Abstract: PURPOSE: To estimate heritability and locate quantitative trait loci influencing axial length.
DESIGN: Classical twin study of monozygotic (MZ) and dizygotic (DZ) twins reared together.
PARTICIPANTS: 893 individuals from 460 families were recruited through the Twin Eye Study in Tasmania (TEST) and the Brisbane Adolescent Twin Study (BATS) and had ocular axial length measured.
METHOD: 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 genome-wide linkage scan data were performed on 318 individuals from 142 BATS families.
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 (genome-wide p=0.0004) on chromosome 5q (at 98 cM). Additional regions with suggestive multipoint LOD scores were also identified on chromosome 6 (LOD score=2.13 at 76 cM; LOD score=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.
Dr Alex Hewitt,
17 Fernwood Way,
Upper Sturt
South Australia, 5165
Australia
Email: hewitt.alex@gmail.com

11 July, 2007

Andrew P. Schachat
Editor-in-Chief
Ophthalmology

Dear Dr Schachat,

RE: Manuscript for consideration titled:
“Genetic dissection of myopia: evidence for linkage of ocular axial length to chromosome 5q.”

Thank you for your recent email and rapid review of our paper. Our manuscript has been formatted to comply with the journal regulations; additionally we have considered the reviewers’ comments and hope that we have addressed each of them adequately:

Reviewer 1:
1. **Issue:** In the abstract the statement that candidate genes exist is frivolous.
   **Action:** Abstract: The sentence “This region contains several candidate genes.” has been removed.

2. **Issue:** In the abstract the Conclusion is a statement of future work.
   **Action:** Abstract Conclusion: has been adjusted to now read “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.”

3. **Issue:** The abstract should be revised to explain that this is an analysis of preexisting genotype data.
   **Action:** Abstract Methods: Sentence changed to now read “Analysis of existing microsatellite marker genome-wide linkage scan data were performed on 318 individuals from 142 BATS families.”

4. **Issue:** Introduction: The sentence that most eyes are emmetropic can be deleted.
   **Action:** Sentence removed

5. **Issue:** In the introduction further explanation as to why myopia is a scientifically important and what genetic research has been performed.
   **Action:** The introduction has been extensively re-written. The sentences beginning “The availability of many effective treatments …”; “However, these
treatments do not rectify the …”; “Furthermore, there are morbidities associated with most treatments, …” have been removed. The paragraphs beginning “High or pathological myopia is…”, “Much work has been performed in the genetic …” and “Many population and twin based studies …” have been inserted.

6. **Issue:** It is not clearly stated that this is analysis of genotyping data which was performed for quantitative trait mapping, to be a re-analysis of pre-existing data.
   **Action:** Methods sentence changed to now read “Analysis of pre-existing genotype data was performed for this study and …”

7. **Issue:** Data for previously identified loci should be shown in the results.
   **Action:** We have inserted the myopia loci details on Figure 2.

8. **Issue:** The failure to replicate the 2p24 locus needs to be discussed.
   **Action:** Discussion section: sentence inserted “The failure to replicate this suggestive locus is a likely 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.”

9. **Issue:** The heading for Table 2 does not match the contents of the table.
   **Action:** Caption changed to now read: Maximum Likelihood Estimates and 95% confidence intervals of axial length calculated using Mx (sex and age effects were in the means model).

10. **Issue:** No citation in the text for Figure 1 could be found.
    **Resolution:** The cross-reference for Figure 1 is located in the methods section under the “Variance component Modeling and Linkage Analysis” subheading.

11. **Issue:** The linkage plots in Figure 2 should include a sufficiently detailed cM scale on the x axis to enable comparison to other work.
    **Action:** The figure has been adjusted to include a cM scale.

**Reviewer 2:**

12. **Issue:** In the Introduction Section, the authors should summarize current knowledge on aetiology of myopia including the genetic, environmental and personnel factors.
    **Action:** as per issue 5.

