Published in final edited form as:

Genet Epidemiol. 2008 July ; 32(5): 454–463. doi:10.1002/gepi.20318.

Genomewide Scan of Ocular Refraction in African-American Families Shows Significant Linkage to Chromosome 7p15

Elise Ciner^{1,‡}, Robert Wojciechowski^{2,3,‡,*}, Grace Ibay², Joan E. Bailey-Wilson², and Dwight Stambolian^{4,5}

¹The Eye Institute of the Pennsylvania College of Optometry, Philadelphia, Pennsylvania

²Inherited Disease Research Branch, National Human Genome Research Institute, NIH, Baltimore, Maryland ³Genetic Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland ⁴Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania ⁵Department of Genetics, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract

Refractive development is influenced by environmental and genetic factors. Genetic studies have identified several regions of linkage to ocular refraction, but none have been carried out in African-derived populations. We performed quantitative trait locus linkage analyses in African-American (AA) families to identify genomic regions responsible for refraction. We recruited 493 AA individuals in 96 families to participate in the Myopia Family Study. Genotyping of 387 microsatellite markers was performed on 398 participants. The mean refraction among genotyped individuals was -2.87 D (SD = 3.58) and myopia of at least 1 D was present in 267 (68%) participants. Multipoint, regression-based, linkage analyses were carried out on a logarithmic transformation of ocular refraction using the statistical package MERLIN-REGRESS. Empirical significance levels were determined via 4,898 whole-genome gene-dropping simulations. Linkage analyses were repeated after clustering families into two subgroups based on admixture proportions as determined by the software package STRUCTURE. Genomewide significant linkage was seen at 47 cM on chromosome 7 (logarithm of the odds ratio (LOD) = 5.87, $P = 0.00005$). In addition, three regions on chromosomes 2p, 3p and 10p showed suggestive evidence of linkage (LOD > 2, $P < 0.005$) for ocular refraction. We mapped the first quantitative trait locus for ocular refraction in an AA population to chr.7p15. Two previous studies in European-derived families reported some evidence of linkage to a nearby region, suggesting that this region may contain polymorphisms that mediate refraction across populations. The genomic region under our linkage peak spans ~17 Mb and contains ~170 genes. Further refinement of this region will be pursued in future studies.

Keywords

ocular refraction; myopia; linkage; genetics; African-American

*Correspondence to: Robert Wojciechowski, IDRB/NHGRI, 333 Cassell Dr., Suite 1200, Baltimore, MD 21231, USA. rwojciec@jhsp.edu.

[‡]These authors contributed equally to this research. E.C. aided in the design of the study, recruited families, collected data and contributed to the article. R.W. performed the analyses and wrote the article.

ELECTRONIC DATABASE INFORMATION

Web resources:

Refseq database: www.ncbi.nlm.nih.gov/RefSeq

Online Mendelian Inheritance in Man (OMIM): www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

INTRODUCTION

Clear vision requires images formed by incident light to be focused at the eye's foveal plane. Refractive errors result from a misalignment between the eye's focal point and the retina. In myopia, images of distant objects formed by the eye's optical system focus in front of the retinal plane, causing blurred distance vision. In its functional opposite, hyperopia, these images focus behind the retina (in the unaccommodating eye). Hyperopia is associated with blurred near vision, eye strain and strabismus. Collectively, refractive errors are the most common causes of visual impairment worldwide. Myopia of at least 1 diopter (D) affects about one quarter of all the Western Europeans and Americans aged 40 and above [Kemp et al., 2004]. However, the predisposition to refractive errors can vary widely between ethnicities and geographical regions [Klein et al., 2003; Saw et al., 2006]. Although data for African-Americans (AAs) are sparse, Katz et al. estimated that about one in five Black residents of Baltimore city was affected with clinically significant refractive error [Katz et al., 1997].

While there is a wide consensus that environmental and behavioral factors influence refractive development and myopization, genetic epidemiology studies have consistently shown that the distribution of ocular refractions within populations is largely determined by genetic factors. Several publications reporting high heritability estimates for ocular refraction provide strong evidence for a genetic influence for refraction across populations [Hammond et al., 2001; Lee et al., 2001; Lyhne et al., 2001; Wojciechowski et al., 2005a,b].

Genetic linkage studies have succeeded in identifying several regions in the genome that may harbor genes influencing refractive error. Most of these studies were conducted on a limited number of families with severe forms of myopia [Naiglin et al., 2002; Paluru et al., 2003, 2005; Young et al., 1998a,b; Zhang et al., 2005]. However, few of these results have been replicated by other groups, reflecting the likelihood that genetic heterogeneity may be the rule for high myopia [Farbrother et al., 2004; Ibay et al., 2004; Mutti et al., 2002]. More recently, population-based designs have been used in linkage studies of myopia and ocular refraction [Hammond et al., 2004; Stambolian et al., 2004, 2005; Wojciechowski et al., 2006]. These studies offer the theoretical advantage of identifying genes that may modulate a trait's expression in a more representative sample of families, thereby increasing generalizability. In a study of the American Ashkenazi Jewish families, Stambolian et al. identified a linkage peak for myopia on chromosome 22q12 (HLOD = 3.54) [Stambolian et al., 2004]. These results have recently been supported by an additional sample from the same Ashkenazi population [Stambolian et al., 2006] as well as by a quantitative trait locus (QTL) linkage analysis of the Beaver Dam Eye Study cohort [Klein et al., 2007].

