most mutations are *de novo*, autosomal dominant loss-of-function mutations or deletions have been reported in families with more than one affected member (Lalani et al., 2006). Known *CHD7* mutations are scattered throughout the gene with no clear genotype-phenotype correlation (Jongmans et al., 2006); this becomes further apparent by the differences in clinical phenotype of sib pairs with identical mutations and mode of inheritance.

In CHARGE syndrome, coloboma is commonly bilateral, and can involve choroid, retina and optic nerve (Tellier et al., 1998; Aramaki et al., 2006; Jongmans et al., 2006; Alazami et al., 2008; McMain et al., 2008); iris colobomas are described less frequently (Aramaki et al., 2006; Jongmans et al., 2006; McMain et al., 2008); and eyelid colobomas are rare. Microphthalmos, optic nerve hypoplasia, myopia, and strabismus are also reported (Aramaki et al., 2006; Jongmans et al., 2006; Lalani et al., 2006; Delahaye et al., 2007; Alazami et al., 2008; Wincent et al., 2008; Jyonouchi et al., 2009) in CHARGE syndrome patients. Here, we focus on colobomatous-microphthalmic findings in genotype-confirmed CHARGE syndrome patients.

In a large cohort of individuals with the *CHD7* mutations reported by Lalani et al., 2006, coloboma of the iris, retina, or optic disc/nerve was observed in 55 out of 62 affected individuals (89%). The study also included a pair of female monozygotic twins carrying the mutation p.(E1271X), both of whom displayed bilateral coloboma in the presence of other phenotypic variation; only one of them, however, survived. Delahaye et al., (2007) reported two familial cases of CHARGE syndrome exhibiting extreme intrafamilial variability and *CHD7* mutations. Each family was comprised of a single mildly affected parent and severely affected children (two boys of each parent). In the first family, the mother carrying the p.(S834F) mutation as well as one of the sons had unilateral (left) optic nerve coloboma whereas, hypoplastic optic nerves were observed in the other son. The father in the second family, who carried a truncating mutation p.(R157X), had no coloboma, whereas one son carrying the same mutation had unilateral retinal coloboma and the coloboma present in the deceased son was not specified. Both families included unaffected siblings without *CHD7* mutations.

Nishina et al., 2012, provided an in-depth ophthalmic evaluation from a multicenter study of 19 Japanese affected individuals who were confirmed for *CHD7* mutations and were retrospectively studied. Coloboma affected the posterior segment of 92.1% of the eyes examined. Fifteen patients presented with retinochoroidal and optic nerve coloboma bilaterally (78.9%) and three unilaterally (7.9%). Coloboma affecting the macula was observed in eight patients bilaterally (42.1%) and five patients unilaterally (13.2%). Only one patient displayed iris coloboma bilaterally and another patient presented with lens coloboma unilaterally. Microphthalmos was present bilaterally in three patients and unilaterally in two patients. The article also reports the patient's refractive errors and BCVA. The authors suggest a correlation between the location of the protein truncation and the severity of the anatomical abnormality in the eye. However, an earlier study had not found any significant genotype-phenotype correlation in a cohort of 107 European patients (Jongmans et al., 2006).

Husu et al., 2013, reported that out of 18 Danish patients with *CHD7* mutations, coloboma and microphthalmia were observed in 17 of the cases. Of 17 affected, one individual presented with unilateral and all others had bilateral coloboma. Coloboma was observed in all structures of the eye i.e., retina (73%), iris and choroid (7%), optic disc (13%). No information was available for one of the affected individuals. Four cases of unilateral and one of bilateral microphthalmia accounted for 28% of the subjects.

In a large French cohort of 92 patients carrying mutations in the *CHD7* gene (Legendre et al., 2017), 67 (73%) were observed to have coloboma. As reported for earlier studies, coloboma of the retina (80%) was more common than iris coloboma (15%).

Recently, prenatal diagnosis of CHARGE syndrome has been conducted in three studies (Busa et al., 2016; Hoch et al., 2017 and Millischer et al., 2019) in which ocular defects have been investigated prenatally. This is particularly important as it would enable better care and management of affected individuals after birth. Busa et al., (2016) screened 12 pregnancies with CHARGE syndrome diagnosis and *CHD7* mutation. At birth, coloboma was observed in 11/12 patients and two had severe microphthalmia along with coloboma. Although the authors were not able to detect ocular abnormalities during the prenatal screening by ultrasound and MRI with the help of a dysmorphologist, they argue that the microphthalmia and coloboma could have been identified by careful examination of the ocular region. Recently, Millischer et al., 2019 performed a retrospective study of 26 suspected cases of CHARGE syndrome, with 20 out of 26 patients positive for the *CHD7* mutation. The MRI features that were most consistently detected were arhinencephaly, dysplasia of the semicircular canals, agenesis and posterior fossa anomalies, whereas, ocular anomalies were observed in only four individuals out of 11 and were overlooked in five cases. Coloboma was identified as a posterior focal bulging of the eyeball, in the MRI axial plane. Ocular asymmetry suggested microphthalmia. Note that the current resolution of MRI does not allow for the imaging of the optic fissure closure defect *per se*. The literature suggests that CHD7 functions by controlling gene expression programs through ATP-dependent chromatin remodeling (Bajpai et al., 2010; Schnetz et al., 2009) and rearrangement of nucleosomes on the DNA (Jiang and Pugh 2009). Zentner et al. have suggested that CHD7 may also play a role in the nucleolus, where it promotes ribosomal RNA biogenesis (Zentner et al, 2010a). Experiments with human pluripotent stem cells have suggested a role for *CHD7* in specification of neural crest fate (Bajpai et al. 2010). Chai et al., (2018) reported that *CHD7* is essential in maintaining neuro-epithelium identity and CNS lineage development by indirectly suppressing the induction of neural crest fates. Furthermore, important roles for *CHD7* in neurogenesis and oligodendrocyte maturation and myelination (He et al. 2016) have been identified using the mouse as a model. In the developing mouse eye, *CHD7* is expressed in the neural ectoderm and surface ectoderm but not in the peri-ocular mesenchymal tissue, and is required for eye morphogenesis, lens development and optic fissure closure (Gage et al., 2015). Finally, Bajpai demonstrated that, in *Xenopus, CHD7* is necessary for normal neural crest development and is essential for activating several components of the neural crest transcriptional circuitry (e.g., *Twist, Slug* and *Sox9*) (Bajpai et al., 2010). Taken together, these studies suggest that *CHD7* may play multiple roles in optic fissure closure and these may vary in different species, as *CHD7* expression,

for example, was not observed in the mouse peri-ocular mesenchyme, which is comprised of neural crest and mesenchymal tissue.

