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Genetic modifiers of retinal degeneration in the rd3 mouse

M Danciger[1,2], **D Ogando**[1], **H Yang**[3], **MT Matthes**[3], **N Yu**[1], **K Ahern**[3], **D Yasumura**[3], **RW Williams**[4], and **MM La Vail**[3]

1*Department of Biology, Loyola Marymount University*

2*The Jules Stein Eye Institute, UCLA School of Medicine*

3*Beckman Vision Center, UCSF School of Medicine, San Francisco, CA*

4*Dept of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN*

Abstract

Purposes—In previous studies of light-induced (LRD) and age-related (ageRD) retinal degeneration (RD) between the BALB/cByJ (BALB) and B6(Cg)-Tyr^{c-2J}/J (B6a) albino mouse strains, we identified RD-modifying quantitative trait loci (QTL). After breeding BALB- and B6a-*rd3/rd3* congenic strains, and finding significant differences in RD, we performed an F1 intercross to determine *rd3* QTL that influence this inherited RD.

Methods—N10, F2 BALB- and B6a-*rd3/rd3* strains were measured for retinal outer nuclear layer (ONL) thickness from 5 to 12 weeks of age. Since 10 weeks showed significant differences in the ONL, F2 progeny from an F1 intercross were measured for ONL thickness. F2 DNAs were genotyped for SNPs by the Center for Inherited Disease Research. Correlation of genotype with phenotype was made with Map Manager QTX.

Results—148 SNPs ~10 cM apart were typed in the F2 progeny and analyzed. Significant QTL were identified on Chrs 17, 8, 14 and 6 (B6a alleles protective) and two on Chr 5 (BALB alleles protective). Suggestive QTL were found as well. For the strongest QTL, follow-up SNPs were analyzed to narrow the critical intervals. Additional studies demonstrated that rd3 disease is exacerbated by light but not protected by the absence of rhodopsin regeneration.

Conclusions—QTL were identified that modulate *rd3*-RD. These overlapped some QTL from previous ageRD and LRD studies. The presence of some of the same QTL in several studies suggests partial commonality in RD pathways. Identifying natural gene/alleles that modify RDs opens avenues of study that may lead to therapies for RD diseases.

INTRODUCTION

Numerous examples exist where siblings and other relatives, or unrelated individuals, have significant variation in the severity and course of their retinal degenerations even though they have inherited the same primary gene mutation. For example, significant variations in disease have occurred in individuals with the same mutation in *RPGR* with X-linked retinitis pigmentosa (RP)[1], in *CRX* with autosomal dominant cone-rod dystrophy[2], in the gene encoding arrestin (*SAG*) with autosomal recessive RP or Oguchi disease[3] and in many other genes with various forms of retinal degeneration (RD) such as *RDS-peripherin*, *RP1*, *PIM-1*

Corresponding author: M Danciger, Dept. of Biology, Research Annex, Loyola Marymount University, Los Angeles, CA 90045-2659, mdancige@lmu.edu, 310 338-7337.

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(RP9), *PRPF31* (RP11), *RHO*, *REP-1* (choroideremia) to mention a few[4-11]. However, with the exception of the identification of the second allele as the modifier in RP11[12-13], little work has been done to identify the background modifier genes responsible for these variations.

Photoreceptor degeneration in the mouse retina has served as a model for human disease in many studies including the use of spontaneous, transgenic, knock-out and knock-in and light-induced models for rod degenerative diseases (See references [14-18] for reviews). The naturally occurring autosomal recessive rd3 model was originally identified by Chang et al in 1993[19] and was later shown to have a variable phenotype on different but mixed and unspecified backgrounds[20, 21]. The variable phenotype was of interest for the possibility of demonstrating significant differences in rd3 disease expressivity on two genetically well-characterized inbred mouse strains that would lay the groundwork for quantitative genetics studies. Since we identified quantitative trait loci (QTL) influencing age-related (ageRD) and light-induced retinal degeneration (LRD) in previous studies of the BALB/cByJ and B6(Cg)-Tyr^{c-2J}/J (formerly C57BL/6J-c^{2J}) albino strains[22, 23], we selected these two backgrounds to determine if the same or different QTL modulated this inherited RD. In addition, the recent discovery of the *rd3* gene and its lack of any known functional domains[24] meant that our endeavor to identify genes that modify rd3 disease could provide insight into the function of this RD gene. Thus, we bred the *rd3* allele through 10 generations and then to homozygosity onto each of the two aforementioned strains. We have found that the degeneration in the N10F2 BALB-*rd3/rd3* retina is significantly faster than in the N10F2 B6a-*rd3/rd3* retina. Therefore, we have been able to begin the process of identifying modifier genes that influence this inherited RD by demonstrating the QTL responsible for the variation in rd3 disease between these two inbred mouse strains.

The identification of genes/alleles that influence RD will provide specific candidates for study in the human as modifiers of variant monogenic RD phenotypes. These modifier genes may also be candidates for susceptibility genes in complex genetic RDs such as AMD and diabetic retinopathy.

