Myopia in African Americans Is Significantly Linked to Chromosome 7p15.2-14.2

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Purpose. The purpose of this study was to perform genetic linkage analysis and association analysis on exome genotyping from highly aggregated African American families with nonpathogenic myopia. African Americans are a particularly understudied population with respect to myopia.

Methods. One hundred six African American families from the Philadelphia area with a family history of myopia were genotyped using an Illumina ExomePlus array and merged with previous microsatellite data. Myopia was initially measured in mean spherical equivalent (MSE) and converted to a binary phenotype where individuals were identified as affected, unaffected, or unknown. Parametric linkage analysis was performed on both individual variants (single-nucleotide polymorphisms [SNPs] and microsatellites) as well as gene-based markers. Family-based association analysis and transmission disequilibrium test (TDT) analysis modified for rare variants was also performed.

Results. Genetic linkage analysis identified 2 genomewide significant variants at 7p15.2 and 7p14.2 (in the intergenic region between *MIR148A* and *NFE2L3* and in the noncoding RNA *LOC401324*) and 2 genomewide significant genes (*CRHR2* and *AVL9*) both at 7p14.3. No genomewide results were found in the association analyses.

Conclusions. This study identified a significant linkage peak in African American families for myopia at 7p15.2 to 7p14.2, the first potential risk locus for myopia in African Americans. Interesting candidate genes are located in the region, including *PDE1C*, which is highly expressed in the eyes, and known to be involved in retinal development. Further identification of the causal variants at this linkage peak will help elucidate the genetics of myopia in this understudied population.

Keywords: genetics, linkage analysis, family studies, genetic association

More people in the world are afflicted with myopia than any other eye disorder. The World Health Organization defines uncorrected refractive errors like myopia as visual impairments and estimates that about 153 million people are living worldwide with uncorrected refractive errors.[1] One quarter of the American population is myopic, and prevalence is rising.[2] Lower-income and disadvantaged populations are particularly at risk because they lack the finances to correct the impairment and thus suffer greater than more affluent populations.

Myopia is a complex disease caused by both genetic and environmental factors, making analysis of the phenotype challenging.[3,4] Multiple environmental factors have been identified, including education level and time outside.[3]

Multiple genetic factors have also been identified to contribute to myopia risk; genetic studies of myopia consist of both family-based linkage studies and population-based association studies. Each method has its advantages/disadvantages. Population-based association studies, specifically genomewide association studies (GWAS), are more effective at identifying common variants with a small to moderate effect on the trait. Many GWAS that have found genomewide significant variants associated with myopia and its quantitative phenotype refractive error.[5–13]

Family-based linkage studies are effective at finding rare, highly penetrant variants. Variants that are rare in the population at large may be common within an individual family. Family-based studies can also offer better coverage of the genome via longer haplotypes. Haplotypes in population-based studies have been broken apart by generations of recombination so only a small number of variants are in linkage disequilibrium (LD). By contrast, the number of

meioses that can occur within a given family is quite small, creating longer haplotypes within a family that can be used to tag rare or ungenotyped causal variants in LD with the linked variants. The drawback is that additional variants along the linked haplotype means identifying the actual causal variant is more difficult. Genomewide significant linked loci have been identified for both refractive error and myopia.[14–26] Multiple studies have reported linkage using common myopia.[18–26]

African Americans have been particularly understudied for myopia. Initial studies showed that African Americans had a lower prevalence of myopia than Caucasians[27]; more recent studies show myopia prevalence in African American children is approximately equal to Caucasians.[28] African American children have been shown to have both a higher percentage of new myopia cases[29] and a higher odds ratio for myopia risk than Caucasian children.[30] Other African groups show various prevalences in children – 3% in Ghana[31] and South Africa,[32] and 10% for African Caribbean children in England.[23] A more recent study by Jiang et al. showed that a parent with myopia was associated with an increase of myopia risk in children of multiple populations, including African Americans.[33] A 2020 review by Grzybowski et al., concluded that myopia prevalences in children are rising in Asia, Europe, and North America but are under 10% in South America and Africa.[34]

Despite ample evidence for myopia prevalence, there have been a paucity of genetic studies with African samples, with zero GWAS and only a handful of microsatellite-based linkage studies.[35,36] Our study is the first genetic analysis using SNP genotypes in African American families with a history of myopia. We used an exome-based microarray for increased coverage of rare variants. A subset of the families used in this study were part of a previous study that found significant linkage to 7p15 with refractive error[36] using microsatellites. Using myopia affection as the phenotype did not result in replication of the signal[35] nor was the signal replicated in meta-analysis with other populations.[16]

Methods

Patient Recruitment

We collected data from 517 individuals from 106 African American families in the Philadelphia metropolitan area as part of the Family Myopia Study. Prospective participants were identified through database review, mailings, clinical visits, interviews, and referrals from private doctors. Eligible families were required to have at least three participants, including at least one parent with myopia, and one myopic sibling. All study participants provided informed consent and protocols adhered to the tenets of the Declaration of Helsinki. The study was approved by the institutional review boards of the University of Pennsylvania and the National Human Genome Research Institute.

All participants received a comprehensive eye examination, including visual acuity, slit lamp biomicroscopy, dilated fundus examination, and manifest refraction. Patients older than 41 years had their refraction measured with manifest refraction, whereas patients 41 years and younger had their refraction measured using cycloplegic refraction. The measurement used in this study was mean spherical equivalent (MSE), measured in diopters (D), which is calculated by adding the spherical component to one-half the cylindrical component and averaging for both eyes.

Genotyping and Quality Control

Five hundred seventeen subject DNA samples were genotyped using an Illumina ExomePlus array at the Center for Inherited Disease Research (CIDR) at Johns Hopkins University (Baltimore, MD, USA). Variants were filtered to a mean call rate of 99%, and any variant with a quality score of 0.15 or less was set to missing. Monomorphic markers were removed using PLINK.[37] Sib-pair[38] was used to identify Mendelian inconsistencies. Markers with a Mendelian inconsistency in a single family were removed from that family; markers with multiple Mendelian error were removed from all families. PLINK and Prest-Plus[39] were used to verify familial relationships by calculating identity by descent (IBD) values.

