Mamm Genome (2008) 19:145–154
DOI 10.1007/s00335-008-9092-2

ORIGINAL CONTRIBUTIONS

Mapping of genetic modifiers of *Nr2e3*$^{rd7/rd7}$ that suppress retinal degeneration and restore blue cone cells to normal quantity

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Received: 28 August 2007 / Accepted: 18 December 2007 / Published online: 20 February 2008

Abstract The retinal degeneration 7 (*rd7*) mouse, lacking expression of the *Nr2e3* gene, exhibits retinal dysplasia and a slow, progressive degeneration due to an abnormal production of blue opsin-expressing cone cells. In this study we evaluated three strains of mice to identify alleles that would slow or ameliorate the retinal degeneration observed in *Nr2e3*$^{rd7/rd7}$ mice. Our studies reveal that genetic background greatly influences the expression of the *Nr2e3*$^{rd7/rd7}$ phenotype and that the inbred mouse strains CAST/EiJ, AKR/J, and NOD.NON-*H2*^{nb1} carry alleles that confer resistance to *Nr2e3*$^{rd7/rd7}$-induced retinal degeneration. B6.Cg-*Nr2e3*$^{rd7/rd7}$ mice were outcrossed to each strain and the F_1 progeny were intercrossed to produce F_2 mice. In each intercross, 20–24% of the total F_2 progeny were homozygous for the *Nr2e3*$^{rd7/rd7}$ mutation in a mixed genetic background; approximately 28–48% of the *Nr2e3*$^{rd7/rd7}$ homozygotes were suppressed for the degenerative retina phenotype in a mixed genetic background. The suppressed mice had no retinal spots and normal retinal morphology with a normal complement of blue opsin-expressing cone cells. An initial genome scan revealed a significant association of the suppressed phenotype with loci on chromosomes 8 and 19 with the CAST/EiJ background, two marginal loci on chromosomes 7 and 11 with the AKR/J background, and no significant QTL with the NOD.NON-*H2*^{nb1} background. We did not observe any significant epistatic effects in this study. Our results suggest that there are several genes that are likely to act in the same or parallel pathway as *NR2E3* that can rescue the *Nr2e3*$^{rd7/rd7}$ phenotype and may serve as potential therapeutic targets.

Electronic supplementary material The online version of this article (doi:10.1007/s00335-008-9092-2) contains supplementary material, which is available to authorized users.

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Introduction

Development of the neural retina is a highly coordinated event that involves transcriptional regulation of mitotic and postmitotic retinal cells to generate several different retinal cell types (Carter-Dawson and LaVail 1979; Young 1985a, b) to produce the mature retina (Fruend et al. 1997; Furukawa et al. 1997; Mears et al. 2001; Ng et al. 2001; Nishida et al. 2003; Zhang et al. 2004).

In this study, we use the *rd7* mouse model, lacking the transcription factor *Nr2e3*, in genetic modifier screens to identify alleles that can moderate or suppress retinal degeneration. The *Nr2e3*$^{rd7/rd7}$ retina is characterized clinically by retinal spots that are apparent at eye opening and histologically by whorls and rosettes that appear at

P12, just prior to retinal maturation. An increase in the number of blue opsin-expressing cone cells and a slow, progressive degeneration of rod and cone photoreceptors are apparent as early as one month of age (Akhmedov et al. 2000; Haider et al. 2001, 2006; Hawes et al. 1999). Mutations in human *NR2E3* result in Enhanced S Cone Syndrome (ESCS) and cause retinal defects similar to those observed in the *Nr2e3*$^{rd7/rd7}$ mouse. ESCS patients have night blindness and an unusual electroretinogram (ERG) caused by a reduction in rod and in L and M cone numbers, and a significant increase in S cones that form aberrant synapses (Haider et al. 2000; Jacobson et al. 1990, 2004; Marmor et al. 1990; Milam et al. 2002).

Nr2e3 is a member of the ligand-dependent family of nuclear hormone receptors and has been shown to be critical for the development and function of rod and cone photoreceptors. Recent studies suggest that NR2E3 directly interacts with NRL and CRX to regulate the expression of rod photoreceptor genes during differentiation while suppressing the expression of cone-specific genes (Chen et al. 2005; Corbo and Cepko 2005; Hong et al. 2004; Peng et al. 2005). Our most recent work suggests that NR2E3 regulates cone cell proliferation and that lack of *Nr2e3* causes the extra blue cones observed in *Nr2e3*$^{rd7/rd7}$ mutant retinas (Haider et al. 2006).

