—Review—

Young Investigator Award

Genetic modifiers of rodent animal models: the role in cataractogenesis

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Abstract: Visual impairment leads to a decrease in quality of life. Cataract is the most commonly observed ocular disease in humans that causes vision disorders. The risk factors associated with cataract development include aging, infections, eye injuries, environmental causes, such as radiation and exposure to ultraviolet rays in sunlight, and genetic mutations. Additionally, several cataract patients display phenotypic heterogeneity, suggesting the role of genetic modifiers in the modulation of severity and onset time of cataractogenesis. However, the genetic modifiers associated with cataract have not been identified in humans yet. In contrast, the identification and mapping of genetic modifiers have been successfully carried out in mice and rats. In this review, we focus on the genetic modifiers of cataract in the rodent models.
Key words: cataract, genetic modifier, lens transparency, mouse model, rat model

Introduction

Cataract, which leads to lens opacity, is one of the most common eye diseases and a leading cause of blindness, accounting for about half of the total visual impairments observed in humans [2, 17, 51, 66]. In developed countries, a large percentage of cataract patients are treated by surgery, which removes the clouded lens and replaces it with an artificial lens [66]. However, in developing countries, a significant number of cataract patients lose their vision due to inadequate access to eye care.

Although around 16 million people worldwide have cataracts, congenital cataracts are rare and are generally caused by an intrauterine infection and other prenatal insults [2, 51, 55]. Previous studies have also reported the association of genetic mutations with the development of several cataracts [55]. It is estimated that about 25% of congenital cataracts are hereditary [51]. The age-related cataract is the most common type [55, 66]. In addition to increasing age, several other risk factors, such as diabetes and exposure to radiation and ultraviolet light, have been identified [55]. Although there is little information available about the loci and genes related to the age-related cataract as compared to the congenital cataract, previous reports have suggested the association of single nucleotide polymorphisms in several genes and genetic compositions of the patients with age-related cataracts [55]. Thus, it can be said that the age-related cataract is a complex disease that occurs through the actions of several quantitative trait loci (QTLs) and environmental insults. In addition, congenital cataract patients show distinct phenotypes despite the same allelic mutation in the same gene [23, 27, 47,

49]. These clinical observations strongly suggest the presence of genetic modifiers that act on cataract pathologies in the genetic backgrounds.

However, such genetic modifiers have not been identified in humans. The major reason why it is difficult to identify the modifier genes is the presence of environmental factors that affect the pathology of cataracts. To identify genetic modifiers associated with cataracts, we propose the use of animal models, in particular mice and rats; they have several advantages that can overcome the weaknesses in the genetic analysis of cataract in humans. There are many rodent models available with mutations in different cataract-associated genes [11, 20, 64, 68], and they can be controlled to avoid environmental insults. These rodent models also allow for the production of chromosomal recombinants by a crossing between the susceptible and resistant strains for cataractogenesis. The chromosomal recombinants can easily perform the genome-wide genotyping by abundant genetic markers. Further, a histopathological analysis at different developmental stages enables a detailed investigation of the severities of the different cataract phenotypes in such models. In this review, we focus on the genetic modifiers for cataract phenotypes that are found in the rodent models, including our recent study.

Lens Structure and Cataract Phenotypes in the Mouse

This section describes the structure of the lens and representative phenotypes of cataracts in mice. Figure 1A shows the histological structure of the mouse eye. The ocular lens is an avascular and transparent central tissue in the eye, and it has a role as the light filter between the cornea and retina and is responsible for accommodation [1]. Figure 1B indicates a schematic illustration of mature lens cells, which are largely divided into two types, lens epithelial cells and lens fiber cells [67]. All of the lens fiber cells are derived from the differentiated lens epithelial cells [1]. During differentiation of the lens epithelial cells, an organelle-free zone is created by digestion of organelles and nuclei of the primary lens fiber cells [67]. Lens opacity is caused by the disruption of the organelle digestion system due to mutations in several genes.

The major intrinsic protein of lens fiber (*Mip*) gene, which is also known as aquaporin 0 protein (AQP0) [10, 61], is one of the major genes responsible for cataracts.