Even though ocular refraction is an inherently continuous metric, most linkage studies have characterized myopia as a dichotomous trait. Binary-trait coding of quantitative phenotypes may cause some loss of information about the trait of interest. By taking the entire distribution of the continuous phenotype into account, QTL linkage methods may be more suitable for identifying regions containing genes that contribute to the whole range of ocular refractions than binary-trait linkage analyses. To date, three genomewide linkage studies have been reported for ocular refraction [Hammond et al., 2004; Klein et al., 2007; Wojciechowski et al., 2006]. In a study of British twin pairs, Hammond et al. [2004] reported evidence for linkage of refraction to four distinct chromosomal regions: 11p (logarithm of the odds ratio (LOD) = 6.1), 3q (LOD = 3.7), 8p (LOD = 4.1) and 4q (LOD = 3.3). The second published study by Wojciechowski et al. [2006] identified a locus in a sample of the Ashkenazi Jewish families on chromosome 1p (LOD = 8.7). More recently, Klein et al. [2007] reported suggestive linkage of refraction to novel QTLs on 1q and 7p.

The current report presents results of a QTL linkage analysis of ocular refraction in AA families ascertained through myopic probands. This is the first linkage study of a refractive phenotype in a population of African descent. We identified a QTL for ocular refraction on chr.7p, which is in the same region of a suggestive linkage signal previously reported in the Beaver Dam Eye Study [Klein et al., 2007].

MATERIALS AND METHODS

ELIGIBILITY CRITERIA AND RECRUITMENT

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the University of Pennsylvania, the Pennsylvania College of Optometry and the National Human Genome Research Institute, National Institutes of Health institutional review boards.

Study participants were primarily recruited at the time of their vision examinations at The Eye Institute of the Pennsylvania College of Optometry. Professional staff provided information and asked myopic patients if they would be willing to speak with someone about the study. Recruitment was also completed through targeted mailings to established myopic patients at The Eye Institute as well as referrals from local optometrists and ophthalmologists.

All participants underwent a comprehensive vision examination including objective refraction. To be eligible for inclusion in the study, families were required to have a minimum of two participating myopic children (≥ 5 years of age), a maximum of one parent with myopia and at least three participating family members. Myopia was defined as a refractive error of at least -1.0 D in each meridian of both eyes. Individuals under age 21 with a refractive error between 0 and -1.0 D were classified as “unknown” and were not included in this study. Also, excluded from the study were individuals who had age-related lens changes or previous cataract surgery, myopia related to prematurity or a history of systemic or ocular disease that may have contributed to refractive error. These included but were not limited to keratoconus, retinitis pigmentosa and connective tissue disorders such as Marfan’s syndrome. Ocular refraction was defined as the spherical equivalent spectacle refraction, averaged between the eyes.

DNA EXTRACTION AND GENOTYPING

Peripheral blood was collected from family members. High molecular weight genomic DNA extraction from the blood samples was performed with a kit (Puregene; Gentra Systems, Inc., Minneapolis, MN, USA). Samples were stored in a DNA repository under a unique code. A genomewide scan was performed at the Center for Inherited Disease Research by the use of automated fluorescent microsatellite analysis. PCR products were sized on an ABI 3700 sequencer. The marker set used was a modification of the Cooperative Human Linkage Center marker set, version 9 (387 markers; average spacing 9 cM; average heterozygosity 0.76). The error rate, which was based on paired genotypes from blind duplicate samples, was 0.06%. The overall missing data rate was 3.6%. All genotyping was performed blind to clinical status.

STATISTICAL METHODS

Error checking—Our genotyping data were subjected to several quality checks to ensure accuracy. Mendelian inconsistencies and potential relationship errors were evaluated and corrected prior to data analysis using SIBPAIR [Duffy, 1997] and the GAS package [Young, 1995]. The accuracy of putative relationships was checked using RelCheck [Boehnke and Cox, 1997; Broman and Weber, 1998]. Mendelian inconsistencies from individuals at

multiple markers that could not be resolved were coded as missing for the purpose of the analysis.

Linkage analysis—Multipoint QTL linkage analyses were conducted using the software package MERLIN, version 1.0.1 [Abecasis et al., 2002]. Multipoint linkage statistics were calculated at marker positions and at two equally spaced intervals between markers. Prior to linkage analysis, refraction data were transformed using a logarithmic transformation to approximate a normal distribution and limit the influence of outliers. The Marshfield clinic database (www.marshfieldclinic.org) was used to determine sex-averaged genetic map locations of the microsatellite markers. Estimates of allele frequencies were determined from the families using a maximum-likelihood algorithm implemented in the MERLIN program.

The MERLIN statistical package implements an extended Haseman-Elston regression-based linkage method through its MERLIN-REGRESS subroutine [Sham et al., 2002]. Given our ascertainment strategy, this analytical method was more appropriate for our study than variance-component models, which can be sensitive to sample selection. The MERLIN-REGRESS method is applicable to extended pedigrees and to pedigrees selected on the basis of trait values, provided correct values for population parameters of the trait distribution are specified [Sham et al., 2002]. For the method to be applicable to ascertained samples, both independent and dependent variables are centered on their population means. Consequently, this regression approach requires the specification of the underlying population mean (μ), variance (V) and heritability (h^2) of the trait.

While the values of μ , V and h^2 for refraction are not known precisely for the underlying population from which the samples were drawn, reasonable estimates of these parameters can be derived from published epidemiological studies of refractive error across ethnic groups [Katz et al., 1997; Kempen et al., 2004; Wojciechowski et al., 2005b]. We therefore specified $\mu = 0$ D (pre-transformation) and $h^2 = 0.6$ in our models, while V was set to the sample variance after log transformation. We also conducted sensitivity analyses of the LOD scores by individually varying these model parameters within plausible bounds to $\mu = +1$ and -1 ; and $h^2 = 0.5$ and 0.7 , as well as halving and doubling the trait's standard deviation (i.e., multiplying V by $\frac{1}{4}$ and 4).

