Linkage Analysis of Quantitative Refraction and Refractive Errors in the Beaver Dam Eye Study

Alison P. Klein,^{1,2,3} Priya Duggal,³ Kristine E. Lee,⁴ Ching-Yu Cheng,^{5,6} Ronald Klein,⁴ Joan E. Bailey-Wilson,⁵ and Barbara E. K. Klein⁴

PURPOSE. Refraction, as measured by spherical equivalent, is the need for an external lens to focus images on the retina. While genetic factors play an important role in the development of refractive errors, few susceptibility genes have been identified. However, several regions of linkage have been reported for myopia (2q, 4q, 7q, 12q, 17q, 18p, 22q, and Xq) and for quantitative refraction (1p, 3q, 4q, 7p, 8p, and 11p). To replicate previously identified linkage peaks and to identify novel loci that influence quantitative refraction and refractive errors, linkage analysis of spherical equivalent, myopia, and hyperopia in the Beaver Dam Eye Study was performed.

METHODS. Nonparametric, sibling-pair, genome-wide linkage analyses of refraction (spherical equivalent adjusted for age, education, and nuclear sclerosis), myopia and hyperopia in 834 sibling pairs within 486 extended pedigrees were performed.

RESULTS. Suggestive evidence of linkage was found for hyperopia on chromosome 3, region q26 (empiric $P = 5.34 \times 10^{-4}$), a region that had shown significant genome-wide evidence of linkage to refraction and some evidence of linkage to hyperopia. In addition, the analysis replicated previously reported genome-wide significant linkages to 22q11 of adjusted refraction and myopia (empiric $P = 4.43 \times 10^{-3}$ and 1.48×10^{-3} ,

respectively) and to 7p15 of refraction (empiric $P = 9.43 \times 10^{-4}$). Evidence was also found of linkage to refraction on 7q36 (empiric $P = 2.32 \times 10^{-3}$), a region previously linked to high myopia.

CONCLUSIONS. The findings provide further evidence that genes controlling refractive errors are located on 3q26, 7p15, 7p36, and 22q11. (*Invest Ophthalmol Vis Sci.* 2011;52:5220–5225) DOI:10.1167/iops.10-7096

Refractive errors in adults are common worldwide. In the Beaver Dam Eye Study (BDES), we observed that 26.2% of eyes were myopic (-0.75 D or more) and 49.0% were hyperopic ($+0.75$ D or more).¹ Genetic and environmental factors both play an important role in the development of refraction errors. Near-work or its surrogate, educational level, is associated with both myopia and increased severity of myopia.^{1–6}

Despite the importance of near-work on the development of myopic refractive errors, it explains only a small portion of the variability in refraction. Familial aggregation studies have demonstrated a strong correlation of refractive errors between twins as well as between family members.^{1,7–12} We reported sibling odds ratios for myopia of 3.36 (95% confidence interval [CI], 1.56–7.12) for brothers, 4.64 (95% CI, 1.91–11.28) for sisters, and 4.52 (95% CI, 2.44–8.37) for brother-sister pairs in the Beaver Dam Eye Study (BDES). Point estimates for sibling odds ratios for hyperopia were slightly lower at 2.47 (95% CI, 1.05–5.28) for sisters, 3.16 (95% CI, 1.60–6.26) for brothers, and 2.95 (95% CI, 1.75–5.00) for brother-sister pairs.¹ Heritability analysis demonstrated strong genetic effects throughout the entire range of refraction, with estimated heritability of refraction of 57.8% after adjustment for age, education, and nuclear sclerosis.¹² Segregation analysis of refraction in the BDES indicated that several genes of small to modest effect may also play a role in refraction.¹⁰

Numerous linkage studies of myopia and quantitative refraction have been conducted. Genome-wide significant evidence of linkage to 2q(MYP12), 4q(MYP11), 12q(MYP3), 17q(MYP5), 18p(MYP2), and Xq(MYP1, MYP13) has been reported for high myopia (≤ -6 D)^{13–18} as well as to chromosome 22q(MYP6)^{19,20} for moderate myopia (≤ -1 D). In addition, genome-wide evidence suggestive of linkage to high myopia has also been reported on 7q36.²¹ The MYP1 locus on Xq28 has been replicated and is associated with Bornholm eye disease.²² Quantitative trait locus (QTL) linkage analysis using the full range of spherical equivalent as a quantitative trait has demonstrated genome-wide significant evidence of linkage to regions on 1p(MYP14), 3q(MYP8), 4q(MYP9), 7p(MYP17), 8p(MYP10), and 11p(MYP7)^{23–25} with confirmation of the 3q(MYP8) and 8p(MYP10) locus,²⁰ according to the guidelines established by Lander and Kruglyak²⁶ for replication: $P < 0.01$.

