**Eye1 and Eye2: Gene Loci That Modulate Eye Size, Lens Weight, and Retinal Area in the Mouse**

Guomin Zhou and Robert W. Williams

**Purpose.** Vision is critically dependent on genetic factors that influence the rate and duration of eye growth. The genetic basis of variation in eye size in mice was explored, and genes that modulate eye weight, lens weight, and retinal area were mapped.

**Methods.** Eyes of approximately 700 mice were weighed. Data were corrected by regression analysis to eliminate effects of sex, age, and body weight. Interval mapping was used to locate quantitative trait loci (QTLs) using recombinant inbred strains and F2 intercrosses between strains C57BL/6J and DBA/2J.

**Results.** Major QTLs were discovered near the centromere of chromosome 5 (Eye1: genomewide P < 0.005) and on proximal chromosome 17 near the mast cell protease 6 gene (Eye2, P < 0.05). Both QTLs have significant effects on eye size, lens weight, and retinal area. The DBA/2J alleles at Eye1 and Eye2 are partially dominant and increase eye weight by as much as 1.0 mg. Analysis of 183 F2 progeny confirmed and refined the chromosomal assignments of both Eye1 and Eye2.

**Conclusions.** Eye1 and Eye2 are the first loci known to control normal variation in eye size in any mammal. The hepatic growth factor gene (Hgf), a potent mitogen expressed in the retina, pigment epithelium, and choroid, is a strong candidate for Eye1. The human homolog of Eye2 should map to chromosome 6p, 16q13.3, or 19q13, whereas that of Eye1 should map to 7q. (Invest Ophthalmol Vis Sci. 1999;40:817-825)

Myopia is a pervasive and extremely common abnormality in modern human populations that is generally caused by excessive growth of the posterior segment of the eye relative to the refractive power of the cornea and lens. The onset and progression of myopia are strongly influenced by environmental factors, including premature birth, sustained near vision, and form deprivation. However, susceptibility to these factors is clearly under partial genetic control. Our understanding of the molecular processes responsible for the maturation of the vertebrate eye is progressing rapidly. Nonetheless, little is known about the critical set of genes that modulate rates and the duration of normal eye growth. These unidentified genes make a key contribution to vision by ensuring a near perfect match between developmental changes in the refractive power of the cornea and lens, the size and shape of the eye, and the position of the retina. We have begun to map the quantitative trait loci (QTLs) that control normal variation in the architecture of the eye, lens, and retina of laboratory mice. Like most heritable quantitative traits, variation in eye size is undoubtedly controlled by a substantial number of QTLs. A subset of QTLs have unusually large effects on phenotypes. Not only are these the most important QTLs, but they tend to be comparatively easy to map. For example, we recently mapped a QTL that has a pronounced effect on retinal ganglion cell number in mice to chromosome 11. In this study we used similar quantitative genetic methods to search for major-effect QTLs that influence eye size, lens weight, and retinal area in both BXD recombinant inbred strains and reciprocal F2 intercrosses.

**Methods**

**Inbred Strains**

Inbred strains, including two common strains, C57BL/6J and DBA/2J, and the entire set of 26 BXD recombinant inbred strains, were obtained from the Jackson Laboratory (Bar Harbor, ME). These strains were generated by crossing two inbred lines—one of which has a relatively small eye (C57BL/6J), or B for short) and the other of which has a large eye (DBA/2J, or D for short). Each of the 26 BXD strains is completely inbred, and each has a genome that is made up of a unique recombinant mosaic of chromosomal segments derived from the two parental strain (see Ref. 19; methods reviewed in detail at http://nervenet.org/papers/shortcourse98.html). At any particular gene locus, a given BXD strain will have either a BB or a DD genotype. For simplicity, BB and DD genotypes are truncated to single Bs and Ds. If we genotype all BXD strains at a
particular gene locus, then the string of 26 genotypes, 1 per strain, defines a unique 26-letter code known as a strain distribution pattern. For example, DDDDBDDDBDBDBDBBDDBBDDBDBD is the strain distribution pattern that uniquely defines the proximal end of Chr 5 in BXD mice. Mapping a quantitative trait such as variation in eye size involves comparing variation in eye weight with the strain distribution pattern. If every strain with a D genotype at a particular locus happens to have large eyes, and if every strain with a B genotype happens to have small eyes, then we can be reasonably confident that a QTL modulating eye size is close to the locus defined by the particular strain distribution pattern. One of the key advantages of recombinant inbred strains is that environmental perturbations and technical errors can be greatly diminished by phenotyping many individuals with the same genotype (i.e., many individuals belonging to the same strain). As shown in Table 1, we have studied an average of 12.5 animals per strain. Most of these BXD strains were bred for several generations in our colony. All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**F2 Intercross**