Many examples of variable expressivity of disease phenotypes exist in the literature, demonstrating that primary disease-associated mutations manifest relative to the genetic background in which they occur. These variations in disease phenotypes can result from allelic heterogeneity, environmental influences, genetic modifiers, or a combination of these factors (Hokanson et al. 1999; Mucci et al. 2001; Vineis 2001; Whalley 2001; Zielenski 2000). There are several heritable retinal diseases in humans such as Bardet-Biedl Syndrome (BBS) (Carmi et al. 1995; Riise et al. 1997), retinitis pigmentosa 1 (RP1) (Jacobson et al. 2000), *Crx* mutation-associated retinal disease (Chen et al. 2002), and ocular retardation associated with *Chx10* (Wong et al. 2006), all of which appear to be affected by genetic modifiers (reviewed by Haider et al. 2002). Interestingly, it has recently been shown that the *RET* gene, associated with Hirschsprung disease (HSCR), can also act as a modifier gene for the HSCR phenotype in patients with BBS as well as patients with Down syndrome and congenital hypoventilation (de Pontual et al. 2007). QTLs have also been identified that affect light-induced retinal degeneration (Danciger et al. 2004), and some modifier genes in *Drosophila* have been shown to be dosage-sensitive interactors with the neural patterning gene *Rap/Fzr* (Kaplow et al. 2007). Modifier genes are likely to function in the same or parallel pathway as the primary gene mutated in a given disorder. As such, they can potentially have significant impact on disease manifestation, progression, and ultimately phenotypic outcome. However, due to genetic heterogeneity it is difficult to identify modifier genes in humans. Identification of modifiers in mouse models, on the other hand, is tractable. Discovering genetic modifiers in mouse may provide insights into both the function and pathologic consequences of the primary disease genes and the pathway(s) in which they function and, therefore, identify new therapeutic targets.

In a previous study (Akhmedov et al. 2000), we observed that the expression of retinal degeneration in homozygous *Nr2e3*$^{rd7/rd7}$ mice was variably attenuated by genetic background. In this study, we confirm that the expression of the *Nr2e3*$^{rd7/rd7}$ mutant phenotype is highly dependent on genetic background. Furthermore, our studies show that the resistant alleles confer complete suppression of the phenotype in combination with all three genetic backgrounds tested. Suppressed mice have normal retinal morphology and photoreceptor function and a restoration of the normal number of blue opsin-expressing cone cells. Several loci that are associated with resistance to retinal degeneration in mice homozygous for the *Nr2e3*$^{rd7/rd7}$ mutation were detected. Interestingly, none of the loci overlapped in the different intercrosses examined, indicating that a large number of factors are able to ameliorate the disease phenotype caused by a mutation in *Nr2e3*. In addition, a suggestive locus that confers resistance to retinal degeneration in *tub* mice (Ikeda et al. 2002) appears to also suppress retinal degeneration in *Nr2e3*$^{rd7/rd7}$ mice, indicating the potential for a common modifier for the degenerative process in retinas.

Materials and methods

Animals

The mice used in this study were bred and maintained under standard conditions either in the research vivarium at The Jackson Laboratory or at the University of Nebraska Medical Center. Mouse strains used for this study were B6.Cg-*Nr2e3*$^{rd7/rd7}$, C57BL/6J (B6), AKR/J, CAST/EiJ, and NOD.NON-*H2*^{nb1}. B6.Cg-*rd7/rd7* mice were outcrossed to AKR/J, CAST/EiJ, and NOD.NON-*H2*^{nb1}. F_1 offspring from each of these outcrosses were intercrossed to generate F_2 mice, which were genotyped as previously described to identify those mice that were homozygous carriers of the *Nr2e3*$^{rd7/rd7}$ mutation (Haider et al. 2001).

Clinical examination

F_2 mice were evaluated by indirect ophthalmoscopy using the Keeler Vantage Indirect Ophthalmoscope with at 60-diopter lens for fundus examinations. Pupils were dilated with 1% atropine. Fundus photographs were taken using a Kowa Genesis small-animal camera. All homozygous

Nr2e3$^{rd7/rd7}$ F_2 mice were examined at 8 weeks and 20 weeks. In addition, at minimum, three F_2 *Nr2e3*$^{rd7/rd7}$ mutant and suppressed mice from each intercross were examined at 1 year of age.

Immunohistochemistry and histologic staining

Immunohistochemical analysis was performed on 6-μm serial sections of eyes from F_2 wild-type controls and phenotypically affected and suppressed *Nr2e3*$^{rd7/rd7}$ mice. Paraffin-embedded eyes were fixed with either 4% PFA or methanol/acetic acid (3:1) and maintained in a dorsal/ventral orientation. After blocking with 2% normal goat or horse serum (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (PBS), sections were incubated with a polyclonal blue opsin antibody (1:200, Chemicon, Millipore, Billerica, MA) at 4°C overnight. The next day samples were rinsed and incubated in a secondary antibody (anti-rabbit Alexa488 at a 1:400 dilution, Molecular Probes, Invitrogen, Carlsbad, CA). Images from sections were collected on a Zeiss Axioplan II fluorescent microscope equipped with the appropriate bandpass filter.

