An Allele of Microtubule-Associated Protein 1A (*Mtap1a*) Reduces Photoreceptor Degeneration in *Tulp1* and *Tub* Mutant Mice

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PURPOSE. To identify genes that modify photoreceptor cell loss in the retinas of homozygous *Tulp1tm1Pjn* and *Tubtub* mice, which exhibit juvenile retinitis pigmentosa.

METHODS. Modifier loci were identified by genetic quantitative trait locus analysis. F2 *Tulp1tm1Pjn/tm1Pjn* mutant mice from a B6-*Tulp1tm1Pjn/tm1Pjn* × AKR/J intercross were genotyped with a panel of single nucleotide polymorphism markers and phenotyped by histology for photoreceptor nuclei remaining at 9 weeks of age. Genotype and phenotype data were correlated and examined with Pseudomarker 2.02 using 128 imputations to map modifier loci. Thresholds for the 63%, 10%, 5%, and 1% significance levels were obtained from 100 permutations. A significant, protective candidate modifier was identified by bioinformatic analysis and confirmed by crossing transgenic mice bearing a protective allele of this gene with *Tulp1*- and *Tub*-deficient mice.

RESULTS. A significant, protective modifier locus on chromosome 2 and a suggestive locus on chromosome 13 that increases photoreceptor loss were identified in a B6-*Tulp1tm1Pjn/tm1Pjn* × AKR/J intercross. The chromosome 2 locus mapped near *Mtap1a*, which encodes a protein associated with microtubule-based intracellular transport and synapse function. The protective *Mtap1a129P2/OlaHsd* allele was shown to reduce photoreceptor loss in both *Tulp1tm1Pjn/tm1Pjn* and *Tubtub/tub* mice.

CONCLUSIONS. It was demonstrated that the gene *Mtap1a*, which modifies hearing loss in *Tubtub/tub* mice, also modifies retinal degeneration in *Tubtub/tub* and *Tulp1tm1Pjn/tm1Pjn* mice. These results suggest that functionally nonredundant members of the TULP family (TUB and TULP1) share a common functional interaction with MTAP1A. (*Invest Ophthalmol Vis Sci.* 2012;53:1663–1669) DOI:10.1167/iovs.11-8871

Retinal degenerative diseases are highly variable in onset, severity, and rate of progression. Retinitis pigmentosa (RP), with an incidence of roughly 1:4000 in the United States, is a prominent example of this variability.[1] RP consists of a heterogeneous group of retinal dystrophies characterized by rod and cone photoreceptor cell degeneration, usually through apoptotic cell death. Clinical features of the disease include night blindness, which is often accompanied by a gradual loss of peripheral visual field and eventual loss of central vision. Variation in the clinical presentation of the disease is partly explained by the existence of >48 RP genes, each with multiple alleles that may cause substantial differences in disease phenotype (Retinal Information Network, http://www.sph.uth.tmc.edu/Retnet/).[1] However, variation has been documented even among family members of similar age with identical RP mutations, suggesting contributions from other genetic or environmental factors.[2–12] Modifier genes, which influence the phenotypic expression of genes in monogenic and multigenic traits, are a potential source of variation in such cases.[13] Identifying modifier genes in RP may help to explain variation in disease presentation and therapeutic response, improve understanding of functional pathways that underlie the RP phenotype, and reveal important targets for clinical intervention.

Our interest in RP gene modifiers arose from studying two members of the tubby-like protein (TULP)[14] gene family that are associated with retinal degeneration. The TULP gene family consists of four members: *Tub*, *Tulp1*, *Tulp2*, and *Tulp3*. Homozygous mutations of *Tub* (*Tubtub*) and *Tulp1* in mice cause increased apoptotic photoreceptor cell death and early-onset retinal degeneration; *Tub* mutants also exhibit cochlear degeneration and late-onset obesity.[15–19] In humans, a disruption in *Tulp1* has been identified as the causative factor of RP type 14, a rare, autosomal recessive form of RP that is genetically heterogeneous.[20–23] Indeed, 23 pathogenic *TULP1* mutations were identified from the literature in a recent study of human patients with RP or Leber congenital amaurosis.[24] Much evidence suggests that TUB and TULP1 function in vesicular transport in neurons, because disruption of either *Tub* or *Tulp1* leads to abnormal accumulation of vesicles in the photoreceptor matrix at the level of the inner segments,[25,26] and deficiency of *Tulp1* results in structural and functional defects at the photoreceptor synapse.[27,28] These defects are thought to be responsible for the observed photoreceptor cell apoptosis. However, other studies point to a role of the TULP family[14] in insulin signaling,[29] transcriptional regulation,[30,31] and phagocytosis.[32,33] Thus, it remains to be determined which of the proposed functions of TUB and TULP1 are of greatest relevance in the healthy and diseased retina.

