Genetic Modifiers of Retinal Degeneration in the *rd3* Mouse

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Purpose. 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, RD-modifying quantitative trait loci (QTLs) were identified. After breeding BALB- and B6a-*rd3/rd3* congenic strains and finding significant differences in RD, an F1 intercross to determine *rd3* QTLs that influence this inherited RD was performed.

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. One hundred forty-eight SNPs ~10 cM apart were typed in the F2 progeny and analyzed. Significant QTLs were identified on chromosomes (Chrs) 17, 8, 14, and 6 (B6a alleles protective) and two on Chr 5 (BALB alleles protective). Suggestive QTLs were found as well. For the strongest QTLs, 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. QTLs were identified that modulate *rd3*-RD. These overlapped some QTLs from previous ageRD and LRD studies. The presence of some of the same QTLs 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. (*Invest Ophthalmol Vis Sci.* 2008;49:2863–2869) DOI:10.1167/iovs.08-1715

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There are numerous examples of siblings and other relatives, or unrelated individuals, who 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* (RP9), *PRPF31* (RP11), *RHO*, and *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 of human disease in many studies, including the use of spontaneous, transgenic, knockout and knockin, and light-induced models for rod degenerative diseases (for reviews, see Refs. 14–18). The naturally occurring autosomal recessive *rd3* model was originally identified by Chang et al.[19] in 1993 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 (QTLs) 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 whether the same or different QTLs modulate 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 found that the degeneration in the N10F2 BALB- *rd3/rd3* retina is significantly faster than that in the N10F2 B6a-*rd3/rd3* retina. Therefore, we were able to begin the process of identifying modifier genes that influence this inherited RD by demonstrating the QTLs 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 B6a mouse is derived from the C57BL/6J strain and differs only by a mutation that inactivates 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 to 7 ft-c. The temperature of the vivarium was maintained between 20°C and 22°C. The 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 the side of the rack, and by position on the shelf (seven positions from side to side). The mice were maintained on a low-fat diet (5001 rodent diet), and breeding pairs were kept on a high-fat diet (5015 mouse diet; both LabDiet, PMI, Richmond, IN) with chow and water ad libitum).

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* x 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* control animals.

Quantitative Trait

The eyes were enucleated from the mice immediately after euthanatization 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, Fort Washington, PA) and Araldite 502 (Tousimis, Rockville, MD) and bisected along the vertical meridian through the optic nerve head. Single 1-μm, toluidine blue–stained 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 Statement for the Use of Animals in Ophthalmic and Vision 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). 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 were proximal chromosome (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 on request. Physical distances were taken from the mouse build 36.1 National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/ Bethesda, MD). Once the QTLs were identified, the remaining SNPs from the original set and within the QTLs were analyzed, along with a new set of follow-up SNPs typed by CIDR. Each of the SNPs used 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 a DNA isolation kit (Puregene; 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 $P < 0.05$. 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 screening, a test of 500 permutations of all marker genotypes together was performed. LRS were converted to LOD scores by dividing by 4.6 (2 $\times$ the natural log of 10). Once the QTLs were identified, genotypes of additional markers in the QTLs 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 evaluate systematically the candidate genes in the QTL regions (operationally defined as the 1-LOD confidence interval), we exploited the Hamilton Eye Institute mouse eye expression data set available online at GeneNetwork (www.genenetwork.org/ hosted in the public domain by the University of Tennessee Health Science Center, Memphis, TN). We specifically used a mouse microarray (BXD M430v2 RMA data release of September 2006; $n = 132$ arrays; Affymetrix, Santa Clara, CA) 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 E. E. Geisert et al.). 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 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 there was, $P = 0.03$, 7.8×10^{-12}, 0.0005, and 0.008, respectively. (The magnitude of probabilities 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 every week. Thus, the probabilities 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, and 0.01, respectively. On the other hand, for the B6 congenic whereas $P = 0.0006$ 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 probability between the 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

