Am. J. Hum. Genet. 75:448–459, 2004

Genomewide Linkage Scan for Myopia Susceptibility Loci among Ashkenazi Jewish Families Shows Evidence of Linkage on Chromosome 22q12

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Mild/moderate (common) myopia is a very common disorder, with both genetic and environmental influences. The environmental factors are related to near work and can be measured. There are no known genetic loci for common myopia. Our goal is to find evidence for a myopia susceptibility gene causing common myopia. Cycloplegic and manifest refraction were performed on 44 large American families of Ashkenazi Jewish descent, each with at least two affected siblings. Individuals with at least −1.00 diopter or lower in each meridian of both eyes were classified as myopic. Microsatellite genotyping with 387 markers was performed by the Center for Inherited Disease Research. Linkage analyses were conducted with parametric and nonparametric methods by use of 12 different penetrance models. The family-based association test was used for an association scan. A maximum multipoint parametric heterogeneity LOD (HLOD) score of 3.54 was observed at marker D22S685, and nonparametric linkage analyses gave consistent results, with a *P* value of .0002 at this marker. The parametric multipoint HLOD scores exceeded 3.0 for a 4-cM interval, and significant evidence of genetic heterogeneity was observed. This genomewide scan is the first step toward identifying a gene on chromosome 22 with an influence on common myopia. At present, we are following up our linkage results on chromosome 22 with a dense map of >1,500 single-nucleotide–polymorphism markers for fine mapping and association analyses. Identification of a susceptibility locus in this region may eventually lead to a better understanding of gene-environment interactions in the causation of this complex trait.

Introduction

Myopia is the most common eye condition in the world; therefore, its public health importance and economic impact are enormous. Presumably because of anatomical distortions in the enlarged myopic globe, myopia, especially in its extreme degrees, is associated with vision-threatening retinal detachment, macular degeneration, and glaucoma (Curtin 1985). Myopia has been shown to raise the risk of glaucoma by a factor of 2.3 in a population ranging in age from 49 to 97 years (David et al. 1985; Mitchell et al. 1999) and has also been reported to be highly associated with the development of cataract (Weale 1980; Harding et al. 1989). Precise prevalence rates for myopia are difficult to ascertain because of the lack of a uniform definition of myopia. Nonetheless, the 1971–1972 National Health and Nutrition Examinations Survey estimated myopia prevalence in the United States to be 25% (Sperduto et al. 1983). A similar prevalence has been reported in other U.S. adult population studies (Wang et al. 1994; Katz et al. 1997). Females are reported to have an earlier onset and a slightly higher prevalence rate than males (Goss and Winkler 1983; Wang et al. 1994; Katz et al. 1997). Whites have a higher prevalence rate than African Americans (Katz et al. 1997). The Chinese and Japanese populations have very high prevalence rates of >50%–70% (Saw et al. 1996). Ashkenazi Jews, the target population of the present study, have consistently demonstrated a higher prevalence rate of myopia than the general white population in both U.S. and European population surveys; Orthodox Jewish males, in particular, show increased susceptibility (Baldwin 1981; Zylbermann et al. 1993).

Myopia is usually first diagnosed at age 8 or 9 years, with progression typically slowing dramatically by the middle to late teenage years. Although myopia can develop and progress after age 21 years, the age at onset of most myopia is usually within this range (Goss and Winkler 1983; Saw et al. 1996). Despite many decades of research, little is known about the precise molecular defects and abnormal biochemical pathways that result in myopia. Animal models of visual deprivation result-

Received May 4, 2004; accepted for publication June 25, 2004; electronically published July 23, 2004.

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0002-9297/2004/7503-0010$15.00

ing in aberrant eye growth and myopia (Wallman et al. 1987; Mutti et al. 1996) suggest that the neural control of eye growth is partly localized to the retina itself, but how retinal signals could directly control the growth of the eye is unknown.

Several lines of evidence have convincingly established the importance of genetic factors in the etiology of myopia. Familial aggregation studies have reported a greater prevalence of myopia in children of myopic parents, compared with children of nonmyopic parents (Sorsby et al. 1966; Keller 1973; Krause et al. 1993; Hui et al. 1995). In another study, the prevalence of myopia among 7-year-old children was 7.3% when neither parent was myopic, 26.2% when one parent was myopic, and 45% when both parents were myopic (Yap et al. 1993). Several twin studies have demonstrated a very high heritability for myopia (estimates range from 60% to 90%) (Teikari et al. 1988; Hammond et al. 2001; Lyhne et al. 2001). In a large study of 78 pairs of MZ twins and 40 pairs of DZ twins, Sorsby et al. (1966) found that the correlation coefficient approached unity for MZ twins, 0.5 for DZ twins, and 0 for the control pairs. Risk for myopia development is influenced by the environment, especially by the amount of near work (Chen et al. 1985; Tokoro 1988; Simensen and Thorud 1994; McBrien and Adams 1997). Observational studies of this risk factor do not fully explain the excessive familial clustering of myopia.

A number of studies have mapped several high myopia (−6.00 diopters [D] or lower) loci in a small number of families and have found evidence for linkage on 18p11.31 (7 families, 64 subjects), 12q21-23 (1 family, 22 subjects), 17q21-22 (1 family, 22 subjects), and 7q36 (23 families, 140 subjects) (Young et al. 1998*a*, 1998*b*; Naiglin et al. 2002; Paluru et al. 2003). However, we previously found no strong evidence of linkage of loci in these regions to a set of 38 Ashkenazi Jewish families with mild/moderate myopia (−1.00 D or lower) (Ibay et al., in press). In these families, a few individuals were classified as having severe myopia (−6.00 D or lower), but the majority of cases were less severe, suggesting that different loci may be involved in mild/moderate myopia.

