Genetic Dissection of Myopia

Evidence for Linkage of Ocular Axial Length to Chromosome 5q

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Purpose: To estimate heritability and locate quantitative trait loci influencing axial length.

Design: Classic twin study of monozygotic and dizygotic twins reared together.

Participants: Eight hundred ninety-three individuals from 460 families were recruited through the Twin Eye Study in Tasmania and the Brisbane Adolescent Twin Study (BATS) and had ocular axial length measured.

Methods: Structural equation modeling on the entire sample was used to estimate genetic and environmental components of variation in axial length. Analysis of existing microsatellite marker genomewide linkage scan data was performed on 318 individuals from 142 BATS families.

Main Outcome Measure: Ocular axial length.

Results: The heritability estimate for axial length, adjusted for age and sex, in the full sample was 0.81. The highest multipoint logarithm of the odds (LOD) score observed was 3.40 (genomewide $P = 0.0004$), on chromosome 5q (at 98 centimorgans [cM]). Additional regions with suggestive multipoint LOD scores were also identified on chromosome 6 (LOD scores, 2.13 at 76 cM and 2.05 at 83 cM), chromosome 10 (LOD score, 2.03 at 131 cM), and chromosome 14 (LOD score, 2.84 at 97 cM).

Conclusion: Axial length, a major endophenotype for refractive error, is highly heritable and is likely to be influenced by one or more genes on the long arm of chromosome 5. *Ophthalmology 2008;115:1053–1057 © 2008 by the American Academy of Ophthalmology.*

Uncorrected refractive error is one of the leading causes of visual impairment and blindness in the world.[1] Although the optics of the eye can be simplified, there are many components influencing refraction, and despite marked variation in ocular size as well as shape across vertebrates, almost every camera-alike eye is generally emmetropic.[2] The determinants of refractive error include the refractive indices, curvature and position of the cornea and lens, and relative position of the retina; however, of all the potential variables affecting refrac-

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tion, axial length and corneal curvature are the major contributors.[3]

High or pathological myopia is generally defined as a refractive spherical power of −6 diopters or higher and is associated with complications such as increased risk of cataract, glaucoma, and retinal detachment.[4] An inverse relationship is known to exist between axial length and refraction, whereby longer eyes are typically more myopic. Given the increasing prevalence of myopia, significant economic costs involved in its treatment, and negative impact on quality of life, more research into the etiology of refractive error is warranted.[5]

Much work has been performed investigating the genetic aspects of myopia.[6] For example, Stickler syndrome, which can occur due to mutations in collagens II and XI, is frequently associated with very high myopia and greatly increased axial length.[7] Two different loci on the X chromosome have been implicated in myopia (MYP1, MYP13), one of which can be associated with optic nerve hypoplasia or cone dystrophy.[8–10] Additionally, there are at least 12 putative autosomal loci for nonsyndromic myopia.[6] Family-based linkage analysis of high myopia has revealed evidence for genetic contributions on chromosomes 2q, 4q, 7q, 12q, 17q, and 18p (MYP2–5, MYP11–12).[11–16] Investigation within Ashkenazi Jewish families, a genetically founded population, has revealed evidence for linkage of mild myopia to chromosome 22q (MYP6) and for refractive error in general to chromosome 1p (MYP14).[17,18] In 2004, Hammond et al, using 221 dizygotic twin pairs, reported evidence for linkage of refractive error to chromosomes 11p, 3q, 4q, and 8p (MYP7–10).[19]

Many population- and twin-based studies have also investigated the presence of environmental stimuli for myopia. Environmental factors that have been proposed to cause myopia include near work, such as reading; high IQ; and academic qualification. Alternatively, spending more time outdoors (e.g., playing sports) has been proposed as an environmental influence for preventing myopia (Ophthal Physiol Opt 26[suppl 1]:30, 2006).[20,21] Such findings open the exciting prospect of identifying individuals with a genetic risk that is amenable to environmental modification.

The significant association of refraction with axial length implies that the identification of quantitative trait loci (QTLs) influencing its development would be useful in the molecular dissection of myopia. Although axial length has been found to have a higher heritability value than refraction (with estimates up to 94%),[22–25] only one linkage study on this trait has been performed.[26] In 2005, Biino et al found suggestive linkage of axial length to chromosome 2p24.[26] We investigated the heritability of axial length in our sample of Australian twins and identified potential QTLs in a subset of 318 individuals from 142 families who had undergone genomewide linkage analysis.

