Biochemical and Biophysical Research Communications 496 (2018) 231–237

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pde6b^{rd1} mutation modifies cataractogenesis in *Foxe3*^{rct} mice

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ARTICLE INFO

Article history:
Received 17 December 2017
Accepted 4 January 2018
Available online 6 January 2018

Keywords:
Cataract
Foxe3
Genetic modifier
Pde6b
rd1 mutation

ABSTRACT

The *Foxe3*^{rct} mutation, which causes early-onset cataracts, is a recessive mutation found in SJL/J mice. A previous study reported that cataract phenotypes are modified by the genetic background of mouse inbred strains and that the *Pde6b*^{rd1} mutation, which induced degeneration of the photoreceptor cells, is a strong candidate genetic modifier to accelerate the severity of cataractogenesis of *Foxe3*^{rct} mice. We created congenic mice by transferring a genomic region including the *Foxe3*^{rct} mutation to the B6 genetic background, which does not carry the *Pde6b*^{rd1} mutation. In the congenic mice, the cataract phenotypes became remarkably mild, and the development of cataracts was suppressed for a long time. Moreover, we created transgenic mice by injecting BAC clones including the wild-type *Pde6b* gene into the eggs of SJL-*Foxe3*^{rct} mice. Although the resistant effect for cataract phenotypes in transgenic mice was less than that in congenic mice, the severity and onset time of cataract phenotypes were clearly improved and delayed, respectively, compared with the phenotypes of the original SJL-*Foxe3*^{rct} mice. These results clearly show that the development of early-onset cataracts requires at least two mutant alleles of *Foxe3*^{rct} and *Pde6b*^{rd1}, and another modifier associated with the severity of cataract phenotypes in *Foxe3*^{rct} mice underlies the genetic backgrounds in mice.

1. Introduction

Congenital cataracts exhibiting lens opacity at an early age is a profound eye disease in humans, and it is estimated that 10–39% of cases are caused by genetic factor(s) [1]. Over the last few decades, many mutations responsible for human congenital cataracts have been identified on the genes encoding lens structural proteins, enzymes and transcription factors [2]. Although these gene mutations evidently cause cataractogenesis, pathological variations have been frequently observed among patients despite identical gene mutations [3,4]. These findings suggest the presence of genetic background effects causing heterogeneous pathologies among human patients, indicating that there are modifier genes associated with major causative genes for cataracts.

The effects of modifier genes have been frequently reported in mouse models for cataracts [5–8]. We previously showed that the lens opacity of Rinshoken cataract (*Foxe3*^{rct}) mice, which were isolated spontaneously from the SJL/J (SJL) strain, is caused by a 22-

Abbreviations: Foxe3, forkhead box E3; *Pde6b*, phosphodiesterase 6B; cGMP, rod receptor, beta polypeptide; RP, retinitis pigmentosa; *rd1*, retinal degeneration 1; BAC, bacterial artificial chromosome; tg, transgenic; RT-PCR, reverse transcription PCR; MIP, major intrinsic protein of lens fiber; *Gapdh*, glyceraldehyde-3-Phosphate Dehydrogenase; ACTB, Actin beta; HRP, horse radish peroxidase; IgG, immunoglobulin G; ORF, Open reading frame; GFP, green fluorescence protein; DsRed, *Discosoma* sp. red fluorescent protein; cDNA, complementary DNA; HE, hematoxylin-eosin; DMEM, Dulbecco's Modified Eagle Medium; qRT-PCR, quantitative RT-PCR; ANOVA, analysis of variance; SD, standard deviation; cGMP, cyclic guanosine monophosphate; *Maf*, avian musculoaponeurotic fibrosarcoma oncogene homolog; *Prx*, periaxin; *Tbc1d20*, TBC1 domain family, member 20; *Glrx2*, glutaredoxin 2.

