The Molecular Genetics of Human Myopia: An Update

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Abstract

Myopia, or nearsightedness, is the most common human eye disorder in the world, and is a significant global public health concern. Along with cataract, macular degeneration, infectious disease, and vitamin A deficiency, myopia is one of the most important causes of visual impairment worldwide. Severe or high-grade myopia is a leading cause of blindness because of its associated ocular morbidities of retinal detachment, macular choroidal degeneration, premature cataract, and glaucoma. Ample evidence documents the heritability of the non-syndromic forms of this condition, especially for high-grade myopia, commonly referred to as myopic spherical refractive power of 5–6 diopters or higher. Multiple high-grade myopia genetic loci have been identified, and confirmatory studies identifying high-grade and moderate myopia loci have also occurred. In general, myopia susceptibility genes are unknown with few association studies performed, and without confirmation in other research laboratories or testing of separate patient cohorts.

Keywords

myopia; International Myopia Conference Sek-jin Chew lecture; human molecualr genetics; mapping studies; association studies

Prevalence Rates and Public Health Concern

Myopia is the most common human eye disorder in the world, and its public health and economic impact are considerable.[1] The prevalence of myopia varies because of varied definitions, but in the U.S. adult population the estimated prevalence of at least 25% is supported by multiple studies.[2–8] Females are reported to have an earlier onset and a slightly higher prevalence than males.[3,6,7] Whites have a significantly higher prevalence than African Americans. [9–11] Chinese and Japanese populations have high myopia prevalence rates of > 50–70%.[10,12,13] Ashkenazi Jews, especially Orthodox Jewish males, have shown a higher prevalence than other white U.S. and European populations.[11,14]

Myopia is a significant public health problem, as it is associated with increased risk for visual loss.[1,7,15–18] Myopic chorioretinal degeneration is the 4th most frequent cause of blindness leading to visual services and disability registration, and accounts for 8.8% of all causes of blindness. [18] An estimated 5.6% of blindness among schoolchildren in the U.S. is attributable to myopia.[18]

Substantial resources are required for optical correction of myopia with spectacles, contact lenses, and, more recently, surgical procedures such as LASIK. [19,20] The market for optical

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aids in the US was estimated to exceed $8 billion in annual sales in 1990; most dollars were spent for the correction of myopia. [19,20] The development of methods for preventing or slowing the onset of myopia, or for limiting its progression is of high significance. [1]

Determinants of Refractive Status

The human eye refractive state depends on the coordinated contributions of the refractive powers of the cornea and lens, axial length (AL), refractive indices of the aqueous and vitreous, and the person's age.[21] The aqueous and vitreous humor effects are constant - both have a refractive index of 1.336. [21] Thus, the major refractive components are the cornea, lens, and AL. The size, shape, and power of all are determined largely by inheritance. [22] Conformational factors such as intrauterine environment, bony orbits, and eyelids may influence eye shape and growth. [21]

Refractive error in the adult population follows a leptokurtic distribution with the peak around emmetropia (plano spherical refraction). [21] Individual refractive components such as dioptric lenticular and corneal powers, anterior chamber depth (in mm), and AL (in mm) follow more bell-shaped distributions. The average refractive error at birth is approximately +1.00 to 2.00 diopters (D) of hyperopia (far-sightedness), and the AL measures approximately 17 mm. By adulthood, the AL grows to about 24 mm.[23] The corneal diameter of the infant is 10mm compared to the adult size of 12mm. Due to steep curvature, corneal power (keratometry) averages +51.00D at birth and flattens to ~ +44.00 D by 6 weeks of age.[24] Lenticular power averages +34.00 D at birth and decreases to +28.00 D by 6 months of age, and to +21.00 D by adulthood.[24] The process of emmetropization, where the refractive components change in a complementary and coordinated fashion as the eye grows, results in minimal changes in refractive error, as the radius of curvature of the cornea decreases, the refractive powers of the lens and cornea decrease, and the AL increases.[15,25] The postnatal human eye normally maintains an AL of within 2% of its optimal focal point.[26] Usually emmetropia- where a clear image is focused on the retina- is reached at by 12 to 18 months, with no substantial refractive change in normal eyes after 16 years.[24]

