Quantitative Trait Locus Mapping for Age-Related Cataract Severity and Synechia Prevalence Using Four-Way Cross Mice

Norman Wolf, Andrzej Galecki, Ruth Lipman, Sbu Chen, Michael Smith-Wheelock, David Burke and Richard Milley

PURPOSE. The goal of this study was to map mouse quantitative trait loci (QTL) that influence the development of murine age-related cataract and synechias, by using a genetically heterogeneous mouse population bred by a four-way cross.

METHODS. The test population consisted initially of 510 mice bred as the progeny of (BALB/c x C57BL/6j)F1 females and (C3H/HeJ x DBA/2j)F1 males. Each mouse was examined by slit lamp at 18 and 24 months of age and scored for degree of lens opacity on a 0 to 4+ scale, and the presence or absence of additional anterior chamber disease was noted. The presence of synechias was confirmed by histology. Each mouse was genotyped at 96 maternal and 92 paternal loci, and the significance of association between genotype and eye lesions was tested by permutation analysis.

RESULTS. Significant QTL with effects on lens opacity at 24 months were detected on mouse chromosomes 4, 11, and 12. The effects were additive, and severe cataracts were seen in 80% of the mice with all three high-risk alleles, but in only 28% of the mice with all three low-risk alleles. The risk of synechias was associated with paternal chromosome 1 and on both the maternal and paternally inherited chromosome 4. Mice with all three high-risk alleles had a 68% risk of synechias, compared with a 0% incidence in mice with all three counteralleles.

CONCLUSIONS. A four-way cross population of mice can be used to map polymorphic loci that influence cataract severity and synechias prevalence in late life. The results provide a first step toward identification of the individual genes involved and may lead to a greater understanding of the development of the eye) has been reported in only a few instances, although it is apparently a universal phenomenon, probably because mice are rarely maintained into old age. The anatomical location of late life cataract in mice, mostly nuclear and posterior cortical, resembles that in otherwise healthy aging humans, and the incidence of lesions increases substantially in the last third of the lifespan in mice as in humans.

In previous studies, a four-way cross mouse population developed by mating (BALB/c x C57BL/6j) F1 females with (C3H/HeJ x DBA/2j)F1 males has been used to map quantitative trait loci (QTL) that influence lifespan and T-cell subset levels, indicating that these lesions are under the control of genetic alleles that show polymorphisms among the common laboratory mouse stocks. Thus, an analysis of the genetics of cataract incidence in aging mice may provide a useful model for the human condition and provide a baseline for comparison of those genetic influences on the time of initiation, progression, and severity of this lesion.

In the present study we used these mice to seek evidence of QTL that modulate the severity of lens opacity at 18 and 24 months of age. We also sought evidence for QTL that influence the risk of iris adhesions (synechias) detected through resistance to dilation of the pupil and supported by subsequent histologic examinations.

MATERIALS AND METHODS

Animals and Housing

(BALB/c x C57BL/6j)F1 female and (C3H/HeJ x DBA/2j)F1 male mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and mated to produce the four-way cross test population. These animals were maintained three to four per cage under specific pathogen-free conditions, with quarterly examinations of sentinel mice for evidence of pinworms or viral infection. Six hundred two mice were bred for a study of the genetics of lifespan and T-cell subsets, and of these 510 were examined by slit lamp first at age 18 months (range, 17–19 months), and of these 418 were tested again at 24 months (range, 23–25 months). The animals were all fully pigmented in coat color, iris, ciliary body, and retina. The cages were regularly rotated among the shelves of each housing rack to equalize exposure to the regulated fluorescent room lighting that was on from 6 AM to 6 PM. Room temperature was regulated at 74 ± 4°F. All the animals were main-
tained until they died of natural causes. The study protocol adhered to the requirements in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Slit Lamp Examinations for Degree of Lens Opacity**