To gain insight into how disruptions in TULP family members lead to retinal degeneration, we previously performed a quantitative trait locus (QTL) analysis by outcrossing B6.Cg-*Tubtub* mutant mice to strain AKR/J, and assessed the progression of photoreceptor degeneration in F2 progeny.[34] In homozygous *tub* F2 mice, a suggestive protective locus ($P < 0.05$) from AKR/J was identified on chromosome 2. Likewise, here, in a similar cross with *Tulp1tm1Pjn/tm1Pjn* mutant mice, a

significant locus ($P < 0.01$) from AKR/J is identified in the same region on chromosome 2. In both studies, the photoreceptor protective locus on chromosome 2 overlaps the *moth1* (modifier of tubby hearing1) locus, which our laboratory had previously identified as *Mtap1a*.[35,36]

Crossing the *Tulp1*$^{tm1Pjn/tm1Pjn}$ and *Tub*$^{tub/tub}$ mutant mice to transgenic mice carrying a 129P2/OlaHsd *Mtap1a*$^{moth1-r}$ allele resulted in a significant protective effect ($P < 0.0005$ and $P < 0.04$, respectively) against photoreceptor loss in the F2 generation in transgene-positive (tg+) *Tulp1*$^{tm1Pjn/tm1Pjn}$ and B6.Cg-*Tub*$^{tub/tub}$ mutant mice. These results indicate that, although *Tulp1* and *Tub* are not functionally redundant, they both exhibit a genetic interaction with *Mtap1a*. Because MTAP1A is implicated in cytoskeletal processes at the neuronal synapse,[37] these findings further support the hypothesis that TULP family members are involved in synapse maintenance[36] or architecture.[28,38]

METHODS

Experimental Animals

All mice were treated in accordance with the Animal Care and Use Committees at each of the contributing institutions and in compliance with the Association for Research in Vision and Ophthalmology (ARVO) statement for ethical care and use of animals.

Mouse Production and Mapping

Mutant mice from an intercross of homozygous B6.129 $\times$ 1-*Tulp1*^{tm1Pjn} $\times$ AKR/J were genotyped by PCR to identify *Tulp1*^{tm1Pjn} homozygotes (designated as *Tulp1*$^{tm1Pjn/tm1Pjn}$ mice elsewhere in this study). The eyes from 9-week-old F2 *Tulp1*$^{tm1Pjn/tm1Pjn}$ intercross mice were processed for histologic observation and scored for the loss of photoreceptor nuclei (1, mild; 2, moderate; 3, severe). Mice were genotyped (KBiosciences, Beverly, MA) with a panel of single nucleotide polymorphism (SNP) markers spaced at approximate 20-cM intervals. The genotype and phenotype data were analyzed with Pseudomarker 2.02[39] using 128 imputations. One hundred permutations were performed to determine thresholds for the 63%, 10%, 5%, and 1% significance levels.

To confirm the chromosome 2 QTL, additional F2 *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice were collected, and follow-up genotyping on these mice combined with the initial cohort was performed in a fashion similar to that described by Danciger and colleagues.[40] Briefly, DNA was genotyped with simple sequence length polymorphic (SSLP) markers at 10-cM intervals in chromosomal regions, demonstrating possible QTL loci, and analyzed using Pseudomarker 2.02 as described earlier.

Microscopy

Whole eyes were enucleated, fixed in 37.5% methanol, 12.5% glacial acetic acid in phosphate buffered saline, embedded in paraffin, and sectioned at 6-μm intervals. The sections were mounted on commercial slides (Superfrost Plus; Fisher Scientific, Pittsburgh, PA) and stained with hematoxylin and eosin. Sections were analyzed by brightfield illumination on a light microscope (Leitz DMRO; Leica Microsystems, Buffalo Grove, IL) and images were collected with a commercial software package (FireCam software; Leica Microsystems). Brightness and contrast of images were optimized with a digital imaging package (Photoshop 7.0; Adobe Systems, San Jose, CA).

Quantification of Retinal Degeneration

To quantify the degree of retinal degeneration, we followed a previously published protocol.[35] The section in which the optic nerve was at its greatest width was selected from serial sections, and carefully photographed. For a quantitative measure of retinal degeneration the number of rows of nuclei contained within the outer nuclear layer (ONL) were counted at three points within a 200-μm square near the optic nerve head and the values were averaged. Because the thickness

of the inner nuclear layer (INL) is not affected by the *Tulp1* mutation,[34] INL thickness was measured to identify oblique sections, which were subsequently removed from the data set.

