Genetic Mapping of Myopia Susceptibility Loci

Maria Schäche,[1,2] *Andrea J. Richardson,*[1,2] *Kelly K. Pertile,*[1,2] *Mohamed Dirani,*[1,2] *Katrina Scurrah,*[3,4] *and Paul N. Baird*[1,2]

Purpose. Myopia (short sightedness) is a complex trait influenced by as yet unidentified genetic factors. To date, there have been four myopia susceptibility loci (MYP7 to -10) identified in twin studies, but these are yet to be independently verified. In an independent yet ethnically and phenotypically similar twin cohort, linkage to these chromosomal regions was sought.

Methods. Participants were 223 dizygotic twin pairs from the Australian Twin Registry who were assessed for evidence of linkage, by using polymorphic microsatellite markers spanning MYP7-10. Data were analyzed by using Haseman-Elston regression analysis.

Results. No evidence of linkage of myopia or its underlying biological components such as eye length to the MYP7-10 regions was found in this twin cohort.

Conclusions. This is the first study to assess for linkage in a secondary myopia twin cohort and highlights the problems associated with applying linkage results from complex traits to the other populations. (*Invest Ophthalmol Vis Sci.* 2007;48: 4924–4929) DOI:10.1167/iovs.07-0572

Refractive errors are a complex group of optical abnormalities occurring when the image entering the eye focuses anterior (myopia [MIM 160700] or near sightedness) or posterior (hypermetropia [MIM 238950] or far sightedness) to the retina, resulting in blurred vision. Myopia is the most commonly studied refractive error and affects more than 25% of the adult population in the United States.[1] The incidence of myopia varies with race, age, and gender with rates as high as 80% reported in urbanized regions of Asia.[2–6]

The development of myopia is a complex process determined by the coordinated effects of ocular biometric components such as lens power, axial length (length of the eye), corneal curvature (average of horizontal and vertical corneal meridians), and anterior chamber depth (distance from the posterior surface of the cornea to the anterior surface of the lens). These components can be quantitatively measured using ocular biometric techniques and provide a powerful means for understanding the underlying causes of myopia. Measurements of the refractive power of the eye are typically represented by the spherical equivalent (SphE), expressed in Diopter Spheres (DS). Classifications of refractive error include hypermetropia ($> +0.5$ DS), low-moderate myopia (-0.5 to -6 DS) and high myopia (< -6 DS), with each category having different clinical manifestations.

The etiology of ocular refraction has not been fully elucidated, but it is clear from findings in twin- and family-based studies that myopia, in particular, has underlying hereditary components.[7–12] Although the exact nature of this genetic component has yet to be elucidated, there have been several susceptibility loci identified from familial linkage studies. Commonly, such studies have focused on high myopia and have considered it to be a binary trait. The first locus reported for familial nonsyndromic high myopia was in 1998 when Young et al.[13] identified MYP2 on 18p11. Subsequent studies have identified further loci on 12q21-23 (MYP3), 7q36 (MYP4), 17q21 (MYP5), 4q22-27 (MYP11), 2q37 (MYP12), Xq23-25 (MYP13), and 10q21 (MYP15).[14–21] A single linkage study for common myopia has reported a susceptibility locus on 22q12 (MYP6).[22] Of these linkage studies only three (MYP2, -3, and -6) have been replicated in independent family studies and positional candidate genes sequenced for a further three loci (MYP2, -3, and -11).[19,23–28] To date, no sequence variants have been identified in genes from these myopia susceptibility loci that are associated with myopia or refractive error. Six genes (*TGFB1*,[29] *COL1A1*,[30] *COL2A1*,[31] *TGIF*,[32] *HGF*,[33] and lumican[34]) have been associated with myopia by single nucleotide polymorphism (SNP) analysis, but these results have not been replicated in independent studies.[35–38]