Types of Myopia

"Juvenile-onset" myopia most often develops and progresses between the ages of 10 and 16 years, whereas "pathologic" or high–grade myopia usually begins to develop in the perinatal period, and is associated with rapid refractive error myopic shifts before 10–12 years of age. [16,27–29]

"Pathologic" or high-grade myopia (refractive spherical dioptric power of −5.00 D or higher) is a major cause of legal blindness in many developed countries.[6,11,14,18,30–32] It affects 27% to 33% of all myopic eyes, corresponding to a prevalence of 1.7% to 2% in the general population of the U.S.[8] High myopia is especially common in Asia.[18,31,33] In Japan, pathologic or high myopia reportedly affects 1–2% of the general population.[10] Comparative prevalence rates from different countries show variability, but confirm that myopia affects a significant proportion of the population in many countries.[8,16,18,31–33]

Ocular Morbidity

Many investigations have reported on the association of high myopia with cataract, glaucoma, retinal detachment (RD), and posterior staphyloma with retinal degenerative changes. [15,26,34–47] High myopia is associated with progressive and excessive elongation of the globe, which may be accompanied by degenerative changes in the sclera, choroid, Bruch's membrane, retinal pigment epithelium, and neural retina. Various funduscopic changes within the posterior staphyloma develop in highly myopic eyes. These changes

include geographic areas of atrophy of the retinal pigment epithelium (RPE) and choroid, lacquer cracks in Bruch's membrane, subretinal hemorrhage, and choroidal neovascularization (CNV). Among these fundus lesions, macular CNV is the most common vision-threatening complication of high myopia.[37–41,43]. Clinical and histopathologic studies have documented CNV in 4–11% of highly myopic eyes. Relative to emmetropic eyes, an approximate 2-fold increased risk of CNV was estimated for eyes with −1.00 to −2.00 D of myopia, a 4-fold increase with −3.00 to − 4.00 D, and a 9-fold increase with −5.00 to −6.00 D.[14,36,43,48] Poor visual outcome following myopic CNV is not uncommon, often affecting relatively young patients.

RD risk is 3–7 times greater for persons with myopia greater than 5.00 D, relative to myopia of less than −5.00 D. [14,26] Myopia of −5.00 to −10.00 D is associated with a higher likelihood of RD.[14,25] The lifetime risk for RD is estimated to be 1.6% for patients with less than −3.00 D of myopia and 9.3% for those with myopia greater than −5.00 D.[46] A sub-group with lattice degeneration and greater than −5.00 D of myopia have a lifetime risk of 35.9%.[46] The prevalence of lattice degeneration increases with higher myopia using AL as a biometric parameter reflective of refractive error.[14,25,49,50]

Glaucoma was observed in 3% of patients with myopia who had ALs of less than 26.5 mm, in 11% with ALs between 26.5 and 33.5 mm, and in 28% of those with longer lengths.[47]

Role of Environment in Myopic Development

The prevalence of myopia in some populations has increased dramatically from one generation to the next in increasingly industrialized settings, or with increased level of educational achievement.[14,51–54] Assessing the impact of inheritance on myopic development may be confounded by children adopting parental behavioral traits associated with myopia, such as higher-than-average near-work activities (i.e. reading).[55] Observational studies of this risk factor do not fully explain the excessive familial clustering of myopia, however. A detailed assessment of confounding effects and interactions between hereditary and environmental influences in juvenile-onset myopia has shown that near work describes very little of the variance in refractive error compared to parental myopia. [56] In addition, near work exerted no confounding influence on the association between parent and child myopia, indicating that children do not become myopic by adopting parental reading habits. More importantly, there was no significant interaction between parental myopia and near work; reading was weakly and equally associated with myopia regardless of the number of myopic parents. This suggests that children inherit myopia as a trait from parents.