Because of the high prevalence of mild/moderate myopia (−1.00 D or lower), one expects this type of myopia to be genetically heterogeneous in a population. It is expected that several genes, acting additively or in concert, may be responsible for myopia in some families, whereas other genetic combinations may explain the disorder in other subgroups. Therefore, one approach to reduce heterogeneity is to pursue families from a relatively genetically isolated population that has emerged from a small number of founders (Bear et al. 1981). This approach increases the likelihood that the subjects genotyped will have similar underlying genetic predispositions, improving the ability to detect the effects of a particular gene. Similar approaches have been used for mapping schizophrenia in isolated populations of Finland and Iceland (Stefansson et al. 2002; Gasperoni et al. 2003) and in Ashkenazi Jews (Fallin et al. 2003).

To further comprehend the genetic basis of mild/moderate myopia, we have undertaken a genomewide scan of extended Ashkenazi Jewish families living in the Lakewood, NJ, area. Analysis was performed by use of a binary affection status based on age-dependent clinical criteria. We chose to analyze myopia as a qualitative (binary) trait rather than as a quantitative trait for several reasons. First, if there are different genes underlying the physiological changes in the eye that cause myopia and hyperopia (the two extremes of the refraction distribution), then consideration of one extreme at a time in a dichotomous trait will reduce heterogeneity and increase power. Second, the effects of age on refraction are quite complex, with most individuals developing myopia in childhood. However, age-at-onset data are very unreliable in adults because of recall bias and differential eye-screening patterns in various age cohorts. Finally, these families were highly selected for having large numbers of individuals with clinically significant myopia, with the concomitant effect that there are not large numbers of persons with normal or hyperopic refractions. This bias in selection from the distribution of the quantitative trait can impact the analyses. For all these reasons, the analysis of myopia as a binary trait seemed most reasonable.

Subjects and Methods

Subject Recruitment and Evaluation

Ascertainment of families.—The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review boards of the University of Pennsylvania and the National Human Genome Research Institute (National Institutes of Health). Informed consent was obtained from the subjects after an explanation of the nature and possible consequences of the study. To gather participants for the study, we used a mass mailing of 3,900 letters to contact all the known Orthodox Jewish families living in Lakewood, NJ. Questionnaires were sent with letters explaining the study. If willing to participate, individuals completed and returned questionnaires that requested their contact and physician information. Second and third mailings were sent to individuals who did not respond—either positively or negatively—to the first mailing. The total number of questionnaires returned was 1,310. All Jewish individuals included in the study were of Ashkenazi heritage. Individuals who returned questionnaires were called, and family histories were obtained by telephone. Criteria for participation in this study included the following: (1) the proband must be affected and must have

Table 1

Characteristics of Ashkenazi Jewish Families Used in Linkage Analysis

Category	Results
No. of families analyzed (no. of individuals)	44 (964)
Total no. of individuals genotyped	399
Total no. of affected individuals genotyped	306
Average no. of individuals per family (range)	22 (9–65)
Average no. of affected individuals per family (range)	10.4 (2–32)
Average no. of genotyped individuals per family (range)	9.1 (4–25)
Average spherical equivalence, in D, in affected individuals (SD)	−4.67 (±3.24)
Average spherical equivalence, in D, in unaffected individuals (SD)	+0.64 (±1.39)
Average age, in years, of examined individuals (SD)	35.4 (±19.4)

a family history of myopia in either a parent or the proband's children; (2) only one parent (as opposed to both parents) of the proband should be myopic; and (3) the family must be willing to participate. These enrollment criteria were designed to preferentially select families that are consistent with a dominant mode of inheritance of a fairly high-penetrance susceptibility allele (Durner et al. 1992). Bilineal pedigrees were avoided because of the difficulty in determining the inherited gene transmitted to the offspring. After a family met the above criteria, medical records were obtained for each member. Data collection included all study-eligible parents, cousins, grandparents, siblings, children, aunts, and uncles of each proband.

Phenotypic evaluation.—Eligibility for family participation in the study required an index case that met the following criteria: (1) cycloplegic refraction of −1.00 spherical equivalent (as long as there was −1.00 D or lower in each meridian if astigmatism was present) or lower in those <50 years of age; (2) manifest refraction of −1.00 spherical equivalent (as long as there was −1.00 D or lower in each meridian if astigmatism was present) or lower in those ⩾50 years of age; and (3) no history of a systemic or ocular disease that might predispose to myopia, including premature birth. The same classification scheme was used to determine affection status for all individuals in the pedigrees, and subjects who did not meet this standard were regarded as unaffected. If a subject was reported to have been myopic but this diagnosis could not be confirmed with either medical records, measurement of the prescription of an old pair of eyeglasses, or current physical examination, the individual was treated as being of "unknown" phenotype.