Ten ungenotyped individuals were added to the data set to connect disjointed pedigrees. The existence of these individuals was confirmed by family history, but they were either unwilling or unavailable to participate. Their phenotypes and genotypes were coded as unknown.

The single-nucleotide polymorphism (SNP) data was merged with a previous set of 367 microsatellite genotypes; 493 individuals from the exome-based array had microsatellite data. The final data set consisted of 527 individuals with 98,631 markers.

Myopia Affection Classification

Subjects were classified as either affected or unaffected with myopia. Individuals with MSE ≤ -1.0 D were coded as affected. Adult participants (21 and over) with an MSE of ≥ 0.0 D were coded as unaffected or unknown if the MSE was between -1.0 and 0.0 D in order to avoid potential misclassification errors. We used extra caution when coding children as unaffected, because normal childhood developmental changes can result in misclassification. Children ages 5 to 10 years were coded as unaffected only if their MSE was ≥ 2.0 D and as unknown between -1.0 and 2.0 D. Children aged 11 to 20 years were coded as unaffected if their MSE was ≥ 1.5 D and as unknown between -1.0 and 1.5 D. All children were affected if their MSE was ≤ -1.0 D. These thresholds were based on ophthalmological guidelines as to what levels of childhood refraction are most indicative of myopia in adulthood and the large number of children deemed to be unknown is designed to allow for uncertainty in these projections. The final data set contained a total of 527 individuals (295 as affected, 100 as unaffected, and 132 as unknown/missing). The data were 58.06% female individuals (306 female individuals to 221 male individuals). The average MSE was -2.78 D with a standard deviation of 3.60. The mean age of the entire data set was 40.37 with a standard deviation of 19. The mean age of the adults (21 years and above) was 47.42 (standard deviation of 14.83) and the mean age of the children (20 years and below) was 14.15 (standard deviation of 3.62).

Allele Frequency Estimation

Allele frequencies for the data set were calculated in Sib-pair.[38] Estimating allele frequencies directly from an ethnically homogeneous data set properly controls type I error rates in parametric linkage studies.[40–42]

Parametric Linkage Analysis

We performed both variant-based and gene-based parametric linkage analyses. In linkage analyses, all individuals

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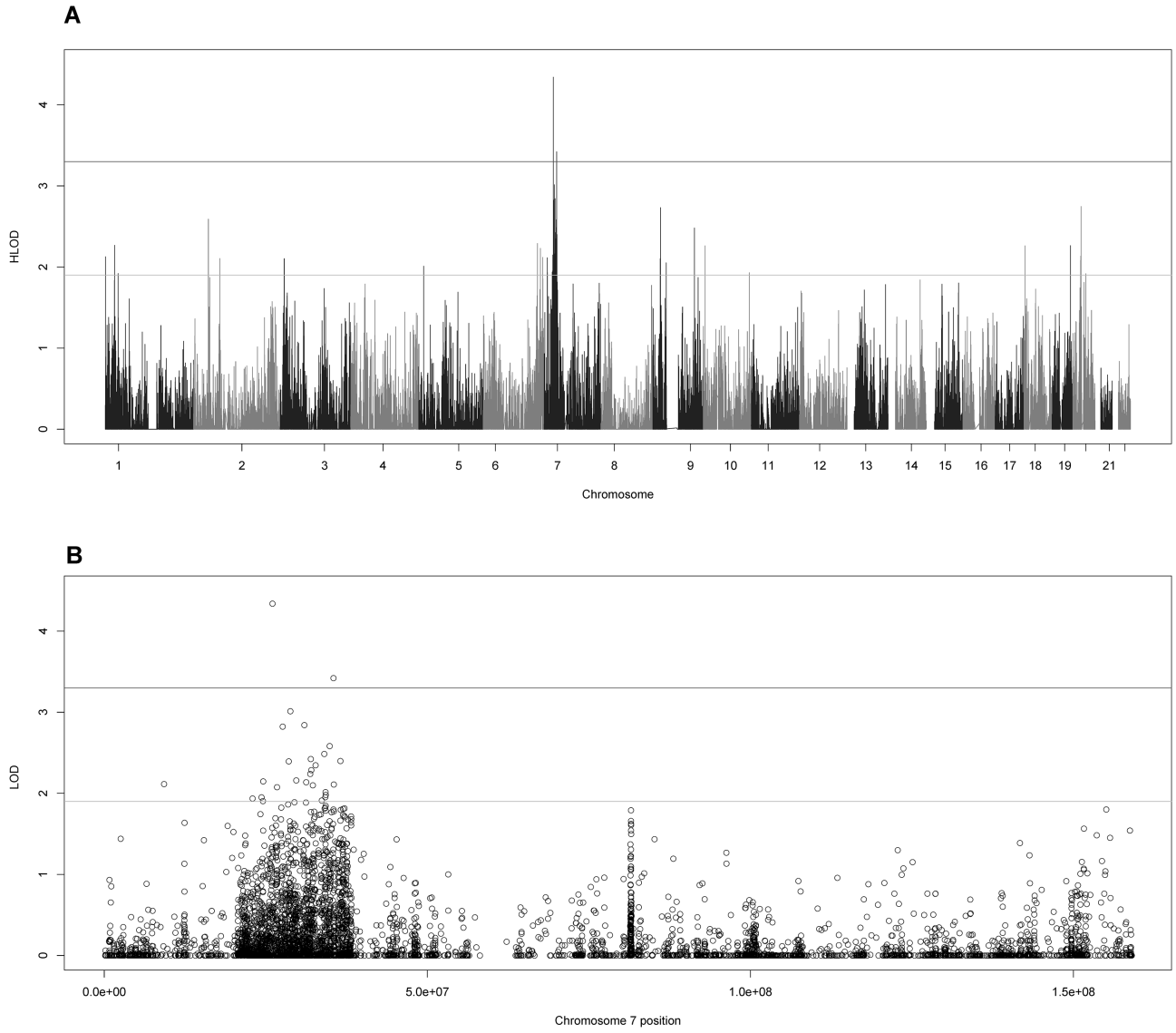

Figure 1. HLOD scores for variant-based two-point linkage analysis. (**A**) The genomewide HLOD scores (**B**) the HLOD scores for chromosome 7. In both, the lines at 3.3 and 1.9 represent the respective significant and suggestive thresholds as suggested by Lander and Kruglyak.