Materials and Methods

Clinical Assessment

Subjects were recruited through the Twin Eye Study in Tasmania and the Brisbane Adolescent Twin Study (BATS).[27,28] This study was approved by the human ethics committees of the University of Tasmania, Royal Victorian Eye and Ear Hospital, and Queensland Institute of Medical Research. Informed consent was obtained from parents with the child's assent or from adult participants before testing.

A comprehensive ophthalmic examination in each subject was preceded by a thorough interview that included questions pertaining to relevant social, medical, and ocular histories. After instillation of 1 drop of oxybuprocaine hydrochloride (0.4%) local anesthetic eyedrops, axial length was measured using ultrasound biometry with the Ocuscan biometer (Alcon, Fort Worth, TX). The axial length for each eye was calculated from the mean of 10 consecutive measurements. One eye of one subject was excluded from analysis due to the presence of a posterior staphyloma and extreme axial elongation (28.22 mm, vs. 24.34 mm in the contralateral eye without staphyloma).

Zygosity Testing and Genotyping

Genomic DNA was obtained by either buccal swabbing or venous blood collection. Zygosity in twins of the same sex was determined by genotyping at least 9 independent autosomal microsatellites using the profiler and polymorphisms. An additional sex marker (*amelogenin*) was analyzed in the BATS samples. Zygosity was confirmed by an average of 400 additional markers in the subset used for linkage analysis (see below).[29]

Analysis of preexisting genotype data was performed for this study and comprised the compilation of 3 entire-genome scans, performed in 2 separate laboratories.[30] A total of 386 short tandem repeat markers were screened at the Center for Inherited Disease Research (Baltimore, Maryland), whereas 2 series of genotyping for 410 markers were performed at the Australian Genome Research Facility (Melbourne, Australia). A total of 35 markers from the Center for Inherited Disease Research and Australian Genome Research Facility scans were shared and used to assess genotyping quality.[30] Three markers (D3S1304, D4S403, and D4S391) were found to have an unusually high number of inconsistencies between genotyping centers, and as a consequence, the data at these loci were not merged.[30] As described previously, several steps were taken to ensure the integrity of data and that the genotyping results were adequately combined.[30,31] The final stage of ensuring data integrity involved the merging of genotyping datasets, for which there was a total of 796 markers from the Center for Inherited Disease Research and/or the Australian Genome Research Facility. Possible genotyping errors and inconsistently typed markers were assessed and removed using the Merlin program (University of Michigan, Ann Arbor, MI).[32] Marker location was determined using the deCODE map.[33]

Variance Component Modeling and Linkage Analysis

Best-fit structural equation modeling was performed using the Mx software package (M. Neale, Richmond, VA) to determine the proportion of variance explained by genetic and environmental effects. Models containing additive and dominant genetic variation as well as nonshared environment variations were considered for the twin as a whole and for right and left eyes separately (Fig 1). A bivariate general sex limitation additive–dominant–environment model for the axial length of each twin's right and left eyes was used. Using age as a covariate, the most parsimonious model that did not significantly worsen the fit was chosen. Given that the variance for axial length differed significantly between males and females, a sex limitation model was applied.

The software package Merlin was used to perform the univariate quantitative trait linkage analysis,[32] whereas bivariate linkage analysis was performed using the program Mendel (Am J Hum