RESULTS

QTL Analysis of Genetic Modifiers of *Tulp1*$^{tm1Pjn/tm1Pjn}$ Retinal Degeneration

To detect genetic modifiers of *Tulp1*$^{tm1Pjn/tm1Pjn}$ retinal degeneration, we first conducted pilot studies of F2 *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice from a B6-*Tulp1*$^{tm1Pjn/tm1Pjn}$ $\times$ AKR/J intercross to identify an age at which the greatest range of ONL phenotypes could be detected. The ONL phenotype of the oldest mice examined, 21 weeks of age, consisted of only one or two rows of photoreceptor nuclei and exhibited little variation (data not shown). Thus, 9-week-old mice were selected for QTL analysis. A total of 72 age-matched F2 *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice from a B6-*Tulp1*$^{tm1Pjn/tm1Pjn}$ $\times$ AKR/J intercross were identified by PCR genotyping and the eyes processed for histologic analysis. Although the retinas of wild-type and heterozygous mice were arranged in orderly layers (Fig. 1A) with a prominent INL and ONL, the retinas of *Tulp1*$^{tm1Pjn/tm1Pjn}$ $\times$ AKR/J mutant mice were abnormal, with a marked reduction in the number of nuclei in the ONL, indicating photoreceptor cell loss (Figs. 1B–F, arrows). Strikingly, the number of ONL nuclei among these animals varied considerably. This variation in ONL nuclei was specific for homozygous *Tulp1*$^{tm1Pjn/tm1Pjn}$ mice and was not observed in randomly sampled retinas from heterozygous or wild-type littermates (data not shown).

Photoreceptor cell loss in each mutant mouse was scored by assigning a value based on the distribution of photoreceptor nuclei remaining (1, mild; 2, moderate; 3, severe loss) and the animals were genotyped with SNP markers. Although QTL analysis identified no statistically significant modifier loci with the first cohort of 72 animals, peaks on chromosomes 1, 2, 6, 12, and 13 nearly reached the significance level for suggestive loci ($P < 0.63$; $n = 72$; Fig. 2A). Therefore, 44 additional mutant mice were collected, and SSLP markers were tested at 10-cM intervals across chromosomes 1, 2, 6, 12, and 13 for all samples combined ($n = 116$ mice). Analysis of the combined data indicated a significant modifier locus on chromosome 2 ($P < 0.01$) and a suggestive locus on chromosome 13 ($P < 0.63$) (Fig. 2B). The locus on chromosome 2 slowed the rate of photoreceptor cell degeneration (Fig. 2C), whereas the locus on chromosome 13 enhanced the rate of photoreceptor cell loss (Fig. 2D).

Identification of Candidate Modifier Genes on Chromosome 2

The region contained under the highest peak on chromosome 2 comprised an interval of approximately 45 Mb (115 to 160 Mb). We expected modifier genes within this interval to show nonsynonymous coding sequence differences between the strain backgrounds that were intercrossed, C57BL/6J and AKR/J. Since this interval contained too many genes to analyze individually, candidate quantitative trait genes (QTGs) were identified by a "virtual" approach using bioinformatic tools as described.[40] Searching the critical interval with the PosMed virtual positional cloning database (http://omicspace.riken.jp/PosMed/) and the keyword "retina" produced a list of 161 genes known to be expressed or function in the retina. To further narrow the list of candidate genes, each gene from the PosMed list was evaluated as to whether mutant alleles were associated with an abnormal eye phenotype using the MGI Phenotypes database (http://www.informatics.jax.org/phenotypes.
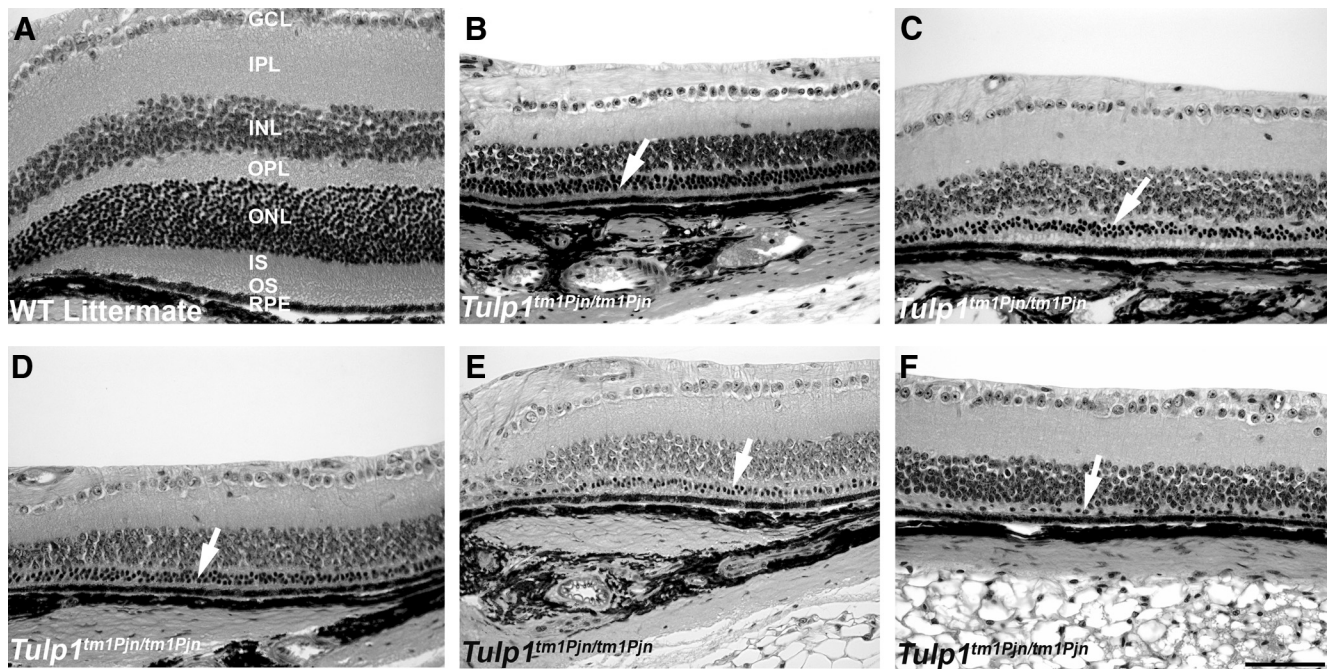
FIGURE 1. Variation of ONL thickness in *Tulp1*-deficient mice is variable in a segregating genetic background. Wildtype (WT) F2 progeny from a B6-*Tulp1*$^{tm1Pjn/tm1Pjn}$ × AKR/J intercross showed normal thickness and arrangement of retinal layers (**A**). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium. F2 *Tulp1*$^{tm1Pjn/tm1Pjn}$ progeny showed attenuated ONL, IS, and OS layers, with considerable variability in the ONL appearance (**B–F**). *Arrows*: ONL layer in *Tulp1*-deficient progeny. Bar: 50 μm.