(including those with missing/unknown phenotypes) are included in the analyses. This is because individuals with missing/unknown phenotypes with genotype information will provide information about allele transmission (thus contributing to the overall LOD score of the variant) and even individuals with no phenotype or genotype information may be needed to provide relationship information to connect relatives with data and avoid disjointed pedigrees. Analyses assumed an autosomal dominant mode of inheritance, with a disease allele frequency of 0.01 and a penetrance of 0.9 for disease allele carriers and 0.1 for noncarriers. The 0.1 penetrance for noncarriers (the phenocopy rate) allow for a 10% chance that an individual that has myopia for reasons other than a high-risk variant (e.g. environmental factors or polygenic inheritance).

Variant-based analyses were two-point linkage analyses performed between the phenotype and each individual SNP, using TwoPointLods.[43] Gene-based analyses used the collapsed haplotype pattern (CHP) method in SEQLinkage[44] to build multi-allelic pseudomarkers, which corresponded to a gene. Two-point linkage analysis was then performed on the pseudomarkers using Merlin.[45] We performed two sets of gene-based analysis: one analysis using rare variants only (MAF $\leq$ 0.05) and one analysis using all variants.

Family-Based Association Analysis

We used the family-based association test (FBAT)[46] to perform variant-based and gene-based analysis association analyses. We also used the rare variant transmission disequilibrium test (RV-TDT)[47] by choosing the most informative trio out of the extended families - two parents (one affected and one unaffected) and affected child trio with the highest genotyping rate.

Functional Annotation

All variants were annotated using wANNOVAR[48,49] and CRAVAT,[50,51] which provide information about SNP location, function, and frequency across multiple populations. They

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Table 1. All Significant and Suggestive HLOD Scores From Variant Based Linkage

CHR	rsID	POS	HLOD	GENE	FUNC	EXON	FREQ	SIFT	POLYPH	FATHMM	CADD	REVEL
7	rs4719841	25997536	4.34	MIR148A; NFE2L3	Intergenic	.	0.27	.	.	.	.	.
7	rs235397	35372749	3.42	LOC401324	ncRNA	.	0.20	.	.	.	.	.
7	rs6462100	28754095	3.01	CREB5	Intronic	.	0.40	.	.	.	.	.
7	rs7797330	30895010	2.84	INMT-MINDY4	ncRNA	.	0.38	.	.	.	.	.
7	rs7779240	27562660	2.82	EVX1; HIBADH	Intergenic	.	0.15	.	.	.	.	.
20	rs3746736	23424613	2.75	CSTL1	Exonic	nonsyn	0.20	T	B	T	0.003	0.086
9	rs10757225	21555445	2.73	MIR31HG	ncRNA	.	0.18	.	.	.	.	.
2	rs1920511	41792845	2.59	SLC8A1; LINC01913	Intergenic	.	0.33	.	.	.	.	.
7	rs10270663	34786398	2.58	NPSR1-AS1	ncRNA	.	0.20	.	.	.	.	.
7	rs1427483	33959239	2.49	BMPER	Intronic	.	0.29	.	.	.	.	.
9	rs61757558	117118379	2.48	AKNA	Exonic	nonsyn	0.06	D	B	T	22.6	0.019
7	rs2270219	31877261	2.42	PDE1C	Intronic	.	0.23	.	.	.	.	.
7	rs3735400	36438709	2.40	ANLN	Exonic	nonsyn	0.12	D	D	T	29.7	0.204
7	rs6462088	28504566	2.40	CREB5	Intronic	.	0.24	.	.	.	.	.
7	rs2011974	32611392	2.35	AVL9	Intronic	.	0.34	.	.	.	.	.
6	rs214950	152708310	2.29	SYNE1	Exonic	nonsyn	0.15	D	B	T	7.324	0.104
7	rs10266620	31957550	2.29	PDE1C	Intronic	.	0.26	.	.	.	.	.
1	rs7550997	26596080	2.27	CEP85	Exonic	nonsyn	0.18	T	B	T	15.09	0.043
1	rs8564	26605069	2.27	CEP85	UTR3	.	0.18	.	.	.	.	.
1	rs7544	26607726	2.27	SH3BGRL3	UTR3	.	0.18	.	.	.	.	.
1	rs10493030	26561856	2.27	CEP85	Intronic	.	0.18	.	.	.	.	.
1	rs10902732	26606174	2.27	SH3BGRL3; CEP85	Intergenic		0.18	.	.	.	.	.
1	rs11247900	26612460	2.27	UBXN11	Exonic	syn	0.18	.	.	.	.	.
1	rs11577318	26601570	2.27	CEP85	Intronic	.	0.18	.	.	.	.	.
1	rs17163746	26564230	2.27	CEP85	Intronic	.	0.18	.	.	.	.	.
1	rs17163749	26568165	2.26	CEP85	Intronic	.	0.18	.	.	.	.	.
10	rs61729846	5920244	2.26	ANKRD16	Exonic	nonsyn	0.20	D	D	T	26.1	0.690
18	rs387462	3458997	2.26	TGIF1	downstream	.	0.36	.	.	.	.	.
7	rs6952967	31795856	2.24	PDE1C	Intronic	.	0.47	.	.	.	.	.
6	rs791183	160610124	2.23	SLC22A1; SLC22A2	Intergenic	.	0.40	.	.	.	.	.
7	rs1420123	29647662	2.16	PRR15; LOC646762	Intergenic	.	0.27	.	.	.	.	.
7	rs1029602	24571485	2.15	NPY; MPP6	Intergenic	.	0.38	.	.	.	.	.
7	rs4291168	31178749	2.14	ADCYAP1R1; NEUROD6	Intergenic	.	0.18	.	.	.	.	.
20	rs6036107	22403287	2.13	LOC284788; LINC00261	Intergenic	.	0.33	.	.	.	.	.
1	rs2228579	1223385	2.13	SCNN1D	Exonic	nonsyn	0.33	T	B	T	0.003	0.036
6	rs34544438	167438292	2.12	FGFR1OP	Exonic	nonsyn	0.07	T	B	T	0.268	0.119
7	rs1285407	9266388	2.11	NXPH1; PER4	Intergenic	.	0.12	.	.	.	.	.
7	rs12113424	35423720	2.11	LOC401324; HERPUD2	Intergenic	.	0.19	.	.	.	.	.
2	rs13424561	73868446	2.11	NAT8	Exonic	nonsyn	0.11	T	B	T	0.166	0.01
3	rs36117895	11400019	2.10	ATG7	Exonic	nonsyn	0.12	D	P	T	25.3	0.227
9	rs10511687	20764870	2.10	FOCAD	Exonic	nonsyn	0.32	T	B	T	14.75	0.069
7	rs6415258	32192596	2.10	PDE1C	Intronic	.	0.27	.	.	.	.	.
7	rs212837	26695215	2.08	C7orf71; SKAP2	Intergenic	.	0.35	.	.	.	.	.
1	rs12138111	26590432	2.07	CEP85	Intronic	.	0.19	.	.	.	.	.
18	rs77600482	3460731	2.06	TGIF1; GAPLINC	Intergenic	.	0.15	.	.	.	.	.
9	rs10973446	37638744	2.05	TOMM5; FRMPD1	Intergenic	.	0.45	.	.	.	.	.
18	rs381234	3464650	2.02	TGIF1; GAPLINC	Intergenic	.	0.38	.	.	.	.	.
7	rs731844	34150264	2.02	BMPER	Intronic	.	0.39	.	.	.	.	.
5	rs7715811	13769974	2.01	DNAH5	Intronic	.	0.39	.	.	.	.	.
5	rs1502050	13779743	2.01	DNAH5	Intronic	.	0.38	.	.	.	.	.
7	rs10224983	34180326	1.98	BMPER	Intronic	.	0.15	.	.	.	.	.
7	rs961652	34111660	1.95	BMPER	Intronic	.	0.30	.	.	.	.	.
7	rs16480	24311069	1.95	STK31; NPY	Intergenic	.	0.38	.	.	.	.	.
7	rs2033670	22929061	1.94	SNHG26; FAM126A	Intergenic	.	0.33	.	.	.	.	.
10	rs7071768	129903016	1.93	MKI67	Exonic	nonsyn	0.47	T	B	T	0.001	0.022
1	rs10908292	36764770	1.92	THRAP3	Intronic	.	0.39	.	.	.	.	.
20	rs5741809	36956026	1.92	BPI	Exonic	nonsyn	0.14	T	B	T	0.001	0.011
7	rs2392246	33571828	1.91	BBS9	Intronic	.	0.12	.	.	.	.	.
7	rs976681	24530016	1.91	NPY; MPP6	Intergenic	.	0.36	.	.	.	.	.

