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A Susceptibility Locus for Myopia in the Normal Population Is Linked to the *PAX6* Gene Region on Chromosome 11: A Genomewide Scan of Dizygotic Twins

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Myopia is a common, complex trait with considerable economic and social impact and, in highly affected individuals, ocular morbidity. We performed a classic twin study of 506 unselected twin pairs and inferred the heritability of refractive error to be 0.89 (95% confidence interval 0.86–0.91). A genomewide scan of 221 dizygotic twin pairs, analyzed by use of optimal Haseman-Elston regression methods implemented by use of generalized linear modeling, showed significant linkage (LOD >3.2) to refractive error at four loci, with a maximum LOD score of 6.1 at 40 cM on chromome 11p13. Evidence of linkage at this locus, as well as at the other linkage peaks at chromosomes 3q26 (LOD 3.7), 8p23 (LOD 4.1), and 4q12 (LOD 3.3), remained the same or became stronger after model fit was checked and outliers were downweighted. Examination of potential candidate genes showed the *PAX6* gene directly below the highest peak at the 11p13 locus. *PAX6* is fundamental to identity and growth of the eye, but reported mutations usually result in catastrophic congenital phenotypes such as aniridia. Haplotype tagging of 17 single-nucleotide polymorphisms (SNPs), which covered the *PAX6* gene and had common minor allele frequencies, identified 5 SNPs that explained 0.999 of the haplotype diversity. Linkage and association analysis of the tagging SNPs showed strong evidence of linkage for all markers with a minimum χ^2_1 of 7.5 ($P = .006$) but no association. This suggests that *PAX6* may play a role in myopia development, possibly because of genetic variation in an upstream promoter or regulator, although no definite association between *PAX6* common variants and myopia was demonstrated in this study.

Introduction

Refractive error is a common, complex trait measured on a continuous scale, with myopia affecting 25%–61% of the population (Wang et al. 1994; Saw et al. 1996). Its incidence may be increasing rapidly (Tay et al. 1992), with not only economic implications but also a significant risk of permanent vision loss in individuals with high myopia ("pathological myopia"), which is also becoming more common (Rosenberg and Klie 1996; Dandona and Dandona 2001). Susceptibility loci have been identified in familial autosomal dominant high myopia (Young et al. 1998*a*, 1998*b*; Naiglin et al. 2002; Paluru et al. 2003), although, to date, these loci have not been associated with lower degrees of juvenile-onset myopia from population-based samples ("simple myopia") (Mutti et al. 2002*b*). High myopia may be influenced by certain candidate genes, such as those involved in Marfan (MIM #154700) or Stickler (MIM #108300) syndromes, but high myopia accounts for only 2%–3% of cases of myopia (Dandona and Dandona 2001). To our knowledge, no susceptibility loci for simple myopia have been published elsewhere.

The mechanism of myopia development is not fully understood. Simple myopia results from a failure of emmetropization; excessive eye growth causes images from distant objects to be focused in front of the retina. Debate about the relative contributions of genes and environment (in particular, close work) has continued (Mutti et al. 1996). Cross-sectional studies suggest that the majority of individual variation around the population mean is attributable to genetic variance (Hammond et al. 2001; Mutti et al. 2002*a*). The rapid growth in the incidence of myopia in Southeast Asia (Tay et al. 1992), however, suggests that recent generational differences are more likely to result from environmental mechanisms, such as increased literacy, than from changes in allele frequencies. There are many potential candidate genes controlling both the fundamental development of the eye and variations in eye growth.

The *PAX6* gene (MIM 607108) is a member of the paired-domain Pax family and encodes transcriptional

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Table 1

Potential Candidate Genes under Peak Area (Maximum 1-LOD, Equating to a LOD Score >5.1) on Chromosome 11 (35–45 cM)

Symbol	Locus	Name	Known Eye Phenotype(s)
BBOX1	11p14.2	γ-Butyrobetaine, 2-oxoglutarate dioxygenase	…
KCNA4	11p14	Potassium voltage-gated channel	…
C11orf8	11p14-p13	Chromosome 11 open reading frame 8, fetal brain protein 239	…
BDNF	11p13	Brain-derived neurotrophic factor	Neuroprotectant in vertebrate eyes
TCL2	11p13	Leukemia, acute T-cell	…
ELP4	11p13	PAX6 neighbor gene	Aniridia (?)
PAX6	11p13	PAX6	Aniridia, Peters anomaly, cataract, keratitis
EVR3	11p13-p12	Familial exudative vitreoretinopathy	Autosomal dominant phenotype with retinal traction and detachment
WT1	11p13	Wilms tumor	Distributed through neural retina; disruption causes small eyes in mice
GA17	11p13	Dendritic cell protein	…
CSTF3	11p13	Cleavage stimulation factor	…
HIPK3	11p13	Homeodomain interacting protein kinase 3	…

regulators involved in oculogenesis and body development, aspects of which are conserved throughout phylogeny (Simpson and Price 2002). The gene is widely expressed in the developing eye, both in neuroectoderm and surface ectoderm, as well as in the adult eye (Asher-Padan and Gruss 2001). Most *PAX6* mutations described elsewhere are deletions resulting in premature protein truncations, haploinsufficiency, and catastrophic congenital phenotypes, rather than minor normal variations. For example, *PAX6* has been the sole gene implicated in aniridia (MIM #106210), a semi-dominantly inherited condition (Glaser et al. 1992) that is characterized by severe iris hypoplasia with other associated abnormalities and that usually results in marked visual impairment (van Heyningen and Williamson 2002). Aniridia is a progressive condition, which suggests that *PAX6* may also play a maintenance role in the adult eye and that it is not involved only in oculogenesis. To our knowledge, *PAX6* has not been implicated in nonpathogenic myopia development in previous studies.