A handheld slit lamp (model SL-14; Kowa, Tokyo, Japan) set at a 30° angle was used to determine the degree of lens opacity in each dilated eye of each mouse at 18 and then (for survivors) again at 24 months of age. After dilation with 1% tropicamide (Mydriacyl Ophthalmic Solution; Alcon, Fort Worth, TX), the animals were hand-held unanesthetized and were presented to the slit lamp observer (NW) randomly and without his knowing the age, sex, or genetic background. The degree of opacity in each eye was rated, as in our previous studies, as 0, 1, 2, 3, or 4+, with the latter number assigned to completely opaque, mature cataracts. Each eye was scored independently, and the mean score for both eyes was used as the index of lens opacity, except in a few cases in which failure of dilation permitted scoring of only one eye. Because the animals were entered into the study at monthly intervals over a period of 24 months, the series of eye examinations was conducted at 3-month intervals and included both 18- and 24-month-old mice except at the beginning and end of the series. A second, control group of 41 similarly produced four-way cross mice were subsequently examined at 4 to 8 months of age as a control for the age-related appearance of cataract.

**Determination of QTL Location in Individual Mice**

Maternal and paternal inheritance genetic maps were constructed with the recombination data from the 421 UM-HET3 animals used in this study, plus an additional 179 animals that were bred and genotyped in the same population. Genetic maps derived from the UM-HET3 cross were considered appropriate for the interval mapping calculations, because they reflect the actual genetic distances observed in the study population. The large number of chromosomal recombination breaks underlying the map—the equivalent of 600 backcross mice from each parental cross—is comparable to that of the most commonly used interspecific mapping panels. The genetic distances and map order were calculated on computer (MapManager QTX; available at http://www.mapmanager.org/mmtQTX.html; provided in the public domain by the Roswell Park Medical Institute, Buffalo, NY); and separate genetic maps were constructed for maternal and paternal recombination values. All the typed markers were used in the initial map construction to form linkage groups for each chromosome. After initial linkage group assignments, each chromosome was reanalyzed by using the Kosambi map function to yield the recombination distances between markers. The genetic distances within the four-way cross population examined in this study differ, to various degrees, from the genetic maps established by the interspecific common resource crosses. This reflects, in part, the differing male- and female-specific recombination rates across the genome and any interstrain differences in genetic recombination. In addition, systematic genotyping errors result in the appearance of local map distance expansion. Physical map locations (in base pairs) were identified for each single sequence repeat genetic marker by using the draft sequence of the mouse genome, build version 3 (MGsV5; GenBank accession number CAA010000000; http://www.ncbi.nlm.nih.gov/genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). Genotyping was performed by standard PCR amplification of genomic DNA from each animal using marker loci obtained from the Mouse Simple Sequence Length Polymorphism Database. Polyacrylamide gels were scored using an automated sequencer, as described (ALFExpress; Amersham Biosciences, Arlington Heights, IL). Analyses at marker loci were performed on 375 mice for which cataract scores were available at both ages (reasons for eliminations are discussed later). Genotyping error rates were estimated by subsampling approximately 5% of the population at random and independently regenotyp-
Table 1. Significant Associations between Marker Loci and Cataract Score at Age 24 Months

| Locus    | MGI Ref* | Position† | Sequence‡ (Mb) | Allele-Specific Mean Scores§ | P(e)|| P(e), Adjusted¶ |
|----------|----------|-----------|----------------|-------------------------------|-----|-----------------|
| D4Mit155 | MGI:92781| 50        | 100.28         | D2 = 2.95; C3 = 2.45           | 0.001| 0.001          |
| D5Mit25  | MGI:94329| 61        | 111.80         | B6 = 2.88; C = 2.58           | 0.07 | 0.06           |
| D11Mit12 | MGI:89087| 2         | 12.25          | C3 = 2.88; D2 = 2.58          | 0.06 | 0.042          |
| D12Mit34 | MGI:89444| 29        | 64.43          | B6 = 2.87; C = 2.56           | 0.016| 0.028          |