MATERIALS AND METHODS

MICE

BALB/cByJ (BALB) and B6(Cg)-Tyr^{c-2J}/J (B6a) mice were originally purchased from the Jackson Laboratories (Bar Harbor, ME) and maintained through many generations in our vivarium before study. The B6(Cg)-Tyr^{c-2J}/J mouse is derived from the C57BL/6J strain and differs only by a mutation inactivating the tyrosinase gene (c) that makes the strain albino. N10F2 BALB- *rd3/rd3* albino mice were bred by crossing Rb(11.13)4Bnr/J (formerly Rb4Bnr) mice obtained from Jackson Laboratories (Bar Harbor, ME) with BALB mice, backcrossing to BALB for 9 more generations while selecting for progeny carrying the *rd3* allele using PCR markers that closely flank the *rd3* gene, and then intercrossing to produce homozygous *rd3/rd3* BALB congenics (99.9% BALB). N10F2 B6a-*rd3/rd3* congenics were produced in the same manner using the B6a strain to cross with Rb(11.13)4Bnr/J. All mice were kept under a 12-12 hour cyclic light cycle with an in-cage illuminance of 2-7 ft-c. The temperature of the vivarium was maintained between 20° and 22° C. Cages were kept on four shelves of free-standing, double-sided, five-shelf racks (never on the top shelf). Each week, the cages were rotated by shelf, by side of the rack and by position on the shelf (seven positions from side to side). Mice were maintained on a low fat diet (#5001 Rodent diet) and breeding pairs were kept on a high fat diet (#5015 Mouse diet) with chow and water *ad lib* (Lab Diet - http://www.labdiet.com/indexlabdiethome.htm).

Both N10F2 B6a- and N10F2 BALB-*rd3/rd3* control mice were aged to 5, 6, 7, 8, 9, 10, 11 or 12 weeks before testing for RD. The B6a-*rd3/rd3* strain was also tested at 14 weeks. For the

quantitative genetics study, a reciprocal (BALB-*rd3/rd3* × B6a-*rd3/rd3*)F1 intercross was made and 431 F2 progeny were aged to 10 weeks along with 24 B6a-, 43 BALB- and 57 F1-*rd3/rd3* controls.

Quantitative trait

Eyes were enucleated from mice immediately after euthanasia by carbon dioxide asphyxiation, fixed in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in phosphate buffer, embedded in a mixture of Epon 812 (EMS, Ft. Washington, PA) and Araldite 502 (Tousimis, Rockville, MD) and bisected along the vertical meridian through the optic nerve head. Single 1-μm, toluidine blue-satined sections were taken along the vertical meridian of each eye, as described previously[25], and the sections were aligned so that Müller cell processes crossing the inner plexiform layer were continuous throughout the section, or nearly so, to ensure that the sections were not oblique. On each section, 54 measurements of the thickness of the outer nuclear layer (ONL) were made, 3 measurements each spaced 50 μm apart taken at nine 0.25-mm intervals both in the superior and inferior hemispheres starting from the optic nerve head. Means of the 54 measurements from each retinal section were used to score the mice for the quantitative trait. All procedures involving the mice adhered to the ARVO Resolution on the Use of Animals in Research and the guidelines of the Loyola Marymount University Committee on Animal Research.

Genotyping

Genotyping included the typing of the F2 progeny for 148 SNPs spanning the genome selected from a larger set provided by the Center for Inherited Disease Research (CIDR). [CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, Contract Number N01-HG-65403.] The SNPs comprised an approximate 10-cM map based on recombination frequencies within the 431 F2 progeny. The average distance between markers was 10.7 cM. For each chromosome, the most proximal marker genotyped was within 13.1 Mb of the centromere (mouse chromosomes are acrocentric), internal markers were no more than 16 cM apart, and the most distal markers were within 10.5 Mb of the telomere. The exceptions to the above were: proximal Chr 2, where the first marker and the second marker were 52 cM apart, and Chr X, where the proximal marker was 32.4 Mb from the centromere. A list of the markers used for this study is available upon request. Physical distances were taken from the mouse build 36.1 NCBI database (http://www.ncbi.nlm.nih.gov/). Once QTL were identified, remaining SNPs from the original set and within the QTL were analyzed along with a new set of follow-up SNPs typed by CIDR. Each of the SNPs employed was homozygous within a strain and different between the two strains studied (polymorphic). Other pairs of mouse strains may not have alleles that are polymorphic for each of the SNPs in this panel.

Mouse genomic DNAs

Genomic DNAs were isolated from livers with the Puregene® DNA isolation kit (Gentra systems, Minneapolis, MN) according to their protocol.

Data analysis

Data were analyzed with Map Manager QTX20b[26]. With this program, a likelihood ratio statistic (LRS) correlating genotype with phenotype was listed for each of the 148 marker genotypes with a $P \leq 0.05$. The one with the highest LRS was analyzed by interval mapping of all the markers on its chromosome. The marker at the peak of this QTL was put into the background for adjustment of the next evaluation. Peak markers from both the first and second interval maps were then put into the background for the next determination, and so on. To determine significance levels for this genome-wide screen, a test of 500 permutations of all

marker genotypes together was performed. LRS were converted to LOD scores by dividing by 4.6 (2 × the natural log of 10). Once QTL were identified, genotypes of additional markers in the QTL were evaluated only by marker regression with background adjustment. The Map Manager program is not designed for interval mapping of markers very close together such as were analyzed for the follow-up study.