Electroretinography

After at least 6 h of dark adaptation, mice were anesthetized with an intraperitoneal injection of normal saline solution containing ketamine (15 mg/g) and xylazine (7 mg/g body weight). Electroretinograms (ERGs) were recorded from the corneal surface of one eye after pupil dilation (1% atropine sulfate) using a gold loop electrode referenced to a gold wire in the mouth. A needle electrode placed in the tail served as ground. A drop of methylcellulose (2.5%) was placed on the corneal surface to ensure electrical contact and to maintain corneal integrity. Body temperature was maintained at a constant temperature of 38°C using a heated water pad. All stimuli were presented in a Ganzfeld dome (LKC Technologies, Gaithersburg, MD) whose interior surface was painted with a highly reflective white matte paint (No. 6080; Eastman Kodak, Rochester, NY). Stimuli were generated with a Grass Photic Stimulator (model PS33 Plus; Grass Instruments, Worcester, MA) affixed to the outside of the dome at 90° to the viewing porthole. Dark-adapted responses were recorded to short wavelength ($\lambda_{max} = 470$ nm; Wratten 47A filter) flashes of light over a 4.0 log unit range of intensities (0.3 log unit steps) up to the maximum allowable by the photic stimulator. Light-adapted responses were obtained with white flashes (0.3 step) on the rod-saturating background after 10 min of exposure to the background light to allow complete light adaptation. Responses were amplified (Grass CP511 AC amplifier, ×10,000; 3 dB down at 2 and 10,000 Hz) and digitized using an I/O board (model PCI-1200; National Instruments, Austin, TX) in a personal computer. Signal processing was performed with custom software (LabWindows/CVI; National Instruments). Signals were sampled every 0.8 ms over a response window of 200 ms. For each stimulus condition, responses were computer averaged with up to 50 records for the weakest signals. A signal rejection window could be adjusted during data collection to eliminate electrical artifacts.

Linkage analysis

A genome scan was performed using 60–80 dinucleotide repeat markers at approximately 20-cM intervals for each scan (Supplementary Table 1). R/QTL v1.04-53 was used for linkage analysis (Broman et al. 2003). Three main analyses were performed. Using a one-QTL model, a whole-genome scan was performed. One thousand permutations were performed to determine the thresholds for selection of candidate QTLs (Doerge and Churchill 1996). QTLs above 1% threshold were treated as strong QTLs ($p \leq 0.01$ was considered significant), while those at 63% or greater were considered suggestive QTLs (Lander and Kruglyak 1995). Next, a genome-wide pairwise QTL scan was performed using 2-cM spacing and 128 imputations; $p \leq 0.001$ was considered significant. For this analysis, observed markers were used in combination with pseudomarkers on each chromosome to create a 2-cM simulated spacing with 128 imputations (Sen and Churchill 2001). All possible pairs of QTL locations on each chromosome were tested for association with the phenotype. The likelihood from the full model (pseudomarker pair and the interaction between them) and the null model (no genetic effect) was compared and LOD scores were calculated. In addition, LOD scores from comparing the full model likelihood and the additive model likelihood (with only the main effects of pseudomarkers and but no interaction) were also calculated for detection of QTL*QTL interaction. Finally, QTLs and QTL*QTL interactions identified from a single QTL scan and pairwise scan were fitted into multiple-regression models.

Results

Suppression of *rd7* retinal spotting observed in some F_2 *Nr2e3*$^{rd7/rd7}$ intercross mice from three divergent genetic backgrounds

Through our previous studies we generated congenic B6.Cg-*Nr2e3*$^{rd7/rd7}$ mice by backcrossing the mutation that arose spontaneously on a mixed genetic background onto the C57BL/6J (B6) inbred strain for ten consecutive generations. To determine whether alleles from other genetic

backgrounds could delay or ameliorate the retinal degeneration observed in homozygous *Nr2e3*$^{rd7/rd7}$ mice, we intercrossed the *Nr2e3*$^{rd7/rd7}$ mutant with three different strains: AKR/J, CAST/EiJ, and NOD.NON-*H2*^{nb1}. For a recessive mutation, one would expect approximately 25% of the F_2 population to be homozygous for the segregating mutation. We observed that 20–24% of F_2 mice were homozygous for the *Nr2e3*$^{rd7/rd7}$ allele within each intercross, with the CAST/EiJ intercross having the lowest number of genotypically affected mice (Table 1). Approximately 28–49% of the F_2 *Nr2e3*$^{rd7/rd7}$ mice from each intercross did not exhibit the retinal spotting normally observed in B6.Cg-*Nr2e3*$^{rd7/rd7}$ mice (Table 1, Fig. 1). Suppressed mice aged to 1 year also showed normal fundi (data not shown). This is in contrast to what we observe in an inbred B6 background in which all mice that are homozygous for the mutation are phenotypically affected. The phenotypic distribution suggests that multiple modifiers are likely to be conferring resistance/susceptibility to the clinical phenotype.

Nr2e3$^{rd7/rd7}$ modifiers suppress both aberrant blue cone proliferation and retinal degeneration

F_2 *Nr2e3*$^{rd7/rd7}$ mice that did not show a clinical phenotype (e.g., retinal spotting) from each intercross were evaluated by histology (hematoxylin and eosin staining) and immunohistochemistry with a blue opsin antibody to determine whether there was partial or complete suppression of the blue cone proliferation that leads to retinal dysplasia. Consistent with the clinical observations, the F_2 mice that did not show retinal spotting displayed a normal retinal morphology as well (Fig. 2). Suppressed mice aged to 1 year also exhibited a normal retinal morphology suggesting a true suppression of the phenotype and not a delay of retinal dysplasia and subsequent degeneration (Fig. 2). In contrast, the clinically affected F_2 *Nr2e3*$^{rd7/rd7}$ mice had retinal dysplasia with whorls and pseudorossette formation similar to that observed in B6.Cg-*Nr2e3*$^{rd7/rd7}$ mice.