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https://doi.org/10.1016/j.bbrc.2018.01.031
0006-291X/

bp deletion in the eye-specific enhancer region of forkhead box E3 gene (*Foxe3*) [9]. The cataract phenotypes in *Foxe3*^{rct} mice are also modified by the genetic background. A previous linkage analysis study used intersubspecific backcross progeny generated by mating SJL-*Foxe3*$^{rct/rct}$ homozygous mutants and MSM/Ms (MSM) mice, an inbred strain derived from the Japanese wild mouse *Mus musculus molossinus* [10]. The phenotypes of the progeny segregated for early- and late-onset cataracts even though the genotype of the *Foxe3*^{rct} locus on chromosome 4 was *rct*/*rct* homozygous. Moreover, a recessive modifier (*mrct*) was mapped to chromosome 5 that accelerates the severity and onset time of cataract phenotypes in *Foxe3*$^{rct/rct}$ mice [10].

Although there are many genes in the candidate region on chromosome 5 for *mrct*, we suspect phosphodiesterase 6B, cGMP, rod receptor, beta polypeptide encoding gene (*Pde6b*), which is a known gene responsible for retinitis pigmentosa (RP) in humans [11,12] and mice [13,14]. The primary reason that we predict *Pde6b* to be a strong candidate for *mrct* is that SJL mice have the retinal degeneration 1 (*rd1*) nonsense mutation (*Pde6b*^{rd1}, c.1090C > A: p.Y347X) in *Pde6b*, which is responsible for RP in multiple inbred mouse strains [14,15]. In this study, we produced congenic mice and bacterial artificial chromosome transgenic (BAC-tg) mice for *Pde6b*. Phenotypic analysis of congenic and BAC-tg mice revealed that the *Pde6b*^{rd1} mutation is a modifier associated with acceleration of lens opacity in mice through additive effects with the *Foxe3*^{rct} mutation.

2. Materials and methods

2.1. Mice

SJL-*Foxe3*^{rct} mice have been previously reported [9,10]. The B6.SJL-*Foxe3*$^{rct/rct}$ congenic strains were produced by transferring the SJL segments including a *Foxe3*^{rct} mutation of interest onto the genetic background of C57BL/6JJcl (B6) mice (CLEA Japan, Tokyo). The (B6 × SJL-*Foxe3*$^{rct/rct}$) F_1 mice were backcrossed with B6 mice, and those carrying the *Foxe3*^{rct} mutation were selected at every generation by genotyping, as previously described [9]. After 12 backcrosses, *Foxe3*$^{rct/+}$ heterozygous mice were intercrossed to produce *Foxe3*$^{rct/rct}$ homozygotes. The congenic intervals were detected by genotyping with polymorphic microsatellite markers. The BAC-tg mice were produced by microinjection of a MSM-derived BAC clone [16], MSMg01-521B08, into the fertilized eggs of SJL-*Foxe3*$^{rct/rct}$ mice. The preparation of BAC DNA was performed as previously described [17]. The BAC-tg mice were genotyped using three PCR primer sets designed for the T7 and SP6 promoters of the pBACe3.6 vector and a microsatellite in *Pde6b* intron 6–7 (Supplementary Table 1). All procedures involving animals met the guidelines described in the Proper Conduct of Animal Experiments as defined by the Science Council of Japan and were approved by the Animal Care and Use Committee on the Ethics of the Tokyo Metropolitan Institute of Medical Science and Tokyo University of Agriculture.

2.2. Materials

The primary and secondary antibodies used in this study are listed in Supplementary Table 2. The GFP-tagged FOXE3 and DsRed-tagged PDE6B constructs were produced by subcloning fragments of the *Foxe3* and *Pde6b* open reading frames (ORFs) into the *EcoR*I/*Sal*I site of the pAcGFP1-C1 and pDsRed-Monomer-C1 expression cloning vectors (Clontech, Mountain View, CA), respectively. The ORFs of *Foxe3* and *Pde6b* were amplified by PCR from eye cDNA of B6 mice using the Foxe3_ORF and Pde6b_ORF primer sets (Supplementary Table 1).