The list of all significant and suggestive variants from the variant-based linkage analyses, as sorted by HLOD. Here, the headers represent: CHR = chromosome, rsID = rsID of the SNP, POS = physical position in base pairs of the SNP, HLOD = heterogeneity LOD score across all 106 families, GENE = Gene location of the SNP (if intergenic then the two closest genes), FUNC = function of the SNP (e.g. exonic, intronic), EXON = if exonic, the exonic function of the SNP (nonsyn = nonsynonymous, syn = synonymous), FREQ = frequency of the variant in gnomAD Africans, SIFT = SIFT prediction (T = tolerated, D = damaging), POLY = PolyPhen2 prediction score (B = benign, P = possibly damaging, D = damaging), FATHMM = FATHMM prediction (T = tolerated), CADD = CADD phred score $\geq$ 10 corresponds to 10% most deleterious substitutions in genome, $\geq$ 20 corresponds to 1% most deleterious substitutions in the genome, etc.), REVEL = REVEL score (corresponds to proportion of trees in random forest algorithm that classified variant as pathogenic).

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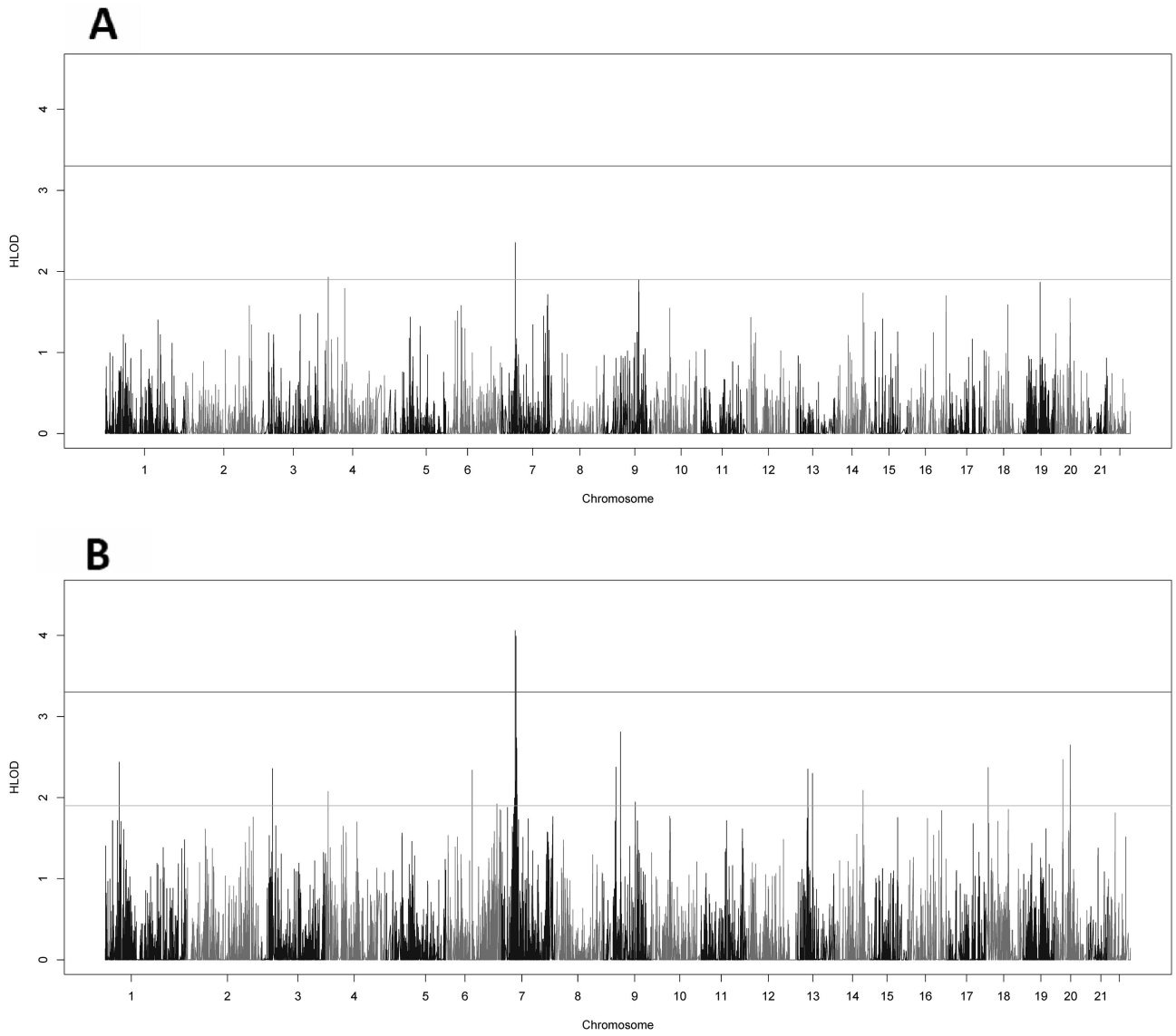