shtml) and PubMed listings (http://www.ncbi.nlm.nih.gov/pubmed/). Candidate genes known to influence eye development, degeneration, or function were selected, yielding 15 candidate genes (Table 1). Additionally, the *Mtap1a* gene, which we have found to modify cochlear and possibly retinal degeneration in homozygous *Tub*$^{tub/tub}$ mice, was identified within the critical region.

Comparison of the C57BL/6J and AKR/J alleles in the MGI Strains, SNPs, and Polymorphisms database (http://www.informatics.jax.org/strains SNPs.shtml) gave no result for the *Vsx1* and *E2f1* alleles. Of the other candidate genes in the critical region, 13 showed no allelic differences between the two strains or showed sequence changes that were limited to introns or synonymous coding changes. *Mertk*, which has been suggested to interact with *Tub* and *Tulpl*,[33] was among the genes that showed no coding sequence changes. By contrast, the C57BL/6J and AKR/J alleles of *Mtap1a* differ at multiple nucleotides corresponding to nonsynonymous coding changes,[36] suggesting that it is the modifier gene on chromosome 2.

An Allele of *Mtap1a* Modifies Retinal Degeneration in *Tulp1*$^{tm1Pjn/tm1Pjn}$ Mutant Mice

Our laboratory has previously reported a modifier of *Tub* hearing (*moth1*) on chromosome 2 in strains AKR/J, CAST/Ei, and 129P2/OlaHsd.[35] Subsequent fine mapping and sequencing identified multiple polymorphisms in the microtubule-associated protein 1A (*Mtap1a*) gene. A C57BL/6J mouse carrying a transgenic *Mtap1a*$^{moth1-r}$ allele from the 129P2/OlaHsd strain (*Mtap1a* transgene) was mated to *Tub*$^{tub/tub}$ mice and, subsequently, the *Mtap1a* transgene was found to rescue hearing loss in *Tub*$^{tub/tub}$ mice.[35] To test whether the *Mtap1a* transgene also attenuates photoreceptor cell loss in *Tulp1*$^{tm1Pjn/tm1Pjn}$ mice, *Tulp1*$^{tm1Pjn/tm1\ Pjn}$ mutant mice were mated to *Mtap1a* transgene positive mice, and the F1s were intercrossed to generate F2 progeny. Retinas of F2

Tulp1$^{tm1Pjn/tm1Pjn}$ mice with the *Mtap1a* transgene (tg+) and without the *Mtap1a* transgene (tg−) were compared histologically at 9 weeks of age (Figs. 3A, 3B). The number of ONL nuclei remaining in the central retina of *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice with and without the *Mtap1a* transgene were counted. The average number of photoreceptor nuclei remaining in mutant retinas lacking the *Mtap1a* transgene averaged 112 ± 7.3 (Fig. 3A), whereas the number of photoreceptor nuclei remaining in the mutant retinas carrying the *Mtap1a* transgene averaged 177 ± 12.2 (Fig. 3B). The degree of photoreceptor cell loss was significantly decreased in mutant mice carrying the *Mtap1a* transgene ($P < 0.0005$, Student's *t*-test, $n = 6$ each) (Fig. 3C).