* Sequence reference number for Mouse Genome Informatics database.
† Distance in cM from centromere.
‡ Physical position, build version 3 (MGSv3; GenBank accession number CAAA01000000).
§ Average score, on a scale of 0 (least severe) to 4 (most severe), for mice with the indicated alleles at the locus.
|| Experiment-wise significance level for residual of cataract score.
¶ Experiment-wise significance level for residual of cataract score (i.e. after adjustment for trend in mean with assay date).

deviation of 1.08 (SEM = 0.05). Among the 418 surviving mice at 24 months of age (reduced by death or removal for fighting, illness, or lesions) 32 mice were not assessed in either eye for cataract, and 12 more were assessed in only one eye because of pupillary nondilation (see reference to synechia later). In the remaining mice, the mean score was 2.71, with a standard deviation of 0.82 (SEM = 0.04). The relative incidences of cataract development in the five categories used were as follows: At age 18 months, grades 0, 1, 2, 3, and 4 were assigned to 142, 270, 280, 264, and 47 eyes, respectively (counts from left and right eyes were pooled). At age 24 months, the counts were 2, 70, 251, 312, and 156, respectively. Of the mice tested at 24 months of age, 42% were males, and there was no significant difference between the two genders (P = 0.69). Of the 375 animals examined in both eyes at both ages, there was a significant, but modest, correlation between scores at the two ages (R = 0.35, P < 0.001), indicating substantial interanimal variation in the rate of cataract progression between 18 and 24 months of age. Photographic examples of lens opacities graded 0 through 4+ are available in our previous publications.4,7

A genome scan approach was used to detect marker loci with significant associations with lens opacity scores, by using an experiment-wise P < 0.05 as the principal significance criterion. This criterion, based on a permutation method, gives an indication of the likelihood that even one such gene–trait association could emerge by chance alone, given the observed data set, if the null hypothesis were correct, and thus adjusts the acceptance threshold to control type I errors for multiple comparisons of the many marker loci tested. No locus achieved this criterion for the 18-month opacity scores; the strongest association was seen for D1Mit318 (17 cM from the centromere on chromosome 1), for which P < 0.08. Opacity scores determined at 24 months, however, were associated with marker loci on four chromosomes, as summarized in Table 1.

Two of these loci—D4Mit155, located 49.6 cM distal from the centromere of chromosome 4, and D12Mit34, 29 cM from the centromere on chromosome 12—met the significance criterion of P < 0.05. The other two loci, on chromosomes 5 and 11, did not meet the P < 0.05 significance criterion, but did have suggestive levels of association at P < 0.07. Table 1 also shows the mean opacity scores for each allele at the loci in question. For example the D2 allele at D4Mit155, derived from the paternal grandfather DBA/2, was associated with a mean increase of 0.5 scale units compared with the counter allele C3, derived from the paternal grandmother C3H/HeJ.

Figure 1 shows the distribution of opacity scores in population subgroups that differ at the D4Mit155 and D12Mit34 alleles. For the D4Mit155 locus, opacity scores of 3 or greater were recorded in only 37% of the mice with the C3 allele, but in 61% of the mice with the D2 allele. For the D12Mit34 locus, opacity scores of 3 or greater were recorded in only 43% of the mice with the C allele, but in 58% of the mice with the B6 allele. A plot (not shown) of trend of the mean opacity scores against date of assessment revealed a small but statistically significant (0.07 units/calendar month; R² = 0.04, P < 0.001 by linear regression against date of examination) increase in scores in groups of mice tested in the second year of the evaluation schedule, both in 18-month-old mice and in 24-month-old mice. Because all these examinations were conducted by the same individual (NW), it was not possible to determine whether this shift represented a shift in the correspondence between tissue disease and score assigned, or an actual alteration in the extent of opacity, due perhaps to external causes, despite careful monitoring of environmental conditions. To determine whether this shift had an important effect on gene–trait associations, we developed a variant of the opacity score that was independent on date of assay, calculated at the residual of the original opacity score with respect to the regression equation, and then repeated the genome scan cal-

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932926/)
Cataract assessment was dubious or impossible for limited iris dilation, and those with severe restriction that made the mean opacity score was 3.2 with relatively high cataract grade. At 24 months, for example, bilateral in approximately half of the cases and was associated of synechia at 24 months was somewhat more common in male than in female mice (29% vs. 16%, \( P < 0.001 \)). Extra care was taken in examination of eyes with defects and was 2.6, and 15 (28%) of the 54 mice had opacity scores of 3 or higher. In contrast, the group with all three high-risk alleles had a mean cataract score of 3.3, and 40 (80%) of the 50 mice had scores of 3 or higher.

