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Genetic modifier loci of mouse *Mfrp*^{rd6} identified by quantitative trait locus analysis

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Abstract

The identification of genes that modify pathological ocular phenotypes in mouse models may improve our understanding of disease mechanisms and lead to new treatment strategies. Here, we identify modifier loci affecting photoreceptor cell loss in homozygous *Mfrp*^{rd6} mice, which exhibit a slowly progressive photoreceptor degeneration. A cohort of 63 F2 homozygous *Mfrp*^{rd6} mice from a (B6.C3Ga-*Mfrp*^{rd6}/J × CAST/EiJ) F1 intercross exhibited a variable number of cell bodies in the retinal outer nuclear layer at 20 weeks of age. Mice were genotyped with a panel of single nucleotide polymorphism markers, and genotypes were correlated with phenotype by quantitative trait locus (QTL) analysis to map modifier loci. A genome-wide scan revealed a statistically significant, protective candidate locus on CAST/EiJ Chromosome 1 and suggestive modifier loci on Chromosomes 6 and 11. Multiple regression analysis of a three-QTL model indicated that the modifier loci on Chromosomes 1 and 6 together account for 26% of the observed phenotypic variation, while the modifier locus on Chromosome 11 explains only an additional 4%. Our findings indicate that the severity of the *Mfrp*^{rd6} retinal degenerative phenotype in mice depends on the strain genetic background and that a significant modifier locus on CAST/EiJ Chromosome 1 protects against *Mfrp*^{rd6}-associated photoreceptor loss.

Keywords

eye disease; retinal degeneration; MFRP; QTL analysis; modifier genes

1. Introduction

Genetic approaches hold great promise toward revealing the molecular basis of degenerative diseases such as retinitis pigmentosa (RP). RP consists of a heterogeneous group of retinal dystrophies characterized by the progressive death of rod and cone photoreceptor cells (Ayuso and Millan, 2010; Ferrari et al., 2011). The clinical phenotype includes night blindness with deterioration of peripheral visual field and, in many cases, eventual loss of central vision. The causal association of 57 genes with RP (June 2013, Retinal Information

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Appendix A. Supplementary data
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Network, http://www.sph.uth.tmc.edu/Retnet/) highlights the success of genetic approaches in the 23 years since a mutation in the first gene associated with autosomal dominant RP, *RHO*, was documented (Dryja et al., 1990). Despite this success, the molecular processes that link RP-causing mutations and pathological progression remain obscure in most cases.

Part of the difficulty of elucidating the molecular mechanisms underlying the pathologies observed in RP is the considerable variation in the onset, severity and clinical presentation of affected individuals. A major source of variation is genetic heterogeneity. Mutations in any one of the 57 disease-causing genes can yield the clinical RP-like phenotype, each with distinct disease characteristics. Allelic variation, affecting different domains within a gene, is also thought to contribute to differences in phenotypic outcome. However, phenotypic variation is observed even among family members with identical mutations, suggesting an effect of environment or interaction of genetic modifiers with the disease gene (Ayuso et al., 1996; Bandah-Rozenfeld et al., 2010; Berson et al., 2001, 1991a,b; Chang et al., 2007; Jacobson et al., 2000; Langmann et al., 2010; Passerini et al., 2007; Paunescu et al., 2007; To et al., 2002). Modifier genes are able to influence the phenotypic expression of genes in both monogenic and multigenic traits, and may interact directly with the mutant gene or influence the pathological pathways that are induced by the disease allele(s) (Genin et al., 2008; Haider et al., 2002). Therefore, identifying modifier genes for RP may help to explain the variation in disease presentation and therapeutic response, improve our understanding of functional pathways that underlie the retinal degenerative phenotype, and reveal important targets for clinical intervention.

Identifying modifier genes in rare human diseases such as RP is challenging due to small population sizes, environmental variability, and heterogeneous genetic backgrounds. For example, the population size of patients carrying the most common autosomal dominant RP (adRP) allele in North America, the P23H mutation of rhodopsin, is estimated to be ~900 individuals (2013 estimate of North American population × RP incidence × fraction of RP attributed to adRP × fraction of adRP linked to *RHO* × fraction of *RHO* RP ascribed to P23H = 470,000,000 × 0.00033 × 0.2 × 0.27 × 0.11).[https://www.cia.gov/library/publications/the-world-factbook/] (Daiger et al., 2007; Sullivan et al., 2006). Population sizes are expected to be smaller for less common alleles and for autosomal recessive RP genes. Even if all individuals with the P23H *RHO* RP mutation were assessed, variation in phenotype due to non-genetic factors, including age, diet, light exposure history, and differences in clinical assessment would confound efforts to establish gene associations. These difficulties are compounded by the substantial genetic variation in human populations. Although modifier genes have recently been revealed in analyses of very large adRP families with similar genetic backgrounds (Venturini et al., 2012) and in X-linked RP (Fahim et al., 2011), success with identifying modifiers of autosomal recessive RP has been limited.