Simulations and empirical significance levels—The MERLIN-REGRESS procedure provides locus-specific, nominal LOD scores and significance levels based on the χ^2 distribution and asymptotic theory [Sham et al., 2002]. We previously reported that the LOD scores estimated by MERLIN-REGRESS tend to be inflated relative to expectations, yielding anti-conservative asymptotic P -values [Wojciechowski et al., 2006]. Empirical significance levels can, however, be assessed through a gene-dropping simulation procedure implemented in the MERLIN software package [Abecasis et al., 2002]. Briefly, MERLIN generates random data sets of markers in founders while preserving family structure, population marker allele frequencies, marker spacing, the pattern of missing genotype data and individual phenotypic information [Terwilliger et al., 1993] (www.sph.umich.edu/csg/abecasis/Merlin/reference/simulation.html). Markers are then dropped through the pedigrees, by following Mendelian segregation rules, and allowing for recombination events using the same genetic map as specified in the original data. Since the markers are generated and transmitted independently from trait values, these simulations yield empirical distributions of linkage statistics under the null hypothesis of no linkage, given sample-specific pedigree structures, phenotypic distribution and marker information. The simulation procedure offers the possibility to assess both locus-specific (i.e., pointwise) and genomewide significance levels in a given sample.

We performed 4,898 whole-genome simulations for each model used. The maximum LOD score was then determined for each replicate across all alternate parameter specifications, and the empirical genomewide P -value was estimated as the proportion of simulated data sets in which the maximum LOD score was equal to or exceeded the one obtained using the original data set. Locus-specific empirical P -values were estimated by comparing pointwise LOD scores in the original data set with the distribution of all LOD scores from our simulations. This procedure yielded ~5.44 million LOD scores (i.e., 4,898 replicates $\times$ 1,111 chromosomal locations) under the null distribution. By using all locations in our simulations to estimate locus-specific P -values, we assumed that the distribution of LOD scores under the hypothesis of no linkage was identical across loci. Our simulations showed that empirical P -values obtained by using all LOD scores are equivalent to P -values determined independently for each locus (data not shown). However, by pooling simulation results from all locations, greater precision is obtained at the tail end of the distribution (i.e., for small P -values). Due to the substantial computational and data-management burden of conducting extensive simulation studies, we limited the number of simulations to 4,898 whole-genome replicates, which necessitated more than 3 months of processing time on the NHGRI Beowulf computing cluster equipped with 2.4 Ghz AMD Opteron 250 dual processors.

Replication regions—We evaluated 13 regions that have shown evidence of linkage to refraction (at a pointwise $P < 0.005$) in three previous studies [Hammond et al., 2004; Klein et al., 2007; Wojciechowski et al., 2006]. Of these, five loci showed significant statistical evidence of linkage to ocular refraction according to criteria proposed by Lander and Kruglyak [1995] (i.e., genomewide $P < 0.05$). The other eight regions showed suggestive evidence of linkage but did not meet the criteria for genomewide statistical significance [Lander and Kruglyak, 1995]. We defined “replication” regions as the areas that spanned 20 cM on either side of the center of these reported linkage peaks. When these linkage peaks were within 20 cM of the chromosome telomeres, the replication regions extended from 20 cM centromeric to the peak to the chromosomal terminus. Two loci on chr.1 reported by Klein et al. [2007] were 41 cM apart and were combined into one replication region. We then determined the maximum LOD score within each of these replication regions. To assess the statistical evidence for replication within these regions, we compared the magnitude of the region-specific maximum LOD scores to the distribution of the maxima in the same regions in our simulations. Empirical P -values for replication were computed in the same manner as genomewide P -values with the unit of observation being the replication region instead of the entire genome. It is perhaps more typical in linkage studies to determine the minimum empirical pointwise P -value in such a replication region and declare that replication has been achieved if any pointwise P -value is less than or equal to 0.01. However, we feel that this is too liberal, given the large number of tests being performed in each replication region. We believe that our more conservative method of determining the empirical P -value of the maximum LOD score within the region is more appropriate. To limit the number of comparisons, we confined our replication analyses to putative ocular refraction QTLs [Hammond et al., 2004; Klein et al., 2007; Wojciechowski et al., 2006] and omitted loci previously shown to be linked to the qualitative traits myopia or hyperopia. Technically, these analyses represent “replication” only for the five loci that exhibited genome-wide significant in the original studies. However, we also present the results for the loci with previously “suggestive” evidence for linkage to determine whether our study shows any support for linkage in these regions.

Subgroup analysis based on admixture proportions—AAs represent an admixed group primarily descended from the West African and, to a lesser degree, the European ancestral populations [Parra et al., 1998, 2001]. Because genetic causes of complex traits are often population specific, we repeated the linkage analyses after clustering the families into

two groups based on genetically determined ancestry using the software program STRUCTURE [Falush et al., 2003; Pritchard et al., 2000]. Allele frequencies were estimated separately, using the maximum-likelihood method, in each sub-group prior to stratified linkage analysis. STRUCTURE utilizes available marker data and a Bayesian clustering algorithm to infer individual membership to a pre-specified number of genetic ancestry clusters (k). All autosomal markers from the linkage panel were used in the STRUCTURE clustering. Although we could not directly estimate proportions of the European/African ancestry in our sample without knowing the marker distribution of these ancestral populations, it is likely that families would cluster on the basis of their admixture proportions (in the absence of any cryptic population substructure). Our use of non-independent family members violated the assumption of independence implicit in the clustering method implemented in STRUCTURE. Nonetheless, our primary goal was to reduce sample heterogeneity by clustering families into more homogeneous groups based on their ancestral origins, thereby reducing the likelihood of confounding by ethnicity [Thompson et al., 2006]. Clustering families into distinct subpopulations based on marker information is therefore a reasonable approach.