A classic twin study was undertaken, in which MZ and DZ twins were used to establish the heritability of refractive error. This was followed up with a genomewide linkage study for the continuous trait by use of unselected DZ sib pairs; the follow-up was conducted to ascertain potential susceptibility loci for myopia. On the basis of the linkage analysis results, an association study for the *PAX6* gene was then performed.

Materials and Methods

Refractive Error Measurement

Refractive error was measured in 506 volunteer twin pairs as part of the Twin Eye Study, by use of a Humphrey 670 Autorefractor (Hammond et al. 2001). Refractive error was determined by the mean spherical equivalent (SE) for the two eyes of each individual, measured in diopters (D). The 280 DZ and 226 MZ twin pairs were part of the Twins UK Registry (Spector and MacGregor 2002) and were unselected as to eye traits, having volunteered for the registry unaware of any potential eye studies or hypotheses. Twins were excluded if they had previous interventions that might have affected refraction, such as cataract or refractive surgery. After approval of the study by the hospital research ethics committee, informed written consent was obtained from all subjects and whole blood was taken for DNA extraction. Zygosity was determined by a standardized questionnaire (Martin and Martin 1975) and confirmed by DNA short-tandem-repeat fingerprinting, in cases of doubt. Individuals in the study were considered to be randomly ascertained, because sampling was not based on a subject's eye data.

Heritability Analysis

We have previously reported heritability of refractive error in this cohort (Hammond et al. 2001), but we repeated the analysis for the measure of mean SE used in the genomewide scan presented in this study. In short, quantitative-model fitting to twin data is based on comparisons of the covariances (or correlations) between MZ and DZ twin pairs; it allows for the partitioning of observed phenotypic variance into additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components. MZ twins are assumed to share the same A and D genetic variance, and DZ pairs, on average, share half of the A variance and a quarter of the D variance; C is assumed to be the same for both MZ and DZ twin pairs (the "equal environment" assumption) (Neale and Cardon 1992; Snieder et al. 1997).

Table 2

Details of the 17 SNPs Selected for *PAX6* Gene from Public Domain

SNP	Public ID	Location	Position (bp)	Major Allele	Minor Allele	MAF	Target Sequence
1	rs3026401	3′ UTR	31771833	A	G	.219	TGTAAGAAATGA[A/G]TACCATATAGGG
2	rs608293	3′ UTR	31772589	A	G	.091	CCCTTAAATGGT[A/G]AACAACTGGTTT
3	rs662702	3′ UTR	31773379	G	A	.065	GGAACTGTCAGA[G/A]AAGGGCTATGTG
4	rs1506	3′ UTR	31774607	T	A	.199	TCCACTTACAGC[T/A]GGGTGTAGATCT
5	rs2071754	Intron 12	31776891	A	G	.152	CTGTGGCCAGTG[A/G]AAGGACTAGCTC
6	rs3026389	Intron 12	31777838	G	C	.152	TCCACATACAGA[G/C]CGGTATGGGGAA
7	rs667773	Intron 10	31779671	C	T	.065	TAACCTGTCCCA[C/T]CTGATTTCCAGG
8	rs2239789	Intron 9	31780205	A	T	.465	TTATAAGGAAAA[A/T]TGATGATTTGGC
9	rs628224	Intron 8	31783483	C	T	.152	CTTCTAAAGTAA[C/T]GAACTGTCTCCG
10	rs592859	Intron 8	31783644	G	C	.091	CTTAGGTTTATC[G/C]TGGGGGTGGGGG
11	rs694617	Intron 4	31789827	C	A	.909	GGGAGGGCTGCT[C/A]TCTAAGTCGGGG
12	rs1894620	5′ UTR	31798825	G	C	.095	AGGCAGGAACGA[G/C]AGGGTGAGGCCC
13	rs1806159	5′ UTR	31800936	G	A	.087	CCACTGGACAAT[G/A]TTATTTTAAAGG
14	rs1540320	5′ UTR	31801752	G	A	.087	GTCAGCCCCTCT[G/A]TCCCCCGGGCAG
15	rs1806158	5′ UTR	31803097	G	C	.087	AAGAATGCGGCC[G/C]ACAGAGCTGGGC
16	rs1806180	5′ UTR	31804574	C	T	.087	ATCTGGGATTTT[C/T]CTGTTTTCCTCC
17	rs1806155	5′ UTR	31805434	C	A	.091	TCTAGGCCCAGA[C/A]TAGAGTGGCCAG

NOTE.—MAFs are results from this study. tSNPs are underlined.

The variance of A, C, D, E, and age were estimated by use of normal theory and maximum-likelihood methods implemented in the software package Mx (Neale 1997). The significance of the variance components A, C, D, and age was assessed by removing each sequentially and testing the deterioration in model fit. Submodels were contrasted to full models by use of nested comparisons and an asymptotic χ^2 fit statistic. If there was no significant change in model fit, then the parameter could be removed, leading to a parsimonious model explaining the variance and covariance. Data handling and preliminary analyses were conducted by use of STATA (StataCorp 2003).

Genomewide Linkage Study

A genomewide linkage analysis was performed on 221 of the 280 DZ twin pairs for which there were genomic marker data from the heritability study. The genomic scan was based on DNA extracted from venous blood samples of the study subjects. Scans involved the use of standard fluorescence–based genotyping methodologies (Reed et al. 1994; Pritchard et al. 1995) for the analysis of 737 highly polymorphic microsatellite markers from the ABI Prism linkage mapping set (Applied Biosystems) and the Généthon Genetic Linkage Map (Dib et al. 1996), as has been described elsewhere (Wilson et al. 2003). The estimated genotyping error rate was <1%.