We generated reciprocal F2 intercrosses using the same parental strains (C57BL/6J and DBA/2J) that had previously been used to generate the BXD strains. The primary use of these F2 crosses was to validate QTLs mapped with BXD strains. Using F2s we have also been able to more accurately assess the strength of the effects that QTLs have on eye size, lens weight, and retinal area. An average of half of the F2 animals are from each strain, and for the Chr 17 locus D17Mit46: RGC, retinal ganglion cell.

<table>
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<tr>
<th>Strain</th>
<th>Eye Weight (mg)*</th>
<th>Eye Weight (mg)†</th>
<th>No. of Cases</th>
<th>Mean Difference (mg)</th>
<th>Lens Weight (mg)</th>
<th>Retinal Area (mm²)</th>
<th>RGC Number (×1000)</th>
<th>Body Weight (g)</th>
<th>Brain Weight (mg)</th>
<th>Genotype Chr 5</th>
<th>Genotype Chr 17</th>
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<td>13</td>
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<td>19.4 ± 0.3</td>
<td>19.3</td>
<td>15</td>
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<td>18</td>
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</table>

The column labeled Mean Difference gives the difference in milligrams between the average for a particular strain and the average of all strains (18.8 mg). Lens weight, retina area, body weight, and brain weight values are all corrected by multiple linear regression to eliminate variance associated with sex and age. Data on retinal ganglion cell numbers (RGC No.) are taken from Williams et al.

* Fresh eye weight after correction for multiple regression for differences in sex, age, body weight, and brain weight. All cases were normalized to 75-day-old 22g female mice with a brain weight of 420 mg.

† Fresh eye weight after multiple regression correction for variance associated only with sex and age. Data are normalized to 75-day-old female mice.

TABLE 1. Eye Weight, Lens Weight, Retinal Area, and Retinal Ganglion Cell Number in BXD Strains
D2B6F2 progeny (n = 76). Eyes of all F1 and F2 progeny were dissected and weighed.

**Husbandry and Age**
Mice were maintained at 20°C to 24°C on a 14-hour/10-hour light-dark cycle in a pathogen-free colony. Most animals were fed a 6%fat NIH31 diet at the Jackson Laboratory and a 5% fat Agway Prolaboratory 3000 rat and mouse chow diet at the University of Tennessee. The average age of BXD animals was approximately 106 days, whereas that of the F2 mice was 98 days.

**Fixation**
Mice were deeply anesthetized intraperitoneally with Avertin (1.25% 2,2,2-tribromoethanol and 0.8% tert-pentyl alcohol in water, 0.5 ml to 1.0 ml). Most mice were perfused through the heart with 0.1 M phosphate-buffered saline followed by 1.25% glutaraldehyde and 1.0% paraformaldehyde in 0.1 M phosphate buffer, and then by 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M buffer.