The identification of modifier genes in mouse models, which allows for the control of environmental, genetic and experimental variation, is an attractive complementary approach to human studies (Hamilton and Yu, 2012). Modifier genes can be discovered in relatively small cohorts (typically 50–300 animals) with well-characterized genetic backgrounds that can be readily generated by crossing inbred strains. A growing number of genes that modify

ocular disease phenotypes have been successfully identified in mice, such as *Mtap1a*, which modifies retinal degeneration associated with *Tub* and *Tulp1* mutations (Ikeda et al., 2002; Maddox et al., 2012); *Rpe65*, which modifies RP conferred by mutant *Rho* alleles (Samardzija et al., 2006); and *Tyr*, which modifies retinoschisis caused by *Rs1* mutations (Johnson et al., 2008) [for additional examples, see (Hamilton and Yu, 2012)].

The study presented here identifies candidate genetic loci that modify the phenotype of homozygous *Mfrp*^{rd6} mice, which exhibit a progressive retinal degeneration as observed in RP (Chang et al., 2002; Hawes et al., 2000; Kameya et al., 2002). A similar phenotype has been described in homozygous *Mfrp*^{rdx} mice (Fogerty and Besharse, 2011). In humans, *MFRP* mutations are associated with RP in an ocular syndrome that also includes posterior microphthalmos, foveoschisis and optic nerve head drusen (Ayala-Ramírez et al., 2006; Crespí et al., 2008; Mukhopadhyay et al., 2010; Neri et al., 2012; Zenteno et al., 2009). A distinct *MFRP*-associated syndrome characterized by nanophthalmos without RP has also been described (Sundin et al., 2008, 2005). A prominent feature of homozygous *Mfrp*^{rd6} and homozygous *Mfrp*^{rdx} mice is the appearance of small discrete spots throughout the fundus, which are likely to correspond to subretinal innate immune cells (Fogerty and Besharse, 2011; Hawes et al., 2000). Similar spots have been documented in individuals with *MFRP*-associated disease (Neri et al., 2012; Zenteno et al., 2009), suggesting that innate immune cells may contribute to disease pathology. *Mfrp* encodes a transmembrane protein of unknown function that has been proposed to modulate or regulate Wnt/Frizzled signaling (Kameya et al., 2002; Katoh, 2001). In this study, we show that the severity of the *Mfrp*^{rd6} retinal degenerative phenotype varies with genetic background. We identify modifier loci that account for this variation and apply bioinformatics to narrow the list of candidate genes that may explain the observed effects.

2. Methods

2.1. Experimental animals

Mice provided with acidified water and JL Rat and Mouse/Auto 4F (5K54) diet (LabDiet, St. Louis, MO) were housed in cages exposed to a 12 h light–dark cycle in The Jackson Laboratory Research Animal Facility. All mice were treated in accordance with the Animal Care and Use Committee at The Jackson Laboratory and in compliance with the Association for Research in Vision and Ophthalmology (ARVO) statement for ethical care and use of animals.

2.2. Mouse production and genotyping

Mutant F2 progeny from an intercross of F1 hybrids of homozygous B6.C3Ga-*Mfrp*^{rd6}/J and CAST/EiJ mice were genotyped by PCR to identify homozygous *Mfrp*^{rd6} mice, which possess a 4 bp deletion in the splice donor sequence of *Mfrp* intron 4 (Kameya et al., 2002). The PCR primers rd6delF (5′-CACTACCACCCCAGCAAGGAC-3′) and rd6delR (5′-CTTCTCCAGAGAGTGCCCTTG-3′) flanking the mutation were used to generate 91 and 87 bp products from the *Mfrp* wild-type and *rd6* alleles, respectively, using the following cycling conditions: 97 °C, 3 min; [95 °C, 15 s; 55 °C, 30 s; 72 °C, 30 s] × 50; 72 °C, 3 min; 11 °C hold. The resulting PCR products were resolved by gel electrophoresis in a mixture of

3% Metaphor® and 1% SeaKem® LE agarose (Lonza Rockland, Rockland, ME). Mice were additionally genotyped by allele-specific PCR as described (Chang et al., 2013) to test for *Crb1rd8*, a common mutation found in laboratory mice (Chang et al., 2013; Mehalow et al., 2003) that is known to influence ocular phenotypes (Mattapallil et al., 2012). For QTL analysis, mice were genotyped by KBio-sciences (Beverly, MA) with a panel of single nucleotide polymorphism (SNP) markers spaced ~20 cM apart on all chromosomes. Additional PCR analyses with the MIT markers *D1Mit33* and *D1Mit353* were used to refine the candidate modifier locus on Chromosome 1. Finally, to strengthen bioinformatics analysis, B6.C3Ga-*Mfrp*^{rd6}/J mice were screened with a 150-marker SNP panel developed at The Jackson Laboratory that distinguishes C57BL/6J and C57BL/6N.