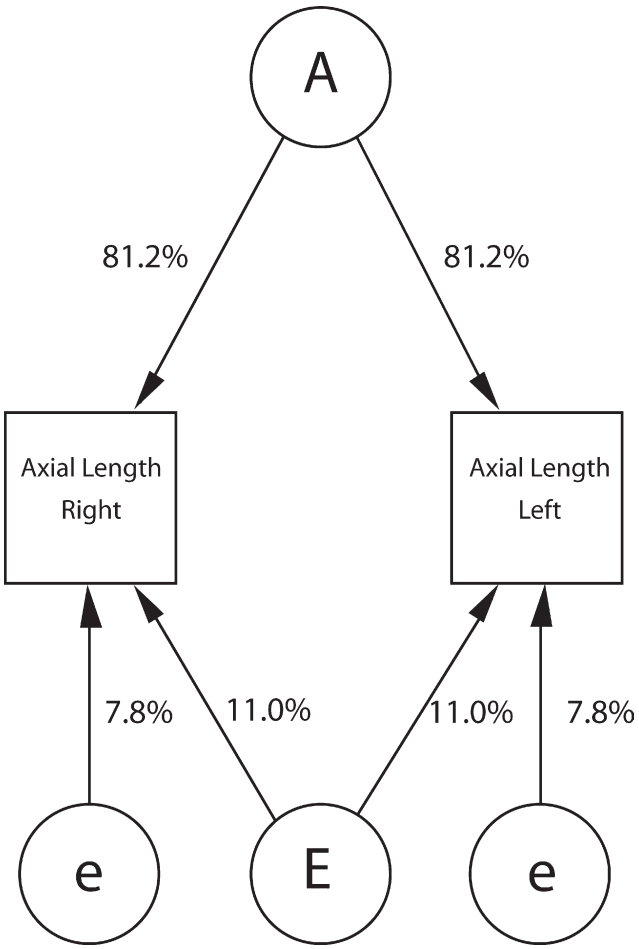

Figure 1. Path diagram illustrating parameter specification in the bivariate AE model: additive genetic factor (A) and unique environmental factor (E) and specific (e) components of variance for axial length of both eyes in each twin.

Genet 69[suppl]:A1886, 2001). As described elsewhere, the estimated proportion of alleles shared identically by descent was regressed on the squared sum and squared differences of trait values of relative pairs.[30] A gene-dropping simulation was performed using Merlin, and after 1000 simulations, logarithm of the odds (LOD) scores of 1.23 and 2.76 were found to be suggestive of or significant for genomewide linkage, respectively.[33]

Table 2. Maximum Likelihood Estimates and 95% Confidence Intervals (CIs) of Axial Length Calculated Using Mx

Zygosity	No. of Twin Pairs	Right Eye Equated to Left Eye	95% CI
MZF	86	0.816	0.760–0.855
MZM	45	0.797	0.713–0.851
DZF	77	0.297	0.099–0.459
DZM	77	0.436	0.187–0.595
DZFM	148	0.419	0.285–0.529
All MZ	131	0.808	0.763–0.844
All DZ	302	0.385	0.284–0.475

DZF = dizygotic females pairs; DZFM = dizygotic opposite-sex pairs; DZM = dizygotic males pairs; MZF = monozygotic females pairs; MZM = monozygotic males pairs.

Results

Axial length measurements were obtained from a total of 893 individuals from 460 families. There were 433 complete twin pairs of 131 monozygotic and 302 dizygotic twin pairs and 27 singletons (Table 1). Mean ages of female and male Twin Eye Study in Tasmania participants were 27 years (standard deviation [SD], 18.4; range, 5–83) and 21 years (SD, 16.8; range, 5–68), respectively. Mean ages in the BATS sample were 21 (SD, 2.3; range, 16–27) and 20 (SD, 2.5; range, 16–25) for female and male subjects, respectively. Axial length did not differ significantly between right and left eyes or between twin 1 and twin 2 ($\Delta\chi^2$ = 12.8, P = 0.85, respectively) but was slightly lower in females than in males (22.9±0.9 and 23.3±0.8, respectively; P<0.001). Age and sex were included as fixed effects in all subsequent models.

We used the maximum likelihood estimates to obtain the optimal age-corrected estimate of the correlations combining data from both eyes and both studies, having first shown that these were homogenous and, hence, could be equated across studies and between eyes (Table 2). Nevertheless, it is possible that some different genetic effects could be operating on right and left eyes, so we performed a bivariate genetic analysis. The ratio of correlation coefficients between the monozygotic and dizygotic twins was >2, suggesting that dominant genetic effects may be operating, but these could be eliminated from the model without significant loss of fit. Likewise, genetic influences specific to each eye could be eliminated. The most parsimonious model comprised one in which a single additive genetic factor accounted for 81.2% of variance in axial length of each eye (Table 3 [available at http://

Table 1. Descriptive Statistics of Axial Length (Millimeters) and Age for Sample by Sex