In the BDES, we used 385 short tandem repeat polymorphisms (STRP) markers and found evidence suggestive of linkage to regions on 1q and 22q. Our results confirmed linkage to 22q(MYP6) for quantitative refraction.²⁷ However, linkage

From the Departments of ¹Oncology and ²Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland; the ³Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; the ⁴Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison Wisconsin; and the ⁵Statistical Genetics Section, Inherited Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, Maryland.

⁶Present affiliation: Department of Ophthalmology and Department of Epidemiology and Public Health, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

Supported by National Eye Institute Grants EY06594 (RK, BEKK) and EY015286 (BEKK) from the National Eye Institute; grants from Research to Prevent Blindness (RK, BEKK); and the Intramural Research Program of the National Human Genome Research Institute. Some of the results of this paper were obtained by using the software program S.A.G.E., which is supported by U.S. Public Health Service Resource Grant RR03655 from the National Center for Research Resources. Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, Contract HHSN268200782096C. APK has full access to the data and takes responsibility for the integrity of the data and the data analysis.

Submitted for publication December 20, 2010; revised March 25, 2011; accepted April 19, 2011.

Disclosure: A.P. Klein, None; P. Duggal, None; K.E. Lee, None; C.-Y. Cheng, None; R. Klein, None; J.E. Bailey-Wilson, None; B.E.K. Klein, None

Corresponding author: Alison P. Klein, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, School of Medicine, 1550 Orleans Street, Baltimore, MD 21231; aklein1@jhmi.edu.

studies using more densely spaced SNPs (single-nucleotide polymorphisms) have been shown to provide greater power than STRP panels to detect linkage, especially when genotype information on pedigree founders is missing or unavailable, as in the BDES.^{28,29} Therefore, we conducted additional SNP genotyping in the BDES population and conducted linkage analysis using the combined SNP and STRP marker set to identify genetic loci harboring genes for quantitative refraction, myopia, and hyperopia, using Haseman-Elston regression methods.

METHODS

Study Population

The BDES, which began in 1987, is a longitudinal population-based cohort study of age-related eye diseases. This study has been approved by the Institutional Review Board of the University of Wisconsin School of Medicine and conforms to the Declaration of Helsinki. A detailed description of the BDES, including the protocols used to measure spherical equivalent, has been published elsewhere.^{30–32} The baseline examination of the BDES included 4926 participants of the 5924 eligible individuals who were 43 to 86 years of age and resided in the township of Beaver Dam, Wisconsin. Follow-up examinations were completed every 5 years. As part of the examination, refraction and best corrected visual acuity measured with a modified Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol was obtained. After dilation, slit lamp photographs were taken of the lens of each eye to assess the severity of nuclear sclerosis and cortical and posterior subcapsular opacities. Photographs were then graded by trained, masked observers according to a standard protocol.³³ Family relationships were recorded and medical, social, and lifestyle information, including years of education, was obtained at the baseline examination. Confirmation of family relationships was obtained at the first follow-up examination (1993 and 1995), and 2783 persons were classified into one of 602 pedigrees. Complete age, sex, education, and spherical equivalent data were recorded for 2138 individuals at the baseline examination. Using phenotype data from the BDES, we previously published familial correlations, heritability analysis, and segregation analysis of spherical equivalent.¹² After excluding noninformative pedigrees (i.e., parent-offspring pairs), we conducted analyses on the 834 sibling pairs within 486 extended pedigrees.