RESULTS

Our AA sample consisted of 493 individuals (of which 398 were genotyped) in 96 families (Table I). The mean spherical equivalent refractive error in the sample was -2.87 D (SD = 3.58), and 267 (68%) of the genotyped individuals had myopia of at least 1 D.

A maximum LOD score of 5.87 (empirical pointwise $P = 0.000065$) was found at 47 cM on chr.7 between markers *D7S1808* and *D7S817* (Table II, Fig. 1). Three additional linkage peaks were seen at 64 cM on chr.2 (LOD = 2.08, empirical pointwise $P = 0.0048$), 39 cM on chr.3 (LOD = 2.12, empirical pointwise $P = 0.0045$), and 67 cM on chr.10 (LOD = 2.06, empirical pointwise $P = 0.0049$). Only the chr.7 linkage signal reached genome-wide statistical significance at the $P < 0.05$ level (empirical genomewide $P = 0.026$).

SENSITIVITY ANALYSES

We explored the effect of altering population parameter values on our linkage statistics by repeating the analyses assuming different values of μ , V and h^2 in the statistical models. Varying h^2 between 0.5 and 0.8 did not have an appreciable effect on the maximum LOD score or its associated empirical genomewide P -value (Table III). Increasing the assumed population variance (V) by a factor of 4 (i.e., doubling the assumed population standard deviation) decreased the maximum LOD score compared to our initial statistical model, as did varying μ from 0 D to either +1 D or -1 D. However, all of these models resulted in genomewide empirical P -values that were statistically significant or approached statistical significance at $P \leq 0.05$. Hence, our results are robust to alternate parameter specifications within reasonable population ranges for ocular refraction.

REPLICATION REGIONS

Results of our replication analyses are presented in Table IV. The MYP9 locus on chr.4 was originally reported to show genome-wide significant linkage with refraction by Hammond et al. [2004]. We found some evidence for replication of linkage to this region in our data (replication $P = 0.065$). However, this area did not reach the $P < 0.01$ criterion recommended by Lander and Kruglyak [1995] for declaring definitive replication. We also did not find significant evidence of replication of linkage to any of the other four loci that were found to have significant linkage in other studies of refractive error.

For the previously published loci that had “suggestive” evidence of linkage, our chromosome 7 linkage peak was within 20 cM of an area of suggestive linkage (multipoint empirical $P = 0.0006$ at *D7S3051*) reported by Klein et al. [2007] (empirical replication $P = 0.00002$). In addition, we had some evidence supporting a putative refraction QTL on chromosome 2 (replication $P = 0.022$) which was initially reported to have suggestive evidence of linkage in the Ashkenazi Jewish families [Wojciechowski et al., 2006].

SUBGROUP ANALYSIS BASED ON ADMIXTURE PROPORTIONS

Using the program STRUCTURE [Falush et al., 2003; Pritchard et al., 2000], two distinct clusters of families were found using $k = 2$ and an estimated admixture proportion of 80%. Forty-two genotyped individuals in 6 families clustered in one group, with each individual deriving >80% (mean = 94%) of his/her genome from the putative subpopulation 1. The remaining 356 (90 families) clustered to the other group. Each individual in this group was estimated to derive at least 70% (mean = 91.5%) of his/her genome from putative ancestral population 2. These subgroups did not differ significantly with respect to their mean refractions ($t = 0.14$, $df = 396$; $P = 0.89$) or sample variances ($F = 0.96$, $df = (34, 362)$; $P = 0.93$). After conducting linkage analyses separately in both clusters, each subset of families still showed evidence of linkage to the chr.7 QTL (LOD = 3.017 at 50 cM for 6 families in cluster 1; LOD = 2.805 at 45 cM for 90 families in cluster 2), although the peak heights were reduced compared to that of the combined sample (Fig. 2).

DISCUSSION

We conducted regression-based, multipoint, linkage analyses of ocular refraction in an American population of African descent. We found statistically significant evidence of linkage of ocular refraction to a QTL on chromosome 7p15. Our maximum linkage signal (LOD = 5.87) was observed at map position 47 cM, and the region of LOD scores exceeding 2 extended from 39 to 62 cM.

Suggestive linkage evidence of ocular refraction to a nearby region has been reported by Klein et al. in an analysis of the Beaver Dam Eye Study cohort [Klein et al., 2007]. Although the linkage peaks from the current analysis and the Beaver Dam study are separated by approximately 18 cM, there is substantial overlap in the linkage profiles between studies. The Klein et al. study localized this locus to chr.7p21 (multipoint empirical $P = 0.0006$ at ~29 cM) [Klein et al., 2007], while our linkage region is centered at 7p14–15 (~47 cM). Moreover, the simulation-based empirical P -value for replication of this region was highly significant (replication region $P = 0.00002$). Although our choice of a 40 cM interval centered on previously reported linkage peaks as a basis for replication was rather arbitrary, alternative interval specifications (of ± 15 and 25 cM) yielded similarly significant results (data not shown).