Coloboma in COACH syndrome due to mutations in TMEM67

Joubert syndrome (JS, OMIM#213300) is a developmental disorder characterized by brainstem malformation, cerebellar vermis hypoplasia/dysplasia, ataxia, hypotonia, mental retardation, neonatal breathing abnormalities, and oculomotor apraxia. It is caused by mutations in more than 35 genes that encode proteins involved in primary cilia and cilium basal body establishment. Additional clinical features in JS subtypes include nephronophthisis, renal cystic dysplasia, hepatic fibrosis, ocular coloboma, retinal dystrophy, and polydactyly. One of the subsets of JS, sometimes referred to as Joubert syndrome related disorders - JSRD, is COACH syndrome, caused by mutations in the transmembrane protein 67 (*TMEM67)/MKS3* gene (Verloes and Lambotte, 1989 and Smith et al., 2006). An autosomal recessive condition, COACH syndrome is characterized by Cerebellar vermis hypoplasia, Oligophrenia (developmental delay/mental retardation), Ataxia, Coloboma, and Hepatic fibrosis. Multiple transcript variants encoding different protein isoforms have been found for this gene. Chorioretinal coloboma, a partially penetrant phenotype of COACH syndrome, is also strongly associated with *TMEM67* mutations. Mutations causing COACH syndrome are spread throughout the *TMEM67* gene. Missense mutations in *TMEM67* largely cause COACH syndrome, whereas truncating mutations are more likely to cause a related condition, Meckel syndrome, type 3 (OMIM#607361) (Otto et al, 2009). No correlation exists, however, between mutation position/type and the coloboma phenotype.

Otto et al., 2009, performed mutation screening of 120 unrelated individuals with JS and identified *TMEM67* recessive mutations in five patients belonging to four independent families. Retinal coloboma was observed in three of these patients and a blind sibling pair. Brancati et al., (2009) reported eight patients carrying recessive *TMEM67*/*MKS3* mutations out of 12 affected individuals diagnosed with COACH syndrome. Of these eight, chorioretinal or optic nerve colobomas were detected in five patients (42%) and two other cases revealed abnormalities (enlarged optic cup or pale optic disc) upon fundoscopy. Subsequently, Doherty et al., (2010), showed that *TMEM67* mutations account for 9% of families in a large JSRD cohort, and that *TMEM67* mutations are observed almost exclusively in the COACH syndrome subtype of JSRD.

Iannicelli et al., (2010), performed mutation analysis of *TMEM67* in 341 probands (265 were JSRD and 76 Meckel syndrome fetuses). Among the 265 JSRD patients, *TMEM67* compound heterozygous mutations were identified in eight out of ten probands with a phenotype of JS plus liver disease and six of these eight probands had coloboma (unilateral or bilateral). No coloboma was reported in 12 of the fetuses from terminated pregnancies carrying *TMEM67* mutation out of 76 Meckel syndrome cases.

In a Northern European patient cohort (51 cases) diagnosed with JS, Kroes et al., (2016) identified five (~10%) patients with colobomatous microphthalmia carrying recessive *TMEM67* mutations. Bachmann-Gagescu et al., (2015), also reported a significant genotype-phenotype correlation between *TMEM67* mutations causing JS and coloboma. Suzuki et al.,

2016 analyzed a cohort of 27 families of Japanese descent diagnosed with JS and found *TMEM67* gene mutation in seven of them, however none displayed coloboma. Kang et al. (2016) identified four Korean patients with recessive mutations in *TMEM67* out of seven nephronophthisis patients with hepatic fibrosis. All four of these patients had JS and congenital hepatic fibrosis compatible with COACH diagnosis (Gentile et al., 1996).

Recently, Brooks et al., (2018) reported a comprehensive ophthalmic evaluation of JS patients and observed high prevalence of coloboma in those patients carrying mutations in *TMEM67* gene. Out of 22 patients genotypically confirmed for *TMEM67* mutation, retinal coloboma was observed in 13, retina and optic nerve coloboma in four, and optic nerve coloboma alone in one, for a total of 18 coloboma affected individuals. Conversely, coloboma was observed only rarely in JSRD due to mutations in other genes.

The *TMEM67* gene product MECKELIN, is predicted to be a 7-transmembrane receptor-like protein and contains an extracellular cysteine-rich region (Smith et al., 2006). MECKELIN (also referred to as MKS or MKS3) is localized to the transition zone of the cilium in protein complexes with other JS and NePHronoPhthisis (NPHP) proteins. The protein complexes localized at ciliary transition zone can be categorized into three main protein families- MKS, NPHP and CEP290 group. These protein complexes extensively collaborate for the proper assembly and functioning of the transition zone and are further composed of multiple interacting proteins including – MKS, B9D1, B9D2, TCTN1, TCTN2, TCTN3, CC2D2A, TMEM17, TMEM67, TMEM107, TMEM216, TMEM231, TMEM237, NPHP1, NPHP4, CEP290, NPHP5, RPGRIP1L, RPGRIP1, RPGR, LCA5, AHI1, CEP162, TMEM138, JBTS17 and TMEM80 (Gonçalves and Pelletier 2017). MECKELIN has also been detected at the plasma membrane in cell lines and primary cells. Silencing of *TMEM67/MKS3* and of the related *MKS1* gene was shown to regulate centrosome number and cilia length in IMCD-3 cell cultures (Dawe et al., 2007). The role of *TMEM67* in eye development has not yet been studied and more importantly, the functional relationship between primary cilium defects and optic fissure fusion defects remains unknown.

