REVIEW

Ocular coloboma: a reassessment in the age of molecular neuroscience

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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.

Congenital ocular colobomata (from the Greek *koloboma*, meaning ''mutilated'' or ''curtailed'') are caused by defects in embryogenesis. The incidence of coloboma depends upon the population studied, ranging from (per 10 000 births) 0.5 in Spain,[1] 1.4 in France,[2] and 2.6 in the USA[3] to 7.5 in China.[4] Coloboma has been reported in 0.6–1.9% of blind adults in Canada[5] and 3.2–11.2% of blind children worldwide.[6] Typically, colobomata are clefts caused by absent tissue in the inferonasal quadrant of the eye, but subtype, severity, and visual prognosis vary, depending on location and associated eye defects.

The underlying aetiology of the phenotype is the failure of the ectodermal optic vesicle fissure to close.[7] This leads to colobomata affecting one or more areas of the eye including the cornea, iris, ciliary body, lens, retina, choroid, and optic nerve. Eyelid coloboma has also been described, but this is thought to arise from failure of the mesodermal folds to fuse at about 7–8 weeks of gestation. Information on the molecular mechanisms underlying coloboma pathogenesis is beginning to emerge based upon animal studies of coloboma and Mendelian genetic disorders, chromosomal abnormalities, toxic environmental agents such as drug usage, and dietary deficiency. The aim of this review is to highlight which genes are emerging as important in coloboma formation and how environment may also influence gene expression to cause coloboma.

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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.[8–10] 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 posteriorly along the optic stalk, with completion by 7 weeks.[11] Failure of part of the fetal fissure to close results in the clinical entity recognised as coloboma. The molecular mechanisms controlling these tissue events are largely unknown.

CLINICAL FEATURES

The typical, most frequently observed, ocular coloboma is seen in the inferonasal quadrant (fig 1A).[10] 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.[12] 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.[4] 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

Table 1 Human diseases with eye coloboma and known genetic locus

OMIM number	Disease	Type of coloboma	Inheritance	Chromosome location	Gene	Reference
Genes or genetic loci linked to ocular coloboma						
274270	DPD deficiency	I, C, M	AR	1p22	*DPD*	Van Gennip *et al*[113]
157170	Holoprosencephaly 2	I, M	AD	2p21	*SIX3*	Wallis *et al*[81]
235730	Hirschsprung syndrome	I	AD	2q22	*ZFHX1B*	Gregory-Evans *et al*[102], Hurst *et al*[114]
601110	CGDS type IV	I	AR	3q27	*ALG3*	Korner *et al*[115]
180500	Reiger syndrome type 1	I, M	AD	4p25	*PITX2*	Ozkei *et al*[116]
121050	CCA syndrome	R,C	AD	5q23–q31	*FBN2*	Bard *et al*[117]
154500	Treacher Collins	I, O, L	AD	5q32–q33.1	*TCOF1*	Treacher Collins Syndrome Collaborative Group[118]
600725	**Uveo-retinal coloboma**	**I, R, C, M**	**AD**	**7q36**	***SHH***	Schimmenti *et al*[57]
109400	Basal cell nevus/Gorlin	I	AD/sporadic	9q22.3	*PTCH*	Hahn *et al*[58]
236670	Walker-Warburg	O, M	AR	9q34.1	*POMT1*	Beltran-Valero de Bernabe *et al*[119]
213300	Joubert syndrome I	R, C	AR	9q34.3		Saar *et al*[120]
180250	RBP deficiency	I	AD	10q24	*RBP4*	Seeliger *et al*[65]
120330	Renal-coloboma	I, R, O,M	AD	10q24.3–25q.1	*PAX2*	Eccles and Schimmenti[58]
120200	**Ocular coloboma**	**R, C, O**	**AD**	**11p13**	***PAX6***	Azuma *et al*[74]
608091	Joubert syndrome II	R, C, O	AR	11p12–q13.3		Keeler *et al*[121]
163950	Noonan syndrome	I, R, O	AD	12q24.1–q24.3	*PTPN11*	Carvalho *et al*[122]
251600	**Microphthalmia**	**R, C, M**	**AR**	**14q24.3**	***CHX10***	Percin *et al*[76]
600165	**Microphthalmia**	**I, R, M**	**AD**	**15q12–q15**		Morrison *et al*[109]
180849	Rubinstein-Taybi	I, O	AD/del	16p13.3	*CREBBP*	Guion-Almeida and Richieri-Costa[123]
177075	**Cataract/microcornea**	**I**	**AD/trans**	**16q22–q23**	***MAF***	Jamieson *et al*[78]
249000	Meckel-Gruber	I	AR	17q22–q23		MacRae *et al*[124]
166750	Oculo-oto-dental	I, R	AD	20q13.1		Vieira *et al*[14]
305600	Goltz syndrome	I, C, O	X-linked dom	Xp22.31		Gorski[125]
304050	Aicardi syndrome	O, I	X-linked dom	Xp22		Ropres *et al*[126]
300472	Corpus callosum defect	I, O	X-linked rec	Xq13.1	*IGBP1*	Graham *et al*[127]
309800	Lenz syndrome	I, O, C, M	X-linked rec	Xq27–q28	*BCOR*	Ng *et al*[128]
304120	Oto-palato-digital type2	I	X-linked dom	Xq28	*FLNA*	Robertson *et al*[129], Stratton *et al*[130]
Chromosomal aberrations associated with coloboma						
120200	**Iris coloboma**	**I**	**–**	**2p25–pter del**		Arias *et al*[131]
243310	Coloboma, ptosis, MR	I	–	2p12–q14 inv		Pallotta[132]
218650	Craniosynostosis	R, C, O	–	2q24–2q31 del		Nixon *et al*[133]
194190	Wolf-Hirschhorn	I	–	4p16.3 del		Zollino *et al*[134]
180500	4q26 deletion syndrome	I	–	4q23–q27 del		Motegi *et al*[135]
–	7q deletion syndrome	R, C	–	7q34–ter del		Taysi *et al*[136]
147791	Jacobsen syndrome	I, R, C	–	11q23–q25 del		Pivnick *et al*[137]
214800	CHARGE association	I, R, C, O	–	8q21.1 del	*CHD7*	Vissers *et al*[138]
–	16q syndrome	I	–	16q23–16q24.2 del		Werner *et al*[139]
115470	Cat eye syndrome	I, C, O, L	–	22q11 inv dup		McTaggart *et al*[140]
192430	VCFS/Di George	I, R, C, M	–	22q11.22 del		Morrison *et al*[141]
300337	Hypomelanosis of Ito	I	Mosaicism	Xp11.2 trans		Bartholomew *et al*[142]