**Fresh versus Fixed Eye Weights**
Both eyes of each mouse were removed, and the optic nerve, vessels, and muscles were cut away. Eyes were weighed to the nearest 0.1 mg, with a repeatability of better than 0.3 mg. We studied eyes taken from eight or more animals from each strain, and averaged data from right and left eyes. Eyes of a total of 380 BXD and parental strains were weighed (Table 1). To assess shrinkage caused by fixation and other postmortem changes, we compared weights of more than 310 fixed and unfixed freshly enucleated eyes from other strains of mice not otherwise described in this article. One or both eyes were enucleated before being perfused, cleaned, and weighed within minutes. Freshly dissected eyes were subsequently placed in fixative and weighed again 1 hour to 5 days later. The weights of fixed eyes—whether perfused or immersion-fixed—were generally 6.1% less than those of fresh eyes. The correlation between fresh and fixed eye weights is high (r = 0.96). The majority of eye weights listed in this article are adjusted to those expected of fresh material immediately after death.

**Eye Weight and Linear Dimensions**
Quantitative genetic studies such as ours require the analysis of comparatively large numbers of animals. The advantage of weighing eyes rather than obtaining a set of linear measurements is that it is easier, faster, and often more accurate, particularly when eyes are as small as those of mice.** Weights that are repeatable to within 1% can be obtained in a minute or less. Differences in eye weight have been used by other investigators as a simple and reliable indicator of eye growth.

**Retinal Wholomeounts and Lens Weight**
Retinal wholemounts were prepared from a subset of 136 cases representing all the BXD strains and the two parental strains. The cornea was removed, and the retina was gently separated from the choroid plexus and the ciliary body. Five radial cuts extending 1 mm to 2 mm into the retina toward the optic disc were made, and the retina was flattened onto a slide and overslipped in Gelvatol. Low-power drawings of retinal wholemounts were traced onto a digitizing tablet, and retinal areas were determined with a precision of better than 0.5 mm². Lens weights were also obtained for a subsample of 122 cases.

**Mapping Eye Weight QTLs**
Simple and composite interval mapping were performed using the program Map Manager QT and a data set of recombinant inbred strain genotypes originally compiled by R. W. Elliott and B. Taylor (see Refs. 19 and 26 at http://nervenet.org/papers/ShortCourse98.html). A nonredundant set of 590 loci that define a genome with a total length of approximately 1640 centiMorgans (cM) was used for QT analysis. Genomewide significance was estimated by comparing the peak likelihood ratio statistic (LRS = 4.61 × LOD) of the correctly ordered data with those computed for 20,000 random permutations of the data. Permutations were run using the equations of Churchill and Doerge implemented by Map Manager QT on Macintosh 8600/300 computers. Procedural details are provided in Williams et al. An analysis of the permutations allowed us to estimate appropriate genomewide statistical criteria. Files used for mapping are available at http://nervenet.org/neuron.html. Mapping data have been deposited with the Mouse Genome Database.

**Genotyping**
Genomic DNA was extracted from the spleen using a high salt procedure. DNA was amplified by the polymerase chain reaction (PCR). A set of microsatellite markers on Chr 5 (D5Mit346, D5Mit294, and D5Mit122) and two markers on Chr 17 (D17Mit121, D17Mit46) were typed in the parental strains, all BXD strains, and the F2 progeny, using a modified version of the protocol of Love et al. and Dietrich et al. Our main objective in this analysis was not to perform a genomewide scan (this would have necessitated typing approximately 100 markers) but rather to corroborate intervals that harbored QTLs in the closely related BXD recombinant inbred set and to measure effect size of QTLs. Each 10-μl PCR contained 1× PCR buffer, 1.92 mM MgCl₂, 0.25 U of Taq DNA polymerase, 0.2 mM of each deoxynucleotide, 132 nM of the primers, and 50 ng of genomic DNA. The microsatellite primer pairs were purchased from Research Genetics (Huntsville, AL). A loading dye (60% sucrose, 1.0 mM cresol red) was added to the reaction before the PCR. PCR products were carried out in 96-well microtiter plates. We used a high stringency touchdown protocol in which the annealing temperature was lowered progressively from 60°C to 50°C in 2°C steps over the first 6 cycles. After 30 cycles, PCR products were run on 2.5% Metaphor agarose gels (FMC, Rockland ME), stained with ethidium bromide, and photographed. Genotypes were entered into Map Manager QT and Microsoft Excel 98. Positions of previously mapped loci are taken from the Mouse Genome Informatics server at www.informatics.jax.org/locus.html.