Trait Definitions

Spherical equivalent and covariates including age and education were assessed at the baseline examination of the BDES. Automated refractive error measurements were obtained for ~96% of the eyes; for the remainder (4%) when corrected visual acuity was 20/40 or worse, the refractions were performed using ETDRS protocol. Refraction measurements from the current prescription (<1% of eyes) were used if other data were not available.¹ The following individuals were excluded: those with an intraocular lens in any eye, those with best corrected visual acuity of 20/200 or worse in at least one eye, and those with data missing for at least one eye. Spherical equivalent was calculated from the refraction measurements. The average of spherical equivalent in the right and left eyes was used in the linkage analysis, because there is very high correlation of spherical equivalent between the two eyes in most individuals.⁷ Thirteen participants with differences in spherical equivalent $> \pm 4$ D between the eyes were coded as unknown, to remove individuals from the dataset with very different interocular spherical equivalent measurements. Myopia was defined as spherical equivalent of < -1 D in either eye, with spherical equivalent < -0.5 D in the opposite eye. Nonmyopes were individuals with spherical equivalent > -0.25 D in both eyes. Individuals meeting none of the criteria were coded as unknown. Hyperopes were defined as individuals with spherical equivalent $> +1$ D in either eye with spherical equivalent $> +0.5$ D in the opposite eye. Nonhyperopes were individuals with spherical equivalent < 0.25 D in both eyes. Individuals meeting none of the criteria were coded as unknown.

Age, education, and nuclear sclerosis have been shown to have strong effects on spherical equivalent and were significant covariates in our heritability analysis, therefore linkage analyses were conducted on unadjusted spherical equivalent and spherical equivalent after adjustment for these covariates. Age (in years), education (in years), and nuclear sclerosis were modeled as quantitative predictors. Nuclear sclerosis was graded on a five point scale, with 1 being no nuclear lens opacity and 5 being severe,³³ and the sum of the grading of the right and left eyes was used in the analysis. The maximum likelihood estimates of the covariate effects of age and education were derived from the segregation models,¹⁰ and traits were adjusted before analysis.

Genotype Data

DNA samples from 2231 individuals were sent for genotyping at the Center for Inherited Disease Research (CIDR). Samples were first genotyped using 385 STRP markers. The results of our linkage analysis of quantitative spherical equivalent using these markers has been published previously.²⁷ Given that SNP markers have been shown to provide increased information content and thereby greater power to detect linkage, all remaining available samples were regenotyped (Linkage Panel I; Illumina, San Diego, CA). Genotype data on 6008 SNPs were generated on 2170 samples with a minimum call rate per sample of 96%. Markers were dropped if they had either poorly defined ($n = 148$) or atypical ($n = 10$) clusters, leaving 5850 high-quality SNPs, of which 5525 were located on the autosomes. All SNPs had a call rate $> 96\%$. For the SNP markers, we then used Haploview 4.0 to identify linkage disequilibrium (LD) blocks, as LD can cause false-positive linkage signals (<http://www.broad.mit.edu/mpg/haploview/>). The Broad Institute, Massachusetts Institute of Technology, Cambridge, MA). A total of 525 singletons and 43 trios from 431 families were used for LD calculations. SNP markers were excluded if they had (1) Hardy-Weinberg Equilibrium $P < 0.001$, (2) Mendelian errors > 1 per marker, or (3) call frequency < 0.98 . Blocks were defined based on solid spine of LD (i.e., the spine was extended if $D' > 0.8$). Within an LD block, the SNP with the highest minor allele frequency was retained. After quality control of the marker data, we merged the SNP and STRP marker sets based on the Marshfield genetic map (research.marshfieldclinic.org/genetics/; Marshfield Clinic, Marshfield, WI). The Marshfield genetic position of the 385 microsatellite markers was obtained from the UCSC Genome Browser (<http://genome.ucsc.edu/>; provided by the University of California at Santa Cruz) and UniSTS database (<http://www.ncbi.nlm.nih.gov/unists/>; National Center for Biotechnology Information [NCBI], Bethesda, MD; and the website of Mammalian Genotyping Service at Marshfield, sponsored by the National Heart Lung blood institute (<http://www.marshfieldclinig.org/mgs/>). We could not obtain a precise genetic position for marker *D2S1780*, and thus this marker was dropped. To obtain a common genetic map for SNPs and STRPs we used 7756 microsatellite markers with known Marshfield genetic position from the USCS website to interpolate the genetic position for each SNP by using the physical position. Relationship errors were identified using PREST³⁴ and RELPAL (S.A.G.E., ver. 6.0) and residual Mendelian errors using MARKERINFO (S.A.G.E., ver. 6.0²⁹), and SIBPAIR.³⁵ We obtained clear evidence of misspecified relationships in 20 pedigrees, and these errors were resolved by removing the problematic individuals or re-assigning the relationship. Our final dataset consisted of 4892 SNP markers and 384 STRP markers on the 22 autosomes.