Figure 2. Genome wide HLODs scores for gene-based two-point linkage analysis. (**A**) The gene-based HLOD scores using only the rare variants (MAF $\leq$ 0.05) and (**B**) the gene-based HLOD scores using all variants. The lines at 3.3 and 1.9 represent the respective significant and suggestive thresholds as suggested by Lander and Kruglyak.

also provide protein predictions from multiple programs such as SIFT,[52–56] PolyPhen2,[57] CADD,[58,59] and REVEL.[60]

Gene Expression in Human Ocular Tissues

To identify high-priority candidate genes, we examined ocular tissue expression of the significant/suggestive genes from our analyses. Gene expression in human ocular tissues was inspected in the publicly available web resources eyeIntegration[61] and The Ocular Tissue Databases.[62] The eyeIntegration database provides the largest RNA-seq based transcriptome database of healthy human eye tissues and hundreds of Genotype-Tissue Expression (GTEx) tissue samples.[63,64] The Ocular Tissue Database contains microarray expression data of 10 normal human ocular tissues.[65] We compared the expression of our significant and suggestive genes in the eyes against two reference tissues (whole blood and pan-body synthetic subtissues). The pan-body synthetic set was comprised of a stratified sample of 54 tissues in the GTEx data set.

Results

Variant-Based Linkage Results

Two variants exhibited genomewide significant linkage to myopia using a definition of genomewide significant as (H)LOD $\geq$ 3.3 and genomewide suggestive as (H)LOD $\geq$ 1.9 in accordance with the recommendations of Lander and Kruglyak.[66] The rs4719841 is in the intergenic region between *MIR148A* and *NFE2L3* at 7p15.2 (HLOD = 4.34), and rs235397 is located in the noncoding RNA *LOC401324* at 7p14.2 (HLOD = 3.43; Fig. 1A). Twenty-five suggestively linked SNPs were found at 7p15.2-14.2 (Fig. 1B). Thirty-three suggestive SNPs were found on other chromosomes, with the largest concentration at 1p36.1-36.11 (Table 1).

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Functional Annotation of Variants

The rs4719841 is intergenic and has an MAF in the gnomAD database of 0.2656 and 0.26 in our data set. The rs235397, in the noncoding RNA *LOC401324* at 7p14.2, has an MAF of 0.2 both in gnomAD and our data set. The only exonic variant in 7p15.2-14.2 was rs3735400 (HLOD = 2.22). The rs3735400 is in *ANLN* (7p14.2), is a nonsynonymous exonic variant, has a Combined Annotation Dependent Depletion (CADD) score over 29 and predicted damaging by SIFT and PolyPhen2, and has an MAF of 0.1201 in the gnomAD 0.13 in our data set.

Gene-Based Linkage Results

The gene-based analysis using rare variants (MAF $\leq$ 0.05) produced no genomewide significant results and four genome-wide suggestive genes (Fig. 2A). The two top genes were located at 7p14.3 - *INMT-MINDY4* (HLOD = 2.35) and *MINDY4* (HLOD = 1.99).

Using all variants, the genomewide significant signal is recovered at the 7p14.3 band (Fig. 2B). This makes sense, as the variants identified in the variant-based analysis were common (MAF > 0.05). The genomewide significant genes were *CRHR2* (HLOD = 4.06) and *AVL9* (HLOD = 3.99). There were an additional 23 suggestive genes, with 7 genes in the 7p14.3-14.2 region (Table 2, Supplementary Fig. S1).

Association Results

No genomewide significant (5×10^{-8}) results were found in the association analyses. The most significant FBAT variant *P* value was rs887468 (1.2×10^{-5}) in *PSORS1C3* at 6q21.33. The most significant FBAT gene-based *P* value was *KCNS1* (4.97×10^{-5}) at 20q12. The most significant RV-TDT *P* value was the intergenic SNP rs11929331 (2.5×10^{-4}) between *LINC01994* and *ATP11B* at 3q26.44.