13. **Issue:** More discussion should be devoted to the potential inherited patterns such as autosomal dominate or recessive in the chromosome 5q region.
    **Action:** Discussion: Sentence inserted: “Since estimates of additive and dominant (or recessive) QTL linkage are highly confounded in sibpair linkage analysis, we do not have the power to speculate on the mode of inheritance of the gene(s) in the 5q region.”
14. **Issue:** Further discussion could be provided regarding candidate genes such as CSPG2, including function of proteins and their potential role in the pathogenesis of myopia.
   **Action:** Discussion: Sentences inserted “SPG2 is one of the principal constituents of the extracellular matrix …” and “It has a key role in tissue morphogenesis participating in cell adhesion, proliferation …”

**Reviewer 3:**

15. **Issue:** In the introductory section the statement that "This homeostatic mechanism of emmetropisation is reflected in the distorted normal distribution of refractive error, with the large kurtosis suggesting compensatory factors are involved." could be rephrased.
   **Action:** Introduction: For simplicity this sentence has been removed.

16. **Issue:** For Table 3, it also might help if the comparison for each model vs. the unconstrained hypothesis were provided rather than the comparison for each model and its immediate precursor.
   **Action:** This was performed and so as to remove ambiguity the caption of Table 3 has been altered to now read: “Models were tested against the full model in order from no male specific …”

17. **Issue:** No recessive models were explicitly mentioned and this should be discussed.
   **Action:** Please refer to issue 13.

18. **Issue:** Table 3 should be included as a regular table rather than in the supplementary material.
   **Action:** Table 3 has now been included as part of regular text.

19. **Issue:** The term quasi independent sib-pairs should be explained.
    **Action:** Methods Section: The following text has been added after the sentence where the term quasi independent sib-pairs is used: “In sibships of size $s$, the number of possible sib pairings is $s(s-1)/2$; e.g. for $s=3$ the number of pairs is 3; for $s=4$, pairs =6. Each of these pairs contributes information for linkage, but because such pairings are not independent of each other (for example, for $s=3$, individual 1 pairs with both siblings 2 and 3) we refer to them as QISPs.”

20. **Issue:** Simulations to calculate an empiric p value should be performed.
    **Action:** Empirical p values were calculated using Merlin and the sentence commencing “A gene dropping simulation …” has been inserted into the Methods Section.

21. **Issue:** The threshold for suggestive results also increases in allele sharing methods, and the authors might wish to incorporate this into the discussion.
**Action:** Discussion Section: Sentence inserted: “Nonetheless, it must be acknowledged that the threshold for suggestive results also increases in allele sharing methods and as such all loci require replication.”

22. **Issue:** It would be useful to have a brief summary of the levels of agreement between genotypes from the various centres.  
**Action:** Methods: Sentence inserted: “Three markers (D3S1304, D4S403 and D4S391) were found to have an unusually high number of inconsistencies between genotyping centres and as a consequence the data at these loci were not merged.”

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Author(s): Gu Zhu, Alex Hewitt, Jonathan B. Sadd, Lisa S. Kearns, Shayne A. Brown, Jane R. Mackinnon, Christine Y. Chen, Christopher T. Hammond, Jamie E. Craig, Grant W. Montgomery, Nicholas G. Martin, David A. Mackey

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Title of Article: GENETIC DISSECTION OF MYOPIA: EVIDENCE FOR LINKAGE OF OCULAR AXIAL LENGTH TO CHROMOSOME 5q.

Author(s): GU ZHU, ALEX W. NEWITT, JONATHAN B. RUDDE, LISA S. KEARNS, SHAYNE A. BROWN, JANE R. MACKINNON, CHRISTINE Y. CHEN, CHRISTOPHER J. HAMMOND, JAMIE E. CRAIG, GRANT W. MONTGOMERY, NICHOLAS G. MARTIN, DAVID A. MACKREY

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Genetic dissection of myopia: evidence for linkage of ocular axial length to chromosome 5q.

RUNNING HEAD: Axial length genome-wide linkage

Gu Zhu,1 Alex W Hewitt,2,3 Jonathan B Ruddle,2 Lisa S Kearns,2 Shayne A Brown,2 Jane R MacKinnon,4 Christine Y Chen,2 Christopher J Hammond,5 Jamie E Craig,3 Grant W Montgomery,1 Nicholas G Martin,1 David A Mackey,2,6

1 Genetic Epidemiology Unit, Queensland Institute of Medical Research, Brisbane, Australia;
2 Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, East Melbourne, Australia;
3 NHMRC Centre for Clinical Eye Research, Department of Ophthalmology, Flinders University, Flinders Medical Centre, Adelaide, Australia;
4 Ophthalmology Department, Royal Hospital for Sick Children, Glasgow, Scotland;
5 Twin Research and Genetic Epidemiology Unit, St. Thomas’ Hospital, London, UK;
6 Department of Ophthalmology, Royal Hobart Hospital, University of Tasmania, Hobart, Australia.