**Genome Scan for Alleles that Modulate Risk of Synechia**

Many of the older mice showed evidence of additional anterior segment damage by slit lamp examination. In general, corneas became thicker and more opaque. In addition, 89 of the 410 mice examined at age 24 months had anterior segment damage that prevented full iris dilation, even with repeated application of 1% tropicamide. This suggestive evidence for the presence of synechia at 24 months was somewhat more common in male than in female mice (29% vs. 16%, \( P = 0.001 \) by Fisher exact test). Failure to dilate was also noted in 42 of the mice examined at 18 months of age. The lesion was noted to be bilateral in approximately half of the cases and was associated with relatively high cataract grade. At 24 months, for example, the mean opacity score was 3.2 ± 0.7 in the mice with dilation defects and was 2.6 ± 0.8 in the mice without such a sign (\( P < 0.0001 \)). Extra care was taken in examination of eyes with limited iris dilation, and those with severe restriction that made cataract assessment dubious or impossible were not scored for.
cataracts (44 eyes in 32 mice, mostly from the 24-month age group). Histologic sections of the eyes of 19 mice with restricted dilation in vivo were subsequently examined after death and in each case were found to have manifestations of synechia. In contrast, only 6 of 19 necropsied mice that did not present restricted dilation at 24 months presented histologic evidence of synechia. Because the eye sections were taken from mice that died naturally, it is probable that the adhesions developed in the latter six animals sometime after the 24-month examination. Figures 4A and 4B show representative sections from a 25- and a 29-month-old mouse, with iris-lens and ciliary body-lens adhesions, respectively, as has been described by Smith et al. Slit lamp examinations also noted iris thinning resulting in radial slits in 15 mice and brown pigment spots on the anterior lens surface in 6, all correlated with minimal dilation of the pupil.22–24

A genome scan was conducted to seek QTL with an effect on the incidence of synechia. Because the outcome was a binary variable (presence or absence of synechia), the QTL scan used a logistic regression model in place of the ANOVA model typically used for continuous traits. The results gave statistically significant support for three segregating loci, which are summarized in Table 2. Two of the significant effects involved markers on chromosome 4: a paternal allele pair at D4Mit155 and a maternal allele pair at D4Mit84. The third QTL is located on chromosome 1, is linked to D1Mit493, and reveals segregation of paternal alleles. It is not possible to conclude from the current data whether risk of synechia is affected by loci at a single locus on chromosome 4 (with segregation from both maternal and paternal chromosomes), or instead reflects two different effector loci, one of which is polymorphic in the sires and the other polymorphic in the dams. We note that neither the synechia allele on chromosome 4, nor that on the maternal chromosome 4 (see Table 2), had a significant effect on cataract severity at age 24 months (compare Table 1). Both cataract severity and synechia prevalence are influenced by alleles inherited on the paternal chromosome 4. The upper and middle portions of Figure 2 show interval plots for synechia prevalence at various regions of the maternal and paternal chromosome 4. On these plots, regions for which LOD scores exceed approximately 1.7 are those for which the association was significant with experiment-wise criteria. At this relatively low level of resolution, the genetic data are consistent with those in several models, ranging from a single locus, near the middle of chromosome 4, with effects on cataracts and synechia, to multiple loci on maternal and paternal chromosome 4, some with effects on synechia and others with effects on cataract severity.