Our linkage findings at chr.7p are further supported by a study of familial high myopia. In a 2005 published abstract, Paget et al. [2005] reported significant linkage in 12 of 50 French families to a ~10 cM region bounded by markers *D7S507* and *D7S529* (multipoint parametric LOD = 4.02). The maximum two-point LOD score was 2.80 at marker *D7S673* ($\theta = 0$). This would localize the putative disease locus to 7p15.3 between 28 and 40 cM (using the Marshfield linkage map), which is approximately 10 cM from our maximum linkage signal at 7p15.1-2. These results should, however, be considered preliminary until published in a peer-reviewed journal. Nevertheless, these studies in independent samples provide strong evidence that this region harbors a gene that influences refractive development across ethnic groups. Moreover, this locus has been identified in samples with varying degrees of refractive error. The Klein et al. [2007] cohort was randomly sampled from the Beaver Dam, WI, adult population, whereas the French study ascertained families

containing at least two members with high myopia [Paget et al., 2005]. In the current study, families were selected to segregate individuals with mild and moderate levels of myopia (i.e., ≤ -1.0 D). Hence, this putative locus may be responsible for modulating refraction across a wide range of phenotypes.

Previous analyses have yielded evidence of linkage to a number of different loci, whether refractive errors were defined as qualitative (i.e., myopia or hyperopia) [Lam et al., 2003; Naiglin et al., 2002; Paluru et al., 2003, 2005; Stambolian et al., 2004, 2005, 2006; Sundin et al., 2005; Young et al., 1998a,b; Zhang et al., 2005; Paget et al., 2005] or continuous (i.e., ocular refraction) traits [Hammond et al., 2004; Klein et al., 2007; Wojciechowski et al., 2006]. However, few of the linkage regions have been replicated in independent samples [Farbrother et al., 2004; Ibay et al., 2004; Mutti et al., 2002]. These inconsistent results suggest that numerous loci may be involved in mediating refractive development in humans. Most binary-trait linkage studies of non-syndromic refractive errors considered only pathological, highly penetrant, forms of myopia or hyperopia. It is likely that families exhibiting severe phenotypes carry rare or private mutations that may not be at play in more prevalent, milder, forms of the condition. In addition, previous linkage studies of severe, non-syndromic, myopia used families ascertained on the basis of a strong autosomal dominant inheritance pattern. Although numerous studies have shown high heritability for ocular refraction, myopia does not generally follow a simple Mendelian mode of transmission [Klein et al., 2005]. It is thus improbable that the same genetic variants that confer susceptibility to pathological myopia in highly aggregated families would also be responsible for a large proportion of the phenotypic variation within the population at large. Our families, although ascertained for multiple affected siblings, and containing few genotyped non-myopic members, were not significantly enriched for severe myopia.

The chr.7 linkage region in the current study was originally reported in studies of the European-derived populations [Klein et al., 2007; Paget et al., 2005]. This raises the possibility that variants within a common locus may influence the distribution of refraction across populations and ethnic groups. It should be noted, however, that previous genomewide QTL analyses of the Ashkenazi Jewish [Wojciechowski et al., 2006] families and a sample of the British twins [Hammond et al., 2004] did not show evidence for linkage of refraction to this chromosomal region. Hence, genes in this region may not have risk alleles that are of high enough frequency to play a major role in variation of refractive development in all human populations.

Population-genetic studies have estimated the proportion of the European admixture in the AA community to be roughly 15–20% (although significant regional differences in admixture proportions also exist) [Parra et al., 1998, 2001]. Parra et al. estimated the proportion of European admixture among AAs residing in Philadelphia to be 13% [Parra et al., 1998]. Given its proximity to a QTL identified in the Beaver Dam Eye Study [Klein et al., 2007] sample, our linkage signal might have been confined to families with high European admixture in which the causative polymorphism was transmitted through a Caucasian lineage. Our stratified analysis showed that the chr.7 linkage signal was maintained, although attenuated, in both sets of families clustered on the basis of admixture proportions. Thus, we found no evidence of confounding by degree of admixture at this locus. This supports the assertion that this locus is not population specific and that the linkage signal is not solely the result of population stratification due to the Caucasian admixture.

In addition to the chr.7 locus, we identified three secondary linkage signals (Fig. 1, Table II), although none of these approached genomewide significance at the $P < 0.05$ level. These regions included 2p22-21 (LOD = 2.08, empirical pointwise $P = 0.0048$), 3p25-24 (LOD =

2.12, empirical pointwise $P = 0.0045$) and 10p21-11 (LOD = 2.06, empirical pointwise $P = 0.0049$). The chr.2 peak at 64 cM is adjacent to an area that showed suggestive evidence of linkage to ocular refraction (LOD = 1.70, $P = 0.003$ at 55 cM) in an Ashkenazi Jewish sample [Wojciechowski et al., 2006]. Interestingly, in a large population-based linkage study of the Sardinian families, Biino et al. [2005] found suggestive evidence of linkage of ocular axial length to 2p24 (41 cM). This raises the possibility that a gene at or near this location may mediate refractive error through its effect on eye length. Although this chromosomal region has shown some evidence for linkage to refraction-related phenotypes in three separate studies, conclusions as to its role in refractive variation must be tempered. Specifically, none of these studies individually produced a LOD score at this locus that met genomewide statistical significance criteria.