Role of Genetics in Myopic Development

Multiple familial aggregation studies report a positive correlation between parental myopia and myopia in their children, indicating heritable myopia susceptibility.[57–61] Children with a family history of myopia had on average less hyperopia, deeper anterior chambers, and longer vitreous chambers even before be-coming myopic. Yap et al. noted a prevalence of myopia in 7-year-old children of 7.3% when neither parent was myopic, 26.2% when one parent was myopic, and 45% when both parents were myopic. [61]

Multiple familial studies support a high genetic effect for myopia.[62–65] Naiglin et al. performed segregation analysis on 32 French multiplex families with high myopia, and determined an autosomal dominant (AD) mode of inheritance.[66] The λs for myopia (the increase in risk to siblings of a person with a disease compared to the population prevalence) has been estimated to be 4.9–19.8 for sibs for high myopia (−6.00 spherical D or greater), and 1.5–3 for low or common myopia (approximately −1.00 to −3.00 spherical D), suggesting a definite genetic basis for high myopia, and a strong genetic basis for low

myopia.[67–68] A high degree of familial aggregation of refraction, particularly myopia, was reported in the Beaver Dam Eye Study population after accounting for the effects of age, sex, and education.[69] Segregation analysis suggested the involvement of multiple genes, rather than a single major gene effect.

Twin studies provide the most compelling evidence that myopia is inherited.[70–74] Multiple studies note an increased concordance of refractive error as well as refractive components (AL, corneal curvature, lens power) in monozygotic twins compared to dizygotic twins. [70–73] Sorsby et al. noted a correlation coefficient for myopia of 0 for control pairs, 0.5 for dizygotic twins, and almost 1.0 for monozygotic twins in a study of 78 monozygotic twin pairs and 40 dizygotic twin pairs. [72] Twin studies estimate a high heritability value for myopia, (the proportion of the total phenotypic variance that is attributed to genetic variance) of between 0.5–0.96.[70–74]

Ocular Refractive Component Genetics

Refraction is determined by coordinated contributions of ocular biometric components such as AL, anterior chamber depth (ACD), corneal curvature (keratometry readings in diopters), and lens thickness. Multiple reports have examined familial aggregation and heritability of ocular components. [63,73–75] These may be intimately related to the phenotype of myopia, and the genetic effects of these traits should be examined.

Several studies have reported an inverse relationship of AL to refraction (the longer the eye, the more myopic the refractive error). [76–80] AL is the largest contributor to the determination of refractive error. [81,82] Estimates of heritability for AL range from 40–94%. [63,73,74,84] A study of 3 large Sardinian families found modest evidence for linkage on chromosome 2p24 with a LOD score of 2.64. [84] A chromosome 5q locus for AL was noted in a recent whole genome twin study. [83] AL includes ACD, and studies have shown that increased ACD has an inverse relationship as well to refractive error.[80] The heritability reports for ACD range from 70–94% [63,73,74,80,84], and the same Sardinian study found modest linkage evidence to chromosome 1p32.2 with a LOD score of 2.32.[84]

Corneal curvature steepness is more likely to result in myopia, as hyperopic eyes are more likely to have flatter corneal curvatures. [76,84] Heritability estimates for corneal curvature range from 60 to 92%. [63,73,74,81] The same Sardinian study noted modest linkage of corneal curvature to chromosomes 2p25, 3p26, and 7q22 with LOD scores ranging from 2.34 to 2.50. [81] Increased lens thickness correlates with increased myopia. [80] A di- and monozygotic twin study showed 90–93% heritability for lens thickness.[73]

Molecular Genetic Studies of Human Myopia

Much of the current information on human myopia molecular genetics can be drawn from our studies of familial high myopia, usually defined as myopic spherical refractive error of at least - 5.00 D. An X-linked recessive form of myopia, named the Bornholm (Denmark) eye disease (BED), was designated the first myopia locus (MYP1-OMIM 310460) on chromosome Xq28. [86] Collaborating with BED researchers, we made comparative molecular genetic haplotype and sequence analyses of a large Minnesota family of Danish descent that showed significant linkage of myopia to chromosome Xq27.3-q28. The phenotype of both families appears to be due to a novel cone dysfunction, and not simple myopia. [87] The genetic etiology of each family appears to be distinct, as the haplotypes were different. [87] A report by Michaelides et al. confirms the different X-linked cone dysfunction syndrome with an associated high myopia phenotype as distinct from the BED in 4 families. [88] We identified the first AD locus for non-syndromic high myopia within a 7.6 cm region on chromosome 18p11.31 (MYP2, OMIM 160700) in 7 U.S. families. [89]