Mtap1a Modifies Retinal Degeneration in *Tub*$^{tub/tub}$ Mutant Mice

Mtap1a maps to a region located at 121 Mb on mouse chromosome 2, near the peak location (*D2Mit17*, 122 Mb) of a suggestive QTL for retinal degeneration in an intercross of B6.Cg-*tub/tub* × AKR/J mice[34] (Fig. 4A). Due to the ability of the 129P2/OlaHsd *Mtap1a*$^{moth1-r}$ transgene to slow the rate of photoreceptor loss in *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice, we investigated whether the same allele of *Mtap1a* was capable of modifying retinal degeneration in homozygous *Tub*$^{tub/tub}$ mutant mice as well. *Mtap1a* transgenic mice were intercrossed with B6.Cg-*tub/tub* mice and the retinas of the F2 generation were examined. Retinas of B6.Cg-*tub/tub* mice with and without the *Mtap1a* transgene were compared histologically at 22 weeks of age. The later time point was selected because retinal degeneration in B6.Cg-*tub/tub* is slower than that in *Tulp1*$^{tm1Pjn/tm1Pjn}$ mutant mice. The numbers of ONL nuclei remaining in the central retina of B6.Cg-*tub/tub* mice with and without the *Mtap1a* transgene were counted. The average number of photoreceptor nuclei remaining in
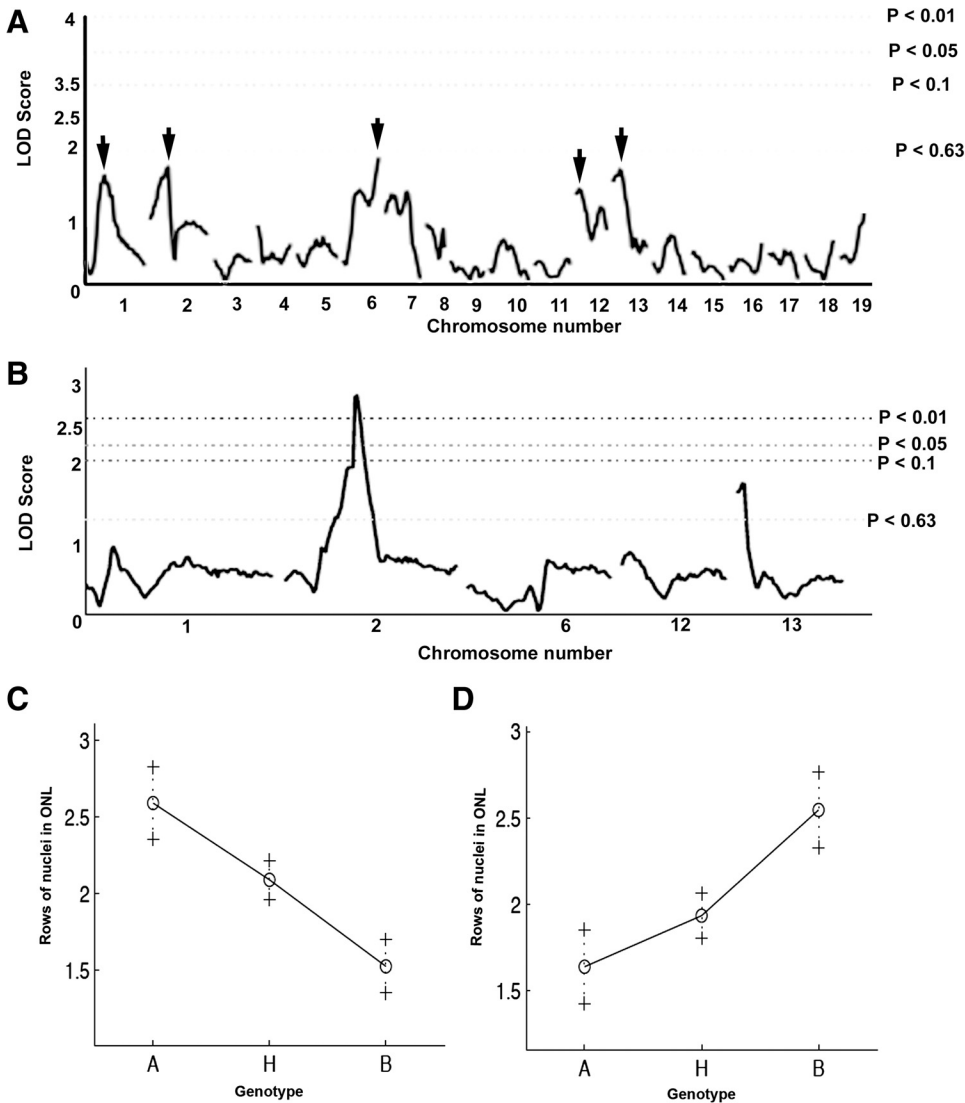
FIGURE 2. QTL analysis revealed a significant modifier locus on chromosome 2 and a suggestive modifier locus on chromosome 13. Analysis of 72 F2 mice at an approximately 20-cM SNP interval yielded five nearly suggestive loci (**A**). Analysis of an additional 44 F2 mice at an approximately 10-cM SNP interval revealed a significant modifier on chromosome 2 and a suggestive modifier on chromosome 13 (**B**). Effect plots of homozygous (A, AKR/J; B, B6) and heterozygous (H) phenotypes indicated a protective and sensitizing effect of the modifiers on chromosomes 2 and 13, respectively (**C**, **D**). LOD: log of the odds ratio.

