Identification of Novel Genetic Loci for Intraocular Pressure

A Genomewide Scan of the Beaver Dam Eye Study

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Objective: To identify genetic loci that control intraocular pressure (IOP).

Methods: We performed a genomewide scan of IOP, using 486 pedigrees ascertained through a population-based cohort, the Beaver Dam Eye Study. Linkage analysis was performed using the modified Haseman-Elston regression models and variance components linkage analysis.

Results: Seven regions of interest were identified on chromosomes 2, 5, 6, 7, 12, 15, and 19. The novel linkage region on chromosome 19p had an empirical multipoint P value of 6.1 \times 10^{-5}. Two of the regions (2 and 19) were especially interesting since each has been identified as a potential linkage region for blood pressure.

Conclusions: The results of this genomewide scan provide evidence that a quantitative trait locus may influence elevated IOP and may colocalize with blood pressure loci. These loci may control systemic pressure reflected in the eye and vascular system.

Clinical Relevance: Glaucoma is a leading cause of blindness in the world, and the identification of genes that contribute to this disease is essential. Elevated IOP is a principal risk factor for primary open-angle glaucoma and an intriguing quantitative trait that may strongly influence the development of disease.

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Primary open-angle glaucoma is a progressive eye disease that often results in blindness. Worldwide, an estimated 37 million people are blind, 12% of whom have blindness attributable to glaucoma.1 Intraocular pressure (IOP) is a physiologic characteristic that is present in every eye and is essential to maintain the structural and functional integrity of the eye. Higher IOP is associated with higher risk of damage to the optic nerve and can result in irreversible vision loss or blindness. Elevated IOP is a primary risk factor for the development of glaucoma.

Several family studies have shown that genetic variation contributes to the development of primary open-angle glaucoma.2-12 Genetic regions that influence glaucoma have also been identified in large population-based studies.3,10 To fully understand the genetic basis of primary open-angle glaucoma, we focused on the principal risk factor, IOP. The goal of this study was to identify quantitative trait loci controlling IOP across its range of values and thereby influencing the development of glaucoma. The identification of genes that contribute to variation in IOP may help to elucidate the pathologic features and mechanisms that result in vision loss due to glaucoma.

Methods

Population

The Beaver Dam Eye Study is a population-based cohort study established in 1988, and a detailed description is available elsewhere.13 Eligibility requirements for inclusion in the study were age between 43 and 84 years and residence in Beaver Dam, Wis. In 1988-1990, a total of 5924 eligible persons was identified by a private census of the community, of whom 4926 (83.1%) participated fully in the baseline evaluation. Family relationships and pedigrees were constructed from participant information and later confirmed at the first follow-up visit (1993-1995). Of the 4926 individuals who enrolled at baseline, 2336 individuals had known familial relationships in the catchment area of the study. For 2044 individuals, we have complete IOP measurements at baseline; DNA for genotypic analysis was available on 1979 individuals. Since the pedigrees were derived from an entire township and eligibility for entry into the study was restricted.
to generally older individuals, most of the pedigrees are not “deep” or multigenerational. Instead, these pedigrees are “hedges” with multiple siblings, cousins, and spouses but limited parental or grandparental phenotypic information. There are 486 pedigrees (of 602 original pedigrees) used for analysis after excluding families uninformative for linkage (ie, parent-offspring trios). The mean ± SD size of all the pedigrees is 10.5 ± 12.3, and for individuals with both IOP phenotype information and genotype information, it is 4 ± 5. All individuals gave informed consent and the institutional review board at the University of Wisconsin approved all protocols. The National Human Genome Research Institute institutional review board also approved the statistical analyses of these existing data.

**CLINICAL EVALUATION**

Detailed medical histories and eye examinations were performed on all participants, including assessment of IOP and glaucoma. Intraocular pressure was measured using a Goldmann applanation tonometer after instilling a drop of fluorescein combined with a topical anesthetic (Flouress; Barnes-Hind Armour Pharmaceutical Co, Kanalakee, Ill) in each eye. The examinations were carried out by trained observers who participated in quality control programs to maintain consistency and validity of measurements.14 Blood pressure was measured according to the Hypertension Detection and Follow-up Program protocol.15 This method entails measuring the blood pressure 3 times, the second and third times with a random-zero sphygmomanometer. The mean of these latter 2 blood pressures was used.

