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Mouse models of congenital cataract

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Abstract

Mouse mutants affecting lens development are excellent models for corresponding human disorders. The mutant *aphakia* has been characterised by bilaterally aphakic eyes (Varnum and Stevens, J Hered 1968;59:147–50); the corresponding gene was mapped to chromosome 19 (Varnum and Stevens, Mouse News Lett 1975;53:35). Recent investigations in our laboratory refined the linkage of 0.6 cM proximal to the marker *D19Mit10*. Several candidate genes have been excluded (*Chuk1, Fgf8, Lbp1, Npm3, Pax2, Pitx3*). The *Cat3* mutations are characterised by vacuolated lenses caused by alterations in the initial secondary lens fibre cell differentiation. Secondary malformations develop at the cornea and iris, but the retina remains unaffected. The mutation has been mapped to chromosome 10 close to the markers *D10Mit41* and *D10Mit95*. Several candidate genes have been excluded (*Dcn, Elk3, Ldc, Mell8, Tr2-11*). The series of *Cat2* mutations have been mapped close to the γ-crystallin genes (*Cryg*; Löster et al., Genomics 1994;23:240–2). The *Cat2*^{nop} mutation is characterised by a mutation in the third exon of *Crygb* leading to a truncated γB-crystallin and the termination of lens fibre cell differentiation. The *Cat2* mutants are interesting models for human cataracts caused by mutations in the human *CRYG* genes at chromosome 2q32–35.

Key words Aphakia, Hereditary cataract, Linkage analysis, Mouse model, Nuclear opacity, Vacuolated lens

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Cataracts are frequent diseases in man and often observed in animal models. Hereditary cataracts in man occur with an incidence of about 1–2 in 10 000; in about one-third cases a positive family history is found.[1] Because of the small size of the families that are usually available for a detailed genetical investigation, it is necessary to look for appropriate animal models to identify genes responsible for cataract formation and to analyse the mechanisms leading to the opacification of the lens. From a genetical point of view, the mouse is one of the best-characterised model systems, and observable pathological alterations are comparable to those in man. A systematic approach to collecting murine cataract mutations was initiated about 20 years ago[2] using paternal treatment of germ cells by X-ray[2] and later ethylnitrosourea.[3] Among the offspring, dominant cataract mutations were identified by slit lamp observations and subsequent genetic confirmation. The Neuherberg collection of cataracts contains now about 150 lines of independent origin and distinct phenotypes.[4,5]

Mouse models affecting early eye and lens development

One of the most important genes in eye development is the paired-box gene *Pax6*, which is affected in various alleles of the mouse and rat *Small eye (Sey)* mutants.[6,7] In homozygous *Sey* mice, eyes and nasal cavities do not develop; the animals die soon after birth. The histological analysis of homozygous mutants demonstrated the presence of optic vesicles, but the ectoderm does not give rise to a lens. The failure in lens development is attributed to a defect in the inductive interaction between the optic vesicle and the overlying ectoderm.[8]

Sey/Pax6 maps to mouse chromosome 2. The molecular analysis of the *Sey* mutations revealed a point mutation leading to a stop codon in the *Sey* allele and a transversion at a splice site of the *Pax6* gene in the *Sey*^{1Neu} allele.[6] Moreover, the analysis of the radiation-induced *Sey*^{H} allele demonstrated a large deletion including *Pax6* and a second gene, *reticulocalbin*. This encodes a Ca^{2+}-binding protein of the endoplasmatic reticulum and might contribute to the early lethality of *Sey*^{H} homozygotes.[9] The series of *Sey/Pax6* mutants in mouse are excellent models for several human inherited eye diseases such as aniridia, Peters' anomaly and also several forms of early-onset cataracts. A review of human *Pax6* mutations was published recently.[10]

Besides *Pax6*, *Pax2* is the second *Pax* gene that is expressed in the eye. A *Pax2* null mutant was reported[11] that exhibits an extension of the pigmented retina into the optic stalk, failure of the optic fissure to close resulting in coloboma, and the ipsilateral formation of the optic tracts without formation of the optic chiasm. Some malformations of the inner ear have been observed in addition to the ocular dysmorphology.

