Evidence of Shared Genes in Refraction and Axial Length: The Genes in Myopia (GEM) Twin Study

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Purpose. Axial length has been shown to explain up to 50% of the total variance in refraction, with axial length and refraction having a major genetic component. However, no study has attempted to determine whether the correlation between axial length and refraction is explained by shared genetic or environmental factors.

Methods. All twins from Victoria aged 18 years or older were invited to participate in the Genes in Myopia (GEM) twin study through the Australian Twin Registry (ATR). Each twin completed a general questionnaire and underwent dilated objective refraction assessment and measurement of axial length.

Results. A total of 612 twin pairs (1224 twins) aged from 18 to 86 years were examined in the GEM twin study. Axial length correlated negatively with refraction ($r = -0.64$ in the men, $r = -0.68$ in the women; $P < 0.01$). The sex limitation ADE (A, additive genetic; D, dominant genetic; E, unique environmental factors) model provided the best-fit genetic model for both measures. Of the variation in spherical equivalence in both the men and the women, approximately 50% were due to genetic factors influencing axial length.

Conclusions. From these findings, it is likely that axial length and refraction share common genes in their etiology. The GEM twin study has provided a basis and direction for future research into identifying the gene(s) in axial length that will ultimately improve our understanding of the etiology of refractive error, particularly myopia. (*Invest Ophthalmol Vis Sci.* 2008;49:4336–4339) DOI:10.1167/iovs.07-1516

Myopia, or short-sightedness, is a complex refractive error that affects approximately 20% to 30% of individuals in Western populations and over 80% in selected regions of South-East Asia.[1] The prevalence of myopia is expected to grow, with approximately one-third of the world's population (2.5 billion people) predicted to have myopia by the 2020. Therefore, myopia poses serious implications at both the public health and economic levels.

There has been a clear consensus that both genetic and environmental risk factors, such as near work, play a role in the development of myopia. However, the latter risk factors are thought to explain approximately 10% of the total variance in myopia.[2] Evidence to support a major genetic component in myopia has been shown through family and twin studies, with heritability estimates as high as 90%.[3] Moreover, to date, 14 myopia loci have been identified (MYP 1 to 14); however, no gene(s) have so far been reported.

Myopia can be explained as a mismatch between the point where light rays intersect and the ocular axial dimensions, particularly axial length. As a result, intersecting light rays focus in front of, rather than at the photoreceptor retinal layer, thus producing a less distinct or blurred image. The hypothesis of a mismatch of refractive power and axial length was supported by an earlier study by Sorsby and Leary,[4] who provided longitudinal refractive data on 68 children aged 3 to 8 years at their initial examination and who were then reassessed approximately 6.5 years later. Children were then defined in two groups, the first group showing normal development or closer development to emmetropia and the second group who were showing signs of myopia. The first group ($n = 49$ children) showed a stable decrease in the amount of hypermetropia during the 6.5-year period with a mean decrease of 0.09 D and mean increase in axial length of 0.14 mm per year. However, in the second group ($n = 19$ children), a greater decrease in their hypermetropia was evident, with a mean decrease rate of 0.38 D per year, and their mean increase in axial length was almost double (0.24 mm per year) that of the first group.

To obtain a perspective on the development of refractive error, it is important to consider the proposed process of emmetropization, which typically occurs in the first 7 to 9 years of life.[5–9] This process was first described by Straub in the late 1800s,[7] to explain the process whereby the optical powers of the cornea and lens accommodate to match the continuing growth of the eye (increasing axial length) during early childhood by decreasing its amount of neonatal refractive error (hypermetropia). Therefore, it is postulated that myopia develops when the reduction of the refractive power of the cornea and lens falls short of matching the axial elongation during the early development of emmetropia.[10–12] Moreover, population-based studies have found a negative correlation between axial length and refraction. For instance, in a recent study, Ip et al.[13] assessed refraction and ocular biometric measurements in 2353 children 12 years of age and found that axial length accounted for approximately half of the variation in refraction. Therefore, axial length measurement of the human eye represents one of the most important ocular dimensions when exploring the components of the eye contributing to the development of refractive error.

Previous twin studies have provided substantial evidence to support a genetic component in both refraction and axial length, with the largest and most recent twin study reporting heritability estimates as high as 88% and 94% for refraction and axial length, respectively in males.[14] Family studies have also shown that children of myopic parents are at a significantly higher risk (up to four times higher) of development of myopia than are children of nonmyopic parents.[15–22] In addition, family studies have also supported a genetic basis to axial length, with one study,[23] reporting that even before the onset of myopia, children with myopic parents had longer axial length (23.08 mm) than did children (aged between 6–12 years) with one or no myopic parents (22.72 mm). This finding remained

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significant after adjusting for diopter-hours of near work and school performance. It is well established that axial length is a major contributor in the development of refractive error. However, there have been only two previous linkage studies[24,25] that have investigated axial length as a quantitative trait locus (QTL). The first study identified suggestive linkage to chromosome 2, area p24, whereas in a more recent study, Zhu et al.[25] identified suggestive QTLs on the long arm of chromosome 5 and on chromosomes 6, 10, and 14.

