Confirmation of Linkage to Ocular Refraction on Chromosome 22q and Identification of a Novel Linkage Region on 1q

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Objective: To localize genes influencing ocular refraction in subjects in the Beaver Dam Eye Study. Previous studies establish that myopia clusters within families and linkage to myopia has been demonstrated on 2q, 4q, 12q, 17q, 18q, 22q, and Xq. Few studies have examined genetic effects across the entire range of refraction, though linkages to 1p, 3q, 4q, 8p, and 11p have been reported, and our previous analysis of the Beaver Dam Eye Study demonstrated substantial heritability for refraction (68%).

Methods: We conducted nonparametric sibling-pair and genome-wide linkage analyses on spherical equivalent adjusting for age, education, and nuclear sclerosis, in 834 sibling pairs in 486 extended pedigrees.

Results: We identified a novel region of suggestive linkage on 1q (multipoint, \(P_{\text{H11021}} = 0.00019\)) and replicated the 22q region (multipoint, \(P = 0.0033\)) previously linked to myopia. Additionally, there was some evidence of linkage to 7p (multipoint, \(P = 0.0023\)).

Conclusion: Refraction is a complex trait influenced by both genes and environment. Our work confirms a previously reported linkage region on 22q and identifies 2 novel regions of linkage on 1q and 7p.

Clinical Relevance: Further, genetic research is needed to finemap this trait to identify the causative gene. Modifying the actions of such a gene might lead to a reduction in the risk of refractive error.

Arch Ophthalmol. 2007;125:80-85

For a human eye to see sharply in the distance, the image must be focused on the retina. When this is the case for the unaided eye, the eye is said to be emmetropic and the refractive error is 0. Small variations around this state are usually minimally symptomatic, but eyes with greater refractive error usually require optical correction to achieve optimal vision. Aside from the inconvenience of using glasses, contact lenses, or refractive surgery to achieve optimal vision, refractive errors of even moderate degrees may precede other vision-threatening ocular conditions, such as retinal detachment, glaucoma, and complications at the time of ocular surgery. Refractive errors are common in general; in the Beaver Dam Eye Study cohort, 26.2% of eyes were myopic (\(-0.75\) diopters [D]) and 49.0% were hyperopic (\(+0.75\) D). A number of environmental factors have been reported to be associated with refraction, the most notable being education.1-5

The first evidence that supported a familial component to refraction came from studies demonstrating familial aggregation of refractive errors. These studies conducted in diverse populations all found a strong correlation of refractive errors between family members.6-9 The majority of these studies examined the clinical traits of myopia and nonmyopia. Numerous linkage studies have been conducted to identify genomic regions that may carry myopia predisposition genes. Linkage to chromosomes 2q (logarithm of odds [LOD] score = 5.67), 4q (LOD = 3.61), 7q36 (LOD = 2.81), 12q21 through 12q23 (LOD = 3.85), 18p11 (LOD = 9.59), and Xq23 (LOD = 2.75) has been found for high myopia (\(-6\)D).10-14 A rare X-linked recessive form of high myopia, Bornholm eye disease, has been mapped to chromosome Xq28.15-17 Moderate myopia (\(\leq -1\) D) has shown evidence suggestive of linkage to 3q (heterogeneity LOD [HLOD] score = 1.84), 6q (HLOD = 1.92), 8p (HLOD = 2.03), 20q (HLOD = 1.59), and Xq13 through Xq21 (HLOD = 2.34), and significant evidence of linkage to 22q (HLOD = 3.54).18-19

While linkage has been established for the clinical traits of high myopia and mod-
erate myopia, the potential for genetic effects through the entire range of refraction has not been well studied. Twin studies have indicated a high heritability for quantitative refraction,8 and there is evidence showing that refraction is highly correlated between siblings.6,20 Hammond et al21 reported heritabilities of 84% to 86% for refraction (spherical equivalent) as a continuous trait in a model with additive genetic components and environmental components. Linkage analysis of refraction as a quantitative trait in the 221 dizygotic twin pairs in the same cohort provided evidence of linkage to regions on chromosomes 3q26 (LOD = 3.7), 4q12 (LOD = 3.3), 8p23 (LOD = 4.1), and 11p13 (LOD = 6.1). The same Ashkenazi Jewish population used by Stambolian et al28 to study myopia also provided genome-wide significant evidence for linkage to chromosome 1p36 and some evidence of linkage to 2p, 4q, and 9p when refraction (spherical equivalent) was examined as a quantitative trait.23 Additionally, the chromosome 8p23 region reported by Hammond et al22 was independently confirmed by Stambolian et al28 (HLOD = 2.04) in an Amish family study examining the qualitative trait of myopia.