COMPETING INTERESTS:
No authors of this paper have any other commercial or competing interests related to this research.

CORRESPONDENCE:
Associate Professor DA Mackey.
Centre for Eye Research Australia
Royal Victorian Eye and Ear Hospital
32 Gisborne Street,
East Melbourne,
Victoria, Australia 3002

Telephone: +61 3 9929 8713
Fax: +61 3 9929 8711
Email: D.Mackey@utas.edu.au
ABSTRACT
PURPOSE: To estimate heritability and locate quantitative trait loci influencing axial length.

DESIGN: Classical twin study of monozygotic (MZ) and dizygotic (DZ) twins reared together.

PARTICIPANTS: 893 individuals from 460 families were recruited through the Twin Eye Study in Tasmania (TEST) and the Brisbane Adolescent Twin Study (BATS) and had ocular axial length measured.

METHOD: 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 genome-wide linkage scan data were performed on 318 individuals from 142 BATS families.

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 (genome-wide p=0.0004) on chromosome 5q (at 98 cM). Additional regions with suggestive multipoint LOD scores were also identified on chromosome 6 (LOD score=2.13 at 76 cM; LOD score=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.

KEY WORDS:
Refractive error; twin study; hypermetropia; genetic linkage

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-like eye is generally emmetropic.\textsuperscript{2} The determinants of refractive error include the refractive indices, curvature and position of the cornea and lens, as well as the relative position of the retina; however, of all the potential variables affecting refraction, axial length and corneal curvature are the major contributors.\textsuperscript{3}

High or pathological myopia is generally defined as a refractive spherical power of $\sim 6$ diopters or higher and is associated with complications, such as increased risk of cataract, glaucoma and retinal detachment.\textsuperscript{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, the significant economic costs involved in its treatment, and the negative impact on quality of life, more research into understanding the aetiology of refractive error is warranted.\textsuperscript{5}

Much work has been performed investigating the genetic aspects of myopia.\textsuperscript{6} For example Stickler syndrome, which can occur due to mutations in collagen II and collagen XI, is frequently associated with very high myopia and greatly increased axial length.\textsuperscript{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.\textsuperscript{8-10} Additionally, there are at least 12 putative autosomal loci for non-syndromic myopia.\textsuperscript{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).\textsuperscript{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). In 2004, Hammond and colleagues using 221 dizygotic (DZ) twin pairs, reported evidence for linkage of refractive error to chromosomes 11p, 3q, 4q and 8p (MYP7-10).

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 sport, has been proposed as an environmental influence for preventing myopia. 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 (QTL) 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%), only one linkage study on this trait has been performed. In 2005 Biino and colleagues found suggestive linkage of axial length to chromosome 2p24. 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 genome-wide linkage analysis.
METHODS:

Clinical Assessment:

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

A comprehensive ophthalmic examination in each subject was preceded by a thorough interview which included questions pertaining to relevant social, past medical and ocular history. Following instillation of one drop of oxybuprocaine hydrochloride (0.4%) local anesthetic eye drops, axial length was measured using ultrasound biometry with the Ocuscan biometer (Alcon, Fort Worth, Texas, USA). 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 (28.22mm versus 24.34mm in the contralateral eye without staphyloma).