2.3. Mouse phenotyping

Eyes from 20-week-old F2 *Mfrp*^{rd6} homozygotes were assessed histologically as previously described (Maddox et al., 2012), with the exception that microscopy was performed with a 40× objective on a Leica DMLB microscope (Leica Microsystems, Buffalo, NY) and images were captured with a DMC-1 digital microscope camera (Polaroid, Minnetonka, MN). Retinal sections in which the optic nerve was at its widest were selected for imaging. Retinal degeneration was quantified by manually counting the number of nuclei in a 450-pixel wide region of the outer nuclear layer (ONL) positioned roughly parallel to the image frame at ~170–230 μm from the edge of the optic nerve.

2.4. QTL analysis

2.4.1. Genome-wide one-dimensional scan—Phenotype and genotype data were imported into R/qtl, version 1.23–16 (Broman et al., 2003), which enables genome-wide one-and two-dimensional (pair-wise) scans, multiple locus modeling and inclusion of covariates. The distribution of ONL nuclei counts was not statistically different from normality (Shapiro–Wilk goodness-of-fit, $p = 0.367$). Therefore the data were not transformed, as they met the modeling assumption criteria. One thousand permutations were performed to determine the thresholds for QTL detection in genome-wide scans (Doerge and Churchill, 1996). Four thresholds, 1%, 5%, 10% and 63%, were calculated from the permutation results. QTL with a log of the odds ratio (lod) score above the 5% threshold were considered significant, while those above the 63% threshold were considered suggestive (Lander and Kruglyak, 1995).

2.4.2. Genome-wide pair-wise scan—Pair-wise scans were performed using 2-cM spacing. All possible pairs of QTL locations on each chromosome were tested for association with the number of cell bodies remaining within a fixed region of the ONL as described above. As permutations are extremely resource intensive, genome-wide two-dimensional scan significance thresholds were based on previously recommended $P < 0.05$ thresholds for a mouse intercross (Broman and Sen, 2009).

2.4.3. Multiple QTL modeling—QTL along with possible QTL × QTL interactions derived from a single QTL scan and pair-wise scans were fit into multiple regression models in the presence of significant covariates, if any. By doing so, variations of the phenotype in the models were estimated. The *p* values for terms in the multiple regression models were

calculated. Terms were dropped sequentially until all of the terms in the model were significant at the 1% level for both the main QTL effects and their interaction effects.

3. Results

3.1. Variation in Mfrprd6 retinal degeneration

To test whether the *Mfrp*^{rd6} phenotype varies with strain genetic background, we examined homozygous *Mfrp*^{rd6} F2 mice from a (B6.C3Ga-*Mfrp*^{rd6}/J × CAST/EiJ) F1 intercross. At 20 weeks of age, the ONL of these mice was thinner than that of heterozygous control mice and varied in thickness (Fig. 1A). The number of nuclei in a 450-pixel wide region (67 μm) of the ONL at a constant distance centered ~200 μm from the optic nerve was counted manually in each of 63 specimens. The frequency distribution of the ONL nuclear count in these samples (Fig. 1B) is consistent with a normal distribution (see Methods). The use of ONL count as a quantitative phenotype does not correct for section obliqueness, which can affect the number of nuclei counted within a fixed area of the histological section. Experimental variation in ONL thickness due to section obliqueness is unlikely to exceed 15–20%, as eyes are oriented similarly prior to embedding, and variation of this magnitude is expected to have a minimal effect on QTL analysis.

3.2. QTL analysis

The genotypes of 100 genome markers in 63 F2 mice were determined by whole genome SNP analysis. Although genotyping quality was generally excellent, four of the markers showed a lod score of >3.0 with the checkAlleles() function of R/qtl and two resulted in a larger predicted recombination estimate than expected. These markers were excluded from further analysis. Three of the excluded markers were located on Chromosome 9 near the *Mfrp* gene, consistent with SNP variation due to the congenic region of B6.C3Ga-*Mfrp*^{rd6}/J. Two of the excluded markers were located on Chromosome 1, where preliminary studies had indicated a candidate modifier locus. Therefore, additional genotyping was performed with the MIT markers *D1Mit33* and *D1Mit353* and included in the final QTL analysis. A genome-wide one-dimensional scan (Fig. 2) of autosomal markers indicated a significant modifier locus on Chromosome 1 (exceeding the p=0.01 threshold calculated for a genome-wide scan; Fig. 2, solid line) and suggestivemodifiers on Chromosomes 6 and 11 (exceeding the p=0.63 threshold; Fig. 2, dashed-dotted line). Further testing failed to reveal an influence of sex on the phenotype, but left open the possibility that analysis of a larger cohort might reveal an influence of gender. There was no evidence for interaction among modifier loci, leading to a simple three-QTL model.