A logistic regression analysis was conducted to see whether the effects of any of these three QTL that modulate synechia prevalence are conditional on the alleles inherited at one or both of the other loci and revealed no evidence of epistatic interaction. The effects thus appear to be additive. Table 3 shows the incidence of synechia in each of the eight genotype classes. The incidence ranges from 0% among mice with the most favorable combination of alleles to 68% among mice with the opposite set of alleles. As a control for age relationship of the diseases reported earlier, a group of 17 female and 24 male four-way cross mice 4 to 8 months of age were examined by slit lamp. None showed evidence of either lens opacities or synechia formation. This provides a 95% confidence level of 0% to 8.6% probability that cataracts in the 18- and 24-month-old study groups developed only with advanced age—that is, neither congenitally nor early in life.
some 5. The QTL on chromosomes 4 and 11 involve segregation of alleles inherited from the father, whereas the QTL on chromosomes 5 and 12 involve segregation of maternal alleles. The risk of a second lesion, an iris-to-lens synechia severe enough to interfere with dilation of the pupil, was found to be influenced by alleles inherited on the paternal chromosome 1 and on both maternal and paternal versions of chromosome 4. The effects of these inherited polymorphisms appear to be additive and, taken together, can have a very strong influence on disease risk (i.e., from 28% to 80% for different combinations of the three most significant cataract genes, and from 0% to 68% for the three significant synechia-related genes). As noted earlier, these are age-related lesions. They were not present in young adult mice of the four-way cross and were more severe in 24-month-old than in 18-month-old animals. It is further noted that cataracts in young adult mice have not been seen in the many ophthalmologic observations made over time in each of the four grandparent strains of the four-way cross animals (Smith RS, personal communication, October 2003).

We chose to use the mean cataract score as our principal end point for estimating the propensity of each mouse to develop cataracts, rather than to perform separate QTL calculations for left eye and right eye scores separately, because it is highly unlikely that there are genetic variants that influence cataract rate in one eye only. Using information from both eyes therefore provided a higher degree of confidence as to the cataract risk in the individual mouse and improved statistical power for identifying QTL. Because there is a fairly high degree of correlation between the severity of cataracts in left and right eyes across individual mice, genome scans using left- or right-eye data alone do not provide completely independent hypothesis tests. Nonetheless, it is of interest to note that association between D4Mit155 and cataract severity remains significant (experiment-wise significance level; P = 0.001) when evaluated using either left- or right-eye data alone. When each eye is considered separately, the other three associations shown in Table 1 remain significant using nominal significance criteria (0.001 < P <

### Table 2. Significant Associations between Marker Loci and Synechia Proportion at Age 24 Months

<table>
<thead>
<tr>
<th>Locus</th>
<th>MGI Ref</th>
<th>Position</th>
<th>Sequence (Mb)</th>
<th>P(c)</th>
<th>Proportions for Indicated Allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Mit493</td>
<td>MGI:105693</td>
<td>62</td>
<td>110.59</td>
<td>0.006</td>
<td>D2 = 30, C3 = 13</td>
</tr>
<tr>
<td>D4Mit84</td>
<td>MGI:92920</td>
<td>38</td>
<td>74.51</td>
<td>0.001</td>
<td>C = 34, B6 = 7</td>
</tr>
<tr>
<td>D4Mit55</td>
<td>MGI:92889</td>
<td>17</td>
<td>44.55</td>
<td>0.001</td>
<td>D2 = 33, C3 = 8</td>
</tr>
</tbody>
</table>

* Sequence reference number for Mouse Genome Informatics database.
† Distance in cM from centromere.
‡ Physical position, build version 3 (MG Sv3; GenBank accession number CAAA01000000).
§ Experiment-wise significance level.

### Discussion

The results presented herein document the usefulness of a gene-mapping strategy that uses segregation in mice bred as a four-way cross among laboratory inbred strains to determine the genetic control of body size, 25 T-cell subsets, hormone levels, risks of specific neoplastic lesions, and, in the present study, two forms of late life eye lesions. An experimental plan that involves assessment of multiple traits precludes selection of progenitor stocks on the basis of extreme values in any one of the traits to be examined, because inbred stocks that differ dramatically in one of the traits may or may not differ in other traits in the test battery. An experimental plan devoted to mapping genes for many different outcomes cannot rely on breeding from mouse stocks that differ in only one of the outcomes of interest.