Zygosity Testing and Genotyping:

Genomic DNA was obtained by either buccal swab or venous blood collection. Zygosity in twins of the same sex was determined by genotyping at least nine independent autosomal microsatellite using the profiler and polymorphisms. An additional sex marker (amelogenin) was analysed in the BATS samples. Zygosity was
confirmed by an average of 400 additional markers in the subset used for linkage analysis (see below).\textsuperscript{30}

Analysis of pre-existing genotype data were performed for this study and comprised the compilation of three whole-genome scans, performed in two separate laboratories.\textsuperscript{31} A total of 386 short tandem repeat (STR) markers were screened at the Centre for Inherited Disease Research (CIDR; Bethesda, USA), whilst two series of genotyping for 410 markers were performed at the Australian Genome Research Facility (AGRF; Melbourne, Australia). A total of 35 markers from the CIDR and AGRF scans were shared and were used to assess genotyping quality.\textsuperscript{31} Three markers (D3S1304, D4S403 and D4S391) were found to have an unusually high number of inconsistencies between genotyping centres and as a consequence the data at these loci were not merged.\textsuperscript{31} As described previously, several steps were taken to ensure the integrity of data and that the genotyping results were adequately combined.\textsuperscript{31,32} The final stage of ensuring data integrity involved the merging of genotyping datasets for which there was a total of 796 markers from CIDR and/or AGRF. Possible genotyping errors and inconsistently typed markers were assessed and removed using the Merlin program.\textsuperscript{33} Marker location was determined using the deCODE map.\textsuperscript{34}

\textit{Variance component Modeling and Linkage Analysis:}

Best-fit structural equation modeling was performed using the Mx software package to determine the proportion of variance explained by genetic and environmental effects.\textsuperscript{35} Models containing additive (A) and dominant (D) genetic variation as well non-shared (E) environment variations were considered for the twin as a whole and
for right and left eye separately (Figure 1). A bivariate general sex limitation ADE 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 was significantly different between males and females, a sex limitation model was applied.

The software package Merlin was used to perform the univariate quantitative trait linkage analysis,\textsuperscript{36} while bivariate linkage analysis was performed using the program Mendel.\textsuperscript{37} As described elsewhere, the estimated proportion of alleles shared identical by descent was regressed on the squared sum and squared differences of trait values of relative pairs.\textsuperscript{31} A gene dropping simulation was performed using Merlin; and following 1,000 simulations logarithm of the odd (LOD) scores of 1.23 and 2.76 were found to be suggestive or significant for genome-wide linkage, respectively.\textsuperscript{34}

**RESULTS:**

Axial length measurements were obtained from a total of 893 individuals from 460 families. There were 433 complete twin pairs of 131 monozygotic (MZ) and 302 DZ twin pairs, and 27 singletons (Table 1). The mean age of female and male TEST participants was 27 years (SD=18.4; range 5 to 83 years) and 21 years (SD=16.8; range 5 to 68 years), respectively. The mean age in the BATS sample was 21 years (SD=2.3; range 16 to 27 years) and 20 years (SD=2.5; range from 16 to 25 years) for the 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 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 combing 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 MZ and DZ twins was greater than two suggesting 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 where a single additive genetic factor accounted for 81.2% of variance in axial length of each eye (Table 3). 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 BATS. A total of 42 additional siblings (ten from eight MZ families, and 32 from 134 DZ families) were added to maximise the total linkage information. Thus, 318 individuals were included in the linkage analysis comprising 219 quasi independent sib-pairs (QISPs). In sibships of size s, the number of possible sib pairings is \( s(s-1)/2 \); e.g. for s=3 the number of pairs is 3; for s=4, pairs =6. Each of these pairs contributes information for linkage, but
because such pairings are not independent of each other (for example, for s=3, individual 1 pairs with both siblings 2 and 3) we refer to them as QISPs. An average of 574 (range=204 to 777) microsatellite markers for sib-pairs were typed with an average distance between markers of 7.68 cM. Age was the sole characteristic which 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 to 26 years). There is currently no genotype information available for any of the TEST sample.

The highest multipoint linkage peak for axial length was identified on chromosome 5q with a LOD score of 3.4 (genome-wide p=0.0004) indicating significant linkage (Table 4). The region of interest spans approximately 10cM and is flanked by markers D5S641 and D5S1725. Additional peaks with LOD scores greater than 2 indicating suggestive linkage were observed on chromosomes 6, 10 and 14 (Figure 2).

