Caucasian Families Exhibit Significant Linkage of Myopia to Chromosome 11p

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PURPOSE. Myopia is a common visual disorder caused by eye overgrowth, resulting in blurry vision. It affects one in four Americans, and its prevalence is increasing. The genetic mechanisms that underpin myopia are not completely understood. Here, we use genotype data and linkage analyses to identify high-risk genetic loci that are significantly linked to myopia.

METHODS. Individuals from 56 Caucasian families with a history of myopia were genotyped on an exome-based array, and the single nucleotide polymorphism (SNP) data were merged with microsatellite genotype data. Refractive error measures on the samples were converted into binary phenotypes consisting of affected, unaffected, or unknown myopia status. Parametric linkage analyses assuming an autosomal dominant model with 90% penetrance and 10% phenocopy rate were performed.

RESULTS. Single variant two-point analyses yielded three significantly linked SNPs at 11p14.1 and 11p11.2; a further 45 SNPs at 11p were found to be suggestive. No other chromosome had any significant SNPs or more than seven suggestive linkages. Two of the significant SNPs were located in BBOX1-ASI and one in the intergenic region between ORA47 and TRIM49B. Collapsed haplotype pattern two-point analysis and multipoint analyses also yielded multiple suggestively linked genes at 11p. Multipoint analysis also identified suggestive evidence of linkage on 20q13.

CONCLUSIONS. We identified three genome-wide significant linked variants on 11p for myopia in Caucasians. Although the novel specific signals still need to be replicated, 11p is a promising region that has been identified by other linkage studies with a number of potentially interesting candidate genes. We hope that the identification of these regions on 11p as potential causal regions for myopia will lead to more focus on these regions and maybe possible replication of our specific linkage peaks in other studies. We further plan targeted sequencing on 11p for our most highly linked families to more clearly understand the source of the linkage in this region.

Keywords: myopia, linkage analysis, family studies, case-control study

Myopia, or nearsightedness, is a condition caused when light is focused in front of the retina rather directly on it, causing distant images to appear blurry. It affects approximately 25% of Americans. Prevalence rates in the United States were significantly higher in the early 2000s compared with the early 1970s (41.6% to 25.0%); this trend holds true in Caucasian individuals at the rate of 43.0% to 26.3%.1

Myopia is a complex trait and is influenced by both genetic and environmental factors.2–5 Environmental studies have identified both positive (near work level) and negative (outdoor activity) correlations with myopia.2 Multiple genetic studies have identified risk variants for myopia including population-based genome-wide association studies (GWAS).3–8 Almost all of the variants identified through GWAS are common (minor allele frequency > 0.05) and have a small effect size. Family-based linkage studies are more effective than population-based studies at identifying rare variants with large effect sizes because family-based studies need fewer individuals to detect rare variants with sufficient power. Rare variants may be common within a given family but rare within the population as a whole. Unlike population-based association studies, family-based linkage studies are not subject to population stratification due to differing allele frequencies in different ethnic groups, because each family is analyzed as its own separate unit and not all in a group as in an association study. Each family receives its own independent logarithm of the odds
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families identified genomic regions likely to be harboring high-
onpathogenic myopia (MSE) find linked genes with variants that increase myopia risk. Here, we used dense exome-based arrays. Here, we use dense exome single coding regions, currently made possible using inexpensive exome genotyping. The 273 individuals with DNA samples were genotyped at the Center for Inherited Disease Research at Johns Hopkins University using an Illumina ExomePlus array. Blind duplicates and HapMap controls were distributed across plates for concordance checking. Affected and unaffected individuals were evenly distributed across plates, but family members were kept on the same plate. Samples with suspected mixtures of other sample DNA or unusual X and Y patterns (e.g., XXY individuals or people with major deletions; none identified) or sex mismatch were identified and dropped before release. SNP

METHODS

Data Collection and Phenotype Classification

Caucasian individuals were recruited from 56 families living in Pennsylvania and New Jersey. Phenotype data were collected on 412 individuals, and genotype data were collected on 273 of these individuals. The average age was 38.58 years, with an SD of 19.67; 56% of the subjects were female. Families ranged from two to four generations. Families were identified through mailings, eye clinic interviews, and referrals from private optometrists and ophthalmologists. Eligibility was dependent on the following criteria: (1) at least three participating family members; (2) only one myopic parent, and (3) at least two myopic siblings. Children had to be at least 5 years of age to participate. Medical records were obtained for each consenting member and/or refractions were done in the absence of adequate records. Data were collected on all eligible and consenting relatives of each proband. All study participants provided informed consent. Protocols were carried out in accordance to the Declaration of Helsinki. The study was approved by the institutional review boards of the National Human Genome Research Institute and the University of Pennsylvania.