The results presented herein indicate that the severity of spontaneous late-life cataract is modulated by alleles in at least three segregating loci on mouse chromosomes 4, 11, and 12, with strong evidence suggestive of a fourth locus on chromosome 5. The QTL on chromosomes 4 and 11 involve segregation of alleles inherited from the father, whereas the QTL on chromosomes 5 and 12 involve segregation of maternal alleles. The risk of a second lesion, an iris-to-lens synechia severe enough to interfere with dilation of the pupil, was found to be influenced by alleles inherited on the paternal chromosome 1 and on both maternal and paternal versions of chromosome 4. The effects of these inherited polymorphisms appear to be additive and, taken together, can have a very strong influence on disease risk (i.e., from 28% to 80% for different combinations of the three most significant cataract genes, and from 0% to 68% for the three significant synechia-related genes). As noted earlier, these are age-related lesions. They were not present in young adult mice of the four-way cross and were more severe in 24-month-old than in 18-month-old animals. It is further noted that cataracts in young adult mice have not been seen in the many ophthalmologic observations made over time in each of the four grandparent strains of the four-way cross animals (Smith RS, personal communication, October 2003).

### Table 3. Additive Effects of QTL on Synechia Prevalence

<table>
<thead>
<tr>
<th>Locus</th>
<th>D1Mit493</th>
<th>D4Mit55</th>
<th>D4Mit84</th>
<th>Synechia Proportion (%)</th>
<th>Number of High-Risk Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>C3</td>
<td>B6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>C</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>D2</td>
<td>B6</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
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<td>C3</td>
<td>B6</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>D2</td>
<td>B6</td>
<td>11</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>C3</td>
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<td>18</td>
<td>2</td>
<td></td>
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<tr>
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<td>C</td>
<td>32</td>
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<td></td>
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<tr>
<td>D2</td>
<td>D2</td>
<td>C</td>
<td>68</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Data are the proportion of cases with synechia at 24 months of age in each of the eight genotypic classes defined by the three QTL with significant effects.
0.05, depending on the locus and the eye involved), but do not meet the more stringent experiment-wise criteria of P < 0.001.

We also considered the possibility that the presence of synechia in some eyes may have affected our results by making it impossible to score cataract severity in the affected eyes. We noted that mice with unilateral synechia tended to have higher than average cataract severity in the eye that could be evaluated, and it is possible that mice with synechia-prevented lens readings would have had an even greater mean cataract score if it had been possible to observe the obscured eye. Such an effect, however, would only diminish the association between specific marker loci and cataract score (a type II error), by diminishing the amount of phenotypic information available and thus would weaken the statistical significance. Whether the pathophysiological processes that contribute to cataract risk also predispose to synechia and whether one of these lesions typically precedes the other when both are present are beyond the ability of this study to discriminate.

This QTL mapping approach is only the first step toward identifying the specific genes in which mutations or differential gene expression may modulate these late-life lesions. As shown in Figure 2, the available information gives only a rough approximation of the position of the gene, or genes, in each of the rather broad chromosomal regions identified by the genome scan. Each 10- to 20-cM region of the genome is likely to contain a number of loci that could, in principle, alter one or more of the cellular and metabolic pathways that mold lens and iris development and protect these structures from various sources of age-related damage. It is nonetheless of some interest to note that some of the chromosomal regions delineated in our mapping study also contain loci previously found to influence lens development and/or to modulate risks of early or later life cataract and related ocular disease in mice. Pertinent examples of the latter follow.