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.

colobomata 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 colobomata 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 missing. In some cases although the retina is present, it is hypoplastic and gliotic.[18] Typically occurring in the inferonasal quadrant, it may extend to include the optic nerve (fig 1C). Macular coloboma, which is not due to defects in optic fissure closure, should not be confused with chorioretinal coloboma. Usually, chorioretinal colobomata 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 colobomata 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 to gross anomaly (fig 1D) unrecognisable as an optic nerve head.[28] Visual deficit attributable to optic nerve head coloboma 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.

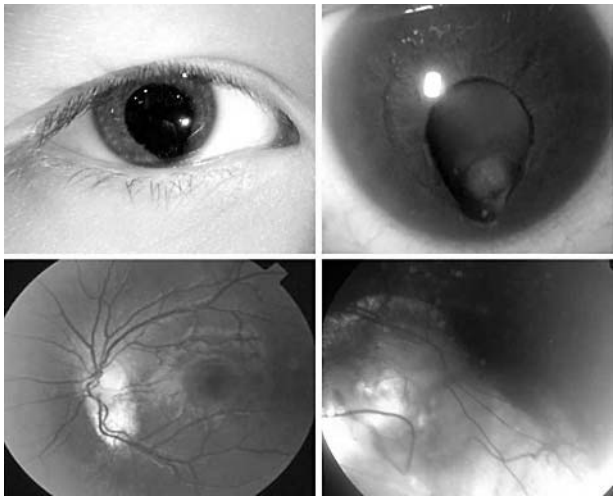

Figure 1 Clinical photographs of human ocular colobomata. (A) Typical iris coloboma; (B) iris coloboma with cataract; (C) small choroidal coloboma below optic nerve head; (D) large chorioretinal coloboma affecting retina and optic nerve head.