Molecular and cellular studies of optic fissure closure

Identification of genes involved in optic fissure closure using animal models

One of the first efforts to identify genes involved in optic fissure closure in an unbiased fashion was reported by Brown et al., 2009. The authors performed gene expression microarray analysis of mouse tissue samples micro-dissected from the fusing margins of the optic fissure corresponding to before (embryonic day (E)10.5), during (E11.5) and after (E12.5) optic fissure fusion. Data analysis identified 250 probe sets that represent 168 annotated genes and 54 predicted genes. Of the 168 annotated candidate genes, 83 have been experimentally mutated in mouse or zebrafish models, out of which, five exhibit coloboma, 22 have other eye phenotypes and 21 have no reported eye phenotype. Association with eye or eye development for 35 of the mutated genes could not be determined from published reports. A high percentage of annotated genes from the screen were confirmed to be expressed during eye development. Of the 78/168 genes for which *in situ* hybridization (ISH) studies exist at relevant time points, 70 are expressed in the eye, four are not expressed in the eye, and expression in the eye is undetermined for the remaining four.

Similar efforts have been reported in mouse (Cao et al., 2018), chick (Hardy et al., 2019) and zebrafish (Richardson et al., 2019) to identify genes involved in optic fissure closure. Cao et al., (2018), used laser-assisted microdissection to collect optic cup tissue samples from E11.5 mouse embryos from central-nasal, central-temporal retina and ventral optic fissure, to provide a comparative gene expression profile of the three different regions. Microarray data analysis highlighted 36 probe sets corresponding to 32 genes that showed significantly different expression levels between nasal and temporal retina and include many known genes (e.g., *Foxg1*, *Foxd1*, *Hmx1*, *Efna5*, *Epha5*, and *Tenm3*), validating the robustness of the technique. These data also revealed several new nasal-temporal differentially expressed genes, including those encoding cell adhesion molecules (*Cdh9*, *Cdh11* and *Sema5a*), transcription factors (*Sall1*, *Zfhx4*, *Onecut2*, *Tfec*, and *Glis3*), and a predicted noncoding RNA (3110039M20Rik). The authors further performed differential gene expression analysis comparing the optic fissure transcription profile with nasal and temporal retinal gene expression to identify optic fissure- specific genes. Using this strategy, novel and already known optic fissure signature genes were identified, including *Vax1*, *Vax2*, *Vax2os*, *Ntn1*, *Smoc1*, *Aldh1a3*, *Cyp1b1*, *Ptchd1*, *Zfp503*, *Laminins*, *Bmpr1b*, *Bmp7* and *Tenm3*. A number of genes that had not been reported previously to be expressed in the optic fissure were confirmed by ISH and 11 were conclusively established as new optic fissure-specific genes: *Afap1l2*, *Adamts16*, *Bmf*, *Slitrk1*, *Cp*, *Ror2*, *Tfec*, *Cplx3*, *Neto1*, *Shtn1*, and *Flrt2*.

In 2019, Hardy et al., reported on a similar developmental profiling strategy applied to the chick eye, albeit using a distinct and complementary approach to laser capture microdissection (Hardy et al, 2019). The authors manually microdissected tissue in/around the optic fissure margins, the ventral eye, the dorsal eye and the eye as a whole from Hamburger-Hamilton stage (HH.St) 25–26 (pre-fusion), HH.St27–28 (initiation of fusion) and HH.St28–30 (during active optic fissure fusion) embryos. After RNA sequencing (RNA-Seq) profiling and analysis, they compiled a list of genes enriched in the optic fissure; these include both known coloboma genes (e.g., *PAX2, VAX1*) and genes not well established to be important in optic fissure closure. Among the latter, the gene with the highest expression at all stages is *Netrin-1* (*NTN1*), which is also differentially regulated during mouse optic fissure closure (Brown et al, 2009). NTN1 belongs to a family of laminin-related secreted proteins important for axonal guidance. Knockout of *Ntn1/ntn1a* function in mouse and zebrafish produces optic fissure closure defects— an additional proof that optic fissure gene profiling strategies can identify genes of potential clinical relevance for coloboma.

Lastly, Richardson et al., (2019) performed RNA-Seq of dorsal retina versus tissue around the optic fissure and identified candidate genes that are differentially expressed at developmental time points corresponding to before (32 hpf), during (48 hpf), and after (56 hpf) optic fissure closure in zebrafish. This screen also suggests an important role for *ntn1a* in optic fissure closure, that was further confirmed via morpholino-mediated depletion and ocular coloboma appearance in zebrafish morphants. Notably, *ntn1a* expression is completely downregulated at the optic fissure margins prior to fusion.

A major difference (other than the obvious species difference) between the Richardson et al., (2019) and the Hardy et al., (2019) data sets compared Brown et al., (2009) and Cao et al., (2018) is the use of RNA-Seq technology instead of microarrays. Unlike hybridization-based

approaches, RNA-Seq is not limited to detecting transcripts that correspond to known genomic sequences and is applicable to non-model organisms with genomic regions that are yet to be sequenced.

