Targeted Disruption of FSCN2 Gene Induces Retinopathy in Mice

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PURPOSE. To investigate the morphology and function of photoreceptors in mice with mutation of the FSCN2 gene.

METHODS. A mouse line was generated carrying the 208delG mutation (point mutation, or p-type) and another with replacement of exon 1 by the cDNA of a green fluorescent protein (GFP knock-in, or g-type). The expression of retinal mRNA was determined by reverse transcription (RT)-polymerase chain reaction (PCR) and in situ hybridization performed on retinal sections. Morphologic analyses of the retinas were performed by light microscopy (LM) and transmission electron microscopy (TEM) and functional analyses by electroretinogram (ERG).

RESULTS. mRNA of FSCN2 was not detected in the retinal mRNA extracted from FSCN22/2 and FSCN2208G2/2 mice. Both FSCN22/2 and FSCN2208G2/2 mice had progressive photoreceptor degeneration with increasing age detected by LM and structural abnormalities of the outer segment (OS) detected by TEM. Both FSCN22/2 and FSCN2208G2/2 mice had depressed rod and cone ERGs that worsened with increasing age.

CONCLUSIONS. These results indicate that haploinsufficiency of the FSCN2 gene may hamper maintenance and/or elongation of the OS disks and result in photoreceptor degeneration, as in human autosomal dominant retinitis pigmentosa. (Invest Ophthalmol Vis Sci. 2005;46:2905–2915) DOI:10.1167/iovs.04-0856

Retinitis pigmentosa (RP) is an ocular disease causing severe loss of central and peripheral vision.1,2 The main clinical features of RP are early onset of night blindness followed by a decrease in the electroretinogram (ERG), constriction and gradual loss of visual fields, and a decrease in visual acuity. There is no effective treatment for the disease, although some researchers are studying gene therapy3 or the implantation of an artificial retina.4 RP can have an autosomal dominant (ADRP), autosomal recessive (ARRP), or X-linked (XLRP) inheritance pattern. Thirteen genes that cause ADRP have been mapped and cloned by genetic analyses.5 Approximately 30% to 40% of patients with ADRP in the United States have mutations in the rhodopsin or RPE65 gene.5 Macular degeneration (MD) is characterized by a reduction of visual acuity, abnormal color vision, and a central scotoma.6 Hereditary MD can have an autosomal dominant (ADMD) or autosomal recessive (ARMD) inheritance pattern. To date, seven genes that lead to MD have been cloned and mapped.7,8 There is also no effective treatment for hereditary MD, although some therapeutic procedures are being tried.7,8

Wada et al.9,10 reported that mutations in the retinal fascin gene (FSCN2) were the cause of ADRP (14 patients from 4 unrelated families) and ADMD (5 patients from 2 unrelated families) in a Japanese cohort. All affected members were heterozygous for the 208delG mutation. The human FSCN2 gene is located at q25 on chromosome 1711 and encodes 516 amino acids.12 It is expressed specifically in the photoreceptors of the retina,12,13 and its products induce actin-binding and bundling.12 These functions indicate that the FSCN2 gene plays an important role in the morphogenesis of photoreceptors, especially in the outer segments (OS).

We generated a mouse line carrying the 208delG mutation (point mutation, or p-type) and another with a replacement of exon 1 by the cDNA of a enhanced green fluorescent protein (GFP knock-in, or g-type), to investigate a possible mechanism of the retinopathy in patients with mutations of the FSCN2 gene. Heterozygotes with both types of mutations showed RP-like photoreceptor degeneration with increasing age but did not show MD-like changes. Retinal mRNA extracted from FSCN22/2 mice lacked mRNA of FSCN2. These results indicate that haploinsufficiency14 of the FSCN2 gene may cause ADRP as in human 208delG heterozygotes.

MATERIALS AND METHODS

Animals

All animal procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Gene Targeting