		Female					Male				
Source	**Traits**	*N*	*Mean*	*SD*	*Minimum*	*Maximum*	*N*	*Mean*	*SD*	*Minimum*	*Maximum*
TEST	AL_R	305	22.9	0.98	20.1	28.9	208	23.3	0.82	20.5	26.3
BATS	AL_R	188	23.0	0.81	20.8	25.3	192	23.4	0.67	21.5	25.2
TEST	AL_L	305	22.9	0.95	20.7	28.0	208	23.2	0.85	20.7	26.5
BATS	AL_L	188	22.9	0.80	20.8	25.0	192	23.2	0.70	20.6	25.1
TEST	Age	305	27	18.4	5	83	208	21	16.8	5	68
BATS	Age	188	21	2.3	16	27	192	20	2.5	16	25

AL_L = axial length, left eye; AL_R = axial length, right eye; BATS = Brisbane Adolescent Twin Study; SD = standard deviation; TEST = Twins Eye Study in Tasmania.

aaojournal.org]). A corresponding individual environmental factor accounted for 11% of variance in each eye; these influences are likely to be genuine environmental stressors or risk factors, like close work, that affect both eyes. The remaining variance, 7.8% for each eye, represents environmental influences specific to each eye; these will include errors of measurement but also any developmental or exposure asymmetries that affect left and right eyes differentially.

Linkage analysis was based on 142 twin families from the BATS. A total of 42 additional siblings (10 from 8 monozygotic families and 32 from 134 dizygotic families) were added to maximize the total linkage information. Thus, 318 individuals were included in the linkage analysis comprising 219 quasi-independent sib pairs. In sibships of size s, the number of possible sib pairings is $s(s-1)/2$—for example, for $s = 3$ the number of pairs is 3, and for $s = 4$, pairs = 6. Each of these pairs contributes information for linkage, but because such pairings are not independent of each other (e.g., for $s = 3$, individual 1 pairs with both siblings 2 and 3), we refer to them as quasi-independent sib pairs. An average of 574 (range, 204–777) microsatellite markers for sib pairs were typed with an average distance between markers of 7.68 centimorgans. Age was the sole characteristic that differed between the full twin sample and those for whom genotyping data were available. The mean age of genotyped subjects was 20.7 (SD, 2.5; range, 14–26). There is currently no genotype information available for any of the Twin Eye Study in Tasmania sample.

The highest multipoint linkage peak for axial length was identified on chromosome 5q with an LOD score of 3.4 (genomewide $P = 0.0004$), indicating significant linkage (Table 4 [available at http://aaojournal.org]). The region of interest spans approximately 10 centimorgans and is flanked by markers D5S641 and D5S1725. Additional peaks with LOD scores > 2 indicating suggestive linkage were observed on chromosomes 6, 10, and 14 (Fig 2 [available at http://aaojournal.org]).

Discussion

In this study, we measured axial length in 433 twin pairs and confirmed that it has a high heritability. The effects of additive and dominant genes explained approximately 80% of the total variance for axial length in this twin sample. We used a distinct model to partition the variance contributing to this ocular trait. In modeling both the factors common to and unique to each eye as well as each person, all latent variables were analyzed. It has been common either to disregard the measurements of one eye[25] or to take the mean of both eyes.[19,27] To an extent, our proposed path model and decomposition overcome the issue of 4 eyes in a twin study.

A subsequent genome scan was performed on a subset of 318 individuals (including 42 extra sibs) from 142 families. The 42 extra sibs increased the number of quasi-sib pairs from 134 to 219, thereby maximizing the number of informative family members to detect linkage. Although a number of chromosomal locations potentially important in determining ocular axial length were identified, we found strong evidence for a QTL on chromosome 5q. Because estimates of additive and dominant (or recessive) QTL linkage are highly confounded in sib-pair linkage analysis, we do not have the power to speculate on the mode of inheritance of the gene(s) in the 5q region. Interestingly, this region has previously been implicated in causing Wagner syndrome, a rare dominantly inherited vitreoretinopathy for which myopia is an associated feature.[34,35] An interesting candidate gene in this region is the extracellular matrix gene chrondroitin sulfate proteoglycan 2 (*CSPG2;* Online Mendelian Inheritance in Man no. 143200) at chromosome 5q14, and splice variants in *CSPG2* have been implicated in Wagner syndrome.[35] CSPG2 is one of the principal constituents of the extracellular matrix and has been identified in a variety of ocular tissues including the sclera.[36] It has a key role in tissue morphogenesis, participating in cell adhesion, proliferation, and migration, making it an excellent candidate for variation in ocular axial length.[37]

We did not replicate the finding of suggestive linkage to 2p24 as identified by Biino et al in their cross-sectional study of a geographically and culturally isolated population in eastern–central Sardinia.[26] The failure to replicate this suggestive locus is a possible reflection of the different study populations, with one being a genetic isolate, and not merely a reflection of poor marker coverage or power in our study.