Linkage Analysis

Multipoint genomewide linkage analyses were performed by use of the unadjusted mean SE of both eyes (in D) and optimal Haseman-Elston regression methods, implemented by use of a generalized linear model (GLM) (Barber et al. 2004). This method, implemented in STATA (StataCorp), is algebraically equivalent to other likelihood techniques (Kruglyak and Lander 1995; Almasy and Blangero 1998) but has the advantage of being robust to deviations in multivariate normality by freely estimating the coefficient of variation (i.e., the mean and variance-corrected residual error) and by utilizing a robust Huber estimate of variance (Barber et al. 2004). Regression diagnostics were used to check the reliability of model fit, including an iterative regression (IR) routine applied to GLM by use of the Huber estimate, followed by biweight iterations (StataCorp 2003). On a genomewide basis, any observed divergence between GLM and GLM_{IR} results indicated potentially poor-fitting models for specific regions.

The map positions and ordering of all marker loci were determined from the Généthon Genetic Linkage Map. Approximate support intervals were generated by use of a 1-LOD drop-interval approach. Twin pairs formed independent families, and no additional sibs were considered in the analysis.

Association Analysis

Of the known genes observed under the linkage peak on chromosome 11p13 (see table 1), only *PAX6* is directly implicated in the identity and growth of the eye (Dominguez et al. 2004) and has a well-documented role in oculogenesis. We therefore undertook a three-step candidate-gene association study (Weale et al. 2003) by (1) characterizing the haplotypic structure of the gene, (2) identifying a subset of highly informative SNP markers in the region, referred to as "tagging SNPs" (tSNPs), and (3) typing the tSNPs for the clinical sample and

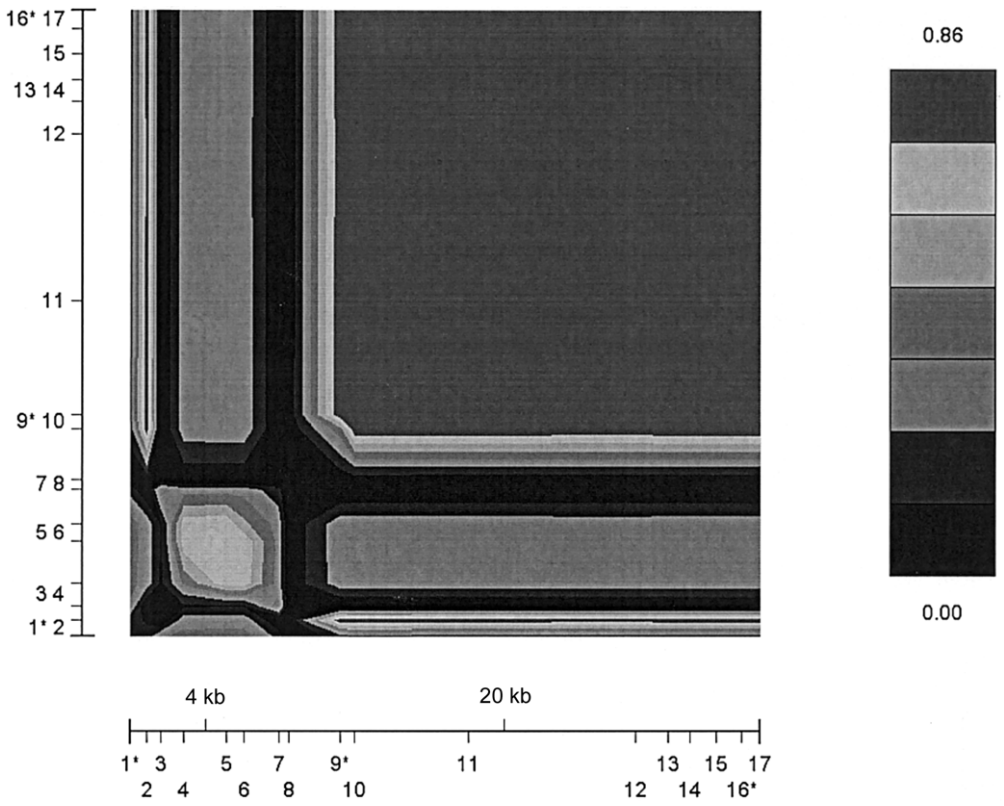

Figure 1 LD map for the 17 *PAX6* SNPs genotyped ($n = 22$) over a 32-kb region. The LD statistic plotted is r^2. The plot orientation is 3′→5′, with SNPs 1, 9, and 16 (rs3026401, rs628224, and rs1806180) also genotyped by the HapMap Project.

testing these for linkage and association, by use of the quantitative transmission/disequilibrium test (QTDT) (Abecasis et al. 2000*a*).

PAX6 *SNP Genotyping*

SNPs were selected from public SNP databases (SNP Consortium and National Center of Biotechnology Information) by criteria that included population-frequency validation, multiple submitters, high-profile submitters, and absence of repeats masking in the sequence. To assess linkage disequilibrium (LD) in a 34-kb region on chromosome 11p13, 17 SNPs, covering 8 kb upstream and 4 kb downstream of the *PAX6* gene, were genotyped for 22 individuals. Details of these 17 SNPs are given in table 2.

The PCR method is based on the accumulation of fluorescence during the PCR process by degradation of an internally quenched allele-specific probe bound to the same template (Heid et al. 1996). Allelic discrimination is achieved by the use of two oligonucleotide probes, each complementary to one of the two alleles and labeled with a different fluorescent reporter dye. Applied Biosystems designed the PCR primers and allele-specific minor groove binder (MGB) probes and optimized the reaction conditions for all 17 SNPs. The company also supplied the reagents and consumables used for TaqMan PCR in our study.

The PCR reaction was performed with the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) in a volume of 25 μl, containing 12.5 μl of TaqMan 2 × Universal PCR Master Mix, 20–50 ng of genomic DNA, and the optimized primer/probe solution as supplied by Applied Biosystems. The PCR program included an initial step at 95°C for 10 min, followed by 40 cycles at 92°C for 15 s and 60°C for 1 min. Endpoint reading of fluorescence and analysis of the genotypes were performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems).