A further model, the mouse *Pax2*^{1Neu} mutant, exhibits defects of optic nerve development, the retinal layer of the eye and several defects of the kidney and brain.[12] The mutation causes a frameshift in the 5'-region of *Pax2* and stop codon 26 amino acids downstream predicting a nonfunctional protein. This mouse mutation is exactly the same as a mutation in man leading to an identical phenotype.[13]

Recently, knock-outs of the genes *sonic hedgehog (Shh)* and *retina and anterior fold homeobox* (*Rax*, but also known as *Rx*) have been reported. The analysis of the complex and very severe *Shh*$^{-/-}$ phenotype in the mouse demonstrated that one function of *Shh* is the definition of a midline axis during early development. This is also important for the paired formation of the eye anlagen. In *Shh* mutant embryos the optic vesicles are fused at the midline and the optic stalks are deficient or absent.[14]

Homozygous mouse embryos carrying a null allele of the *Rax* gene have no visible eye structures, whereas mice heterozygous for the loss of *Rax* are apparently normal. The abnormal phenotype of the homozygous mutants is visible as early as E9.0 and E10.5 by a failure to form the indentation (sulcus opticus) that gives rise to the optic cup. It demonstrates clearly that *Rax* gene function is required for eye formation from its initial stage.[15] The *Rax* gene maps to mouse chromosome 18.[16] From the present data it seems obvious that at least four genes – *Shh, Pax2, Pax 6* and *Rax* – are necessary to provide the correct information at the right place(s) to induce ocular development. However, there is growing evidence for some additional master control genes including members of the *Six* and *Eya* gene families.[17,18] From these data, it is now becoming clear that an entire genetic cassette may be used for early eye development in vertebrates.[19] Unfortunately, no mutants are yet available for these newly discovered genes.

The important second step, the formation of lens vesicle, might be addressed by some further autosomal recessive mutations such as *aphakia* (*ak*, for details see next section), *eyeless, myelencephalic blebs* or *head blebs*. The *eyeless* mutant *ey1* was defined to be responsible for anophthalmia;[20] its chromosomal localisation is unknown. In homozygous mutants, lens invagination at E10 is abnormal, the lens is smaller than normal and often improperly centred in the optic cup.[21]

The first morphological alteration in mouse mutants referred to as *myelencephalic blebs (my)* becomes visible at E9.5. A large area of extracellular matrix is formed between the optic vesicle and the overlying presumptive lens ectoderm. At E12 the lens capsule is ruptured, and at E14 the cornea and other structures of the eye were not found.[22] The mutation is located on mouse chromosome 3.[23] Phenotypically similar to *my*, but mapped to mouse chromosome 4, is the *head blebs* mutation (*heb*).[24]

Aphakia: arrest of lens formation at the lens stalk stage

The homozygous mouse mutant *aphakia (ak)* is characterised by bilaterally aphakic eyes without a pupil. The abnormality in eye development of homozygous *ak* mice was first observed at the early lens vesicle stage. At this stage, affected animals can be distinguished from normal littermates by groups of cells in the cavity of the lens vesicle.[25] It was suggested that the release of the cells into the vesicle might be caused by a malorientation of the mitotic figures in the lens placode and early eye cup.[26] These cells appeared to persist and to be included in the lens vesicle.[27] Additionally, the extracellular matrix material that forms a firm attachment between the lens cup and the optic cup during invagination of the lens cup is abnormal in mutant embryos.[28] The irregular lens development is arrested at the lens stalk stage.[29] The lens stalk is normally present transiently during detachment of the lens vesicle from the surface epithelium.[30,31] All other malformations observed at later stages are likely consequences of these initial defects. Lens-specific proteins were found in the *ak/ak* mice only in small amounts from E14 onward.[27,32,33]

Moreover, we investigated the expression pattern of the developmental control genes *Pax2, Pax6* and *Six3* and the lens-specific gene *Cryaa* in *ak* mice. The *ak* mutation does not affect the expression pattern of *Pax2*, which is important for the developmental regulation of the posterior eye. The remaining high expression of *Pax6* in the persisting lens stalk is consistent with the hypothesis that the lens does not develop beyond a rudimentary lens vesicle. *Six3* is expressed in the *ak/ak* mutants throughout the remnant lens structure, but cannot be observed in the lens stalk. The presence of *Pax6* and the absence of *Six3* transcripts in the lens stalk of the *ak/ak* mutant mice is an additional line of evidence that the lens stalk is related more to corneal epithelium than to lens-derived tissue.[34]

The mutation *ak* was mapped to mouse chromosome 19 at a distance of 8 cM from the marker *brachymorphic (bm)*.[35] Our results revealed as a best fit the gene order *Pax2* – (10.3 cM) – *D19Mit4/D19Mit91* – (0.7 cM) – ***ak/*** *D10Mit9* – (0.6 cM) – *D19Mit10*. These data excluded *Pax2* as a candidate gene and are a prerequisite for isolating the *ak* gene. Based on their chromosomal position *Chuk1, Fgf8, Lbp1* and *Npm3* were tested as candidate genes; none of them revealed differences in the DNA coding sequence between wild-type and the *ak/ak* mutants. On the basis of our fine mapping, *Pitx3*[36] has to be excluded as a candidate gene for *ak*.