Previously we have demonstrated substantial familial aggregation of spherical equivalent in the Beaver Dam Eye Study population after accounting for the effects of age, sex, and education with correlations of 0.344 for sibling pairs and 0.171 for parent-offspring pairs,7 which corresponds to a heritability of approximately 68%. However, segregation analysis of refraction in the Beaver Dam Eye Study did not support the involvement of a single major gene throughout the entire spectrum of refraction; it did indicate that several genes of small to modest effect may play a role in refraction.7 Therefore, to localize the genes responsible for the familial aggregation of refraction in this population, we conducted nonparametric genome-wide linkage analyses using SIBPAL (S.A.G.E. version 4.6)24 on data from 881 sibling pairs within 486 extended pedigrees (2231 genotyped individuals) using 385 autosomal microsatellite markers. This work represents the first population-based linkage study of quantitative refraction.

STUDY POPULATION

The Beaver Dam Eye Study is a population-based study of age-related eye diseases that began in 1987 and has been continuously funded by the National Eye Institute. This study conforms to the Declaration of Helsinki and has been approved by the internal review boards of the University of Wisconsin School of Medicine, National Human Genome Research Institute, and Johns Hopkins School of Medicine. Design of the Beaver Dam Eye Study, including the standardized protocols used to measure refraction, has been described in detail elsewhere.25-27 Baseline examination of the Beaver Dam Eye Study, conducted between 1988 and 1990, involved 4926 participants (of the 5924 eligible individuals who resided in the township of Beaver Dam, Wis) aged 43 to 86 years. Follow-up examinations have been conducted every 5 years. Relevant parts of the examination included refraction and best-corrected visual acuity using a modification of the Early Treatment of Diabetic Retinopathy Study protocol. Slitlamp photographs were taken of the lens of each eye after the pupils were pharmacologically dilated. The photographs were graded by trained, blinded observers, according to a standard protocol,28 to assess the severity of nuclear sclerosis. Medical, social, and lifestyle information including years of education were obtained. Family relationship information was obtained from all participants at the baseline examination. During the first follow-up visit, conducted between 1993 and 1995, family relationships including extended pedigree information were confirmed. Of the 5924 eligible individuals, 2783 had available information on familial relationships and could be classified into 1 of 602 pedigrees. Of these individuals, 2138 had complete age, sex, education, and refraction data at the baseline examination. We previously published familial correlations and segregation analysis of spherical equivalent using phenotype data from the Beaver Dam Eye Study.7 After excluding pedigrees not informative for linkage (ie, parent-offspring pairs), analysis was performed on the remaining 834 sibling pairs within 486 extended pedigrees, with a total of 2231 genotyped individuals using 385 autosomal microsatellite markers. The mean family size was 10.5 individuals and ranged from 2 to 102 members. This includes several larger pedigrees that were split because they exceeded the complexity allowed for by the analysis program. However, because we are examining only sibling pairs and no sibling pairs were split, this did not impact the results of the analysis.

TRAITS DEFINITION

Refraction and covariates including age and education were assessed at the baseline examination of the Beaver Dam Eye Study. For the entire Beaver Dam cohort, automated refractive error measurements were obtained for approximately 96% of the eyes; for most of the remainder (4%), the refractions were performed using the Early Treatment Diabetic Retinopathy Study protocol.3 Refraction, both adjusted and unadjusted, is not normally distributed. In an attempt to normalize the data, Box-Cox transformations of the trait (or adjusted trait) were performed (additional transformations were also examined) using the

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For the genetic analysis, the average of refraction in the right and left eyes was used because there is very high correlation of refraction between the 2 eyes in most individuals.7 Thirteen participants with differences in refraction greater than 4 D between the 2 eyes were coded as “unknown” to remove individuals from the data set with very different refraction measurements in each eye. This somewhat arbitrary cutoff was loosely based on the SD of the measurements and what in our judgment identified persons in whom 1 eye may have had an altered refraction due to injury or other external effect. Because of the independent effects of age, education, and nuclear sclerosis on refraction, both in multivariate modeling as well as in our prior segregation analysis, analyses were conducted both with and without adjustment for these covariates. However, because refraction adjusted for age, education, and nuclear sclerosis had the overall highest heritability compared with unadjusted refraction or refraction adjusted for only age and education, we chose to focus our analysis on this composite trait. Age, education, and nuclear sclerosis were modeled as quantitative predictors. Nuclear sclerosis was graded on a 5-point scale, with 1 being no nuclear lens opacity and 5 being severe;26 the sum of the grading of the right and left eyes was used in analysis. The maximum likelihood estimates of the covariate effects of age and education were derived from the segregation models; traits were adjusted prior to analysis.