To test the relative importance of the modifier loci, the three-QTL model was examined by multiple regression analysis, in which the effect of eliminating terms one at a time on model quality was explored. As shown in Table 1, the candidate loci on Chromosomes 1 and 6 contribute significantly to the observed phenotypic variation and are required for the model, whereas that on Chromosome 11 may be omitted. Together, the three candidate loci are estimated to account for ~30% of the phenotypic variation, while the loci on Chromosomes 1 and 6 account for a combined 26%.

3.3. Effect plot and confidence interval of the Chromosome 1 modifier locus

The effect plot (Fig. 3A) of *D1Mit33*, the marker closest to the Chromosome 1 modifier locus, indicates that the CAST/EiJ sequence protects against the loss of photoreceptors in homozygous *Mfrp*^{rd6} mice. The plot linearity shows that the Chromosome 1 modifier effect is additive, arguing against dominance of the CAST/EiJ or C57BL/6J allele. The peak marker on Chromosome 1 likely to contain modifier genes at the 95% Bayes credible interval is shown in Fig. 3B. This interval maps to 54.7–76.3 centimorgans of Chromosome 1, which relates to a genomic distance of ~44 Mbp (Chromosome 1124 Mb–168 Mb, GRCm38 build).We designate this locus as retinal degeneration 6 modifier 1 (*Rd6m1*).

3.4. Identification of candidate modifier genes within the Rd6m1 locus

Bioinformatics analysis of the C57BL/6J and CAST/EiJ inbred strains was used to provide useful leads for modifier genes within the *Rd6m1* locus. To justify an analysis based on C57BL/6J data, we first determined whether B6.C3Ga-*Mfrp*^{rd6}/J is congenic with this strain. Although B6.C3Ga-*Mfrp*^{rd6}/J was derived by backcrossing to C57BL/6J, the discovery of the *Crb1*^{rd8} mutation during introgression (Mehalow et al., 2003) suggests that at least one generation was conducted with a strain related to C57BL/6N, the apparent source of the *Crb1*^{rd8} allele (Mattapallil et al., 2012). We therefore examined B6.C3Ga-*Mfrp*^{rd6}/J with a collection of 150 SNP markers that differ in C57BL/6N relative to C57BL/6J and are distributed throughout the genome. Of 145 markers that yielded products, 143 were homozygous for the C57BL/6J allele. A single marker on Chromosome 6 was heterozygous for the C57BL/6N allele and a single marker close to the *Mfrp* locus was homozygous for the C57BL/6N allele. These results indicate that B6.C3Ga-*Mfrp*^{rd6}/J is at least 95% congenic with C57BL/6J, supporting bioinformatics analysis based on C57BL/6J data.

The Mouse Genome Informatics (MGI) Gene Query tool (GRCm38 build of the C57BL/6J mouse reference genome) indicated that 385 genes are located within the *Rd6m1* modifier locus, including 343 protein-coding and 42 noncoding RNA genes (Supplemental Table 1). Comparison of the C57BL/6J (GRCm38 build) and CAST/EiJ genome sequences using the Wellcome Sanger Mouse SNP query tool (http://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1303) yielded 259 genes in the candidate locus with single nucleotide polymorphisms or insertion/deletions that are predicted to result in one or more protein coding sequence changes (CDS Change, Supplemental Table 1; Supplemental Data File 1). Fourteen genes containing a variation in a splice donor or acceptor sequence (*Cd55*, *Tnnt2*, *RP23-302C16.3*, *Crb1*, *Cfh*, *Gm15486*, *Myoc*, *Ysk4*, *Elk4*, *Plekha6*, *Gm10530*, *Serpinc1*, *Cenpl*, *Rxrg*), six genes predicted to contain a frameshift (*Eif2d*, *Gm10188*, *Gm17678*, *Dennd1b*, *Tor1aip1*, *Blzf1*), and two genes predicted to gain a premature stop codon (*Gm4204*, *Gm10530*) are of particular interest. These variations may be the most likely to alter the structure and function of the encoded polypeptides. An accession-based query of the 385 genes within the *Rd6m1* modifier interval in PosMed (http://biosparql.org/PosMed/) using a keyword containing terms for retina, retinal pigment epithelium, Wnt/Frizzled signaling, or immune processes yielded 154 unique genes that may be relevant to *Mfrp* expression or function in the eye (PosMed, Supplemental Table 1). Of these, 116 genes overlapped with CDS Change genes (Fig. 4). To filter this list further, differentially expressed genes within the modifier locus were found by reanalyzing published microarray

data comparing C57BL/6J and CAST/EiJ retinas (Jelcick et al., 2011). This subset contained 80 genes (RNA Change, Supplemental Table 1), 33 of which were common to both the CDS Change and PosMed gene subsets (Fig. 4).