CONCLUSION

In the first linkage study of ocular refraction in an AA population, we found genomewide significant evidence that a QTL for ocular refraction maps to chr.7p15. This linkage evidence is supported by previous findings in two separate studies of refraction phenotypes in European-derived families [Klein et al., 2007; Paget et al., 2005]. The chromosome 7 linkage region in the current study is bounded, approximately, by markers *D7S1802* (33 cM, 20.5 Mb) and *D7S2846* (57 cM, 37.9 Mb). This area spans roughly 17.5 Mb and contains about 170 known and predicted genes as referenced in the Refseq database. Sixty-two genes within this region are referenced in the Online Mendelian Inheritance in Man database as being implicated in human genetic disorders or quantitative trait variation. To our knowledge, none of the genes within our linkage region have been directly implicated in either syndromic or non-syndromic forms of myopia or hyperopia. This region merits further scrutiny to investigate plausible candidate genes for myopia and/or refractive error control.

Acknowledgments

This study was supported in part by U.S. Public Health National Eye Institute grant EY12226 (D.S.) and in part by funds from the intramural program of the National Human Genome Research Institute, NIH (J.E.B.-W., G.I., and R.W.). R.W. also received a William C. Ezell-CIBA Vision Fellowship from the American Optometric Foundation. Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to the Johns Hopkins University (contract number N01-HG-65403). The authors thank the families for their participation in the study. We are also grateful to the staff of The Eye Institute of the Pennsylvania College of Optometry and local optometrists and ophthalmologists for their aid in participant recruitment.

REFERENCES

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet.* 2002; 30:97–101. [PubMed: 11731797]
- Biino G, Palmas MA, Corona C, Prodi D, Fanciulli M, Sulis R, Serra A, Fossarello M, Pirastu M. Ocular refraction: heritability and genome-wide search for eye morphometry traits in an isolated Sardinian population. *Hum Genet.* 2005; 116:152–159. [PubMed: 15611866]
- Boehnke M, Cox NJ. Accurate inference of relationships in sibpair linkage studies. *Am J Hum Genet.* 1997; 61:423–429. [PubMed: 9311748]
- Broman KW, Weber JL. Estimation of pairwise relationships in the presence of genotyping errors. *Am J Hum Genet.* 1998; 63:1563–1564. [PubMed: 9792888]
- Duffy DL. Sibpair: a program for nonparametric linkage/association analysis. *Am J Hum Genet.* 1997; 61 Suppl:A197.
- Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics.* 2003; 164:1567–1587. [PubMed: 12930761]

- Farbrother JE, Kirov G, Owen MJ, Pong-Wong R, Haley CS, Guggenheim JA. Linkage analysis of the genetic loci for high myopia on 18p, 12q, and 17q in 51 U.K. families. *Invest Ophthalmol Vis Sci*. 2004; 45:2879–2885. [PubMed: 15326098]
- Hammond CJ, Andrew T, Tat MY, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genome-wide scan of dizygotic twins. *Am J Hum Genet*. 2004; 75:294–304. [PubMed: 15307048]
- Hammond CJ, Snieder H, Gilbert CE, Spector TD. Genes and environment in refractive error: the twin eye study. *Invest Ophthalmol Vis Sci*. 2001; 42:1232–1236. [PubMed: 11328732]
- Ibay G, Doan B, Reider L, Dana D, Schlifka M, Hu H, Holmes T, O'Neill J, Owens R, Ciner E, Bailey-Wilson JE, Stambolian D. Candidate high myopia loci on chromosomes 18p and 12q do not play a major role in susceptibility to common myopia. *BMC Med Genet*. 2004; 5:20. [PubMed: 15291966]
- Katz J, Tielsch JM, Sommer A. Prevalence and risk factors for refractive errors in an adult inner city population. *Invest Ophthalmol Vis Sci*. 1997; 38:334–340. [PubMed: 9040465]
- Kempen JH, Mitchell P, Lee KE, Tielsch JM, Broman AT, Taylor HR, Ikram MK, Congdon NG, O'Colmain BJ. The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Arch Ophthalmol*. 2004; 122:495–505. [PubMed: 15078666]
- 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. [PubMed: 15671267]
- 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. [PubMed: 17210856]
- Kleinstei RN, Jones LA, Hullett S, Kwon S, Lee RJ, Friedman NE, Manny RE, Mutti DO, Yu JA, Zadnik K. Refractive error and ethnicity in children. *Arch Ophthalmol*. 2003; 121:1141–1147. [PubMed: 12912692]
- Lam DS, Tam PO, Fan DS, Baum L, Leung YF, Pang CP. Familial high myopia linkage to chromosome 18p. *Ophthalmologica*. 2003; 217:115–118. [PubMed: 12592049]
- Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995; 11:241–247. [PubMed: 7581446]
- 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. [PubMed: 11709020]
- Lyhne N, Sjolie AK, Kyvik KO, Green A. The importance of genes and environment for ocular refraction and its determiners: a population based study among 20–45 year old twins. *Br J Ophthalmol*. 2001; 85:1470–1476. [PubMed: 11734523]
- Mutti DO, Semina E, Marazita M, Cooper M, Murray JC, Zadnik K. Genetic loci for pathological myopia are not associated with juvenile myopia. *Am J Med Genet*. 2002; 112:355–360. [PubMed: 12376937]
- Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idider A, Rascol O, Malecaze F, Calvas P. A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. *J Med Genet*. 2002; 39:118–124. [PubMed: 11836361]
- Paget, S.; Julia, S.; Levy, D.; Mendes, F.; Mathis, A.; Malecaze, F.; Calvas, P. A new susceptibility locus of familial high myopia on chromosome 7p15.3. ARVO Annual Meeting; 1–5 May 2005; Fort Lauderdale. 2005. ARVO abstracts
- Paluru P, Ronan SM, Heon E, Devoto M, Wildenberg SC, Scavell G, Holleschau A, Makitie O, Cole WG, King RA, Young TL. New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. *Invest Ophthalmol Vis Sci*. 2003; 44:1830–1836. [PubMed: 12714612]
- 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. [PubMed: 15980214]
- Para EJ, Kittles RA, Argyropoulos G, Pfaff CL, Hiester K, Bonilla C, Sylvester N, Parrish-Gause D, Garvey WT, Jin L, McKeigue PM, Kamboh MI, Ferrell RE, Pollitzer WS, Shriver MD. Ancestral proportions and admixture dynamics in geographically defined African Americans living in South Carolina. *Am J Phys Anthropol*. 2001; 114:18–29. [PubMed: 11150049]