Although ocular refraction is an inherently quantitative trait, most linkage studies have characterized myopia as a binary trait. Given the evidence suggesting that myopia is a polygenic trait, attempts to identify susceptibility loci need to consider the phenotype as a continuous quantitative variable with a range of refraction measurements. To our knowledge, there have only been two linkage studies that have considered ocular refraction as a quantitative trait.[39,40] The first, published by Hammond et al.,[39] identified myopia susceptibility loci on 11p13 (MYP7), 3q26 (MYP8), 4q12 (MYP9), and 8p23 (MYP10) using spherical equivalent as the phenotype. Theirs is the only linkage study into myopia that has used a twin-based cohort. This finding has been replicated in the 8p23 region (MYP10) in an Amish population by using myopia as a binary trait.[17] The second study assessing ocular refraction as a quantitative trait identified a single susceptibility locus on 1p36 in Ashkenazi Jewish families.[40] To date, there have been no linkage studies assessing ocular biometric measurements such as ocular axial length, corneal curvature, and anterior chamber depth as underlying quantitative traits for ocular refraction.

We have reported heritability of ocular refraction and ocular biometric measurements in a twin-based population.[9] The twins used for this heritability study were recruited as part of the Genes in Myopia (GEM) Twin Study. The GEM Twin Study recruitment protocol involved inviting all twins older than 18 years registered in the state of Victoria (Australia) to participate. Invitations were sent to potential participants via a mailing through the Australian Twin Registry (ATR). A total of 4158 twin pairs (8316) including 4562 women and 3754 men were

From the [1]Centre for Eye Research Australia, the [3]Department of Physiology, and the [4]Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, University of Melbourne, Melbourne, Australia; and the [2]Vision Cooperative Research Centre, Sydney, Australia.

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Corresponding author: Maria Schäche, Centre for Eye Research Australia, The University of Melbourne, 32 Gisborne Street, East Melbourne VIC 3002, Australia; mschache@unimelb.edu.au.

invited to participate. Of the 4158 pairs contacted, 15.1% consented to participate, 29.4% declined to participate, and 55.5% were nonresponders. The recruitment phase of the GEM Twin Study is now complete.

In the present study, we report the results of quantitative trait locus (QTL) linkage analysis in the dizygotic twins from the GEM Twin Study. A QTL is a chromosomal region containing genes associated with a quantitative or continuos trait. A quantitative trait exhibits a wide range of phenotypes (measurements) that is an indication of complex inheritance patterns with many genetic and environmental effects. This is the first genetic study using both refraction and ocular biometric measurements as quantitative traits and represents only the second myopia linkage study in a twin-based population. Findings from the previous twin-based study by Hammond et al.[39] formed the basis of our analysis, in which we sought to assess whether the MYP7-10 susceptibility loci for ocular refraction could be replicated in an independent twin population. Extension of the analysis to include axial length, corneal curvature, and anterior chamber depth as quantitative traits was also performed.

METHODS

Subjects

All subjects were recruited by mailouts through the Australian Twin Registry (ATR) based in Melbourne, Australia. All twins older than 18 years living in Victoria were invited to participate. Individuals with secondary ocular disorders, such as amblyopia, strabismus, lens opacification, glaucoma, retinopathy, or keratoconus, which may affect refraction measurements, were excluded as were those with known connective tissue disease such as Marfan or Stickler Syndrome.

All subjects underwent a comprehensive eye examination including visual acuity assessment, subjective, and objective (dilated) refraction, slit lamp examination, and ocular biometry as described by Dirani et al.[9] Each subject also completed a standard questionnaire consisting of information on demographics, medical and family history, ocular history, and zygosity. All subjects provided informed consent before examination, and approval for the study was granted by both the Royal Victorian Eye and Ear Hospital (RVEEH) Human Research and Ethics Committee and the Australian Twin Registry (ATR). The protocol adhered to the guidelines in the Declaration of Helsinki.