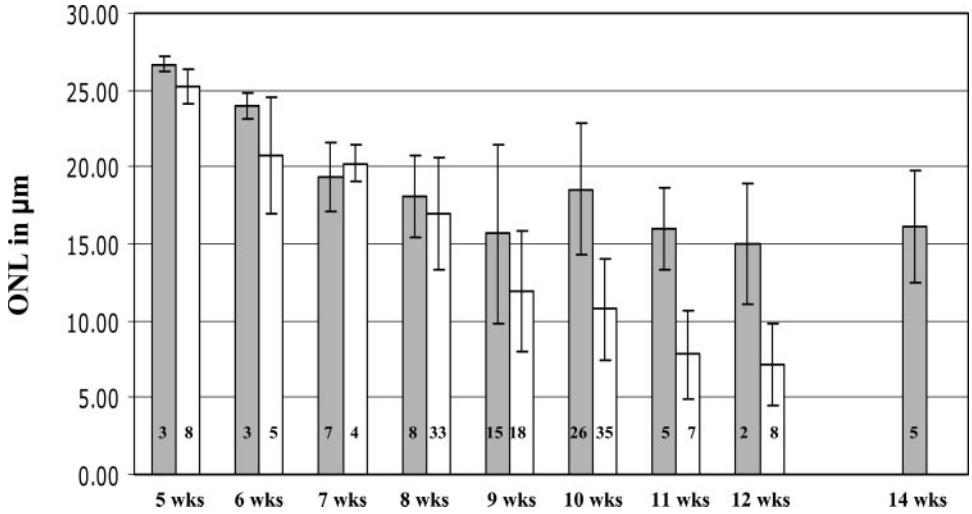

FIGURE 1. Average retinal ONL thickness of BALB-*rd3/rd3* (□) and B6a-*rd3/rd3* (▨) strains at ages 5 to 14 weeks. The numbers in the bars represent the number of mice providing data at each time point.

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 a significant difference in ONL thickness between the two strains (7.8×10^{-12}) and because there was still 10 to 11 μm of ONL left in the more susceptible BALB-*rd3/rd3* strain. Four hundred thirty-one F2 progeny were bred from an F1(BALB-*rd3/rd3* x 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 QTLs ($P < 0.33$), 3.48 for significant QTLs ($P < 0.05$), and 5.17 for highly significant QTLs ($P \leq 0.001$). Highly significant QTLs were found on Chrs 17, 5 (two), 8, 14, and 6 and suggestive QTLs on Chrs 7 and 1. The two QTLs on Chr 5 had BALB alleles that protected against RD; the rest of the QTLs 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 QTLs on Chrs 17, 5, and 8.

A second peak on Chr 5 was detected with the 10-cM map, 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 whether any genes were acting together to influence *rd3*-RD in a significant, synergistic way,

TABLE 1. QTL from *rd3*-RD, BALB-*rd3/rd3* × B6a-*rd3/rd3* Intercross

Sig*	Chr	LOD Score at Peak of Interval Map	SNPs Flanking QTL at ≤1 LOD	Mb from Centromere†	Critical Area (Mb)	% Effect (% Total Genetic Effect)‡	Best-Fitting Inheritance Model
HS	96 cntrls§	11.9	—	—	—	40 (100)	Additive
HS	17	19.3	rs6397584	27.2	21.8	19 (47.5)	Additive
			rs13483016	49.0			
HS	5	19.7	rs13478402	96.4	21.8	−16 (−40)‖	Recessive
			rs13478483	118.2			
HS	8	14.5	rs6237645	112.9	17.8	10 (25)	Additive
			rs3697596	130.7			
HS	14	7.3	rs6290836	11.3	36.2	4 (10)	Additive
			rs13482170	47.5			
HS	6	5.3	rs13478697	32.8	35.4	3 (7.5)	Additive
			rs13478841	78.2			
S/HS	5	5.15	rs6187409	63.1	33.3	−3 (−7.5)	Recessive
			rs13478402	96.4			
Sugg	7	2.7	rs8255275	46.0	93.2	1 (2.5)	Recessive
			rs3663988	139.2			
Sugg	1	2.7	Centromere	0	111.5	1 (2.5)	Recessive
			rs3685919	111.5			

Analysis with Map Manager QTXb20. Quantitative trait based on the mean of all ONL thickness measurements.

