Ocular coloboma: a reassessment in the age of molecular neuroscience

C Y Gregory-Evans, M J Williams, S Halford, K Gregory-Evans

Congenital colobomata of the eye are important causes of childhood visual impairment and blindness. Ocular coloboma can be seen in isolation and in an impressive number of multisystem syndromes, where the eye phenotype is often seen in association with severe neurological or craniofacial anomalies or other systemic developmental defects. Several studies have shown that, in addition to inheritance, environmental influences may be causative factors. Through work to identify genes underlying inherited coloboma, significant inroads are being made into understanding the molecular events controlling closure of the optic fissure. In general, severity of disease can be linked to the temporal expression of the gene, but this is modified by factors such as tissue specificity of gene expression and genetic redundancy.

NORMAL EYE DEVELOPMENT

The processes that occur during formation of the vertebrate eye are well documented and include (i) multiple inductive and morphogenetic events, (ii) proliferation and differentiation of cells into mature tissue, and (iii) establishment of neural networks connecting the retina to the higher neural centres such as the superior colliculus, the geniculate nucleus, and the occipital lobes. At around day 30 of gestation, the ventral surface of the optic vesicle and stalk invaginates leading to the formation of a double-layered optic cup. This invagination gives rise to the optic fissure, allowing blood vessels from the vascular mesoderm to enter the developing eye. Fusion of the edges of this fissure starts centrally at about 5 weeks and proceeds anteriorly towards the rim of the optic cup and stalk invaginates leading to the formation of a double-layered optic cup.

CLINICAL FEATURES

The typical, most frequently observed, ocular coloboma is seen in the inferonasal quadrant (fig 1A). Colobomata in other quadrants are atypical and the embryologic basis for these is unclear. Ocular colobomata are frequently seen in association with other developmental defects. In the eye, coloboma is often associated with microphthalmos and anophthalmia. Systemically, a large number of congenital defects are associated with coloboma (table 1), including craniofacial anomalies such as cleft lip, skeletal defects such as thumb hypoplasia, and genitourinary anomalies such as horseshoe kidney.

An interesting sub-classification has been proposed, based on corneal diameter and axial length. Colobomata are subdivided into those with cysts, those with microphthalmos (small axial length), those with microcornea and normal axial length, and those with coloboma only. Such a classification can aid in determining visual prognosis, but does not take into account the effects on vision of chorioretinal and optic nerve colobomata, which can occur in the absence of cysts, microphthalmos, or microcornea. Since different types of

Abbreviations:
BMP, bone morphogenetic protein; CGN, Coloboma Gene Network; HPE, holoprosencephaly; MIA, multiple incomplete ascertainment; RA, retinoic acid; RPE, retinal pigment epithelium; SIA, single incomplete ascertainment; VAD, vitamin A deficiency
coloboma can occur in individual families it is also unlikely that this classification identifies aetiologic subtypes.

Iris coloboma
A complete iris coloboma involves the pigment epithelium and stroma giving rise to the so-called “keyhole” pupil (fig 1B), which can be unilateral or bilateral.\(^{13}\) A partial coloboma involves only the pupillary margin making the pupil oval. Occasionally, the coloboma only affects the iris pigment epithelium and can be seen only on transillumination.\(^{14}\) Although isolated iris coloboma is observed, it is often associated with coloboma in other parts of the eye. Occasionally surgical repair is indicated either for cosmetic reasons or for photophobia.\(^{15–17}\)

Chorioretinal coloboma
Colobomata affecting the posterior segment of the eye can be unilateral or bilateral. If the fetal fissure fails to close posteriorly, then a coloboma affecting the retinal pigment epithelium (RPE), neurosensory retina, or choroid may occur. The defect is essentially a bare sclera with the overlying RPE, retina, or choroid.\(^{18}\) Typically occurring in the inferonasal quadrant, it may extend to include the optic nerve (fig IC). Macular colobomas, which is not due to defects in optic fissure closure, should not be confused with chorioretinal coloboma. Usually, chorioretinal colobomas are asymptomatic despite significant upper visual field defects. It has been proposed that 8.1–43% of cases can be complicated by retinal detachment\(^{19–21}\) and surgical correction has variable success.\(^{22–23}\) Rarely, chorioretinal coloboma give rise to subretinal neovascularisation,\(^{24}\) especially if involving the optic nerve head.\(^{25–27}\)

### Optic nerve
The severity of optic disc involvement varies from no involvement to an obviously enlarged optic cup and optic disc (fig 1D), which can be associated with congenital forebrain anomaly. Optic nerve atrophy correlates with the severity of this anomaly. Two special cases are optic nerve pits\(^{29}\) (which can be associated with central serous retinopathy) and the morning glory disk anomaly\(^{30}\) (which can be associated with congenital forebrain anomalies). It is as yet uncertain whether these are types of coloboma, in the sense that they derive from failure of the optic fissure to close.