Zygosity was determined using a series of questions recommended by the Australian Twin Registry.[41] Participants were asked if they were identical twins and if people (besides family and long-term friends) could easily tell them apart. The majority (over 95%) of the twins was aware of their zygosity, either due to their upbringing, physical and psychological similarities, or previous DNA testing that had been performed by other research teams. In cases of doubt (20 individual twins) standardized genotyping using a panel of 12 polymorphic microsatellite markers was performed by the Australian Genome Research Facility (Melbourne, Australia).[42] This procedure allowed unambiguous determination of zygosity in all recruited twin pairs.

Refraction in both eyes was determined by spherical equivalent (SphE) measurements expressed in diopters sphere (DS). Myopia was defined as equal to or worse than −0.5 DS and hypermetropia as equal to or worse than +0.5 DS. Emmetropia was defined as between −0.499 and +0.499 DS. Ocular biometry measurements for axial length (anterior-posterior diameter), corneal curvature, and anterior chamber depth were taken with an optical biometer (IOLMaster; Carl Zeiss Meditec, Oberkochen, Germany). A minimum of three measurements were taken and averaged to ensure consistency and reproducibility.

Genotyping

After eye examinations, a blood sample was collected from each subject via venipuncture, and DNA was extracted by standard techniques.[43] A total of 223 dizygotic twin pairs were used for subsequent genotyping and linkage analysis. The choice of polymorphic microsatellite markers (denoted using the standard D-chromosome–S-identification number nomenclature) to use for genotyping was based on those previously used by Hammond et al.[39] identifying MYP7, -8, -9 and -10 on chromosomes 11p13, 3q26, 4q12, and 8p23, respectively. Polymorphic microsatellite markers spanning chromosomal regions where the reported LOD score was greater than 2.0 in the Hammond et al.[39] study were chosen. The exception to this was on 4q12, where uninformative markers *D4S1547* and *D4S407* were replaced by *D4S392* located at the same chromosomal position. In total, 11 markers in MYP7 (11p13), 6 in MYP8 (3q26), 8 in MYP9 (4q12), and 9 in MYP10 (8p23) were chosen for genotyping. Genotyping involved the use of standard fluorescence-based genotyping methodologies and was performed by the Australian Genome Research Facility (AGRF) on an automated DNA sequencer (model 377Applied Biosystems, Inc., Foster City, CA).

Statistical Analysis

As previously reported, we observed no statistically significant differences in the mean SphE , axial length, corneal curvature, and anterior chamber depth between the right and left eyes in our twin cohort. Therefore, we performed all our genetic analysis on measurements from the right eye only.[9] Considering all four phenotypes as quantitative traits, multipoint Haseman-Elston regression analysis was performed as implemented in GeneHunter to calculate LOD scores.[44] The map positions and order of all markers was confirmed using the Ensembl online database.[45]

Initial LOD scores were generated using ocular refraction as a quantitative trait. Given that the twin cohort in the Hammond et al.[39] study consisted entirely of female twins older than 49 we decided to repeat our analysis using only women and only those older than 49, to determine how this would affect the LOD scores. In addition, we examined whether the LOD scores were due to myopia or hypermetropia by subdividing our cohort into myopic and hypermetropic groups and repeating the analysis. As an extension of this study and that of Hammond et al. we examined whether the MYP7, -8, -9, or -10 loci also contains a QTL for axial length, corneal curvature, and anterior chamber depth, by using available genotyping data.

RESULTS

The twin cohort used for analysis consisted of 223 dizygotic twin pairs who were recruited as part of the GEM Twin Study. The twins were 18 to 80 years of age (mean, 51.6 ± 15.2). They consisted of 32.3% men and 67.7% women with 143 pairs being of the same gender and 80 pairs being of opposite genders. Refraction and ocular biometric measurements for this cohort are summarized in Table 1. The refraction measurements showed a leptokurtic distribution (+6.87 DS), with a skew (−1.75) toward negative refraction. These 223 twin pairs did not differ significantly in their refraction and ocular biometric measurements when compared with the total GEM Study Twin cohort (n = 612 pairs).[9] Refraction and ocular biometric measurements for two subcohorts of the 223 pairs are also shown in Table 1. The first subcohort consisted of female-only twins (n = 108 pairs) and the second of those over the age of 49 years (n = 134 pairs).