AQP0 is the most abundant membrane protein in lens fiber cells, constituting approximately 29.6% of the total membrane protein content of these cells, and plays a role in lens homeostasis via water channel activity [3, 10]. In mice, four mutant mice and a *Mip* knock-out mouse have been established [44, 53, 54, 56] that develop semidominant cataracts and show severe and mild phenotypes in homozygous and heterozygous alleles, respectively. Recently, we identified a missense mutation in *Mip* in the Nodai cataract (*Nat*) mouse. As an example of cataract phenotype, we showed that the *Nat* mouse developed semidominant cataracts, similar to other *Mip* mutations (Figs. 1C and F) [61]. The *Nat*/+ heterozygous and *Nat*/ *Nat* homozygous mutants also develop cataracts. The detection of cataract phenotypes from just the appearance was difficult in *Nat*/+ mice (Fig. 1C), but the lens opacity of the nuclear region and swelling of lens fiber cells were observed at three weeks of age (Figs. 1D and E). In contrast, the cataract phenotype of *Nat*/*Nat* mice could easily be detected at the same age just from the appearance (Fig. 1F). Severe lens opacity could be observed in *Nat*/*Nat* mice (Fig. 1G). The lens fiber cells showed disorganization, swelling, and vacuolation (Fig. 1H) [61]. *MIP* mutations lead to dominant and recessive cataracts in humans [9, 10, 14, 24, 25, 29, 32, 33, 48, 57, 58, 69–72]. Therefore, these mutant mice might be useful models of human cataracts caused by *MIP* mutations.

Genetic Modifiers of Cataractogenesis in Mice and Rat

Although we showed typical cataract phenotypes in mice, their severities and onset times were often modulated by differences in the genetic backgrounds among the mouse and rat strains. Several modifier loci and genes accompanying the genetic backgrounds were identified by genetic mapping studies (Table 1).

The phenotypic modulations of cataracts were identified in mice with mutations in connexins, lens membrane proteins that play crucial roles in intracellular homeostasis [5]. Gap junction proteins, alpha 3 gene (*Gja3*) and alpha 8 gene (*Gja8*), are expressed in the lens fibers, and mutations in both genes can cause recessive and/or dominant cataracts in humans [13, 24, 34, 52], mice [8, 21, 50, 59, 65], and rats [31, 68]. Effects of genetic background were characterized in *Gja3* and *Gja8* knockout (KO) mice. In these mice, the severity of cataract phenotype is different between the genetic backgrounds of
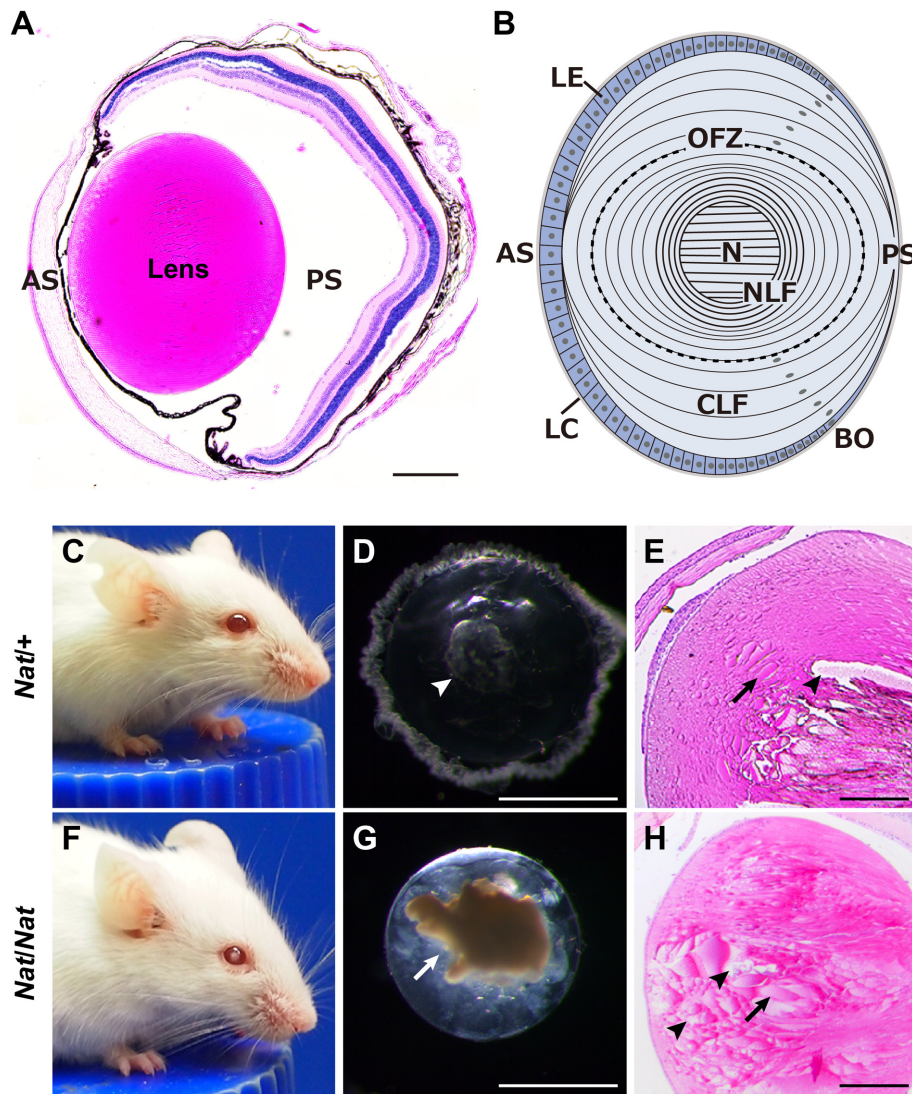
Fig. 1. Lens structures and cataract phenotypes in the mouse. (A) The entire eye section of a C57BL/6J mouse at 6 weeks of age, stained by hematoxylin and eosin (H&E). AS, anterior segment; PS, posterior segment. Scale bar=200 μm. (B) Schematic representation of lens structure (partially modified from the study of Cvekl and Zhang [12]). Lens tissue is composed of lens epithelium (LE), cortical lens fiber (CLF), and nuclear lens fiber (NLF). An organelle-free zone (OFZ) is generated in the NLF area (demarcated by dashed circle). N, nucleus; LS, lens capsule; and BO, bow. (C and F) Gross phenotypes of $Mip^{Nat/+}$ heterozygous (C) and $Mip^{Nat/Nat}$ homozygous (F) mutants [61]. Low- (D and G) and high-magnification (E and H) images of histological phenotypes in the lens from the $Mip^{Nat/+}$ (D and E) and $Mip^{Nat/Nat}$ (G and H) mice [61]. White arrowhead and arrow indicate mild disorganization of the lens fibers and severe lens opacity, respectively (D and G). Black arrows and arrowheads indicate swelling and vacuolation of lens fiber, respectively (E and H). Scale bars=1,000 μm (D and G) and 250 μm (E and H).