Identification of candidate QTG

To systematically evaluate candidate genes in QTL regions (operationally defined as the 1-LOD confidence interval) we exploited the Hamilton Eye Institute mouse eye expression data set available on-line at GeneNetwork (www.genenetwork.org). We specifically used the mouse BXD Affymetrix M430v2 RMA data release of September 2006 (n= 132 arrays) that includes data for 18 conventional strains including BALB/cByJ, C57BL/6J, and an additional 64 recombinant inbred strains (see http://www.genenetwork.org/dbdoc/Eye_M2_0906_R.html for details on the generation of this data set by EE Geisert and colleagues). Four of the stains in this data set have retinal degeneration—C3H/HeJ, BXD24/TyJ, FVB/NJ, and MOLF/EiJ; a feature that was exploited to determine whether or not particular transcripts are associated with photoreceptors.

RESULTS

Differences in *rd3*-caused retinal degeneration between strains

Figure 1 shows the progression of photoreceptor loss (expressed as ONL thickness) in the BALB- and B6a-*rd3/rd3* strains. Based on the Student's t-test, there was no significant difference in ONL thickness between the two strains from 5 to 8 weeks. However, at 9, 10, 11 and 12 weeks, the *P* values were 0.03, 7.8×10^{-12}, 0.0005 and 0.008, respectively (The magnitude of *P* values was influenced by the number of mice tested). For the BALB-*rd3/rd3* strain, the ONL thickness dropped significantly throughout the 12-week study period although not at every week. Thus, the *P* values for the BALB congenic between 5 and 7, 6 and 8, 7 and 9, 8 and 10, 9 and 11 and 10 and 12 weeks were 1.5×10^{-5}, 0.03, 0.0005, 1.4×10^{-10}, 0.02, 0.01, respectively. On the other hand, for the B6 congenic while the *P* values were 0.0006 and 0.06 for 5 *vs*. 7 and 6 *vs*. 8 weeks, respectively, there was no significant difference between ONLs from 7 weeks on. For example, the *P* between ONL of B6a-*rd3/rd3* retinas at 7 weeks and at 9 weeks was 0.12 and between 7 and 14 weeks, 0.08; between 8 and 10 and 8 and 14 weeks 0.80 and 0.29, respectively, and between 10 and 14 weeks, 0.26.

Quantitative genetics

We selected the 10-week age for QTL study because there was significant difference in ONL thickness between the two strains ($P = 7.8 \times 10^{-12}$) and because there was still 10-11 μm of ONL left in the more susceptible BALB-*rd3/rd3* strain. 431 F2 progeny were bred from an F1 (BALB-*rd3/rd3* × B6a-*rd3/rd3*) intercross, aged to 10 weeks and measured for mean ONL thickness. A set of 877 SNPs was genotyped for the 431 DNAs by CIDR and from these, 148 were selected for analysis by the Map Manager program. The program is most efficient for interval mapping (estimating the breadth, significance and strength of a QTL) when markers are approximately 10 cM apart. Control parental and F1 markers were also analyzed in the program to establish the degree of genetic variance (as opposed to environmentally caused variance). To determine the levels of significant LOD scores, 500 permutations were performed on the 431-DNA × 148-marker matrix establishing LOD scores of 2.15 for suggestive QTL ($P \leq 0.33$), 3.48 for significant QTL ($P \leq 0.05$) and 5.17 for highly significant QTL ($P \leq 0.001$). Highly significant QTL were found on Chrs 17, 5 (two), 8, 14 and 6; suggestive QTL on Chrs 7 and 1. The two QTL on Chr 5 had BALB alleles that protected against RD; the rest of the QTL had B6a protective alleles. Table 1 shows the peak LOD scores and the nearest flanking

markers of ≥ 1 LOD lower significance. Figure 2 shows the three strongest QTL on Chrs 17, 5 and 8.

Using the 10-cM map, a second peak on Chr 5 was detected but not well delineated from the first peak. Therefore, to distinguish the two peaks, a 5-cM map of Chr 5 was created for the analysis. To determine if any genes were acting together to influence *rd3*-RD in a significant, synergistic way, we used the interaction function in Map Manager QTX. For an intercross, this function tests every marker as an additive and dominant allele against every other marker as additive and dominant (four interactions per pair of markers). The interaction likelihood ratio statistic (IX) needed for significance is about 20 (LOD score of 4.35) for an intercross (QTX manual). When this function was performed with an exclusion probability of $\leq 10^{-5}$ (as the manual recommends), no interactions were found.

Follow-up genotyping

The five highly significant QTL identified (Table 1) all span broad regions of the respective chromosomes from 17.8 Mb for the Chr 8 QTL to 36.2 Mb for the Chr 14 QTL. In order to prioritize the search for candidate quantitative trait genes (QTG) by reducing the 1-LOD-support area, additional SNPs within the QTL were genotyped by CIDR. Since the minimum strength of a QTL necessary to make future recombinant progeny testing feasible is ~10% of the variance, we focused on the QTL of Chrs 17 (19%), 5 (17%) and 8 (10%). Recombinant progeny testing involves the breeding of a QTL region from one of the parental strains into the background of the other to create a congenic for physically refining a QTL or for testing a candidate QTG; this has not been done so far in this study.

Table 2 shows the 1-LOD-support interval for the refined QTL of Chrs 17, 5 and 8. Comparing Tables 1 and 2, the critical areas of the three QTL were reduced from 21.8, 21.8 and 17.8 Mb to 16.7, 7.8 and 11.6 Mb, respectively. These are still very large regions that must be refined to areas encompassing a manageable number of genes to evaluate.