**DNA EXTRACTION AND GENOTYPING**

For 76% of individuals, DNA was extracted from buffy coat separated at the time of the blood draw from the second or third Beaver Dam visit and stored at −80° C. For 24% of individuals, DNA was extracted from frozen whole blood cells from the first examination. A genomewide scan was performed at the Center for Inherited Disease Research using automated fluorescent microsatellite analysis. Polymerase chain reaction products were sized using a capillary sequencing platform. The marker set consisted of 404 short tandem repeat markers with an average spacing of 9 centimorgans (cm) throughout the genome. The marker set is a modified version of the Marshfield Genetics (Marshfield, Wis) version 8 screening set with an average heterozygosity of 0.76. The overall missing data rate was 3.5%.

**STATISTICAL METHODS**

**Error Testing**

The data were deposited into a Web-based secure database, GeneLink.16 The Center for Inherited Disease Research released the data after running GAS (Genetic Analysis System) to check for mendelian inconsistencies and to identify any systematic laboratory or binning problems. Relationship errors were further identified using PREST17 and RELCHECK,18 which both examine extended pedigrees. The reclassifications of errors resulted in a reduction of 2435 sib pairs to 2427 genotype-verified sib pairs in the full data set. We also identified any residual mendelian errors using MARKERINFO in SAGE version 4.6.19 All errors were corrected prior to any analyses.

**Linkage Analyses**

For quantitative analyses, the higher IOP measurement from either eye was used. Intraocular pressure was normally distributed and did not need additional transformations. We adjusted for covariates (age, sex, systolic blood pressure, and treatment for IOP) outside of the linkage analysis programs. Using linear regression, we modeled variation in IOP due to these covariates. Then, for each individual, we calculated the predicted deviation from the mean due to that individual’s specific values of the covariates and subtracted this from the mean, thus creating a mean-adjusted residual deviation. This adjusted value was then used in our linkage analysis. Intraocular pressure was assessed for deviations from normality using normal quantile plots and measures of skewness and kurtosis. Allele frequencies at marker loci were estimated from founders using the maximum likelihood option in the program FREQ (SAGE version 4.6). Nonparametric linkage analysis was conducted using the Haseman-Elston regression as implemented in SIBPAL (SAGE version 4.6). A weighted combination of squared trait difference and squared mean-corrected trait sum (option W4) was used. This method adjusts for the nonindependence of sib pairs and the nonindependence of squared trait sums and differences. We performed up to 1 million Monte Carlo permutations using SIBPAL to determine the empirical P values.

In addition, we performed variance components analysis using nuclear families in Merlin (version 0.10.2).20 Prior to running variance components in Merlin, our pedigrees were reduced to nuclear families using an option in the statistical program, Mega2.21 Merlin performs multipoint variance components linkage analysis under the assumption of no dominance and calculates the locus-specific heritability for each trait. No ascertainment correction was necessary since the Beaver Dam Eye Study is a population-based cohort. Allele frequencies were calculated in Merlin for founders only. We also performed multipoint variance components using the entire pedigrees in SOLAR (version 2.1.4)22 since it is not limited by larger pedigree size and included covariates in the analysis. Allele frequencies were estimated in FREQ, and identity-by-descent calculations were done within SOLAR for these analyses.