the 129SvJae and C57BL/6J strains [18, 19]. The lens opacity in the KO mice with the 129SvJae genetic background is more severe than that observed in KO mice with the C57BL/6J genetic background [18, 19]. The modifier locus of the cataract phenotype caused by *Gja8*

mutation was mapped in the genetic background of a rat strain. Although Upjohn Pharmaceuticals Limited (UPL) rats develop early-onset cataracts in homozygotes and late-onset cataracts in heterozygotes, the phenotype was suppressed by a modifier locus on chromosome 5 of the

Table 1. Loci and genes associated with modulation of cataract phenotype in mouse and rat models

Species	Causative gene	Mutation	Susceptible strain	Resistant strain	Modifier gene	Reference
Mouse	*Gja3*	Targeted deletion	129SvJae	C57BL/6J	Unknown	[19]
	Gja8	Targeted deletion	129SvJae	C57BL/6J	Unknown	[18]
	Cpox	p.Arg380Leu	BALB/c	MSM/Ms	Unknown	[42]
	Gpr161	143 aa deletion at C-terminal region	C3H/HeSnJ	MOLF/Ei	*Foxe3*	[36]
	Foxe3	22-bp deletion of putative cis element	SJL/J, C3H/HeN	C57BL/6J, MSM/Ms	*Pde6b*	[35, 63]
Rat	*Gja8*	p.Arg340Trp	Crj:SD	BN/Sea	Unknown	[68]

BN/Sea strain [68]. Mutations of *GJA3* and *GJA8* also result in various types of cataracts in human patients, even in the same allelic variant [13, 24, 52]. Therefore, the abovementioned mutant mice and rats may contribute to identification of the modifier gene causing heterogeneous pathology in human cataracts.

The Nakano cataract (NCT) mouse model carries a missense mutation in the coproporphyrinogen oxidase (*Cpox*) gene [41]. The intersubspecific backcross progeny of homozygous *nct,* generated by mating with MSM/Ms mice, an inbred strain derived from the Japanese wild mouse *Mus musculus molossinus*, showed two forms of cataract phenotypes, viz., pin-head and diffused subtypes [42]. The genetic loci related to these distinct phenotypes in *nct* were mapped onto chromosomes 3 and 10.