Immunohistochemical analyses using a blue opsin antibody were performed to determine if the suppression in the F_2 *Nr2e3*$^{rd7/rd7}$ mice extended to restoring the normal complement of blue cone cells. F_2 littermates, including wild-type controls, and affected and suppressed *Nr2e3*$^{rd7/rd7}$ mice were evaluated within the same genetic background to distinguish between expression differences that were due to the strain background and those related to the mutation. All F_2 *Nr2e3*$^{rd7/rd7}$-suppressed mice had a normal complement of blue cone cells as well as a normal total number of cone cells (Fig. 3). Furthermore, electroretinography (ERG) at 2–7 months showed no loss of cone function in suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice (Fig. 4). Rod ERGs were also similar between suppressed and control mice (data not shown).

Localization of *Nr2e3*$^{rd7/rd7}$ suppressor loci to multiple chromosomes

A genome scan was performed on each intercross using approximately 100 F_2 mice homozygous for the *Nr2e3*$^{rd7/rd7}$

Table 1 F_2 mice from three different intercrosses generated for genome-wide scans to identify modifiers of *Nr2e3*$^{rd7/rd7}$ retinal phenotype

Strain	Total F_2 mice collected of all genotypes	Total F_2 mice of *Nr2e3*$^{rd7/rd7}$ genotype (%)	Total F_2 mice supppressed (% of *Nr2e3*$^{rd7/rd7}$)	Total affected/total suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice used for genome-wide scan
CAST/EiJ	1046	214 (20.50%)	61 (28.5%)	87/48
AKR/J	513	111 (22%)	55 (49%)	40/55
NOD.NON-*H2*^{nb1}	687	166 (24.20%)	56 (33.70%)	73/51

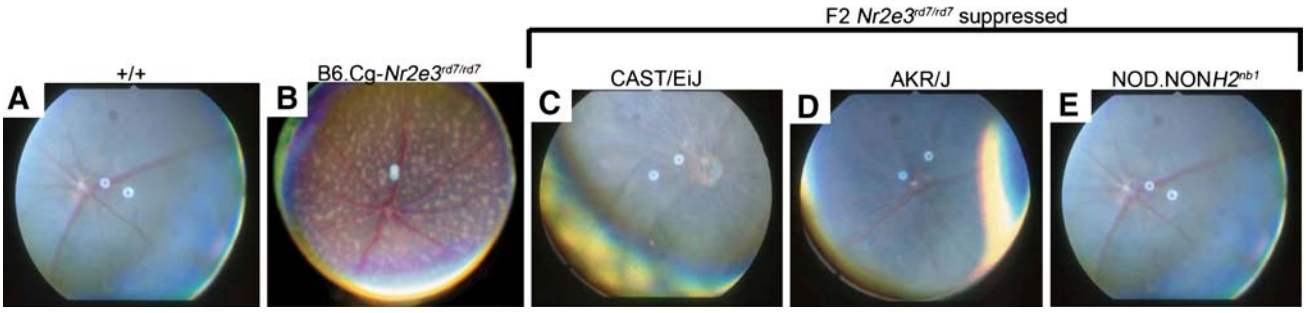

Fig. 1 Fundus photographs depicting normal fundus in suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice. B6 wildtype (**A**) and *Nr2e3*$^{rd7/rd7}$-affected (**B**) fundus photos at 8 weeks shown for comparison. Twenty-week-old F_2 suppressed mice under CAST/EiJ (**C**), AKR/J (**D**), or NOD.NON-*H2*^{nb1} (**E**) genetic backgrounds show no retinal spots