4. Discussion

Our examination of an intercross of F1 hybrids between inbred B6.C3Ga-*Mfrp*^{rd6}/J and CAST/EiJ mice revealed that strain genetic background affects the retinal degenerative phenotype associated with homozygosity for the retinal degeneration 6 mutation, *Mfrp*^{rd6}. QTL analysis of the F2 intercross mice indicated the presence of a significant modifier locus on CAST/EiJ Chromosome 1, *Rd6m1*, which protects against the retinal degeneration phenotype of homozygous *Mfrp*^{rd6} mice on the C57BL/6J genetic background in a primarily additive fashion. Suggestive modifier loci on Chromosomes 6 and 11 were also observed in the one-dimensional genome-wide scan. However, based on multiple regression analysis, only *Rd6m1* and the modifier locus on Chromosome 6 were essential for the simplest QTL model.

The observation that genetic background affects the phenotype of *Mfrp*^{rd6} mutant mice under conditions where environmental and experimental variation is minimized strongly supports the influence of one or more *Mfrp* modifier genes. *Mfrp* thus joins a growing list of ocular disease genes in mice that vary in phenotypic severity depending on genetic background, such as *Crb1*, *Tub*, *Tulp1*, *Nr2e3*, *Rs1*, *Rp1*, and *Rd3* (Danciger et al., 2008; Haider et al., 2008; Ikeda et al., 2002; Johnson et al., 2008; Liu et al., 2009; Maddox et al., 2012; Mehalow et al., 2003). The existence of at least two ocular syndromes associated with human *MFRP* mutations (Mukhopadhyay et al., 2010; Neri et al., 2012; Sundin et al., 2005) may reflect an influence of genetic background. In support of this hypothesis, phenotypic variation has been noted among family members with the same *MFRP* mutation (Mukhopadhyay et al., 2010). However, a more recent report claimed that the phenotype of a common *MFRP* deletion variant was similar among individuals in the same family as well as between families, arguing against the hypothesis (Neri et al., 2012). In these studies, interpreting the role of genetic background in *MFRP*-associated disease is problematic due to the small population size. Our finding that the *Mfrp*^{rd6} phenotype in mice depends on genetic background argues for future studies of larger patient cohorts to test if similar effects modify disease phenotypes in humans with *MFRP* mutations.

The discovery of *Rd6m1* prompted us to explore bioinformatics approaches to find candidate modifier genes. From the 385 annotated genes within the modifier interval, 33 genes were identified that possess coding sequence changes in CAST/EiJ compared to C57BL/6J, have semantic association with pathways or tissue and cell types relevant to postulated *Mfrp* function, and are differentially expressed in the CAST/EiJ and C57BL/6J retinas. Although a broader subset of genes focused on differential expression changes or coding sequence changes might also be productive, these 33 genes may serve as a good starting point for future studies. For example, one of these genes, *Cfh*, is a mouse ortholog of *CFH*, which encodes complement factor H and is a risk determinant for age-related macular degeneration (AMD) (Hageman et al., 2005; Klein et al., 2005). Mutations in *CFH* that both increase and decrease AMD risk have been documented (Hageman et al., 2005; Klein et al., 2005;

Tortajada et al., 2009). The CAST/EiJ gene includes 14 missense polymorphisms and a change in a putative splice acceptor site (Supplemental Data File 1), which may alter the CFH protein in away that protects against photoreceptor loss. Thus, the *Cfh* gene in CAST/EiJ mice may be among the most promising candidates within the critical region for modifying the *Mfrp*^{rd6} phenotype.