mutant retinas lacking the *Mtap1a* transgene averaged 76 ± 18.2 (Fig. 4B), whereas the number of photoreceptor nuclei remaining in mutant retinas carrying the *Mtap1a* transgene averaged 126 ± 14.4 (Fig. 4C). Statistical analysis indicated the degree of photoreceptor cell loss was markedly decreased in the mutant B6.Cg-*tub/tub* mice carrying the *Mtap1a* transgene ($P < 0.04$, Student's *t*-test, $n = 5$ each) (Fig. 4D).

Discussion

This study has identified a significant locus on AKR/J chromosome 2 that modifies the progression of retinal degeneration in *Tulp1*[tm1Pjn/tm1Pjn] mice. Mating of *Tulp1*[tm1Pjn/tm1Pjn] mice to mice carrying an *Mtap1a* transgene derived from the 129P2/OlaHsd strain indicated that the protective gene within the chromosome 2 locus was *Mtap1a*. We demonstrated a similar protective effect of the 129P2/OlaHsd *Mtap1a*[motb1-r] allele on photoreceptor loss in *Tub*[tub/tub] mice. Our results indicating that *Mtap1a* is a genetic modifier of both *Tulp1* and *Tub* raise the possibility that two members of the TULP protein family (TULP1 and TUB) share a common functional interaction with MTAP1A. This interaction may be a characteristic feature of sensory neurons because the protective allele of *Mtap1a* mod-

ulates the *Tub*[tub/tub] mutant phenotype in both the ear[35] and retina.

We combined QTL analysis and bioinformatic techniques to identify candidate genetic modifiers. Although QTL analysis is a powerful tool for identifying large chromosomal regions that influence a given phenotype, the identification of individual genes within the QTL that specifically influence the phenotype of interest is rare. This is inherent to this type of analysis because, as the region of interest becomes smaller, the number of crossovers observed within the region become correspondingly infrequent. Moreover, the imperfect phenotype–genotype correspondence of a QTL dictates that quantitative statistical analysis must be performed to define the most likely candidate regions. The expense to generate, house, and genotype enough mice to obtain sufficient crossovers to delineate the critical region required to narrow the candidate gene pool to a single or a few genes is often prohibitive. Even well-designed studies using large numbers of mice with closely spaced genotypes can fail to minimize the critical region to a manageable number of candidate genes. For this reason, we chose to filter our results through the PosMed database as reported by Danciger and colleagues,[40] and to further filter the results through reports of phenotypic relevance of these genes to retinal development, degeneration, and function and by the pres-