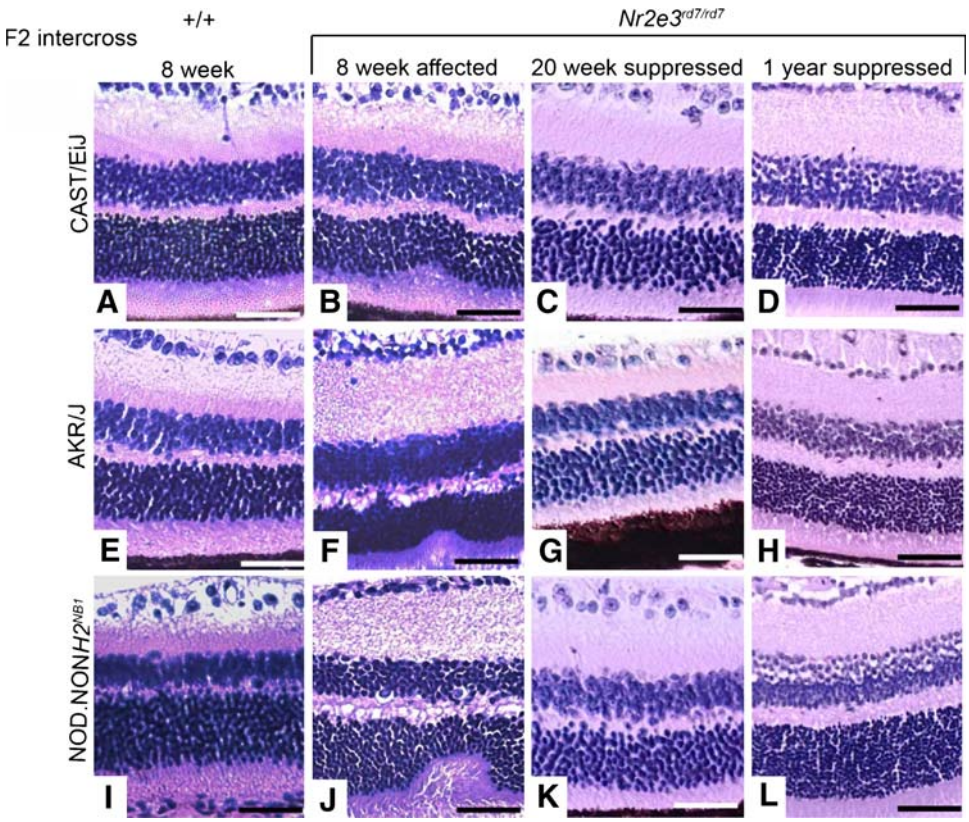

Fig. 2 Hematoxylin and eosin staining of retinal sections showing morphology of F_2 wild-type control and F_2 *Nr2e3*$^{rd7/rd7}$ mice from intercrosses between C57BL/6 and CAST/EiJ (**A–D**), AKR/J (**E–H**), or NOD.NON-*H2*^{nb1} (**I–L**). Retinal dysplasia is not observed in suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice under any of these genetic backgrounds at 20 weeks (**C,G,K**) or 1 year of age (**D,H,L**)

mutation. All mice were genotyped and phenotyped at 8 and 20 weeks. We collected at minimum 400 F_2 animals to identify approximately 100 samples for each genome scan (Table 1). In each scan, 60–80 short tandem repeat polymorphisms (STRPs) were tested at approximately 20-cM intervals across the mouse genome to identify regions associated with the suppressed phenotype.

The genetic data were analyzed using R/QTL v1.04-53 software that allows for detection and localization of quantitative trait loci (QTLs) using a multiple regression model to estimate statistical significance in support of linkage as well as a permutation analysis (Doerge and Churchill 1996; Lander and Kruglyak 1995; Sen and Churchill 2001). Candidate loci were then evaluated against the whole genome using a multiple-regression model. Through these analyses we identified two significant single suppressor loci on chromosomes 8 and 19 for the Cast/EiJ strain and two suggestive loci for the AKR/J strain on chromosomes 7 and 11 (Fig. 5, Table 2). Because the chromosome 11 AKR/J locus overlapped with one identified for suppression of retinal degeneration in the *Tubby* mouse (Ikeda et al. 2002), in the same background strains we decided to evaluate whether this region alone could confer resistance to retinal degeneration in *Nr2e3*$^{rd7/rd7}$ mice. Incipient congenic B6.AKR-Chr11 *D11Mit30-D11Mit360* mice (N6) were mated with B6.Cg-*Nr2e3*$^{rd7/rd7}$ mice to generate F_1 and subsequently F_2 mice that were homozygous for the *Nr2e3*$^{rd7/rd7}$ mutation and heterozygous or homozygous for the AKR alleles on chromosome 11. Similar to our mapping cross, approximately 45% of these mice exhibit a suppressed phenotype histologically ?tul?> (data not shown), suggesting a modifier with incomplete penetrance on chromosome 11 or, alternatively, the incipient congenic may be harboring AKR/J alleles in other regions that interact with B6 alleles to modify the *Nr2e3*$^{rd7/rd7}$ phenotype.

Discussion

Several factors can influence the phenotypic outcome resulting from a disease-associated mutation. These include alternate alleles (allelic heterogeneity), environmental influences, genetic modifier loci, or a combination of these factors (Friedman et al. 2000; Hokanson et al. 1999; Mucci et al. 2001; Vineis 2001; Whalley 2001; Zielenski 2000). For example, recent studies reveal allelic effects in patients with compound heterozygous mutations in *NR2E3* which lead to an atypical mild ESCS phenotype (Lam et al. 2007), and a heterozygous mutation in the first zinc finger of *NR2E3* has been associated with autosomal dominant retinitis pigmentosa (Coppieters et al. 2007). It is possible that both of these phenotypes could be influenced by modifier genes because typically patients