- Parra EJ, Marcini A, Akey J, Martinson J, Batzer MA, Cooper R, Forrester T, Allison DB, Deka R, Ferrell RE, Shriver MD. Estimating African American admixture proportions by use of population-specific alleles. *Am J Hum Genet.* 1998; 63:1839–1851. [PubMed: 9837836]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics.* 2000; 155:945–959. [PubMed: 10835412]
- Saw SM, Goh PP, Cheng A, Shankar A, Tan DT, Ellwein LB. Ethnicity-specific prevalences of refractive errors vary in Asian children in neighbouring Malaysia and Singapore. *Br J Ophthalmol.* 2006; 90:1230–1235. [PubMed: 16809384]
- Sham PC, Purcell S, Cherny SS, Abecasis GR. Powerful regression-based quantitative-trait linkage analysis of general pedigrees. *Am J Hum Genet.* 2002; 71:238–253. [PubMed: 12111667]
- Stambolian D, Ciner EB, Reider LC, Moy C, Dana D, Owens R, Schlifka M, Holmes T, Ibay G, Bailey-Wilson JE. Genome-wide scan for myopia in the Old Order Amish. *Am J Ophthalmol.* 2005; 140:469–476. [PubMed: 16084785]
- Stambolian D, Ibay G, Reider L, Dana D, Moy C, Schlifka M, Holmes T, Ciner E, Bailey-Wilson JE. 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. [PubMed: 15273935]
- Stambolian D, Ibay G, Reider L, Dana D, Moy C, Schlifka M, Holmes TN, Ciner E, Bailey-Wilson JE. Genome-wide scan of additional Jewish families confirms linkage of a myopia susceptibility locus to chromosome 22q12. *Mol Vis.* 2006; 12:1499–1505. [PubMed: 17167407]
- Sundin OH, Leppert GS, Silva ED, Yang JM, Dharmaraj S, Maumenee IH, Santos LC, Parsa CF, Traboulsi EI, Broman KW, Dibernardo C, Sunness JS, Toy J, Weinberg EM. Extreme hyperopia is the result of null mutations in MFRP, which encodes a Frizzled-related protein. *Proc Natl Acad Sci USA.* 2005; 102:9553–9558. [PubMed: 15976030]
- Terwilliger JD, Speer M, Ott J. Chromosome-based method for rapid computer simulation in human genetic linkage analysis. *Genet Epidemiol.* 1993; 10:217–224. [PubMed: 8224802]
- Thompson CL, Rybicki BA, Iannuzzi MC, Elston RC, Iyengar SK, Gray-McGuire C. Reduction of sample heterogeneity through use of population substructure: an example from a population of African American families with sarcoidosis. *Am J Hum Genet.* 2006; 79:606–613. [PubMed: 16960797]
- Wojciechowski R, Congdon N, Bowie H, Munoz B, Gilbert D, West S. Familial aggregation of hyperopia in an elderly population of siblings in Salisbury, Maryland. *Ophthalmology.* 2005a; 112:78–83. [PubMed: 15629824]
- Wojciechowski R, Congdon N, Bowie H, Munoz B, Gilbert D, West SK. Heritability of refractive error and familial aggregation of myopia in an elderly American population. *Invest Ophthalmol Vis Sci.* 2005b; 46:1588–1592. [PubMed: 15851555]
- Wojciechowski R, Moy C, Ciner E, Ibay G, Reider L, Bailey-Wilson JE, Stambolian D. 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. [PubMed: 16501916]
- Young A. GAS (Genetic Analysis System), (Version 2.0). Oxford, UK: 1995.
- Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD, Wilkin DJ, King RA. A second locus for familial high myopia maps to chromosome 12q. *Am J Hum Genet.* 1998a; 63:1419–1424. [PubMed: 9792869]
- Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS, Atwood LD, Wilkin DJ, King RA. Evidence that a locus for familial high myopia maps to chromosome 18p. *Am J Hum Genet.* 1998b; 63:109–119. [PubMed: 9634508]
- 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. [PubMed: 16052171]

Fig. 1.

Quantitative trait locus (QTL) linkage statistics for refractive error in an African-American families. Top panel: multipoint MERLIN-REGRESS LOD scores. The horizontal dashed line represents the threshold for genomewide significance ($\text{LOD} = 5.2$) determined through gene-dropping simulations. The maximum LOD score was 5.87 (empirical pointwise $P = 0.00005$, genomewide $P = 0.026$) at 47 cM on Chr.7, between markers *D7S1808* and *D7S817*. Bottom panel: empirical pointwise $-\log_{10} P$ -values corresponding to LOD scores in top panel. Horizontal dashed lines show pointwise P -value thresholds of 0.01, 0.001 and 0.0001. Vertical dashed lines show boundaries between chromosomes.