Assessment of PAX6 *Haplotypic Structure*

LD measures r^2 and D' (Hedrick 1987) were used to assess pairwise LD between markers, both in the immediate vicinity of *PAX6,* by use of 17 public-domain SNPs with common minor allele frequencies (MAFs $\geq$7%), and in a 250-kb region on either side of *PAX6,* by use of HapMap data. tSNPs were originally identified by use of haplotype-diversity criteria (Johnson et al. 2001). However, because of the high levels of disequi-

librium found in the gene, other, more recent, and potentially more efficient marker-based selection criteria were used, such as association r^2 (Weale et al. 2003), which, in this case, also produced the same tagging solutions. Haplotype tagging in the 34-kb region identified five tSNPs, which explained 0.999 of the haplotype diversity arising from 17 SNPs with common MAFs (see fig. 1). The five tSNPs were subsequently genotyped in the original sample of 221 DZ twin pairs.

Results

The 506 twin pairs included in the heritability study were aged 49–79 years, with a mean age of 62.2 years (SD 5.7 years). Their mean refractive error (SE) was +0.39 D (SD 2.38), with a range of −12.12 D to +7.25 D. The distribution was leptokurtic (5.87 D), with a left skew (−0.91 D), in keeping with other population studies of refractive error. There were no significant differences between MZ and DZ twins for age (62.4 years vs. 62.0 years) and refractive error (mean 0.36 D vs. 0.40 D). Age accounted for only ~1% of the variance and so was excluded from further analysis.

Refractive errors for twin 1 versus twin 2 for mean SE are plotted in figure 2. The pairwise intraclass correlation coefficient was 0.89 for MZ twins and 0.49 for DZ twins, suggesting a significant genetic contribution to the variance. Univariate model–fitting results for mean SE are displayed in table 3 and show the best-fitting model to be the one that explained the variation of SE due to additive genetic effects (A) and to unique environmental effects (E). The effects of shared family environment (C) and dominant genes (D) could be eliminated without significant deterioration in model fit. The parameter estimates for mean SE obtained by this model suggested a very high heritability of 0.89 (95% CI 0.86–0.91), with the rest of variance (0.11 [95% CI 0.09–0.14]) explained by individual environmental effects. These results do not differ significantly from those of our previous heritability analysis, in which right and left eyes were studied separately (Hammond et al. 2001).

Table 3

Model-Fitting Results for Univariate Analysis of Mean SE

Model	χ^2	$\Delta\ \chi^{2a}$	Δ df[a]	P^b
ACE	13.390	…	…	…
ADE	14.119	.729	…	…
AE	14.119	.729	1	.4
CE	135.425	121.306	1	<.001

[a] Change between submodel and full model.
[b] Probability that $\Delta\chi^2 = 0$.

The 221 DZ twin pairs included in the genomewide linkage analysis did not significantly differ from the whole cohort (mean age 61.4 years, mean SE +0.31 D). Maximum evidence of linkage in the twins was observed at chromosome 11p13 (LOD 6.1), with further loci at chromosomes 3q26 (LOD 3.7), 4q12 (LOD 3.3), 8p23 (LOD 4.1), and 11q23-24 (LOD 2.9), as shown in figure 3. Evidence for these loci became stronger after model-

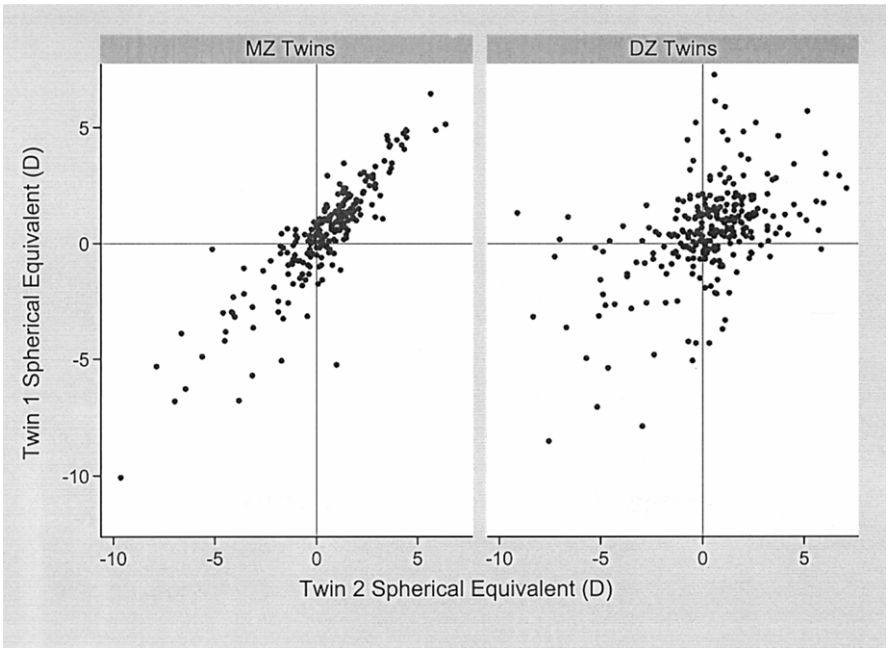

Figure 2 Scatter plots of refractive error measured as mean SE for each twin (in D), plotted for twin 1 versus twin 2 in 226 MZ and 280 DZ twin pairs.