Studies of optic cup morphogenesis and optic fissure margins fusion using live zebrafish

Investigation of the morphogenetic dynamics of optic cup formation and optic fissure margins fusion was initiated as early as the 1980s (Hero 1989, 1990 and Hero et al., 1991). In seminal studies performed on the *cinnamon* mouse, Hero used transmission electron microscopy (TEM) to show how early appositional junctions are established, followed by subsequent creation of cell-cell junctions in apposing RPE cells during optic fissure margins fusion. Given the limitations of working with developing mouse embryos, the focus has shifted towards the use of developing zebrafish. Imaging optic cup morphogenesis in live zebrafish presents with many advantages over fixed tissues. In one of the earliest efforts, Kwan et al., (2012) showed how live imaging eliminates fixation artifacts, yields relatively accurate size and shape measurements, and allows concomitant tracking of many cell types. These “4-dimensional” data sets, coupled with advanced cell tracking and the use of segmentation software, have revealed several unexpected findings: 1) although cell division contributes to the growth of the optic cup, it is largely dispensable for eye formation; 2) optic vesicle evagination persists longer than it had been previously appreciated and cells move in a “pinwheel” fashion, with retinal precursors involuting around the rim of the optic cup; and 3) cells that are adjacent early in the process and, presumably, subject to similar extracellular cues, can settle in disparate locations in the final optic cup structure.

Recent studies have shed light for the first time on morphogenetic events shaping the optic cup (Heermann et al., 2015 and Bryan et al., 2016), optic stalk (Gordon et al., 2018) and fusion of optic fissure margins (Bernstein et al., 2018), in real time. These studies have been instrumental in understanding optic cup morphogenesis up to the point of the optic fissure margins organizing around the fissure. Such understanding would not have been possible studying fixed tissues at different “static” time points.

Heermann et al., (2015) describe the lens-averted epithelium as a source of presumptive stem cells that flow around the distal rims of the optic cup to their destination in the ciliary marginal zone and contribute to the growing neuroretina. This epithelial flow contributes to optic cup morphogenesis and, when inhibited by BMP, ectopic neuroretina forms in the RPE domain, leading to failed fissure closure and coloboma. Using live imaging, Bryan et al., (2016) determined the important role of laminin extracellular matrix in optic cup morphogenesis. They showed that in *lama1* mutant zebrafish, optic cup morphogenesis, optic stalk constriction, invagination, and formation of a spherical lens are affected. Also, *lama1* mutants exhibit loss of epithelial polarity and altered adhesion leading to defective tissue architecture and disorganized retina. Similarly, Nicolás-Pérez et al., (2016) provided evidence for the role of contractile actomyosin network dynamics mediated by *lamc1* and extracellular matrix in shaping the optic cup. They elegantly demonstrated that optic cup morphogenesis requires clustered myosin accumulation inside basal neuroepithelial cells and attachment of these cells to an underlying extracellular matrix. In addition, Sidhaye and Norden (2017) have shown that active migration of connected epithelial cells into the retinal

neuroepithelium, driven by cell-matrix contacts, together with basal shrinkage of retinal neuroepithelium are crucial for optic cup formation. Finally, Gordon et al., (2018) for the first time employed a combination of four-dimensional imaging, cell tracking, and molecular genetics in zebrafish to understand the morphogenesis of the optic stalk in relation to the optic fissure. Using the *ptch2* mutant in which the Hedgehog (Hh) signaling is overactive, they show how cell motility required for optic fissure and stalk formation is impaired via non-cell-autonomous and cell-autonomous mechanisms.

More recent studies are focused on understanding the actual fusion process of the optic fissure margins. Although *Drosophila* dorsal lip/pore closure has been the system of choice for studying epithelial sheet fusion, investigations of optic fissure margin fusion have been gaining traction in vertebrate models. Bernstein et al., (2018) suggest that optic fissure closure is accomplished by breaking down of the basement membrane along the fissure margins, and by subsequently establishing basement membrane continuity along the dorsal and ventral surfaces of the fissure. Fissure closure is finally accomplished by cell protrusions and movements of partially polarized retinal cells into the fissure space to initiate the fusion and intercalation of various tissues into the fissure space.

Conclusion and future directions

One of the most important aspects of understanding the genetics of human optic fissure closure defects is to disentangle the primary drivers of fusion per se and other, more "secondary" causes such as morphogenetic defects and non-cell autonomous effects from surrounding tissues. Tools such as *in vivo* imaging (especially if this were to extend into a mammalian system), tissue-specific genetic manipulations, and complementary *in vitro* cell culture models will likely be crucial in disentangling these potential mechanisms. We anticipate that the knowledge gained by studying optic cup formation and optic fissure closure will have important implications for human disease and will likely be generalizable to other developmental processes involving epithelial sheet fusion.

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Highlights

- Uveal coloboma, a rare potentially blinding condition affecting between 0.5 and 2.6 per 10,000 births.
- Isolated and syndromic cases of uveal coloboma.
- Current knowledge on the genetics of human uveal coloboma with a specific focus on CHARGE and COACH syndromes.
- Approaches to identify genes involved in optic fissure closure using animal models.
- Live imaging of zebrafish eye development to understand optic fissure closure.