The *vl* mouse, which was spontaneously isolated from C3H/HeSnJ, exhibits congenital cataract and neural tube defects (NTDs), which are caused by a truncating mutation in the G protein-coupled receptor 161 gene (*Gpr161*) [36]. Matteson *et al.* [36] reported an 85% decrease in the cataract incidence rate in F_2 individuals, obtained from mating between C3H/HeSnJ-*Gpr161*[vl/vl] and MOLF/Ei mice, that carry homozygous *vl* alleles. MOLF/Ei is one of the inbred strains derived from wild mice trapped in Japan. This observation strongly suggested that the MOLF/Ei mice possess resistance loci to cataractogenesis resulting from *vl* mutation.

Matteson *et al.* identified three modifier loci, namely *Modvl1*, *2*, and *3*, which are linked to the incidence of cataract and/or NTD. Among these modifiers, the *Modvl3* locus was found to be associated with modulation of the cataract phenotypes of the *vl* mice [36]. They speculated that the Forkhead box E3 (*Foxe3*) gene was a strong candidate gene for *Modvl3* due to the presence of c.68C>T mutation in *Foxe3* in C3H/HeSnJ mice. The c.68C>T mutation is a missense mutation that substitutes a proline residue with a leucine residue at position 23 in the FOXE3 protein (p.Pro23Leu). This proline is a

highly conserved residue in mammals, and a mutation in this p.Pro23Leu positively influences the transcriptional activity of FOXE3 [36]. *Foxe3* has been shown to play a crucial role in lens development [37, 38]. In mice, *Foxe3* is initially expressed in the undifferentiated lens placode, and later, *Foxe3* expression persists in the relatively undifferentiated anterior lens epithelium [38, 62]. In the early developing lens, *Gpr161* shows similar localization to *Foxe3* [36]. Although molecular interactions have not been identified between them, Matteson *et al.* [36] suggested that *Gpr161* and *Foxe3* act on the same or interacting pathways involved in lens development. Therefore, the phenotypic modulation might occur via the additive effects on both these genes having similar functions.

Li *et al.* mapped the additional modifier (*Modv4*) for *vl* cataract on chromosome 15 [30]. They narrowed down a candidate interval on chromosome 15 using subcongenic lines and screened three candidate genes (*Cdh6*, cadherin 6; *Ank*, progressive ankylosis; and *Trio*, triple functional domain) for *Modv4*, based on expression analysis and polymorphism between C3H/HeSnJ and MOLF/Ei. Li *et al.* also suggested that at least one of these three candidate genes for *Modv4* affects the cataract phenotype in *vl* mutation [30].