**RESULTS**

**Eye Weights of Parental Strains**
At 75 days of age, the eyes of the parental strains, C57BL/6j and DBA/2J, weighed 18.9 ± 0.2 mg and 20.2 ± 0.2 mg, respectively. The difference between these strain averages is 1.3 mg (6.5%) and is highly significant (F₁, ₅ = 10.1, P = 0.0026). Eye
weights of the reciprocal F1 hybrids—B6D2F1 and D2B6F1—are 20.8 ± 0.3 mg and 20.6 ± 0.3 mg, respectively, an insignificant difference. The high values in both F1 hybrids suggest that collectively the alleles inherited from the DBA/2J parent are dominant. Relative to body size (Table 1), eyes of these mice are 10 times larger than those of humans (0.1% versus 0.01% of body weight).23 Eye size is clearly a crucial parameter in maintaining reasonable sensitivity under scotopic conditions.

**Effects of Age, Gender, Body Weight, and Brain Weight on Eye Size of BXD Mice**

Eye weights for all BXD cases were corrected by multiple linear regression analysis to minimize extraneous effects of variation in body weight, brain weight, sex, and age on the eyes (Table 1). Age, sex, body weight, and brain weight collectively account for approximately 57% of the variance in eye weight. The line of best fit is described by the following equation: eye weight (fixed in milligrams) = 1.04 + 5.53 (logarithm of age in days) + 0.0658 (body weight in grams) + 0.0119 (brain weight in milligrams) + 0.1555 (if female).

Using this relationship, the predicted fixed eye weight of a 75-day-old, 22-g female mouse with a brain weight of 420 mg is 18.0 mg. The final estimate, after correcting for fixation (+6.1%), is 19.1 mg. The logarithm of age is the most important single predictor of eye size in sexually mature mice ($P = 0.0001$, $r_\text{ratio} = 10.4$). Brain weight ($P = 0.0001$, $r_\text{ratio} = 5.1$) and body weight ($P = 0.01$, $r_\text{ratio} = 2.6$) are also significant predictors in a multiple regression analysis. Once the effect of body weight was factored out, it became apparent that sex had no independent or specific effect on eye weight ($P = 0.39$). A similar multiple linear regression was used to adjust retinal area and lens weight.

**Heritability of Eye Weight Variation**

An ANOVA of all BXD mice indicates that the broad-sense heritability for variation in eye weight computed using Hegmann and Possidente’s\textsuperscript{34} equation is approximately 48%.

**Differences in Eye Weight among BXD Strains**

Mean (± SE) eye weights of the 26 BXD strains averaged 18.8 ± 0.3 mg and ranged from a low of 15.6 mg in BXD21 to a high of 20.2 mg in BXD11. This range extends far below the value of the parental strain C57BL/6J, which has the smaller eye (18.9 mg). Six strains have eye weights significantly less than 18.0 mg. In contrast, none of the BXD strains has an eye weight greater than that of the DBA/2J parent (20.2 mg). Mean strain values below 18 mg are presumably due to the inheritance of low alleles from both parental strains. This indicates that a small number of loci may have relatively large effects on eye weight.

**Two Major Modes**

Strain averages are characterized by several prominent modes centered at the values of 17.5, 18.3, 19.4, and 20.1 mg. (Fig. 1). This is illustrated clearly in the probability density distribution seen in Figure 1. In essence this figure is a smoothed histogram of average eye weights for all 26 strains. This multimodal distribution suggests that one or more QTLs generate a difference of 1 mg or more among the BXD strains (compare with Fig. 4 of Ref. 35).