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>896 (45)</td>
</tr>
<tr>
<td>Female</td>
<td>1083 (55)</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>92 (4.6)</td>
</tr>
<tr>
<td>Treatment for IOP</td>
<td>48 (2.4)</td>
</tr>
<tr>
<td>Age, y, median (range)</td>
<td>64 (43-86)</td>
</tr>
<tr>
<td>IOP, mm Hg, median (range)</td>
<td>16.0 (6-36)</td>
</tr>
<tr>
<td>Adjusted IOP*, mm Hg, median (range)</td>
<td>11.9 (2-32)</td>
</tr>
<tr>
<td>SBP, mm Hg, median (range)</td>
<td>131 (81-221)</td>
</tr>
<tr>
<td>DBP, mm Hg, median (range)</td>
<td>78 (42-127)</td>
</tr>
<tr>
<td>Normotension</td>
<td>936 (47)</td>
</tr>
<tr>
<td>Hypertension (SBP&gt;139 mm Hg or DBP&gt;89 mm Hg and/or taking BP-lowering medication)</td>
<td>1041 (53)</td>
</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; IOP, intraocular pressure; SBP, systolic blood pressure.

*Adjusted for age, sex, systolic blood pressure, and treatment for IOP.

Table 1 provides clinical characteristics for the Beaver Dam Eye Study participants with available IOP measurements and genotype information. Forty-five percent of the individuals were male, and the median age was 62
years for men and 65 years for women. The median IOP measurement was 16 mm Hg and did not differ between sexes. The mean and median IOP measurements (adjusted and unadjusted) were very similar, supporting a normal distribution for this quantitative trait. In addition, we saw no deviation from normality using quantile plots or measures of skewness and kurtosis. One thousand forty-one individuals were classified as having hypertension (systolic blood pressure >139 mm Hg and/or diastolic blood pressure >89 mm Hg and/or taking blood pressure–lowering medication). The mean systolic blood pressure was 133 mm Hg and the mean diastolic blood pressure was 77 mm Hg. Our previous segregation analysis showed that age, sex, treatment for IOP, and systolic blood pressure were important covariates in our models, and they were included in all analyses. In total, we had 1979 individuals with both genotype and IOP phenotype information. Of these 1979 participants, there were 1059 full-sib pairs, 59 half-sib pairs, 1567 cousin pairs, 686 avuncular pairs, and 1 grandparent-grandchild pair. Among the 1524 sibships with genotype data, 1293 had 0 parents with phenotype data, 181 had 1 parent with phenotype data, and 50 had 2 parents with phenotype data.

The genomewide scan using Haseman-Elston regression of sib pairs identified 7 regions with an empirical multipoint linkage signal of less than 0.01 (chromosomes 2, 5, 6, 7, 12, 15, and 19). The Figure graphically displays the multipoint linkage regression results for all chromosomes, and Table 2 details the empirical multipoint P values and variance components P values of significance. The strongest evidence for linkage was

![Multipoint graphs of the \(-\log_{10}\) empirical P value for intraocular pressure adjusted for age, sex, treatment for intraocular pressure, and systolic blood pressure. The horizontal lines depict P value = 0.01 (−log[P value]=2.0) and P value = 0.00074 (Lander and Kruglyak suggestive linkage). CM indicates centimorgan.](image-url)
identified on chromosome 19 near marker D19S586, with single-point and multipoint empirical P values of 2.0 × 10^{-3} and 6.1 × 10^{-5}, respectively. This novel linkage region reached suggestive evidence for linkage according to the highly conservative Lander and Kruglyak thresholds.24 The chromosome 19 region spans approximately 20 cM with a 1 logarithm of the odds (LOD) drop physical interval from 15 cM to 35 cM.

This same region, near marker D19S586, was also identified in variance components linkage analysis of the nuclear family data (P value = .005; locus-specific h^2 = 28.4%). Of the 6 additional regions identified by Haseman-Elston regression, 5 were also supported with variance components analysis of the nuclear family data (Table 2) with locus-specific heritability ranging from 20% to 28%. Additionally, we performed variance components linkage analysis using extended pedigree data; however, we did not identify any regions that reached a LOD score of 2.0 or greater. Using the entire pedigree, we identified 3 regions with a LOD score of 1.0 or greater: chromosome 15 (71 cm) with a LOD score of 1.39 and a locus-specific heritability of 22%, chromosome 12 (88 cm) with a LOD score of 1.28 and a locus-specific heritability of 20%, and chromosome 7 (64 cm) with a LOD score of 1.43 and a locus-specific heritability of 20%.