This locus was confirmed in Chinese Hong Kong and Italian Sardinian cohorts. [90,91] Using the Hong Kong cohort, investigators identified TGIF-beta as the implicated gene for MYP1 using limited SNP association studies and exonic sequencing. [90] Our group fully sequenced TGIF-beta in our cohort of original MYP1 families, and found no associations with high myopia affection status. [92] An Australian lab also investigated the association of TGIF with refraction and ocular biometric measurements in a Caucasian case-control population, and found no association. [93] No association of TGIF to high myopia case control studies was found in Japanese and Chinese cohorts. [94,95] A second locus for AD high myopia mapped to a 30.1 cM region on chromosome 12q21–23 (MYP3, OMIM 603221) in an American family of German-Italian descent also in our laboratory. [96] This locus was confirmed in a high myopia Caucasian British cohort, and in a large German family. [97,98] A statistically suggestive third locus for AD high myopia was reported on chromosome 7q36 in a Caucasian French cohort (MYP4, OMIM 608367).[99] A fourth AD locus on chromosome 17q21–23 (MYP5, OMIM 608474) was determined in a large multigenerational English-Canadian family in our laboratory. [100] We identified a locus for AD high-grade myopia on chromosome 2q37 (MYP12, OMIM 609994) in a large, multigenerational U.S. Caucasian family. [101] This locus was replicated in an Australian study of three multigenerational AD moderate myopia families. [102] We recently published a new locus (MYP17) on chromosome 10q21.2 in a Hutterite (Austrian /German) colony. [103] Loci on chromosomes Xq23–25[104] (MYP13, OMIM 300613) and 4q22–27[105] (MYP11, OMIM 609994) have also recently been identified by Zhang et al. in ethnic Chinese families. [104,105] Loci identified to date for isolated non-syndromic high-grade myopia are primarily AD and highly penetrant.

At least two studies have shown nominal or no linkage of low to moderate myopia to many of the known high myopia loci. Mutti et al. genotyped 53 common myopia families (at least one child with more myopia than −0.75D in each meridian) using the highest intra-interval LOD score microsatellite markers for the chromosome 18p and 12q loci and did not establish linkage. [106] Ibay et al. found no strong evidence of linkage to the chromosomes 18p, 12q, 17q, and 7q in a cohort of 38 Ashkenazi Jewish families with mild/moderate myopia (−1.00D or more). [107] These studies suggest that different genes account for mild/ moderate myopia susceptibility or development, or that the genes effects are too small to be detected with the relatively small sample sizes. However, a recent study showed replication of our chromosome 2q37 locus in a cohort of Caucasian Australians with moderate to low myopia. [102]

Three whole-genome mapping studies have identified several candidate gene intervals for common, juvenile-onset myopia using spherical refractive error data. The results of these studies demonstrate the potential for determining molecular genetic factors implicated in myopia at all levels of severity. These studies however, used microsatellite genotyping instead of SNP technology, a limited cohort sample size, and two used homogenous isolated populations. One study was a genome screen of 44 families of Ashkenazi Jewish descent. [108] Individuals with at least −1.00D of myopic spherical refractive error were classified as affected. Their strongest signal localized to chromosome 22q12 (MYP6, OMIM 608908) - (HLOD = 3.56; NPL = 4.62). Eight additional regions (14q, 4q22-q28, 8q22.2, 10q22, 11q23, 13q22, 14q32, and 17qter) had nominal linkage evidence. Hammond et al. evaluated 221 dizygotic twin pairs with moderate myopia and found significant linkage to 4 loci, with a maximum LOD score of 6.1 on chromosome 11p13-MYP7, OMIM 609256).[74] Other identified loci mapped to chromosomes 3q26-MYP8, OMIM 609257 (LOD 3.7), 4q12-MYP9, OMIM 609258 (LOD 3.3), and 8p23-MYP10, OMIM 609259 (LOD 4.1). This group found that the PAX6 gene at the chromosome 11p13 locus showed linkage with 5 SNPs, but no association. They suggested that PAX6 (a major eye development gene) may play a role in myopia development, possibly due to genetic variation in an upstream

promoter or regulator. This group later reported that neither the master control genes PAX6 or SOX2 were implicated in common myopia in a large population study cohort. [109] A report replicated the chromosome 8p23 myopia locus (MYP10) in an isolated Pennsylvania Amish population of 34 families. [110] These identified loci are prioritized in our association studies.