The FSCN2 genomic clone was obtained from a 129SVJ mouse library. A fragment lacking a guanine at nucleotide 208 of the mouse FSCN2 gene, corresponding to the human 208delG mutation of FSCN2, was generated by polymerase chain reaction (PCR). Mixtures of 256-bp PCR product with one primer pair (5'-ACAAGACAGAGGAGCGACAG-CATTG-3', p21, and 5'-CTCTCTGCAGTAGTTAGCGGTTCG-3', p18) and an 186-bp PCR product with the other primer pair (5'-CGACGCTACTCTGACAGAGGTTG-3', p19, and 5'-ATCCATCT-CACGGGCCCCAGTGCA-3', p30) were used as templates to create a 419-bp fragment lacking guanine, with the sense primer p21 and the antisense primer p30. The fragment replaced the BstXI/SmaI region (284 bp) on the 5' genomic flanking sequence (10.5 kb) subcloned from the Department of Ophthalmology and Molecular Genetics, Tohoku University School of Medicine, Miyagi, Japan; the 2Department of Cell Biology, Japanese Foundation for Cancer Research (JFCR) Cancer Institute, Tokyo, Japan; and the 3Mouse Functional Genomics Research Group, Institute of Physical and Chemical Research (RIKEN), Genomic Sciences Center, Yokohama, Kanagawa, Japan.

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into the EcoRI and KpnI sites of a vector (pBlueScript; Stratagene, La Jolla, CA). The floxed pMC1-neo poly(A) fragment, the 3′ genomic flanking sequence (2.3 kb), and the DTA (diphtheria toxin A-chain) fragment were ligated at the 3′ end of this fragment. An 853-bp fragment was created in which exon 1 was replaced by the cDNA of enhanced green fluorescein protein (EGFP; BD-Clonetech, Palo Alto, CA), in frame. The mixture of a 118-bp PCR product obtained with one primer pair (5′-AGATACCGAGATGCTGGCTCAGG-3′, p17, and 5′-TGCATGGGACTTCTTCTTGAGGCTCGAGCATG-3′, p15) and a 762-bp PCR product with the other primer pair (5′-GCTAGCTCTAAAAGATGGGTAGCGAAGGGAAGGA-3′, p61, and 5′-GGGTAGGACTGCTGCGG-3′, p66) was used as a template to amplify the 853-bp fragment, obtaining EGFP with the sense primer p17 and the antisense primer p66. The fragment was replaced with the Nhel/KpnI region (1521 bp) on the 3′ genomic flanking sequence (2.7 kb) and ligated DTA fragment at the 5′ end of this fragment. Finally, we ligated pMC1-neo poly(A) and 5′ genomic flanking sequence (8.8 kb) on the 3′ end of the fragment. Each targeting vector was linearized with the NotI site and electroporated into J1 embryonic stem (ES) cells. Twelve clones of 120 G418-resistant clones that had a point mutation (p17) and 17 clones of 140 G418-resistant clones in which exon 1 was replaced by EGFP (p16) underwent homologous recombination, as determined by Southern blot analysis. Two p17-positive clones and one p-type-positive clone were injected into C57BL/6 blastocysts, resulting in the birth of male chimeric mice. Germine transmission of the disrupted FSCN2 alleles was determined by mating these chimeric mice with C57BL/6 females.

RT-PCR Analysis

Poly(A) mRNA was extracted from retinas of 8-week-old wild-type, heterozygous, and homozygous mice with a kit (FastTrack; Invitrogen, Carlsbad, CA). cDNA synthesis and PCR were performed with a cDNA cycle kit (Invitrogen). Two primer pairs were used for the detection of the wild-type FSCN2 mRNA. Primers for detecting the 343-bp fragment were 5′-gttcgaagtcgctgccctcaggggctgagc (p25) and 5′-tacccacacgttgtgtctgact-3′ (p24) and for primers detecting the 720-bp fragment were 5′-gagattgaacctcctgacgctggacgg (p101) and 5′-ttctccccacagcggattcgctgact-3′ (p32). The primer pair for β-actin detection as control was 5′-ctctccacagccgccagcag-3′ and 5′-tgctctcagggagggagta g-3′.

In Situ Hybridization

Wild-type, FSCN2+/p, and FSCN2+/m mice were perfused with 4% paraformaldehyde (PFA) in PBS, and the eyes were enucleated and fixed overnight at 4°C with 4% PFA in PBS. The eyes were rinsed in PBS three times, passed through a sucrose gradient (10%, 20%, and 30% in PBS) and then incubated in 30% sucrose-optimal cutting temperature compound, and sectioned at 12-μm thickness with a cryostat. Sections were counterstained by 4′,6-diamino-2-phenylindole (DAP) for 20 minutes, washed by PBS three times, and examined.

Retinal Histology

Specimens were perfused and fixed with 2% PFA plus 2.5% glutaraldehyde (GA) in cacodylate-buffer for 1 week. The eyes were rinsed in PBS twice and were dehydrated in a graded ethanol series. The specimens were infiltrated with mixtures of chloroform and paraffin three times and then embedded in paraffin. The sections were cut at 3 μm along the vertical meridian and stained with hematoxylin and eosin. The thicknesses of the outer nuclear (ONL) and inner nuclear (INL) layers, the number of cells in the ONL and INL, and the length of the OS were measured by light microscopy, as described.18 The measurements were made at several equidistant loci, beginning at the optic nerve head.