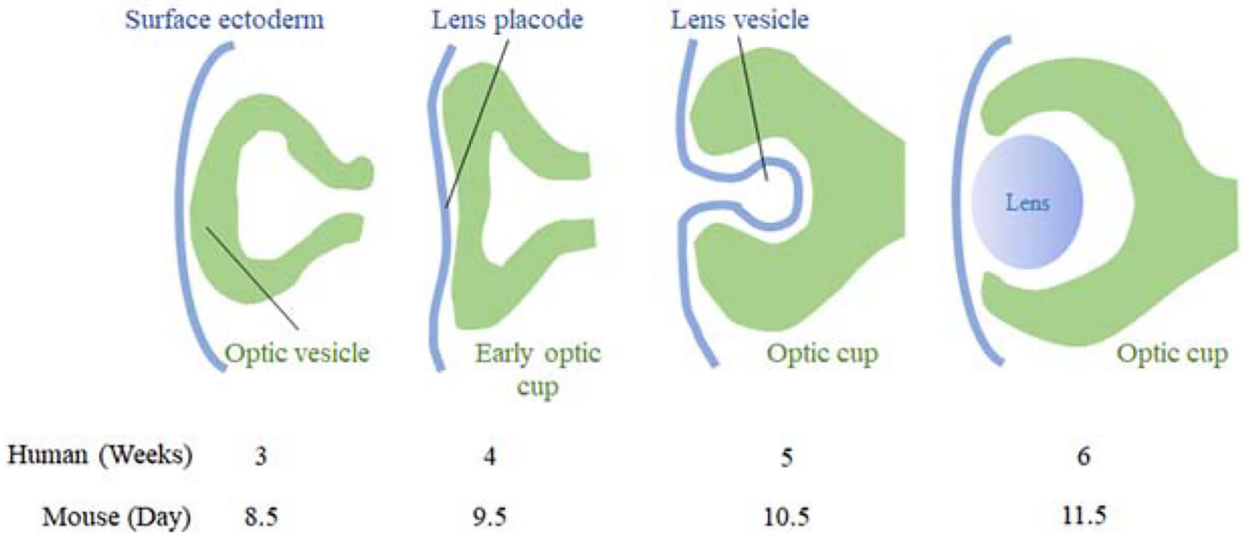


Figure 1.
Schematic of embryonic eye development.

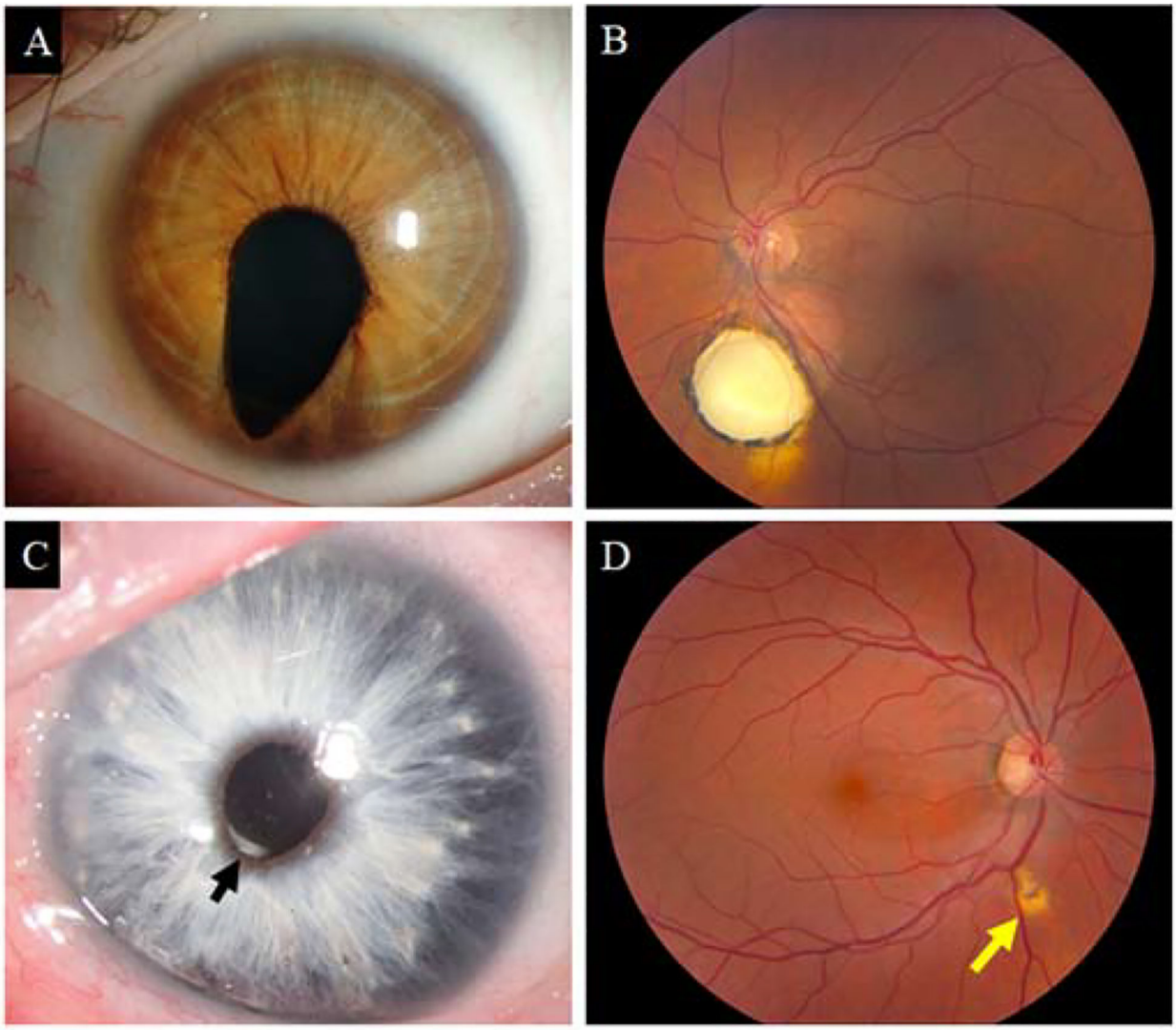
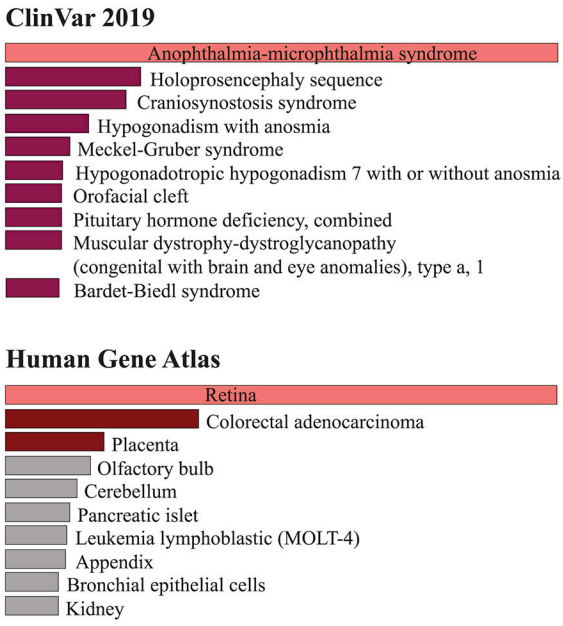


Figure 2.