There is now a substantial body of evidence to indicate a significant correlation between axial length and refraction. Furthermore, the importance of eye growth (axial elongation) in the development of refractive error has been clearly demonstrated, and a large amount of data are also available to support a genetic involvement in both refraction and axial length. However, in no single study so far have investigators sought to examine whether this association is in part explained by shared genetic or environmental factors. Answering this question would be helpful in our understanding of the etiology of refractive error, particularly myopia. To our knowledge, the GEM twin study represents the first time that this approach has been undertaken to explore the relationship between axial length, and myopia.

Methods

Subjects and Recruitment

All twins from Victoria 18 years if age or older of both sexes were invited to participate in the GEM twin study. Twin recruitment was facilitated by the Australian Twin Registry (ATR) located at the University of Melbourne, Victoria, Australia. The ATR is a national twin registry with more than 31,000 registered twin pairs. All registered twins in Victoria of the criterion age received a letter of invitation, an information sheet, and a consent form from the ATR. When both twins agreed to be included, they were contacted by the GEM study team to arrange appointment times for examination.

Ethics approval for the GEM twin study was provided by the Royal Victorian Eye and Ear Hospital (RVEEH) Human Research and Ethics Committee. In addition, the ATR approved the GEM study. Written, informed consent was obtained from each twin before testing. This protocol adhered to the tenets of the Declaration of Helsinki, and all privacy requirements were met.

Study Protocol

Each twin underwent a general questionnaire and comprehensive eye examination, including a dilated objective refraction and axial length measurement. A detailed outline of the GEM testing protocol is presented elsewhere.[26] In brief, dilation was achieved through a single drop of tropicamide 1%. After dilation (~15 minutes) autorefraction was undertaken (KR 8100 model autorefractor; Device Technologies, Melbourne, Australia). Partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Oberkochen, Germany) was used to obtain ocular measurements on axial length of the eye (anteroposterior diameter). For both autorefraction and axial length measurements, a total of three readings were taken for each eye and the average value recorded. For autorefraction measurements, results for each eye were converted to their spherical equivalent (SE) (half the amount of cylinder plus the spherical component). Myopia was defined as an SE equal to or worse than −0.50 D.

Zygosity

A series of questions (recommended by the ATR) were used to determine zygosity,[27] with these questions being validated as having a 95% accuracy in determining correct zygosity.[28] Most twins recruited into the GEM twin study were aware of their zygosity, mainly through prior zygosity testing in other twin studies. In cases in which zygosity was uncertain ($n = 20$ twins), standardized genotyping using a panel of 12 polymorphic markers (Linkage Mapping Set version 2; Applied Biosystems, Foster City, CA)[29] was performed by the Australian Genome Research Facility (AGRF), Melbourne. The results of this genotyping were in complete agreement with the zygosity as previously determined by the examiner based on the series of twin questions and the assessment of physical characteristics in all cases.

Modeling of Variance Components

Genetic modeling is primarily used to quantify the proportion of phenotypic variance attributable to either genetic or environmental factors. The phenotypic variance is then separated into additive genetic effects (A), nonadditive genetic effects (dominance or epistatic interactions [D]), common shared environment (C), and individual specific environmental effects and measurement error (E). Fitting a model with all parameters specified, parameters were then removed in a step-wise manner. Twice the difference in log likelihoods between the full and submodels is distributed as χ^2 with the degrees of freedom equal to the difference in degrees of freedom between the two models (likelihood ratio test).[30]

A gender-specific model with additive genetic, nonadditive, and unique environmental parameters (ADE) was fitted to axial length, since the intrapair correlation for monozygotic (MZ) twins was more than double the intrapair correlation between dizygotic (DZ) twin pairs. Given the formulas, C is ($C = 2r_{DZ} - r_{MZ}$), where r_{MZ} is the intrapair correlation for MZ twins and r_{DZ} is the intrapair correlation for DZ twins. Therefore, when the MZ intrapair correlation is more than double that of the DZ intrapair correlation, C would be estimated at 0. The sex limitation model was applied in the analysis, as the variances for measured variables were significantly different between the men and women. Heritability was defined as the phenotypic variance that can be explained by additive and nonadditive genetic effects.

A bivariate Cholesky decomposition model [31] was fitted to axial length and refraction, to determine the extent to which genetic and environmental effects influencing axial length also influence refraction. In brief, the Cholesky model allows decomposition of variation in myopia into that due to genetic and environmental influences common with axial length and those specific to myopia. The approach to modeling is such that, initially, a model is specified that has all possible

Table 1. Total Number of Twins Recruited in the GEM Twin Study

Sex	MZ	DZ	Total
Male/male	117 (33.9%)	49 (18.4%)	166 (27.1%)
Female/female	228 (66.1%)	132 (49.4%)	360 (58.8%)
Male/female	N/A	86 (32.2%)	86 (14.1%)
Total	345 TP	267 TP	612 TP
Males	234 (33.9%)	184 (34.5%)	418 twins
Females	456 (66.1)	350 (65.5%)	806 twins
Total	690 twins	534 twins	1224 twins

Data are the number (%) of total pairs. TP, twin pairs.