### AETIOLOGY
Coloboma and genetic abnormality
An extraordinary number of conditions are associated with coloboma (74 entries in OMIM; see www.ncbi.nlm.nih.gov.\(^{31}\)}

---

**Table 1** Human diseases with eye coloboma and known genetic locus

<table>
<thead>
<tr>
<th>OMIM number</th>
<th>Disease</th>
<th>Type of coloboma</th>
<th>Inheritance</th>
<th>Chromosome location</th>
<th>Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>274270</td>
<td>DPF deficiency</td>
<td>I, C, M, A, R</td>
<td>AR</td>
<td>1p22</td>
<td>DPF</td>
<td>Van Gennip et al(^{11})</td>
</tr>
<tr>
<td>157170</td>
<td>Holoprosencephaly 2</td>
<td>I, M</td>
<td>AD</td>
<td>2p21</td>
<td>SIAX</td>
<td>Wallis et al(^{12})</td>
</tr>
<tr>
<td>235730</td>
<td>Hirschsprung syndrome</td>
<td>I</td>
<td>AD</td>
<td>2q22</td>
<td>ZFHX18</td>
<td>Gregory-Evans et al(^{13}), Hurst et al(^{14})</td>
</tr>
<tr>
<td>601110</td>
<td>CGDS</td>
<td>I, A</td>
<td>AR</td>
<td>3q27</td>
<td>ALG3</td>
<td>Krimer et al(^{15})</td>
</tr>
<tr>
<td>180500</td>
<td>Reiger syndrome type 1</td>
<td>I, M</td>
<td>AD</td>
<td>4p25</td>
<td>PITX2</td>
<td>Ozeki et al(^{16})</td>
</tr>
<tr>
<td>121050</td>
<td>CCA syndrome</td>
<td>R, C</td>
<td>AD</td>
<td>5q23–q31</td>
<td>FRN2</td>
<td>Bard et al(^{17})</td>
</tr>
<tr>
<td>154500</td>
<td>Treacher Collins</td>
<td>I, O, L</td>
<td>AD</td>
<td>5q32–q33.1</td>
<td>TCOF1</td>
<td>Collaborative Group(^{18})</td>
</tr>
<tr>
<td>6000725</td>
<td>Uveo-retinal coloboma</td>
<td>I, R, C, M</td>
<td>AD</td>
<td>7q36</td>
<td>SHH</td>
<td>Schimmiten et al(^{19})</td>
</tr>
<tr>
<td>109400</td>
<td>Basal cell nevus/Gorlin</td>
<td>I</td>
<td>AD/sporadic</td>
<td>9q22.3</td>
<td>PTCH</td>
<td>Hahn et al(^{20})</td>
</tr>
<tr>
<td>236670</td>
<td>Walker-Warburg</td>
<td>O, M</td>
<td>AR</td>
<td>9q34.1</td>
<td>POMT1</td>
<td>Beltran-Valero de Bernabe et al(^{21})</td>
</tr>
<tr>
<td>213300</td>
<td>Jaubert syndrome I</td>
<td>R, C, A</td>
<td>AR</td>
<td>9q34.3</td>
<td>Saar et al(^{22})</td>
<td></td>
</tr>
<tr>
<td>180250</td>
<td>RBP deficiency</td>
<td>I</td>
<td>AD</td>
<td>10q24</td>
<td>RBP4</td>
<td>Seelinger et al(^{23})</td>
</tr>
<tr>
<td>120330</td>
<td>Renal-coloboma</td>
<td>R, C, M, A</td>
<td>AD</td>
<td>10q24.3–25q1.1</td>
<td>PAX6</td>
<td>Eccles and Schimmiten(^{24})</td>
</tr>
<tr>
<td>120200</td>
<td>Ocular coloboma</td>
<td>R, C, O</td>
<td>AD</td>
<td>11p13</td>
<td>PAX6</td>
<td>Azuma et al(^{25})</td>
</tr>
<tr>
<td>608091</td>
<td>Jaubert syndrome II</td>
<td>R, C, O</td>
<td>AR</td>
<td>11p12–q13.3</td>
<td>Keeler et al(^{26})</td>
<td></td>
</tr>
<tr>
<td>163950</td>
<td>Noonan syndrome</td>
<td>R, C</td>
<td>AD</td>
<td>12q24.1–q24.3</td>
<td>PTPN11</td>
<td>Carvalho et al(^{27})</td>
</tr>
<tr>
<td>251600</td>
<td>Microphthalmia</td>
<td>R, C, M</td>
<td>AD</td>
<td>14q24.3–14q31</td>
<td>CHX10</td>
<td>Peric et al(^{28})</td>
</tr>
<tr>
<td>600165</td>
<td>Microphthalmia</td>
<td>I, R, M</td>
<td>AD</td>
<td>15q12–15q15</td>
<td>Morrison et al(^{29})</td>
<td></td>
</tr>
<tr>
<td>180849</td>
<td>Rubinstein-Taybi</td>
<td>I, O</td>
<td>AD/DEL</td>
<td>16p13.3</td>
<td>CREBBP</td>
<td>Guillon-Almeida and Richieri-Costa(^{30})</td>
</tr>
<tr>
<td>177075</td>
<td>Cataract/microcornea</td>
<td>I</td>
<td>AD/TRANS</td>
<td>16q22–q23</td>
<td>MAF</td>
<td>Jamieson et al(^{31})</td>
</tr>
<tr>
<td>249000</td>
<td>Meckel-Gruber</td>
<td>I</td>
<td>AR</td>
<td>17q22–q23</td>
<td>Mackae et al(^{32})</td>
<td></td>
</tr>
<tr>
<td>166750</td>
<td>Oroculo-oral-dental</td>
<td>I, R</td>
<td>AD</td>
<td>20q13.1</td>
<td>Vierra et al(^{33})</td>
<td></td>
</tr>
<tr>
<td>305600</td>
<td>Goltz syndrome</td>
<td>I, C, O</td>
<td>X-linked rec</td>
<td>Xp21.33.1</td>
<td>Gorski(^{34})</td>
<td></td>
</tr>
<tr>
<td>304050</td>
<td>Aicardi syndrome</td>
<td>O, I</td>
<td>X-linked rec</td>
<td>Xp22</td>
<td>Roper et al(^{35})</td>
<td></td>
</tr>
<tr>
<td>300472</td>
<td>Corpus collosum defect</td>
<td>I, O</td>
<td>X-linked rec</td>
<td>Xq13.1</td>
<td>IGGBP1</td>
<td>Graham et al(^{36})</td>
</tr>
<tr>
<td>300510</td>
<td>Lens syndrome</td>
<td>I, C, M</td>
<td>X-linked rec</td>
<td>Xq27–q28</td>
<td>BORC</td>
<td>Nil et al(^{37})</td>
</tr>
<tr>
<td>304120</td>
<td>Oto-palato-digital type2</td>
<td>I</td>
<td>X-linked rec</td>
<td>Xq28</td>
<td>FINA</td>
<td>Robertson et al(^{38}), Stratton et al(^{39})</td>
</tr>
</tbody>
</table>