Strain-Specific Mutation in *Pde6b* Modifies the Cataract Phenotype in Mice

We have also discovered phenotypic modification of cataracts in a *Foxe3*[rct] mouse which had a hypomorphic mutation in *Foxe3* caused by deletion of its *cis* element [62]. In its SJL/J genetic background, the homozygous *Foxe3*[rct] mouse showed a severe cataract phenotype with mild microphthalmia (Figs. 2A, A', and C). The intersubspecific backcross progeny generated by mating SJL/J-*Foxe3*[rct/rct] homozygous mutants and MSM/Ms mice showed segregation for early- and late-onset cata-
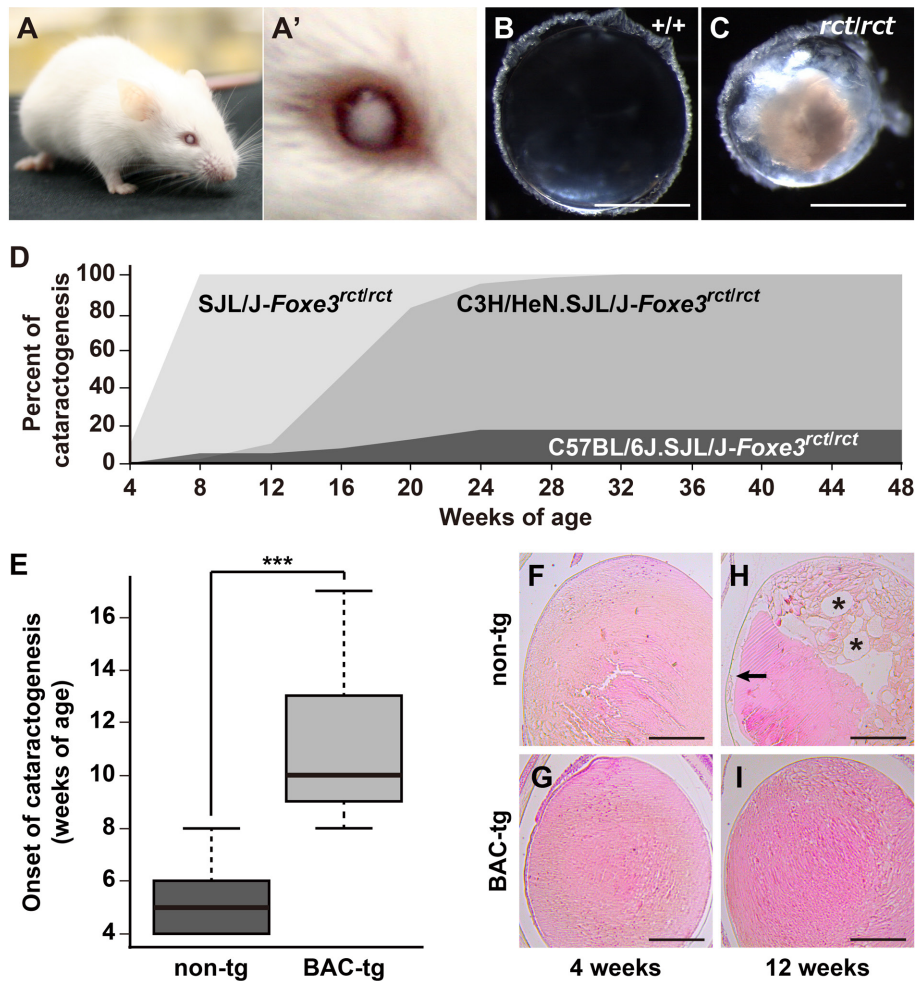
Fig. 2. Modulation of the cataract phenotypes among the mouse strains and identification of a genetic modifier, a *Pde6b^rd1* mutation in the SJL/J genetic background. (A) Gross phenotype of the SJL/J-*Foxe3^rct* mouse and an image of the eye at higher magnification (A'). (B and C) Lens phenotypes of SJL/J-*Foxe3^+/+* (B) and SJL/J-*Foxe3^rct/rct* (C) mice observed under dark-field microscopy at 3 weeks of age. Scale bars=1,000 *μ*m. (D) Differences in onset time of *Foxe3^rct/rct* mice with the SJL/J, C57BL/6J, and C3H/HeN genetic backgrounds. Light grey, grey, and dark grey colors represent the onset time of cataractogenesis in the SJL/J-*Foxe3^rct*, C3H/HeN.SJL/J-*Foxe3^rct*, and C57BL/6J.SJL/J-*Foxe3^rct* congenic mice, respectively. (E) Comparison of onset times for cataract of non-tg and BAC-tg mice in the SJL-*Foxe3^rct/rct* *Pde6b^rd1/rd1* genetic background. The dark grey and light grey boxes indicate age of onset cataract in non-tg and BAC-tg mice, respectively. (F–I) Representative histological phenotypes of the lens in the non-tg and BAC-tg mice at 4 weeks and 12 weeks of age. An arrow indicates the loss of the gap junction between the lens epithelium and fiber. Asterisks show vacuoles on the lens. Scale bars=250 *μ*m.

racts even though the genotype of the *Foxe3^rct* locus on chromosome 4 was *rct* homozygotes [35]. We speculated that phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide (*Pde6b*) encoding gene, which is known to be responsible for retinitis pigmentosa in humans [4, 40] and in mice [6, 46], is the most suitable candidate gene for the modifier of *rct* (*mrct*). The primary reason

behind our speculation was the fact that SJL mice have a retinal degeneration 1 (*rd1*) nonsense mutation (*Pde-6b^rd1*) in *Pde6b* [16, 46]. C57BL/6J.SJL-*Foxe3^rct* and C3H/HeN.SJL-*Foxe3^rct* congenic mice were generated by transferring the *Foxe3^rct* mutation to the genetic backgrounds of C57BL/6J and C3H/HeN mice carrying *Pde6b^+* and *Pde6b^rd1*, respectively. Surprisingly, cata-

ractogenesis was strongly suppressed in *Foxe3^rct* mice in the C57BL/6J genetic background (Fig. 2D). C3H/HeN.SJL/J-*Foxe3^rct* mice also showed a resistant phenotype as compared with that of SJL/J-*Foxe3^rct*, despite harboring *Pde6b^rd1*. Our findings evidently revealed that there are multiple genetic modifiers affecting *Foxe3^rct* in the C57BL/6J and C3H/HeN genetic backgrounds.