One of the most interesting features of *ak/ak* mutant eyes is the persisting lens stalk leading to lens-corneal adhesions; the anterior chamber is not formed. Some other mutations resulting in lens-corneal adhesions and microphthalmia in the mouse are the dominant mutations *Coloboma (Cm)*, located on mouse chromosome 2,[37] *Cat4* on mouse chromosome 8,[38] the recessive mutation *dysgenetic lens (dyl)* on mouse chromosome 4,[39,40] and the mutation causing *eye lens aplasia (elap;* formerly *lap*), which has not yet been assigned to a chromosome.[41] The radiation-induced *Cm* mutation was characterised as a large deletion encompassing about 1.5 cM including the genes *Snap25* (synaptosomal-associated protein, 25 kDa) and *Plcb1* (phospholipase Cβ).[42]

Dominant cataract 3 (*Cat3*) leads to anterior eye malformation

The dominant cataracts $Cat3^{vl}$ and $Cat3^{vao}$ arose independently in the F_1 generation of γ-irradiated mice.[43,44] They belong to the Neuherberg collection of cataracts, which were recovered during studies on genetic risk assessment. It has been shown that $Cat3^{vl}$ and $Cat3^{vao}$ are allelic mutations with an excluded maximum linkage distance of 0.5 cM.[45]

The lens opacities of the $Cat3^{vl}$ and $Cat3^{vao}$ mutants are already developed at birth, as was seen in dissected eyes. At eye opening on postnatal day 12, opaque lenses and microphthalmia are visible to the naked eye. Slit lamp observation reveals that the extent and topography of the opacities do not change throughout the life of the animal. The lenses of the $Cat3^{vl}$ mutants are filled with small and large vacuoles. Pupillary dilatation is limited. The anterior chamber is flattened; anterior synechiae and opacified corneas are frequently observed. The $Cat3^{vao}$ mutants show an opaque area located in the anterior part of the lens, beginning immediately beneath the lenticular capsule and forming a disc-like opacity. The two alleles differ in the appearance of their opacities.[46]

Histological analysis of *Cat3* mutants identified an aberrant cell layer between the anterior epithelium and the primary lens fibres at E12.5 leading to a maldevelopment of the anterior lens epithelium and degeneration of fibres. Preliminary results showed that the *Six3* expression pattern at the anterior lens is shifted in the *Cat3* embryos to the posterior part of the developing lens. After birth, the lens capsule ruptures at the equatorial region, and synechiae with the iris occur.[47]

Genetic analysis with visible markers revealed linkage of *Cat3* with *Steel* (Sl^{gbH}) on chromosome 10 at a genetic distance of 3.2 cM. Using the markers *D10Mit41* and *D10Mit95*, no recombinants were found in approximately 1000 offspring tested. Thus, $Cat3^{vao}$ and these markers are located within 0.3 cM.[46] The linkage data exclude allelism of *Cat3* with cataract genes already mapped to chromosome 10: *To2* was located 6 cM from the centromere,[48] and Cat^{Lop} 22.4 cM distal from Sl^{gbH}.[49] Cat^{Lop} is allelic with Cat^{Fr},[50] a mutation in *Mip*.[51] *Lumican (Ldc), decorin (Dcn), Tr2-11* and *Elk3* are mapped close to *Cat3* and were tested as candidate genes; none of them revealed differences in the DNA coding sequences between wild-type and the *Cat3* mutants.[46,52]

Dominant cataract 2 (*Cat2*) mutations affect terminal lens fibre cell differentiation

Among the various cataract mutants of the Neuherberg collection, the group of *Cat2* mutants is the largest.[45,48] The murine dominant cataract locus *Cat2* was mapped to chromosome 1 between the loci *fuzzy* and *leaden*.[48] Subsequent fine mapping revealed that the genetic distance to the *Cryg* gene cluster is 0.3 ± 0.3 cM.[53] This result, together with the finding of reduced *Cryg* transcripts in mutant lenses,[54] strongly suggested the *Cryg* genes as candidates for the *Cat2* mutations.[53]

The *Cryg* gene cluster comprises six closely related genes (*Cryga*→*Crygf*). Each gene contains three exons and codes for a protein of 20 kDa. In mammals, the *Cryg* genes are specifically expressed in the eye lens and considered to be structural proteins. The γ-crystallin proteins are characterised by four Greek-key motifs (for a recent review on crystallins see Graw[55]). All murine genomic *Cryg* sequences are described[56–60] and were tested as candidate genes for the *Cat2* mutations.