| Chromosomal aberrations associated with retinal coloboma | | | | | | |
|-------------------------------------------------|---|---|---|---|---|
| 120200  | Iris coloboma | I | – | 2p25–pier del | Arias et al\(^{40}\) |
| 243310  | Iris coloboma, ptosis, MR | I | – | 2p12–q14 inv | Pollotta\(^{41}\) |
| 218650  | Craniosynostosis | R, C, O | – | 2q42–q43 del | Nixon et al\(^{42}\) |
| 194190  | Wolf-Hirschhorn | I | – | 4p16.3 del | Zallino et al\(^{43}\) |
| 180500  | 4q26 deletion syndrome | I | – | 4q23–q27 del | Mepeti et al\(^{44}\) |
| 147791  | Jacobsen syndrome | R, C | – | 7q34–ter del | Tayss et al\(^{45}\) |
| 214800  | CHARGE association | I, C, O, A | – | 8q21.1 del | CHD7 | Vissers et al\(^{46}\) |
| 115470  | Cat eye syndrome | I, C, O, L | – | 22q11 inv del | Mccaggart et al\(^{47}\) |
| 192430  | VCFS/Di George | I, R, C, M | – | 22q11 inv del | Morrison et al\(^{48}\) |
| 300337  | Hypomelanosis of Ito | I | Mosaicism | Xp11.2 trans | Barhaoalve et al\(^{49}\) |