Transmission Electron Microscopy

Eyes were perfused and fixed with 2% PFA and 2.5% GA in cacodylate-buffer for 1 week. The anterior segments were removed, and the eyecups were rinsed in 0.1 M PB five times. The eyecups were then osmicated for 1 hour in 2% aqueous solution of osmium tetroxide and dehydrated through ascending ethanol (50%–100%, 10 min/step). After five changes of 100% ethanol, the specimens were passed through propylene oxide twice and left overnight in a 1:1 mixture of propylene oxide and araldite. The specimens were embedded and cured in pure Epon for 6 hours at 80°C. Ultrathin sections were cut with a microtome (EM-Ultracut; Leica, Deerfield, IL), and the sections (80–90 nm) were collected on polyvinyl formal (Formvar)-coated 100-mesh copper grids (SPI, West Chester, PA), and examined with an electron microscope at 80 kV (JEM-1010; JEOL, Tokyo, Japan).

Electroretinograms

For the dark-adapted ERGs, the animals were dark-adapted overnight and prepared for the recordings in dim red light. After dark adaptation for an additional 30 minutes, they were anesthetized by intramuscular injections of xylazine (13 mg/kg) and ketamine (86 mg/kg). The pupils were dilated with 0.1% phenylephrine HCl. The body temperature was maintained close to 38°C with a heating pad. ERGs were recorded with a 1-mm silver-silver chloride wire-loop electrode placed on the cornea, which was anesthetized by topical 1% tetracaine. A stainless-steel needle was inserted subcutaneously on the nose as the reference electrode. Signals were amplified 10,000 times and band-pass filtered from 0.1 to 1000 Hz. The mice were placed in a Ganzfeld bowl, and single-flash ERGs were recorded with increasing light intensities from 0.01 to 10 cd/m² in equal-size, logarithmic steps. Five responses were averaged at each intensity, and the interstimulus interval increased from 10 seconds for 0.01 and 0.1 cd/m² and 60 seconds for 1 and 10 cd/m². Light-adapted ERGs were recorded with 1 and 10 cd/m² flashes on a background of 43 cd/m², to suppress rod responses after 20 minutes of exposure to the background light.

RESULTS

Generation of FSCN2 Knockout and Knock-in Mice

A guanine was deleted from nucleotide 208 of the mouse FSCN2 2 gene, the same deletion that occurs in humans with ADRP or ADMD, for a single-base deleted allele with homologous recombination (Fig. 1A). In another line, exon 1 of the FSCN2 was displaced by GFP for an exon 1-null allele with the same method (Fig. 2A). Each type of mouse (point mutation [p-type], and GFP knock-in [g-type]) was crossed with CAG-Cre transgenic mice to remove the neomycin-resistant genes (Figs. 1A and 2A). The single-base deletion and displacement of exon 1 were confirmed by Southern blot analyses (Figs. 1B, 2B). For LM, transmission electron microscopy (TEM), and ERGs, mice without neomycin-resistant genes were used. FSCN2+/p neom(+)+ and (−), FSCN2+/m neom(+) and (−), FSCN2+/p neom(+) and (−), and FSCN2+/m neom(+) and (−) mice were morphologically normal, viable, and fertile.
In the 8-week-old *FSCN2* neon(−) retina, green fluorescence was detected in the photoreceptors, although no signals were observed in 8-week-old, wild-type retinas (Figs. 2C–H).

**Expression of *FSCN2* mRNA in *FSCN2* Mutant Mice**

RT-PCR was performed to evaluate the expression of mouse *FSCN2* mRNA in samples extracted from 8-week-old wild-type, *FSCN2* neon(+) and neon(−), *FSCN2* neon(+) and neon(−), *FSCN2* neon(+) and neon(−), and *FSCN2* neon(+) and neon(−) mice (Fig. 3A). The mRNA of *FSCN2* was not detected with the pair of primers (p11 and p32) that detected cDNA of *FSCN2* downstream of the GFP poly(A) sequence in *FSCN2* neon(+) and neon(−) mice (Fig. 3B). In *FSCN2* neon(+) and neon(−) mice, the mRNA of *FSCN2* was also not observed with p11 and p32 and was not detected with another pair of primers (p23 and p24) that detected cDNA of *FSCN2* downstream of the deleted guanine (Fig. 3B).