Future work will refine the *Rd6m1* locus and the modifier regions on Chromosomes 6 by generating congenics for use in additional mapping studies. Eventually, as the region is narrowed, combining mouse models of the candidate genes that fall within the critical modifier region with *Mfrp*^{rd6} mice in the same genetic background may shed light on the genetic interactions. Identification of modifier(s), especially those that provide protection for photoreceptor loss, is arguably important as they may provide blueprints for therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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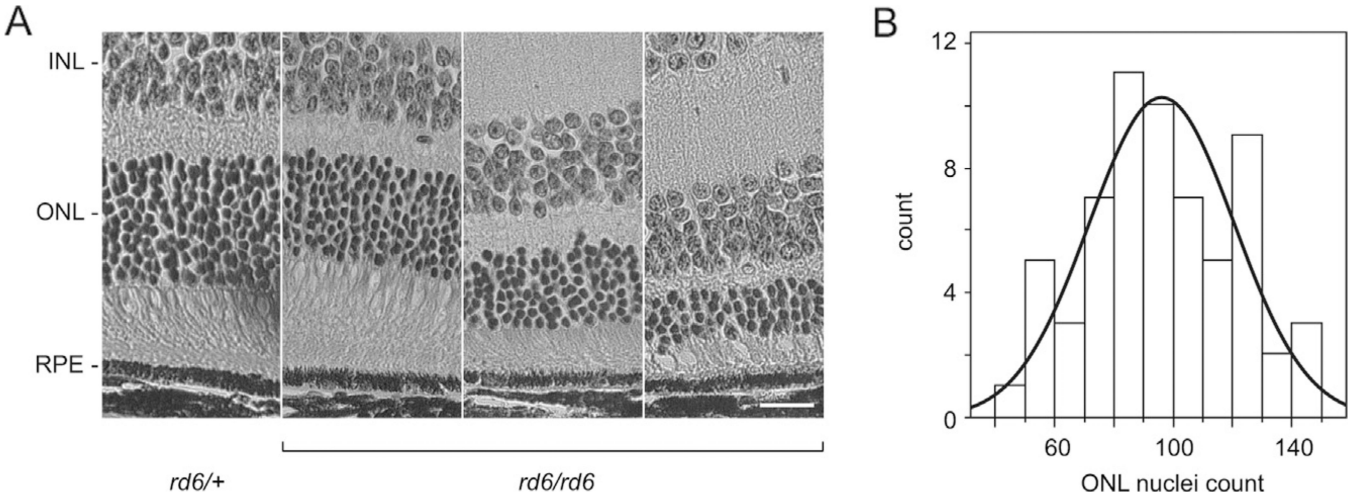

Fig. 1.

Phenotypic analysis of retinas at 20 weeks of age. (A) ONL thickness of F2 homozygous *Mfrp*^{rd6} mice varies in a segregating genetic background obtained from a (B6.C3Ga-*Mfrp*^{rd6}/J × CAST/EiJ) F1 intercross. Heterozygous mice (*rd6*/+, left panel) show normal retinal layers while homozygous *Mfrp*^{rd6} progeny (*rd6/rd6*) showed differing degrees of ONL thinning. The *Mfrp* genotype is indicated. *INL*, inner nuclear layer; *ONL*, outer nuclear layer; *RPE*, retinal pigment epithelium. *Bar*: 20 μm. (B) Frequency distribution of ONL nuclear counts from 63 F2 homozygous *Mfrp*^{rd6} mice. The data fit a normal distribution (solid curve).

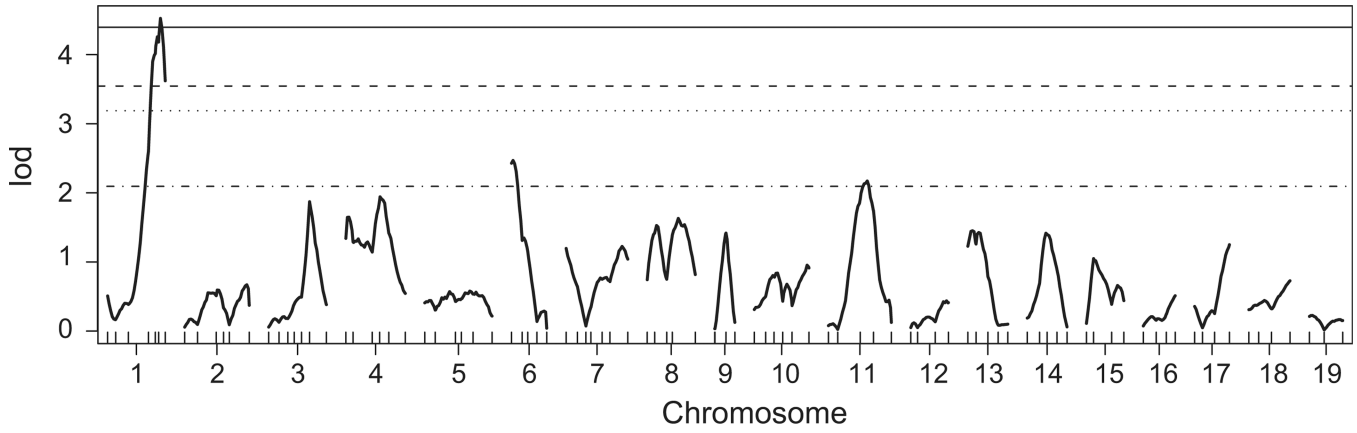

Fig. 2.