Gene Expression and Functional Studies Related to Myopia and Eye Growth

One impediment to correlating genotypic data with actual tissue histopathology in human myopia is that the tissue of interest (i.e. retina/ sclera) can not be directly sampled. Animal models of myopia have been developed to be used as surrogates, although it is unclear how correlative induced myopia in animals may be to physiologic myopia in humans. Animal studies over the past 30 years in juvenile and newborn monkey, tree shrew, and chick models have revealed an active emmetropization mechanism that normally achieves and maintains a match of the eye AL to the eye's optical power so that the photoreceptors are in focus for distant objects. [111–115] This control mechanism begins in the retina, where neurons (perhaps a subset of amacrine cells detect focused versus defocused images. [116] Constant hyperopic defocus uniformly (across species) produces retinal signals that pass, in a signaling cascade that is not well understood, through the retinal pigment epithelium (RPE) and choroid [116] to remodel the scleral extracellular matrix and cause axial elongation. [117,118] This, in turn, reduces the hyperopic defocus, so that this feedback system is self-limiting, resulting in a match of AL to optical power. Genes expressed in retina, RPE, choroid, and/or sclera that are involved in the normal emmetropization process could be involved in myopia development with irregular expression so that the emmetropization mechanism is disrupted, causing the eye to elongate and become myopic. [117,120–122] The retina is suspected of having the highest likelihood of harboring candidate genes that influence scleral growth based on multiple studies, including optic nerve sectioning in animal models. [123] Genes within significant intervals will be ranked using information from human retinal gene expression databases and publications such as Ret Net (http://www.sph.uth.tmc.edu/RetNet/), [124–126] and retinal SAGE libraries. [127]

Mouse Studies of Myopia

Factors that regulate rate and duration of eye growth in mice have revealed two loci (Eye1 and Eye2) that may be responsible for larger eye size. [128–130] Human syntenic homologous regions are chromosomes 6p, 16q13.3, and 19q13 for Eye2, and chromosome 7q for Eye1.

The first knock-out mouse model for relative myopia [131] was based on form-deprivation experiments in chickens, mice, and rhesus macaque monkeys. The immediate early gene transcription factor ZENK (also known as Egr-1) is implicated in the feedback mechanisms for visual control of axial eye growth and myopia development. [132–134] ZENK is up-regulated in retinal amacrine cells when axial eye growth is inhibited by positive lens wear, and is down-regulated when axial growth is enhanced by negative lenses, suggesting that ZENK is linked to an axial eye growth inhibitory signal. ZENK knockout mice had longer eyes and a relative myopic shift relative to heterozygous and wild-type mice with identical genetic background. [131] ZENK maps to a significantly linked loci at chromosome 5q31.1 from our international consortium dataset. We recently selected ZENK SNPs and performed Taqman genotyping association studies in a Duke myopia dataset, and found no association. We did not find sequence variants after mutation screening. (Data not shown.)

Association Studies for Non-syndromic Myopia

The list of hypothesized candidate genes for myopia, based largely on the current understanding of the pathophysiology of syndromic myopia, is relatively small. The vast majority of the work examining the relationship between candidate genes and individual polymorphisms in candidate genes has been performed on single candidates at a time, to the exclusion of other independent or interacting genes. Two myopia susceptibility genes were identified in Chinese cohorts using the family-based transmission disequilibrium test (TDT) approach [135] - the hepatocyte growth factor (which maps to the homologous human mouse Eye 2 locus at chromosome 7q21.1, and myocillin-a gene which has been implicated in juvenile-onset glaucoma, and can be associated with early-onset myopia. [136,137] Our lab has performed candidate gene SNP genotyping of these two genes using TaqMan on our collection of 120 Duke families (primarily Caucasian) to perform TDT family-based association testing, and we found no association with the same SNPs used in the primary studies. (Data not shown) The collagen type 1 alpha 1 gene (COL1A1) is an extracellular matrix gene expressed in the scleral wall, and maps within the MYP5 locus for high myopia on chromosome 17q22-q23.3. A case-control study of a Japanese cohort showed association with high myopia for this gene with 2 SNPs. [138] Another case control study of mixed ethnicities showed a 2 SNP association to the collagen 2 alpha 1 gene (COL2A1), which maps to chromosome 12q13.11, a locus not associated with myopia to date. [139] We have also performed TaqMan SNP genotyping association studies in our family cohort for these two scleral genes using the same SNPs as the original studies, and have found inconclusive evidence for association to either gene. No functional SNP effects were implicated for any of these candidate genes, and it is unclear how ethnic differences play a role in the degree of associative significance.