A, anophthalmia; AD, autosomal dominant; AR, autosomal recessive; C, choroid; del, deletions; dup, duplication; I, iris; inv, inversion; L, lid; M, microphthalmia; MR, mental retardation; O, optic nerve; R, retina; trans, translocation; VCFS, velo-cardiofacial syndrome. Table entries in bold type are eye-specific diseases without systemic defects.
approximately 86% of patients have uveal or iris coloboma. Examples include CHARGE association where multisystem malformation syndromes are associated with most likely due to non-genetic factors. Many non-Mendelian, Environmental causes of coloboma are mostly associated with systemic disease.

Inheritance has yet to be established (table 2). The coloboma inheritance, 14 are autosomal recessive, three are thought to be caused by chromosomal aberrations at 22q11: cat eye syndrome, velocardiofacial syndrome, and DiGeorge syndrome. This suggests there is a gene or genes important for optic fissure closure at this location. In phenotypes where there is no evidence to date, 13 show autosomal dominant inheritance, 14 are autosomal recessive, three are thought to be X-linked, and in seven phenotypes the mode of inheritance has yet to be established (table 2). The coloboma phenotype is therefore genetically heterogeneous and is mostly associated with systemic disease.

**Environmental causes of coloboma**

A large proportion of sporadic, unilateral, coloboma cases are most likely due to non-genetic factors. Many non-Mendelian, multisystem malformation syndromes are associated with colobomata. Examples include CHARGE association where approximately 86% of patients have uveal or iris coloboma and nevus sebaceous of Jadassohn where some patients have iris and choroidal colobomata. The underlying mechanisms are not known for such syndromes, which constitute a significant proportion of coloboma cases.

There are many reports in the literature suggesting environmental associations with coloboma, but without appropriate case-controlled epidemiologic studies the data remain somewhat speculative. There are only a few clear associations which are described below, with appropriate caveats.

A number of studies in humans have led to the suggestion that the use of various drugs during pregnancy may be associated with ocular coloboma. For example, there seems reasonable evidence to support an effect of thalidomide and alcohol as reproducible studies have been documented. Children of expectant mothers treated with thalidomide manifested a number of eye malformations including coloboma (4%) and microphthalmos (7%). Up to 90% of children whose mothers have misused alcohol in pregnancy show ocular manifestations. A small proportion had isolated coloboma, but frequently these patients have microphthalmos, such that now this is now regarded as a specific sign of fetal alcohol syndrome. However, other associations with maternal use of drugs such as LSD and carbamazepine are less convincing, as they are case reports and have not been replicated in other patient populations. Although numerous animal studies have been performed documenting the teratogenic effects of drugs, these studies are sometimes at doses much larger than used in humans and may not be relevant to human disease aetiology.

Several studies have suggested that maternal vitamin A deficiency (VAD) may be a cause of ocular coloboma in Asia. Most recently, a study showed that 16% of pregnant women from South India who gave birth to a child affected with coloboma, had suffered night-blindness that reverted after birth. Evidence in support of a role for VAD is that 50% of pregnant women in parts of South India were found to have mild-to-moderate VAD. However, due to the high frequency of consanguineous marriage in India, it has also been hypothesised that perhaps there is a genetic predisposition to the effects of VAD, leading to a higher prevalence of coloboma. In more westernised countries, however, it seems unlikely that dietary VAD would occur.

Other incidences of ocular coloboma in humans have been reported in association with maternal infections caused by cytomegalovirus; three cases) and toxoplasmosis (six reports). However, further studies are required before these associations are considered bona fide. In animal studies vitamin E deficiency, ionising radiation, and hyperthermia have also been associated with coloboma. These associations at present require further rigorous study as there is no evidence that they cause an effect in humans.

**MOLECULAR BASIS OF COLOBOMA**

Whilst currently the molecular and cellular processes underlying optic fissure closure are poorly understood, this is changing rapidly with a great deal of genetic information being generated from family studies. An impressive number of very useful animal models have been described with an ocular coloboma phenotype (table 3). In the mouse, for example, nine genes have been identified, of which two are orthologous to human coloboma-associated disease genes (Pat1 and Pat6). Thus it is important to consider both human and mouse data in trying to dissect the molecular basis of coloboma.