The majority (>95%) of the twins recruited as part of the GEM Twin Study were of Caucasian background and English-speaking (Anglo-Celtic), therefore the effects of ethnicity in the development of refractive error were not explored. There were no reported twins from African and Asian backgrounds.

Using ocular refraction as the quantitative phenotype linkage analysis was initially performed on the entire cohort of 223 twin pairs. The maximum LOD scores for each chromosomal region analyzed were 0.578 for 11p13, 0.255 for 8q23, 1.126

TABLE 1. Phenotypic Characteristics of the Twin Cohort and Subcohorts Used for QTL Analysis

		SphE (DS)		Axial Length (mm)		Anterior Chamber Depth (mm)		Corneal Curvature (mm)	
	Pairs (*n*)	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Entire cohort	223	+0.33 (2.02)	+5.00–−9.25	23.40 (1.05)	27.15–21.36	3.40 (0.46)	5.22–2.36	44.09 (1.51)	48.01–39.59
Female-female	109	+0.21 (2.14)	+6.25–−14.50	23.19 (0.90)	26.51–21.28	3.38 (0.34)	4.25–2.36	44.31 (1.43)	48.09–39.59
Over 49 years old	134	−0.47 (2.20)	+6.25–−14.50	23.45 (1.12)	26.99–17.24	3.49 (0.35)	5.33–1.95	44.12 (1.50)	48.09–39.59

for 3p26, and 0.051 for 4q21 (Fig. 1). None of these linkage peaks was suggestive of linkage. The contributions of myopia and hypermetropia to the LOD scores are also indicated, but again, none of the linkage peaks reached significance. The study by Hammond et al.[39] suggested that the paired box 6 gene (*PAX6*) may be the causative gene for myopia in the MYP7 chromosomal region (11p13). The *PAX6* gene maps between *D11S904* and *D11S935*, and at this region we obtained a maximum LOD score of 0.237 using ocular refraction as the trait, 0.397 for corneal curvature, and less than 0.001 for both axial length and anterior chamber depth. This result suggests that there is little likelihood of a QTL in the vicinity of *PAX6* and excludes it as a candidate gene for refraction and ocular biometrics in this twin cohort.

Given that our primary goal was to replicate the MYP7-10 regions that were previously identified in an all female dizygotic twin populations over the age of 49, we decided to investigate what effect the influence of age and gender would have on our analysis. Reanalysis of the refraction data using a subset of twins pairs who were female-female resulted in no significant change in the LOD score with a maximum of 0.769 at 3p26 (MYP8). Reanalysis of the ocular refraction data using a subset of twin pairs over the age of 49 again did not significantly affect the LOD score, with a peak of 0.851 observed on 8p23 (MYP10).

As an extension of our analysis and that of Hammond et al.[39] we performed a QTL analysis using the ocular biometric measurements of axial length, corneal curvature, and anterior chamber depth as quantitative traits. This analysis was performed on data from the cohort of 223 twin pairs. Ocular biometric measurements were available for 90% of the twin pairs, with an identical group of twin pairs analyzed for all three traits. The maximum LOD scores were 0.142 for axial length on 8p23, 0.002 for anterior chamber depth on 4q12,