In situ hybridization with a cRNA riboprobe specific for the 3′ end of the mRNA of *FSCN2* was performed on retinal sections of 8-week-old wild-type and *FSCN2* neon(−) mice (Fig. 3A). In the wild-type retina, hybridization signals for mRNA of mouse *FSCN2*...
were found in the inner segment (IS) and the ONL, but no signal was observed in the OS of the photoreceptors and in other layers of the retina (Fig. 3D). Signals for the mRNA of \(FSCN2\) were not detected in any layers, including the IS and the ONL in \(FSCN2^{p/p}\) neo\((/H11002)\) retina (Fig. 3E).

**Light Microscopic Analysis of Retina of \(FSCN2\) Mutant Mice**

The length of the OS, thickness of the ONL, and thickness of the INL were measured in retinal sections from 4-, 8-, 16-, and 24-week-old wild-type, \(FSCN2^{+/H11001}\) neo\((-)\), and \(FSCN2^{p/p}\) neo\((-)\) mice, and the number of nuclei in the ONL and INL were counted as described. At 4 weeks of age, the OS in the central retinal region of \(FSCN2^{+/H11001}\) neo\((-)\) mice were shorter than in the wild-type \((P < 0.05)\), and the ONL was slightly thinner \((P < 0.05)\), with fewer nuclei than in the wild-type mice (Figs. 4A, 4B, 5). \(FSCN2^{2908\text{GFP}}\) neo\((-)\) mice at the same age had much shorter OS than did the \(FSCN2^{+/H11001}\) neo\((-)\) mice, but the thickness and cell counts in the ONL were comparable to those in the \(FSCN2^{+/H11001}\) neo\(-)\) mice (Figs. 4B, 4C, 5). The thickness of the INL of \(FSCN2^{+/H11001}\) neo\(-)\) and \(FSCN2^{2908\text{GFP}}\) neo\(-)\) mice was almost the same as in wild-type mice, and cell counts in the INL of \(FSCN2^{+/H11001}\) neo\(-)\) and \(FSCN2^{2908\text{GFP}}\) neo\(-)\) mice were similar to wild-type (Figs. 4A-C, 6).
At 24-week, the FSCN2+/p neo(-) mice had significantly shorter OS \((P < 0.01)\), thinner ONL \((P < 0.01)\), and thinner INL \((P < 0.05)\), with reduced cell counts in the ONL and the INL compared with those in the wild-type mice (Figs. 4D, 4E, 5A-C, 6). The FSCN2+/p neo(-) mice at the same age had significantly shorter OS and a significantly thinner ONL and INL with fewer nuclei than the FSCN2+/p neo(-) mice (Figs. 4E, 4F, 5, 6). In the p-type neo(+) and (-) mice, the difference in the length of the OS and thickness of the ONL and the number of nuclei in the ONL were not significantly different (data not shown).

The retinal morphologies of the FSCN2+/p neo(-) and FSCN2+/p neo(-) mice were comparable to that of FSCN2+/p neo(-) and FSCN2+/p neo(-) mice, respectively (Figs. 4G–J, 5, 6). No significant differences in the length of the OS, thickness of the ONL, thickness of the INL, or the number of nuclei in the ONL and INL were observed between the g-type neo(+) and (-) mice (data not shown).

**TEM Analysis of Retina of FSCN2 Mutant Mice**

The ultrastructure of the retinas, especially the OS of the wild-type, FSCN2+/p neo(-), and FSCN2+/p neo(-) mice, was examined by TEM. At 4 weeks, the OS of FSCN2+/p neo(-) retina appeared very similar to that of wild-type mice, but the...
OS of FSCN2p/p neo(−) photoreceptors were extremely bent (Figs. 7A–C). There was no significant difference in the alignment of OS discs between wild-type and FSCN2+/p neo(−)/H11002 mice (Figs. 7K, 7L), but misalignment of OS discs was observed at the OS of FSCN2p/p neo(−) mice, especially near the distal end of connecting cilium (Fig. 7M).