Four *Cat2* mutant lines have been characterised by mutations within one of the *Cryg* genes: The mutant line *ENU-436* was obtained in an experiment using ethylnitrosourea as mutagen.[61,62] The lenses of the mutants from this line were characterised as a small nuclear opacity.[61] An A→G transition was identified at position 230 in exon 2 of *Cryga*. The new allele symbol for the line *ENU-436* is suggested as $Cryga^{1Neu}$. The deduced replacement of Asp by Gly at amino acid position 77 affects the connecting peptide between the second and third Greek-key motifs. Computer-assisted analysis predicts that the loss of this hydrophilic and acidic residue affects the α-helical characteristics of the protein.[63]

The spontaneous mutation $Cat2^{nop}$ (formerly *Nop*) has a decreased content of γ-crystallins in the juvenile lenses.[54,64] Using *in situ* hybridisation techniques with a probe detecting all *Cryg* transcripts in embryonic sections, a lower level of *Cryg* transcripts was detected in the mutants beginning at E13.5. However, the first morphological abnormality in the mutant lenses was observed as swelling of lens fibres at E15.5. Progressive degeneration of the lens core followed, leading to a cataracta immatura.[65] Histological investigations at the age of 3 weeks confirmed the preliminary characterisation as a nuclear opacity and revealed additionally polar cataracts with vacuolarisation. In contrast to wild-type lenses, the nuclei of the cortical cells were also observed in the area of the lens nucleus of the $Cat2^{nop}$ lenses.[54]

The molecular analysis detected only in the *Crygb* cDNA a difference between the amplification products from wild-type and the $Cat2^{nop}$ mutants. The final result revealed that in $Cat2^{nop}$ the third exon of the γB-crystallin gene is affected. The deletion of 11 bp starting after position 416 and the insertion of 4 bp lead to a frame shift and finally create a new stop codon. The new allele symbol is suggested as $Crygb^{nop}$. The corresponding γB-crystallin protein is predicted to be truncated after 144 amino acids; the last six amino acids are different from the wild-type γB-crystallin.[63]

The $Cat2^{t}$ mutant was discovered in an experiment using X-rays as mutagenic agent (formerly R-324[44,66]). The lenses of heterozygous and homozygous mutants exhibit swollen epithelial and fibre cells and a ruptured lens capsule. The histological analysis of mutants at the age of 3 weeks demonstrated complete destruction of the cellular organisation of the lens.[66] The molecular analysis resulted in the finding of a C→G exchange at position

Table 1. *Mutations at the γ-crystallin gene cluster in mouse and man*

Designation	Gene	Mutation	Phenotype	References
Mouse				
ENU-463	*Cryga*	230 A→G	Small nuclear opacity	61–63
Cat2^{Nop}	*Crygb*	Deletion in exon 3 of 11 bp and insertion of 4 bp	Nuclear opacity	45, 54, 63, 64
Cat2^{t}	*Cryge*	432 C→G	Total opacity	63, 66
Cat2^{ns}	*Cryge*	Deletion in exon 3 (> 2kb)	Suture and nuclear opacity	44, 45; Klopp and Graw, unpublished
Elo	*Cryge*	Δ403	Impaired elongation of central lens fibres, microphthalmia	69, 70
Human				
CCL	ψ*CRYGE*	Point mutations in the promoter leading to the formation of a truncated protein	Coppock-like cataract	86
PCC	*CRYGB* (linkage)	?	Polymorphic congenital cataract	87
	CRYG?	?	Aculeiform cataract	88

432. This creates a premature stop codon predicting a truncated protein after amino acid 143. The new allele symbol is suggested as *Cryge^{t}*.[63]

The *Cat2^{ns}* mutant (previously *Scat*[67]) occurred spontaneously in the GSF breeding colony and exhibits an anterior suture opacity in heterozygotes and microphthalmia with vacuolated lenses in homozygotes. In histological sections of lenses from 3-week-old mice the heterozygotes exhibit a hydropic swelling of lens epithelium, whereas in homozygotes interruption and degeneration of lens fibres and clefts and folds of the capsule were also observed. A rupture of the posterior lens capsule was also observed in homozygous *Cat2^{ns}* mutants, but not in heterozygotes.[67] However, by exposing these weakly affected heterozygous mutants to UV radiation, rupture of the posterior lens capsule was induced.[68] Molecular analysis revealed a large delection (> 2 kb) within the third exon of *Crgye*; the exact breakpoints have not been determined (Klopp and Graw, unpublished); the mutation is referred to as *Cryge^{ns}*.