Because of the normal developmental changes in refractive error during childhood and concomitant potential problems of misclassification, we took a more stringent approach to classification of affected versus unaffected subjects for the groups of individuals aged 6–10 years and 11–20 years. Individuals with a −1.00 D or lower spherical equivalent were considered affected, as above. However, subjects in the group of individuals aged 6–10 years with a +2.00 spherical equivalent refraction or higher in both eyes were classified as unaffected, since they are not likely to develop myopia. Individuals in this age group with a spherical equivalent between +2.00 and −1.00 were designated as "unknown." Individuals in the group of subjects aged 11–20 years with +1.50 spherical equivalent or higher in both eyes were classified as unaffected. Any individual with a spherical equivalent of between +1.50 and −1.00 in this age group was placed in the "unknown" class. This conservative approach balances the power loss that results from our lack of a good segregation-analysis model of age-dependent penetrance and the concomitant confusion about appropriate genotype probabilities for young unaffected subjects, with the power loss resulting from the classification of normal children as "unknown."

Our ascertainment strategy for the multiply affected pedigrees was to obtain eye records of all affected individuals, the parents of those individuals, and any other family members connecting affected pairs. We also sought unaffected siblings, as well as affected cousins, grandparents, uncles, and aunts. Eye records of subjects were reviewed to determine if cycloplegia was properly utilized for those individuals <50 years of age. Subjects with eye records >2 years old and subjects with improper exams were re-examined by their local eye doctor or one of the authors (D.S.). Although most exams occurred on the side of the family with the history of myopia, a family history of myopia was collected from both parents and from the parents and siblings of each parent (i.e., all grandparents, aunts, and uncles of the proband). Sometimes, members of the family of the unaffected parent of the proband were examined so that we could be certain of their clinical status.

Demographic and clinical characteristics.—The 44 families are generally large, with many families containing both affected sibling pairs and other types of affected relative pairs. The 44 families consisted of 29 three-generation families, 11 two-generation families, and 4 one-generation families (data available only on siblings). A total of 303 affected sibling pairs, 109 affected cousin

pairs, 263 affected avuncular pairs, and 33 affected grandparent-grandchild pairs were genotyped for the genomewide-scan markers. The average family size (table 1) was 22 individuals (range 9–65), and the average number of affected individuals per family was 10.4 (range 2–32). Subjects with unilateral myopia were not included in the study unless the fellow eye was previously enucleated.

To reduce the possibility of non-Ashkenazi grandparents or founders in our sample, ancestry questions were completed for each proband to establish the country or region of origin of the proband's parents and grandparents. Eastern and central Europe account for all of the known regions of origin of proband grandparents. Families were excluded if any grandparent of an affected subject was known to be of non-Ashkenazi descent.

Genotyping

High–molecular-weight DNA was isolated from buffy coats with a kit (Puregene [Gentra Systems]). Samples were stored in a DNA repository under a unique code. A genomewide scan was performed at the Center for Inherited Disease Research (CIDR) by 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 testing and relationship testing.—Mendelian inconsistencies and potential relationship errors were evaluated and corrected prior to data analysis by use of SIBPAIR (Duffy 1997) and GAS (GAS 1995). The accuracy of putative relative pairs was also checked by use of the program RelCheck (Boehnke and Cox 1997; Broman and Weber 1998). Data from individuals who demonstrated Mendelian inconsistencies at multiple markers that could not be resolved by retyping were treated as missing for the purpose of this analysis. In total, 0.32% of the data were treated as missing. Allele frequencies at marker loci were estimated from the married-in, unrelated individuals in the families by use of the SIBPAIR and LINKMEND programs.

Table 2

Model Numbers for the 12 Parametric Models Used in the Genomewide Linkage Analysis

	Model No. with Phenocopy Rate[b] of			
Penetrance[a]	.0	.05	.10	.15
.9	1	2	3	4
.8	5	6	7	8
.584	9	10	11	12

[a] Penetrance of DD/Dd genotypes (gene carriers).

[b] Phenocopy rate of dd genotype (non–gene carriers).

Linkage analyses.—We performed linkage analysis under multiple models. The parametric analyses consisted of 12 models with a dominant mode of inheritance, a frequency of the susceptibility allele of 0.0133, and a combination of different penetrances that included three genotypic penetrances (0.9, 0.8, and 0.584) for the gene carriers and four phenocopy rates (0.0, 0.05, 0.10, and 0.15) for non–gene carriers. These combinations resulted in 12 different parametric models (table 2). The parametric analyses were performed assuming all combinations of three genotypic penetrances for the gene carriers and four phenocopy rates for non–gene carriers. The first model assumed a penetrance of 90% in heterozygous or homozygous susceptibility-allele carriers, and the ninth model assumed a penetrance of 58.4%, with no phenocopies in either model. These two models were based on prior studies of high myopia (Young et al. 1998*a*, 1998*b*; Naiglin et al. 1999, 2002). The fifth model assumed a penetrance of 0.80 with no phenocopies. However, in our study of mild myopia (−1.00 D or lower), we expected that sporadic cases of myopia may be common and may occur in aggregated families. Therefore, we assumed different penetrances for the non–gene carriers (0, 0.05, 0.1, and 0.15), resulting in a total of 12 models for the linkage analyses. All persons <5 years of age were coded as "unknown" for the trait. Intermarker distances of the microsatellite markers were obtained from the Marshfield database (see the Marshfield Center for Medical Genetics "Build Your Own Map" Web site). The sex-averaged distances were used for autosomal markers. Parametric pairwise linkage calculations were performed with the MLINK program of the FASTLINK package (Cottingham et al. 1993; Schaffer et al. 1994), as well as the utility programs MAKEPED, Linkage Control Program, and Linkage Report Program from LINKAGE 5.1 (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986). Recombination fractions were assumed to be equal in men and women. The program HOMOG (Ott 1983) was used to test for evidence of heterogeneity in the presence of linkage in the two-point parametric linkage analysis. Multipoint parametric and nonparametric linkage (NPL) analyses were performed with the GENEHUNTER program (Kruglyak et al. 1996). Because of program memory constraints, three pedigrees were split into smaller ones in the GENEHUNTER multipoint analysis. The parametric analysis in GENEHUNTER used the 12 models described

above, allowing for locus heterogeneity. The X chromosome was analyzed by use of MLINK of the FASTLINK package (Cottingham et al. 1993; Schaffer et al. 1994) for the two-point linkage test and GENEHUNTER-PLUS X (Kruglyak et al. 1996) for the multipoint test.