TABLE 1. Candidate QTGs Identified on AKR/J Chromosome 2

Gene*	Position	GO Molecular Function†	Mutant Eye Phenotype‡
Meis2	115 Mb	Sequence-specific DNA binding transcription factor activity (GO:0003700)	Developmental eye defects
Tyro3	119 Mb	Transmembrane receptor protein tyrosine kinase activity (GO:0004714)	Vision/eye; retinal degeneration
Mtap1a§	121 Mb	Actin binding (GO:0003779)	Possible modifier of *tub/tub* retinal degeneration
Nphp1	127 Mb	Protein binding (GO:0005515)	Vision/eye; retinal degeneration
Mertk	128 Mb	Transmembrane receptor protein tyrosine kinase activity (GO:0004714)	Vision/eye; retinal degeneration
Bcl2l11	128 Mb	Microtubule binding (GO:0008017)	Increased retinal thickness
Pank2	131 Mb	Pantothenate kinase activity (GO:0004594)	Vision/eye; retinal degeneration
Plcb4	135 Mb	Mitogen-activated protein kinase binding (GO:0051019)	Vision/eye; reduced visual processing
Jag1	136 Mb	Notch binding (GO:0005112)	Vision/eye; developmental eye defects
Mkks	136 Mb	ATP binding (GO:0005524)	Vision/eye; retinal degeneration
Bfsp1	143 Mb	Structural constituent of eye lens (GO:0005212)	Vision/eye; cataract
Vsx1	150 Mb	Sequence-specific DNA binding transcription factor activity (GO:0003700)	Vision/eye; impaired retinal electrophysiology
E2f1	154 Mb	Sequence-specific DNA binding transcription factor activity (GO:0003700)	Vision/eye; defects
Gss	155 Mb	Glutathione synthase activity (GO:0004363)	Seasonal sensitivity to light
Src	157 Mb	Nonmembrane spanning protein tyrosine kinase activity (GO:0004715)	Vision/eye; Glaucoma
Rbl1	157 Mb	Transcription factor binding (GO:0008134)	Vision/eye; retinoblastoma

* Candidate genes within the modifier locus were identified from PosMed, MGI Phenotypes, and PubMed databases as described in the text.

† When multiple gene ontology (GO) molecular functions were found, a single informative molecular function was chosen, with the corresponding GO term accession number given in parentheses.

‡ The existence of mutant alleles affecting vision or the eye (Vision/eye) was determined with MGI Phenotypes. Other evidence for an ocular phenotype was obtained from PubMed listings.

§ Nonsynonymous coding difference between strains.

ence of nonsynonymous coding SNPs. The filtering process allowed us to test a subset of biologically relevant genes with known retinal associations within the critical interval for se-

FIGURE 3. *Mtap1a* modifies photoreceptor cell loss in *Tulp1* mutant retinas. Retinal micrographs reveal fewer ONL nuclei *Tulp1*^tm1Pjn/tm1Pjn^ mice lacking a transgenic copy of the 129P2/OlaHsd *Mtap1a*^moth1-r^ gene (*tg*−, **A**) than in those containing the transgene (*tg*+, **B**). Retinal layers are labeled as in Figure 1A. Bar: 50 μm. ONL nuclei counts (mean ± SE) indicate a significantly higher number of photoreceptor cells in *tg*+ mice (**C**). *Asterisks*: $P < 0.0005$.

quence changes. The success of this combined QTL and bioinformatics approach is expected to improve as genetic tools such as the Collaborative Cross and Diversity Outbred mice become available for high-resolution genetic mapping,[41–43] and as the annotations and quality of bioinformatics search engines increase.

Although the modifier locus on chromosome 13 identified in our study did not reach statistical significance, the data raise the possibility of an intriguing modifier gene that enhances the loss of photoreceptor cells. By filtering genes with the same PosMed and bioinformatic criteria we used for chromosome 2 and by screening the results for nonsynonomous coding changes between C57BL/6J and AKR/J (data not shown), we have tentatively identified *Lyst* as a potential candidate gene in the critical interval of chromosome 13. LYST is a widely expressed protein involved in vesicular trafficking, particularly in the biogenesis of lysosomes and related organelles.[44] *Lyst* mutations in *beige* and *gray* mice exhibit lysosomal abnormalities within the retinal pigment epithelium,[45,46] and *LYST* mutations in humans cause Chédiak–Higashi syndrome,[44] a systemic disease with ocular manifestations that include a progressive visual loss consistent with retinal degeneration.[47,48] A spontaneous mouse mutant of the *Lyst* gene is available (*Lyst*^bg-J^), which might be used to test for modifier effects on *Tulp1* and *Tub* retinal degeneration.