Gene Expression in Human Ocular Tissue

The eyeIntegration and Ocular Tissue databases revealed that a vast majority of the significant/suggestively genes were expressed in ocular tissues (Supplementary Table S1, Supplementary Figs. S2, S3). The suggestive genes (including *ANLN* and *PDE1C*) from the variant-based and gene-based analyses were found to have higher expression in most tissues of the eyes compared to whole blood and pan-body synthetic subtissues (Fig. 3, Supplementary Table S1). Other significant and suggestive genes (including *AVL9*, *BBS9*, and *BMPER*) have a higher expression in most of the ocular tissues compared to at least one reference tissue group (Fig. 4, Supplementary Table S1). The Ocular Tissue Database showed that *EPHB2*, *PDE1C*, *NPY*, *EVC*, and *COL12A1* have good expression in the adult human sclera, which may play a role in myopia pathogenesis (see Supplementary Figs. S2, S3). Both databases are primarily derived from European ancestry individuals, so expression may vary in African Americans, but there are no known databases with African American eye expression tissue data.

Table 2. All Significant and Suggestive Genes From the Gene-based Linkage Analysis

CHR	POS	GENE	CUMUL LOD	HLOD	VARIANTS
7	48.34	*CRHR2*	3.98	4.06	All
7	50.78	*AVL9*	3.99	3.99	All
9	59.79	*DNAI1*	2.81	2.81	All
7	52.59	*NPSR1-AS1*	2.74	2.74	All
7	52.05	*BMPER*	1.86	2.73	All
20	54.18	*BPIFA2*	2.64	2.65	All
7	54.00	*SEPT7*	2.34	2.61	All
20	28.26	*PAK7*	1.75	2.47	All
1	49.16	*EPHB2*	2.44	2.44	All
9	43.87	*FOCAD*	2.14	2.37	All
18	8.08	*SMCHD1*	2.37	2.37	All
3	39.61	*EFHB*	1.77	2.36	All
7	48.59	*INMT-FAM188B*	2.35	2.35	Rare
13	36.59	*CSNK1A1L*	2.15	2.35	All
6	92.24	*COL12A1*	2.34	2.34	All
13	52.86	*SETDB2*	1.94	2.30	All
7	50.30	*PDE1C*	2.21	2.21	All
20	55.10	*CEP250*	1.93	2.10	All
14	97.59	*SERPINA9*	1.35	2.09	All
4	9.39	*EVC*	0.92	2.08	All
7	54.69	*KIAA0895*	1.34	2.04	All
7	46.21	*LOC646762*	1.49	1.99	All
7	48.61	*FAM188B*	1.99	1.99	Rare
9	111.62	*ZNF462*	1.95	1.95	All
4	9.39	*EVC*	1.93	1.93	Rare
6	178.62	*PACRG*	0.72	1.92	All
1	49.16	*EPHB2*	1.92	1.92	All
7	50.30	*PDE1C*	0.31	1.91	All
9	123.13	*AKNA*	1.90	1.90	Rare

The list of all significant and suggestive genes from the gene-based linkage analyses, as sorted by HLOD. Here the headers represent: CHR = chromosome, POS = genetic position in cM of the gene, GENE = gene, CUMUL LOD = cumulative LOD score for the gene across all 106 families, HLOD = heterogeneity LOD score for the gene across all 106 families, VARIANTS = type of variants used in this test (All = all variants were used, Rare = only rare variants (MAF $\leq$ 0.05) were used).

Discussion

We have identified a genomewide significant linkage between four markers (2 SNPs and 2 genes) at 7p15.2-14.2 and myopia in African American families. This is the largest genetic analysis of myopia in the African American population and the first using SNP genotypes. This is the first study to report a myopia risk locus in African Americans. African Americans have been severely understudied with respect to myopia and refractive error. We note that the 7p15 linkage signal is not entirely novel, as we have previously identified a genomewide significant linkage to refractive error at 7p15 in a subset of these African American families[36] and replicated it in a Caucasian data set.[67] Both those studies used a different phenotype (quantitative refractive error scores) and the African American study used only microsatellite data and pointedly did not find significant linkage anywhere when myopia affection (the trait used in this study) was the analyzed trait.[35] The risk locus identified at 7p14.2 is entirely novel; it was not identified in the microsatellite study. Further, the increased granularity of the SNP data (especially with the addition of the rare exonic variants), allowed for the location of regions and genes where the linked variants are accumulating, which cannot be done in a study with sparse microsatellite data only. This allowed us to go beyond defining a single linked region and offer specific genes within that linked region that might be causal, which is discussed in detail in the following paragraphs.