Analytical Methods

Autosomal nonparametric linkage analyses were conducted using the Haseman-Elston regression as implemented in SIBPAL (S.A.G.E. ver. 6.0).³⁶ Allele frequency estimates were obtained from the sample data by using FREQ (S.A.G.E.) The results reported include multipoint and single-point, nominal P values from SIBPAL.³⁶ Because of the non-normality of these data, allele-sharing among the pairs was permuted with Monte Carlo simulations of up to 2,000,000 replicates, to obtain empirical P values. We report both the nominal and permuted P values.

TABLE 1. Demographic of the Study Population: A Subset of the Beaver Dam Eye Cohort

	Individuals (<i>n</i>)	Mean	Standard Deviation	Range
Age, y	1897	62.47	10.67	43 to 86
Refraction, spherical equivalent diopters	1897	0.46	2.14	−12.13 to +8.38
Myopia, <i>n</i> (%)	1569	406 (25.9%)	NA	NA
Hyperopia, <i>n</i> (%)	1498	894 (59.7%)	NA	NA
Education, y	1894	11.25	2.31	2 to 21
Nuclear sclerosis, <i>n</i>	1897	4.97	1.65	2 to 10
Sex, <i>n</i>		NA	NA	NA
Male	862			
Female	1035			

We used the criteria proposed by Lander and Kruglyak²⁶ for declaring genome-wide significance ($P = 2.2 \times 10^{-5}$) and evidence suggestive ($P = 7.4 \times 10^{-4}$) of linkage, as well as for replication ($P = 0.01$) in the interpretation of our results as these thresholds control the genome-wide type 1 error rates while allowing for correlation between markers. Regions that provided some evidence of linkage ($P < 0.001$) but did not meet Lander and Kruglyak criteria are also summarized.

RESULTS

A detailed description of the subset of the Beaver Dam Cohort included in our linkage study is presented in Table 1. Overall, there were 1897 individuals in 482 pedigrees. Mean refraction (spherical equivalent) among these individuals was +0.46 D, mean age was 62.47 years, and mean years of education was 11.25. Overall, the mean sum of the nuclear sclerosis grade in the right and left eye was 4.97. Within these families, 1569 individuals were classified as meeting our criteria of myopic ($n = 406$, 25.9%) or nonmyopic ($n = 1136$, 74.1%). In addition, 1498 individuals could be classified as hyperopic ($n = 894$, 59.8%) versus nonhyperopic ($n = 602$, 40.3%).

The results of our linkage analysis are presented in Table 2 and in Figures 1 and 2. We had evidence of replication to several previously reported genome-wide significant linkage regions. Genomewide evidence suggestive of linkage to hyperopia was found on 3q23 (empiric $P = 5.34 \times 10^{-4}$ at *D3S1763*). Spherical equivalent adjusted for age, education, and nuclear sclerosis provided evidence of linkage to chromosome 22q11 (empiric $P = 4.43 \times 10^{-3}$ at rs737923). There was evidence of linkage to myopia in this same region (empiric

$P = 1.48 \times 10^{-3}$ at rs737923). In addition, in our data, spherical equivalent was linked to 7q36 (empiric $P = 2.32 \times 10^{-3}$ at rs2536007), a region that provided genome-wide evidence suggestive of linkage to 7q36.²¹ A second peak on chromosome 7q15 provided evidence of linkage to refraction ($P = 9.43 \times 10^{-3}$). We first reported evidence of linkage in the region in an earlier microsatellite marker analysis.²⁷

In addition to replication of the above-mentioned peaks, evidence of linkage was also detected in several additional regions. Chromosome 2, region q12 showed an indication of linkage to refraction (empiric $P = 1.06 \times 10^{-3}$). Two regions on chromosome 4 also demonstrated an indication of linkage to refraction in the Beaver Dam population. Adjusted refraction was linked to a region on 4q26 (empiric $P = 1.29 \times 10^{-3}$ near marker rs291079). Both refraction and myopia provided some evidence of linkage to 4q31 (refraction empiric $P = 4.16 \times 10^{-3}$ at marker rs978752, myopia empiric $P = 4.24 \times 10^{-3}$ at marker *D4S1625*). Chromosome 6, region q15, gave evidence of linkage to adjusted refraction (empiric $P = 1.15 \times 10^{-3}$ at marker rs2610715). A region on 12q24 was linked to adjusted refraction (empiric $P = 4.19 \times 10^{-3}$ at marker rs918044). For each of these peaks, we provide the results of the additional trait measurement (refraction, adjusted refraction, and myopia or hyperopia) that provided some evidence of linkage ($P < 0.001$) in Table 2. Consistent results were obtained across quantitative trait parameterizations as shown in Figure 1.