Refraction, both adjusted and unadjusted, is not normally distributed. In an attempt to normalize the data, Box-Cox transformations of the trait (or adjusted trait) was performed (additional transformations were also examined) using the

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transformation parameter estimates obtained in segregation analysis of these same data. Additional transformations of the data were also examined. While this did bring the distribution of refraction closer to normal, substantial deviations from normality were still present.

ANALYTICAL METHODS

Relationship errors were identified using PREST29 and RELPAL (S.A.G.E version 4.6).24 After the relationships with errors were reassigned (reclassifications resulted in reduction of 2435 sib-pairs to 2427 genotype-verified sibpairs in the full data set), we also identified any residual mendelian errors using MARKERINFO (S.A.G.E version 4.6)29 and SIB-PAIR.30 Non-parametric linkage analyses were conducted using the Haseman-Elston regression as implemented in SIBPAL.24 Allele frequency estimates were obtained from the sample data using FREQ (S.A.G.E. version 4.6).24 The results reported include multipoint and single-point nominal P values from SIBPAL.24 Because of the non-normality of these data, permutation tests using Monte Carlo simulations of up to 1 million replicates were performed for both the single-point and multipoint analysis. These are referred to subsequently as exact P values.

RESULTS

An overview of the subset of the entire Beaver Dam cohort included in our linkage study, individuals with genotype and phenotype data, is described in Table 1. Overall, the mean refraction (spherical equivalent) was +0.44 D. The average age of this group was 62 years and the mean number of years of education was 11.2. Overall, the mean sum of the nuclear sclerosis grade was 4.9.

The results of our single-point linkage analysis of transformed refraction after adjustment for age, education, and nuclear sclerosis using SIBPAIR24 are shown in Figure 1; regions of interest are also detailed in Table 2.

Table 1. Demographics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No.</td>
<td>M 835 F 985</td>
</tr>
<tr>
<td>Refraction (spherical equivalent), diopters</td>
<td>0.44 ± 2.11 (−12.13 to + 8.38)</td>
</tr>
<tr>
<td>Age, y†</td>
<td>62.14 ± 10.6 (43 to 86)</td>
</tr>
<tr>
<td>Education, y†</td>
<td>11.27 ± 2.31 (2 to 21)</td>
</tr>
<tr>
<td>Nuclear sclerosis‡</td>
<td>4.97 ± 1.65 (2 to 10)</td>
</tr>
</tbody>
</table>

* A subset of the Beaver Dam Eye cohort.
† Individuals on whom refraction measurements are also available.
‡ Reflects the sum of nuclear sclerosis grade in the right and left eyes.

Table 2. Sibling Pair Linkage Analysis Results for Transformed Refraction Adjusted for Age, Education, and Nuclear Sclerosis

<table>
<thead>
<tr>
<th>Chromosome Location</th>
<th>Closest Marker to Minimum P Value</th>
<th>Single-point Empirical P Value</th>
<th>Single-point Exact P Value</th>
<th>Multipoint Empirical P Value</th>
<th>Multipoint Exact P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q24</td>
<td>D1S1589</td>
<td>0.00636</td>
<td>0.00229</td>
<td>0.00845</td>
<td>0.0266</td>
</tr>
<tr>
<td>1q41</td>
<td>D1S2141</td>
<td>0.000501</td>
<td>0.00362</td>
<td>0.000234</td>
<td>0.00191</td>
</tr>
<tr>
<td>6q16</td>
<td>D6S1056</td>
<td>0.00959</td>
<td>0.0149</td>
<td>0.00129</td>
<td>0.00560</td>
</tr>
<tr>
<td>7p21</td>
<td>D7S3051</td>
<td>0.00202</td>
<td>0.00899</td>
<td>0.000602</td>
<td>0.00224</td>
</tr>
<tr>
<td>16q21</td>
<td>GATA18A08</td>
<td>0.00115</td>
<td>0.00455</td>
<td>0.00327</td>
<td>0.00738</td>
</tr>
<tr>
<td>22q11</td>
<td>D22S345</td>
<td>0.00153</td>
<td>0.00515</td>
<td>0.00120</td>
<td>0.00330</td>
</tr>
</tbody>
</table>

Figure 1. Single-point SIB-PAIR24 linkage analysis of transformed refraction adjusted for age, education, and nuclear sclerosis.
We found evidence of linkage on chromosome 22 near marker D22S345 (exact $P$ value = .005), replicating the findings of Stambolian et al.\textsuperscript{18} Additionally, 2 adjacent regions on chromosome 1 met the criterion for suggestive linkage.\textsuperscript{31} The first peak was in a region near marker D1S2141 (exact $P$ value = .00036). A second peak on chromosome 1 near marker D1S1589 also provided suggestive evidence for linkage (exact $P$ value = .00022). Two additional regions, one on chromosome 7 near marker D7S3053 and one on chromosome 16 near marker GATA184A08, also provided modest evidence of linkage (exact $P$ values = .0089 and .0045, respectively).