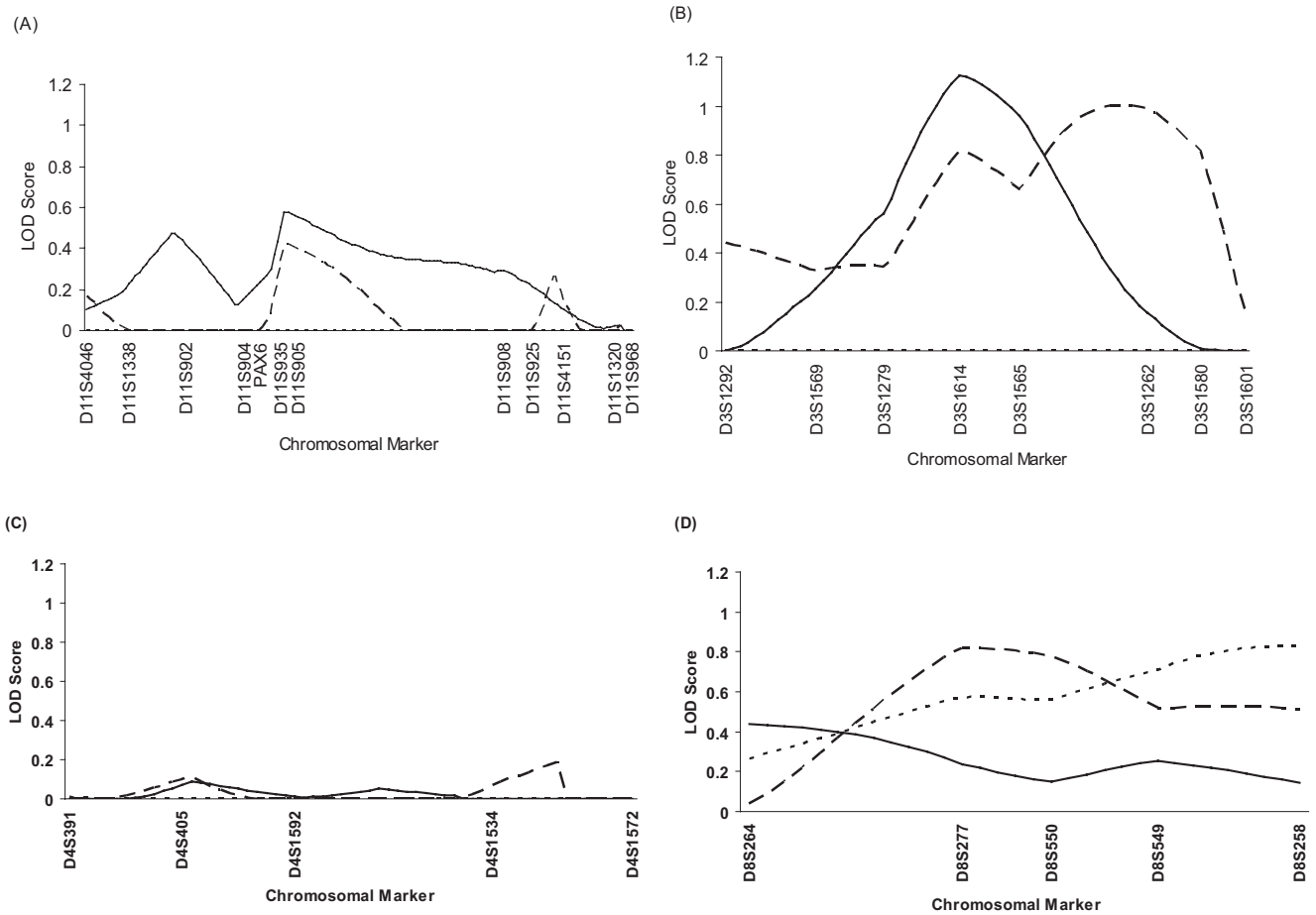

FIGURE 1. QTL linkage analysis results with SphE as the quantitative phenotype for MYP7 (11p13), MYP8 (3q26), MYP9 (4q12), and MYP10 (8p23). Analysis with myopic twin pairs only (*dotted line*), hypermetropic twin pairs only (*dashed line*), and all twin pairs (*solid line*) is shown. In cases in which the LOD score was 0, the plotted line lies on the *x*-axis and hence is not clearly visible.

and 1.178 for corneal curvature also on 4q12 (Fig. 2). Again, these results did not reach statistical significance.