In view of the uncertain mode of inheritance of common myopia, we also used a nonparametric approach that was based on an allele-sharing statistic, NPL_{all}—which estimates the statistical significance of alleles shared identical-by-descent (IBD) between all affected family members—as implemented in GENEHUNTER (Kruglyak et al. 1996). This statistic uses hidden Markov models in an optimized version of the Lander-Green algorithm (Lander and Green 1987) to calculate the multipoint inheritance distribution conditional on the genotypes at all marker loci. The NPL_{all} score statistic, a normalized version of the S_{all} statistic of Whittemore and Halpern (1994), is the average number of permutations that preserve a collection of marker alleles obtained by choosing one allele from each affected person. The size of the score increases sharply as the number of affected individuals sharing a particular founder allele IBD increases. We calculated the overall NPL score for the data set by use of equal weights for all pedigrees. The significance level for the NPL score is calculated by use of a perfect-data approximation on the basis of the exact approach (Kruglyak et al. 1996).

Family-Based Association Analysis

Linkage analysis can detect susceptibility loci over large genetic regions, but the resolution is reduced when applied to complex disease, for which both phenocopies and etiologic heterogeneity are known to exist. Frequently, linkage analysis implicates segments of a chromosome that are too large for direct sequencing and contain many genes that could be involved in disease etiology (Hauser and Boehnke 1997). Joint detection of linkage and allelic association within these large chromosomal regions could refine the search for susceptibility genes, since linkage disequilibrium is generally expected to be maintained over smaller chromosomal regions (Pericak-Vance 1998). Falk and Rubenstein (1987) proposed that valid tests of the allelic association between an observed genetic marker and an unobserved trait locus can be constructed by use of the parental genotypes and the genotype data from the affected offspring; these tests are immune from confounding due to admixture. Family-based association tests (FBATs) "are a class of tests that utilize within- and between-family marker-inheritance patterns to test for association and that are safeguarded, by design, from confounding caused by spurious associations" (Lake et al. 2000, p. 1515 [commenting on Ewens and Spielman 1995]).

Terwilliger and Ott (1992) noted that the alternative hypothesis for family-based tests is a composite for two genetic parameters, the recombination fraction (denoted as θ, ranging from 0 to 0.5) and a measure of allelic association between the marker locus and the unobserved putative disease gene (denoted as δ). In this situation, the null hypothesis (H_0) stipulates that there is no linkage or no association (i.e., disequilibrium due to linkage)—that is, for H_0, $\delta = 0$ or $\theta = 1/2$. The alternative hypothesis also involves both parameters: for H_A, $\delta \neq 0$ and $\theta < 1/2$. Rejecting the null hypothesis requires the presence of both linkage and association between the locus tested and the susceptibility gene. This approach protects against type I error arising from spurious association due to admixture, since the test has no power to detect the alternative hypothesis if either condition fails (Laird et al. 2000).

Even when, for H_0, $\delta = 0$ and $\theta < 1/2$, which tests for association in the presence of linkage, the alternative hypothesis, H_A, remains the same: $\delta > 0$ and $\theta < 1/2$. Thus, FBATs can be used to test for association, given some evidence for linkage (Lake et al. 2000), which would be necessary for fine mapping in a given chromosomal region.

Recently, Rabinowitz and Laird (2000) developed a broad class of FBATs that separate the question of test statistic from the problem of adjusting for admixture. This approach extends the statistic to permit "tests of different genetic models, tests of different sampling designs, tests involving different disease phenotypes, tests with missing parents, and tests of different null hypotheses, all in the same framework" (Lake et al. 2000, p. 1515). The approach was implemented by Xu et al. (2000) in the FBAT program, which tests for linkage and association in family data of arbitrary structure.

The program FBAT (Laird et al. 2000; Horvath et al. 2001; FBAT Web Page) was used to evaluate the association between common myopia and loci on chromosome 22. The null hypothesis of "no association in the presence of linkage" was examined by use of an empirical variance test (EV-FBAT) that adjusts for correlation between sibling genotypes, as well as marker genotypes of different nuclear families drawn from a single pedigree. The myopia trait was defined as $T_{ij} = Y_{ij} - \mu$, where Y_{ij} is a dichotomous indicator of affection status, and μ is the prevalence of the disorder or the weighting parameter that minimizes the variance of the test statistic. Although both affected and unaffected offspring were included in the pedigree file, the trait was defined as $T_{ij} = 1$ for affected offspring and $T_{ij} = 0$ for unaffected and missing offspring (μ was set to zero), thereby allowing only the affected subjects to contribute to the test statistic. Including the unaffected offspring in the data helped determine the distribution of the offspring genotypes when parental genotype data are missing. At least 10 informative families were set as the minimum

number necessary to compute any test statistic. The dominant model was used for this study, although the additive model performs just as well, even when the true genetic model is not additive (Horvath et al. 2001). Both multiallelic (testing alleles simultaneously) and biallelic (testing each allele separately against all others) tests were conducted.