This region (24 cM) among white sib pairs27 These are sibling pairs with hypertension diagnosed before 60 years of age and without type 1 diabetes mellitus. More recently, a study of pulse pressure (the difference between systolic and diastolic blood pressure) in the Family Blood Pressure Program, which includes black, Asian, Hispanic, and white families, found evidence for linkage (LOD = 3.1) to this same locus among black and white individuals.28 Our evidence for a quantitative trait loci for IOP coupled with these studies of hypertension, systolic blood pressure, or pulse pressure all suggest that this region shows strong evidence of harboring a quantitative trait gene related to both IOP and systemic blood pressure.

Although the linkage peak on chromosome 2 was not substantiated using variance components, this region is still of strong interest since it also appears to colocalize with a blood pressure locus. In the Quebec Family Study, Rice and colleagues26 found suggestive evidence for linkage of systolic blood pressure (LOD = 2.26; P value = .00062) within the same 20-cM region on chromosome 2. Additionally, in a study of pulse pressure in Mexican American individuals from the San Antonio Family Heart Study, a linkage peak of 1.28 was found at marker D2S1790, which is within 4 cM of our locus.29 This region on chromosome 2 has also been identified as a glaucoma locus, GLC1B. In an initial study of 6 families with glaucoma,
Stoilova and colleagues\(^2\) found significant linkage with a maximum parametric LOD score of 6.48. Recently, a study of a single 4-generation LOD region on chromosome 6 (exact nonparametric linkage = 0.005) that overlaps with GLC1B, confirming this as a glaucoma locus.\(^3\) Our findings suggest that this region on chromosome 2 may harbor a single gene that influences systemic blood pressure, with strong effects on glaucoma, or possibly 2 or more genes in close proximity that influence pressure and glaucoma independently.

It is not surprising that 2 of our linkage regions appear to colocalize with blood pressure loci. A previous analysis in the Beaver Dam Eye Study demonstrated a significant correlation of IOP with systolic and diastolic blood pressures at baseline and follow-up.\(^4\) For a 10–mm Hg increase in systolic blood pressure or diastolic blood pressure, there was a 0.21–mm Hg increase (95% confidence interval, 0.16–0.27 mm Hg) and 0.43–mm Hg increase (95% confidence interval, 0.35–0.52 mm Hg) in IOP, respectively. This study showed that systemic blood pressure and IOP may be intertwined and intimates that a common mechanism or gene may be controlling pressure in the eye and vascular system.

Interestingly, we did not replicate our own pilot linkage analysis of IOP in the Beaver Dam Eye Study.\(^5\) In our previous study, we performed linkage analysis using 263 sibling pairs from 102 pedigrees previously selected for age-related maculopathy and identified 2 interesting potential linkage regions on chromosomes 6 (P value = .008) and 13 (P value = .0007). However, after expanding our study to include more individuals (n = 1979) and pedigrees (n = 486) and using a population-based sample not selected for any other traits, these previous linkage regions were no longer significant. This is not particularly surprising since the initial pilot study used a minimal sample of individuals compared with our more complete, current study, and neither region on chromosome 6 or 13 was significant using Lander and Kruglyak\(^6\) thresholds of significance. However, this further highlights the importance of replication or confirmation studies for any important epidemiologic or genetic findings.

It was revealing that for this quantitative trait the most powerful method of analysis was Haseman-Elston regression using sibling pairs. Analysis of complete pedigrees using variance components found limited evidence for linkage. This is most likely due to the type of pedigrees in the Beaver Dam Eye Study. Unlike more traditional linkage studies that aim to maximize information using multigenerational pedigrees with affected individuals across generations, the Beaver Dam Eye Study pedigrees were derived from a population-based cohort study, and age at enrollment was 43 to 86 years. Although these pedigrees are large, they most often lack a parental generation for both phenotype and genotype information and comprise predominantly sibs and cousin pairs. In this case, the major contributors to linkage information were sib pairs or individuals within a nuclear family. The use of the entire hedge pedigree may have introduced heterogeneity for this complex trait from extreme branches of the pedigrees where variation was controlled by different IOP genes. We know from studi-