Recessive mutations of the mouse tyrosinase-related protein 1 (tyr1) gene, which may be related to or identical with iso, the iris stroma atrophy gene located at 38 cM on chromosome 4, lead in DBA/2 mice to a condition involving glaucoma, cataract, anterior synechia, and iris discoloration and damage, as well as brown pigment deposition on the anterior lens surface in the DBA/J mouse. This is reportedly due to release of toxic intermediates from the melanin synthesis pathway.25,24,20,27 It is thus noteworthy that, as reported earlier, the D2 alleles included in this region on chromosome 4 are associated with both cataract and synechia in our own population, as well as the presence of iris thinning and brown spots on the anterior lens surface, similar to the developments at 18 to 21 months of age in DBA/2 mice reported by Chang et al.25 and by Anderson et al.25,26 Aged mice of the (AKR × DBA)F1 genotype have also been reported to have both glaucoma and severe cataracts.25

The chromosome 4 region, found in our work to modulate both lens and iris disease, also contains a FOX3 gene cluster including the gene dyl, implicated in lens development (locus Fox3, forkhead box E3, MGI:1355369, 50 cM from the centromere, sequence position 112.71 Mb). Mutation at the dyl locus results in anterior segment abnormalities that include failure to divide in lens epithelial cells28 and mutations in a human orthologue lead to ocular dysgenesis and cataract, a condition known as Peters’ anomaly.29,30 Although the late-life lesions studied in our mice do not closely resemble the congenital abnormalities produced by these dyl mutations, it is possible that subtler alterations in the pathways regulated by the dyl product or by other loci in this cluster contribute to cataracts and/or synechia in old age. This region of mouse chromosome 4 also contains the Prdx1 locus (peroxiredoxin 1, MGI:199523, 47 cM, sequence position 114.45 Mb), the product of which is found in the anterior tissues of the eye and protects against oxidative damage.31 The mouse fourth chromosome also contains the Galt locus (MGI:95638, 20 cM, sequence position 41.52 Mb), deletion of which results in moderate galactosemia. Reduced expression of this gene product may increase exposure of lens cells to galactose, a known cataractogenic agent.31,32 The region of mouse chromosome 12 identified in Table 1 by D12Mit54 also bears a gene that causes developmental microphthalmia when combined with the or(1) allele of the Chx10 homeobox gene (locus Cdx10, ceh-10 homoj neo domain–containing homologue, 38 cM, sequence position 79.3 Mb).33 The human homologue of the Chx mutation is located on 1q4 and results in early cataracts, as well as microphthalmia and iris abnormalities.34 Finally, if the residual set of significance values is used, D5Mit25 is joined by a close new position, D5Mit95—each of these now at P < 0.06 and both located near the midpoint of chromosome 5, the site of Cryb2/aey2. The latter result in a T-to-A transversion at the sixth exon, producing a mature cataract by 8 weeks of age.35 It is clear that a good deal of additional work is needed to test the idea that the QTL that we found to correlate with late-life presence of eye lesions in the HET mice were indeed the result of alterations in the sequences and subsequent modified expressions of one or more of these candidate genes.

The recent appearance of a rough draft of the laboratory mouse genome will provide a dramatic advance in the ability to identify the underlying DNA sequence variation responsible for the identified QTL.36 Known genes, expressed sequence tags (ESTs), and computationally identified candidate transcription units are identified and annotated in easily accessible formats (e.g., UCSC Genome Browser, http://genome.ucsc.edu/; provided by the University of California at Santa Cruz; Ensembl, www.ensembl.org/Mus_musculus/; and www.ncbi.nlm.nih.gov/genome/guide/mouse/ provided by NCBI). As a next step, refinement of each QTL interval will be based on single-nucleotide polymorphism (SNP) identification and genotyping of the HET animals. The available genomic sequence can be easily searched for candidate SNP variants between the progenitor strains in most genomic intervals. In addition, QTL localization will benefit from the use of common inbred strains as the source of genetic variation in the UM-HET3 cross. An analysis of several strains (including three of the four of the progenitor strains in the UM-HET3 cross) shows a mosaic pattern of sequence variation across large regions of the genome.37 After SNP typing across the QTL candidate region, ancestral haplotype segments of the genome can be identified. Haplotype segments that match the QTL inheritance pattern in the HET population can be used to refine the location of the causative gene variants.

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References