gov/entrez/query.fcgi?db = OMIM) either as Mendelian inherited traits or as chromosomal aberrations (tables 1 and 2). A key feature is that the majority of inherited coloboma cases, and especially those associated with chromosomal aberrations, are associated with systemic disease. To date, 27 genetic loci have been mapped to specific chromosomal regions and 21 of the genes have been identified (table 1). Eleven chromosomal aberrations have been documented and three of these overlap with known coloboma-associated genes (*SHH*, *CHX10*, *MAF*). Interestingly, three syndromes which include coloboma are due to chromosomal abnormality at 22q11: cat eye syndrome, velo-cardiofacial 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 mapping information 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[31] and nevus sebaceous of Jadassohn where some patients have iris and choroidal colobomata.[32] 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,[33–37] 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%).[38] 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.[39] However, other associations with maternal use of drugs such as LSD[40] and carbamazepine[41] are less convincing, as they are case reports and have not been replicated in other patient populations.[42] 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.[43 44] 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.[44] 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.[44] 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.[45] 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),[46 47] however, further studies are required before these associations are considered bona fide. In animal studies vitamin E deficiency,[48] ionising radiation,[49 50] and hyperthermia[51 52] 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 (*Pax2* and *Pax6*). 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.[53] 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 in both humans and mice has led us to propose a CGN (Coloboma Gene Network) model (fig 2). Similar gene expression networks have proven useful in studies of fetal development[54] and in understanding disease pathogenesis, for example in cancer.[55] There are two key genes that underpin this network, *Sonic hedgehog* (*SHH*) and *PAX6*. Rare mutations in these genes are associated with coloboma phenotypes, however, both these genes act as transcriptional regulators of many other genes that are also associated with coloboma. It should also be noted that some of the coloboma phenotypes are rare and mutation-specific, but nonetheless provide insights into coloboma formation.

Table 2 Familial coloboma without genetic localisation

OMIM number	Disease	Type of coloboma	Inheritance pattern	Reference
Autosomal dominant conditions				
184705	Steinfeld syndrome	I, R	AD	Nothen *et al*[143]
602499	**Macrophthalmia**	**I, R, O**	**AD**	Toker *et al*[144]
120433	Coloboma/cleft lip and palate/MR/deafness	I, R, C, O, M	AD	Ravine *et al*[145]
102490	Acro-reno-ocular syndrome	I, C, O	AD	Aalfs *et al*[146]
113620	Branchio-oculo-facial	I, R, O, M	AD	Richardson *et al*[147]
280000	Chime syndrome	R	AD	Shashi *et al*[148]
142500	**Heterochromia iridis**	**I**	**AD**	Morrison *et al*[149]
147920	Kabuki syndrome	I, R, C, O	AD	Ming *et al*[150]
157980	MOMO syndrome	R	AD	Moretti-Ferreira *et al*[151]
155145	Pai syndrome	I	AD	Rudnik-Schoneborn and Zerres[152]
601707	Curry-Jones syndrome	I, M	AD	Temple *et al*[153]
201350	Biemond syndrome type 2	I, R, M	AD, AR	Verloes *et al*[154]
Autosomal recessive conditions				
601706	Yemenite deaf-blind (severe)	I, C	AR	Bondurand *et al*[155]
223370	Dubowitz syndrome	I, M	AR	Tsukahara and Opitz[156]
218340	Temtamy syndrome	I, R, C	AR	Temtamy *et al*[157]
216820	**Ocular coloboma**	**R, C**	**AR**	Pagon *et al*[158]
229400	Frontofacionasal dysostosis	I, L	AR	Gollop *et al*[159]
220210	Ritscher-Schinzel syndrome	I, R	AR	Leonardi *et al*[160]
251505	**Microphthalmia**	**R, C**	**AR**	Porges *et al*[161]
222448	Donnai-Barrow syndrome	I	AR	Avunduk *et al*[162]
216360	COACH syndrome	I, C, O	AR	Verloes and Lambotte[163]
274205	Hypoplastic thumb, coloboma	C	AR	Ward *et al*[164]
601427	Anterior chamber cleavage	I	AR	Jung *et al*[165]
244300	Kapur-Toriello	I	AR	Kapur and Toriello[166]
215105	Chondrodysplasia punctata	R	AR	Toriello *et al*[167]
X-linked conditions				
258865	Oral-facial-digital type VIII	R, C	X-linked	Gurrieri *et al*[168]
302380	Catel-Manzke syndrome	I	X-linked	Wilson *et al*[169]
600122	Verloes syndrome	C	Probably X-linked	de Die-Smulders *et al*[170]
Inheritance pattern not yet determined				
234100	Hallermann-Streiff syndrome	I, C, O, M	Sporadic	Cohen[171]
163200	Nevus sebaceus of Jadassohn	I, C, L	Sporadic	Baker *et al*[82]
136760	Frontonasal dysplasia	R, C	Sporadic	Temple *et al*[172]
165630	Organoid nevus phakomatosis	R, C	Mosaicism	Neumann *et al*[173]
601359	Sebaceous nevus syndrome	I	Mosaicism	Dodge and Dobyns[174]
107550	Aortic arch anomalies	R	Twins	Levin *et al*[175]
–	**Familial iris coloboma**	**I**	**Pre-mutation**	Barros-Nunez *et al*[176]

For abbreviations see table 1.