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

† Mb positions were taken from 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 is the % effect for this locus divided by total % effect of controls (40%) rounded to the nearest whole number.

§ The 96 control subjects 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.

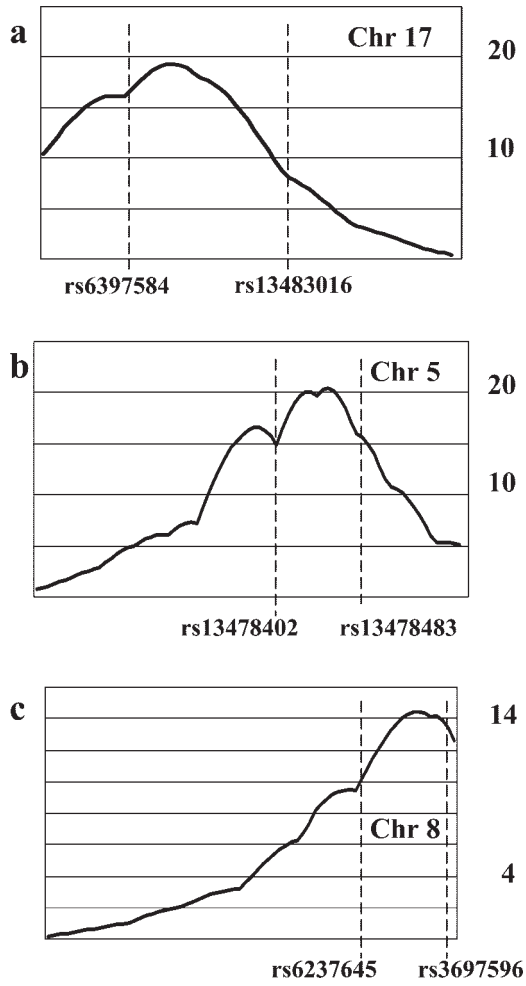

FIGURE 2. Interval maps for the three strongest QTLs. The *x*-axis is the region of the chromosome corresponding to the QTLs and the *y*-axis is the LOD score. Peak LOD scores of each of the three QTLs are well above the 5.17 LOD that marks the 0.001 level of significance. *Dashed lines* circumscribe 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.

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 approximately 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 QTLs 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. To prioritize the search for candidate quantitative trait genes (QTG) by reducing the 1-LOD-support area, additional SNPs within the QTLs 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 QTLs 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 QTLs of Chrs 17, 5, and 8. A comparison of Tables 1 and 2 shows that the critical areas of the three QTLs 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 co-isogenic 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. Figure 4 shows that, as expected, the wild-type B6a strain had no significant loss of ONL thickness after light exposure, whereas the BALB strain did. This result 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

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	30.5	16.7
			rs13483008	47.2	
Chr5	13	24.7	rs13459186	110.4	7.8
			rs13478483	118.2	
Chr8	10	16.2	rs13479995	116.6	11.6
			rs6310608	128.2	

* Mb positions were taken from NCBI mouse build 36.1.