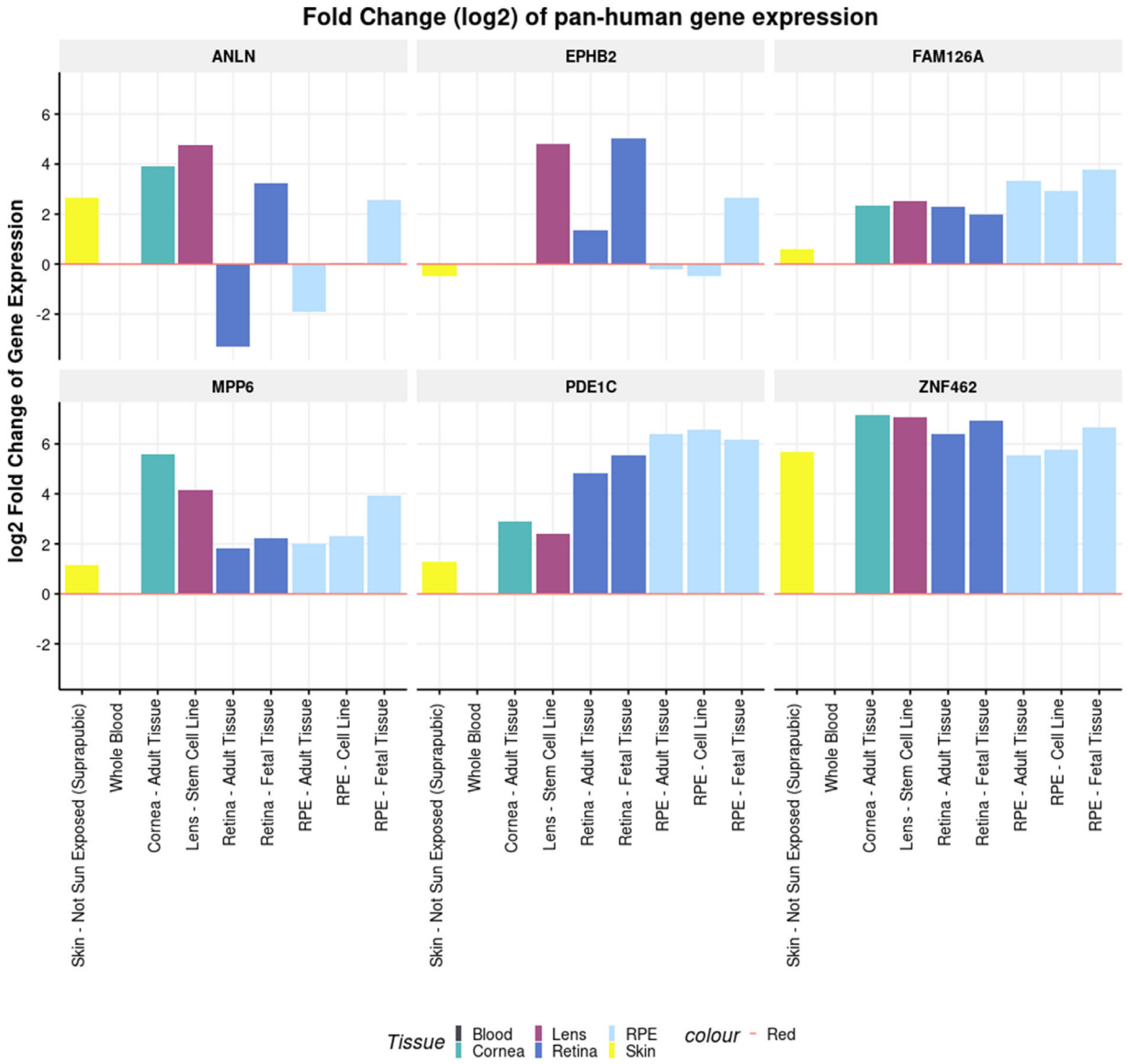

Figure 3. Pan-human tissue differential expression of *ANLN*, *FAM126A*, *MPP6*, *PDE1C*, *EPHB2*, and *ZNF462*. The x-axis shows the different types of tissues used in the test. The y-axis shows the log2 fold change of gene expression. The differential expression is being shown relative to the reference tissue (whole blood).

The risk locus identified at 7p15 is known as *MYP17*, and, despite multiple replications, the causal gene remains unknown. Our significant linkage signals in the variant-based analysis are in an intergenic region between the transcription factor *NFE2L3* and the noncoding RNAs *MIR148A* at 7p15.2 and *LOC401324* at 7p14.2. None of these genes have any previously known connection to eye disorders. The significant genes found by the gene-based analysis (*CRHR2* and *AVL9*) were located slightly downstream at 7p14.3 and have no known association with eye disease. *AVL9* and *CRHR2* expressions were enriched in most ocular tissues versus reference tissue groups.

Linkage peaks are often broad and locus heterogeneity and other factors add to the uncertainty. Thus, the true causal variant(s) may lie anywhere within the linked region. Thus, it is important to examine the entire linked 7p region for candidate genes, which does identify multiple good candidates.

The rs3735400 (HLOD = 2.4) in the anillin (*ANLN*) gene were predicted damaging by both SIFT and PolyPhen2 and had a CADD score over 29. Thus, the variant is likely to have a deleterious effect on gene/protein function. Anillin is an actin binding protein that is involved in cell migration, cell growth, and cytokinesis; it regulates actin cytoskeletal dynamics and has been implicated in multiple cancers.[68] Its expression was shown to be elevated in eye tissue.

PDE1C (7p14.3) exhibited suggestive evidence of linkage in the gene-based test (HLOD = 2.2) and had four suggestive intronic variants. *PDE1C* is a phosphodiesterase that has been reported to be involved in avian and rodent retinal development.[69,70] *PDE1C* is expressed significantly higher in both adult and fetal retina tissue and adult and cell line RPE compared to whole blood and pan-body tissues. *BMPER* (7p14.3) was found to be suggestively linked in both the variant-based and gene-based analyses. *BMPER* has been