Recent studies have demonstrated that the earliest developmental processes are controlled by a complex network of transcriptional factors, cell cycle regulators, and diffusible signalling molecules. These act in concert to form different ocular compartments, regulate cell proliferation, migration, and apoptosis, and specify cell identities. Mutations in some of these proteins or the genes they regulate leads to ocular coloboma. Evaluation of such genes associated with ocular coloboma phenotype (table 3). In the mouse, for example, nine genes have been identified, of which two are orthologous to human coloboma-associated disease genes (Pat1 and Pat6). Thus it is important to consider both human and mouse data in trying to dissect the molecular basis of coloboma.

Figure 1 Clinical photographs of human ocular colobomata. (A) Typical iris coloboma; (B) iris coloboma with cataract; (C) small choroidal coloboma below optic nerve head; (D) large chorioretinal coloboma affecting retina and optic nerve head.
SHH/Shh regulated genes and coloboma

Sonic hedgehog is a secreted protein that regulates embryonic morphogenesis through an intracellular signalling network. It is expressed in the floorplate of the neural tube and when disrupted in mouse leads to cyclopia and neural tube defects. The Shh^-/- mouse is therefore lethal due to severe neurological maldevelopment. Interestingly, the Shh^-/- mouse is indistinguishable from wild-type, yet in humans SHH heterozygous mutations lead to holoprosencephaly (HPE3). The HPE3 eye phenotype ranges from cyclopia, anophthalmia, and microphthalmia to coloboma. Intriguingly though, a 12 bp deletion in SHH has been shown to
cause isolated colobomatous microphthalmia affecting the iris, retina, and choroid without holoprosencephaly, highlighting the genotype-phenotype specificity.57

Sonic hedgehog regulates a number of genes which have also been directly associated with ocular coloboma (fig 2). Heterozygous mutations in PAX2, for instance, cause renal-coloboma syndrome, resulting in coloboma of the uveal tract and in some cases microphthalmia.58 In homozygous Pax2 null mutant mice, the optic fissure fails to close, resulting in bilateral coloboma at birth.59 Pax2 is expressed early in the ventral half of the optic vesicle and in the lips of the closing optic fissure, extending ventrally to the optic stalk. Interestingly, it has been suggested that in the Pax2 mutant mice there is no contact-dependent dissolution of the basal lamina of the neuroepithelium at the fissure edges, and therefore closure is inhibited. Thus, these studies suggested that Pax2 has a direct role in optic fissure closure.

Shh also regulates two closely-related homeobox genes, Vax1 and Vax2. In Vax1−/− mutants there is ocular coloboma, optic nerve agenesis, and abnormal projections of the retinal ganglion cells to the brain consistent with the normal expression of Vax1 in the optic stalk.60 In these mice, Vax1 was also shown to negatively regulate both Pax6 and Rx gene expression, but had no effect on Pax2 expression. Vax2 knockout mice exhibit coloboma, consistent with the exclusive expression pattern of Vax2 in the ventral part of the developing eye.61 This is similar to Pax2 mutants where the basal lamina persists preventing optic fissure closure. Neither Pax2 nor Tbx5 expression patterns were altered by the absence of Vax2, suggesting that the coloboma was a direct consequence of Vax2 inactivation. The human VAX1 or VAX2 genes are, therefore, good candidate genes for ocular coloboma, however, no mutation screens have been reported to date, perhaps because these genes have only recently been identified.

Bf-1 (Foxg1b) is a winged-helix transcription factor and is normally expressed early at the time of optic vesicle evagination and later in the optic cup and stalk. Targeted disruption of the gene in mice leads to absence of the optic stalk and an expanded retina62 in addition to brain defects. The eyes are not spherical in shape and there is a large ventral coloboma. In the absence of Bf-1 there is an increase in Pax6, a loss of Pax2, and a localised deficit of Shh expression around the base of the optic vesicle. To date, no mutation screens have been reported in human eye disease.

The signalling molecule retinoic acid (RA), a derivative of vitamin A, has been shown to regulate eye development.63 There is evidence to suggest that in some populations dietary deficiency of vitamin A and its derivatives seems to be linked to ocular coloboma in humans64 and mammals.65 RA up-regulates the retinoid binding protein gene (RBP4) and a missense mutation in RBP4 results in iris coloboma and retinal dystrophy in a sib-pair.66 Patched-1 (Pch) and Shh expression are negatively regulated by RA.67 Absence of RA results in coloboma68 and mutation of human PTCH leads to iris and lid colobomata in association with multiple basal cell carcinomas, craniofacial defects, and skeletal abnormalities.69 Furthermore, in Xenopus eye development RA has been shown to upregulate Vax2, again implicated in coloboma.65 These studies would support a role for RA in the signalling pathway controlling closure of the optic fissure.