**Mapping QTLs That Modulate Eye Size**

Mapping QTLs using the BXD strains is in essence a search for those marker loci (if any) for which $B$ alleles and $D$ alleles match up with BXD strains that have low and high eye weights (for methodological details and rationale see http://nervenet.org/papers/shortcourse98.html). In this case we detected an excellent match between variation in eye weight and the strain distribution pattern of $B$ and $D$ alleles at the marker $D5Mit346$ on proximal Chr 5. Figure 2 illustrates this concordance. The average eye weight of 11 strains with the $B$-genotypes at this particular locus was 17.9 ± 0.33 mg, whereas that of 15 strains with the $D$-genotype was 19.8 ± 0.14 mg. The correlation coefficient between eye weight and alleles at $D5Mit346$ is 0.78 (Table 1; for purposes of computing correlations, $B$ alleles at Mendelian loci were assigned a value of 0 and $D$ alleles were
FIGURE 2. Variation in eye weight is tightly correlated to the marker locus D5Mit346. The upper half of this figure illustrates one of the gels used to genotype BXD strains. Genomic DNA isolated from each of the strains was amplified using PCR primers that are specific for sequences that flank the D5Mit346 microsatellite locus on proximal Chr 5. The allele inherited from the DBA/2J strain is longer (174 bp) than that inherited from the C57BL/6J strain (120 bp). As indicated under each lane by Bs and Ds, each of the BXD strains is homozygous for one or the other of the two parental alleles. Numbers at the top of each lane are the strain ID numbers (i.e., BXD1, BXD2, BXD5). The lower part of the figure lists mean eye weights for each strain (also see Table 1). Note the excellent concordance between B alleles and strains with smaller eyes.

assigned a value of 1). This suggests that as much as 38% of the genetic variance and 15% to 20% of the total phenotypic are generated by Eye1. This is probably an overestimate of this QTL’s influence.36

As assessed by simple interval mapping, the QTL on proximal Chr 5 has a LRS of 24.9 (Fig. 3). This is a remarkably high value and indicates the presence of a QTL with a major effect. The map of the likelihood of linkage as a function of position

FIGURE 3. Linkage between variation in eye weight and proximal Chr 5. The x axis represents the entire genetic length of Chr 5—from the proximal end (left) near marker D5Mit346 to the distal end of the chromosome at approximately 60 cM (far right). The y axis represents the strength of linkage assessed using the LRS computed at 1-cM intervals using an interval mapping procedure without any adjustment for secondary QTLs using data for the 26 BXD strains. The peak LRS of 24.9 is approximately 1 cM distal to D5Mit346. The horizontal white bar indicates the 2-LOD confidence interval of the position of the Eye1 QTL. The inset histogram in the upper right corner shows the distribution of peak LRS scores for a set of 20,000 permutations of eye weight mapped across the entire genome. Only 116 of the permutations (0.58%) attained an LRS as high as or higher than that of Eye1. In contrast, the LRS for Eye2 is approximately 10, and the genomewide probability of achieving this level by chance is shown to be approximately 0.4. Eye2 was subsequently confirmed by analysis of F2 progeny, and the cumulative data for this locus have a genomewide P of < 0.05. Three criterion levels (P = 0.5, P = 0.05, and P = 0.005) are shown both on the histogram and on the LRS plot.
along Chr 5 has an unusually sharp profile (Fig. 3, left side). The 2-LOD confidence interval, the chromosomal region in which the QTL is located with a probability of greater than 95%, is 6 cM to 8 cM. We have named this QTL Eye1. Less than 0.6% of permutations gave peak LRS scores as high as the correctly ordered eye weight data set (Fig. 3, inset).

Composite Interval Mapping to Detect Additional QTLs

Eye1 is the only QTL defined unambiguously by our data set by means of simple interval mapping. Composite interval mapping is a refinement on simple interval mapping in which effects of well-mapped QTLs are accounted for while searching for additional loci. This method eliminates variance generated by prior QTLs and allows QTLs with smaller effects to be resolved. When we controlled for variation generated by Eye1, the best concordance was found with marker loci between Hba-ps4 (9.2 cm) and Tpx1 (22.8 cm). The LRS score peaks at 10.5 (genomewide \( P = 0.4 \), approximately 2 cM distal to Hba-ps4. This interval falls short of the significance level which the QTL is located with a probability of greater than 95%. In this case, nonautosomal C57BL/6J genotypes are associated with a higher mean value.