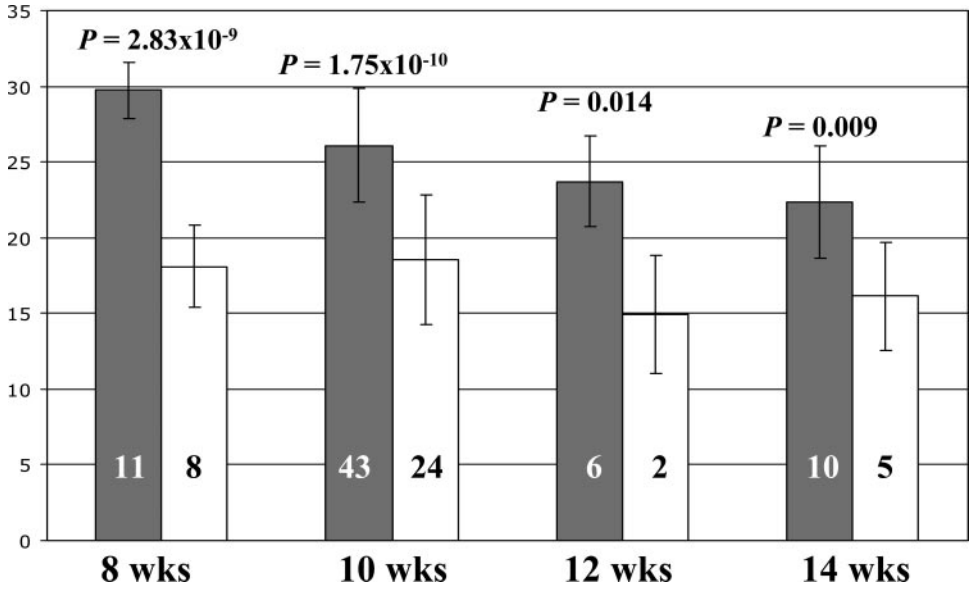

FIGURE 3. Average retinal ONL thickness in micrometers ± SD in B6 pigmented (▨) and B6a albino (□) mice at ages 8 to 14 weeks. *P* by Student's *t*-test. Numbers in bars are the number of mice providing the data.

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 were more susceptible to RD than those *rd3/rd3* mice that were *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 QTLs that modulate rd3 disease have been identified. The QTLs 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 QTLs 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 QTLs reflect B6a alleles that are protective against the disease (relative to the BALB alleles), the two QTLs on Chr 5 reflect BALB alleles that are relatively protective.

Additional informative SNPs within the critical areas of the QTLs on Chrs 17, 5, and 8 were genotyped and analyzed by marker regression. The results reduced the critical areas of these QTLs 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/, provided in the public domain by the Institute of Physical and Chemical Research [RIKEN], Japan), the number of candidate genes re-

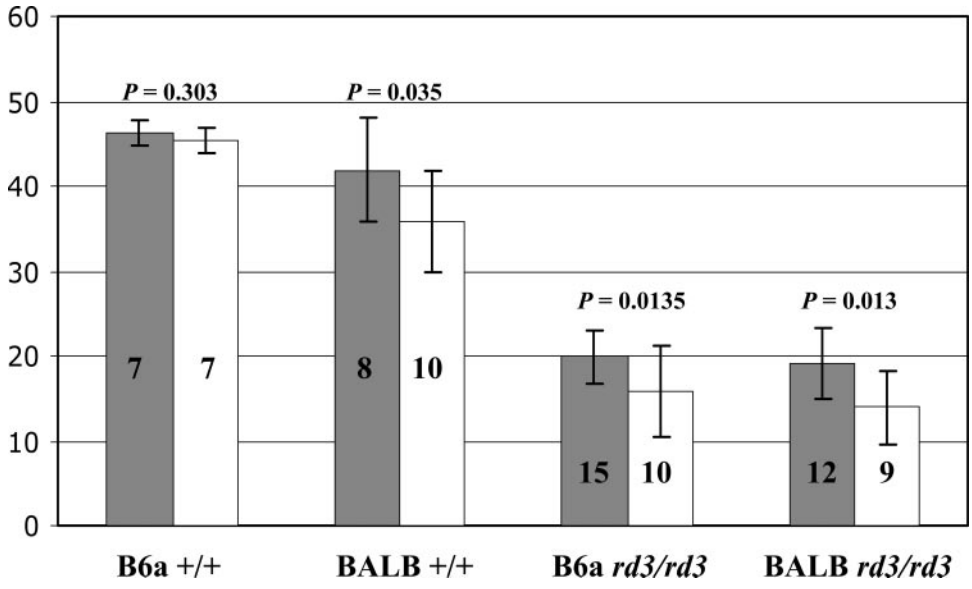

FIGURE 4. Average retinal ONL thickness in micrometers ± SD after 6 days of constant exposure to 70 to 120 ft-c of light with overhead white fluorescent lighting. (▨) Unexposed mice at age 48 days; (□) 6 days of light exposure to mice at 42 days (age 48 days at measurement). Numbers in bars are the number of mice providing the data. *P* by Student's *t*-test.