2.3. Phenotypic analyses

For observation of lens opacity, pupils of wild-type (B6), B6.SJL-*Foxe3*$^{rct/rct}$, SJL-*Foxe3*$^{rct/rct}$ (non-tg) and SJL-*Foxe3*^{rct} BAC tg (BAC-tg) mice were dilated with Mydrin-P (Santen Pharmaceutical, Osaka), and both eyes were observed after 5 min. For histological analysis,

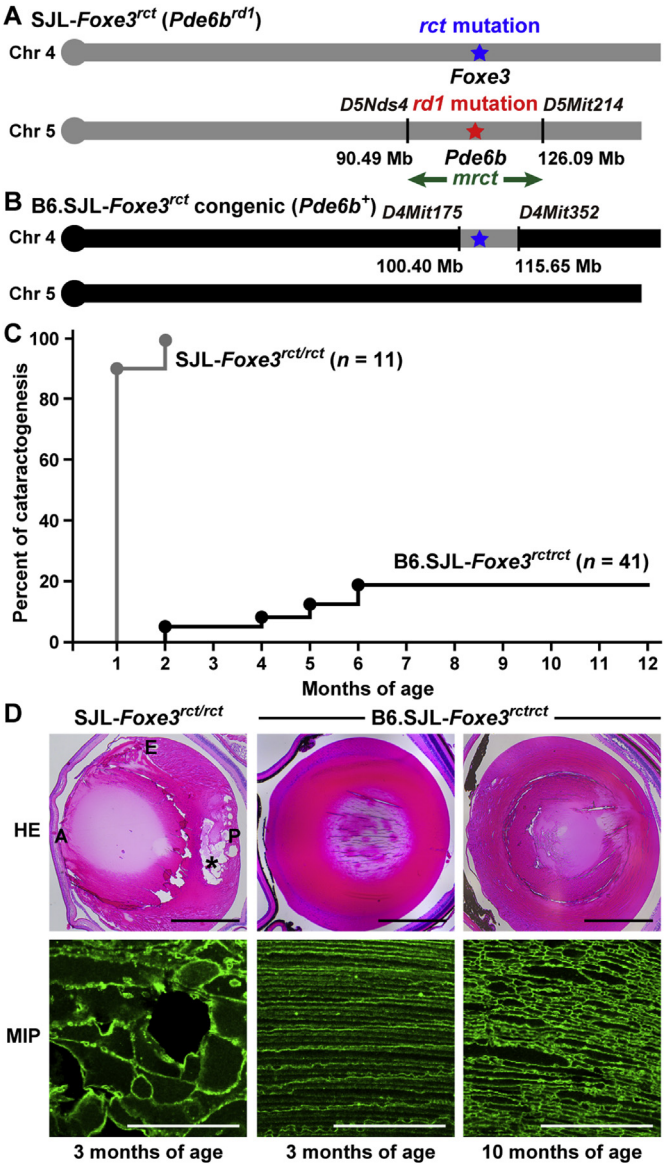

Fig. 1. Reduction of cataract phenotypes in *Foxe3*^{rct} mice by transferal of genetic background. (**A** and **B**) Creation of B6.SJL-*Foxe3*^{rct} congenic mice. Schematic representation of mouse chromosome (Chr) 4 and 5 illustrating the position of the *Foxe3*^{rct} mutation (blue stars) and the *Pde6b*^{rd1} mutation (red star), respectively, in SJL- (**A**) and B6.SJL-*Foxe3*^{rct} mice (**B**). The green bi-directional arrow in **A** represents the maximum genomic interval of the *mrct* locus on Chr 5. The gray area of Chr 4 in **B** represents the SJL-derived chromosomal segment carrying the *Foxe3*^{rct} mutation. (**C**) Comparison of the onset time of cataracts among the SJL- and B6.SJL-*Foxe3*^{rct} mice. The survival analysis graph shows the temporal incidence rate for cataractogenesis in the SJL- (gray line) and B6.SJL-*Foxe3*^{rct} (black line) mice. (**D**) Representative phenotypes of lens in SJL- and B6.SJL-*Foxe3*^{rct} mice at 3 and 10 months of age identified using hematoxylin-eosin staining (HE) and immunofluorescent staining for anti-MIP antibody of lens sections. The images stained with the anti-MIP antibody are magnified lens fiber cells from the equator region (E) of the lens. Asterisks indicates a large vacuole on the lens. A, anterior region; P, posterior region. Scale bar = 100 μm (HE images) and 50 μm (immunofluorescent images). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)