To confirm the *Pde6b^rd1* mutation as one of the modifiers for *Foxe3^rct*, we produced bacterial artificial chromosome transgenic (BAC-tg) mice for *Pde6b*. The BAC-tg mice exhibited a significant delay in cataractogenesis as compared with non-tg mice (Fig. 2E) [63]. Degeneration and vacuolation in the anterior and equator regions of the lens and swelling of lens fibers were evident in non-tg mice (Fig. 2F). In contrast, the lens of BAC-tg mice showed slight swelling of fiber cells, and this phenotype was observably milder as compared with that of non-tg mice (Fig. 2G). At 12 weeks of age, the lens of non-tg mice exhibited loss of gap junctions, severe disorganization, and the presence of a large number of vacuoles (Fig. 2H). Although BAC-tg mice showed degeneration in their lens fibers, the phenotype was clearly milder as compared with that of non-tg mice even at 12 weeks of age (Fig. 2I).

It is unlikely that *Foxe3* and *Pde6b* interact and are a part of the same regulatory pathway in cataractogenesis; this is because both proteins have distinct functions and show completely distinct localizations in the eye [63]. Therefore, we speculate that phenotypic modulation of *Foxe3^rct* mice probably leads to the noninteractive additive effects on different functional mutations.

Many mutations of *FOXE3* are related to ocular diseases, including dominant or recessive cataracts [23, 47]. Diverse pathologies have been reported in patients harboring the *FOXE3* mutation, which might be caused by its mutational position and/or genetic background. In mice, a dysgenetic lens mutant (*Foxe3^dyl*) harboring two missense mutations and knock-out of *Foxe3* exhibited a persistent lens-ectoderm connection and corneal opacity, which are partially similar to the human pathology [7, 38]. In contrast, the *Foxe3^rct* allele does not lead to a *Foxe3^dyl*-like phenotype and has a very weak effect on cataractogenesis in mice with the C57BL/6J genetic background. Therefore, the *Foxe3^rct* allele requires other genetic factor(s), including *Pde6b^rd1*, to develop early-onset and severe cataracts in mice, implying that the FOXE3 hypomorph is a potential genetic modifier acting on human cataracts. We predict that *Foxe3^rct* is a useful model to understand ocular heterogenetic pathologies of *FOXE3* mutations in humans.

Modification of the Inheritance Pattern of Cataracts by Species Differences

We have previously reported that Kyoto Fancy Rat Stock 4 (KFRS4) rats, the first and only rat model of the *Mip* mutations, develop severe nuclear cataracts caused by a frameshift mutation that leads to complete loss of the MIP protein [64] (Figs. 3A–C). As mentioned above, humans and mice carrying heterozygous *MIP*/*Mip* mutations including null alleles develop dominant cataracts [48, 54, 57]. However, the cataract phenotype was found to be inherited in a recessive manner in *Mip* null rats. The lens transparency and lens fibers are clearly maintained in heterozygous *Mip* null rats at 96 weeks (2 years) of age (Figs. 3D–F). These findings indicated a difference in phenotypes and inheritance modes for cataracts in humans/mice compared with rats and suggested the presence of a genetic modifier(s) that plays a role in resistance to cataractogenesis caused by MIP haploinsufficiency. Although we currently have no clear evidence to explain the rat-specific modifier, our preliminary microarray data detected some differentially expressed genes between mouse and rat eyes (unpublished data). Moreover, several pseudogenes in the genome are divergent among mammals, implying that several genes are degenerated into a pseudogene in humans and mice, whereas a few of these genes may be functional only in rats [15, 28]. Although further study is necessary due to investigation of only one allele, we speculate that highly expressed genes and/or rat-specific functional genes may act as resistant modifiers and prevent cataract development in rats.