In addition to the 3q23 region for hyperopia and the 22q11 and 4q26 regions for myopia, several additional regions of linkage were detected for these qualitative traits. There was some evidence of linkage to hyperopia at 16q13 (empiric $P =$

TABLE 2. Results of SIBPAL Linkage Analysis

Location (MYP locus)	Trait	Nearest Marker	cM	Nominal <i>P</i>	Empirical <i>P</i>	Previous Linkage Evidence (Population, Trait)
2p25	Myopia	rs1309	21.5	4.32E-04	1.35E-03	
2q12	Refraction adjusted	rs931326	116.3	4.10E-04	2.14E-03	
	Refraction	rs931326	116.3	2.07E-04	1.06E-03	
3q26 (MYP8)	Hyperopia	D3S1763	175.6	8.53E-04	5.34E-04	UK Twins, Quantitative Refraction ²³
4q26	Refraction adjusted	rs291079	128	3.46E-04	2.88E-03	
4q31	Refraction	rs978752	143.5	1.31E-03	4.16E-03	
	Myopia	D4S1625	144.6	1.54E-03	4.24E-03	
6q15	Refraction adjusted	rs2610715	94.8	1.95E-04	1.15E-03	
7p15 (MYP17)	Refraction adjusted	rs1859805	36	8.85E-04	2.66E-03	French and Algerian Families, High Myopia ³⁷
	Refraction	rs1859805	36.1	1.46E-04	9.43E-04	
7q36	Refraction adjusted	rs2536077	164.1	7.19E-04	4.60E-03	French and Algerian Families, High Myopia ²¹
	Refraction	rs2536077	164.1	4.01E-04	2.32E-03	
12q24	Refraction adjusted	rs918044	147.2	4.17E-04	4.19E-03	
16p13	Hyperopia	rs741175	19.2	2.72E-03	2.94E-03	
16q24	Myopia	rs452176	131.7	7.61E-04	1.25E-03	
22q11 (MYP6)	Refraction adjusted	rs737923	9.1	6.28E-04	4.43E-03	US Ashkenazi Jewish Families, Myopia ¹⁹
	Refraction	rs737923	9.1	1.39E-03	8.52E-03	
	Myopia	rs737923	9.1	4.91E-04	1.48E-03

FIGURE 1. SIBPAIR linkage analysis of refraction (red) and adjusted refraction (green). The y-axis is $-\log$ (exact P value). Horizontal lines: thresholds for evidence of replication ($P < 0.01$) and evidence suggestive ($P = 0.0074$) of linkage.³¹

2.94×10^{-3} at rs741175). For myopia, there was some evidence of linkage to regions on 2p25 (empiric $P = 1.35 \times 10^{-3}$ at rs1309) and 16q24 (empiric $P = 1.25 \times 10^{-3}$ at rs452176).

DISCUSSION

Refraction, like most complex traits, is probably influenced by multiple genetic factors. This supposition is supported by the fact that many regions of genetic linkage have been reported for both quantitative refraction and myopia. Given the inherent difficulties in replicating linkage findings when there is locus heterogeneity, it is not surprising that many of these reported linkage regions have not yet been replicated. However, in our current linkage analysis of refraction and refractive errors we were able to replicate several previously reported linkage peaks.

There is considerable debate regarding what constitutes evidence of replication in linkage studies. While statistical criteria, $P < 0.01$, have been established, it is less clear how differences in phenotype definition and chromosomal location of maximum LOD scores across studies should be evaluated. For example, can evidence of linkage in a population-based family study of quantitative refraction replicate a linkage peak identified in families with high myopia? It is well established that refractive errors are etiologically complex. This complexity may arise, in part, from the different mutations within the same gene that lead to differences in the severity of refractive errors and/or interactions between genes and environmental

factors that influence the severity of the phenotype. It is possible that genes involved in the more severe forms of myopia also play a role in the more modest forms of refractive errors. Therefore, we feel that replication of linkage peaks can occur when different phenotype definitions are used. Furthermore, linkage signals are often quite broad (>10 Mb) and the location of the causal gene varies with respect to the maximum LOD score. Therefore, it is unclear how far apart a replication linkage peak can be from the initial linkage signal; however, overlap of the linkage regions is supportive of replication.