Participants underwent comprehensive eye examinations that included medical/ocular health history, visual acuity, slit-lamp biomicroscopy, dilated fundus examinations, and manifest refraction. For subjects under 41 years of age, cycloplegic refraction using 0.5% cyclopentolate or 1% tropicamide was performed; older subjects used manifest refraction. If participants were unable to be examined at a clinic, refraction data were obtained from medical records or eyeglass prescriptions. Refraction was measured using MSE in diopters, obtained by adding the spherical component of the refraction to one-half the cylindrical component and averaging for both eyes.

Individuals with an MSE of −1.00 D or lower in both eyes (based on an actual eye examination, medical records, or eyeglass prescriptions, if the person was not available for examination) were coded as affected. Individuals with an MSE of 0.00 D or greater were coded as unaffected, and any individuals with an MSE between 0.00 and −1.00 D were coded as having an unknown or missing phenotype. Any individual that had a history of systemic or ocular disease that might cause a predisposition to myopia was coded as unknown regardless of their MSE measurements.

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 5 to 21 years. Individuals with a −1.00 D or lower spherical equivalent were considered affected, as above. Individuals were considered unaffected if the MSE refraction was > +3.00 D (5–9), > +2.00 D (10–17), and > +0.50 (18–21) in both eyes because they are not likely to develop myopia. Individuals that fell between their given upper limit and −1.00 D were designated as “unknown” due to the anticipated refractive errors changes toward less hyperopia in children. This conservative approach balances the power loss that results from 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.” Classification resulted in 261 affected individuals, 68 unaffected individuals, and 83 unknown individuals. The average MSE was 2.88 D, with an SD of 3.40.

Exome Genotyping and Quality Control

The 273 individuals with DNA samples were genotyped at the Center for Inherited Disease Research at Johns Hopkins University using an Illumina ExomePlus array. Blind duplicates and HapMap controls were distributed across plates for concordance checking. Affected and unaffected individuals were evenly distributed across plates, but family members were kept on the same plate. Samples with suspected mixtures of other sample DNA or unusual X and Y patterns (e.g., XXY individuals or people with major deletions; none identified) or sex mismatch were identified and dropped before release. SNP
clustering was performed on all SNPs in project, and SNP genotypes with genotype quality (GC) score less than 0.15 were recoded as missing genotypes. Autosomal SNPs with less than 85% call rate, cluster separation of less than 0.3, and heterozygote rate greater than 80% were dropped prior to laboratory release. We later further filtered markers using a 95% call rate, resulting in a mean call rate of 99%.

We merged the phenotype and family relationship information with the genotype data and included an additional 118 ungenotyped individuals in the data set for use in the linkage analysis. The ungenotyped individuals included some individuals who provided phenotype information but did not provide samples or failed genotyping. However, the majority of these ungenotyped individuals consisted of individuals who were known to exist (based on family history) but who did not participate in the study because they chose not to or were deceased. These individuals (who were coded as having an unknown phenotype and unknown genotypes) were added to connect disjointed pedigrees and ensure proper familial relationships. Mendelian error checking was performed on the pedigrees using the sib-pair, and any SNP with more than one Mendelian error was dropped. PLINK was used to detect sex discrepancies; samples that did not appear sufficiently matched to their recorded sex were dropped. PREST-PLUS was used to identify duplicate samples. SNPs with more than one error in blind duplicates or HapMap controls were dropped. Heterozygosity rates across samples were checked, and outlier samples were excluded. We also examined the samples for chromosomal abnormalities and autosomal SNPs with a sex difference in allelic frequency $>0.2$ or sex difference in heterozygosity $>0.3$ were dropped. Monomorphic variants were dropped. We merged this exome data with 367 microsatellite markers from the same genotyped individuals to increase power in the multipoint analyses; microsatellites are highly informative. All markers were mapped to the Rutgers Genetic Map version 3. The final set of markers for analysis contained 67,145 SNPs and 367 microsatellites. Hardy-Weinberg equilibrium was tested for all linked variants.