The influence of eye pigment, light and rhodopsin regeneration on rd3 disease

Pigmented B6-*rd3/rd3* and albino B6a-*rd3/rd3* retinas were compared at 8, 10, 12 and 14 weeks of age (Fig. 3). The B6a albino carries a mutation in the tyrosinase *c* gene. The results show substantially less degeneration in the pigmented strain verifying previous reports of protection against *rd3*-RD by eye pigment[20, 21]. However, in the previous cases, the backgrounds of pigmented and albino strains were not documented. In our case, we have isolated the pigment variable on two otherwise identical coisogenic strains.

Since protection by eye pigment suggests that rd3 disease is sensitive to light, we tested the influence of constant light on the BALB- and B6a-*rd3/rd3* strains. The B6a and BALB backgrounds have different alleles of the retinal light damage modifier *Rpe65*. We compared retinal ONL of the congenic *rd3/rd3* strains to that of the wild-type BALB and B6a strains after 6 days of constant light exposure and compared that to the same strains with no constant light exposure. Fig. 4 shows that, as expected, the wild-type B6a strain had no significant loss of ONL thickness after light exposure while the BALB strain did. This has been shown to be due to a decrease in the amount of RPE65 protein brought about by the 450met variant present in the B6a strain. Less RPE65 protein makes for slower regeneration of rhodopsin and protection of photoreceptors[23, 27-29]. On the other hand, the presence of homozygous *rd3* alleles made each of the congenic strains equally sensitive to the light insult. The mice were tested from 6 to 7 weeks of age because the ONL of the BALB- and B6a-*rd3/rd3* strains were not significantly different at those ages. Thus, even though rd3 retinal degeneration is exacerbated by light, the RPE65 met450 variant does not appear to influence the progression

of disease. This conclusion is supported by the absence of a detectable QTL in our intercross study at the locus of the *Rpe65* gene, distal Chr 3.

Since the function of the *rd3* gene is unknown[24], and since modulation of rhodopsin regeneration by the RPE65 met 450 allele does not influence *rd3*-RD, we investigated whether the complete absence of rhodopsin regeneration and therefore, the absence of phototransduction, influenced the disease. Figure 5 shows that *rd3/rd3* mice homozygous for the *rd12* allele are more susceptible to RD than those *rd3/rd3* mice that are *rd12*/+ or +/+. The *rd12* allele has a null mutation in exon 3 of the *Rpe65* gene and is, therefore, comparable to a knockout[30]. Eliminating the RPE65 isomerohydrolase produces no protective effect against rd3 RD suggesting that rhodopsin regeneration and/or phototransduction are not necessary for progression of the disease.

DISCUSSION

By means of a quantitative genetics study, several highly significant QTL that modulate rd3 disease have been identified. The QTL on Chrs 17, 5 and 8 are each responsible for ≥ 10% of the variance in the course of RD between the B6a-*rd3/rd3* and BALB-*rd3/rd3* strains. Several more on Chrs 14, 6 and 5 are highly significant as well, but account for only a small percentage of the variance. Still more QTL on Chrs 7 and 1 account for only 1% of the variance each and are only suggestive. Although the B6a-*rd3/rd3* strain is less sensitive to the disease process than the BALB-*rd3/rd3* strain, and most of the QTL reflect B6a alleles that are protective against the disease (relative to the BALB alleles), the two QTL on Chr 5 reflect BALB alleles that are relatively protective.

Additional informative SNPs within the critical areas of the QTL on Chrs 17, 5 and 8 were genotyped and analyzed by marker regression. The results reduced the critical areas of these QTL somewhat, although the remaining areas were still quite large – 16.7, 7.8 and 11.6 Mb, respectively (Table 2). With such large areas, the number of candidate QTG genes to evaluate is prohibitive. Even with the use of the virtual positional cloning web-based program Positional Medline (PosMed) (http://omicspace.riken.jp/PosMed/), the number of candidate genes remained very high. Thus, we entered the refined 1-LOD intervals for each of the Chr 17, 5 and 8 QTL with the keyword ‘retina’ and the program identified 96, 31 and 32 candidate genes, respectively. To mention a few, candidate QTG in the Chr 17 QTL included the photoreceptor-specific genes *Guca1a*, *Guca1b* and *Rds-peripherin*; candidates in the Chr 5 QTL, included several crystallin genes; and candidates in the Chr 8 critical area included the genes encoding beta-carotene 15,15’ monooxygenase (*Bcmo1*) and phospholipase C gamma-2 subunit (*Plcg2*), both expressed in retina.

As an additional approach to identifying candidate genes in the three strong QTL intervals, we used a large mouse eye expression data set available on-line at GeneNetwork (see Methods). We used advanced search parameters to select transcripts with high expression in these intervals. This was accomplished by entering search strings such as “*Mb=(chr17 30.5 47.2) Mean=(10 20)*” into the “*All*” query field. This search generated a list of transcripts with genes located on Chr 17 between 30.5 and 47.2 Mb that also have steady-state hybridization signals with values between 10 and 20 $\log_2$ units (10 units is more than 2X above the average expression level). This particular query for the Chr 17 interval yielded 112 probe sets representing transcripts derived from 81 unique genes and ESTs. Corresponding searches for the refined intervals on Chr 5 and Chr 8 yielded 35 and 37 unique genes/ESTs, respectively. Lists of candidates were further winnowed to a very small subset of genes known to have expression tightly coupled to photoreceptors as judged by expression differences of 2X or greater between wildtype and RD strains. The following represents gene/ transcripts that met these criteria: Chr 17 interval - *Guca1b* (non-RD strains had an average expression 36.8 X the

average of RD strains), *Guca1a* (11.4X) *Rrp1b* (3.5 X), *Pla2g7* (2.9 X), and *Zfp472* (2.6 X); Chr 5 interval: *Ccdc64* (3.4 X); Chr 8 interval: *Wwox* (2.4 X).

