An International Collaborative Family-Based Whole-Genome Linkage Scan for High-Grade Myopia

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Purpose. Several nonsyndromic high-grade myopia loci have been mapped primarily by microsatellite markers and a limited number of pedigrees. In this study, whole-genome linkage scans were performed for high-grade myopia, using single nucleotide polymorphisms (SNPs) in 254 families from five independent sites.

Methods. Genomic DNA samples from 1411 subjects were genotyped (Linkage Panel IVb; Illumina, San Diego, CA). Linkage analyses were performed on 1201 samples from 10 Asian, 12 African-American, and 221 Caucasian families, screening for 5744 SNPs after quality-control exclusions. Two disease states defined by sphere (SPH) and spherical equivalence (SE; sphere+cylinder/2) were analyzed. Parametric and nonparametric two-point and multipoint linkage analyses were performed using the FASTLINK, HOMOG, and MERLIN programs. Multiple stratified datasets were examined, including overall, center-specific, and race-specific. Linkage regions were declared suggestive if they had a peak LOD score ≥ 1.5.

Results. The MYP1, MYP3, MYP6, MYP11, MYP12, and MYP14 loci were replicated. The novel region q34.11 on chromosome 9 (max NPL= 2.07 at rs913275) was identified. Chromosome 12, region q21.2-24.12 (36.59 cM, MYP3 locus) showed significant linkage (peak HLOD = 3.48) at rs337663 in the overall dataset by SPH and was detected by the Duke, Asian, and Caucasian subsets as well. Potential shared interval was race dependent—a 9.4-cM region (rs163016–rs1520724) driven by the Asian subset and a 13.43-cM region (rs163016–rs1520724) driven by the Caucasian subset.

Conclusions. The present study is the largest linkage scan to date for familial high-grade myopia. The outcomes will facilitate the identification of genes implicated in myopic refractive error development and ocular growth. (*Invest Ophthalmol Vis Sci.* 2009;50:3116–3127) DOI:10.1167/iovs.08-2781

Myopia, or nearsightedness, is the most common eye disorder. High-grade levels of myopic refractive error (consensus threshold of −5.00 D sphere or more severe [more minus]) are associated with blindness, due to an increased risk of premature cataracts, glaucoma, retinal detachment, and chorioretinal degeneration.[1–7] The prevalence of myopia varies in different countries. Multiple studies have reported approximate prevalence rates of 17% in Australia, 26% in the United States, and 27% in Western Europe.[7–9] Higher prevalence rates of 71% to 96% have been reported in Asian countries such as Japan, Taiwan, Hong Kong, and Singapore.[10–12] Myopia is a significant global public health problem.

Multiple familial studies support a high genetic etiology of myopia.[13–16] Naiglin et al.[17] performed segregation analysis on 32 French multiplex families with high–grade myopia and determined an autosomal dominant (DOM) mode of inheritance. A high degree of familial aggregation of refractive error, and myopia in particular, was reported in the Beaver Dam Eye Study population after accounting for the effects of age, sex, and education.[18] Segregation analysis suggested the involvement of multiple genes, rather than a single major gene effect. Twin studies provide the most compelling evidence that myopia has a strong genetic component, with estimated heritability estimates ranging from 0.5 to 0.96.[14,19–22] The sibling recurrence risk ratio, λ_s (the increase in risk to siblings of a person with a disease compared with the population prevalence), for myopia has been estimated to be approximately 4.9 to 19.8 for sibs for high-grade myopia (−6.00 spherical D or greater), and approximately 1.5 to 3 for low-grade or common myopia (approximately −1.00 to −3.00 spherical D), suggesting a definite genetic basis for high-grade myopia, and a moderate to strong genetic basis for low-grade myopia.[23,24]

At least 20 genetic loci have been identified for high-grade and moderate myopia, usually using genome-wide microsatellite markers. Much of the current information on the genetic basis of human high-grade myopia is derived from studying a small number of extended pedigrees.[25–40] Loci identified to date for isolated nonsyndromic high-grade myopia are primarily DOM and highly penetrant. Whole-genome mapping studies have identified several candidate gene intervals for common, moderate myopia in twin or case–control datasets primarily. The results of these studies demonstrate the potential for determining molecular genetic factors implicated in myopia at all levels of severity.[36,41–43] However, at least two studies have shown nominal or no linkage of low- to moderate-grade myopia to many of the known high-grade myopia loci.[44,45] The studies

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suggest that different genes account for mild to moderate myopia susceptibility or development, or that the genetic effects are too small to be detected with the relatively small sample sizes.

The present study is an international genotyping collaboration combining pedigrees from five sites. To our knowledge, this is the first large-scale linkage study of high-grade myopia. The purpose of this study was to map new high-grade myopia loci and to replicate and re-examine known myopia loci.

Materials and Methods

Patients and Families

The dataset consists of 254 multiplex families (at least two affected individuals per family) with a total of 1411 subjects (47% male). Families were ascertained independently at five international sites: Cardiff University (CARD), Duke University Medical Center in the United States (DUK), National Eye Clinic, Kennedy Institute in Denmark (HEL), University of Melbourne in Australia (MEL), and Toulouse University in France (TOU). All study sites obtained the appropriate institutional review board human subject research study approvals before initiating recruitment. Individuals were not included in the study if there was known ocular disease or insult that could predispose to myopia, such as retinopathy of prematurity, retinal dystrophy, corneal keratopathy, or any genetic syndromes that include myopia as a clinical phenotypic component. All subjects provided consent according to the Declaration of Helsinki and underwent a complete eye examination by licensed ophthalmologists or optometrists. Each subject completed clinical and family history questionnaires developed at each study site. Objective measurements for refractive error were documented for most subjects. Axial length data were not available for the CARD and HEL datasets. Keratometry data from some subjects were obtained in all sites except CARD. For the overall dataset, limited axial length and keratometry measurements were available (25% and 35%, respectively). The descriptive statistics of shared clinical phenotypes and subject age from each center were obtained.

Two refractive error phenotypes, determined by the sphere (SPH) or spherical equivalence (SE) (sphere + cylinder/2), were created for each individual. Dichotomous affection status was based on having a SPH or SE ≤ -5.00 D for the least-affected eye. Unaffected individuals were primarily defined as having a SPH or SE > -5.00 D for both eyes for all analyses. We are aware of the arbitrary designation of unaffected individuals, which may affect the results of parametric linkage analyses in particular. Therefore, we verified the findings by analyzing the same datasets with different designations of unaffected status. The details are described in the linkage analysis section that follows later.

Sample Preparation and SNP Genotyping

All subject genomic DNA samples were assembled at the Duke University Center for Human Genetics (Duke CHG), with a sample concentration of 75 ng/μL, in a total volume of 110 μL. The majority of genomic DNA samples were extracted from blood (77.36%), whereas the remaining samples were derived from buccal mucosa (22.16%) or saliva (0.48%) specimens. All samples were shipped to the National Institutes of Health Center for Inherited Disease Research (CIDR) for genotyping. The genotyping platform used in this study was the Linkage Panel IVb of 6008 genome-wide SNPs (Illumina, San Diego, CA). The CIDR genotyping protocol required each 96-well DNA sample plate to include two CEPH DNA control samples and four replicates of subject DNA samples. The total number of samples genotyped in this study was 1411 subject DNA samples, 81 anonymous DNA replicates, and 87 CEPH DNA control samples. The genotype data were transferred to the Duke CHG for data analysis.