Of the previously reported putative loci for myopia, we found suggestive linkage for axial length close to *MYP3* (Online Mendelian Inheritance in Man no. 603221) and *MYP9* (Online Mendelian Inheritance in Man no. 609258), where our LOD scores were 1.81 and 1.38, respectively. Linkage to the *MYP3* locus at 12q21–q23 was originally demonstrated in a large German/Italian family with high myopia.[12] In a classic twin study of refractive error, Hammond et al observed evidence for linkage at 4q12 (*MYP9*).[19] It was also noteworthy that a suggestive LOD score of 1.59 was identified in our sample at 14q32, a locus to which a recessive form of isolated microphthalmia has been mapped.[38] Nonetheless, it must be acknowledged that the regions of suggestive linkage are especially in need of replication.

A limitation of this work was that the genomewide analysis was performed in only a subset of examined individuals. Increasing the sample size for such analysis should ensure a stable point estimation for particular QTLs, and this is currently underway. Although beyond the scope of this work, the incorporation of other refractive parameters such as spherical equivalence or keratometry would also be useful, and clearly, it is premature to test for associations without first replicating these linkages in another sample.

Refractive error incurs a significant direct cost to the community, and its increasing incidence rates as a major public health concern. At one extreme of the refractive continuum, myopia is associated with significant ocular comorbidity. Although the heritability of myopia as a complex disease is generally acknowledged, the underlying genes are as yet unknown, and the many putative linked loci remain to be replicated. Dissecting the refractive error phenotype provides a powerful means by which to improve the current understanding of its molecular etiology.

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Table 3. Tests of Alternative Models of Sources of Variation in Ocular Axial Length

		Hypotheses Tested					
Parameters	%	*Fix A′_m*	*f = m (ade)*	*f = m (ADE)*	*Fix d_(fm)*	*Fix a_(fm)*	*Fix D_(fm)*
A_f	57.5	68.6	68.2	72.2	72.2	72.2	**81.2**
D_f	24.9	13.8	13.8	8.7	8.7	8.7	**—**
E_f	10.9	10.9	10.8	11.3	11.3	11.3	**11.0**
a_f	0.3	0.3	0.7	0.7	0.7	—	**—**
d_f	0.0	0.0	0.0	0.0	—	—	**—**
e_f	6.4	6.4	6.5	7.1	7.1	7.1	**7.8**
A_m	52.0	72.4	72.8	72.2	72.2	72.2	**81.2**
D_m	26.8	6.6	6.7	8.7	8.7	8.7	**—**
E_m	0.0	11.4	11.6	11.3	11.3	11.3	**11.0**
M_m	11.6	—	—	—	—	—	**—**
a_m	1.2	1.2	0.8	0.7	0.7	—	**—**
d_m	0.0	0.0	0	0.0	—	—	**—**
e_m	8.4	8.4	8.2	7.1	7.1	7.1	**7.8**
−2LL	2899.6	2899.9	2902.6	2908.8	2908.8	2911.0	**2911.2**
df	1774	1775	1778	1781	1782	1783	**1784**
$\Delta\chi^2$		0.3	2.7	6.2	0.0	2.2	**0.2**
Δdf		1	3	3	1	1	**1**
P value		0.584	0.440	0.102	1.000	0.138	**0.655**

_f = female; LL = log likelihood; _m = male.
Boldface, most significant.
Models were tested against the full model in order: no male specific (A′_m), no sex limitation [f = m (ade)], no specific dominance [f = m (ADE)], no specific additive [a_(fm)], and no dominance [D_(fm)] effects. The best fitted model was AEe.