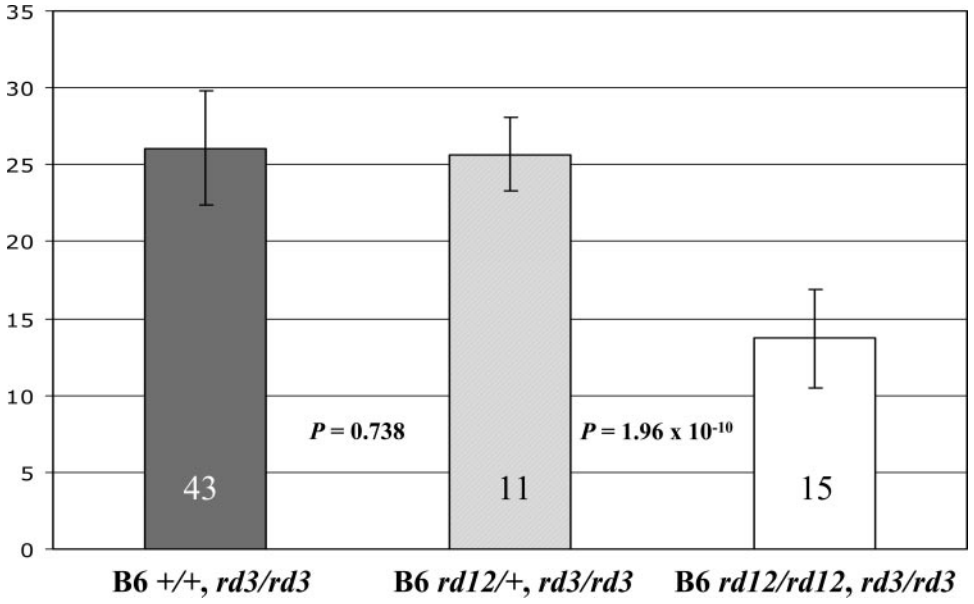

FIGURE 5. Average retinal ONL thickness in micrometers ± SD of pigmented B6-*rd3/rd3* mice with variation in the *rd12* allele. All mice were 10 weeks of age when tested. *P* by Student's *t*-test. Numbers in bars are the number of mice providing the data. The *rd12* allele contains a null mutation that creates an effective knockout of the RPE65 protein when homozygous.

mained very high. Thus, we entered the refined 1-LOD intervals for each of the Chr 17, 5, and 8 QTLs with the keyword retina, and the program identified 96, 31, and 32 candidate genes, respectively. To mention a few, candidate QTGs 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 β-carotene 15,15′ monooxygenase (*Bcmo1*) and phospholipase C γ-2 subunit (*Plcg2*), both expressed in the 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 online from the GeneNetwork (see the Methods section). 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 twofold 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 expressed sequence tags (ESTs). Corresponding searches for the refined intervals on Chrs 5 and 8 yielded 35 and 37 unique genes and 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 twofold or greater between wild-type and RD strains. The following represents genes/transcripts that met these criteria: Chr 17 interval, *Guca1b* (non-RD strains had an average expression of 36.8× the average of *rd1*-RD strains), *Guca1a* (11.4×) *Rrp1b* (3.5×), *Pla2g7* (2.9×), and *Zfp472* (2.6×); Chr 5 interval: *Ccdc64* (3.4×); Chr 8 interval: *Wwox* (2.4×).

It should be noted that these 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 QTGs 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 QTLs identified using the B6a and BALB strains with age-RD and LRD QTLs previously identified with the same two strains.[22,23] The Chrs 8 and 14 *rd3* QTLs overlapped QTLs in the age-RD study but the age-RD QTLs were weaker. None of the *rd3* QTLs was present in the LRD study. Expanding the comparison to QTL studies with different strains, we found overlapping QTLs in several cases: the Chrs 17 and 14 QTLs were present in an AxB age-RD study,[31] but the ageRD QTLs 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 QTLs suggest a broad and fundamental role in RD for some QTGs.

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 whether 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 an 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 QTGs, particularly in the strong QTLs on Chrs 17, 5, and 8 will provide

avenues of study that may lead to future therapies for human RDs.

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