Results

Linkage Analysis

Twelve penetrance-phenocopy models (table 2) were tested in the two-point parametric analyses, fixing the probability of being affected for non–gene carriers (phenocopy rate) to 0.0, 0.05, 0.10, or 0.15, by use of penetrances of either 0.90, 0.80, or 0.584 in gene carriers. Eight markers showing LOD scores >1.0 were identified when linkage homogeneity was assumed (D1S1665, D4S1647, D5S820, D13S285, D14S1426, D17S1308, D22S683, and DXS9900). Model 3 (0.90 penetrance, 0.10 phenocopy rate) (fig. 1) resulted in moderate support for linkage to myopia at D14S1426 (LOD = 1.67; 14q32), D22S683 (LOD = 1.45; 22q12), and D13S285 (LOD = 1.35; 13q33) (fig. 1). When the phenocopy rate was reduced to zero (model 1), the LOD score for D14S1426 increased to 2.12, whereas the LOD score for D22S683 was reduced to 1.01. When the penetrance rate was either 0.8 or 0.584, with no phenocopies (models 5 and 9), the LOD score for D4S1647 increased to 1.64, compared with a LOD score of 1.12 for model 3. Allowing for sex-specific recombination rates resulted in slightly larger maximum two-point LOD scores (2.0 at D22S683 and 2.1 at D14S1426, under model 3).

Nominal evidence of heterogeneity was detected at D14S1426 (heterogeneity LOD [HLOD]= 1.70; α = 85% of linked families) by use of the program HOMOG for two-point linkage analysis, under model 3 (α is defined as the estimate of the proportion of linked families, but it is well known that this estimate is not accurate in the case of complex traits, although the test for linkage in the presence of heterogeneity is robust and powerful [Greenberg and Abreu 2001; Whittemore and Halpern 2001; Hodge et al. 2002; Vieland and Logue 2002]). Tests for linkage in the presence of heterogeneity (HLOD in HOMOG) revealed that five additional markers had HLOD scores >1.4: D4S1647 (HLOD = 1.45; α = 0.35), D4S2394 (HLOD = 1.43; α = 0.2), D10S2327 (HLOD = 1.71; α = 0.15), GATA193A07 (HLOD = 1.85; α = 0.25), and D22S683 (HLOD = 1.47; α = 1.0).