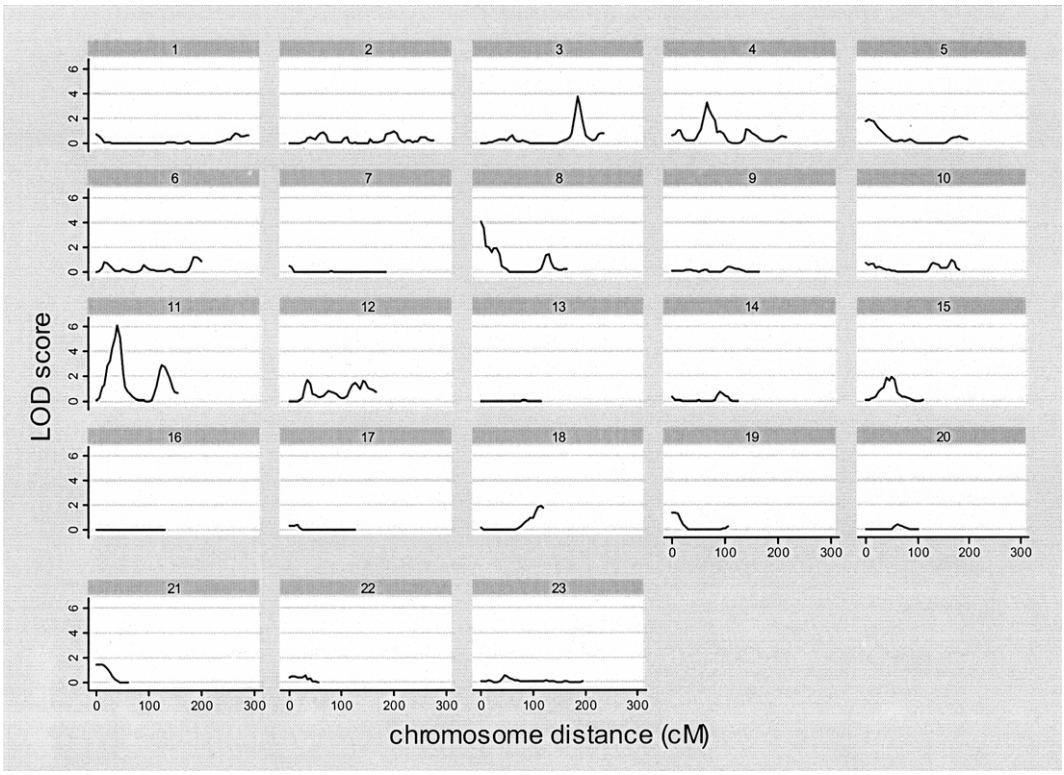

Figure 3 Linkage peaks in genomewide scan of 221 twin pairs

fit residuals were checked and after unduly influential outliers were downweighted by use of GLM_{IR}, as displayed in table 4. The linkage results for the four peaks with LOD scores >3.2 are displayed in figure 4, with marker information. To examine whether this linkage pertained to myopia, as opposed to hyperopia (farsightedness), twin pairs were divided into myopes (mean SE ≤ 0; 85 pairs) and hyperopes (mean SE >0; 136 pairs) by use of the arbitrary threshold of zero. Figure 4 demonstrates that the linkage signal was maintained in the myopic group, despite the smaller numbers (albeit with a lower LOD score), as well as in the hyperopic group. This suggests that the locus is likely to confer myopia susceptibility across the whole spectrum of refractive errors.

The nearest markers to the maximum LOD score of 6.1 at 40 cM from the p-terminal on chromosome 11p13 were D11S904 (37 cM; two-point LOD score 4.28) and D11S935 (49 cM; two-point LOD score 2.2). Support intervals, which used the maximum 1-LOD approach, equated to 35–45 cM, in which there are 44 described genes or potential genes. Some possible candidate genes are listed in table 1. The most likely candidate gene in this area is *PAX6,* at 39–42 cM, directly beneath the highest linkage peak at 40 cM. We therefore attempted to determine whether *PAX6* was associated with myopia in this population.

Analysis of the tSNP genotypes and haplotypes by use of QTDT showed strong evidence of linkage for all markers (minimum $\chi^2_1 = 7.5$; $P = .006$), as detailed in table 5. There was marginal evidence for population stratification for SNP1 and SNP3 (and the resulting haplotypes). All five SNP polymorphisms were in Hardy-Weinberg equilibrium, and no evidence for phenotypic association with any of the SNPs or haplotypes was found by use of tests of total association or tests of association robust to population stratification (Abecasis et al. 2000*b*). Tests of linkage, controlling for association, also did not significantly reduce the observed linkage signal.

Discussion

These results show a high heritability for refractive error of 0.89. This is the first study of myopia, to our knowledge, that has demonstrated significant linkage to susceptibility loci by use of a genomewide screen. In this population-based sample, we have found four loci with LOD scores >3.2. A QTL in the vicinity of *PAX6* that predisposes individuals to myopia has not been reported elsewhere; to date, only major developmental eye abnormalities have been associated with mutations in this gene. This means that *PAX6* may play an important role postnatally in continued eye growth.