Quality Control and Data Cleaning

The CIDR genotyping protocol instituted several quality control measures to determine and provide the final set of markers released for analysis. These included reproducibility rate determinations comparatively, obtained by using the replicated sample genotyping calls, Mendelian inconsistency rate determinations and calls, Y-linked marker genotyping to confirm the sex status associated with a particular DNA sample, and the GeneCall score which is a quality measure for each genotype used in the Illumina genotyping system. In particular, the GeneCall score measures how close a genotype is to the center of the cluster of other samples assigned to the same genotypes, compared with the centers of the clusters of the other genotypes. This measure ranges from 0 to 1. The higher the GeneCall score, the more reliable the genotype. A set of 5928 SNPs were released for linkage analysis, of which 5903 markers had GeneCall scores greater than 0.15. These were used for additional data cleaning.

For additional data quality assurance, 700 markers with approximately equal intermarker distances across the genome were selected to examine family relationships using the RELPAIR[46,47] and PREST[48] programs. After correcting for family relationship errors, Mendelian consistency was checked again with the PEDCHECK software program.[49] Either missing genotypes were assigned to the family with Mendelian inconsistencies in certain members, or the individual was dropped from further analysis if the family designations were ambiguous and not reconcilable.

All SNPs were tested for Hardy-Weinberg equilibrium (HWE). Two datasets with unrelated samples were formed in which one affected individual sample per family was randomly selected to cluster within a designated affected group, and one unaffected individual sample per family was selected to add to a designated unaffected group. An exact test implemented in the Genetic Data Analysis (GDA) program was used to test HWE in which 3200 permutations were performed to estimate the empiric *P*-value for each marker.[50] To correct for multiple testing, *q*-values generated from the Q-VALUE program (http://faculty.washington.edu/jstorey/qvalue/ provided in the public domain by the University of Washington, Seattle) were computed for all markers.[51] A marker was declared significantly deviated from HWE if the *q*-value was less than 20%. Markers that were out of HWE were excluded in the linkage analysis.

Whole Genome Linkage Analysis

As described earlier, we delineated two refractive error phenotypes per individual based on SPH and SE criteria. The same analysis procedures were performed for both phenotypes. Two-point parametric linkage analyses using the FASTLINK and HOMOG programs (http://linkage.rockefeller.edu/ provides in the public domain by Rockefeller University, New York, NY) were performed, and HLOD scores were generated. Since the mode of inheritance is unknown for high-grade myopia, both DOM and autosomal recessive (REC) genetic models were assumed in the parametric analysis with disease allele frequencies of 0.01 for both models. The penetrance settings of an individual who was deemed affected by the susceptibility genotypes were based on the assumed genetic models and were consistent with the goal of conducting affecteds-only parametric linkage analysis. To examine robustness to phenotype misclassification in the unaffected, we examined three different classifications of unaffected states while affected individuals remained the same. The designations used were as follows: (1) for the primary dataset: individuals with SPH or SE > -5.00 D of both eyes were designated as unaffected; (2) for the dataset *without unaffecteds*: unaffected individuals were excluded by designating the unaffected status as an unknown phenotype; (3) for the dataset of the *emmetropia as an unaffected* designation: individuals with a refractive error of -0.50 D $\leq$ (SPH or SE) ≤ 0.50 D of both eyes were designated as unaffected; others who were not affected were classified as having unknown status.

The MERLIN program was used to perform both two-point and multipoint parametric and nonparametric linkage analyses (NPL).[52] The same DOM and REC models with various unaffected states, as just described, were assumed in the MERLIN parametric analysis. The MERLIN program generates the number of bits for each family based on the family structure and cannot handle families with a large bit size. Ten multigenerational pedigrees were trimmed to bit sizes less than 24 for the analysis. In addition, it is known that linkage disequilibrium (LD) may inflate the type I error of multipoint linkage analysis.[53] Using the MERLIN program, we selected the option to take into account the marker–marker LD to reduce its impact on the LOD score. The threshold of the square of the Pearson correlation coefficient (r^2) between markers was set at 0.16 in the multipoint linkage analysis.[53]

Multiple stratified datasets were analyzed in addition to the overall dataset because of possible genetic and ethnic admixture influences. For example, DUK Family 66 is known to be an X-linked dominant high-grade myopia family and thus presumptively has a different genetic transmission state than other complex forms of high-grade myopia.[26] The multicenter derived dataset was predominantly Caucasian (91.2%), and also had Asian (4.8%) and African-American (5%) samples from the DUK dataset. Four categories of datasets for whole-genome linkage scans were formed and studied: (1) an overall dataset; (2) an overall dataset without DUK Family 66; (3) center-specific datasets; and (4) race-specific datasets. For the chromosomes that had significant LOD scores ≥ 1.5 in any of the whole genome scans, additional detailed analyses were conducted, such as generating family-specific LOD scores and excluding African-Americans from the overall and DUK datasets.

Results

Clinical and Marker Data Summary

Table 1 summarizes sample sizes and related ocular phenotypes by ascertainment sites and disease states (affected, unaffected, and unknown) in the final dataset used for analyses after rigorous quality control procedures. The subject sample sizes for affected, unaffected, and unknown (due to missing SPH or SE) groups are slightly different between disease states defined by SPH and SE. The total number of subject samples used in the analysis is 1201 (243 families), ranging from 529 samples (132 families) from the DUK site to 14 samples (1 family) from the MEL site. In the primary dataset, the SPH-defined disease states have 551 affected, 492 unaffected, and 158 subjects without SPH measures. The SE-defined disease states have 503 affected, 447 unaffected, and 251 subjects without SE measures. All 503 SE-defined affected subjects were designated as affected in the SPH group. In the dataset of emmetropia as an unaffected designation, there were 184 by the SPH definition and 182 by the SE definition.

The mean and standard deviation of subject age and ocular measurements of interest including sphere, cylinder, axis, axial length, and keratometry were obtained for the right and left eyes, respectively, for each disease state. Table 1 presents the mean, SD, and sample size for the affected (A) and unaffected (U) groups based on the SPH definition. All ocular measures were in similar scale for affected and unaffected groups across ascertainment sites except MEL, due to the small sample size. The MEL cohort had a slightly higher mean cylinder (e.g., 3.08 ± 2.78 OD and 2.65 ± 2.56 OS in affecteds) and lower SPH (e.g., −5.50 ± 2.06 OD and −5.35± 5.21 OS in affecteds) relative to the other centers. The majority (87.1%) of participants were older than 18 years. Of the 1201 subjects analyzed, 78 unaffected subjects were younger than 18 years at the time of ascertainment, and their SPHs ranged from −4.75 to +2.50 D (19 with SPH < −3.00 D). Unaffected subjects younger than 18 years could develop a greater degree of myopia. The potential misclassification of these subjects is a limitation of the study. However, this is a relatively small proportion of the sample. We also examined the effect of different definitions of unaffected status, as described earlier.