SHH/*Shh* regulated genes and coloboma

Sonic hedgehog is a secreted protein that regulates embryonic morphogenesis through an intracellular signalling network.[56] It is expressed in the floorplate of the neural tube and when disrupted in mouse leads to cyclopia and neural tube defects.[57] 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

Table 3 Animal models with ocular coloboma

Locus or breed	Genotype	Type of coloboma	Species	Syntenic human locus	Reference
Vax2	$Vax2^{-/-}$	**I, R, C, O**	**Mouse**	**2p13.3**	Barbieri *et al*[61]
Pax2	*Pax2* 1bp ins; $Pax2^{Neo/+}$; *Krd*	I, R, C, O	Mouse	10q24.31	Torres *et al*[59], Favor *et al*[177], Keller *et al*[178]
Vax1	$Vax1^{-/-}$	I, R, C, O	Mouse	10q22.3	Hallonet *et al*[60]
Pitx2	$Pitx2^{-/-}$	O	Mouse	4q25	Gage *et al*[179]
Bf-1 (*Foxg1b*)	$Bf\text{-}1^{-/-}$	I, R, C, O	Mouse	14q12	Huh *et al*[62]
Jnk1/2	$Jnk1^{-/-}/Jnk2^{-/+}$	R, C	Mouse	10q11.2/5q35.3	Cekan *et al*[50]
Pax6	$Pax6^{lacZ/+}$	**I, CO**	**Mouse**	**11p13**	Stull and Wikler[67], Singh *et al*[180]
Jag1	$Cm^{+/-}$; $Jag1^{+/-}$	I	Mouse	20p12.2	Wilson[181], Xue *et al*[182]
AP-2	$Ap\text{-}2\alpha^{-/-}$	A, C, R	Mouse	6p24.3	West-Mays *et al*[183]
Onc1	$Onc1^{-/+}$	O	Mouse	–	Hawes *et al*[184]
CALB/Rk	Multigenic	O	Mouse	–	Hawes *et al*[184]
CEA	**Recessive**	**O, C**	**Dog**	**–**	Barnett[185]
BW	**Bmn-wys**	**O, C, M**	**Rat**	**–**	Wyse and Hollenberg[186]
Charolais	**Dominant**	**O, C, R**	**Cow**	**–**	Falco and Barnett[187]
MOC	**Complex trait**	**L, R, O**	**Cat**	**–**	Barnett and Lewis[188],Belhorn *et al*[189]
Co	X-linked	Whole eye	Chicken	–	Abbott *et al*[190]

For abbreviations see table 1.