Clinical presentations of uveal coloboma. (A) Typical iris coloboma of a left eye. Note the inferonasal positioning of the coloboma, corresponding to the position of the optic fissure. (B) Typical chorioretinal coloboma inferior to the optic nerve in a patient with excellent visual acuity. (C) Microform of iris coloboma in a patient with Waardenburg syndrome, type 2A. Note slight peaking of the pupil of the inferonasal quadrant (arrow). (D) Microform of a chorioretinal coloboma in the asymptomatic mother of a patient with bilateral nonsyndromic coloboma.



Name	Genes involved	Adjusted p-value
Hippo signaling pathway	SOX2, YAP1, BMP4, FZD5, GDF6, BMP7, ACTB, ACTG1	0.0001
Proteoglycans in cancer	SHH, FZD5, PTCH1, FLNA, PTPN11, ACTB, ACTG1, FGFR1	0.0004
Signaling pathways regulating pluripotency of stem cells	SOX2, BMP4, FZD5, PAX6, FGFR2, FGFR1	0.0037
TGF-beta signaling pathway	BMP4, CREBBP, PITX2, GDF6, BMP7	0.0039
Basal cell carcinoma	BMP4, SHH, FZD5, PTCH1,	0.0110
Adherens junctions	CREBBP1, ACTB, ACTG1, FGFR1	0.0153
Pathways in cancer	BMP4, SHH, CREBBP, FZD5, PTCH1, RARB, MITF, FGFR2, FGFR1	0.0256
Axon guidance	SHH, PTCH1, SEMA3E, PTPN11, BMP7	0.0441
Hedgehog signaling pathway	SHH, PTCH1, LRP2	0.0461
Leukocyte transendothelial migration	CLDN19, PTPN11, ACTB, ACTG1	0.0486

Figure 3.

Pathway analysis was performed using online tool Enrichr (https://amp.pharm.mssm.edu/Enrichr/enrich#) where ninety genes described Table 1 and 2 were uploaded to the site. The genes were assigned to the most relevant disease phenotype by the ClinVar and Cell Types by Human Gene Atlas options. KEGG 2019 Human option was used to identify the significant pathways that were arranged in the order of decreasing *P* value as shown in the table.

Table 1:

Classification of coloboma genes based on MAC phenotype. The number preceding the gene name denotes the OMIM nomenclature# (CHX10:MCOP2).

MAC phenotype	OMIM Nomenclature	OMIM #. Gene	
Isolated microphthalmia	MCOP	1. ch 14q32, 2. CHX10 3. RAX 4. GDF6	5. MFRP 6. PRSS56 7. GDF3 8. ALDH1A3
Syndromic microphthalmia	MCOPS	1. NAA10 2. BCOR 3. SOX2 4. ANOP1 5. OTX2 6. BMP4 7. HCCS	8. 6q21 9. STRA6 10. - 11. VAX1 12. RARB 13. HMGB3 14. MAB21L2
Isolated microphthalmia with coloboma	MCOPCB	1. Chr.X 2. 15q12-q15 3. CHX10 4. -	5. SHH 6. GDF3 7. ABCB6 8. STRA6 9. TENM3

Table 2.

Genes associated with non-syndromic coloboma

Gene	MAC phenotype	OMIM	Inheritance	Syndrome/associated phenotype -*	Reference
ABCB6	Microphthalmia, Coloboma of iris, retina and choroid	605452	AD	-	Wang et al., 2012
ALDH1A3	Microphthalmia, retinal coloboma	600463	AR	-	Fares-Taie et al., 2013; Yahyavi et al., 2013
ATOH7	Microphthalmia	609875	AR	Persistent hyperplastic primary vitreous	Khan et al., 2012
CRYAA	Coloboma of iris, Cataract	123580	AD	-	Beby et al., 2007
FZD5	Microphthalmia, Coloboma of iris, retina and choroid	601723	AD	-	Liu et al., 2016
IPO13	Microphthalmia, coloboma of iris, cataract	610411	AR	-	Huang et al., 2018
LCP1	Coloboma of iris and choroid	153430	AD	-	Rainger et al., 2017
MAF	Coloboma of iris, Cataract	177075	AD	Cataract	Jamieson et al., 2002
Mir204	Coloboma of iris	610942	AD	Retinal dystrophy	Conte et al., 2015
PAX6	Coloboma of iris, retina, choroid and optic nerve Anophthalmia	607108	AD AR	Aniridia, Morning glory disc anomaly, Peter's Anomaly, Anterior segment dysgenesis, Cataract with late-onset corneal dystrophy, Foveal hypoplasia, Keratitis, Optic nerve hypoplasia.	Azuma et al., 1996; Azuma et al., 2003; Glaser et al., 1994
RARB	Microphthalmia	180220	AR, AD	diaphragmatic hernia, pulmonary hypoplasia, and cardiac defects	Srour et al., 2013
RAX	Microphthalmia, anophthalmia, coloboma of optic nerve	601881	AR	-	Voronina et al., 2004
RBP4	Microphthalmia, Coloboma of iris, choroid and retina	180250	AD AR	Retinal dystrophy, comedogenic acne syndrome	Cukras et al., 2012; Chou et al., 2015.
SALL2	Microphthalmia, Coloboma of iris, retina and choroid	602219	AR	-	Kelberman et al., 2014
SHH	Microphthalmia with Coloboma of retina, choroid, iris and retina	600725	AD	Holoprosencephaly	Schimmenti et al. 2003; Nanni et al., 1999
SIX6	Coloboma of iris, choroid and optic nerve	606326	AR	Optic disc anomalies, macular atrophy and reduces retinal ganglion cell differentiation	Aldahmesh et al., 2013; Yariz et al., 2015
STRA6	Microphthalmia, anophthalmia, coloboma	610745	AR	-	Casey et al., 2011
TENM3/ ODZ3	Microphthalmia, iris coloboma	610083	AR	-	Aldahmesh et al., 2012
VSX2	Microphthalmia, anophthalmia, iris coloboma	142993	AR	cataracts	Kohn et al., 1988; Bar-Yosef et al., 2004

*No associated phenotype reported with this gene.