A total of 5,928 markers and 9,295,104 genotypes were provided by CIDR for data analysis. The markers had an average coverage of 0.65 cM (or 521 kb) intermarker distance. Missing genotype rates were higher for buccal (0.28%) and saliva (0.25%) than blood DNA (0.18%). All anonymous duplicated samples and CEPH samples were removed before analysis. After the QC analysis, individual families were excluded, individuals within families with significant Mendelian errors were corrected, and individual genotypes were deleted. Families were excluded on the basis of (1) unresolved Mendelian relationship errors and (2) having a single family member affected post QC-procedures which renders a family uninformative for linkage. We excluded six families (three DUK and three TOU) from analysis due to sample relationship errors found by RELPAIR and PREST, and five Duke families that were uninformative for linkage analysis. We also excluded sporadic samples showing family relationship error and their immediately family members: 59 samples from DUK, 3 from HEL, and 3 from TOU. Seven sample switches were corrected after confirmation or regenotyping new DNA samples in consultation with clinical investigators at each site. A total of 128 markers from eight families were reassigned as missing genotypes due to Mendelian inconsistencies detected with the PEDCHECK analysis. Three markers (rs1981193 and rs1464816 from chromosome 1 and rs952382 from chromosome 5) were significantly out of HWE, based on a 20% q-value cutoff threshold. In total, we excluded 131 markers from the analysis. The final dataset used in this report was based on 243 families (132 DUK, 46 CARD, 40 TOU, 24 HEL, and 1 MEL), 1201 samples, and 5744 markers. This dataset contained 10 Asian, 12 African-American, and 221 Caucasian families.

Linkage Regions for High Myopia Defined by the SPH Phenotype

Sixty-three markers across 19 chromosomes displayed two-point HLOD scores ≥ 2, and 18 linkage regions from 12 chromosomes contained peak multipoint HLOD or NPL ≥ 1.5 from various analyses. (Tables 2, 3) Figure 1 depicts the two-point and multipoint linkage results from both parametric analyses using a DOM model and nonparametric linkage analyses for the overall dataset. The most notable linkage interval was 12q21.2-24.12, a 36.59-cM (89.57-126.16 cM) region with a significant peak marker at rs337663 (HLOD = 3.48; 101.97 cM) in the overall dataset. This linkage region contains four markers with two-point HLOD scores ≥ 2 from various datasets (rs2063239, rs20508, rs1849929, and rs4213). For instance, the SNP rs2063639 had the highest-scoring two-point HLOD of 4.8 from the DUK subset. (Table 2) This interval overlaps with regions identified by multipoint parametric linkage analyses for the DUK (peak HLOD = 3.62, rs1489895), Asian (peak HLOD = 1.55, rs930248), and Caucasian (peak HLOD = 2.34, rs337663) subsets, as well as with the DUK subset after nonparametric linkage analysis (peak NPL = 2.07, rs337663). The chromosome 12 linkage interval can be contracted to a shared 9.94-cM region (12q21.31-22) from rs163016 (95.21 cM) to rs1520724 (105.15 cM) among four datasets (overall, DUK, Asian, and Caucasian). Notably, all peak markers (rs1489895, rs337663, and rs930248) from the four datasets were located within this 9.94-cM interval (Table 3). However, the Asian dataset delineated the smallest interval size for this locus, which may be the result of a smaller subset. If we exclude the Asian dataset, the overlapping region expands to 13.43 cM and is less affected by dataset size.

Other linkage regions of interest are on chromosomes 2 (2p24.1 with max LOD = 2.37 at rs1813617 and 2q37.1 with

TABLE 1. Ascertainment Site Demographics

	DUK		CARD		TOU		HEL		MEL	
	A	U	A	U	A	U	A	U	A	U
Families (*n*)	132		46		40		24		1	
Samples (*n*)	(529)		(238)		(272)		(148)		(14)	
Sample size by SPH (by SE)	252 (238)	245 (255)	153 (145)	81 (72)	71 (56)	97 (76)	67 (60)	66 (40)	8 (4)	3 (4)
Age at exam	37.33 ± 17.06 (230)	40.32 ± 22.65 (208)	40.97 ± 16.72 (153)	51.59 ± 19.26 (81)	41.11 ± 17.27 (66)	36.79 ± 21.62 (95)	42.82 ± 20.13 (67)	47.38 ± 18.63 (66)	20.17 ± 13.72 (6)	63.00 ± 19.08 (3)
Sphere										
OD (D)	−9.94 ± 5.91 (251)	−1.21 ± 2.47 (240)	−9.19 ± 3.03 (152)	−1.29 ± 1.83 (78)	−10.88 ± 5.38 (70)	−1.23 ± 1.85 (97)	−9.38 ± 4.40 (65)	−0.59 ± 1.81 (66)	−5.50 ± 2.06 (6)	−2.33 ± 0.72 (3)
OS (D)	−9.91 ± 5.63 (250)	−1.19 ± 2.44 (243)	−9.08 ± 3.09 (153)	−1.17 ± 1.82 (78)	−10.22 ± 5.19 (69)	−1.12 ± 2.11 (91)	−9.66 ± 3.77 (67)	−0.77 ± 1.78 (63)	−5.35 ± 5.21 (5)	−1.58 ± 1.89 (3)
Cylinder										
OD (D)	1.07 ± 1.09 (251)	0.55 ± 0.78 (240)	1.13 ± 1.01 (149)	1.04 ± 1.15 (69)	1.23 ± 1.1 (61)	0.5 ± 0.64 (78)	1.45 ± 1.35 (59)	0.79 ± 0.7 (49)	3.08 ± 2.78 (6)	2.17 ± 0.76 (3)
OS (D)	0.99 ± 1.02 (250)	0.54 ± 0.74 (243)	1.13 ± 0.89 (147)	0.89 ± 0.98 (70)	1.35 ± 1.18 (57)	0.55 ± 0.67 (78)	1.35 ± 1.18 (63)	0.82 ± 0.67 (48)	2.65 ± 2.56 (5)	1.75 ± 1.64 (3)
Axial length										
OD (μm)	26.99 ± 2.20 (112)	23.88 ± 1.30 (53)	—	—	26.93 ± 2.54 (53)	23.89 ± 1.78 (73)	—	—	24.61 ± 0.01 (2)	25.05 (1)
OS (μm)	27.01 ± 2.23 (110)	23.70 ± 1.23 (51)	—	—	26.98 ± 2.69 (52)	23.88 ± 1.83 (73)	—	—	24.7 ± 2.93 (2)	23.56 (1)
Keratometry										
OD (D)*	43.14 ± 5.14 (110)	43.62 ± 1.42 (62)	—	—	43.59 ± 3.21 (39)	43.36 ± 1.52 (65)	42.62 ± 1.69 (53)	43.15 ± 1.31 (55)	41.87 ± 1.76 (3)	45.25 ± 0.00 (2)
OS (D)*	42.97 ± 5.29 (113)	42.86 ± 4.39 (64)	—	—	43.66 ± 2.13 (38)	42.80 ± 5.11 (65)	42.85 ± 1.44 (53)	43.22 ± 1.33 (58)	43.46 ± 0.08 (3)	44.75 ± 1.41 (2)

Data include number of families and total sample size per site and per measurement (in parentheses), disease state (A, affected; U, unaffected), refractive phenotype mean SPH and SE, subject age at examination (years), and all other measured biometric parameters, with the mean and standard deviation.

* The mean ± SD of the keratometry measurements was based on the average of the keratometry 1 (K1) and keratometry 2 (K2) readings of each participant for the DUK, HEL, and MEL sites. For the TOU site, only the K1 reading was used.