Discussion

This study is the first to use both ocular refraction and biometric measurements as quantitative traits and represents only the second myopia linkage study of a twin-based population. The previous twin-based study by Hammond et al.[39] identified MYP7-10 as quantitative trait loci for ocular refraction; hence, we sought to replicate these findings in our (independent) twin cohort. We found no evidence of significant linkage to MYP7-10 when considering ocular refraction, axial length, corneal curvature, and anterior chamber depth as quantitative traits. Furthermore, there was no evidence for a QTL in the vicinity of the *PAX6* gene, which has been proposed by Hammond et al. as a potential susceptibility gene for ocular refraction, in particular myopia.

Given the difference in the findings between the GEM Twin Study and those published by Hammond et al.[39] in twin study in the United Kingdom, it is important to determine how comparable the two cohorts are phenotypically. Both cohorts are of similar size (223 pairs in our study; 221 pairs in Hammond et al.) and ethnicity (Caucasian), but differ in the age range and gender mix. The age range of our cohort was wider than that used by Hammond et al., who assessed only twins older than 49. Our subanalysis based on this age range in our twins did not alter our findings, suggesting that age is not a significant contributor to the LOD scores. The twins in both studies also differ in gender mix in that the cohort in Hammond et al. was entirely female and the GEM Twin Study had no gender selection. Given the findings of our subanalysis using only female–female twin pairs, we can conclude that gender does not appear to influence LOD scores significantly. Although it should be noted that the twin pair numbers used in the GEM Study gender- and age-selected subcohorts were smaller than those in that of Hammond et al. When considering ocular refraction, both cohorts report a similar mean and range for the SphE measurements (mean, -0.02 ± 2.17 DS; range, -14.5 to $+6.25$ DS in our study; average $+0.39 \pm 2.38$ DS; range, -12.12 to $+7.25$ DS in Hammond et al.). There were no measurements reported for axial length, corneal curvature, or anterior chamber depth by Hammond et al., and so we are unable to comment on these traits. Given the apparent phenotypic similarities between the two cohorts, we can conclude that the different findings between the two studies are unlikely to reflect cohort makeup.

The question now remains as to why, given the phenotypic similarities, the genetic results are so different between our study and that by Hammond et al. An obvious explanation is that the causative genes for ocular refraction and ocular biometrics in our twin cohort lie in a different chromosomal region outside the MYP7-10 loci. The exact nature of these genes is likely to be elucidated only by undertaking a genome-wide scan in our twin cohort. It should also be considered that perhaps environmental or even epigenetic effects influence