Our data support linkage to refractive errors, particularly hyperopia on 3q26. Genomewide significant linkage for refraction to this region was first reported in 506 dizygotic twins enrolled in the Twin Eye study. A maximum LOD of 3.7 was observed at marker *D3S1614* with markers *D3S1279* (168.1 Mb) and *D3S1565* (175.3 Mb) flanking the linkage regions.²³ To examine whether this linkage peak was due to genetic sharing among myopes versus hyperopes, Hammond et al.²³ also conducted qualitative analysis refractive errors in these same families. Although there was little evidence to support linkage to this region in myopes ($\text{LOD} < 1$), there was support for linkage of this region to hyperopia ($\text{LOD} \sim 2.5$). This finding is consistent with ours of genome-wide evidence suggestive of linkage to this region for hyperopia. Our minimum empiric $P = 5.34 \times 10^{-4}$ was observed at marker *D3S1763*, with our linkage region spanning marker rs937478 (159.8 Mb) to rs1920122 (169.5 Mb), which overlaps that of Hammond et al. However, there was only slight evidence of linkage in the

FIGURE 2. SIBPAIR linkage analysis of myopia (blue) and hyperopia (orange). The y-axis is $-\log$ (exact P value). Horizontal lines: denote thresholds for evidence of replication ($P < 0.01$) and evidence suggestive ($P = 0.0074$) of linkage.³¹

region to quantitative refraction, with a minimum empiric $P =$ of 0.036 near marker rs920417. Hammond et al. conducted follow-up linkage and association analysis of the 3q26 locus and found evidence of association for three genes, *MFN1* (179.0 Mb), *SOX2OT* (181.3 Mb) and *PSARL* (183.5 Mb).³⁸ These genes are located downstream of our linkage region. Follow-up studies are needed to determine whether genetic variation in the genes explains the linkage in these regions or additional genes are responsible for the linkage signal on 3q26.

While we had previously reported linkage on 22q to refraction,²⁷ here we demonstrate linkage in this region to myopia as well as refraction, but little evidence of linkage to hyperopia. This finding is consistent with the initial report by Stambolian et al.¹⁹ of genome-wide significant evidence of linkage in this region to myopia, with an LOD (HLOD) of 3.54, at marker *D22S685* (physical position 34.4 Mb) and a linkage region spanning *D22S689* (28.7 Mb) and *D22S445* (37.4 Mb). However, our linkage peak is located adjacent to that reported by them, with a minimum $P = 4.43 \times 10^{-3}$ at rs737923 (19.1 Mb) with the region spanning rs2097596 (17.9 Mb) to rs374225 (20.0 Mb) for myopia and from rs3747026 (18.2 Mb) to rs1476445 (19.6 Mb) for adjusted refraction.¹⁹ There is additional evidence of linkage to refraction in our data at rs5762174 (27.9 Mb, empiric $P = 1.31 \times 10^{-3}$). Given that linkage signals can extend for several megabases and the location of the peak around a causal locus can vary markedly due to genetic heterogeneity and marker information content, these results could represent the same locus or they could be due to multiple loci

on chromosome 22. In addition, the families studied by Stambolian et al. were highly ascertained for multiple myopes in each family, whereas the BDES families were population based. However the consistent linkage signals for myopia and refraction to this region of chromosome 22 strongly indicate that at least one major locus resides in this region.

Evidence suggestive of linkage to high myopia (> -6 D) on 7q36 was first reported by Naiglin et al.²¹ In their analysis of 21 French and 2 Algerian families, they reported a maximum LOD of 2.81 at *D7S550*, with the region of linkage extending from *D7S798* (152.7 Mb) to *D7S2423* (157.4 Mb). Our linkage region for refraction ranged from rs1547958 (150.6 Mb) to rs1389240 (156.0 Mb), overlapping the region that they reported. However, there was no evidence supporting linkage to myopia in this region for our families.