An analysis of F2 progeny confirms that Eye1 is tightly linked to D5Mit346. Individuals with the BB genotype at D5Mit346 have a mean eye weight of 20.06 ± 0.17 mg, whereas individuals with BD and DD genotypes have eye weights of 20.72 ± 0.09 mg and 20.74 ± 0.14 mg, respectively. These three genotypes can be readily analyzed using a single-factor ANOVA, and the result is a highly significant effect of genotype (\( F_{2,180} = 8.5, P = 0.0003 \)) at D5Mit346. Because there is no significant quantitative difference between BD and DD genotypes, it is highly likely that the D allele at Eye1 is dominant. The Pearson-product moment correlation between eye weight and a dominant model of gene action (BB genotypes scored as 0, and both BD and DD scored as 1) is 0.26, and when the ANOVA is recomputed with just two classes (BB versus BD and DD), the statistics are strengthened substantially (\( F_{1,181} = 17.0, P = 0.000056 \)). The analysis of F2 animals allowed us to estimate the size of the effect of Eye1 on eye weight. (Note that effects of allele substitutions depend on population structure, allele frequencies, norms of reaction, and other complex gene-gene and gene-environment interactions.) A simple analysis of the difference between mice raised in a uniform environment indicates that Eye1 is responsible for approximately 7% of the total phenotypic variance in eye weight of an age-adjusted population of F2 progeny. The additive effect of a single B to D allele substitution is approximately 0.4 mg. As expected, this is a more modest effect size than that suggested by the difference between BXD strains at D5Mit346 (17.9 mg and 19.8 mg for BB- and DD-genotypes).

Variation in eye size among the F2 intercross progeny also strongly supports the presence and position of the second eye weight QTL, Eye2, on proximal Chr 17. Mean eye weights of the three genotypes at the marker microsatellite D17Mit46 in the mast cell protease 6 gene (Mcp6 at 12 cM) differ significantly—BB = 19.64 mg, BD = 20.09 mg, DD = 20.24 mg (\( F_{2,180} = 3.79, P = 0.024 \), two-tailed). The moderately large eye size of heterozygotes suggests partial dominance (\( F_{1,181} = 7.1, P = 0.009 \) dominant model). A comparable ANOVA at the marker D17Mit135, that is closer to ColIa2, reveals no statistical association. Combined BXD and F2 data indicate that Eye2 is likely to map between 10 cM and 16 cM on Chr 17 (Zfp52 to Pim1 interval).

Specificity of Action

From the preceding analysis we can say with confidence that Eye1 and Eye2 modulate weight of the eye and that this effect is independent of body weight or brain weight. However, using these data we cannot assess whether these loci act selectively on anterior or posterior segments of the eye. To test specificity of gene action, we quantified additional eye traits and examined correlations between these traits and genotypes of markers on Chr 5 (D5Mit346) and Chr 17 (D17Mit46).
traits include total retinal area, lens weight, and retinal ganglion cell numbers for the complete set of BXD strains (Table 3).

Retinal area is a good surrogate measure for the size of the posterior segment. Areas range from 16.0 mm² in strain BXD21 to 20.3 mm² in BXD11 with a mean (±SE) value of 18.8 ± 0.4 mm² (Table 1). There is a strong positive correlation between retinal area and D alleles at D5Mit346 (the marker closest to Eyel). The correlation is 0.70—a highly significant value. This indicates that Eyel has an appreciable effect on retinal area—approximately 0.7 mm² per D allele—and by inference that Eyel has an effect on the size of the posterior segment. The correlation with lens weight is 0.64 (Table 3; +0.28 mg per D allele), and this value is also significant. Finally, the correlation with the ganglion cell population is significant (r = 0.45; P = 0.02). Regression analysis suggests an effect of approximately 2500 cells per D allele. Given these relatively high correlations it is likely that the Eyel gene product has widespread direct or indirect effects on many aspects of eye development and growth.