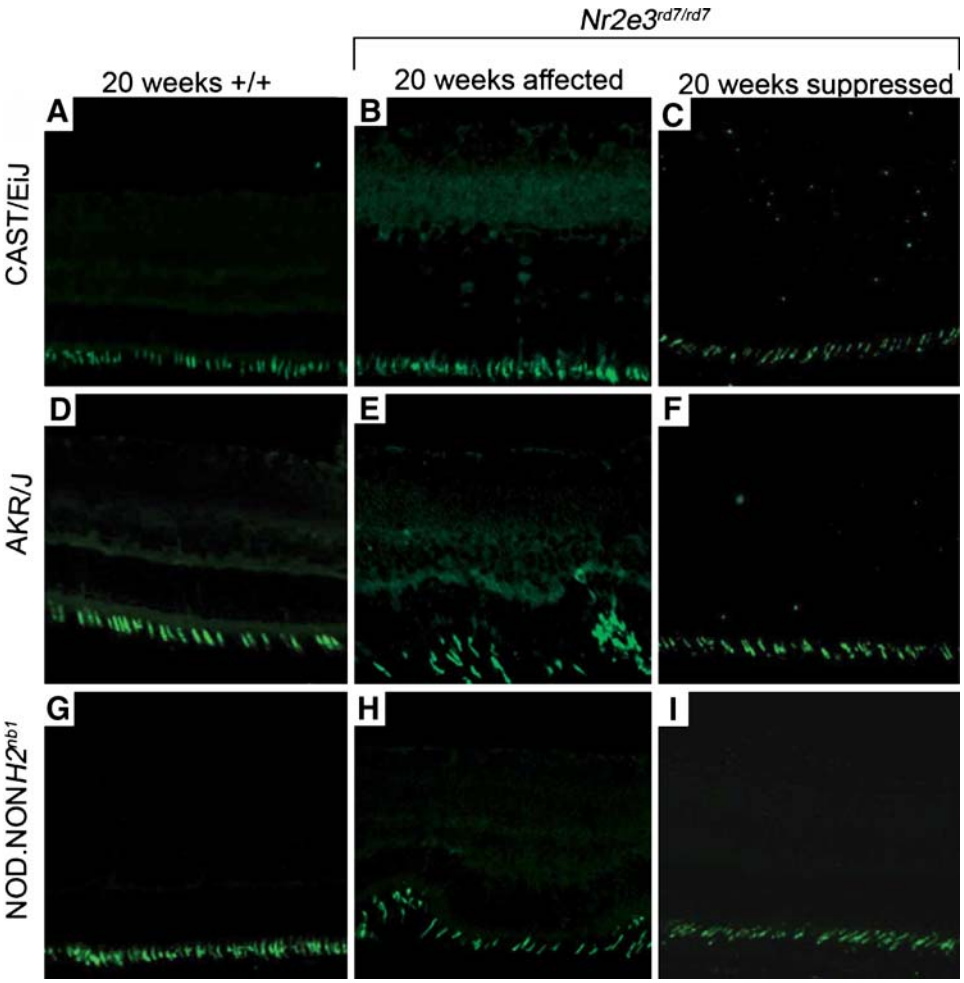

Fig. 3 Blue cone population is restored to normal in suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice. Retinal sections were collected from 20-week-old F_2 *Nr2e3*$^{rd7/rd7}$ and control (+/+) mice and labeled with blue opsin. **A–C** CAST/EIJ. **D–F** AKR/J. **G–I** NOD.NON-*H2*^{nb1}. **A, D, G** are from normal F_2 *Nr2e3*$^{+/+}$ mice; **B, E, H** are sections from affected F_2 *Nr2e3*$^{rd7/rd7}$ mice; and **C, F, I** are sections from suppressed F_2 *Nr2e3*$^{rd7/rd7}$ mice

with homozygous mutations in *Nr2e3*, as well as mice with *Nr2e3* mutations, have disease while heterozyotes are normal. The modifier effect can create a more severe mutant phenotype or suppress the mutant phenotype even to the extent of completely restoring the normal condition, as is seen in the *Nr2e3*$^{rd7/rd7}$ modifiers. Modifier genes can also alter the pleiotropy of a given disease resulting in different combinations of traits. In addition, for any given disorder, multiple modifier genes may be required to act in concert to create a cumulative effect on the expression of a phenotype, or, alternatively, they may act independently to affect a similar modification of the observed phenotype.

In this study, the frequency of mice that were genotypically homozygous for the *Nr2e3*$^{rd7/rd7}$ mutation was slightly but significantly reduced in the CAST/EiJ intercross progeny: 20% compared to the expected 25% Mendelian pattern. This could potentially be due to the fact that the CAST/EiJ strain is highly divergent from B6 and a combination of alleles from the two strains may have a detrimental effect on the survival of mice with the *Nr2e3*$^{rd7/rd7}$ genotype. Although *Nr2e3* has its highest expression in the eye, it is also expressed in other tissues as well (Choi et al. 2006; Genomic Institute of the Novartias Research Foundation 2007; Kitambi et al. 2007). In addition to the potential modifying effects of genetic background on survival, we observed complete suppression of retinal degeneration and restoration of the normal complement of mature blue opsin-expressing cone cells in 28–49% of F_2 *Nr2e3*$^{rd7/rd7}$ mutant mice from intercrosses using three genetically divergent strains of mice. The observed frequency of disease suppression in the AKR/J cross (49%) is close to that expected if two recessive loci were responsible for the suppression. Although we detected two suggestive QTLs in this cross, the low percent variance explained indicates that additional loci may be acting, including perhaps additional susceptibility loci carried by the "resistant" strain AKR. This may also be the case in the CAST/EiJ cross and explain the low percent (28%) of suppressed animals in this cross. Surprisingly, despite 34% of suppressed animals in the NOD.NON-*H2*^{nb1} cross, we did not detect statistically significant QTLs. Considering the number of nonsignificant peaks in the genome scan, it seems possible that there are many loci contributing to the