In addition to the region on 7q36, we also had evidence of linkage to 7p15-21. Our previous linkage analysis of refraction in the BDES using microsatellite markers alone provided some evidence of linkage to this region, with the minimum multipoint $P = 2.2 \times 10^{-3}$ at marker *D7S3051*.²⁷ This linkage region spanned markers rs957960 (18.8 Mb) to rs1725074 (27.1 Mb). Ciner et al.²⁴ have reported linkage to refraction adjacent to this region among 96 African American families. In their analysis they observed a maximum LOD of 5.87 with an associated $P = 5 \times 10^{-5}$ with the linkage peak ranging from markers *D7S1808* (27.9 Mb) to *D7S2846* (38.0 Mb), respectively, which is adjacent to our region.

In addition to the linkage loci identified for refraction, myopia, and hyperopia, several genomewide association studies for myopia have recently been conducted. Linkage studies are better suited to detecting rare alleles associated with a strong effect (high odds ratio or high penetrance) of disease and are robust to allelic heterogeneity (multiple mutations in the same gene which result in the same phenotype). Association studies are better suited to detecting lower-penetrance common variation associated with disease. While variation on 5p15, 11q24, 15q14, and 15q25 have been associated with myopia it is not surprising that we did not detect linkage in these regions, given the relative strengths of linkage versus association methods (Verhoeven VJM, et al. *IOVS* 2010;51:ARVO E-Abstract 2972).³⁹⁻⁴¹

Work is ongoing to refine these linkage peaks and to identify the genes underlying these linkage signals, with particular emphasis on the interesting regions on 3q36, 22q11, 7p21, and 7q36.