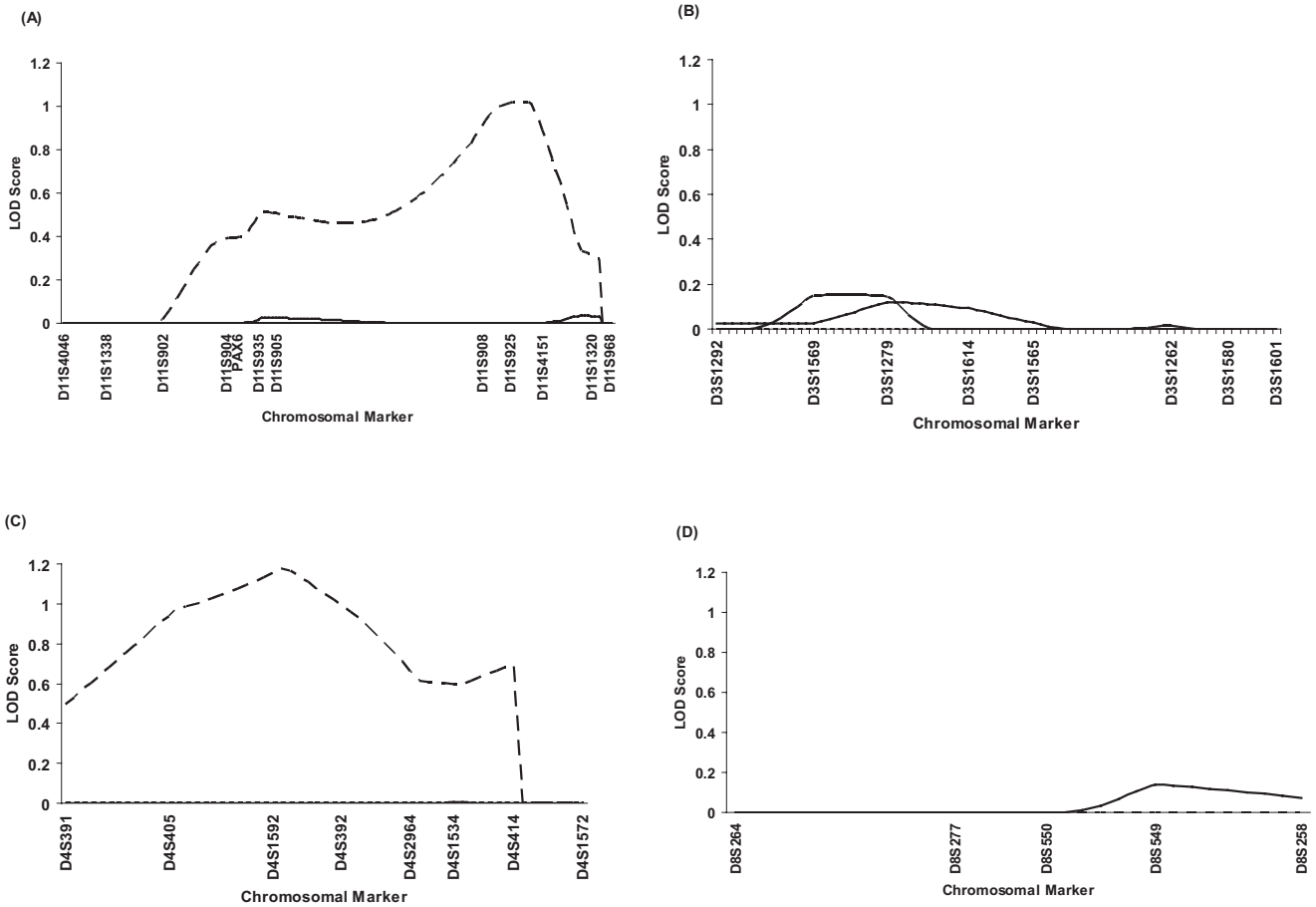

Figure 2. QTL linkage analysis results with axial length (*solid line*), corneal curvature (*dashed line*), and anterior chamber depth (*dotted line*) as the quantitative phenotype for MYP7 (11p13), MYP8 (3q26), MYP9 (4q12), and MYP10 (8p23). In cases in which the LOD score was 0 the plotted line lies on the *x*-axis and hence is not clearly visible.

ocular refraction in the two twin cohorts. At this stage, we cannot definitively explain the genetic differences between the two cohorts. More work is needed to define more clearly the genetic basis of ocular refraction and ocular biometric measurements in our twin cohort.

There have been several studies reporting susceptibility loci for ocular refraction in families but only one other report of a twin cohort. Our study represents only the second genetic study of ocular refraction in twins and presents findings that are different from the initial published study. We found no evidence of a susceptibility locus for ocular refraction at MYP7-10 and have excluded *PAX6* as the causative gene in our dizygotic twin cohort. Although this report presents negative findings, it demonstrates the complexity of ocular refraction at the genetic level and the need for replication of findings from linkage studies.

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