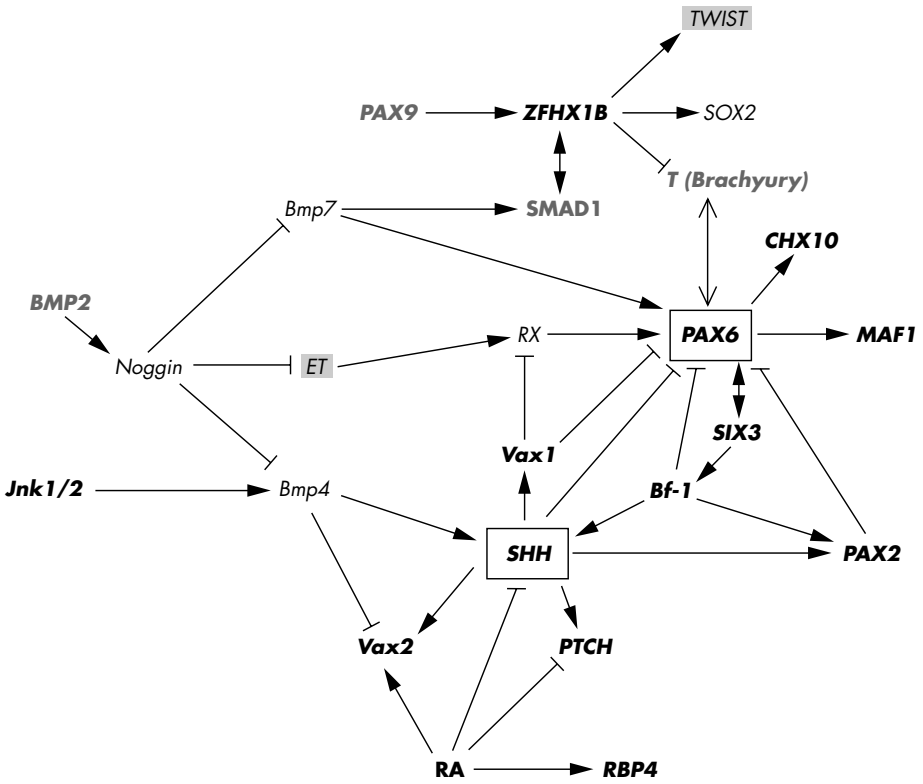

Figure 2 Coloboma gene network (CGN) model. Mutations in genes or signalling molecules that are associated with coloboma are depicted in bold black text. Mutations in genes that are associated with anophthalmia/microphthalmia are in normal text. Mutations in genes that are associated with other eye defects 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.

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 ganglions 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 retina[62] 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 both in humans[44] and mammals.[64] 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.[65] Patched-1 (*Ptch*) and *Shh* expression are negatively regulated by RA.[66] Absence of RA results in coloboma[67] and mutation of human *PTCH* leads to iris and lid colobomata in association with multiple basal cell carcinomas, craniofacial defects, and skeletal abnormalities.[68] Furthermore, in *Xenopus* eye development RA has been shown to upregulate *Vax2*, again implicated in coloboma.[69] These studies would support a role for RA in the signalling pathway controlling closure of the optic fissure.

The c-Jun NH_2-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]

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,[72] however, numerous mutations in the gene have been causally associated with an impressive range of ocular phenotypes,[73] 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[74] and mouse,[75] 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.[76] Although the whole eye is affected by loss of *Chx10* function, the primary genetic defect is specific to the retina.[77] 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.[78] 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*[79] and Maf and Sox2 cooperatively regulate the expression of delta-crystallin during chick lens development.[80] 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.[81] 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.[82] Studies of *Six3* knockout mice show abnormal forebrain development and complete absence of eyes, indicating its central role in eye development.[82] In zebrafish retina *Six3* is directly regulated by *Pax6*,[83] however, *Six3* can also up-regulate *Pax6* during eye field specification early in development.[84] The ability of *Pax6* and *Six3* to induce each other's expression is consistent with their overlapping expression patterns in the developing eye.[82 84] 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*,[85] *Rx*,[86] and *Bmp7*[87 88] 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.[84] 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.[89]

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.[90 91] *Bmp4* is expressed in the optic vesicle and in the trabecular meshwork and optic nerve head cells of mature tissue.[92] Targeted deletion of the mouse *Bmp7* gene results in anophthalmia (also kidney and skeletal defects),[93] whereas heterozygous *Bmp4* mice exhibit microphthalmia (also kidney, skeletal, and craniofacial defects).[94] These data suggest that the bone 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*.[95]

A number of studies show that *Nog* is able to repress the transcription of *Bmp7* and *Bmp4*.[90 96] 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.[97] 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.[98] No 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.[99] SMAD1 interacts with *ZFHX1B*, a zinc finger transcription factor that is expressed in craniofacial mesenchyme and migrating neural crest cells.[100] Targeted deletion of *Zfhx1b* prevents closure of the neural tube and a heterozygous mutation in the human *ZFHX1B* gene results in Hirschsprung syndrome[101] with bilateral iris and retinal colobomata.[102] In *Zfhx1b* knockout mice *Sox2* is absent and *Twist* is markedly suppressed; in man *SOX2* mutations lead to anophthalmia[103] and *TWIST* mutations lead to eyelid abnormalities in Saethre-Chotzen syndrome.[104] Unfortunately, there was no investigation of the eyes of these *Zfhx1b* null mice. However, *ZFHX1B* is expressed in the eye from 7–9 weeks of human development[105] and overexpression of the *Xenopus* gene results in defective eye development.[106] Furthermore, *Zfhx1b* also negatively regulates the mouse *T (Brachyury)* gene.[107] Overexpression of *Pax6* in zebrafish embryos results in greatly reduced eye and forebrain development, whereas overexpression of the zebrafish *T* gene has no effect on the eye, consistent with the absence of any reported disease-causing mutations of *T* in humans. However, simultaneous injection of *Pax6* and *Zf-T* resulted in embryos lacking eyes[108] suggesting that both of these genes are required during 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 with bilateral coloboma were more critically examined, 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%.[2] 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.[111 112] Also data from mouse studies indicate that non-penetrance or a difference in severity for the coloboma phenotype depends on the mouse genetic background.[59 61] 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[4] 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

The URLs mentioned in this paper are: Human PAX6 Allelic Variant Database, http://pax6.hgu.mrc.ac.uk/Tables/tables.htm; and OMIM, www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM.

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