**Allele Frequency Calculations**

Prior studies have shown that separation of family data into major ethnic groups (e.g., European ancestry Caucasians) and

![Figure 1](image1.png)

**Figure 1.** Graph of the genome-wide single variant two-point HLOD scores produced by TwoPointLods. The lines at 1.9 and 3.3 represent the respective suggestive and significant thresholds recommended by Lander and Kruglyak.

![Figure 2](image2.png)

**Figure 2.** Graph of the chromosome 11 single variant two-point HLOD scores produced by TwoPointLods. The lines at 1.9 and 3.3 represent the respective suggestive and significant thresholds recommended by Lander and Kruglyak.
Significant and suggestive SNPs from the single variant two-point linkage analysis from chromosome 11, sorted by HLOD. The significance threshold is 3.3 and the suggestive threshold is 1.9, as recommended by Lander and Kruglyak. Gene annotations were performed by ANNOVAR.

## Parametric Linkage Analyses
Three types of parametric linkage analysis were performed: single variant two-point analysis, collapsed haplotype pattern variant two-point analysis, and multipoint analysis. All analyses assumed an autosomal dominant model with a disease allele frequency of 0.01. Penetrance was 90% for carriers and 10% for noncarriers.

Single variant two-point analyses were performed using an implementation of the Elston-Stewart algorithm by the TwoPointLods software created by Alun Thomas (http://www-genepi.med.utah.edu/~alun/software/). Each family was analyzed individually, and afterward, cumulative LOD scores and HLOD scores were calculated across all families.

Multipoint analyses were performed using SimWalk2. Previous studies have shown that multipoint linkage using a dense marker map with strong intermarker LD often lead to type I error inflation, so we pruned the data by condensing...
SNPs into 1-cM bins. The SNP with the highest minor allele frequency chosen to represent the bins in the subsequent multipoint analyses (because such SNPs will provide the highest information content about segregation of this region of the chromosome in the families). Further LD analysis was performed in Haploviz. Any SNP pairs with an \( r^2 \) value greater than 0.2 had one SNP of the pair removed. Microsatellites were not included in the pruning; they were retained due to their high information level and general low intermarker LD with nearby SNPs.

Collapsed haplotype pattern (CHP) variants were created using the SEQLinkage software. This program uses rare variants to create short regional haplotypes that serve as multiallelic pseudo-markers corresponding to specific genetic regions such as genes (determined by RefSeq). This approach has been shown to not require pruning of markers based on intermarker linkage disequilibrium (required for multipoint linkage). Because SEQLinkage is known to properly control type I error rate when using rare variants, we limited this analysis to SNPs with a MAF \( \leq 0.05 \). The SEQLinkage method can use different informative rare variants in different families to build the family-specific haplotype pseudo-markers, thus allowing retention of information that would otherwise be lost in the pruning process when all rare variants are discarded prior to multipoint linkage. MERLIN\(^{36} \) was used to perform two-point linkage on the pseudo-markers. Variants were annotated with ANNOVAR,\(^{31,32} \) SIFT,\(^{34–39} \) and ClinVar.\(^{40} \)

### RESULTS

The single variant two-point analysis localized a strong linkage signal to 11p (Fig. 1). Three variants exhibited genome-wide significant HLODs, and 45 variants had suggestive HLODs in the regions around 11p15.4, 11p15.1, 11p14.1, and 11p11.2 (Fig. 2). No suggestive variants were observed on 11q. HLOD values \( \geq 3.3 \), and HLODs \( \geq 1.9 \) are considered genome-wide significant and suggestive as recommended by Lander and Kruglyak.\(^{41} \) Two significant variants (rs11029865 and rs7939668) were identified at 11p14.1 (HLOD = 3.31, 3.27) and were located in the noncoding antisense RNA BBOX1-AS1 gene. Both SNPs are common, with sample data MAFs of 0.20 (rs11029865) and 0.40 (rs7939668). Neither of the sample MAFs deviated greatly from the MAF for CEU in 1000Genomes. The final significant SNP was rs11040198 (HLOD = 3.26), located at 11p11.2 in an intergenic region between OR4A47 and TRIM49B. This SNP had a MAF of 0.1506 in the data set with no significant deviation from the 1000Genomes population frequency. Note we have considered all three of these variants to be significant because their LOD scores round to 3.3; however, only one SNP has a LOD that actually exceeds 3.3. All three of these “significant” SNPs are very close together in the 11p region, giving strong evidence of a myopia susceptibility gene in this region.