**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 analysed. It has been common previously to either disregard the measurements of one eye,\textsuperscript{26} or to take the mean of both eyes.\textsuperscript{19,28} To an extent our proposed path model and decomposition overcomes the issue of four 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. Since 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.38 An interesting candidate gene in this region is the extracellular matrix gene *chondroitin sulfate proteoglycan 2* (*CSPG2*; OMIM:143200) at chromosome 5q14. Being expressed in the sclera, splice variants in *CSPG2* have been implicated in Wagner disease.39,40 *CSPG2* is one of the principal constituents of the extracellular matrix and has been identified in a variety of ocular tissues including the sclera.39 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.41

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.27 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 at *MYP3* (OMIM: 603221) and *MYP9* (OMIM: 609258) where our LOD scores were 1.81 and 1.39 respectively. Linkage to the *MYP3* locus at 12q21-q23 was originally demonstrated in a large German/Italian family with high myopia. In a classical twin study of refractive error Hammond and colleagues observed evidence for linkage at 4q12 (*MYP9*). 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. 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 genome-wide analysis was performed in only a subset of examined individuals. Increasing the sample size for such analysis should ensure 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 co-morbidity. 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.
ACKNOWLEDGEMENTS:

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REFERENCES:


This study reports the identification of a significant quantitative trait locus influencing ocular axial length, a major endophenotype for myopia. The finding of a relatively high heritability estimate (81%) is similar to that described previously.
**Table 1:**

Descriptive statistics of axial length and age for sample by sex.

<table>
<thead>
<tr>
<th>Source</th>
<th>Traits</th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>Min.</td>
<td>Max.</td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>TEST</td>
<td>AL_R</td>
<td>305</td>
<td>22.9</td>
<td>0.98</td>
<td>20.1</td>
<td>28.9</td>
<td>208</td>
<td>23.3</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>AL_L</td>
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<td>22.9</td>
<td>0.95</td>
<td>20.7</td>
<td>28.0</td>
<td>208</td>
<td>23.2</td>
<td>0.85</td>
</tr>
<tr>
<td>BATS</td>
<td>AL_R</td>
<td>188</td>
<td>23.0</td>
<td>0.81</td>
<td>20.8</td>
<td>25.3</td>
<td>192</td>
<td>23.4</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>AL_L</td>
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<td>22.9</td>
<td>0.80</td>
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<td>25.0</td>
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</tr>
<tr>
<td>TEST</td>
<td>Age</td>
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<td>27</td>
<td>18.4</td>
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<td>83</td>
<td>208</td>
<td>21</td>
<td>16.8</td>
</tr>
<tr>
<td>BATS</td>
<td>Age</td>
<td>188</td>
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<td>2.3</td>
<td>16</td>
<td>27</td>
<td>192</td>
<td>20</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Abbreviations: TEST, Twins Eye Study in Tasmania; BATS, Brisbane Adolescent Twin Study; AL_R, axial length right eye; AL_L, axial length left eye; N, number; SD, standard deviation; Min., minimum; Max., maximum.
Table 2:
Maximum Likelihood Estimates and 95% confidence intervals of axial length calculated using Mx.

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>Number of twin pairs</th>
<th>Right Eye equated to Left Eye</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZF</td>
<td>86</td>
<td>0.816</td>
<td>(0.760 - 0.855)</td>
</tr>
<tr>
<td>MZM</td>
<td>45</td>
<td>0.797</td>
<td>(0.713 - 0.851)</td>
</tr>
<tr>
<td>DZF</td>
<td>77</td>
<td>0.297</td>
<td>(0.099 - 0.459)</td>
</tr>
<tr>
<td>DZM</td>
<td>77</td>
<td>0.436</td>
<td>(0.187 - 0.595)</td>
</tr>
<tr>
<td>DZFM</td>
<td>148</td>
<td>0.419</td>
<td>(0.285 - 0.529)</td>
</tr>
<tr>
<td>All MZ</td>
<td>131</td>
<td>0.808</td>
<td>(0.763 - 0.844)</td>
</tr>
<tr>
<td>All DZ</td>
<td>302</td>
<td>0.385</td>
<td>(0.284 - 0.475)</td>
</tr>
</tbody>
</table>
Table 3:

Tests of alternative models of sources of variation in ocular axial length. Models were tested against the full model in order from 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>
<thead>
<tr>
<th>Parameters</th>
<th>%</th>
<th>Fix ( A'_m )</th>
<th>( f=m ) (ade)</th>
<th>Fix ( f=m ) (ADE)</th>
<th>Fix ( d_{(fm)} )</th>
<th>Fix ( a_{(fm)} )</th>
<th>Fix ( D_{(fm)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_f )</td>
<td>57.5</td>
<td>68.6</td>
<td>68.2</td>
<td>72.2</td>
<td>72.2</td>
<td>72.2</td>
<td>81.2</td>
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<tr>
<td>( D_f )</td>
<td>24.9</td>
<td>13.8</td>
<td>13.8</td>
<td>8.7</td>
<td>8.7</td>
<td>8.7</td>
<td>-</td>
</tr>
<tr>
<td>( E_f )</td>
<td>10.9</td>
<td>10.9</td>
<td>10.8</td>
<td>11.3</td>
<td>11.3</td>
<td>11.3</td>
<td>11.0</td>
</tr>
<tr>
<td>( a_f )</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( d_f )</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( e_f )</td>
<td>6.4</td>
<td>6.4</td>
<td>6.5</td>
<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
<td>7.8</td>
</tr>
<tr>
<td>( A_m )</td>
<td>52.0</td>
<td>72.4</td>
<td>72.8</td>
<td>72.2</td>
<td>72.2</td>
<td>72.2</td>
<td>81.2</td>
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<tr>
<td>( D_m )</td>
<td>26.8</td>
<td>6.6</td>
<td>6.7</td>
<td>8.7</td>
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<td>-</td>
</tr>
<tr>
<td>( E_m )</td>
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<td>11.4</td>
<td>11.6</td>
<td>11.3</td>
<td>11.3</td>
<td>11.3</td>
<td>11.0</td>
</tr>
<tr>
<td>( M_m )</td>
<td>11.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>( a_m )</td>
<td>1.2</td>
<td>1.2</td>
<td>0.8</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>( e_m )</td>
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<td>7.1</td>
<td>7.1</td>
<td>7.1</td>
<td>7.8</td>
</tr>
</tbody>
</table>

\(-2LL\) 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
\( \Delta df \) 1 3 3 1 1 1 1
\( P \) 0.584 0.440 0.102 1.000 0.138 0.655
Table 4:

Summary of genome-wide linkage results for bi-variate axial length where the multipoint probability was <0.05.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position (cM)</th>
<th>Markers</th>
<th>Bi-variate LOD</th>
<th>$p^*$</th>
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<tbody>
<tr>
<td>4</td>
<td>77.89</td>
<td>D4S392</td>
<td>1.38</td>
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<tr>
<td>5</td>
<td>53.78</td>
<td>D5S1470</td>
<td>1.36</td>
<td>0.0436</td>
</tr>
<tr>
<td>5</td>
<td>98.59</td>
<td>AD5S641</td>
<td>3.40</td>
<td>0.0004</td>
</tr>
<tr>
<td>5</td>
<td>151.19</td>
<td>D5S436</td>
<td>1.84</td>
<td>0.0144</td>
</tr>
<tr>
<td>6</td>
<td>76.36</td>
<td>D6S460</td>
<td>2.13</td>
<td>0.0074</td>
</tr>
<tr>
<td>6</td>
<td>83.79</td>
<td>D6S462</td>
<td>2.05</td>
<td>0.0090</td>
</tr>
<tr>
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<td>D10S547</td>
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<td>0.0496</td>
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<tr>
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<tr>
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<td>0.0195</td>
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<tr>
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<td>59.7</td>
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<td>1.42</td>
<td>0.0383</td>
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<td>14</td>
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<td>0.0208</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>D21S1432</td>
<td>1.37</td>
<td>0.0431</td>
</tr>
</tbody>
</table>

* Multipoint probability
**Figure 1:**
Path diagram illustrating parameter specification in the bi-variate 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.
**Figure 2:**

The genome wide multipoint bi-variate linkage analysis for axial length. Chromosome positions are displayed on the x axis (▲ denotes centromere) and the y axis displays the strength of evidence for linkage (LOD score).
Figure 1

- 81.2% from A to Axial Length Right
- 81.2% from A to Axial Length Left
- 7.8% from Axial Length Right to e
- 11.0% from Axial Length Right to E
- 11.0% from Axial Length Left to e
- 7.8% from Axial Length Left to E