The c-Jun NH2-terminal kinase (Jnk) subfamily of protein kinases are stimulated by cellular stress and pro-inflammatory cytokines. Targeted disruption of either Jnk1 or Jnk2 has no effect on the eye, and it has been assumed that each can compensate for the other. When these knockout mice have been backcrossed to each other, however, a number of interesting effects are seen. Mice which lacked both Jnk1 and Jnk2 (Jnk2−/−Jnk1−/−) died during gestation with neural tube and brain defects.70 Mice which lacked Jnk2, but had a single allele of Jnk1 (Jnk2−/−Jnk1+/−) had no developmental phenotype, whereas the absence of Jnk1 and the presence of only one copy of Jnk2 (Jnk1−/−Jnk2+/−) resulted in retinal coloboma, small lenses, and other developmental defects.71

**Figure 2.** Coloboma gene network (CGN) model. Mutations in genes or signalling molecules that are associated with coloboma are depicted in bold black text. Mutations in genes that are associated anophthalmia/microphthalmia are in normal text. Mutations in genes that are associated with other eye defects are shaded grey. Genes in bold grey text have not been associated with developmental eye defects, but are directly involved in regulation/interaction of downstream coloboma target genes. SHH and PAX6 are boxed as they regulate many genes associated with coloboma. Human genes are in uppercase, and mouse genes are in lowercase.
Gene expression and complementation studies in the Jnk1/2 embryos revealed the signalling pathway of Jnk1/2→Bmp4→Shh→Pax2→coloboma, the first definitive coloboma pathway to be dissected.

**PAX6/Pax6 regulated genes and coloboma**

The PAX6 gene, expressed in the developing central nervous system including the eye, has been shown to be vital to eye development and to be influential at the earliest stages of ocular morphogenesis (master control gene). It was first identified as the candidate gene for aniridia, however, numerous mutations in the gene have been causally associated with an impressive range of ocular phenotypes, all detailed in the Human PAX6 Allelic Variant Database (http://pax6.hgu.mrc.ac.uk/Tables/tables.htm). Of particular interest here, rare missense mutations in PAX6 have been shown to cause optic nerve and chorioretinal coloboma in man and mouse, whereas the more severe aniridia phenotype is commonly associated with nonsense/frameshift mutations, highlighting a genotype-phenotype correlation for PAX6.

There are a number of genes downstream of PAX6 that have also been directly associated with eye coloboma. Mutation of the CHX10 gene for example, leads to iris and chorioretinal colobomata with microphthalmia/anophthalmia. Although the whole eye is affected by loss of Chx10 function, the primary genetic defect is specific to the retina. How this is related to failure of the optic fissure to close is not yet known.

Mutation of the MAF1 gene leads to cataract, microcornea, microphthalmia, and bilateral iris coloboma. The gene is expressed during lens differentiation and regulates crystallin gene expression. However, MAF1 may play a bigger role in anterior segment formation since an iris coloboma has been associated with mutant MAF1 in one study. Cell culture studies have implicated Pax6 in the regulation of Maf and Sox2 cooperatively regulate the expression of delta-crystallin during chick lens development. Whether Maf and Sox2 cooperatively regulate optic fissure closure has not been examined to date.

Mutation of the SIX3 gene causes holoprosencephaly (HPE2; single central incisor and microcephaly, with or without associated brain malformations) with associated ocular defects such as cyclopia, iris coloboma, microphthalmia, or hypertelorism. In the mouse, Six3 is first expressed in the optic vesicles and stalks at E9.5, and then later is limited to the retina and lens. Studies of Six3 knockout mice show abnormal forebrain development and complete absence of eyes, indicating its central role in eye development. In zebrafish retina Six3 is directly regulated by Pax6; however, Six3 can also up-regulate Pax6 during eye field specification early in development. The ability of Pax6 and Six3 to induce each other’s expression is consistent with their overlapping expression patterns in the developing eye. These data suggest that mutation of SIX3 has a role in coloboma formation.