Scleral Gene Expression Studies

We published human and mouse ocular tissue expression studies to report candidate genes that may be relevant to myopic ocular shape change as it relates to scleral wall expansion. This was to develop a catalog of genes with an extracellular matrix associated function in sclera, given that many syndromic forms of myopia have connective tissue-associated gene mutations. Genes that are important for constituent organization and maintenance of connective tissue function may be physiologically important in heritable myopic eye development. To expand the candidate gene catalog for myopia susceptibility genes, our laboratory performed the first comprehensive gene expression studies in human sclera, describing genes expressed in a cDNA library, and using microarray technology and reverse-transcription polymerase chain reaction (RT-PCR) of mRNA extracted from human sclera. [140,141] We also described the first comprehensive effort to determine scleral eye growth genes differentially expressed in developmentally staged mice using microarray gene chip technology.[142] Ocular developmental candidate genes (MFRP, SOX2, CHX10) were screened in extreme states of refractive error/ eye growth.

International High Myopia Consortium

Our laboratory established an international consortium for studying the genetics of high-grade myopia. This was accomplished in collaboration with Drs. Thomas Rosenberg of the Gordon Norrie Disease Centre for Genetic Eye Diseases in Hellerup Denmark, Jeremy Guggenheim at the Cardiff University in Wales UK, David Mackey of the University of Melbourne, Australia, and Patrick Calvas and Francois Malacaze of the Toulouse University in France. We submitted an application to the NIH Center for Inherited Disease Research (CIDR) in July 2005 to request WGG of our combined 250+ multiplex families with

heritable high-grade myopia. CIDR approval was granted, (CIDR contract # N01-HG-65403). This study was approved by all Institutional Review Boards at each study site.

Overall Dataset

Although several linkage scans for high-grade myopia have been reported, all were based on microsatellite markers and a small number of families. We recently completed whole genome genotyping of our international high myopia dataset with a release of 5,928 out of 6008 SNPs attempted using the Illumina Linkage Panel IVb, which has an average SNP inter-marker distance coverage of 0.65cM, or 521kb. Our dataset consisted of 261 multiplex families (at least two affected individuals per family). A total of 1568 samples were released. For experimental samples, a total of 1400 out of 1457 samples were attempted, 81 out of 81 blind duplicates were attempted, and 87 Centre d'tudes du Polymorphisme Humain, France (CEPH) DNA controls (http://ccr.coriell.org/Sections/Collections/NIGMS/CEPHDNAPools.aspx) were used. Total genotypes released was 9,295,104. There was a missing data rate of 0.20% missing genotypes/ total genotypes. The missing data rate varied by sample tissue-type of 0.28% for buccal, 0.25% for saliva, 0.16% for CEPH, and 0.18% for blood. The Mendelian consistency rate was 99.98%, and the blind duplicate reproducibility rate was 99.99%. To our knowledge, this is the largest dataset used for a linkage study for high myopia. Objective measurements for spherical equivalent, sphere, cylinder, axis, and axial lengths were documented. (Some individuals were plano in each eye and therefore had no measurable refractive error.) Keratometry data was obtained by all sites except Cardiff. Axial length was not obtained at the Cardiff and Hellerup sites, and not consistently measured on subjects at the Duke and Toulouse sites. The diagnosis of myopia was determined by the spherical refractive error. Since our target phenotype is high myopia, we defined affected individuals as having a minus spherical refractive error less than or equal to −5.00 diopters for the less affected eye. Unaffected individuals had a plus spherical refractive error greater than or equal to 0 D. The mean number of subjects per pedigree is 5, the range of spherical refractive error is −50.00D to +7.00D. Table 1 summarizes the phenotypic characteristics of the dataset.

Table 2 provides the descriptive statistical summary for each phenotype obtained from all clinical sites.

Both eyes show similar mean and standard deviation (SD) for each phenotypic variable. We also performed polygenic analysis using the SOLAR program to estimate residual heritability for each phenotypic variable. As shown in Table 2, only the right eye cylinder and axis have non-significant residual heritability. Based on our experience with other linkage studies of quantitative traits, the likelihood of identifying linkage regions is higher if the residual heritability of a trait is greater than 20%. From these preliminary data, we believe that spherical equivalence and sphere are the best quantitative traits to analyze. Furthermore, these data prove that these quantitative traits are genetic.