Other smaller and less dense suggestive signals were found throughout the genome. The top 13 HLOD scores were all located on 11p, as were 45 of the 68 overall suggestive variants. The list of suggestive SNPs on chromosome 11 can be found in Table 1, whereas the full list of all other suggestive signals (excluding chromosome 11) can be found in Supplementary Table S1.

CHP variant linkage analysis did not reveal any genome-wide significant regions (Fig. 3). It did reveal four suggestive peaks, with three on 11p (Table 2). The two highest overall HLODs were centered on SAAL1 at 11p15.1 (HLOD = 3.21) and ORE15E1 at 11p15.4 (HLOD = 2.38). SAAL1 also had a suggestive signal in the two-point analysis (rs77235279). The SNP had a MAF of 0.04 and was nonsynonymous exonic, although not predicted as damaging. There was a further suggestively linked CHP variant centered in the MRVI1 gene at 11p15.4 (HLOD = 1.96).

There were no genome-wide significant regions identified in the multipoint analysis; 16 suggestive variants were found (Fig. 4; Supplementary Table S2). The top 14 variants located on

### Table 2. Suggestive Genome-Wide Collapsed Haplotype Pattern Variants

| Chromosome | POS   | Gene    | LOD   | HLOD | \( \alpha \) |
|------------|-------|---------|-------|------|-------------|
| 11p15.1    | 31.7444 | SAAL1   | 3.2119 | 3.2119 | 1.00        |
| 11p15.4    | 11.5924 | OR51E1  | 2.3792 | 2.3792 | 1.00        |
| 18p11.22   | 52.5242 | MTCL1   | 2.0459 | 2.0459 | 1.00        |
| 11p15.4    | 21.145  | MRVI1   | 1.9555 | 1.9555 | 1.00        |

Genome-wide suggestive signals from the collapsed haplotype pattern variants sorted by HLOD. The genome-wide significance threshold is 3.3 and the genome-wide suggestive threshold is 1.9, as recommended by Lander and Kruglyak.\(^{41} \) LODm, cumulative LOD; POSm, position in cM.
20q13.13–20q13.31. This result is near a previous suggestive finding for myopia in Ashkenazi Jewish families at 20p12-q11.1.42 Two further suggestive variants (HLOD = 2.07) were found at 11p15.1.

**DISCUSSION**

This study has identified a cluster of significant and suggestive linkage signals along 11p15-11p11 for myopia in Caucasian families. Suggestive signals were spread throughout the region from both the single-variant and CHP two-point linkage analyses. The only significant signals were identified by the single-variant two-point analysis at 11p14.1 and 11p11.2, but the CHP analyses showed strong evidence that rare variants in 11p15.1-11p15.4 are segregating with myopia in these families. Both the two-point and CHP analyses agree that there is a strong linkage signal on 11p. The significant SNPs are novel and have not been previously reported significant in any previous association or linkage study, so replication to these specific regions are needed, and any interpretations of these specific regions should be viewed with some caution. However, the broader 11p region has a strong history of linkage with myopia and refractive error, including the Myopia-7 (MYP7) region at 11p13-11p15.1,43 11p15.1 in a subset of these Caucasian families,19 and 11p14-q14 in Ashkenazim.42

The SNPs that are individually significantly linked are common variants and possibly suggest that different rare variants within the same gene may be causal in different families or that the causal variants are located in noncoding areas in 11p15-11p11. It is possible that one of these common SNPs could be causal for a common, heterogeneous phenotype like myopia or, more likely, that these SNPs may be tagging (within each family) the segregating haplotype that may contain any rare, potentially causal, variants (probably not on this limited exome-based chip). Targeted sequencing could help to elucidate the source of the linkage signal.

Both the two-point and CHP analyses found significant or highly suggestive linkage signals on 11p. The two-point analyses did have a higher magnitude and number of signals than the CHP analyses, due mainly to the fact that the CHP analyses required SNPs with a MAF ≤0.05. Nevertheless, both analyses showed that the signals on 11p were much higher than elsewhere in the genome (Figs. 1, 3).