A further dominant cataract mutation, *eye lens obsolescence (Elo)*, was characterised recently as a single base pair deletion (position 403) within the *Cryge* gene predicting a truncation of the γE-crystallin within the fourth Greek-key motif after amino acid 145; the last 11 amino acids are changed compared with the wild-type.[69] The new allele symbol is suggested as *Cryge^{elo}*. The first changes in *Cryge^{elo}* embryos were detected at E12.5, when elongation of the central fibres at the basal cytoplasm was impaired. Necrotic cells were found among the central lens fibres, which never reached full maturation length and progressively degenerated thereafter.[70] At E13, the lens fibre cells are morphologically abnormal. The nuclei of the poorly elongated fibre cells are located in the posterior region of the cytoplasm, and dense bodies were noted at nuclear poles. In contrast to the deep cortex and central region, the lens fibre cells in the outer cortex appear to elongate normally from E15 onward. At E18 the *Cryge^{Elo}* lens is conical instead of oval as in the wild-type, and the lens capsule of the *Cryge^{elo}* mice is ruptured in the posterior region. In summary, the developmental analysis of the *Cryge^{elo}* mutants suggested that the primary effect of the deletion in the *Cryge* gene may be specific to fibre cell differentiation rather than to cell proliferation and to inhibited fibre cell elongation.[71]

The mutations observed at the *Cryg* gene cluster in mice are summarised in Table 1 and compared with mutations in human patients suffering from hereditary cataracts. The *Cryg* mutations affect only the lens, but the different cataracts exhibit some diversity. In all cases a *Cryg* gene is altered but the consequences for the lens are different and might be due to distinct functions of the individual γ-crystallins or to their various domains. The comparison demonstrates that the characterisation of the phenotype does not allow the prediction of the mutated gene *per se*.

Further cataract mutations in mice

Several further cataract mutations are now mapped, and their number is rapidly increasing: the *Tcm* mutation, a cataract with iris dysplasia and coloboma,[72] and the *Ccw* mutation, *cataract and curly whiskers*,[73] are localised on mouse chromosome 4. The *nuclear-posterior polar opacity (Npp)* maps to chromosome 5, and *total opacity (To2)* to chromosome 10. The mutation leading to an *opacity due to poor secondary fibre cell junctions (Opj)* was mapped to chromosome 16[48] and affects the *Crygs* gene.[74]

The *total opacity (To3)* is placed on chromosome 7.[73] Mice heterozygous or homozygous for the *To3* mutation have total opacity of the lens with a dense cataract. Additionally, homozygotes exhibit microphthalmia and small eyes. Histological analysis revealed vacuolisation of the lens and gross disorganisation of the fibres; posterior lens rupture was observed only in homozygotes. The *To3* mutation was characterised as a single G→T transversion within the first exon of the *Lim2* gene coding for a lens-specific integral membrane protein, MP19. It was predicted that this DNA change results in a non-conservative substitution of Gly to Val at amino acid 15 of the MP19 protein.[75] *Xcat* is localised at the distal part of the X chromosome,[76–78] but the mutated

gene is not yet identified. The development of the lens is altered from E14 onward. The primary fibres are irregularly arranged and show small foci of cellular disintegration. Progressive degeneration of primary fibres occurs from E15 to E18 and, during late gestation, secondary fibres also begin to degenerate. The lens epithelium and newly differentiating fibres appear normal. Postnatally, most of the lens substance becomes amorphous and the posterior lens capsule is ruptured at P21.[79]

Another cataract model exhibiting rupture of the lens is *'rupture of lens cataract' (rlc)*. It was mapped to chromosome 14,[80] and recently, a similar phenotype, *lr2 (lens rupture 2)*, was mapped nearby.[81] The opacity in *rlc/rlc* mice becomes apparent at 35–60 days of age.[82] Other forms of cataract that start postnatally are the *Nakano* cataract *(nct)* at chromosome 16[83,84] (http://www.informatics.jax.org./bin/fetch_marker?11815), or the *Philly* cataract; the latter was characterised by a mutation within the βB2-crystallin-encoding gene.[85] These examples of various cataract mutants demonstrate the genetic heterogeneity eye disorders in mice and reflect also the diversity in human congenital cataracts.

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