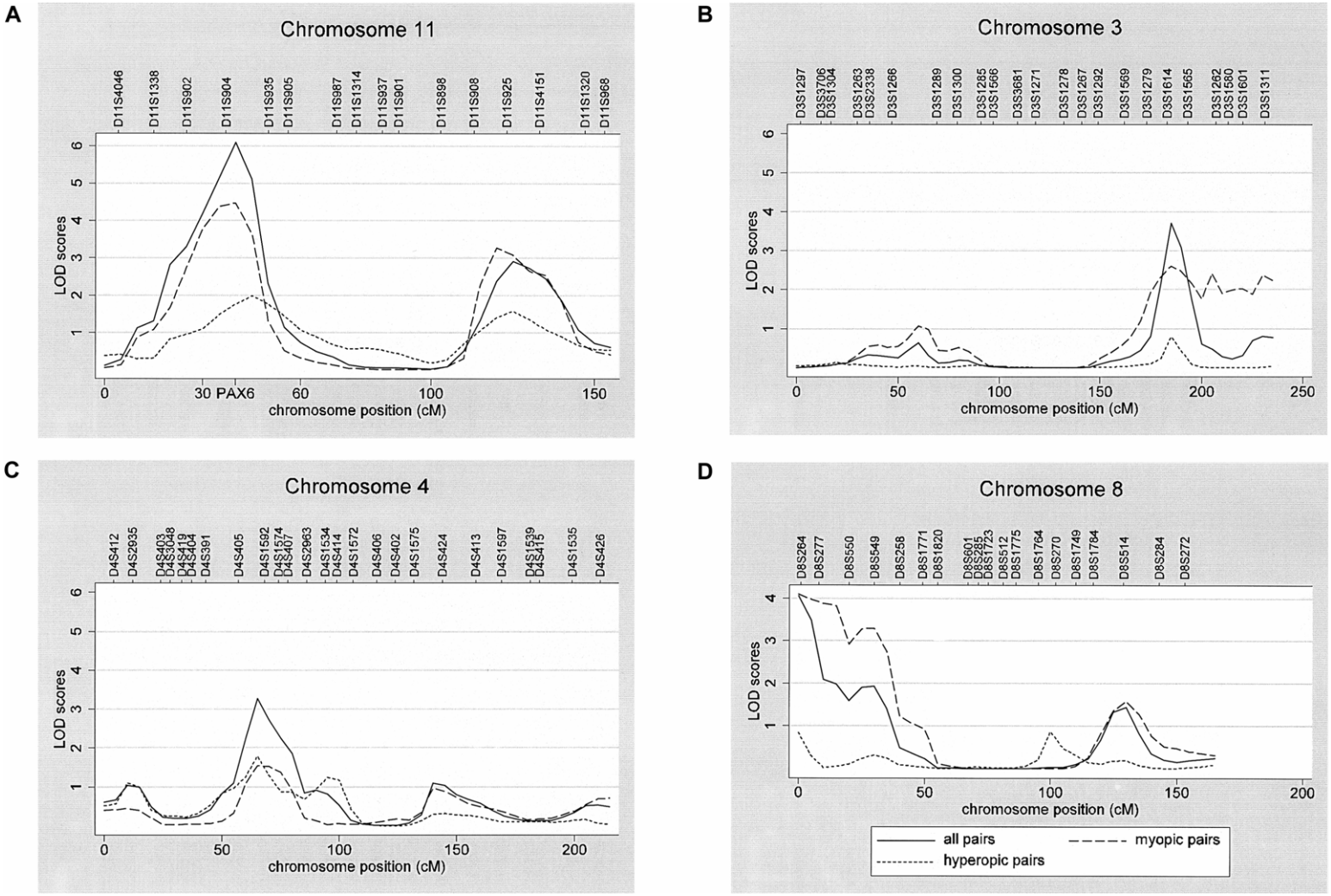

Figure 4 Linkage analysis results for the four peaks with LOD scores >3.2 (on chromosomes 3, 4, 8, and 11) for all sib pairs, "myopic" pairs (mean SE ≤0), and "hyperopic" pairs (mean SE >0). Marker positions are shown, and the position of the *PAX6* gene on chromosome 11 is indicated.

Table 4

Significant Linkage Loci (LOD >2.0) in Genomewide Scan

Chromosome	Position (cM)	LOD Score (GLM)	*P* Value (GLM)	LOD Score (GLM_{IR})	*P* Value (GLM_{IR})
11p13	40	6.1	.0000001	8.0	.000000001
3q26	185	3.7	.00004	5.1	.000001
8p23	0	4.1	.00002	4.8	.000003
4q12	65	3.3	.0001	4.6	.000004
11q23-24	125	2.9	.0003	4.4	.000005

NOTE.—Généthon Map chromosomal positions were used for this analysis.

The strong evidence of linkage to *PAX6* suggests that genetic variants are located in the vicinity of the gene, perhaps within regulatory or promoter regions of *PAX6*. The important role (Kellis et al. 2003) and greater numbers of these variants are increasingly being recognized (Dermitzakis et al. 2003). There have also been advances in the understanding of the control of eye growth: the dosage of *PAX6* may be important, and overexpression of *PAX6* in transgenic mice led to reduced eye size (Schedl et al. 1996). Recent work on *Drosophila melanogaster* eyes has shown that two distinct Pax genes control eye identity and growth separately and that these equate to the *PAX6* and *PAX6(5a)* isoforms of the gene in humans (Dominguez et al. 2004). This raises the exciting possibility that *PAX6* or one of its isoforms may be involved in eye growth in childhood, and, therefore, it may play a role in the etiology of myopia.

Animal studies have suggested that eye growth is regulated by the quality of the retinal image ("emmetropization") (Flitcroft 1998). If humans have the same mechanism, human myopia may occur if a child inherits a dysfunctional emmetropization mechanism (Norton 1994), with the trigger being close work. The exact mechanisms are unclear. Humoral interactions occur between scleral and choroidal layers in response to negative and positive fitted lenses in chicks (Marzani and Wallman 1997), and this effect is also seen in primates (Hung et al. 2000). Retinoic acid may have a mediating role between vision and growth (Mertz and Wallman 2000). Further examination of the four loci significantly linked to myopia in this study may allow further elucidation of the mechanisms of myopia development.

The high heritability of myopia in this twin cohort (0.89 [95% CI 0.86–0.91]) is almost identical to that reported in the Danish twin study (0.91 [95% CI 0.86–0.94) (Lyhne et al. 2001) and is consistent with other family studies (Zadnik et al. 1994; Framingham Offspring Eye Study Group 1996). The possibility of ascertainment bias was reduced because the twins in this study were unselected for refractive error, having volunteered for osteoporosis and other studies. The twins in this study are representative of the general population for many common traits and exposures, such as those in the cardiovascular and musculoskeletal systems (Andrew et al. 2001), supporting the generalizability of these results. Although the LOD scores of these loci are impressive for a common complex trait, we need to confirm them in a replication study to prioritize potential regions of interest for further research.