**Anophthalmia/microphthalmia genes**

A number of genes (Rx, Bmp7, Bmp4, Nog, Sox2), which have been associated with anophthalmia/microphthalmia, interact with or regulate some of the genes associated with a coloboma phenotype and have been included in the CGN network (Fig 2). Pax6 is directly regulated by Shh, Rx, and Bmp7 during different aspects of murine eye morphogenesis such as optic stalk and vesicle formation. Temporal expression studies in Xenopus have suggested that there is a specific network of transcription factors during eye field development. During early eye specification in Xenopus, ET induces sequentially Rx, Pax6, and then Six, and ET itself is strongly repressed by Nog. These data in vivo support the recent finding that mutations in human RX/RAX cause anophthalmia, without systemic defects.

During eye development Bmp7 is expressed in the neuroepithelium of the optic vesicle at day E11.5 and is limited to the presumptive neural retina and developing lens placode. From E12.5 to E13.5, there is expression in the neural retina, lens, and developing cornea. Bmp4 is expressed in the optic vesicle and in the trabecular meshwork and optic nerve head cells of mature tissue. Targeted deletion of the mouse Bmp7 gene results in anophthalmia (also kidney and skeletal defects), whereas heterozygous Bmp4 mice exhibit microphthalmia (also kidney, skeletal, and craniofacial defects). These data suggest that the home morphogenetic protein (BMP) genes have a critical role in eye development. No mutations in the human BMP4 or BMP7 genes have yet been reported in association with anophthalmia/microphthalmia. However, this may be due to redundancy because there are overlapping regions of expression in the developing eye of Bmp4 and Bmp7.

A number of studies show that Nog is able to repress the transcription of Bmp7 and Bmp4. Over-expression of Nog in chick embryos at optic vesicle stages of development results in microphthalmia with concomitant disruption of the developing neural retina, RPE, and lens. At optic cup stages, however, Nog overexpression caused colobomata and ectopic expression of optic stalk markers in the region of the ventral retina and RPE. Transgenic over-expression of Nog in mice prevents the eyelids from opening. These antagonist effects of Nog prevent the appropriate expression of BMPs downstream, and thus have a coloboma/microphthalmia effect similar to targeted deletion of BMPs themselves. In humans six missense mutations in NOG cause proximal symphalangism without eye defects, consistent with the absence of eye defects in the Noggin null mouse.

Mutations have yet been described which have a gain of function that would be predicted to have a microphthalmia/coloboma phenotype.

Another role for Bmp7 is in up-regulation of SMAD1. SMAD1 interacts with ZFHX1B, a zinc finger transcription factor that is expressed in craniofacial mesenchyme and migrating neural crest cells. Simultaneous injection of Zf-Hx1b and a Bmp7 dominant-negative construct into Xenopus embryos resulted in embryos lacking eyes. Bmp7 induces sequentially Rx, Pax6, and then Six, and ET itself is strongly repressed by Nog. These data in vivo support the role of Bmp7 in eye development.

**GENETIC COUNSELLING**

An extensive review of genetic counselling in coloboma cases is beyond the remit of this review, however, a guide for managing familial cases, isolated coloboma, or cases with systemic features is described below. If a familial form of coloboma or a specific syndrome of which coloboma is a part is identified, then counselling follows a conventional method.
based on the applicable Mendelian inheritance (autosomal dominant, recessive, or X-linked). More commonly, and more difficult, are simplex cases where a coloboma patient has no family history.

If a patient has an isolated coloboma then consideration of reported recurrence risks is useful; however, these studies are limited. A study in Scotland, over a 16 year period, reported sibling recurrence risks of 8.1% (single incomplete ascertainment, SIA) and 13.3% (multiple incomplete ascertainment, MIA).109 When bilateral cases were analysed separately, the risk to siblings seemed to be higher (33%) than with unilateral cases. However, when the parents of these simplex cases were considered, many cases were more critically examined. In many cases of occult (often retinal) coloboma were seen, suggesting dominant inheritance. Where both parents were found to be normal, the bilateral recurrence risk dropped to 2.9% (SIA) and 4.3% (MIA). For unilateral coloboma probands no cases of occult disease in parents were seen and the recurrence risk was estimated to be 4.9% (SIA) and 7.9% (MIA). Surprisingly, this suggests that in cases where parents are definitely unaffected, the risk to other siblings is slightly greater in unilateral than bilateral cases. This emphasises the need to thoroughly examine parents prior to counselling, especially in bilateral cases, to ascertain if there could be a dominant pattern of inheritance.