TABLE 2. Summary of Two-Point Parametric Using FASTLINK for Both DOM and REC Models for Markers with HLOD ≥ 2 in at Least One Dataset

Chr	SNPs	DeCode Map (cM)	Model	All Data	Center Specific					Race Specific	
					DUK	CARD	HEL	TOU	MEL	Asian	Cauc
1	RS785495	68.9	DOM	1.37	0.57	**2.15**	−0.24	0.08	0.06	−0.01	0.86
2	RS1813617	41.64	REC	**2.27**	0.14	0.72	0.44	0.80	0.20	0.02	1.09
	RS524586	174.12	DOM	1.40	0.97	**2.09**	0.07	−0.05	−0.08	−0.01	1.43
	RS1378011	221.57	DOM	**2.25**	0.86	0.9	0.01	1.07	0.16	**2.29**	1.60
	RS721357	226.85	DOM	0.18	0.09	**2.16**	0.006	−0.01	−0.12	−0.01	0.32
	RS838715	241	DOM	1.90	0.85	0.08	0.77	0.85	−0.04	**2.26**	1.82
	RS4247179	261.27	DOM	0.85	−0.04	0.09	**2.38**	0.22	0.26	0.14	0.52
3	RS2102313	97.77	DOM	0.54	−0.03	0.15	0.06	**2.40**	0.75	0.02	0.53
4	RS1812590	112.44	DOM	1.07	−0.05	**2.04**	0.03	0.14	−0.1	0.06	1.20
	RS1812590	112.44	REC	1.36	0.24	**2.19**	0.16	−0.05	−0.04	0.04	**2.36**
	RS1495127	125.35	DOM	1	−0.09	0.2	**2.64**	0.07	0.34	0.05	0.83
	RS1320244	126.28	REC	1.35	0.3	1.88	0.14	0.44	0.04	0.15	**3.10**
5	RS171610	12.44	DOM	0.2	**2.46**	−0.06	−0.019	0.50	0.00	−0.01	0.24
	RS378932	14.81	DOM	0.12	**2.70**	−0.05	0.033	0.01	0.01	−0.01	0.07
	RS495237	64.56	REC	1.76	1.75	0.46	−0.06	0.22	0.07	0.00	**2.35**
	RS1553578	64.9	REC	**2.20**	1.37	0.20	0.07	0.21	0.15	0.54	0.83
	RS32222	132.27	DOM	**2.25**	1.9	0.28	0.86	0.41	0.03	0.10	**2.20**
	RS31251	133.46	REC	1.8	0.67	0.41	0.76	1.01	0.00	0.08	**2.71**
	RS9216	164.52	DOM	1	0.07	0.02	**2.56**	0.09	0.05	−0.01	0.43
6	RS561332	27.45	DOM	−0.30	−0.28	**2.04**	0.16	0.00	0.00	0.00	−0.31
	RS1398576	90.11	REC	0.27	0.19	0.36	0.12	0.5	0.17	0.05	**2.19**
7	RS846294	63.67	DOM	1.36	−0.13	0.46	**2.46**	0.52	0.01	−0.02	1.20
	RS1524341	154.87	DOM	1.08	0.08	0.33	**2.17**	−0.01	0.00	−0.01	1.14
	RS243491	159.71	DOM	0.42	−0.15	**2.591**	−0.4	0.66	0.02	0.00	0.42
10	RS2439903	20.05	REC	**2.24**	−0.01	0.64	0.00	0.05	0.09	0.01	1.01
	RS1441027	61.3	REC	1.85	0	1.96	0.03	0.12	0.14	−0.02	**2.48**
12	RS581642	53.89	DOM	**3.18**	1.49	0.06	**3.34**	0.09	0.04	0.28	**2.47**
	RS581642	53.89	REC	1.82	1.58	0.6	1.82	−0.15	0	0.31	**2.88**
	RS1843910	56.15	REC	1.78	**2.31**	0.76	0.08	0	−0.06	0.06	**2.38**
	RS956066	56.41	REC	1.41	0.82	1.04	0.24	0.14	0.2	−0.03	**2.06**
	RS774033	72.1	REC	**2.58**	0.41	0.20	0.20	0.00	0.05	0.14	0.4
	RS1000708	73.92	REC	**2.03**	1.33	0.08	0.57	0.17	−0.02	0.05	0.8
	RS1695105	77.64	DOM	**2.04**	**2.19**	0.1	0.46	0.01	−0.01	0.29	1.68
	RS472197	84.56	DOM	**2.61**	1.9	0.05	1.04	0.05	0.02	0.08	**3.32**
	RS1493829	87.43	DOM	**2.27**	1.82	0.25	0.47	0.01	0.22	0.07	**2.02**
	RS2063239	96.74	DOM	1.86	**4.80**	0.64	−0.02	−0.14	−0.06	1.34	1.90
	RS20508	108.97	DOM	**2.38**	**2.09**	0.01	0.86	0.00	0.07	1.36	**2.26**
	RS1849929	112.36	DOM	**3.07**	**2.23**	0.8	0.76	−0.04	0.15	0.74	**2.51**
	RS4213	140.09	DOM	**2.43**	1.06	0.58	0.58	0.81	−0.01	0.10	**2.36**
13	RS719185	111.86	DOM	0.78	**2.45**	0.06	−0.10	−0.02	−0.01	0.11	0.70
14	RS4961390	13.54	DOM	1	0.12	−0.04	0.30	**2.00**	0.73	0.00	1.24
	RS2065632	62.31	DOM	0.01	−0.18	**2.031**	−0.06	0.14	0.51	0.21	0.21
	RS2205187	73.85	DOM	0.18	−0.01	**2.069**	−0.05	−0.08	0.3	0.06	0.20
15	RS969860	7.36	DOM	1.3	**2.13**	0.14	0.09	−0.01	0.03	0.03	0.80
	RS1433895	38.88	DOM	−0.05	−0.21	0.14	−0.10	**2.10**	0.07	0.17	−0.05
	RS1648282	43.85	DOM	0.14	−0.34	0	−0.03	**2.26**	0.02	−0.01	−0.10
	RS1648312	43.87	DOM	0.02	−0.12	0.03	−0.06	**2.32**	0.2	0.15	0.03
	RS983188	118.39	DOM	0.40	−0.22	**2.021**	0.95	0.40	−0.01	0.10	0.37
16	RS13143	56.82	DOM	1.27	**2.04**	0.27	−0.18	0.44	0.18	0.01	1.22
	RS41103	113.21	DOM	1.01	**2.73**	0.2	−0.02	−0.02	−0.14	0.00	0.89
17	RS2058237	42.6	DOM	**2.248**	1.23	1.52	−0.03	0.10	0.07	0.25	**2.60**
18	RS1389532	55.71	DOM	**2.086**	0.7	**3.122**	1.06	0.54	−0	0.15	1.78
19	RS243404	14.92	DOM	0.54	0.09	**2.914**	−0.06	−0.01	0.07	0.04	0.80
	RS105038	15.02	DOM	0.35	0.06	**2.635**	−0.07	−0.01	0.07	0.06	0.46
	RS714307	31.31	DOM	−0.16	−0.13	**2.731**	−0.29	−0.03	0.08	−0.06	−0.20
	RS1273522	38.27	DOM	0.15	−0.06	**2.114**	0.01	−0.01	−0.06	−0.01	−0.80
	RS2041975	73.01	DOM	**2.05**	1.71	0.2	0.30	0.30	0.15	0.10	1.54
20	RS995480	79.37	DOM	0.21	0.07	−0.07	**2.20**	−0.16	0.05	0.14	0.24
22	RS2056965	39.06	DOM	**2.25**	1.2	**2.338**	0.22	−0.03	0.02	0.00	1.67
	RS2008519	39.12	DOM	**2.17**	**2.48**	0.26	0.35	−0.08	0.02	0.16	1.57
	RS738510	40.44	DOM	0.88	0.43	0.01	**2.04**	−0.05	0.06	0.03	0.78
	RS713912	50.81	DOM	0.58	**2.30**	0.03	−0.04	0.01	−0.07	1.10	0.90
X	RS1264216	80.64	DOM	−0.36	−0.08	**2.024**	−0.37	−0.06	0.00	0.02	−0.36
	RS761544	110.18	DOM	0.71	1.2	0.96	−0.13	−0.03	0.16	0.03	**2.07**
	RS1522889	121.48	DOM	1.4	0.77	1.4	−0.07	−0.03	0.86	0.04	**2.13**
	RS9856	124.34	DOM	0.14	−0.01	**2.335**	−0.08	−0.02	0.12	0.23	0.78
	RS2066918	128.28	DOM	−0.09	−0.17	**2.478**	0.03	−0.10	0.00	0.40	−0.20
	RS1997686	154.29	DOM	1.01	**2.12**	−0.03	−0.11	−0.07	0.23	0.00	0.83
	RS6540401	166.7	REC	**2.00**	0.20	0.03	0.26	0.06	0.35	0.01	0.18
	RS1860929	168.06	REC	**2.42**	0.51	0.46	1.06	−0.15	0.00	0.06	1.13
	RS760109	180.17	REC	**2.03**	0.05	0.15	0.14	0.03	0.15	0.15	0.52