Multiple regression analysis was also used to assess effects of Eye2 in isolation from Eyel. Eye2 affects retinal area (P = 0.015; -0.3 mm² per D allele) and lens weight (P = 0.016; -0.14 mg per D allele). However, this locus has little if any effect on retinal ganglion cell number (P = 0.95). Table 3 shows that correlations are weakly negative; in other words, the D alleles are associated with lower values. This finding suggests that epistatic interactions between Eye2 and as yet undefined loci may have important modulatory effects.

A final question is whether Eyel and Eye2 have selective effects on any specific ocular components after correcting for variation in eye weight. In other words, does either locus have a greater effect on the lens or retina than on the eye as a whole? This question can be addressed by using multiple regression to control for differences in eye weight. There are many interesting ways to perform the multiple regression analysis, and the key data for 28 strains are provided in Table 1. One can, for example, determine the coefficient of the highly significant relationship between retinal area and lens weight. With respect to selectivity of Eyel and Eye2. Neither QTL had selective effects on retinal area or retinal ganglion cell number. However, when we corrected for the derived variable eye weight - lens weight, then Eyel had a significant selective effect on lens weight (P = 0.03; +0.21 mg/D allele). In contrast, Eye2 did not have a selective effect on the lens that we could detect.

**Discussion**

**Synopsis**

We have mapped two QTLs that have pronounced effects on eye weight, lens weight, and retinal area to the proximal parts of both Chr 5 and 17 in mouse. Eyel and Eye2 are the first QTLs in any vertebrate that have been shown to modulate normal variation in eye size. This work is important for two reasons: First, it represents a key step in characterizing genes that normally modulate growth of the eye. Second, our discovery raises the possibility that a human counterpart of the Eyel gene is located on the long arm of Chr 7—the homologous chromosomal segment in humans. Similarly, a homolog for Eye2 may map to human 6p21, 16q13.3, or 19q13. These loci may control normal eye growth in humans and may also influence susceptibility to the development of myopia, and possibly even glaucoma.

**A New Model to Study the Genetic Basis of Human Myopia**

Many of the most serious eye pathologies that afflict humans have murine counterparts that have proved to be useful in understanding disease etiology and, in some instances, in developing treatments. Despite its prevalence and huge cumulative cost to society, myopia is a notable exception, and murine experimental systems have not yet been developed. One reason is that research on myopia is well served by sophisticated primate and bird models. However, complementary genetic models are now needed. It is evident that susceptibility to myopia is under partial genetic control. Heritability estimates for myopia in humans are typically above 25%, and we have found that heritability in mice is roughly 50%. As we have demonstrated, the mouse model can be especially useful in dissecting the genetic mechanisms that modulate eye growth. Many of the fundamental molecular and cellular processes that control eye growth are likely to be shared among mammals, regardless of the reliance that they place on vision.

One aim in this study has been to demonstrate how mice can be used to begin to identify QTLs that normally influence the rate and duration of eye and retinal growth. Normal variation in the size of the eye and retina can be attributed in part to the Eyel gene locus on proximal Chr 5. The Eyel allele inherited from strain DBA/2J is dominant with respect to that inherited from C57BL/6J, and, consequently, heterozygotes have eyes as large as DBA/2J homozygotes. Allelic variants at Eyel modulate eye weight by 1 mg to 2 mg. A 5% decrease in eye weight—from 20 mg to 19 mg—is associated with a 1.7% decrease in linear dimensions. The axial length of the eye should be reduced by approximately 57 μm. If there was no concomitant increase in the refractive power of the cornea and lens (a fixed anterior segment), this reduction would cause a myopia of approximately 7 D (Howland H, personal communication and Ref. 42). Whether or not these size differences are actually associated with refractive error or are balanced fully by other adaptive changes, the variation in eye size has made it possible to use a forward genetic approach (from phenotype to gene) to track down genes that contribute to the molecular control of eye growth.
A Candidate Gene for Eye2