A series of quality control programs were implemented before linkage analyses, which included examining family relationships using the RELPAIR and PREST programs, Mendelian inconsistency testing using PedCheck and PREST software, and Hardy-Weinberg equilibrium testing using the Genetic Data Analysis (GDA) program. [143–146]

The final dataset used for linkage analysis was based on 249 families (137 DUK, 46 CARD, 40 TOU, 25 HEL and 1 MEL) and 5796 markers. The dataset contained 10 Asian, 12 African-American, and 227 Caucasian families.

We performed two point parametric linkage analysis using the FASTLINK and HOMOG programs (http://linkage.rockefeller.edu). [147] Since the mode of inheritance for high

myopia is unknown, parametric analyses were performed using both autosomal dominant (AD) and autosomal recessive (AR) models with disease allele frequencies of 0.01 for both models. The MERLIN program was used to perform both parametric and non-parametric multipoint linkage analysis, and the same AD and AR models were assumed in the MERLIN parametric analysis. [148] It is known that linkage disequilibrium (LD) may inflate the type I error of multipoint linkage analysis. [149] The current version of MERLIN has an option to take into account marker-marker LD to reduce its impact on the LOD score. The threshold of correlation due to LD (r2) between markers was set at 0.16 in the multipoint linkage analysis. Overall, and multiple stratified center and race-specific datasets were analyzed, (DUK, CARD, TOU, MEL, HEL and Asian, African-American, Caucasian).

Overall, 61 markers across 19 chromosomes had two point HLOD scores 2, and 18 linkage regions from 12 chromosomes had peak multipoint HLOD or NPL scores 1.5. SNP rs2063239 on chromosome 12 had the highest two point HLOD score (HLOD=4.80). The multipoint analysis corresponded to the two point results for chromosome 12 delineating a 36.59cM linkage region with the SNP peak marker rs337663 (HLOD=3.48). This region overlaps with regions identified by multipoint parametric linkage analyses for DUK (peak HLOD=3.62, rs1489895), Asian (peak HLOD =1.55, rs930248), and Caucasian (peak HLOD=2.34, rs337663) subsets as well as nonparametric linkage analysis for the DUK subset (peak NPL=2.07, rs337663). The chromosome 12q22 linkage region was contracted to a 9.94cM region from rs163016 to rs1520724 where it is shared by four datasets (overall, DUK, Asian, and Caucasian) (see Figure 1). Other regions of interest emanating from the CIDR linkage study were located on chromosomes 2, 5, 9, and X. Two regions were found in chromosomes 2, 5, and X. Several linkage regions were notable for high lod scores in single datasets, including: chromosomes 5 (1st region) and X (2nd region) from DUK; chromosomes 2 (2nd region), 4, and X (1st region) from CARD; chromosome 14 from TOU; chromosome 1 from HEL; and chromosomes 1 and 3 from the Asian dataset. Replicated MYP loci from this study include: MYP1 on chromosome Xq27.3–28; MYP3 on chromosome 12q21–23; MYP5 on chromosome 17q21–23; MYP9 on chromosome 4q12; MYP12 on chromosome 2q37; MYP14 on chromosome 1q36 and MYP15 on chromosome 1q41. The results of this international consortium genotyping are in press. [150]

Chromosome 12 Linkage

As mentioned earlier, my lab established linkage for the MYP3 locus to chromosome 12q21–23 in 1998 in a single large, multigenerational Caucasian family with autosomal dominant high-grade myopia. [96] This locus was replicated in 3 independent labs. [97] (Eric Yap-Singapore, and Paul Baird-Australia-personal communication) It was recently replicated in a Hong Kong Chinese cohort (Pang CP, et al. Poster 1397–2007 American Society of Human Genetics Meeting, 2007). Our SNP genotyping of the international myopia cohort showed the strongest linkage region at the MYP3 locus.