The failure to find a phenotypic association with a common SNP in the *PAX6* gene could have occurred for one of several reasons: (1) the variant(s) lies within the gene and has been missed by the tSNPs, (2) the variant lies outside the *PAX6* gene, or (3) the linkage signal is a false-positive result. In the first scenario, if the potential causal variant is common, it is unlikely that one or a combination of the tSNPs we selected are not in LD with the polymorphism, since the common haplotype diversity

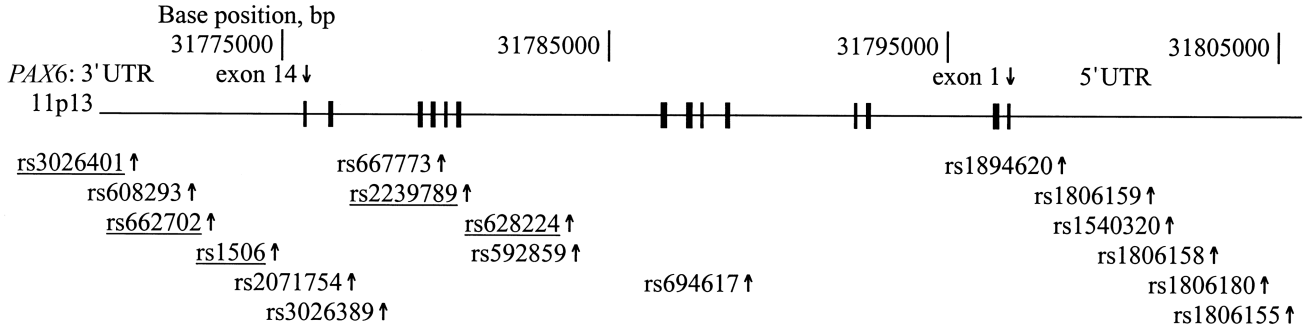

Figure 5 Schematic representation of *PAX6* gene and the positions of SNPs (presented from 3′ to 5′). Exons are represented as vertical lines, and introns are depicted as the horizontal line between the exons. tSNPs that were used in this study are underlined.

Table 5

Results of Linkage, Association, and Combined (Linkage and Association) Tests by Use of QTDT

Marker	χ^2_1 Value for Multipoint Linkage Test	*P* Value for				
		Multipoint Linkage Test	Association Test			Combined Test
			Population Stratification (PS)	Total	Permute (Robust to PS)	
SNP1	7.5	.006	.09	...	...	.006
SNP3	7.5	.006	.05	...	...	.006
SNP4	7.5	.006	...	...	...	.006
SNP8	7.5	.006	...	...	...	.006
SNP9	7.5	.006	...	...	...	.006
Haplotypes	6.8	.009	.008	...	...	.009

captured was >99% (Johnson et al. 2001). In addition, in a sampling experiment in which each of the original 17 SNPs was sequentially dropped (Weale et al. 2003), the three- or four-tSNP solution exactly predicted the dropped SNP ($r^2 = 1$ in all cases). The exception was rs2239789 (SNP8), for which r^2 was 21%. This is because SNP8 lies in a region of the gene with low LD between exons 8 and 9 (see figs. 1 and 5). It is possible that a (combination of) rare variant(s) not in LD with the common tSNPs may be associated with myopia; sequencing would be required for their identification.

However, if common and rare functional polymorphisms within the *PAX6* gene are ruled out, then the alternative explanations for the linkage signal are either a different gene nearby (such as *ELP4* [see table 1]) or a variant in a nearby regulator of *PAX6*. Further detailed examination of *PAX6* and its isoforms and regulatory regions is required.

Conclusion

Myopia is a common trait that is increasing in incidence and is strongly heritable. Four loci have been identified from a genomewide linkage analysis, with the highest peak on chromosome 11p13 (LOD 6.1). We found strong linkage for refractive error in the vicinity of *PAX6,* a candidate gene at this locus. Further mapping is required to confirm whether the linkage to *PAX6* is due to regulatory loci or to linkage to an unrecognized nearby gene.

Acknowledgments

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Electronic-Database Information

The URLs for data presented herein are as follows:

HapMap, http://www.hapmap.org/ (for HapMap data)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *PAX6*, Marfan syndrome, Stickler syndrome, and aniridia)
University of California–Santa Cruz Genome Browser Gateway (July 2003 version), http://www.genome.ucsc.edu/cgi-bin/hgGateway?db=hg8 (for SNPs in fig. 5)

References

Abecasis GR, Cardon LR, Cookson WO (2000*a*) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66:279–292

Abecasis GR, Cookson WO, Cardon LR (2000*b*) Pedigree tests of transmission disequilibrium. Eur J Hum Genet 8:545–551

Almasy L, Blangero J (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198–1211

Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ (2001) Are twins and singletons comparable?: a study of disease-related and lifestyle characteristics in adult women. Twin Res 4:464–477

Ashery-Padan R, Gruss P (2001) Pax6 lights-up the way for eye development. Curr Opin Cell Biol 13:706–714

Barber MJ, Cordell HJ, MacGregor AJ, Andrew T (2004) Gamma regression improves Haseman-Elston and variance components linkage analysis for sib-pairs. Genet Epidemiol 26:97–107

Dandona R, Dandona L (2001) Refractive error blindness. Bulletin of the World Health Organization 79:237–243

Dermitzakis ET, Reymond A, Scamuffa N, Ucla C, Kirkness E, Rossier C, Antonarakis SE (2003) Evolutionary discrimination of mammalian conserved non-genic sequences (CNGs). Science 302:1033–1035

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Dominguez M, Ferres-Marco D, Gutierrez-Avino FJ, Speicher SA, Beneyto M (2004) Growth and specification of the eye are controlled independently by Eyegone and Eyeless in *Drosophila melanogaster.* Nat Genet 36:31–39

Flitcroft DI (1998) Ophthalmologists should consider the causes of myopia and not simply treat its consequences. Br J Ophthalmol 82:210–211

Framingham Offspring Eye Study Group (1996) Familial aggregation and prevalence of myopia in the Framingham Eye Study. Arch Ophthalmol 114:326–332

Glaser T, Walton DS, Maas RL (1992) Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. Nat Genet 2:232–239

Hammond CJ, Snieder H, Gilbert CE, Spector TD (2001) Genes and environment in refractive error: the twin eye study. Invest Ophthalmol Vis Sci 42:1232–1236