Our finding that *Mtap1* is a genetic modifier of both *Tulp1* and *Tub* supports the hypothesis that TUB and TULP1 function in synaptic maintenance or architecture, possibly reflecting their role in vesicular trafficking throughout the photoreceptor cell.[14,25–28,36,49] MTAP1A (also known as MAP1A) is predominantly found at the synapses of adult neurons, where it associates with both filamentous-actin and microtubules, presumably integrating these two components of the cytoskeleton.[37] We previously showed that MTAP1A interacts with DLG4 (previously known as PSD95),[36] a PDZ-domain protein that organizes transmembrane signaling proteins and the cytoskeleton at postsynaptic membranes. In the mouse retina, DLG4 local-
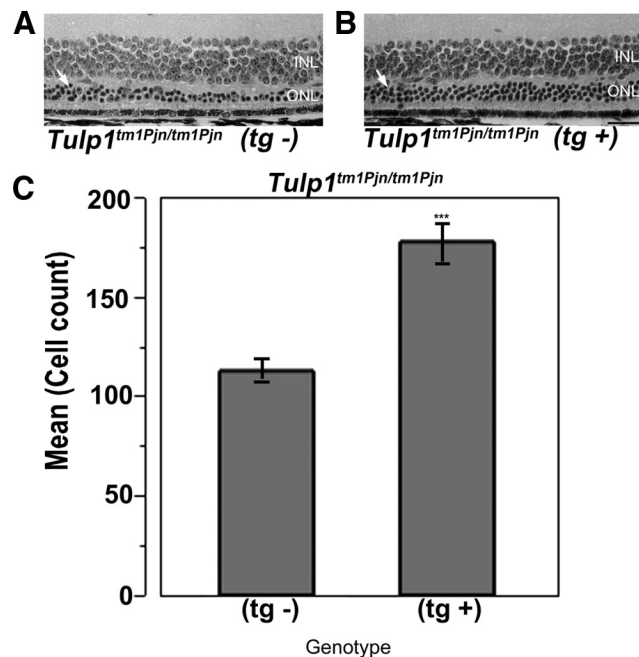
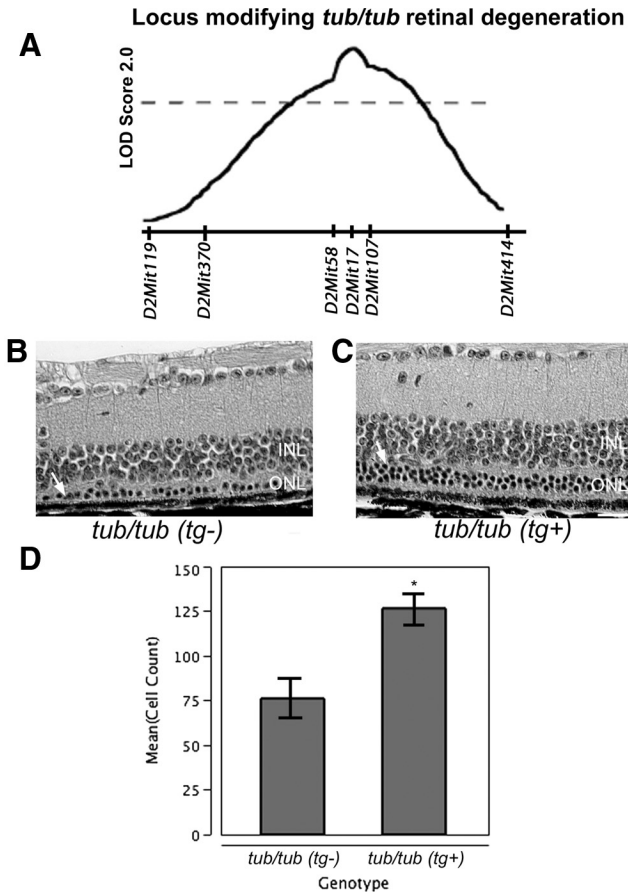## Locus modifying *tub/tub* retinal degeneration

FIGURE 4. *Mtap1a* modifies photoreceptor cell loss in *Tub$^{tub/tub}$* retinas (**A**). Previous QTL studies suggested a retinal-protective locus on chromosome 2, similar to findings with *Tulp1* (reprinted with permission from the International Society for Eye Research/Elsevier Publishers[34]). Retinal micrographs reveal fewer ONL nuclei in *Tub$^{tub/tub}$* mice lacking a transgenic copy of the 129P2/OlaHsd *Mtap1a$^{moth1-r}$* gene (*tg*−, **B**) than in those containing the transgene (*tg*+, **C**). Retinal layers are labeled as in Figure 1A. Bar: 50 µm. ONL nuclei counts (mean ± SE) indicate a significantly higher number of photoreceptor cells in *tg*+ mice (**D**). *Asterisk*: $P < 0.04$.

izes to the presynaptic termini of rod and cone photoreceptor cells.[50] Interestingly, TULP1 has also been shown to interact with dynamin-1, a neuron-specific GTPase that has roles in endocytosis, vesicle formation, vesicular movement at the trans-Golgi network, and plasma membrane and vesicle recycling at neuronal synapses.[38] TULP1 colocalizes with this protein at the photoreceptor synapse and inner segment,[38] and with the actin cytoskeleton of photoreceptor cells.[51] *Tub*- and *Tulp1*-deficient mice mislocalize rhodopsin into extracellular vesicles and alter the cellular distribution of arrestin and transducin, consistent with a trafficking defect.[25,26,49] *Tulp1* mice also show striking defects in the normal distribution of photoreceptor synaptic proteins.[27,28] Together with our observations, these findings suggest a working model in which MTAP1, TUB, and TULP1 in conjunction with components of the cytoskeleton are necessary for maintaining the architecture of the photoreceptor synapse and/or vesicular trafficking at the synapse and inner segment.

Acknowledgments

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