Twelve distinct genetic models for mild myopia (table

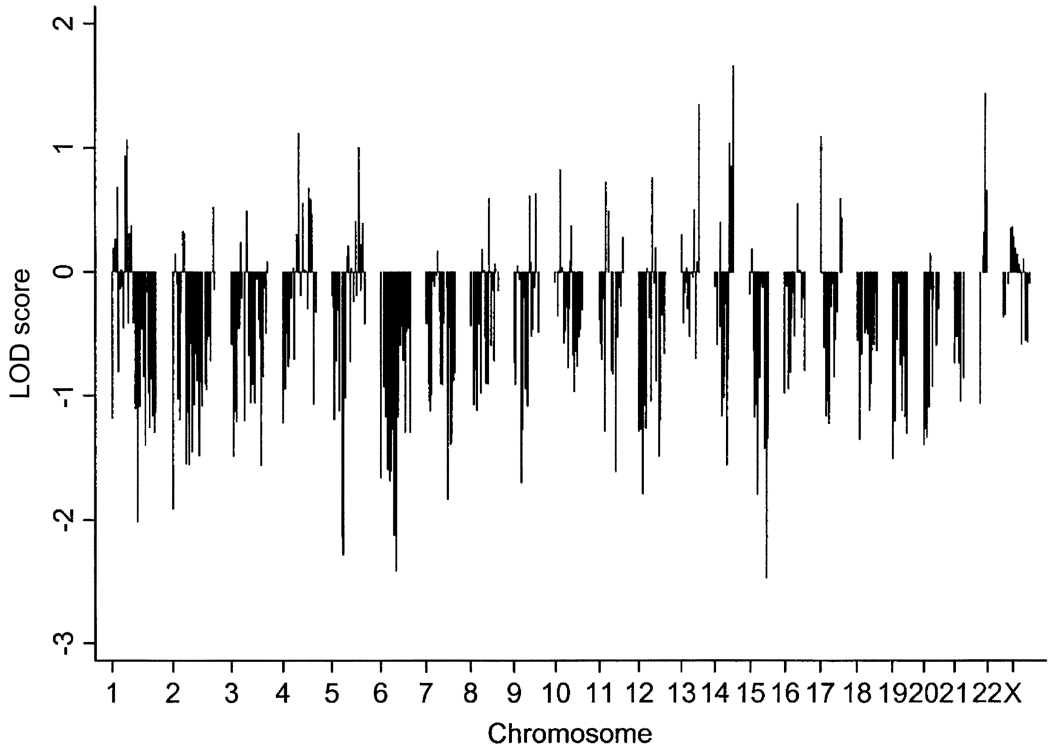
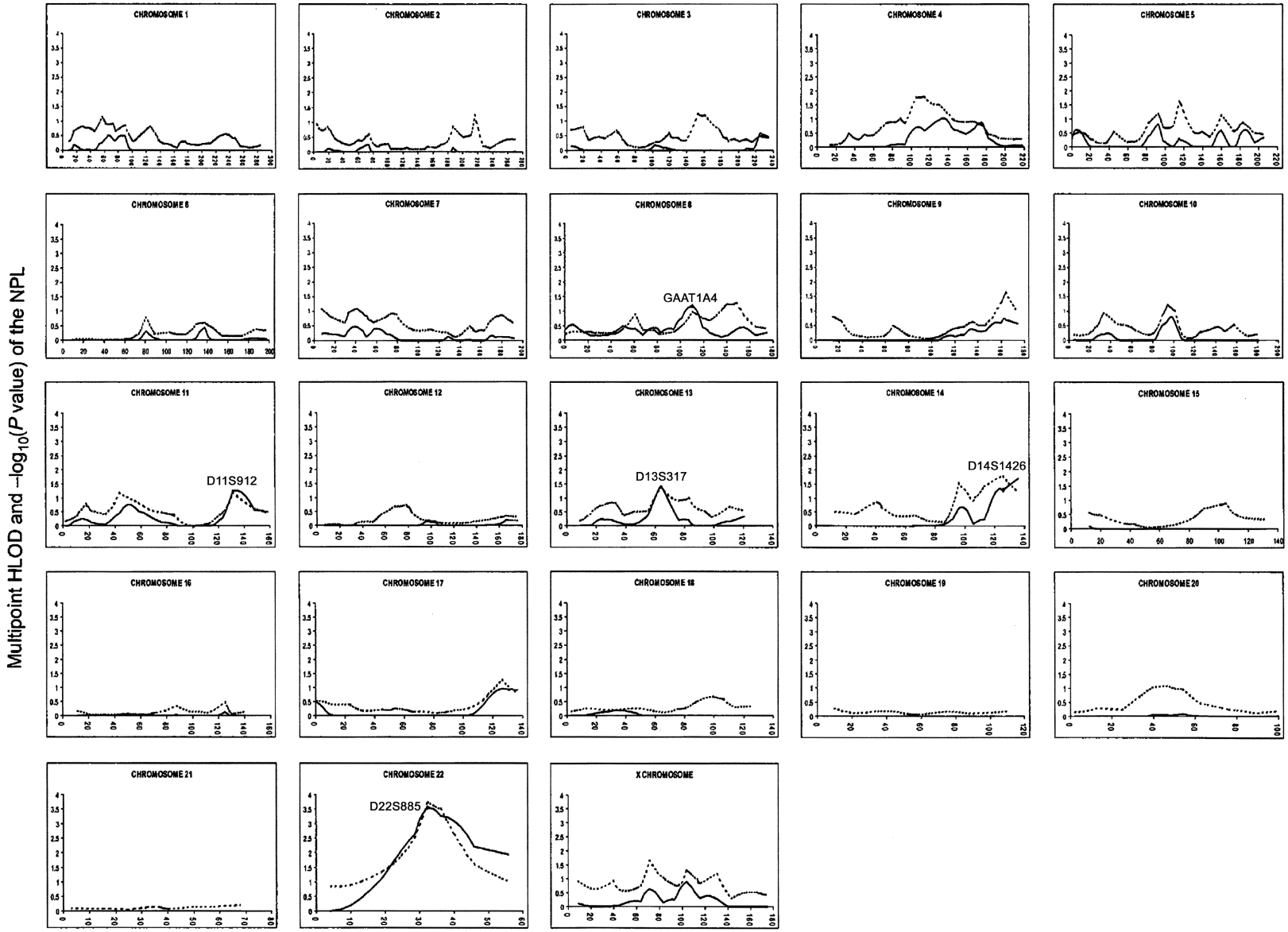


Figure 1 Genomewide parametric two-point LOD scores for model 3 (0.9 penetrance, 0.10 phenocopy rate). LOD scores at θ = 0.3 are plotted along the length (in cM) of the entire genome.


CHROMOSOME 1
CHROMOSOME 2
CHROMOSOME 3
CHROMOSOME 4
CHROMOSOME 5
CHROMOSOME 6
CHROMOSOME 7
CHROMOSOME 8
GAAT1A4
CHROMOSOME 9
CHROMOSOME 10
CHROMOSOME 11
D11S912
CHROMOSOME 12
CHROMOSOME 13
D13S317
CHROMOSOME 14
D14S1426
CHROMOSOME 15
CHROMOSOME 16
CHROMOSOME 17
CHROMOSOME 18
CHROMOSOME 19
CHROMOSOME 20
CHROMOSOME 21
CHROMOSOME 22
D22S885
X CHROMOSOME
Multipoint HLOD and $-\log_{10}(P \text{ value})$ of the NPL
Chromosomes (distances in cM)

2) were tested in the multipoint analyses, in an attempt to thoroughly search for linkage. The results of the multipoint analyses for penetrance-phenocopy model 3 (0.9 penetrance, 0.10 phenocopy rate) are presented in figure 2 and table 3. The strongest evidence for a myopia susceptibility locus was localized at D22S685 (multipoint HLOD = 3.54; $\alpha = 0.40$) in the 22q12.3 region by use of this penetrance model. A marker 4 cM away from this locus, D22S683, was observed to have a multipoint HLOD score of 3.27 ($\alpha = 0.37$). Two families were observed to have individual multipoint LOD scores >1.0 in this region. One of the largest multigenerational families obtained its highest LOD score (1.78) in the interval between D22S685 and D22S683. Suggestive evidence of linkage (multipoint HLOD score >2.0) was also found for two other loci in this region: D22S689 at 22q11.2-12.1 (HLOD = 2.63; $\alpha = 0.35$) and D22S445 at 22q13.1-13.2 (HLOD = 2.2; $\alpha = 0.38$). These loci flanked the region where the peak multipoint parametric HLOD score was observed for chromosome 22q12.3, and the four loci covered a total distance of 17 cM. In the region of chromosome 22q12.3 where parametric multipoint LOD scores under heterogeneity were significant, the NPL score also achieved significance ($P < .001$). The highest NPL scores were 4.60 ($P = .0002$) at D22S685 and 4.34 ($P = .0003$) at D22S683, which supports the results from the parametric linkage analysis.