QTL analysis. Genome-wide one-dimensional scan analysis of 63 F2 *Mfrp*^{rd6} homozygous mice at SNP intervals of ~20 cM yielded a significant modifier locus on Chromosome 1 and suggestive loci on Chromosomes 6 and 11. Sex chromosomes were not included in the analysis as there was no indication of gender effects on the disease phenotype. Genome-wide significance thresholds are indicated at $p = 0.63$ (dotted-dashed line), 0.10 (dotted line), 0.05 (dashed line) and 0.01 (solid line). *lod*: log of the odds ratio.

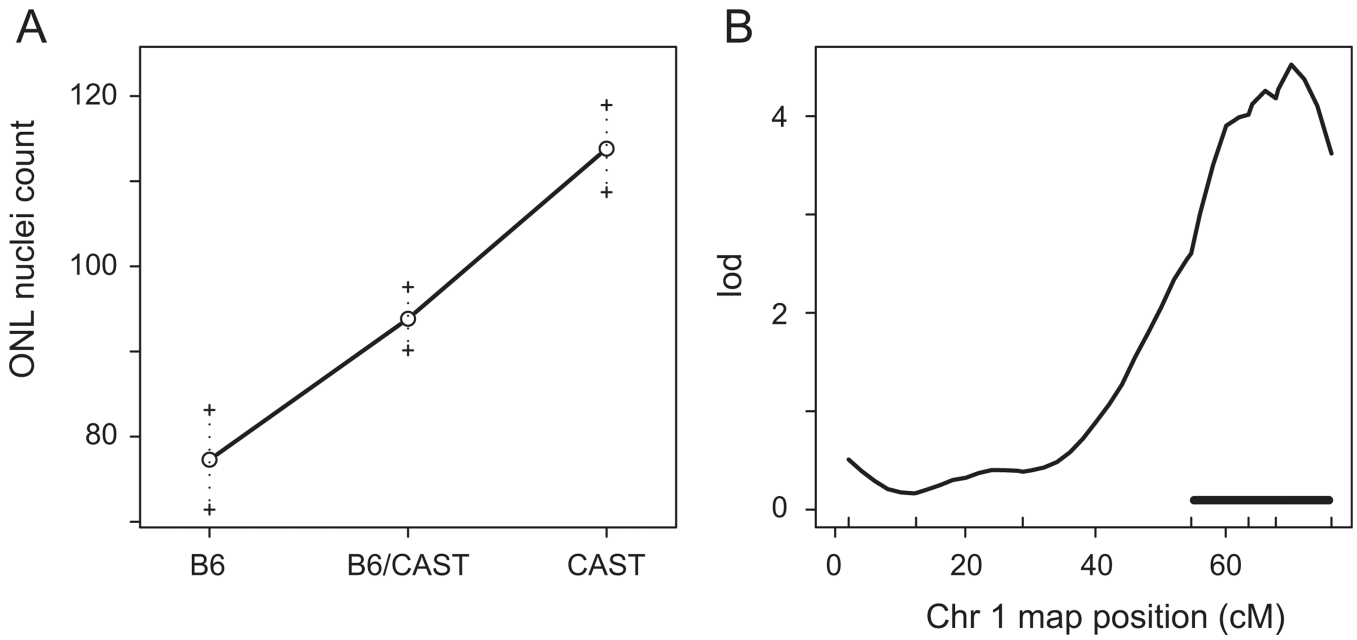

Fig. 3.

Effect plot and confidence interval for the significant modifier locus on Chromosome 1. (A) Effect plot of homozygous (B6, C57BL/6J; CAST, CAST/EiJ) and heterozygous (B6/CAST) phenotypes indicated an additive, protective effect of the modifier locus on Chromosome 1. (B) A confidence interval of ~22 cM was found for the Chromosome 1 modifier locus. *lod*: log of the odds ratio; *ONL*: outer nuclear layer.