Eye2 is tightly linked to the hepatic growth factor (Hgf) gene on proximal Chr 5.[4] HGF is a secreted protein with an unusually broad range of action mediated through the c-Met tyrosine kinase receptor.[44] Once cleaved by plasminogen activator and reassembled as an aβ dimer, HGF stimulates the division, migration, differentiation, and survival of numerous types of cells, including neural crest, central nervous system cells, the retinal pigment epithelium, and corneal epithelial cells.[5] HGF has also been shown to have an important role as neurotropic and neurotrophic factors in the developing nervous system.[46,47] In the past year there has been a surge of interest in the role of Hgf in eye research.[38-40] HGF and its receptor are expressed by the retinal pigment epithelium in fetuses and adults.[51] In humans, HGF is also secreted by Müller cells into the vitreous, and titers are upregulated in patients with diabetic retinopathy.[52] Clearly, allelic variants at the Hgf gene could play a role in eye development. Inactivating the HGF gene by homologous recombination causes mice to die in utero at an early stage,[53-55] but it is possible that normal allelic variants in the Hgf gene produce variation in eye size. It should be possible to test the role of Hgf by carefully studying eyes of Hgf transgenic mice.[56]

Candidate Genes for Eye2

The BXD and F2 data indicate that Eye2 maps in an interval that is proximal to the major histocompatibility complex. The analysis of the F2 progeny strengthens linkage to Mctp6 and appears to rule out the more distal region around D17Mit135 (17 cM). We can thus discount several candidate genes expressed in the eye that appear to be just out of range, namely, peripherin 2 (Prph2 at 18.8 cM), the retina X repressor (Rxrb at 18.5 cM), and procollagen XI alpha 2 (Collla2 at 18.5 cM). We have not yet identified strong candidate genes close to Eye2 that have well-characterized ocular expression. However, this segment of Chr 17 does include the ubiquitously expressed Bak gene (Bcl2 homolog antagonist/killer), a poorly understood cyclin (Ccnd), and a somatostatin receptor expressed in the central nervous system (Smstr).

Other QTLs Affecting Eye Growth

Eye1 and Eye2 are just two of many polygenes that influence the rate and duration of eye growth in the mouse. Our analysis indicates that these loci account for approximately 10% of the total phenotypic variance in eye weight in the particular cross that we used. Given that more than 10,000 genes are expressed in the mammalian eye during development,[37] there are almost certainly a substantial number of additional polymorphic genes that contribute to normal genetic variation in eye growth.[37,56]

Eye Size and Glaucoma

A hallmark of glaucoma is an increase in intraocular pressure that can in some instances be associated with an increase in eye size. We initially assumed that variation in eye weight among mice is an expression of normal variation and that the modest difference between the parental strains C57BL/6J and DBA/2J was not related to any pathology. However, recent work by John and colleagues[39] calls this assumption into question. They demonstrate that DBA/2J mice develop severe pigment dispersion-type glaucoma after 1 year of age. Their discovery is particularly important to us because DBA/2J is one of the parental strains that we used in our mapping study.[60] Almost all mice that we examined were young asymptomatic adults (<4 months old). It is nevertheless possible that the DBA/2J allele of the Eye1 locus contributes both to large eye size and to a predisposition to develop glaucoma.

The Eye1 locus is expected to have a human homolog that maps to the long arm of Chr 7, either in band q21, or with lower probability in band q36. An autosomal dominant locus associated with pigment dispersion and adult-onset glaucoma similar to that seen in DBA/2J mice has been shown by Andersen and colleagues[61] to map to 7q35–q36. The associations between Eye1, glaucoma in DBA/2J mice, and a human pigment dispersion/glaucoma locus on Chr 7 are tantalizing and highlight the potential of comparative genomic analysis of eye development and disease in mice and humans.[62]

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References

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