It should be noted that the above candidates are only "virtual" suggestions and still include a large number of genes. Further, some or all of the modifiers of rd3 may not be photoreceptor specific. Sequencing and mRNA expression studies of many candidate QTG is prohibitive. Therefore, before any genes are studied, it would be more reasonable to reduce the number of candidates by recombinant progeny testing or to identify specific candidates that are provocative for other reasons.

We compared the *rd3*-RD QTL identified using the B6a and BALB strains with age-RD and LRD QTL previously identified with the same two strains[22, 23]. The Chrs 8 and 14 *rd3* QTL overlapped QTL in the age-RD study but the age-RD QTL were weaker. None of the *rd3* QTL were present in the LRD study. Expanding the comparison to QTL studies with different strains, we found overlapping QTL in several cases: the Chrs 17 and 14 QTL were present in an AxB age-RD study[31], but the ageRD QTL were weaker; the Chr 6 QTL was present in an LRD study between BALB and 129S1/SvImJ and was of approximately the same effect[32]. These overlapping QTL suggest a broad and fundamental role in RD for some QTG.

Comparative studies of *rd3*-RD in pigmented and albino mice showed that eye pigment is protective. This has been shown before but on unspecified backgrounds (20, 21) so that the influence of modifiers other than eye-pigment could not be ruled out. In this work, the pigment characteristic was isolated as a variable because the pigmented and albino strains were of the same B6 background. Protection by eye pigment led us to compare constant light-exposed and control rd3 retinas with the result that rd3 disease was exacerbated by light. The facts that disease in both the BALB- and B6a-*rd3/rd3* strains was equally exacerbated by constant light, and that no QTL was present at distal Chr 3 in the intercross study, strongly suggest that the RPE65 met/leu450 variant that influences LRD[23, 27-29] has no influence on *rd3*-RD. On the other hand, the met450 variant was shown to offer some small amount of temporal protection against a different RD caused by the rhodopsin *VPP* transgene[33] and to reduce the accumulation of the lipofuscin A2E in the retinas of Abcr -/- mice[34]. The influence of the absence of the *Rpe65* gene was tested in *rd3/rd3* mice to determine if rhodopsin regeneration and/or phototransduction are involved in the disease process. If so, the absence of RPE65 should have been protective, but it was not. In fact, rd3 disease was worsened by the absence of RPE65. One possible explanation is that when rhodopsin is not regenerated it is destabilized making the photoreceptor more sensitive to the rd3 disease process.

Our conclusions are that rd3 disease involves a RD that is influenced by several modifier genes, is slowed by pigment and exacerbated by light but is not related to rhodopsin regeneration or phototransduction. Identification of the QTG particularly in the strong QTL on Chrs 17, 5 and 8 will provide avenues of study that may lead to future therapies for human RDs.

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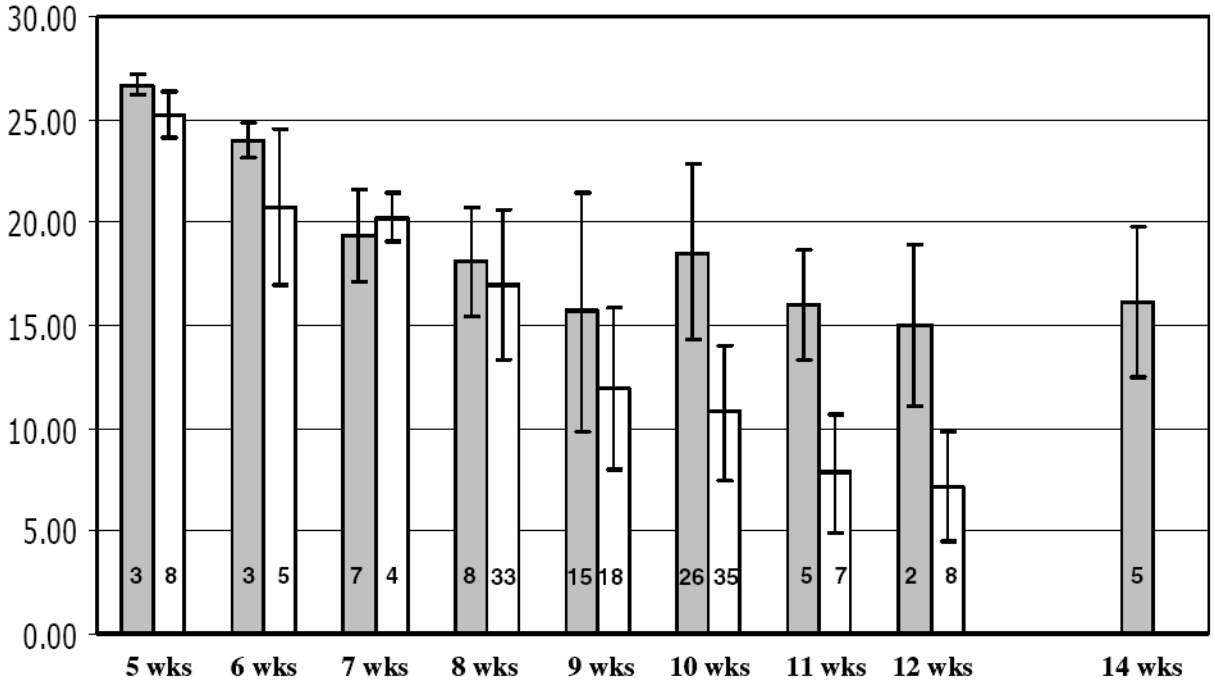

Figure 1.