Data set in bold represent HLOD ≥ 2.
Chr, chromosome.

TABLE 3. Summary of Multipoint Linkage Regions

Chr*	Marker region	DeCode Map(cM)	All Data	Center Specific				Race Specific	
				DUK	CARD	TOU	HEL	Asian	Cauca
1	RS2017143	0					1.22		
	RS878063	3.64					1.11		
1p36.32	RS1870509 (peak)	6.43					**1.80**	**1.83**	
	RS912991	8.53					1.06		
	RS631099	10.08						1.16	
	RS834984	121.8						1.38	
1p21.1	RS1330226 (peak)	126.73						**1.55**	
	RS2057127	137.05						1.17	
2	RS340767	38.32				1.39			
	RS558912	39.5	1.25			1.00			**1.20**
2p24.1	RS1813617 (peak)	**41.64**	**1.91**			**1.69**	**2.37**		**1.84**
	RS1344083	44.57							1.07
	RS952275	44.62	1.00						
	RS2001795	46.46				1.32	1.54		
	RS1000758	89.01			1.24				
2p14	RS2002879 (peak)	92.44			**2.01**				
	RS2058899	95.41			1.18				
	RS959327	225.9	1.37						1.37
	RS997363	231.23					1.14		
2q37.1	RS887062 (peak)	242.05	**2.25**				**2.12**		**2.28**
	RS16747	261.64	**1.44**				1.30		1.44
3	RS1913081	106.8						1.42	
3p12.3	RS1383407 (peak)	107.18						**1.55**	
	RS820273	107.71						**1.22**	
4	RS11736201	96.69			1.48				
	RS1461605	99.66			1.19				
4q24	RS1384401 (peak)	108.02			**1.98**				
	RS1024481 (peak)	109.25			**1.87**				
	RS1948983	119.28			1.10				
	RS1459062	122.05			1.17				
5	RS372169	8.79		1.04					
	RS924674	9.99		**1.56**					
5p16.33	RS639718 (peak)	10.27		**1.57**	**1.85**				
	RS1506030	25.83		**1.04**					
	RS13180426	27.04		1.23					
	RS930047	195.49	1.02						**1.08**
	RS1544926	199.82			1.20				
5q363	RS185493 (peak)	200.73	**1.63**						
	RS1079487 (peak)	201.1							**1.63**
	RS684609 (peak)	202.9			**1.57**				
	RS1487	205.93	1.20		1.32				1.19
9	RS12009	133.08	1.05			1.14			
	RS624903	134.43							1.08
9q34.11	RS913275 (peak)	136.55	**1.84**			**2.07**			**1.60**
	RS1220789	138.31							1.53
	RS1054879	139.65	1.05			1.00			
12	RS1493829	87.43		1.00					
	RS2037581	89.57	**1.06**	**1.14**					**1.05**
	RS998070	91.72							
	RS1163016	95.21						**1.55**	
12q21.33	RS1489895 (peak)	101.18		**3.62**					**2.34**
	RS337663 (peak)	101.97	**3.48**						
	RS930248 (peak)	103.49						**1.55**	
	RS1620724	105.15		1.52					
	RS1544921	113.36						1.24	
	RS1662032	119.05		1.13					
	RS1476470	125.57							1.14
	RS737280	126.16	1.03						
14	RS1542313	63.85				1.10			
14q24.3	RS1125221 (peak)	72.95				1.58			
	RS935340	75.15				1.15			
15	RS1433887	38.84				1.15	1.29		
16q21.1	RS1062124 (peak)	47.98					**2.47**		
	RS877007 (peak)	48.25				**2.71**			
	RS11856	54.65				**1.52**	1.62		
17	RS450433	110.73				**1.45**			
17q26.1	RS717571 (peak)	116.09				**1.52**			
	RS116719	116.48				**1.25**			

(*continues*)

TABLE 3 (*continued*). Summary of Multipoint Linkage Regions

Chr*	Marker region	DeCode Map(cM)	All Data	Center Specific				Race Specific	
				DUK	CARD	TOU	HEL	Asian	Cauca
X	RS714597	116.17			1.16				
Xq24	RS2018368 (peak)	118.64			**2.40**				
	RS767216	129.18			1.03				
	RS560689	165.77		**1.48**					
Xq28	RS985595 (peak)	167.62		**1.69**					
	RS764908 (peak)	169.39		**1.69**					
	RS941400	181.59		**1.22**					

Regions were identified by MERLIN parametric analysis using an autosomal dominant model and nonparametric analysis. Each linkage region has a peak marker with LOD score (HLOD or NPL) ≥ 1.5 and extends to boundary markers with LOD score ≥ 1. The *solid-lined* boxes denote linkage regions from the parametric analysis based on the autosomal dominant model, and the *dashed-lined* boxes are from the nonparametric analysis.

Chr, chromosome; Cauc, Caucasian.

max LOD = 2.25 at rs887062), 5 (q35.3, max LOD = 1.63 at rs185493), and 9 (q34.11, max LOD = 2.07 at rs913275) (Table 3). These intervals have SNPs with LOD scores ≥ 1.5 in the overall dataset, a single ascertainment site, and are primarily associated with the Caucasian subset. Of note, these three linkage regions were determined for chromosome 2 by various stratified datasets. The first chromosome 2 locus (2p24.1) from 38.32 to 46.46 cM (peak NPL = 2.37 at rs1813617) was supported by the overall, TOU, and Caucasian datasets. The second chromosome 2 region (2p14) extends from 89.01 to 95.41 cM (peak HLOD = 2.01 at rs2002879), and was determined in the CARD dataset only by multipoint parametric analysis. The last linkage region (2q37.1) extending from 225.9 to 261.64 cM (peak HLOD = 2.25 at rs887062) was determined in the overall, HEL, and Caucasian datasets (Table 3). Clearly, different ascertainment sites seem to be influencing the various significant linkage regions on chromosome 2.