Table 4. Summary of Genomewide Linkage Results for Bivariate Axial Length Where the Multipoint Probability Was <0.05

Chromosome	Position (cM)	Markers	Bivariate LOD	*P* Value*
4	77.89	D4S392	1.38	0.0414
5	53.78	D5S1470	1.36	0.0436
5	98.59	AD5S641	3.40	0.0004
5	151.19	D5S436	1.84	0.0144
6	76.36	D6S460	2.13	0.0074
6	83.79	D6S462	2.05	0.0090
10	26.51	D10S547	1.31	0.0496
10	131.04	D10S1237	2.03	0.0094
11	7.75	D11S2362	1.71	0.0195
11	59.7	D11S905	1.42	0.0383
11	148.87	D11S1320	1.91	0.0124
12	111.13	D12S393	1.81	0.0154
13	8.24	D13S787	1.78	0.0168
13	43.71	D13S325	1.87	0.0137
13	123.26	AD13S285	1.78	0.0166
14	97.36	D14S1434	2.84	0.0015
14	119.92	D14S292	1.59	0.0260
16	0.72	D16S2616	1.57	0.0271
19	23.69	D19S221	1.68	0.0208
21	0	D21S1432	1.37	0.0431

cM = centimorgan; LOD = logarithm of the odds score.
*Multipoint probability.

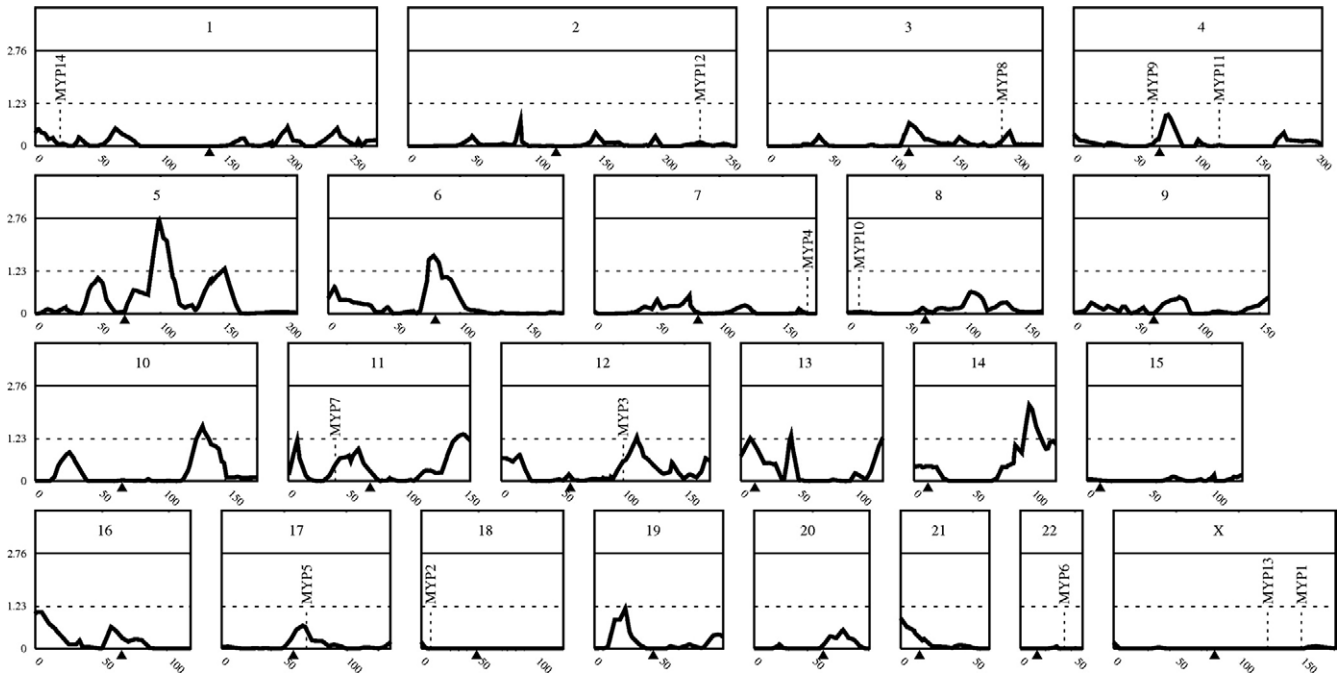

Figure 2. Genomewide multipoint bivariate linkage analysis for axial length. Chromosome positions are displayed on the x-axis (▲, centromere), and the y-axis displays the strength of evidence for linkage (logarithm of the odds score).