In addition, other regions of the genome showed nominal evidence of linkage for myopia in the multipoint parametric analyses under model 3: GAAT1A4 at 8q22.2 (HLOD = 1.17; $\alpha = 0.19$), D11S912 at 11q23 (HLOD = 1.24; $\alpha = 0.19$), D13S317 at 13q22 (HLOD = 1.24; $\alpha = 0.26$), and D14S1426 at 14q32 (HLOD = 1.29; $\alpha = 0.34$).

Penetrance for the heterozygote and homozygote susceptibility-allele carriers was assumed to be 0.9, and the phenocopy rate for the homozygous noncarrier was assumed to be 0.10. Although model 3 showed the highest multipoint LOD score for D22S685, tests with different combinations of penetrances and phenocopies yielded similar results for the 22q12 region (table 4), indicating that, for common myopia, the effect of penetrance on the LOD score in this region is not as strong. An interesting result is that the multipoint HLOD scores on chromosomes 14 and 17 were higher in the model in which penetrance was set at 58.4% than they were in the model in which penetrance was set at 90% (maximum multipoint HLOD scores of 2.11 and 2.2 at D14S1426 and D17S928, respectively).

We did not find any evidence favoring linkage at the previously reported loci for high myopia on chromosomes 7q36, 12q22-q23, 17q21-q22, and 18p11.31. It is interesting that a pseudoautosomal locus (DXS9900) on the X chromosome showed a two-point LOD score of 1.07 at $\theta = 0.3$ for model 1. No other X-chromosome loci were significant in either the two-point or multipoint linkage analysis.

Family-Based Association

The program FBAT was used to test for allelic association between chromosome-22 loci and the dichotomous myopia trait. Moderate evidence for association was observed for D22S683 allele 3 ($S = 36.0$; 16 informative families; $P = .017$) in the test for linkage and association but not in the test for association in the presence of linkage ($S = 41.0$; 14 informative families; $P = .082$). When all the alleles were compared simultaneously, no significant association was observed for either the null hypothesis of no linkage and no association (FBAT) or the null hypothesis of no association in the presence of linkage (EV-FBAT). *P* values ranged from .23 to .85. It should be noted that the chromosome-22 loci were >3.0 cM apart from one another and were unlikely to have been near enough to the putative myopia locus to detect association, if it exists. Although linkage disequilibrium does not generally extend over regions >1 cM in outbred populations (Pericak-Vance 1998), the Ashkenazi Jewish population used in this study is considered to be relatively homogeneous and so refining the search for the underlying trait gene to regions <1 cM may provide more evidence for association.

Discussion

We have performed the first genome scan for common-myopia susceptibility loci among an Ashkenazi Jewish sample of multiplex pedigrees, in hopes of reducing the underlying heterogeneity among myopia linkage samples. Although our strategy of collecting samples from a single ethnic group and selecting families that appeared consistent with dominant inheritance of a highly penetrant susceptibility allele restricted recruitment to a relatively small number of families, we have potentially

Figure 2 Genomewide multipoint heterogeneity LOD scores and $-\log_{10}(P$ value) of multipoint NPL scores for model 3 (0.9 penetrance, 0.10 phenocopy rate). Multipoint HLOD (*solid line*) and $-\log_{10}(P$ value) of the NPL score (*dashed line*) were calculated with the GENEHUNTER software package, version 2.1. In each graph, the marker distances (in cM) are indicated on the horizontal axis. Only loci with an HLOD score >1.0 are identified. Note that the evidence in favor of linkage given by the HLOD score and by the $-\log_{10}(P$ value) of the multipoint NPL score are not exactly equivalent, although they are similar.

Table 3

HLOD Scores >2.0 for Genomewide Analysis of Myopia under Model 3 (0.9 Penetrance, 0.10 Phenocopy Rate) in 44 Ashkenazi Jewish Families: Parametric and Nonparametric Methods

Chromosome	Marker	Location[a]	α	HLOD	NPL	*P*
22q11.2-q12.1	D22S689	28.57	.35	2.63	3.26	.0030
22q12.3	D22S685	32.39	.40	3.54	4.60	.0002
22q12	D22S683	36.22	.37	3.27	4.34	.0003
22q13.1	D22S445	45.82	.38	2.2	2.18	.0231

[a] Sex-averaged locations, in Kosambi cM, obtained by use of the Marshfield Center for Medical Genetics Search Form Web site.

attained a more homogeneous group for detection of linkage. Also, we believe that our strategy of collecting samples from large multigenerational families is advantageous for a complex trait like common myopia. Other studies of complex dichotomous traits have usually used affected sibs and relatively small nuclear families. In contrast, some of the families in our study are large enough to individually attain, under a dominant model, maximum LOD scores exceeding the generally accepted cutoff of 3. Because of the large size of the families we studied, if the genetic basis of the disorder in many of these families is relatively close to dominant inheritance and only one locus is segregating in each family, then we should be able to detect linkage even in the presence of genetic heterogeneity. Our combined sample should also be large enough to identify loci that contribute to possible complex inheritance patterns of myopia when studied by use of a dense enough map of marker loci.