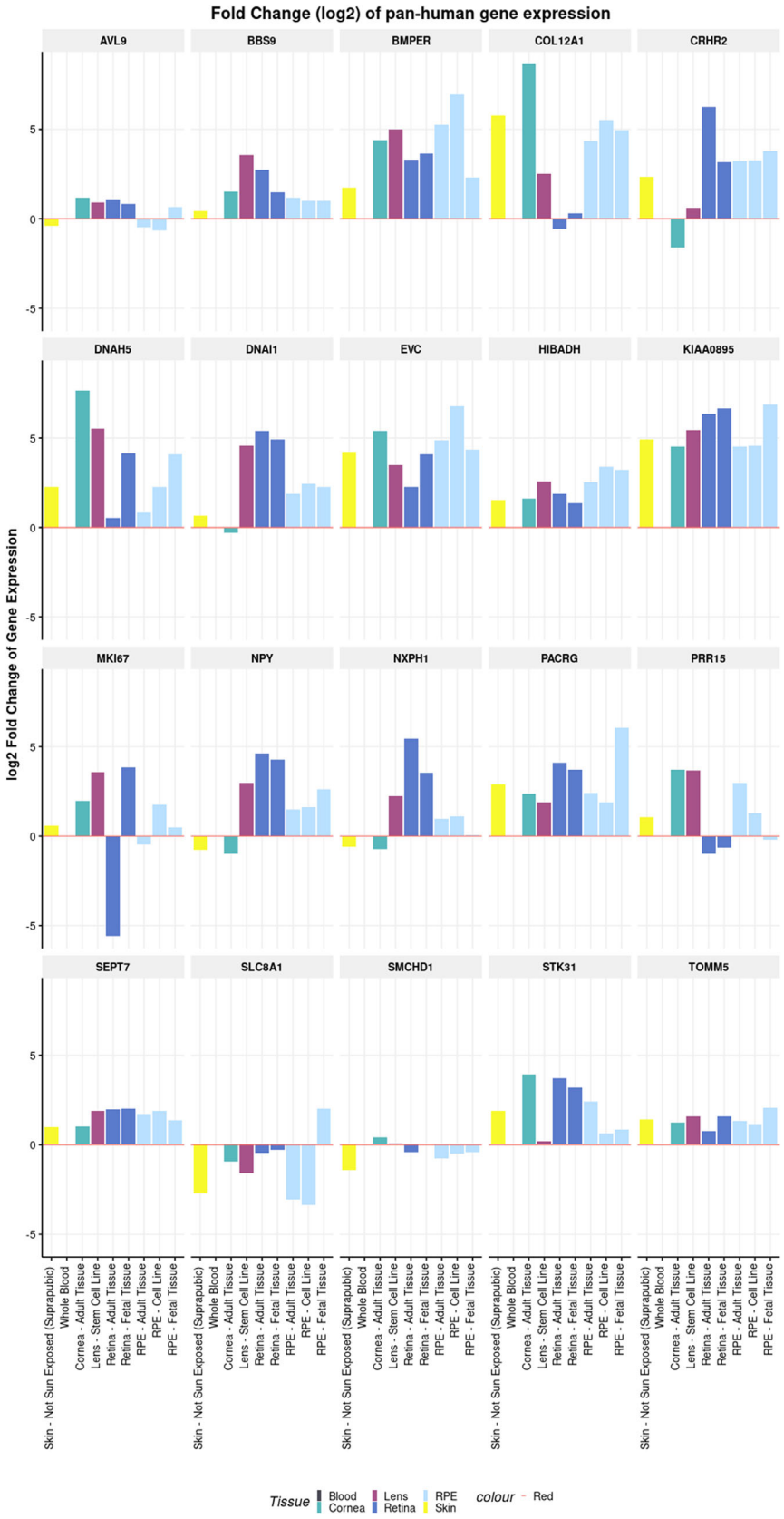

Figure 4. Pan-human tissue differential expression of additional significant and suggestive genes in the two-point linkage analysis. The x-axis shows the different types of tissues used in the test. The y-axis shows the log2 fold change of gene expression. The differential expression is being shown relative to the reference tissue (whole blood).

shown to be functionally involved in eye organogenesis in mice.[71] *BBS9*, which was found to have a genomewide suggestive intronic variant (HLOD = 1.9), is known to cause Bardet-Biedl syndrome, a symptom of which includes retinal degeneration.[72,73] Both genes were expressed higher in ocular tissue than the reference set.

In addition to the significantly linked region on 7p, we also identified several genomewide suggestive linkage signals, the most interesting being located at 1p36. This suggestive signal is a replication of a well-documented myopia/refractive error risk locus, *MYP14*, at 1p36.[15,67,74,75] This is the first time that this linkage to myopia has been documented in African Americans. The causal gene at *MYP14* remains elusive. Six of the 9 suggestive SNPs at 1p36 were intronic SNPs located in *CEP85*. *CEP85* is involved in centromere disjunction and has no known connection to eye disease.[76] Other centromere proteins in the CEP family have been found implicated in eye disease, like *CEP250* in Usher syndrome (which has retinitis pigmentosa as one of its symptoms).[77]

The association analyses did not identify any genomewide significant signals. The top variant was in an intron of the psoriasis gene *PSORS1C3*. The top gene was *KCNS1* at 20q12, a voltage gated potassium channel protein that is functional in lens epithelia.[78] The top SNP in the RV-TDT analysis in an intergenic region between *LINC01994* and *ATP11B* at 3q26.44. The 3q26 is the site of the known myopia locus *MYP8*[79] and *ATP11B* has been shown to be present in mouse retina.[80]

It is not surprising that the linkage and association results tended to disagree, because they are testing for different things. The family-based association analysis relies on the risk allele being shared across families, either identical by state (IBS) or IBD. Linkage tracks co-segregation of haplotypes and the phenotype within a family but does not require that these haplotypes contain alleles IBS across different families.

Although we have given evidence for some interesting candidate genes for the 7p and 1p signals any implication of causality is speculative at this point. Several genes, including the eye development genes *PDE1C* and *BMPER*, are good candidates for future follow-up studies, but additional studies will be needed to determine which variants are responsible for the observed linkage peaks. We also note that any novel linkage findings will need further replication in independent data sets. This study's major limitation was the coverage of the exome-enriched microarray. The causal variant may not have been identified; it is more likely that ungenotyped variants are segregating on the linked haplotypes in each family in LD with the more common variants identified here. This haplotype tagging ability is one of the advantages of family-based linkage studies. This explains why most of the linked variants on the 7p haplotype are common; they are most likely tagging rare variants that were not genotyped on this limited microarray. This also explains why we only identified a genomewide suggestive signal at 7p when using only rare variants in the gene-based tests and why the genomewide significant signal was recovered upon returning the common variants to the analysis. Thus, targeted sequencing of the linked regions, particularly on 7p and 1p, is a necessary future step in determining the causal variant(s).

The expression databases helped to narrow down our candidate genes. We should exercise caution in the interpretation of these findings because the samples from the transcriptome databases were from healthy human eye tissues and their expression profile may differ from the diseased eye. We also do not have a refractive error phenotype on the eyes used in these databases. Further, the tissue used in these databases were primarily from European ancestry individuals, so any expression results must be evaluated within that context.

We have identified a genomewide significant linkage of myopia to the 7p15.2-14.2 region in African Americans. This is the first significant myopia risk locus found in African Americans. This supports a previous study which has linked 7p15 with refractive error.[36,67] We also identified several genomewide suggestive signals, including replication of the *MYP14* locus at 1p36 for the first time in African Americans. We note that linkage analyses like this one identifies large linked regions and this study aimed to identify good candidate genes/variants and which families are most informative. We plan further whole genome sequencing of our most informative families, which will give us increased coverage of the genome, particularly intronic regions. Deep coverage of these linked regions may elucidate the causal genes/variants.

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