Hedrick PW (1987) Gametic disequilibrium measures: proceed with caution. Genetics 117:331–341

Heid CA, Stevens J, Livak KJ, Williams PM (1996) Real time quantitative PCR. Genome Res 6:986–994

Hung LF, Wallman J, Smith EL III (2000) Vision-dependent changes in the choroidal thickness of macaque monkeys. Invest Ophthalmol Vis Sci 41:1259–1269

Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA (2001) Haplotype tagging for the identification of common disease genes. Nat Genet 29:233–237

Kellis M, Patterson N, Endrizzi M, Birren B, Lander ES (2003) Sequencing and comparison of yeast species to identify genes and regulatory elements. Nature 423:241–254

Kruglyak L, Lander ES (1995) Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439–454

Lyhne N, Sjolie AK, Kyvik KO, Green A (2001) The importance of genes and environment for ocular refraction and its determiners: a population based study among 20–45 year old twins. Br J Ophthalmol 85:1470–1476

Martin NG, Martin PG (1975) The inheritance of scholastic abilities in a sample of twins. I. Ascertainments of the sample and diagnosis of zygosity. Ann Hum Genet 39:213–218

Marzani D, Wallman J (1997) Growth of the two layers of the chick sclera is modulated reciprocally by visual conditions. Invest Ophthalmol Vis Sci 38:1726–1739

Mertz JR, Wallman J (2000) Choroidal retinoic acid synthesis: a possible mediator between refractive error and compensatory eye growth. Exp Eye Res 70:519–527

Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K (2002*a*) Parental myopia, near work, school achievement, and children's refractive error. Invest Ophthalmol Vis Sci 43:3633–3640

Mutti DO, Semina E, Marazita M, Cooper M, Murray JC, Zadnik K (2002*b*) Genetic loci for pathological myopia are not associated with juvenile myopia. Am J Med Genet 112: 355–360

Mutti DO, Zadnik K, Adams AJ (1996) Myopia: the nature versus nurture debate goes on. Invest Ophthalmol Vis Sci 37:952–957

Naiglin L, Gazagne C, Dallongeville F, Thalamas C, Idder A, Rascol O, Malecaze F, Calvas P (2002) A genome wide scan for familial high myopia suggests a novel locus on chromosome 7q36. J Med Genet 39:118–124

Neale MC (1997) Mx: statistical modeling. Department of Psychiatry, Medical College of Virginia, Richmond, VA

Neale MC, Cardon LR (1992) Methodology for genetic studies of twins and families. Kluwer Academic Publishers, Dordrecht, The Netherlands

Norton TT (1994) A new focus on myopia. JAMA 271:1362–1363

Paluru P, Ronan SM, Heon E, Devoto M, Wildenberg SC, Scavello G, Holleschau A, Makitie O, Cole WG, King RA, Young TL (2003) New locus for autosomal dominant high myopia maps to the long arm of chromosome 17. Invest Ophthalmol Vis Sci 44:1830–1836

Pritchard LE, Kawaguchi Y, Reed PW, Copeman JB, Davies JL, Barnett AH, Bain SC, Todd JA (1995) Analysis of the CD3 gene region and type 1 diabetes: application of fluorescence-based technology to linkage disequilibrium mapping. Hum Mol Genet 4:197–202

Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SC, Kawaguchi Y, Cordell HJ, Balfour KM (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Rosenberg T, Klie F (1996) Current trends in newly registered blindness in Denmark. Acta Ophthalmol Scand 74:395–398

Saw SM, Katz J, Schein OD, Chew SJ, Chan TK (1996) Epidemiology of myopia. Epidemiol Rev 18:175–187

Schedl A, Ross A, Lee M, Engelkamp D, Rashbass P, van Heyningen V, Hastie ND (1996) Influence of PAX6 gene dosage on development: overexpression causes severe eye abnormalities. Cell 86:71–82

Simpson TI, Price DJ (2002) Pax6: a pleiotropic player in development. Bioessays 24:1041–1051

Snieder H, Boomsma DI, van Doornen LJP (1997) Heritability of respiratory sinus arrhythmia: dependency on task and respiration rate. Psychophysiology 34:317–328

Spector TD, MacGregor AJ (2002) The St. Thomas' UK Adult Twin Registry. Twin Res 5:440–443

StataCorp (2003) Intercooled Stata for Unix Select Edition (SE), version 8. StataCorp, College Station, TX

Tay MT, Au Eong KG, Ng CY, Lim MK (1992) Myopia and educational attainment in 421,116 young Singaporean males. Ann Acad Med Singapore 21:785–791

van Heyningen V, Williamson KA (2002) PAX6 in sensory development. Hum Mol Genet 11:1161–1167

Wang Q, Klein BE, Klein R (1994) Refractive status in the Beaver Dam Eye Study. Invest Ophthalmol Vis Sci 35:4344–4347

Weale ME, Depondt C, Macdonald SJ, Smith A, Lai PS, Shorvon SD, Wood NW, Goldstein DB (2003) Selection and evaluation of tagging SNPs in the neuronal-sodium-channel gene SCN1A: implications for linkage-disequilibrium gene mapping. Am J Hum Genet 73:551–565

Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M, Prince RL, Thompson D, Thompson E, Bailey M, Kleyn PW, Sambrook P, Shi MM, Spector TD (2003)

Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. Am J Hum Genet 72:144–155

Young TL, Ronan SM, Alvear AB, Wildenberg SC, Oetting WS, Atwood LD, Wilkin DJ, King RA (1998*a*) A second locus for familial high myopia maps to chromosome 12q. Am J Hum Genet 63:1419–1424

Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS, Atwood LD, Wilkin DJ, King RA (1998*b*) Evidence that a locus for familial high myopia maps to chromosome 18p. Am J Hum Genet 63:109–119

Zadnik K, Satariano WA, Mutti DO, Sholtz RI, Adams AJ (1994) The effect of parental history of myopia on children's eye size. JAMA 271:1323–1327