References

- Lee KE, Klein BE, Klein R, Fine JP. Aggregation of refractive error and 5-year changes in refractive error among families in the Beaver Dam Eye Study. *Arch Ophthalmol*. 2001;119:1679-1685.
- Wang Q, Klein BE, Klein R, Moss SE. Refractive status in the Beaver Dam Eye Study. *Invest Ophthalmol Vis Sci*. 1994;35:4344-4347.
- Wong TY, Foster PJ, Johnson GJ, Seah SK. Education, socioeconomic status, and ocular dimensions in Chinese adults: the Tanjong Pagar Survey. *Br J Ophthalmol*. 2002;86:963-968.
- Angle J, Wissmann DA. Age, reading, and myopia. *Am J Optom Physiol Opt*. 1978;55:302-308.
- Aine E. Refractive errors in a Finnish rural population. *Acta Ophthalmol (Copenh)*. 1984;62:944-954.
- Saw SM, Chan YH, Wong WL, et al. Prevalence and risk factors for refractive errors in the Singapore Malay Eye Survey. *Ophthalmology*. 2008;115:1713-1719.
- Chen CY, Scurrah KJ, Stankovich J, et al. Heritability and shared environment estimates for myopia and associated ocular biometric traits: the Genes in Myopia (GEM) family study. *Hum Genet*. 2007;121:511-520.
- Sorsby A, Sheridan M, Leary GA. Refraction and its components in twins. *Medical Research Council Special Report Series 303*. London, HMSO; 1962.
- Ashton GC. Segregation analysis of ocular refraction and myopia. *Hum Hered*. 1985;35:232-239.
- Klein AP, Duggal P, Lee KE, Klein R, Bailey-Wilson JE, Klein BE. Support for polygenic influences on ocular refractive error. *Invest Ophthalmol Vis Sci*. 2005;46:442-446.
- Bear JC, Richler A, Burke G. Nearwork and familial resemblances in ocular refraction: a population study in Newfoundland. *Clin Genet*. 1981;19:462-472.
- Klein AP, Suktitipat B, Duggal P, et al. Heritability analysis of spherical equivalent, axial length, corneal curvature, and anterior chamber depth in the Beaver Dam Eye Study. *Arch Ophthalmol*. 2009;127:649-655.
- Young TL, Ronan SM, Alvear AB, et al. A second locus for familial high myopia maps to chromosome 12q. *Am J Hum Genet*. 1998;63:1419-1424.
- Young TL, Ronan SM, Drahozal LA, et al. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am J Hum Genet*. 1998;63:109-119.
- Zhang Q, Guo X, Xiao X, Jia X, Li S, Hejtmancik JF. Novel locus for X linked recessive high myopia maps to Xq23-q25 but outside MYP1. *J Med Genet*. 2006;43:e20.
- Nallasamy S, Paluru PC, Devoto M, Wasserman NF, Zhou J, Young TL. Genetic linkage study of high-grade myopia in a Hutterite population from South Dakota. *Mol Vis*. 2007;13:229-236.
- Paluru PC, Nallasamy S, Devoto M, Rappaport EF, Young TL. Identification of a novel locus on 2q for autosomal dominant high-grade myopia. *Invest Ophthalmol Vis Sci*. 2005;46:2300-2307.
- Zhang Q, Guo X, Xiao X, Jia X, Li S, Hejtmancik JF. A new locus for autosomal dominant high myopia maps to 4q22-q27 between D4S1578 and D4S1612. *Mol Vis*. 2005;11:554-560.
- Stambolian D, Ibay G, Reider L, et al. Genomewide linkage scan for myopia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 22q12. *Am J Hum Genet*. 2004;75:448-459.
- Stambolian D, Ciner EB, Reider LC, et al. Genome-wide scan for myopia in the Old Order Amish. *Am J Ophthalmol*. 2005;140:469-476.
- Naiglin L, Gazagne C, Dallongeville F, et al. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J Med Genet*. 2002;39:118-124.
- Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease: linkage to DNA markers on the distal part of Xq. *Clin Genet*. 1990;38:281-286.
- Hammond CJ, Andrew T, Mak YT, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. *Am J Hum Genet*. 2004;75:294-304.
- Ciner E, Wojciechowski R, Ibay G, Bailey-Wilson JE, Stambolian D. Genomewide scan of ocular refraction in African-American families shows significant linkage to chromosome 7p15. *Genet Epidemiol*. 2008;32:454-463.
- Wojciechowski R, Moy C, Ciner E, et al. Genomewide scan in Ashkenazi Jewish families demonstrates evidence of linkage of ocular refraction to a QTL on chromosome 1p36. *Hum Genet*. 2006;119:389-399.
- Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995;11:241-247.
- Klein AP, Duggal P, Lee KE, Klein R, Bailey-Wilson JE, Klein BE. Confirmation of linkage to ocular refraction on chromosome 22q and identification of a novel linkage region on 1q. *Arch Ophthalmol*. 2007;125:80-85.
- Schaid DJ, Guenther JC, Christensen GB, et al. Comparison of microsatellites versus single-nucleotide polymorphisms in a genome linkage screen for prostate cancer-susceptibility loci. *Am J Hum Genet*. 2004;75:948-965.
- Klein AP, Tsai YY, Duggal P, et al. Investigation of altering single-nucleotide polymorphism density on the power to detect trait loci and frequency of false positive in nonparametric linkage analyses of qualitative traits. *BMC Genet*. 2005;6(suppl 1):S20.
- Lee KE, Klein BE, Klein R, Wong TY. Changes in refraction over 10 years in an adult population: the Beaver Dam Eye study. *Invest Ophthalmol Vis Sci*. 2002;43:2566-2571.
- Lee KE, Klein BE, Klein R. Changes in refractive error over a 5-year interval in the Beaver Dam Eye Study. *Invest Ophthalmol Vis Sci*. 1999;40:1645-1649.
- Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. *Ophthalmology*. 1991;98:1310-1315.
- Klein BE, Klein R, Linton KL, Magli YL, Neider MW. Assessment of cataracts from photographs in the Beaver Dam Eye Study. *Ophthalmology*. 1990;97:1428-1433.
- Sun L, Wilder K, McPeck MS. Enhanced pedigree error detection. *Hum Hered*. 2002;54:99-110.
- Duffy DL. *SIB-PAIR: A Program of Elementary Genetical Analysis*. Herston, QLD, Australia: Queensland Institute of Medical Research; 2002.
- S.A.G.E. *Statistical Analysis for Genetic Epidemiology*. Cork, Ireland: Statistical Solutions Ltd; 2002.
- Paget S, Julia S, Vitezica ZG, Soler V, Malecaze F, Calvas P. Linkage analysis of high myopia susceptibility locus in 26 families. *Mol Vis*. 2008;14:2566-2574.
- Andrew T, Maniatis N, Carbonaro F, et al. Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. *PLoS Genet*. 2008;4:e1000220.
- Li YJ, Goh L, Khor CC, et al. Genome-wide association studies reveal genetic variants in CTNND2 for high myopia in Singapore Chinese. *Ophthalmology*. 2011;118:368-375.
- Hysi PG, Young TL, Mackey DA, et al. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. *Nat Genet*. 2010;42:902-905.
- Solouki AM, Verhoeven VJ, van Duijn CM, et al. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nat Genet*. 2010;42:897-901.