Several linkage regions were determined by a single stratified dataset, including: 5p15.33 and Xq28 from the DUK subset; 2p14 (second region), 4q24, and Xq24 from the CARD subset; 14q24.3 from the TOU subset; 1p36.32 from the HEL subset; and 1p21.1 and 3p12.3 from the Asian subset (Table 3). In particular, the Xq28 locus (165.77–181.59 cM) LOD score associated with the DUK dataset was primarily derived from the DUK family 66 genotyped data, as the LOD score was no longer significant after removing this family from the analysis.

The effect of including African-American samples in the linkage analyses of the overall dataset was small. All linkage regions identified in the overall dataset remained significant after the exclusion of African-American families (Table 3). The peak LOD scores for SNPs at linkage regions on 2p24.1 (rs1813617) and 2q37.1 (rs887062), 5q35.3 (rs185493), 9q34.11 (rs913275), and 12q22 (rs357663) were slightly reduced, with LOD scores ranging from 1.58 to 3.19. Similarly, the African-American dataset had little impact on the linkage regions identified with the analysis of the DUK subset.

Linkage Regions for High Myopia Defined by the SE Phenotype

Figure 2 depicts the results of whole-genome linkage scans with two-point and multipoint analyses of the overall dataset. A similar tabulated summary for both two-point and multipoint analyses based on the SE phenotype is provided in Supplementary Tables S1 and S2, online at http://www.iovs.org/cgi/content/full/50/7/3116/DC1. In total, 39 markers had two-point LOD scores ≥ 2.0 within 16 chromosomes, of which 16 markers were also identified in the analysis based on SPH (Supplementary Table S1). There are 16 linkage regions in 13 chromosomes from all datasets, and regions from chromosomes 2 (2p24.1 and 2q37.1), 4 (q24-26), 5 (p15.33), 9 (q34.11), 12 (q21.31-24.12), 17 (q25.1), and X (q28) overlap with the results from the SPH phenotype analyses (Table 3 and Supplementary Table S2).

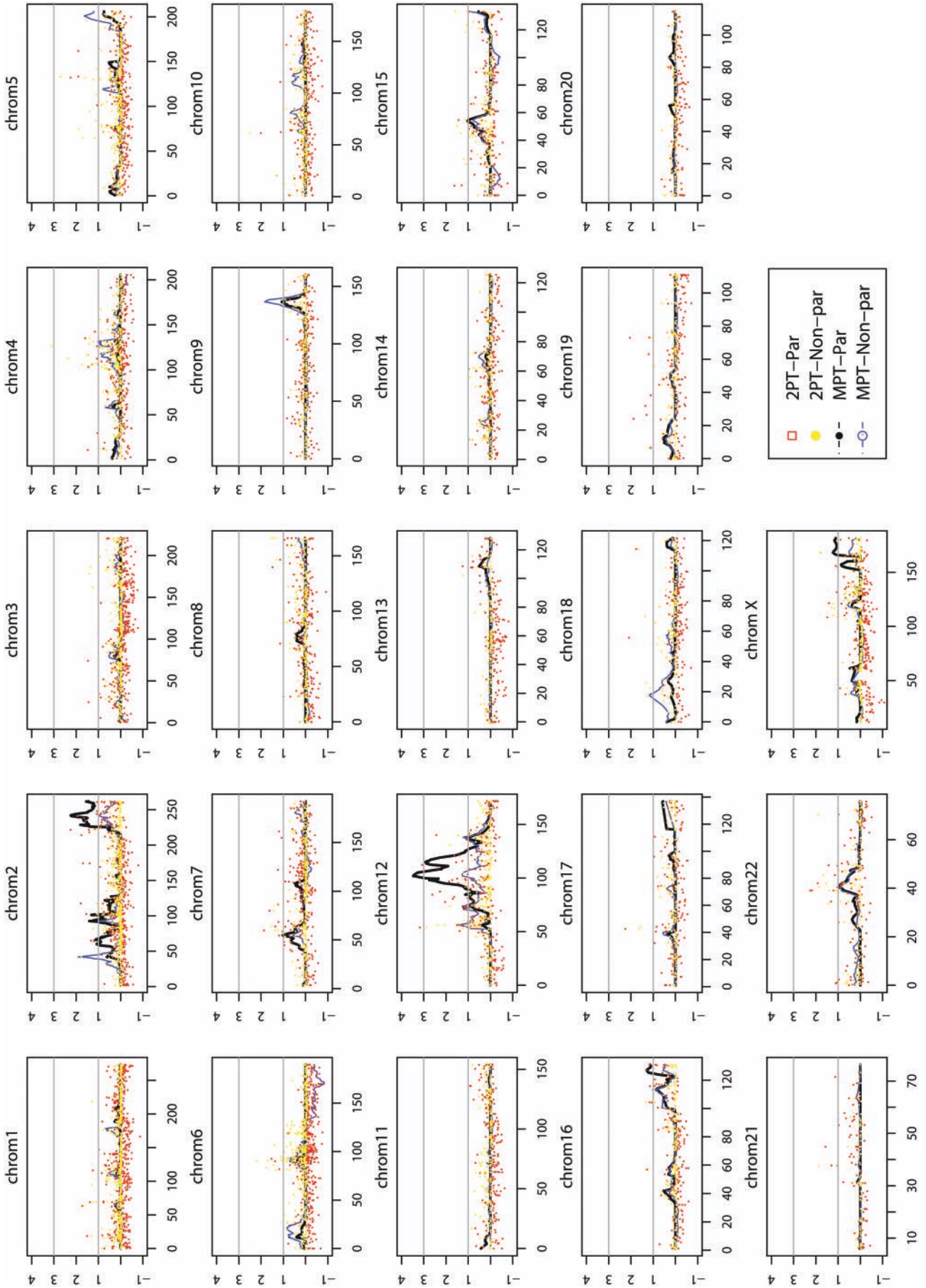
With the SE phenotype analyses, the 12q linkage region remained significant but the peak marker identified shifted slightly downstream to rs746035 at 117.17 cM, with a lower LOD score (HLOD = 2.12) for the overall dataset. Although the DUK, Asian, and Caucasian subsets still showed strong linkage signals with peak LOD scores ≥ 1.5 at 12q, these linkage intervals showed less overlap than the analyses based on the SPH phenotype. For instance, the 12q linkage region for the Asian subset extended from 112.36 to 119.05 cM, which does not overlap with the interval of 95.2 to 108.17 cM determined in the Caucasian subset (Supplementary Table S2).

New suggestive linkage regions that were not identified by SPH-defined high-grade myopia include 6p25.3, 7q36.3, 18q23, 19q12, and 22q12.3, which were found in a single dataset with peak LOD scores >1.5. Regions 6p25.3, 7q36.3, and 22q12.3 had peak LOD scores >2: NPL = 2.14 for rs10793833 at 6p25.3 (DUK), NPL = 2.06 for rs7784332 at 7q36.3 (overall dataset), and NPL = 2 for rs972153 at 22q12.3 (DUK). The 22q12.3 region (37.93–42.08) derived from the DUK dataset was also supported by the DOM parametric analysis with a peak HLOD = 1.66 at the same marker rs972153.