Fig. 2.

Subpopulation-specific QTL LOD scores on chromosome 7 in the African-American population. The short dashes represent cluster 1 (6 families). Long dashes show cluster 2 (90 families). The vertical line shows the location of the peak LOD score for the combined analysis. The maximum LOD score was 3.017 at 50 cM for cluster 1 and 2.805 at 45 cM for cluster 2.

TABLE I

Sample characteristics

No. of families (no. of individuals)	96
No. of individuals	493
No. of genotyped individuals	398
No. of myopic	267
Average age, in years, of examined individuals (SD)	40.4 (19)
Male: female	206:287
Average no. of individuals per family (range)	5.1 (4–16)
Mean spherical equivalent refraction (SD)	−2.87 (3.58)

TABLE II
QTL linkage LOD scores for log-transformed refraction in an African-American population

Chr	Marker	Pos. (cM)	Cytoband	Physical location (Mb)	LOD	Nominal pointwise P^*	Empirical pointwise P^{**}	Empirical genome-wide P^a
2	D2S1788	56	2p22.3	36.17	1.38	0.006	0.015	
	—	58			1.73	0.002	0.0084	
	—	61			1.99	0.0012	0.0057	
	D2S1356	64	2p21	43.29	2.08	0.001	0.0050	0.72
	—	67			1.81	0.002	0.0074	
	D3S1259	37	3p25.2	12.07	1.54	0.004	0.011	
3	—	39			2.12	0.0009	0.0047	0.71
	—	42			2.08	0.001	0.0049	
	D3S3038	45	3p24.3	21.92	1.15	0.011	0.022	
7	D7S1802	33	7p15.3	20.48	1.45	0.005	0.013	
	—	36			2.13	0.0009	0.0046	
	—	39			2.67	0.0002	0.0022	
	D7S1808	42	7p15.2	27.81	2.65	0.0002	0.0023	
	—	45			5.22	<0.000001	0.0001	0.026
	—	47			5.87	<0.000001	0.00005	
	D7S817	50	7p15.1	31.91	4.44	<0.000001	0.00027	
	—	53			4.14	0.00001	0.00038	
	—	55			3.37	0.00004	0.00093	
	D7S2846	58	7p14.1	37.91	2.48	0.0004	0.0028	
	—	62			2.49	0.0004	0.0028	
	—	66			1.78	0.002	0.0077	
10	—	62			1.77	0.002	0.0078	
	D10S1426	63	10p11.22	30.54	1.94	0.0014	0.0061	
	—	67			2.06	0.001	0.0051	0.73
	—	71			1.70	0.003	0.0087	
	D10S1227	76	10q21.1	37.20	1.15	0.011	0.022	
	—	77			1.17	0.01	0.021	

Regions containing LOD scores above 2.0. Dashes in the marker column represent inter-marker regions.

* Nominal, pointwise, P -values were output by MERLIN-REGRESS and are based on the χ^2 distribution and asymptotic theory.

** Empirical P -values were estimated via whole-genome gene-dropping simulations of unlinked markers using the simulate procedure in MERLIN-REGRESS.

α Genomewide significance levels for the maximum LOD score represents the proportion of simulated replicates in which the maximum LOD score exceeded the high LOD score of 5.87. Genomewide significance levels of secondary peaks were calculated as the proportion of replicates that contained one or more LOD scores greater than the regional peak LOD score.

TABLE III

Effect of alternate parameter specification on QTL linkage statistics

Population parameter	Max LOD	Empirical genomewide <i>P</i>
Original model ^a	5.87	0.026
$\mu = +1$ D	4.53	0.053
$\mu = -1$ D	4.76	0.081
$V = 1/4 \times V_0$	6.01	0.052
$V = 4 \times V_0$	4.4	0.05
$h^2 = 0.5$	5.37	0.031
$h^2 = 0.7$	6.1	0.031

Parameters for the trait distribution in the underlying population were μ , mean; V , variance and h^2 , heritability. Alternate values of μ are on the diopter (D) scale before log transformation.

^aThe original statistical model for trait parameters had $\mu = 0$ D, $V_0 = 0.141$ and $h^2 = 0.6$.

TABLE IV

Replication region^a linkage LOD scores

Author (year)	Replication region				African-American population replication results		
	Chr.	Location (cM)	OMIM locus ID	Replication region (cM)	Max LOD	Location (cM)	Empirical <i>P</i>
Hammond et al. (2004)	3	185	MYP8	165–205	0.35	204	0.28
	4	65	MYP9	45–85	1.19	82	0.065
	8	0	MYP10	0–20	0.03	18	0.5
	11	40	MYP7	20–60	0	—	1
Wojciechowski et al. (2006)	11	125	—	105–145	0	—	1
	1	49	MYP14	29–69	0.61	57	0.18
	2	50	—	30–70	2.08	64	0.022
	1 ^b	192	—	172–253	0.02	175	0.95
Klein et al. (2007)	1 ^b	233	—	172–253	0.02	175	0.95
	7	29	—	82–122	0	—	1
	16	81	—	61–101	0.15	64	0.43
	22	19	—	0–39	0.26	29	0.33

^aReplication regions included loci that showed statistical evidence for linkage to refraction in three previous reports. These included five loci that showed genomewide significance (named and indexed by OMIM) and eight that showed suggestive evidence of linkage. Regions in bold showed genomewide statistical significance in the original study.

^bCombined into one replication region spanning from 172 to 253 cM on chr.1.