Concluding Remarks and Future Perspectives

Genetic modifiers are an integral part of the genetic landscape of human diseases [22]. The identification of genetic modifiers will contribute to elucidation of underlying mechanisms behind the phenotypic heterogeneity of Mendelian disorders, through genetic and molecular analyses for the responsible gene and modifier genes. In this review, we have described phenotypic moderations in cataractogenesis caused by particular genetic backgrounds of inbred strains and genetic modifiers, as revealed by studies using mouse models.
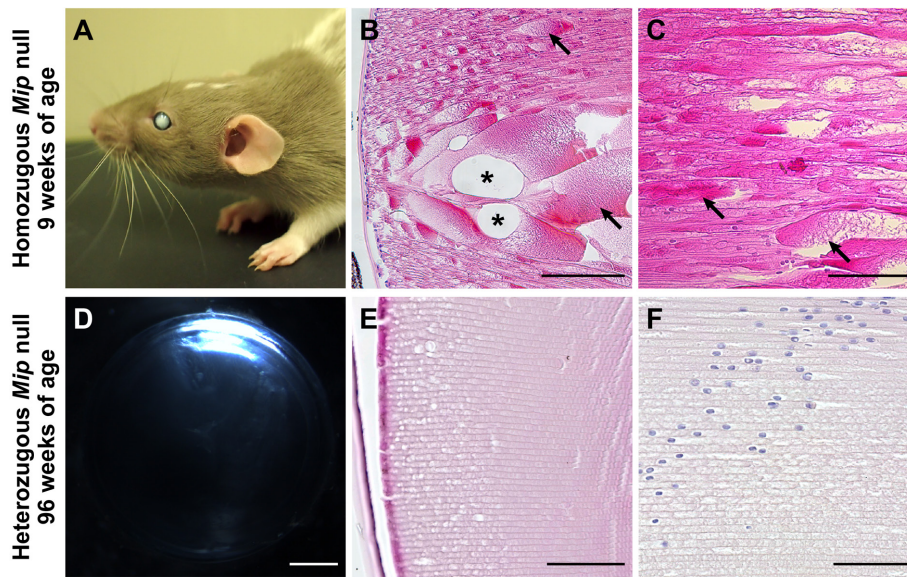
Fig. 3. Cataract phenotypes of *Mip* null mutant rats. (A) Gross phenotype of homozygous *Mip* null rats at 9 weeks of age. (B and C) Representative histological phenotypes of the anterior segment (B) and lens fiber (C) in homozygous *Mip* null rats at 9 weeks of age. (D) Maintenance of lens transparency in heterozygous *Mip* null rats at 96 weeks of age as observed by dark-field microscopy. (E and F) Normal phenotypes of the anterior segment (E) and lens fiber (F) in heterozygous *Mip* null rats at 96 weeks of age. Scale bar=1,000 μm (D) and 100 μm (B, C, E, and F).

Mouse and rat ocular tissues show anatomical and histological similarities to those of humans. As mentioned above, some pathologies caused by *FOXE3* mutations were identical to the mouse phenotype. Further, human genomes show heterogeneity and divergence among individuals, which might be expressed as heterogeneous pathologies of cataracts via multiallelic effects. High-throughput technology, such as the genome-wide association study and whole genome exome sequencing, are predicted to become powerful tools for identification of modifier genes in cataracts. However, human cataracts are caused by various gene mutations, and their pathologies are strongly affected by environmental factors as mentioned above. Thus, we suspect that it might be difficult to clearly identify genetic factors that modify the cataract phenotype in humans. Many rodent models for cataracts have been established that are largely inbred strains that live in well-controlled environments.

Studies have identified two modifiers influencing cataractogenesis by forward genetic analysis using inbred mice [36, 63]. Both studies identified the modifiers in the genetic backgrounds of wild-derived inbred strains, which possess large genetic divergences as compared with the common inbred strains [26, 60]. Other studies, such as those of Miyasaka *et al.*, Okumura *et al.*, and Peters *et al.*, have discovered many genetic modifiers involved in other diseases in the genetic backgrounds of wild-derived inbred strains [39, 43, 45], suggesting that these strains can act as highly useful genetic resources in the identification of the genetic modifiers. Moreover, we showed an example of the differences in the inheritance mode of the cataract phenotype observed among humans, mice, and rats. Identification of the species-specific differences of gene expression profiles caused by evolutionary *cis*-regulatory and *trans*-regulatory divergences, species-specific alternative splicing, and pseudogenization of functional genes may be the key to discerning the interspecies differences of the disease phenotypes.

Acknowledgments

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