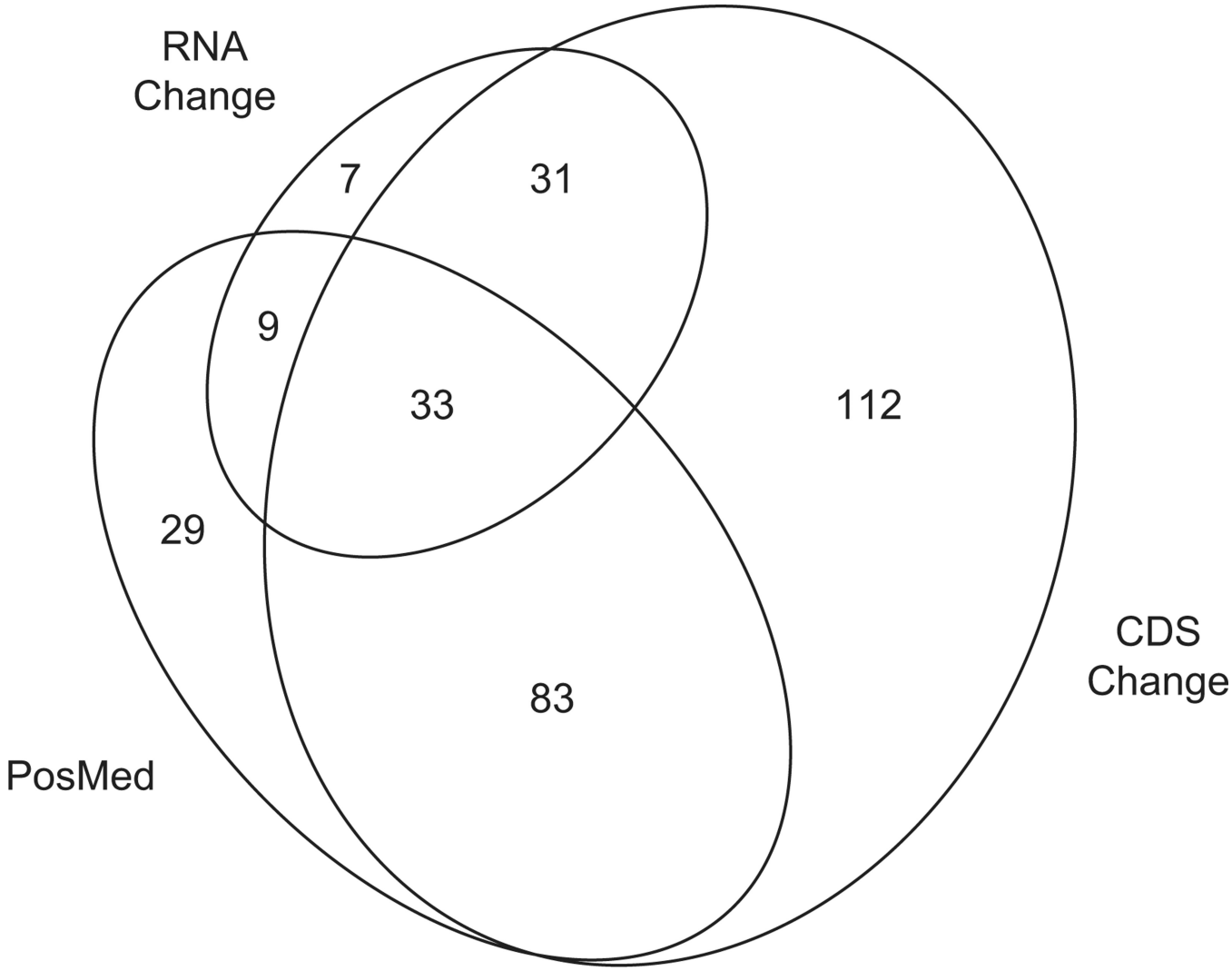

Fig. 4.

Area-proportional diagram showing the intersection of gene subsets derived by filtering 385 annotated genes within the *Rd6m1* modifier locus. The subsets include genes with nucleotide changes in CAST/EiJ compared to C57BL/6J that are likely to affect protein coding (CDS Change, obtained with the Sanger Mouse Genomes Project query tool); genes with a semantic link to the postulated function or tissue expression of *Mfrp* (PosMed); and retinal genes that are differentially expressed (Q value of false discovery rate <0.05) in C57BL/6J compared to CAST/EiJ (RNA Change, obtained from reevaluation of microarray data (Jelcick et al., 2011)). The number of genes in each area of the diagram is indicated. Proportioned ellipses were generated with eulerAPE v2.0.3 (http://www.eulerdiagrams.org/eulerAPE/).

Table 1

Multiple regression analysis of the QTL model.

QTL	df[a]	Type III SS[b]	lod[c]	%Var[d]	F[e]	*p*-value (χ^2)[f]	*p*-value (F)[g]
1@70.1 cM	2	5742	3.31	15.44	7.66	0	0.0012**
6@14.1 cM	2	3760	2.25	10.11	5.01	0.006	0.0099**
11@52.9 cM	2	1739	1.09	4.67	2.32	0.082	0.1079

Values reflect a comparison of the full three-QTL model to the sub-model with the indicated QTL term dropped.

[a] Degree of freedom.

[b] Type III sum of squares.

[c] log of the odds ratio.

[d] Percentage of variance explained.

[e] F statistics.

[f] *p* value for χ^2 distribution.

[g] *p* value for F distribution with significance threshold <0.01,**; <0.05,*.