In a French study, over a 15 year period, congenital eye malformations were considered as a whole group (including microphthalmia, anophthalmia, cataract, and coloboma) and the recurrence risk for first degree relatives of probands was estimated to be 8.9%. However, in 54% of the cases, there were systemic malformations and so the reported recurrence risk is not specific to isolated coloboma, but does highlight the frequent association of coloboma with other phenotypes. The Scottish study also reported that many coloboma cases with systemic features (31 of 40) could not be assigned to a specific syndrome, making assessment of risk difficult. In fact, 11 of the 12 reported cases of coloboma with chromosomal aberrations (table 1) have been in cases associated with multiple systemic defects. Therefore, karyotyping might be of particular value in the genetic counselling of this subgroup, but is unlikely to be of value in isolated coloboma. Another factor relevant here is that clinicians need to be aware that ocular coloboma can be the presenting feature of a great number of systemic developmental disorders, and they should therefore investigate these cases accordingly.

Further refinement in genetic counselling will be based on new information on genes causing coloboma; the potential use of genes in diagnosis and screening is an emerging factor in clinical management. When specific syndromes such as renal-coloboma syndrome are considered, the PAX2 gene should be screened. Similarly, when holoprosencephaly is seen with coloboma, the SIX3 and SHH genes could be screened. For isolated coloboma cases, human and mouse studies suggest that a gene screen could include PAX6, MAF1, VAX1, VAX2, and SHH. For isolated microphthalmia good candidates are CHX10, RX, SOX2, BMP4, BMP7, MAF1, and NOG. Since coloboma and microphthalmia are sometimes seen together, these are not mutually exclusive lists. Candidate gene screens are currently limited, however, because probably most coloboma genes are still not known and gene screening for genetic eye diseases is currently very limited in most countries. The most effective genetic screening is still in those families where a causative mutation has already been established.

Another important principle in this group of patients is that incomplete penetrance and variable expressivity in autosomal dominant cases seems to be the rule rather than the exception.110 Clinical variability may be explained by modifier genes, an influence of the allele in trans, sex, mosaicism, or environmental factors. For example, evidence suggests that disease penetrance can be increased by coinheritance of a specific gene defect with a low-expressed wild-type allele.111 Also data from mouse studies indicate that non-penetrance or a difference in severity for the coloboma phenotype depends on the mouse genetic background.112 Although rapid progress has been made in understanding the basis of incomplete penetrance and the differences in expressivity, they still remain unknown for most genetic disorders. Therefore, patients should be counselled assuming there is full penetrance of the gene defect, unless a specific modifying mechanism has been identified.

CONCLUSIONS

A significant body of information is now emerging on the molecular mechanisms involved in the pathogenesis of ocular coloboma. Although many elements are still missing the skeleton for a classification can now be constructed based on molecular pathogenesis. Coloboma can be classified as a disease of increasing severity, for example as (i) being isolated, (ii) being associated with other ocular anomaly (for example microphthalmos), and (iii) being associated with other CNS anomaly and with systemic manifestations outside the CNS. The first two subsections would incorporate the classification of Hornby and co-workers’ where visual prognosis is linked to severity of ocular malformation and also takes into account the CNS and systemic abnormalities so commonly seen with ocular coloboma.

The key to this subclassification, however, is that it can be correlated with groups of coloboma genes, in particular with the timing of their action. Coloboma-related genes such as SHH and SIX3 which act prior to eye development (that is, before 20 days post conception) are associated with severe neurological deficits and systemic anomalies. Other coloboma genes acting later in eye development (after 20 days post conception) are usually associated with either milder CNS and systemic anomalies (for example TCOF1) or isolated coloboma (for example PAX6, MAF1, CHX10, RBP4). Other factors as well as timing of expression are also important. Site of expression is relevant. For example, SHH is ubiquitously expressed and so it is not surprising that mutation leads to multiple anomalies. Other genes, for example MAF1, are thought to be exclusively expressed in the eye and so mutation leads to an isolated eye phenotype. Genetic redundancy is also a factor in the phenotype associated with a particular gene, for example PAX6 and CHX10 are expressed elsewhere in the developing CNS but mutations are mainly associated with eye anomalies, presumably because their function can be compensated for elsewhere in the CNS. Thus, to a limited extent phenotypic characterisation (the CNS and other systemic anomalies as well as the ocular phenotype) can be helpful in identifying the underlying molecular deficit.

ELECTRONIC-DATABASE INFORMATION

REFERENCES


889

Ocular coloboma


131. Arias M, Gonzalez ME. The short arm of chromosome 2, informative for acid phosphatase (ACP1), malate dehydrogenase (MDH1), and collagen of the iris. Cyto genetic Cell Genet 1984; 37: 40–1A.