Following single-point analysis, multipoint analysis was also performed, the results of which are shown in Figure 2. In our multipoint analysis, evidence for linkage on chromosome 1 near marker D1S2141 was increased (exact $P$ value < .00019). This region appears to be distinct from the adjacent region near marker D1S1589, which only showed limited evidence of linkage in the multipoint analysis (exact $P$ value = .026). Our multipoint analysis further supports a region of linkage on chromosome 22, where there was evidence of linkage near markers D22S345 through D22S685 (multipoint SIBPAL,\textsuperscript{24} exact $P$ value = .0033).

The multipoint analysis provided modest evidence for linkage on chromosome 7 near marker D7S3051 (exact $P$ value = .00224) and modest evidence of linkage on chromosome 6 near marker D6S1056 (exact $P$ value = .0056). We performed linkage analysis of quantitative refraction prior to adjustment for age, education, and nuclear sclerosis and obtained similar results (not shown).

Figure 2. SIBPAL\textsuperscript{24} multipoint linkage results for chromosomes 1 through 22. The dashed lines represent the thresholds for suggestive ($P$ = .00074) and significant ($P$ = .000022) evidence for linkage.\textsuperscript{31} cM indicates centimorgan.
A parametric LOD score of 3.0 has an approximate equivalent P value = .0001. Lander and Kruglyak\(^3\) proposed that a LOD score of 3.3 in parametric LOD score linkage or a P value = .000022 in nonparametric sibpair methods should be considered to give genome-wide significant evidence of linkage. Furthermore, they proposed that a parametric LOD score of 1.9 or a nonparametric sibpair P value = .00074 constituted evidence of genome-wide suggestive linkage. Finally, they stated that a P value \(\leq .01\) was adequate for replication of a previously published genome-wide significant result.

Our analysis provided suggestive evidence of a novel linkage region on chromosome 1 near marker D1S2141. Additionally, some evidence for linkage was found in a novel region on chromosome 7 near marker D7S3051 in our multipoint analysis, while the single-point analysis also indicated linkage to an additional region on chromosome 1 near marker D1S1589. Given that the 2 regions we report on chromosome 1 are approximately 40 centimorgans apart, it is likely these represent distinct linkage peaks.

The results of our linkage analysis provide the first replication of the chromosome 22 region first reported by Stambolian et al.\(^{18}\) Given the large differences between the study populations in the Stambolian study and our study (large Ashkenazi Jewish pedigrees ascertained due to multiple myopic individuals and a population-based sample, respectively), as well as differences in trait definitions (myopia vs quantitative refraction, respectively), our ability to replicate this linkage peak provided strong evidence that a gene associated with either myopia or refraction is present in this region. Our inability to replicate other reported linkage peaks is not surprising given the large difference in our study population from that of the other studies and further suggests that myopia and refraction are oligogenic phenotypes. Our previous segregation analysis suggested that quantitative refraction is a complex trait, and it is therefore reasonable that our results indicate several potential regions of linkage. Unlike most of the previous studies that selected myopic or extreme hyperopic families, the majority of the individuals in our study had emmetropia or had only modest refractive errors. Our power to detect linkage in this population is enhanced owing to the fact that the population of Beaver Dam is relatively ethnically homogenous, with the majority of the individuals reporting Northern European and/or German ancestry.

Work is ongoing to refine these linkage peaks. Denser single nucleotide polymorphism genotyping will be performed in these regions of interest to determine if these regions represent true linkage peaks and to narrow these large linkage regions. However, this work confirms chromosome 22q as a region of linkage for refraction and identifies novel regions of linkage for quantitative refraction on chromosomes 1 and 7.

Submitted for Publication: June 30, 2006; final revision received September 20, 2006; accepted September 21, 2006.

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Financial Disclosure: None reported.

Funding/Support: This work was supported by grants EY06594 (Drs R. Klein and B. E. K. Klein) and EY015286 (Dr B. E. K. Klein) from the National Eye Institute; grants from Research to Prevent Blindness (Drs R. Klein and B. E. K. Klein); and by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health.

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