The multipoint analysis identified a suggestive signal on 11p and a suggestive signal of higher magnitude on 20q (Fig. 4). This discrepancy was likely caused by pruning; only SNPs with the highest MAF (i.e., most informative) were retained, and large amounts of information were lost, resulting in the decreased signal on 11p. The increased evidence for linkage on 20q in the multipoint analysis suggests that the common markers were jointly tagging segregating haplotypes in these families. It is possible that these haplotypes may harbor potentially causal variants, but replication will be needed to rule out the possibility that the linkage on 20q is a false positive.

Given that multiple studies, including this one, have reported significant evidence of linkage to the broader 11p region, we briefly examine a few potential candidate genes implicated in our study. We note that we do not infer causality of any of these genes or variants; we are simply reporting some potential candidates for future work and replication.

Two of the significant SNPs and 13 suggestive SNPs from the two-point analysis were located at 11p14.1 in or near the noncoding RNA BBOX1-A51 gene, which has no known associations to myopia. Another noncoding RNA gene at 11p14.1, BDNF-AS, contained seven suggestively linked SNPs. The target gene, BDNF (at 11p14.1) has well-known protective and healing properties in the retina.44–46

A significantly linked SNP at 11p11.2 was located in the intergenic region between the olfactory receptor OR4A47 and TRIM49B. The protein tyrosine phosphatase receptor PTPRJ (11p11.2) contained two suggestive linkages at exonic SNPs (HLODs = 2.28, 2.21). One suggestive SNP rs322580 (rs1566734) was nonsynonymous and predicted damaging by ClinVar and PolyPhen2. The mitochondrial protein tyrosine phosphatase PTPMT1 (11p11.2) was slightly below the suggestive threshold (HLOD = 1.65) in the CHP analysis. Other protein tyrosine phosphatases such as PTPRR and PPFIA2 (PTPRF) have been associated with myopia and average dioptic sphere measurements.47

11p15 was the site of two highly suggestive linkage signals, both located in the exons of the olfactory receptor OR52H1 at 11p15.4. MRVI1 (11p15.4) was suggestive for linkage in the CHP analyses; one study found it to be overexpressed in keratoconic corneas.48

The mitochondrial protein tyrosine phosphatase PTPMT1 (11p11.2) was slightly below the suggestive threshold (HLOD = 1.65) in the CHP analysis. Other protein tyrosine phosphatases such as PTPRR and PPFIA2 (PTPRF) have been associated with myopia and average dioptic sphere measurements.47
proliferation in joints. Multipoint analysis also found a suggestive linkage signal to 11p15.1. Both findings recapitulate a previous linkage signal found on 11p15.1 in a subset of these Caucasian families.

We did not see any suggestive evidence of linkage at 11p13, the site of the maximum LOD score of 6.1 for the MYP7 locus from the original study of Hammond et al. of dizygotic twins that identified this locus. However, in this study, they actually showed genome-wide significant linkage to the entire 11p13-11p15.1 region and simply suggested \( PAX6 \) as a candidate gene. Later studies have both supported\(^{50,51} \) and disputed\(^{52,53} \) the causality of \( PAX6 \). Simpson et al.\(^{52} \) suggests that no priority be given to \( PAX6 \) over other genes in the region. The significant region of the study of Hammond et al.\(^{45} \) overlaps the location of the genome-wide significant and suggestive linkages on 11p found in this study, and thus our study replicates their linkage. Because it is known that linkage peaks are broad and that the causal genes for a given peak in a particular study may not be located at the position with the highest LOD score, it is possible that the same gene could be responsible for all published linkages in 11p. However, this is speculative, and much additional work is still needed to confirm the existence and identity of such a causal locus in 11p.

We presented genome-wide significant evidence for linkage of a susceptibility gene for myopia on 11p in Caucasian families. Novel genome-wide significant two-point linkage signal sequences were identified at 11p14.1 and at 11p11.2. Highly suggestive signals were identified at 11p15.4 and 11p15.1. Further suggestive signals were observed in all these regions. This replicates evidence of linkage to this region from several previous studies.\(^{19,42,43,52} \) The 11p region contains a large number of good potential candidate genes for future study. Targeted sequencing of the 11p region is planned to search for any possible causal variants.

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