Average retinal ONL thickness in μm ± the standard deviation of BALB-*rd3/rd3* (open bars) and B6a-*rd3/rd3* (gray bars) strains at ages 5 to 14 weeks. The numbers in the bars represent the numbers of mice used for each time point.

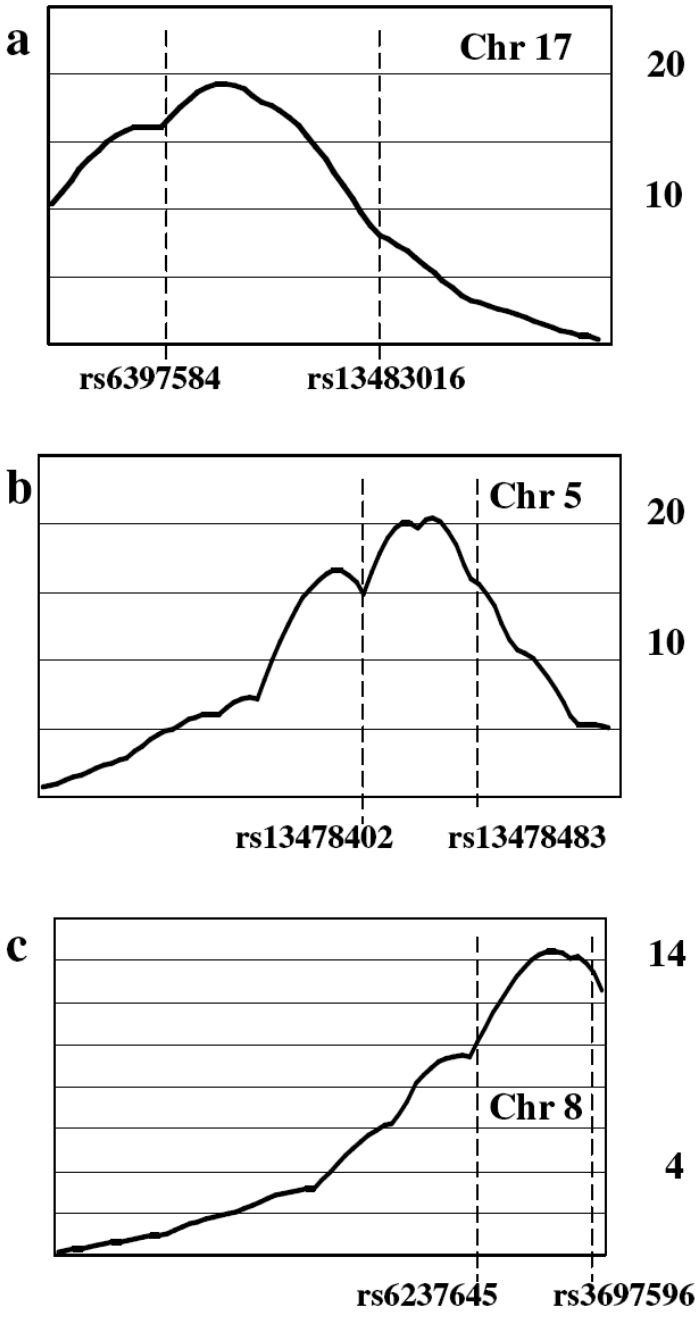

Figure 2.

Interval maps for the three strongest QTL. The X axis is the region of the chromosome corresponding to the QTL and the Y axis is the LOD score. Peak LOD scores of each of the three QTL are well above the 5.17 LOD that marks the 0.001 level of significance. The dashed lines circumscribe the 1-LOD support interval shown in Table 1.

a. Chr 17 QTL **b.** Chr 5 QTL and **c.** Chr 8 QTL. The proximal hump in **b.** represents a second Chr 5 QTL of lesser strength.