At 24 weeks, the retinas of FSCN2+/p neo(−)/H11002 and FSCN2p/p neo(−) mice had considerably shorter OS (Figs. 8A–C). The FSCN2+/p neo(−) retina had much shorter OS and thinner ONL than did the wild-type. The thickness of the INL was almost the same as in the wild-type. (C) Retinal section of 4-week-old FSCN2+/p neo(−)/H11002 mice. The FSCN2+/p neo(−) retina had much shorter OS and thinner ONL than did the wild-type. The thickness of the INL was very similar to that in the wild-type. (D–F) Sections of the central retina of 24-week-old (D) wild-type and (E) FSCN2+/p neo(−)/H11002 mice. The FSCN2+/p mice had significantly shorter OS and thinner ONL and INL, with reduced cell counts in the ONL and INL than did the wild-type. (F) Retinal section of 24-week-old FSCN2+/p neo(−)/H11002 mice. The FSC2+/p neo(−) mice had significantly shorter OS and thinner ONL and INL, with fewer nuclei than did the FSCN2+/p neo(−). (G–J) Retinal sections of central retina from 4-week-old (G) FSCN2+/g neo(−) and (H) FSCN2+/p neo(−)/H11002 mice and 24-week-old (I) FSCN2+/p neo(−) and (J) FSCN2+/p neo(−)/H11002 mice. Hematoxylin-eosin; magnification, ×400.
agreeing with the LM analyses. Misalignment of OS discs was not observed in wild-type and FSCN2/p neo mice, but that of FSCN2p/p neo mice was significantly stronger. The thickness and shape of the connecting cilium were not significantly different among all mice (Figs. 7N–P). There was no significant difference in the morphologic changes between the p-type neo(+) and (-) mice (data not shown).

The ultrastructure of the retinas of FSCN2+/+ and FSCN2+/- mice was comparable to that in FSCN2+/- and FSCN2-/- mice, respectively (Figs. 7G–J). No significant morphologic differences were observed.

**FIGURE 5.** Time course of the changes in the central retina (relative distance from the optic nerve head is 0.2) of (A) thickness of the ONL, (B) nuclei in the ONL, and (C) the length of the OS in the central retina. In both mutant homozygous mice and mutant heterozygous mice, the OS were shorter, the ONL were thinner, and the number of the nuclei in the ONL decreased with increasing age. Data are the mean ± SEM. +/+; wild-type; +/+ FSCN2+/+; p/p, FSCN2p/p; +/g, FSCN2+/g; g/g, FSCN2g/g; n = 6 for each genotype.

**FIGURE 6.** Time course of the changes in the central retina (relative distance from optic nerve head is 0.2) of (A) the thickness of the INL and (B) the nuclei in the INL. In both mutant homozygous and mutant heterozygous mice, the INL was thinner and the number of the nuclei in the INL decreased with increasing age. Data are the mean ± SEM. Labels for genotypes are as described in Figure 5; n = 6 for each genotype.
observed between the g-type neo(+) and (-) mice (data not shown).

**ERGs of FSCN2 Mutant Mice**

ERGs were recorded in the wild-type, FSCN2+/p neo(-), and FSCN2p/p neo(-) mice under dark- and light-adapted conditions and at different ages to evaluate retinal function in vivo. At 4 weeks, the scotopic a- and b-wave amplitudes of FSCN2+/p neo(-) mice were similar to those in wild-type mice (Figs. 8A, 8B, 9A, 9B). But at 24 weeks, both the a- and b-waves were significantly smaller in the FSCN2+/p neo(-) mice than in the wild-type mice (P < 0.01; Figs. 8D, 8E, 9A, 9B). The ERGs of FSCN2p/p neo(-) mice were significantly smaller than those of FSCN2+/p neo(-) at 4 weeks and progressively decreased with increasing age (Figs. 8C, 8F, 9A, 9B).

At 4 weeks, the photopic b-wave amplitudes of FSCN2+/p neo(-) mice were comparable to those of the wild-type mice.
were shorter than in wild-type mice of the same age, and the shortening was greater in older mice. In the TEM analyses, the OS of FSCN2+/b and FSCN2+/r mice were shorter, agreeing with the LM examination. These observations suggest that the formation of discs is delayed in the photoreceptors of FSCN2+/b and FSCN2+/r mice. The thinning of the INL in FSCN2+/b, FSCN2+/p, FSCN2+/r, and FSCN2+/G mice followed photoreceptor degeneration. These findings suggest that the degeneration of bipolar cells in the INL was among the secondary changes.

In contrast, the length of the OS of FSCN2+/p and FSCN2+/G at 4 weeks of age was significantly shorter than that of FSCN2+/b and that of FSCN2+/b at the same age, and the OS were extremely bent in the TEM analyses. The length of the OS of FSCN2+/p and FSCN2+/G also shortened with increasing age.