TABLE 2. Shared Genetic Effects between SE and AL in All Twins

Sex	Additive Genetic Effects	Dominant Genetic Effects	*P*
Male	23%	27%	<0.001
Female	28%	25%	<0.001

$P < 0.01$ indicates significant genetic sharing between SE and axial length.

parameters. Parameters are then removed in a step-wise manner and the subsequent, nested model is compared to the full model to see whether there is a significant difference in fit. Quantitative genetic modeling was achieved by using the Mx statistical program[32] and all descriptive statistics were obtained with commercial software (Statistical Package for the Social Sciences [SPSS], ver. 12.1; SPSS, Chicago, IL).

RESULTS

Demographic Characteristics

Of the recruited twin pairs ($n = 612$ twin pairs), 345 (56.4%) were MZ and 267 (43.6%) were DZ twin pairs (Table 1). There were significantly more female twins than male twins within both the MZ (female, 456 [65.2%]; male, 234 [33.9%]; $P < 0.05$) and DZ twin pair groups (female, 350 [65.5%]; male, 184 [34.5%]; $P < 0.05$) respectively. Overall, there was almost double the number of female (806, 65.8%) than male ($n = 418$, 34.2%) twins ($P < 0.05$), this phenomenon being common in other twin studies.[33] No significant differences in mean SE and axial length (AL) were evident between the right and left eyes of all twins ($P > 0.05$); therefore, only results for the right eye are presented.

Bivariate Cholesky Decomposition Model for the Covariance between AL and SE

The heritability estimates and modeling used for SE and AL in the GEM twin study has been published elsewhere. In brief, the sex-limited ADE model was found to be the best-fit genetic model to explain both measures. Heritability estimates for SE were 88% and 75% in the men and women, respectively and as high as 94% in the men and 90% in the women for AL. Moreover, in the men, SE correlated significantly with AL ($r = -0.64$, $P < 0.01$), with AL explaining 41% (coefficient of determination, $r^2 = 0.41$) of the total variance in SE. Similarly, AL explained more than 40% ($r = -0.68$, $P < 0.01$) of the total variance in the women. A bivariate Cholesky decomposition found that the correlation between AL and SE was due to both genetic and environmental factors common to both measures. Of the variation in spherical equivalence in the men, 23% and 27% were due to either additive genetic or nonadditive genetic factors that influence AL ($P < 0.001$). For the same variation in the women, the proportion explained by these genetic factors were 28% and 25%, respectively ($P < 0.001$; Table 2). Unique environmental effects (men, 17%; women, 54.73%) were also found to be common for SE and AL.

DISCUSSION

The GEM twin study is the first study to ascertain whether the association between AL and myopia is explained in part by shared genetic or environmental factors, as determined through genetic modeling. A large cohort of Australian male and female twins over a broad age range was used in the GEM twin study.

In the GEM twin study, negatively AL correlated with SE (longer eyes being associated with more myopic refractions), explaining approximately 50% of the variance in SE and thus suggesting that AL is, in itself, one of the major determinants of refractive error. The strong correlation (−0.64 and −0.68 in the men and the women, respectively) between AL and refraction reported in the GEM twin study is consistent with previous studies that have found similar correlation coefficients ranging from −0.44 to −0.60.[34–37] The GEM twin study findings also concur with previous studies that have reported longer eyes in myopia[38] and shorter eyes in hypermetropia.[39]

The heritability estimates for SE and AL in the GEM twin study have been discussed elsewhere.[14] In brief, a major genetic component was indicated for both SE and AL, with the gender-specific ADE model being the most parsimonious model to explain the variance for both measures.[14] Our findings (heritability estimate of 75%–88%) concur with that of the largest and most recent study by Hammond et al.,[40] in which a heritability estimate of 84% to 85% was reported in females for refraction. A genetic basis to AL was also found in the GEM twin study and confirmed findings in previous twin studies that collectively support a strong genetic component to AL.[9,41,42]

From the literature, the genetic contribution of each ocular measure, SE and AL, have been quantified through twin and family studies. However, to date, there has been no other study to explore the potential influence of shared genetic and environmental effects on SE and AL. We have found that a large proportion of the correlation between SE and AL is explained by genetic effects for both of the sexes and to a lesser degree by unique environmental factors. These findings provide significant insights into the etiology of refractive error, with the potential that AL and SE may share common genes. However, further research is needed to determine the effects of potential dominant genes in AL. Nonetheless, we may postulate that future linkage analysis of AL may be helpful in identifying genes involved in refractive error.

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