Effect of Unaffected Status Classification on the Linkage Results

In general, similar multipoint parametric linkage curves were obtained among the three datasets with different classifications of unaffected status, as described earlier. The LOD score curves shifted lower for both datasets, with unaffected coded as unknown and the emmetropic state as unaffected. Therefore, fewer linkage regions were determined to be significant for these two datasets (results not shown). However, key linkage regions on chromosomes 2 and 12, determined in the original dataset, remained. For example, Figure 3 displays the chromo-

FIGURE 1. Genome-wide linkage analysis results for high myopia defined as sphere (SPH) ≤ −5.00 D. LOD scores are plotted on the *y*-axes and genetic distance (in centromeres) along each chromosome on the *x*-axes. Four analyses are shown: two-point parametric (2PT-Par), two-point nonparametric (2PT-Non-par), multipoint parametric (MPT-Par), and multipoint nonparametric (MPT-Non-par).


chrom1
chrom2
chrom3
chrom4
chrom5
chrom6
chrom7
chrom8
chrom9
chrom10
chrom11
chrom12
chrom13
chrom14
chrom15
chrom16
chrom17
chrom18
chrom19
chrom20
chrom21
chrom22
chrom X
2PT–Par
2PT–Non–par
MPT–Par
MPT–Non–par

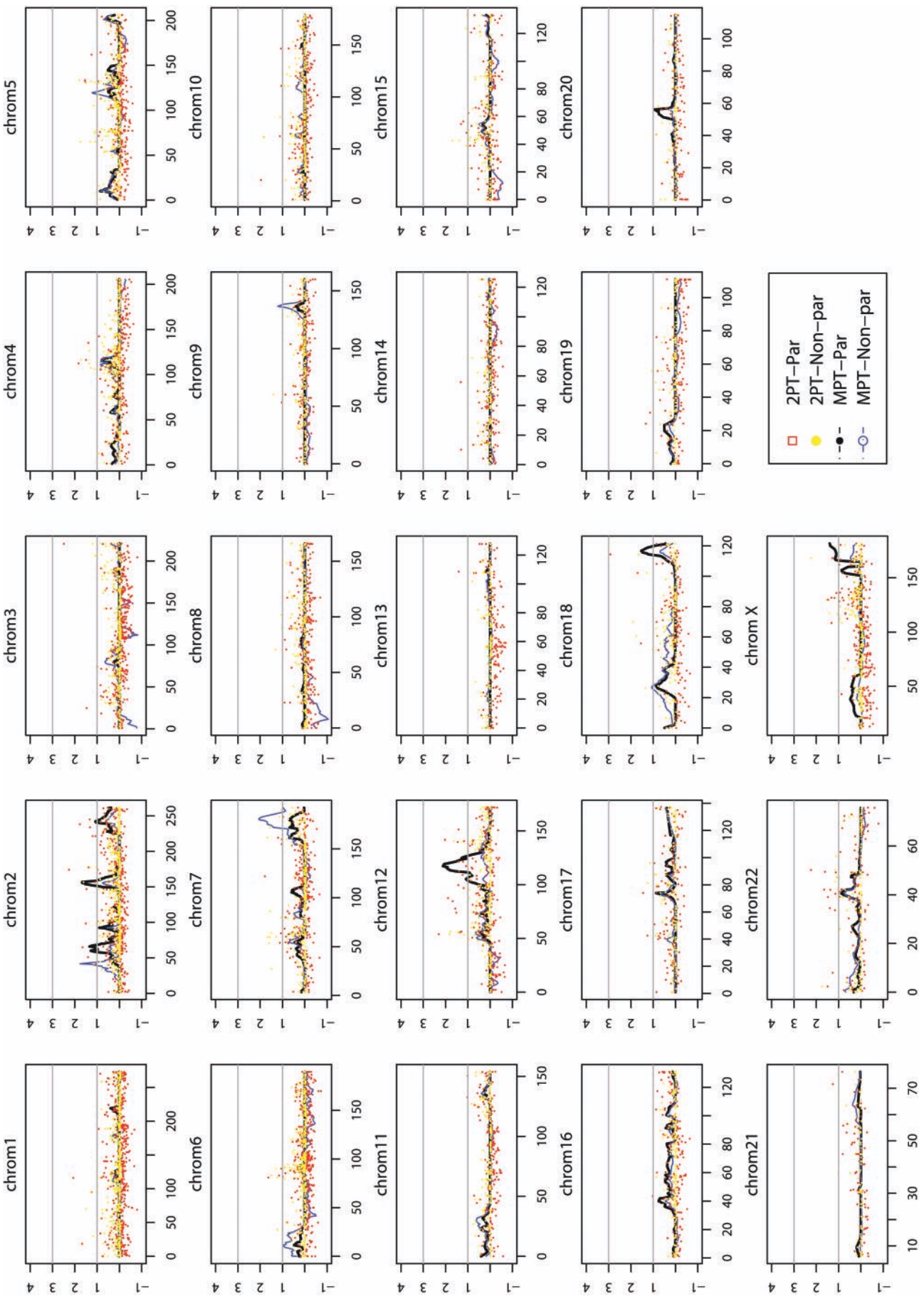


Figure 2. Genome-wide linkage analysis results for high myopia defined as spherical equivalence SE ≤ -5.00 D, plotted as in Figure 1.

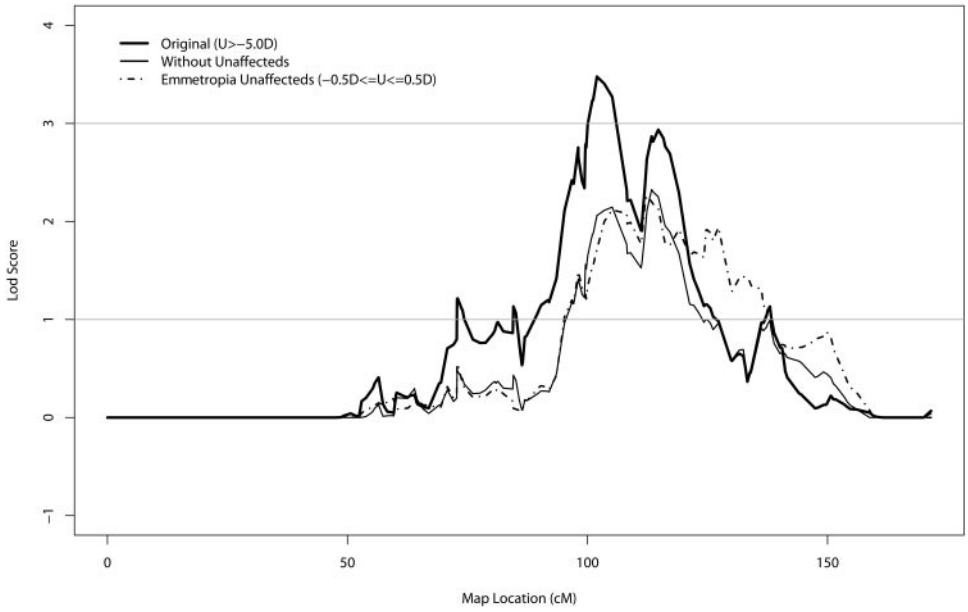


FIGURE 3. Chromosome 12 multipoint parametric linkage results for three datasets with the use of different definitions of unaffected status: (1) the primary dataset with unaffected individuals defined as SPH $<$ -5.00 D; (2) without unaffected phenotype; (3) with emmetropic state (-0.50 D $\leq$ SPH $\leq$ 0.50 D) as unaffected.

some 12 multipoint parametric linkage results from the MERLIN analysis for the overall dataset based on the original classification of SPH (unaffected: SPH $>$ -5.00 D; peak LOD = 3.48 at 101.97 cM), without unaffecteds (peak LOD = 2.33 at 13.36 cM), and with the emmetropic state as unaffected (unaffected: -0.5 D $\leq$ SPH $\leq$ 0.5 D; peak LOD = 2.26 at 112.36 cM). The decrease in LOD scores in the latter two datasets may have resulted primarily from a reduction of samples with known phenotypes. The higher LOD scores in the original dataset suggest that the analysis was not substantially altered from the misclassification of unaffected status, as the unaffected samples used did not contribute a significant number of recombinant events.