The question then arises as to the mechanism causing the photoreceptor degeneration. There is a meshwork of actin filaments at the distal end of the connecting cilium,17–20 Individual actin filaments radiate from the meshwork into the base of OS disks by passing between pairs of ciliary microtubule doublet.21 Rhodopsin is densely packed in the membrane of the photoreceptor connecting cilia and axonemal actin in the connecting cilium, which is spatially colocalized with myosin VIIa and opsin.22 These observations suggest that the actin filament network provides cytoskeletal support and guidance for the growing OS disks, and mediates the trafficking of membrane components including rhodopsin. In situ hybridization demonstrated hybridization signals at the inner segments of photoreceptors in wild-type mice, but no signal was observed in FSCN2+/b mice. The OS disks of FSCN2+/b and FSCN2+/G retinas were misaligned at the distal end of the connecting cilium, and this misalignment was stronger with increasing age. FSCN2 includes actin binding and actin-bundling activity in vitro.24 In vivo, Drosophila fascin (singed) is necessary for actin bundle assembly in developing bristles and in the nurse cell cytoplasm of egg chambers.25 When Drosophila nurse cell cytoplasmic actin bundles are absent, the rapid phase of nurse cell cytoplasm transport is blocked, and mature eggs are only 50% the size of wild-type eggs. In severely singed mutants, the small disorganized actin filament bundles lack structural integrity and allow bristles to bend, like the OS of FSCN2+/p and FSCN2+/G. Taken together, the observations in the FSCN2+/p and FSCN2+/G retinas imply that mutations of FSCN2 may hamper maintenance and/or elongation of OS disks and decrease the function of the photoreceptors.

Recent molecular genetic analyses have shown that inherited retinal degeneration has allelic and nonallelic heterogeneity and that the phenotype depends on the type of mutation. Thus, mutations of peripherin/RDS and ABCA4 genes can lead to MD or RP in humans,24–26 and an identical 1147delA mutation in the arrestin gene is the cause not only of Oguchi disease but also of autosomal recessive RB2.27–29 In our mice, the photopic a- and b-wave amplitudes of FSCN2+/b and FSCN2+/G decreased with increasing age, corresponding to the histologic changes. The 208delG mutation in the FSCN2 gene causes AMD at a young age in humans, with degeneration mainly of the cones, and it also causes ADRP in humans. The photopic b-wave amplitudes of all heterozygous FSCN2+/b and FSCN2+/G mice, however, decreased much later than the decrease in the scotopic b-wave amplitudes. These observations suggest that the photoreceptor degeneration in FSCN2 mutant mice resembles RP, but not MD, in humans. The mouse retina has significantly fewer cones than does the human retina, and there is no manifest macula as in primates. It is unclear whether these anatomic differences and/or other factors affect the phenotypes observed in FSCN2 mutant mice. Further analysis may help to resolve this question.
RT-PCR analyses demonstrated that mouse FSCN2 mRNA was not transcribed in the g-type allele because the transcription was prevented by a poly(A) signal added to the 3' end of the cDNA of GFP. Alternatively, our results showed that all the FSCN2 mRNA was degraded in both FSCN2<sup>p/p</sup> neo<sup>+</sup> and <sup>−</sup> mice. A nonsense-mediated decay mechanism may degrade the mRNA with an abnormal stop codon (codon 140).<sup>30</sup> The p-type mutant mice with a single-base deletion were phenotypically similar to the g-type mutant mice that did not have exon 1 of the FSCN2 gene. These results suggest that the mutant FSCN2 gene (208delG mutation) did not produce FSCN2 protein, and therefore haploinsufficiency of the FSCN2 protein may cause RP in human heterozygotes.

In conclusion, we produced FSCN2 mutant mice by gene targeting techniques. The FSCN2<sup>p/p</sup> and FSCN2<sup>+/−</sup> mice had decreased rod function that worsened with increasing age and was followed by reduction of cone function. In patients with RP, rod photoreceptor abnormalities precede the onset of cone abnormalities typically.<sup>31</sup> Therefore, the phenotypes of our mice may resemble RP, not MD, in human heterozygotes. Our results suggest that haploinsufficiency of FSCN2 may alter maintenance and/or elongation of OS disks and induce photoreceptor degeneration. However, more detailed analyses of photoreceptor morphogenesis in these mouse models are needed to understand the mechanism of photoreceptor degeneration. We suggest that these mice are good models to study...
the mechanisms for the changes found in human ADRP caused by mutations of the FSCN2 gene.

References