AD: Autosomal dominant, AR: Autosomal recessive.

Table 3.

Human syndromes with eye coloboma

Gene	MAC phenotype	OMIM	Inheritance	Syndrome/associated phenotype	Reference
ACTB	Coloboma of iris and retina	102630	AD	Baraitser-Winter syndrome 1, Dystonia, juvenile-onset	Riviere et al., 2012
ACTG1	Coloboma of iris and choroid	102560	AD	Baraitser-Winter syndrome 2, Deafness	Riviere et al., 2012
ALG3	Coloboma of iris	608750	AR	Congenital disorder of glycosylation, type IV (CDGS type IV)	Korner et al., 1999
ALX1	Microphthalmia, coloboma of iris and eye lid	601527	AR	Frontofacionasal dysostosis	Uz et al., 2010
BCOR	Microphthalmia, Coloboma of iris, choroid and optic nerve	300485	XL Recessive	Lenz syndrome	Ng et al., 2004
BMP4	Microphthalmia, anophthalmia, coloboma	112262	AD	Orofacial cleft facial dysmorphism	Bakrania et al., 2008; Reis et al., 2011
BMP7	Microphthalmia, anophthalmia, Coloboma of retina, choroid and optic nerve	112267	AD	Developmental delay, deafness, scoliosis, and cleft palate	Wyatt et al., 2010
C12orf57	Coloboma of iris, retina and choroid	615140	AR	Temtamy syndrome	Temtamy et al., 1996
CHD7	Coloboma of iris, retina, choroid and optic nerve, eye lid (rarely)	608892	AD	CHARGE syndrome	Vissers et al., 2004
CLDN19	Pseudo coloboma?	610036	AR	Hypomagnesemia, renal, with ocular involvement	Khan et al., 2018
CREBBP	Microphthalmia, coloboma of iris, choroid and retina	600140	AD/del	Rubinstein-T aybi syndrome	Ge et al., 1995
CRIM1	Coloboma of iris, retina, choroid and optic nerve	606189	AD		Toker et al., 2003
DPYD	Microphthalmia, coloboma of iris and choroid	612779	AR	Dihydropyrimidine dehydrogenase deficiency	Van Gennip et al., 1994; Meinsma et al. 1995
FAT1	Microphthalmia, coloboma of choroid and retina	600976	AR	Glomerulonephropathy, cutaneous syndactyly	Lahrouchi et al., 2019
FBN1	Coloboma of lens; rarely iris, retina and optic disk coloboma	134797	AR	Marfan syndrome	Nemet et al., 2006
FBN2	Coloboma of retina and choroid	612570	AD	Congenitalcontractural arachnodactyly	Bard 1979
FGFR1	Microphthalmia, anophthalmia, coloboma of iris and eye lid	136350	Somatic Mosaicism	Oculocerebrocutaneous syndrome Encephalocraniocutaneo us lipomatosis	Prontera et al., 2009
FGFR2	Coloboma of iris	176943	AD	Multiple	Graul-Neumann et al., 2017
FLNA	Coloboma of iris, retina and optic nerve	300017	XL Dominant XL Recessive	Multiple	Robertson et al., 2003
FOXA2	Choroidal coloboma	600288	*de novo*	hypopituitarism, hyperinsulinism and endoderm-derived organ abnormalities	Giri et al., 2017

Gene	MAC phenotype	OMIM	Inheritance	Syndrome/associated phenotype	Reference
FOXE3	Microphthalmia, Coloboma of iris, retina and optic disc	601094	AD, AR	Anterior segment dysgenesis, Cataract, aniridia	Khan et al., 2016
FREM1	Anophthalmia, microphthalmia and coloboma of upper eyelid	608944	AR	MOTA syndrome	Marles et al., 1992
GDF3	-	606522	AD	Klippel-Feil Syndrome3, skeletal anomalies	Ye et al., 2010
GDF6	Microphthalmia, Coloboma of iris, retina, choroid and optic nerve	601147	AD AR	Klippel-Feil Syndrome1 Leber congenital amaurosis	Asai-Coakwell et al., 2009
GJA8	Microphthalmia,	600897	AD	Congenital cataracts	Ceroni et al., 2019
HMGB3	Coloboma of iris, retina and choroid	300193	XL	microcephaly, short stature, and intellectual disability	Scott et al., 2014
HMX1	Coloboma of iris, retina and choroid	142992	AR	Oculoauricular syndrome	Schorderet et al., 2008
IGBP1	Coloboma of iris and optic nerve	300139	XL Recessive	Corpus callosum defect, mental retardation, and micrognathia	Graham et al., 2003
KCTD1	Coloboma of iris and eye lid	613420	AD	Scalp-ear-nipple syndrome (Finlay-Marks syndrome)	Sobreira et al., 2006
KMT2D	Coloboma of iris, retina, choroid and optic nerve	602113	AD	Kabuki syndrome	Ming et al., 2003
LINC00237	Coloboma of retina and eye lid	614992	AD	MOMO syndrome	Moretti-Ferreira et al., 1993
LRP2	Coloboma of iris	600073	AR	Donnai-Barrow syndrome	Avunduk et al., 2000
MAB21L2	Anophthalmia, microphthalmia, Coloboma of iris and retina	604357	AD AR	skeletal dysplasia	Rainger et al., 2014
MITF	Microphthalmia, coloboma	156845	AR	COMMAD syndrome	George et al., 2016
MKS1	Microphthalmia, coloboma of iris	609883	AR	Meckel-Gruber syndrome	Slaats et al., 2016
MSX2	Coloboma of iris, retina and choroid	123101	*de novo* duplication	Craniosynostosis 2, Parietal foramina 1	Plaisancie et al., 2015
OTX2	Microphthalmia	600037	AD	Retinal dystrophy, early-onset, with or without pituitary dysfunction	Ragge et al., 2005a
PACS1	Coloboma of iris and optic nerve	615009	*de novo* (c.607C>T)	Schuurs-Hoeijmakers syndrome	Pefkianaki et al., 2018
PAX2	Coloboma of optic nerve	167409	AD	Papillorenal Syndrome	Sanyanusin et al., 1995
PDE6D	Coloboma of optic nerve	602676	AR	Joubert syndrome 22	Thomas et al., 2014
PIGL	Coloboma of retina and choroid	605947	AR	CHIME syndrome	Ng et al., 2012
PITX2	Microphthalmia, coloboma of iris	601542	AD	Rieger syndrome, type1	Ozeki et al., 1999
POMT1	Microphthalmia, coloboma	607423	AR	W alker-W arburg syndrome	Beltran-Valero de Bernabe et al., 2002
PQBP1	Coloboma of retina, choroid and optic disc	300463	XL Recessive	Renpenning syndrome	Martinez-Garay et al., 2007
PRR12	Coloboma of iris.	616633	*de novo* (LOF)		Leduc et al., 2018
PTCH1	Coloboma of iris	601309	AD/Sporadic	Holoprosencephaly, Basal cell nevus syndrome	Chassaing et al., 2016