DISCUSSION

The present study is the first large-scale linkage study of high-grade myopia, and the first to use dense SNP genotyping rather than microsatellite markers. A comprehensive linkage analysis plan is presented in this report by including two different ways of defining myopic refractive error, different linkage analytical methods and genetic models, and multiple stratified datasets. The results are consistent with previous genetic findings of high-grade myopia in two ways. First, the DOM model in general fits better than the REC model for parametric analysis. Most of the linked markers or regions of analytical significance were determined using the DOM model parametric analytical strategy (Table 2; Figs. 1, 2). Linkage regions determined using the DOM model were more congruent with those derived using nonparametric analyses (Table 3). This finding is consistent with segregation analysis in a sample of carefully ascertained high myopia pedigrees with a predominant DOM inheritance pattern.[17] However, it should be noted that DOM inheritance appears to be the exception rather than the rule in less heavily ascertained subject groups.[23,24] Second, several MYP loci were replicated by at least one of the datasets analyzed herein (OMIM; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), including MYP1 (OMIM 310460; Xq28),[25,26] MYP3 (OMIM 603221; 12q21),[28,35] MYP6 (OMIM 608908; 22q12.3),[41,54,55] MYP11 (OMIM 609994; 4q22-27),[42] MYP12 (OMIM 609995; 2q37.1),[35,36] and MYP14 (OMIM 610320; 1p36),[56] as well as a previously reported locus on 5p15.33.[57] Individually, all study sites have not necessarily replicated other MYP loci in their respective datasets. These findings validate the high likelihood that these genetic loci harbor myopia-associated sequence variants and thus further study with association mapping of these regions is warranted.

For replicated chromosomal intervals, the LOD scores of implicated SNPs were consistently lower than those determined using microsatellite markers. These scores may be a result of the highly informative nature of microsatellite markers relative to SNPs. Microsatellite versus SNP genotyping is more labor intensive, however. The expanded dataset in this study compared to other smaller family-based microsatellite studies of high-grade myopia linkage also lends toward more families neutralizing or tempering the overall SNP LOD score due to selective nonlinkage at certain loci relative to others. What is also meant by "replicate" is mainly a comparison of the use of SNP versus microsatellite linkage platforms.

There is controversy regarding which biometric measurement, SPH or SE, to use as the phenotypic definition of myopia. In all our previous reports, we have consistently used SPH, as this was not an approximate but an absolute measurement as proxy for myopia status.[26,28,31,34,35] However, most of the subsequent myopia genetic analyses in the literature have used SE.[33,36,41,43] Consequently, we chose to study both parameters. The number of affected subjects according to SE would necessarily be less than that according to SPH, because of moderate or significant astigmatism contributions. In this circumstance, a subject with astigmatism may not qualify as affected in a dichotomous trait analysis. As our dataset shows, there were fewer affected individuals with a SE-defined phenotype than SPH (503 vs. 551), which may diminish the power to detect linkage signals or provide discrepant linkage regions. Compared to SE, SPH resulted in higher LOD scores and more clearly delineated intervals in all analyses. For example, the overall and stratified dataset analyses of the 12q21 MYP3 locus had high overlap using SPH as the phenotype parameter studied. This was not the case for the corresponding SE analyses (Table 3 and Supplementary Table S2).

Using the SE phenotype, high-grade myopia in three Han Chinese pedigrees from Hong Kong was recently mapped to 5p15.33-p15.2.[57] The locus interval is 17.4 cM, and a maximum two-point LOD score of 4.81 was garnered with the microsat-

ellite marker D5S630. The chromosome 5 linkage peak in our 10 Asian families did not meet the stated threshold of a 1.5 LOD score to qualify as a linkage region of interest in multipoint analysis for either SPH- or SE-defined high-grade myopia phenotypes (Table 3 and Supplementary Table S2). Of interest, the highest LOD score for chromosome 5 was found at rs13157690 at 5p15.2 with a peak HLOD score of 1.34 based on the parametric multipoint analysis for high-grade myopia defined by SPH. This linkage interval of 28.25 cM (defined by HLOD > 1) extends from rs3828570 (0.61 cM of 5p15.33, HLOD = 1.22) to rs879253 (33.93 cM of 5p15.2, HLOD = 1.25). There is interval overlap, but it is wider in this study. Thus, there is suggestive replication of the mapped 5p15.33-5p15.2 locus for high-grade myopia found in the initial Hong Kong Asian cohort.

To date, most refractive error or refractive trait linkage studies were conducted for Caucasian and Asian populations. Little is known for African-American cohorts. Recently, Ciner et al.[58] reported linkage of moderate myopia to 7p15 based on a genome-wide linkage scan with 387 microsatellite markers using SE as a quantitative trait in a cohort of 96 African-American families. In our cohort of 13 African-American families, we did not determine significant LOD scores for high myopia defined by either SPH or SE, perhaps because of the diminished statistical power of our dataset, as almost all families are small with four subjects per family except for one two-generation family with eight participants.

The strongest linked regions noted with this study were 12q21 and 2q37.1, the MYP3 and MYP12 loci previously identified through whole-genome microsatellite genotyping. The two linkage regions were still detectable after modification of unaffected status. Both loci were initially identified in our laboratory, and subsequently replicated in independent laboratories.[28,32,35,36] After analysis, the 12q21 MYP3 locus was contracted to a 9.97 cM region in this study. The original DUK family 10 linked to 12q21 (MYP3 locus) ranked as the second top contributor to the total LOD score for the peak marker rs337663 (DUK family 10 HLOD = 1.15 versus the total HLOD = 3.48).[28] The consistent finding of high-grade myopia linkage to 12q21 with two genotyping platforms (microsatellite and SNP) and multiple dataset types emphasizes the strength of this locus and suggests a major gene effect. In contrast to chromosome 12, chromosome 2 showed relative genetic heterogeneity, as multiple linkage regions were determined from different ascertainment sites. The 2q37.1 MYP12 locus had main contributions from the HEL dataset for both SPH and SE defined phenotypes. Two new chromosome 2 linkage regions (2p24.1 and 2p14) were identified, and were mainly derived from the TOU and CARD datasets, respectively.

9q34.11 is a novel linkage region found in this study. This 6.58-cM region is flanked by markers rs12009 and rs1054879, and was determined by the overall (peak NPL = 1.84), CARD (peak NPL = 2.07), and Caucasian (peak NPL = 1.60) datasets at the same peak marker, rs913275, using the SPH-defined phenotype (Table 3). Of interest, this region approached significance in the CARD dataset as well (peak NPL = 2.13 at rs913275), when the SE-defined phenotype was used (Supplementary Table S2).

In conclusion, a whole-genome, SNP-based linkage panel was used to identify genetic loci involved in myopic refractive error development in a large, family-based, primarily Caucasian, international cohort. The results of this study complement efforts world-wide to determine genes implicated in high-grade myopia and ocular growth.

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