Our strongest signal was localized to 22q12, according to both model-free and parametric multipoint analysis (HLOD = 3.56; NPL = 4.62), even though only a subset of the families showed linkage to this region. The only other region with a LOD score >2.0 was on chromosome 14q, where the two-point LOD score was 2.12 at $\theta = 0.3$. In the two-point analysis, the linkage signal was stronger in this region of chromosome 14 than it was on chromosome 22. However, under the model that gave the highest score of 3.56 for the 22q region (model 3), the multipoint HLOD score on 14q was reduced to ~1.5, but the use of model 9 resulted in a multipoint HLOD score on 14q of ~2.1. This suggests that if there are two loci segregating in these families, they may have different genotype-specific penetrances. Beyond the 14q and 22q findings, seven additional regions (4q22-q28, 8q22.2, 10q22, 11q23, 13q22, 14q32, and 17qter) showed nominal evidence of linkage in at least one analysis.

It has been shown that the critical value of an HLOD score required to yield a specific size of the test (i.e., *P* value) is larger than the equivalent threshold for a homogeneity LOD score and that maximizing over penetrance also increases the type I error rate. Hodge et al. (1997) showed that the critical threshold for a *P* value of .0001 (LOD = 3.0) should be increased to 3.3 when maximizing over penetrance in the linkage analysis. Abreu et al. (2002) showed that the critical value of 3.0 needs to be increased by an additional 0.47 for two-point HLOD scores, and Greenberg and Abreu (2001) showed that the critical threshold of 3.0 must be increased by ~0.7 to adjust for the increased type I error rate of multipoint HLOD scores. Thus, if one adjusts for maximizing over penetrance and for the use of HLOD scores, one gets an adjusted two-point HLOD threshold of 3.77 (3.0 + 0.3 + 0.47; corresponding to a threshold of 3.0 for a two-point LOD score without maximization over penetrance) and an adjusted multipoint HLOD threshold of 4.0 (3.0 + 0.3 + 0.7, corresponding to a threshold of 3.0 for a multipoint LOD without maximization over penetrance). These are conservative adjustments and thus may decrease power unnecessarily. However, requiring a conservative multipoint HLOD threshold of 4.0 (3.3 + 0.3 + 0.7), corresponding to the traditional LOD threshold of 3.0, or an even more conservative multipoint HLOD threshold of 4.3 to achieve genomewide significance (Lander and Kruglyak 1995) suggests that the evidence on chromosome 22 is at least suggestive evidence of linkage.

In view of our results showing several peaks, only one of which had a LOD score >3.0, future emphasis must be placed on the cross-validation of results obtained by different groups, even without individually achieving statistical significance, as a method to narrow the regions of interest. We plan to study additional independent samples of families to increase our power to detect and confirm myopia susceptibility loci. In addition, we believe that the uncertain mode of inheritance of common myopia justifies and necessitates the use of several analysis methods. Although the use of multiple models decreases the actual significance levels of the results (because it increases false-positive rates), it remains a valid approach to the identification of the most likely candidate regions, particularly when the significance levels are appropriately adjusted for the methods used.

In summary, we observed a strong linkage signal for

Table 4

Comparison of Multipoint HLOD Scores for Chromosome 22 by Use of Different Penetrance Models

	HLOD Score for D22S685 (D22S683) with Phenocopy Rate[b] of			
Penetrance[a]	.0	.05	.10	.15
.9	3.18 (3.15)	3.48 (3.41)	3.54 (3.27)	3.44 (2.98)
.8	3.20 (3.11)	3.49 (3.33)	3.51 (3.14)	3.42 (2.85)
.584	3.16 (2.98)	3.42 (3.10)	3.36 (2.80)	3.17 (2.46)

[a] Penetrance of DD/Dd genotypes (gene carriers).
[b] Phenocopy rate of dd genotype (non–gene carriers).

myopia on chromosome 22q, with lower evidence for a second locus on 14q. Several genes highly expressed in the eye lie within the 22q region, including heme oxygenase 1 (Yoshida et al. 1988; Kutty et al. 1994; Morse and Choi 2002), RNA binding motif protein 9 (Norris et al. 2002; Strausberg et al. 2002; Ota et al. 2004), and minichrom maintenance deficient 5 (Paul et al. 1996; Tsuruga et al. 1997). We are currently pursuing candidate-gene studies and SNP-based fine mapping of this region. We believe that identification of one or more susceptibility loci for common myopia will have major public health importance, since it will help us understand the causes of this common eye disorder and may lead to methods to prevent or slow the progression of the disorder.

Acknowledgments

This study was supported in part by U.S. Public Health National Eye Institute grant EY12226. The authors thank the families for their participation in the study and Dr. Reuvain Shanik and Rabbi Yitzchok Rozsansky for their enthusiasm and effort on the project. Genotyping services were provided by CIDR. CIDR is fully funded through a federal contract from the National Institutes of Health to Johns Hopkins University (contract number N01-HG-65403).

Electronic-Database Information

The URLs for data presented herein are as follows:

"Build Your Own Map," Marshfield Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/Map_Markers/mapmaker/MapFormFrames.html

FBAT Web Page, http://www.biostat.harvard.edu/~fbat/default.html (for FBAT software)

Search Form, Marshfield Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/Map_Markers/mapmaker/SearchForm.html (for marker search)

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