Gene	MAC phenotype	OMIM	Inheritance	Syndrome/associated phenotype	Reference
PTPN11	Coloboma of iris, retina and optic nerve	176876	AD	Noonan syndrome	Lee et al., 1992
PUF60	Coloboma of iris, retina and choroid	604819	*de novo*	Verheij syndrome	Graziano et al., 2017
RAB3GAP1	Microphthalmia, anophthalmia, coloboma of iris, choroid, retina, optic nerve	602536	AR	Warburg micro syndrome 1	Aligianis et al., 2005
RERE	Coloboma	605226	*de novo*	Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart	Fregeau et al., 2016
SALL1	Coloboma of retina and choroid	602218	AD	Townes-Brocks syndrome	Botzenhart et al., 2005
SALL4	Microphthalmia, coloboma of iris, choroid and optic nerve	607343	AD	Duane-radial ray syndrome (Okihiro syndrome; acroreno-ocular syndrome)	Borozdin et al., 2004
SCLT1/ TBC1D32	Retinochoroideal lacunae of colobomatous	611399	XL	Orofaciodigital syndrome IX	Gurrieri et al., 1992
SEMA3E	Coloboma of iris, retina and optic nerve	608166	AD	CHARGE syndrome	Lalani et al., 2004
SIX3	Microphthalmia, coloboma of iris, retina, choroid and macula	603714	AD	Holoprosencephaly2, Schizencephaly	Wallis et al., 1999
SMOC1	Microphthalmia, Coloboma of retina and limb anomalies	608488	AR	limb anomalies	Abouzeid et al., 2011; Okada et al., 2011
SMO	Microphthalmia, coloboma of iris	601500	AD	Curry-Jones syndrome	Twigg et al., 2016
SOX2	Anophthalmia, microphthalmia, coloboma of iris, retina and choroid	184429	AD	Optic nerve hypoplasia and abnormalities of the central nervous system	Fantes et al., 2003; Ragge et al., 2005b
SOX3	Microphthalmia, Coloboma	313430	XL (germline mosaicism)	Panhypopituitarism Mental retardation	Jelsig et al., 2018
SPINT2	Coloboma of optic nerve	605124	AR	Congenital sodium diarrhea	Hirabayashi et al., 2018
SRD5A3	Coloboma of iris	611715	AR	Congenital disorder of glycosylation, Kahrizi Syndrome	Cantagrel et al., 2010
TCOF1	Coloboma of iris, choroid, optic nerve and eye lid	606847	AD		Treacher Collins Syndrome Collaborative Group (1996)
TFAP2A	Microphthalmia, coloboma of iris, choroid and optic nerve	107580	AD	Branchio oculofacial syndrome	Milunsky et al., 2008
TGDS	Coloboma of iris	616146	XL recessive	Catel-Manzke syndrome	Ehmke et al., 2014
TMEM67	Coloboma of iris, retina and choroid	609884	AR	COACH syndrome	Verloes et al., 1989
VAX1	Microphthalmia	604294	AR	small optic nerves, orofacial clefting, agenesis of corpus callosum	Slavotinek et al., 2012
WDR11	Coloboma of iris.	606417	AD	Hypogonadotropic hypogonadism with or without anosmia	Kim et al., 2010
WASHC5	Coloboma of iris and retina	610657	AR	Ritscher-Schinzel syndrome (3C syndrome)	Leonardi et al., 2001

Gene	MAC phenotype	OMIM	Inheritance	Syndrome/associated phenotype	Reference
YAP1	Coloboma of iris, retina and choroid	606608	AD	hearing impairment, cleft lip/ palate, and/or mental retardation	Williamson et al., 2014
ZEB2	Coloboma of iris and retina	605802	AD	Mowat-Wilson syndrome	Wakamatsu et al., 2001

AD: Autosomal dominant, AR: Autosomal recessive, XL: X-linked, LOF: Loss of function.

Table 4:

Diagnosis criteria for CHARGE syndrome

Major criteria	Minor criteria
Coloboma	Cranial nerves dysfunction including hearing loss
Choanal atresia and/or cleft lip or palate	Dysphagia (feeding difficulties)
Abnormal external, middle or inner ears, including hypoplastic semicircular canals	Structural brain anomalies
Pathogenic *CHD7* variant	Developmental delay/intellectual disabilities/autism
	Hypothalamo-hypophyseal dysfunction (gonadotropin or growth hormone deficiency) and genital anomalies
	Heart or esophagus malformation
	Renal anomalies
	Skeletal/limb anomalies