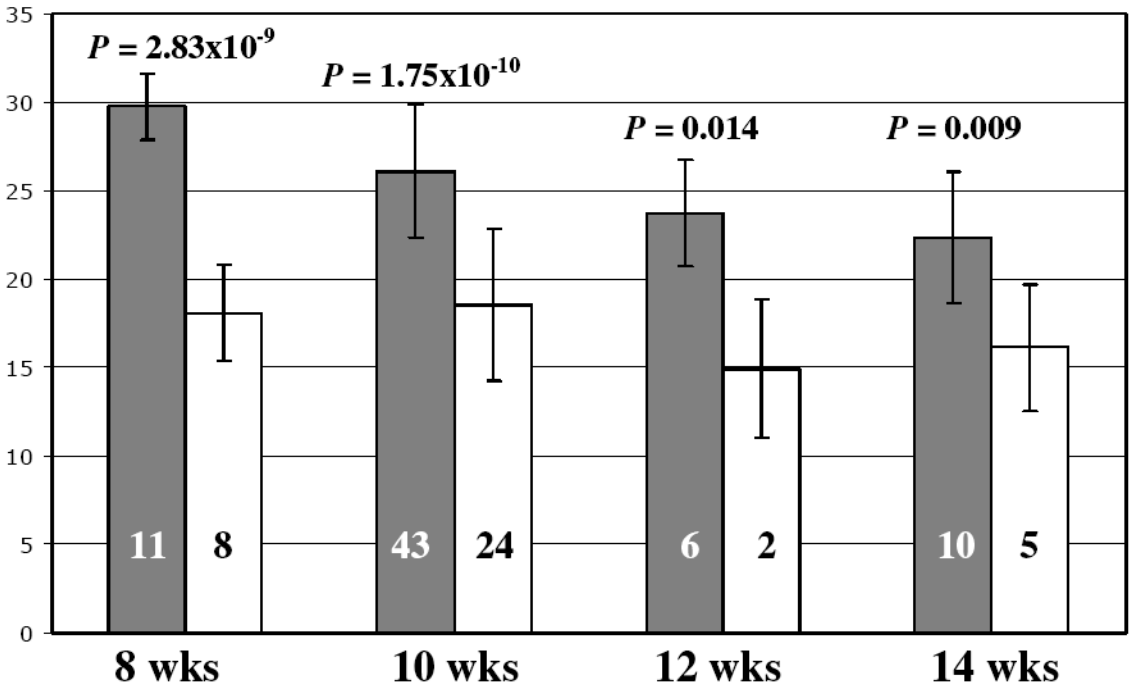

Figure 3.
Average retinal ONL thickness in μm ± the standard deviation of B6 pigmented mice (gray bars) and B6a albino mice (white bars) at ages 8 to 14 weeks. *P* values based on Student's t-test. Numbers in bars = numbers of mice.

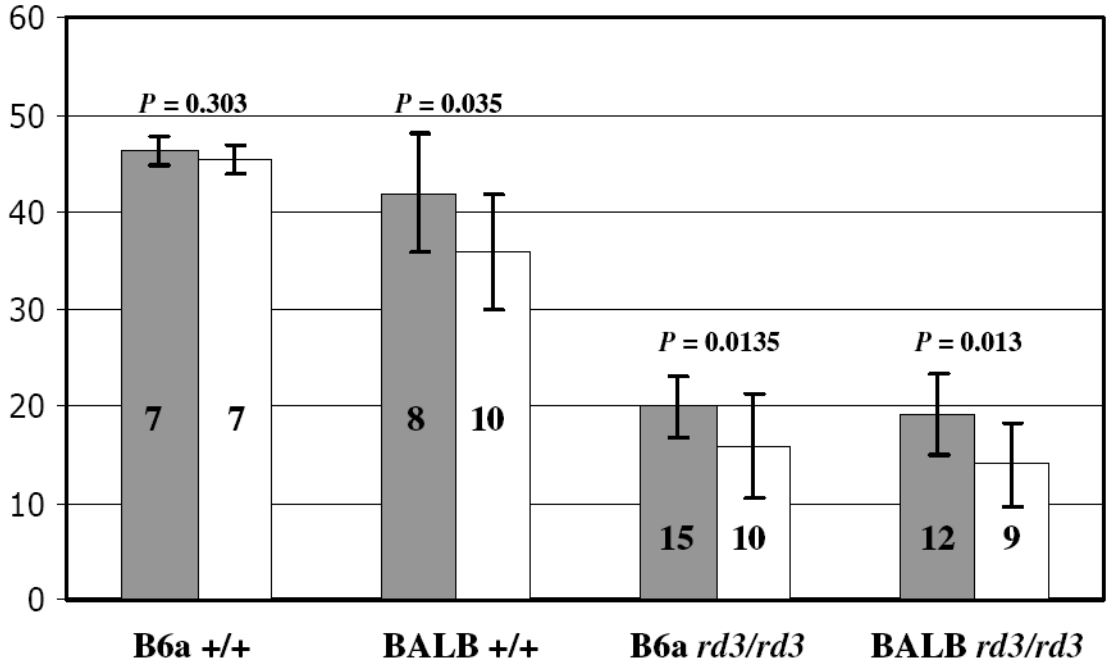

Figure 4.

Average retinal ONL thickness in μm ± the standard deviation after 6 days constant light exposure of 70-120 ft.-C with overhead white fluorescent lighting. Gray bars = unexposed mice at age 48 days; white bars = 6 days of light exposure to mice at 42 days (age 48 days at measurement). Numbers in bars = numbers of mice. *P* values based on Student's t-test.