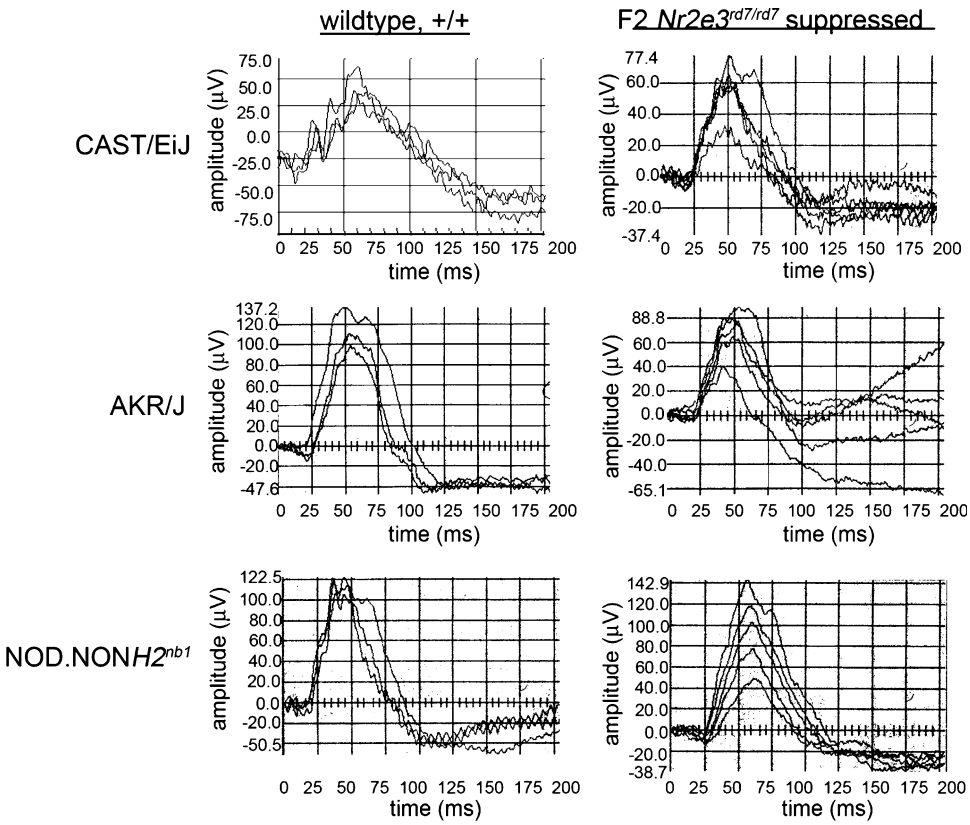

Fig. 4 ERG analysis of suppressed mice under light-adapted conditions demonstrating cone responses. ERG responses from representative 2–7-month-old suppressed mice and their respective control strains are shown. Suppressed mice exhibit normal ERG function

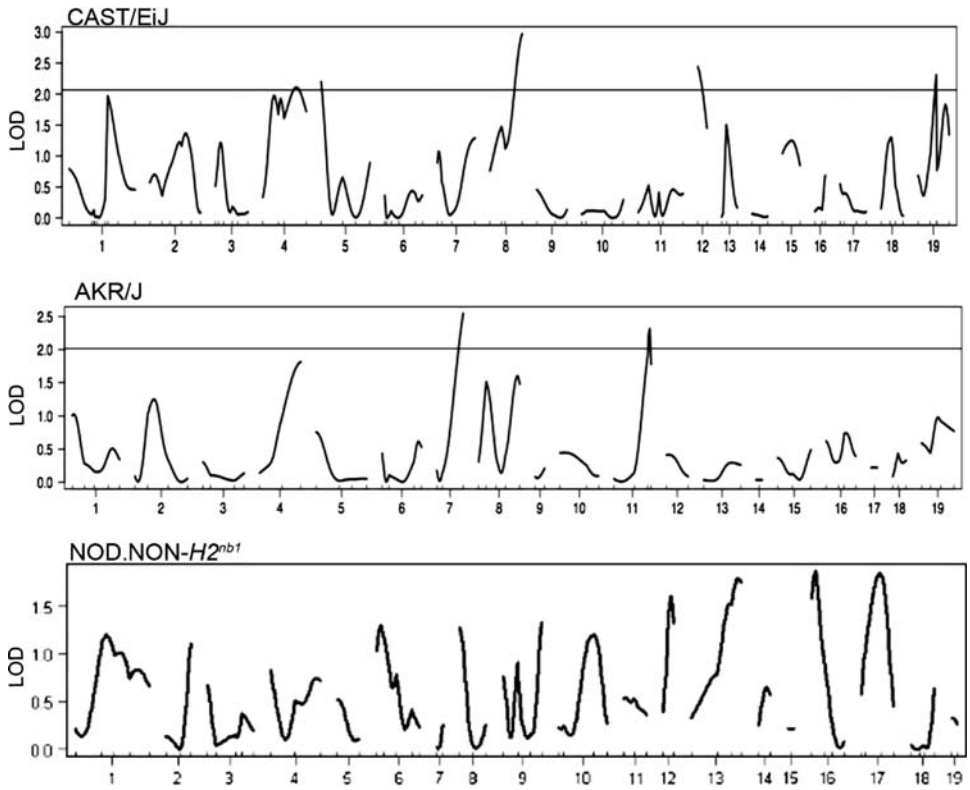

Fig. 5 Genome-wide scan for genetic modifiers associated with the *Nr2e3*$^{rd7/rd7}$-suppressed phenotype identified several significant loci from CAST/EiJ and AKR/J intercrosses while no significant modifying loci were identified for NOD.NON-*H2*^{nb1}. Phenotypes were coded as 0 for *rd7* and 1 for suppressed. The black threshold line in each plot represents a 63% threshold line, which indicates suggestive loci with low or moderate QTL effects. LOD score values are indicated on the *Y* axis and chromosomes are indicated on the *X* axis

Table 2 Summary of linkage data for *Nr2e3* $^{rd7/rd7}$ genetic modifiers

Strain (No. markers)	Chromosome	df	Type III SS	%var	*F* value	*p* value	LOD score
CAST/EiJ (81)	Chr8 @ *D8Mit92*	2	2.7843	9.5	7.1302	8e-04	3.07
	Chr19 @ *D19Mit17*	2	2.2731	7.8	5.8211	0.003	2.53
AKR/J (69)	Chr7 @ D7Mit358	2	1.4967	7.3	3.7427	0.021	1.67
	Chr11 @ D11Mit360	2	1.4141	6.9	3.536	0.026	1.58
NOD.NON-H2^{nb-1} (63)	None identified						

All candidate genetic modifier regions were fit into multiple regression models. df = degree of freedom associated with each term in the model; Type III SS = sum of squares adjusted for all other terms in the model; %var = total variance explained by the adjusted sum of squares; *F* statistics are based on adjusted SS; *p* values are based on the χ^2 distribution

suppression in this cross, thus reducing the power of our analysis. It is noteworthy that the QTLs detected in the different crosses do not seem to overlap, further indicating that many loci are able to compensate for the loss of a functional *Nr2e3* allele. Interestingly, gene-gene interactions among the modifier loci were not detected in any of the intercrosses. However, a larger cohort may be necessary to detect QTL*QTL interactions.

We also demonstrate that the suggestive modifier locus on chromosome 11, identified in the AKR/J intercross, which had not reached statistical significance, is able to independently suppress the *Nr2e3*$^{rd7/rd7}$ disease phenotype. With the elimination of other genetic variables by mating *Nr2e3*$^{rd7/rd7}$ mice to an incipient congenic stock, B6-*Motr1*$^{AKR/J}$, containing the modifier region, suppression of the retinal degenerative phenotype was observed. As with the F_2 mice described in the intercross, *Nr2e3*$^{rd7/rd7}$; *Motr1*$^{AKR/J/AKRJ}$ mice have normal fundus, histology, and blue cone cell number. However, in contrast to the complete suppression of the disease phenotype observed in *Nr2e3*$^{rd7/rd7}$; *Motr1*$^{AKR/J/AKRJ}$ mice, there was only a partial suppression of photoreceptor degeneration in *tubby;Motr1*$^{AKR/J/AKRJ}$ mice. This suggests that either there are two genes on chromosome 11 that are able to rescue a retinal degenerative phenotype or that one gene is able to completely rescue the *Nr2e3*$^{rd7/rd7}$ phenotype and only partially rescue the *tub* phenotype. If the latter scenario were true, it is possible that the modifier gene may be more directly associated with the *Nr2e3* transcriptional pathway while it functions in a more tangential pathway to the *tub* gene. Alternatively, photoreceptor degeneration in *tubby* mice, which occurs very rapidly, may already be initiated before the modifier gene or some downstream factor on which it acts, which is important for cell survival in the adult retina, is turned on.

The identification of genetic modifier genes for *Nr2e3*^{rd7} may provide new insights into the variability in disease phenotype observed in ESCS patients and the biological pathways in which *Nr2e3* functions. For example, while all ESCS patients exhibit a common pattern of retinal dysfunction, clinically evident retinal degeneration varies in severity among these individuals (Favre 1958; Fishman and Peachey 1989; Fishman et al. 1976; Jacobson et al. 1991), with the most severe form referred to as Goldmann-Favre syndrome. Other clinical features that vary among ESCS patients include cystic foveal changes, vitreous degeneration, yellow flecks, macular scarring, peripheral retinoschisis, cataracts, and pigmentary retinopathy (Haider et al. 2000). Clearly, the pathway through which *Nr2e3* functions is affected by different modifying genetic factors. The identification of modifier genes in the *Nr2e3*$^{rd7/rd7}$ mouse model may lead to new treatments either by providing additional information about the genetic contributions to the phenotype for which treatment may already be available or by pointing to additional steps in a biological pathway that may be more amenable to treatment. Once identified, these modifier genes can be evaluated to determine how they are affecting the phenotypic expression in patients with ESCS and the related retinal degenerative disorders that are affected by mutations in *Nr2e3*. In addition, these modifier genes may also identify candidate genes for related retinal degenerative disorders for which mutations have not yet been discovered.

Acknowledgments This study was supported by grants from the National Eye Institute [EY11996 (PMN), NRSA postdoctoral training grant (F32 EY07080–01A (NBH)], the Center for Biomedical Excellence Award through the National Center for Research Resources, National Institutes of Health [NIH 5 P20 RRO18788–02 (NBH)], and the Nebraska Tobacco Settlement Biomedical Research Development, TJL Cancer Core Grant (CA-34196).

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