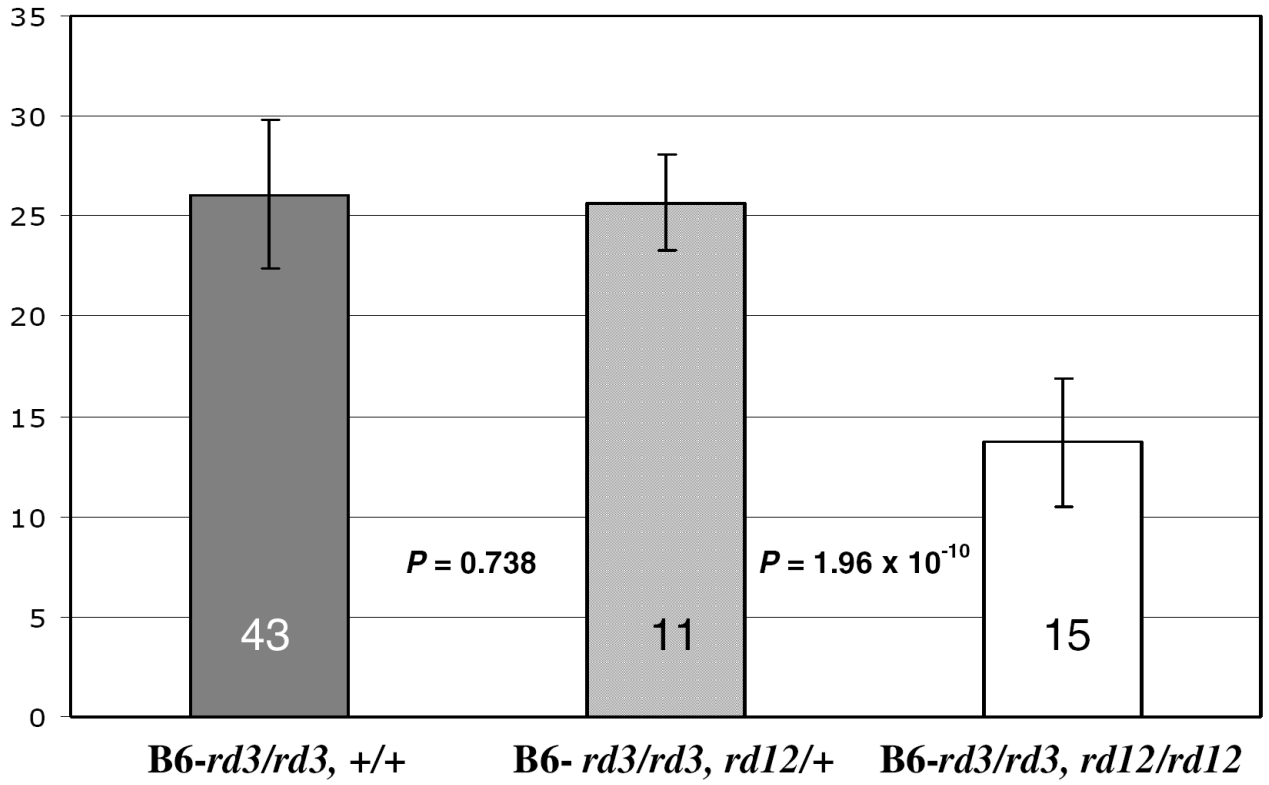

Figure 5.

Average retinal ONL thickness in μm ± the standard deviation of pigmented B6-*rd3/rd3* mice with variation in the *rd12* allele. All mice were 10 weeks of age when tested. *P* values based on Student's t-test. Numbers in bars = numbers of mice. The *rd12* allele contains a null mutation that creates an effective knockout of the RPE65 protein when homozygous.

Table 1

QTL from *rd3*-RD, BALB-*rd3/rd3* × B6a-*rd3/rd3* intercross. Analysis with Map Manager QTXb20. Quantitative trait based on the mean of all ONL thickness measurements.

Sig*	Chr	LOD score at peak of interval map	SNPs flanking QTL at ≥ 1 LOD	Mb† from centromere	Critica l area, Mb	% effect (%total genetic effect)‡	Best fitting inheritance model
HS	96 cntrls§	11.9	---	---	---	40 (100)	additive
HS	17	19.3	rs6397584 rs13483016	27.2 49.0	21.8	19(47.5)	additive
HS	5	19.7	rs13478402 rs13478483	96.4 118.2	21.8	-16(-40)//	recessive
HS	8	14.5	rs6237645 rs3697596	112.9 130.7	17.8	10(25)	additive
HS	14	7.3	rs6290836 rs13482170	11.3 47.5	36.2	4(10)	additive
HS	6	5.3	rs13478697 rs13478841	32.8 78.2	35.4	3(7.5)	additive
S/HS	5	5.15	rs6187409 rs13478402	63.1 96.4	33.3	-3(-7.5)	recessive
Sugg	7	2.7	rs8255275 rs3663988	46.0 139.2	93.2	1(2.5)	recessive
Sugg	1	2.7	Centromere rs3685919	0 111.5	111.5	1(2.5)	recessive

*HS, highly significant; S, significant; Sugg, suggestive.

†Mb positions were taken from the NCBI mouse build 36.1 (http://www.ncbi.nlm.nih.gov/projects/mapview) and were rounded to the nearest 0.1 million bases. Distances shown are from the acrocentric centromere.

‡The % total genetic effect = % effect for this locus divided by total % effect of controls (40%) rounded to the nearest whole number.

§The 96 controls included 24 of each parental congenic and 48 F1 mice.

//The negative or "-" % genetic effect score indicates a B6a-susceptible allele; all other % genetic effect scores indicate B6a-protective alleles.

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Table 2

Refined 1-LOD support interval after follow-up genotyping.

QTL	Number of additional informative SNPs	LOD score at Peak of QTL	SNPs flanking QTL at ≥ 1 LOD	Mb* from centromere	Critical area, Mb
Chr 17	23	19.6	rs3145545 rs13483008	30.5 47.2	16.7
Chr 5	13	24.7	rs13459186 rs13478483	110.4 118.2	7.8
Chr 8	10	16.2	rs13479995 rs6310608	116.6 128.2	11.6

*Mb positions were taken from the NCBI mouse build 36.1

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