These analyses show strong confirmation for linkage of one or more high-grade myopia susceptibility genes to the MYP3 locus. The major contributors to this locus with two peaks are from the Duke and Hellerup sites. The Melbourne, Toulouse and Cardiff sites did not have significant linkage to this locus. Interestingly, a subset of the Cardiff cohort previously showed suggestive microsatellite linkage to this chromosomal interval. For race-specific analysis, Asian and African American categories did not have significant linkage. The strongest consensus linkage region is near 101.97cM, which replicates previous MYP3 locus analyses. This interval was contracted to a 23.5cM region from the original 31 cM microsatellite-derived MYP3 locus. The Duke site provides the highest contribution. Future studies will focus on SNP fine-mapping association analysis of the 23.5 cM interval using Illumina Golden Gate custom chip arrays.

MYP1 Locus on Chromosome Xq27.3–28

We have more fully characterized and refined the interval (Xq27.3–28) for the first high myopia locus MYP1, which is an X-linked high-grade myopia associated with a novel mild cone dysfunction and color vision deficits (protanopia for the Bornholm Eye Disease pedigree, and deuteranopia in my large Minnesota pedigree). [86–88] We have sequence-screened multiple genes (SPRY3, THMLE, TKTL1, RENBP, SYBL1, MECP, CXorf2) for mutations within the MYP1 locus interval. More than 40 of approximately 300 X-linked diseases map to this region. The red and green opsin (visual cone pigment) genes are located in a head-to-tail tandem array on chromosome Xq28. [151] Our most promising candidate gene TEX28 (or CXorf2), is a nested gene within this cone pigment gene array. This gene was more highly expressed in SAGE libraries of the human macular retina relative to peripheral retina (SAGE experiments courtesy of Dr. Cathy Bowes-Rickman-Duke University Eye Center). CXorf2 is comprised of five exons that almost span the entire distance between the protein-coding regions of the opsin genes and a transketolase-related gene, and encodes a polypeptide of 410 amino acid residues. (Figure 2.)

We investigated whether the X-linked myopia phenotype with possible cone dysfunction is a consequence of disruption of the CXorf2 gene. More specifically, we screened CXorf2 for any mutations using automated sequencing, and for any variations in copy number at the genomic DNA level using real-time PCR and the commercial NimbleGen comparative genomic hybridization (CGH) SNP panel for the X chromosome. (NimbleGen Systems, Madison WI).

Primer pairs were designed to amplify the identified exons (including at least 100 bp of each intron-exon boundary) of CXorf2. Polymerase chain reactions were performed on genomic DNA and amplified products were visualized using agarose gel electrophoresis. Amplicons were purified with Quickstep™ 2 SOPE™ Resin (Edge BioSystems, Gaithersburg, MD) and sequenced using BigDye™ Terminator on ABI3730 or 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were trimmed for quality and aligned using Sequencher™ (Gene Codes, Ann Arbor, MI). Normal and affected individual DNA sequences were compared alongside the known reference sequence (University of California Santa Cruz genome browser- http://genome.ucsc.edu/) for CXorf2.

After preliminary screening, we noted a single nucleotide polymorphism (SNP) in the 5' untranslated region (UTR) of exon 2 of the CXorf2 gene that segregates with the affection status in the X-linked myopia phenotype with protanopia. This was revealed in all affected males [4] and a carrier female screened in this pedigree, while the unaffected males [3] and marry-in controls [2] did not show this polymorphism. In addition, further screening of 107 external controls did not reveal this polymorphism. A second X-linked myopia family was provided to us for gene screening by the M. Michaelides and Anthony Moore and colleagues at Moorfields Eye Institute in London England with the same phenotype which also showed this UTR polymorphism in the affected males only. 5'UTRs are deemed to be crucial determinants of mRNA stability and translation efficiency. [152,153] Furthermore, polymorphisms in 5'UTRs have been proven to be associated with disease. [154,155] Hence, we suspect that the polymorphism we observe in CXorf2 could be regulatory in nature, and these results indicate that CXorf2 is a potential candidate gene for this phenotype.

To examine the copy number variation of CXorf2 gene, gene expression assays (Assays-by-Design™) consisting of a mix of unlabeled PCR primers and TaqMan® MGB probe (FAM™ dye-labeled) were designed on CXorf2 sequence to quantitate the pattern of expression at the genomic DNA level. A similar assay targeted on the opsin genes was also designed to validate the sensitivity of the experiment as the number of copies of opsins has

been previously determined by single-strand conformational polymorphism testing in the current pedigree. [87] An assay on GAPDH (housekeeping gene) served as an endogenous reference. All real-time PCR reactions were performed with Taqman® Universal PCR Master Mix on ABI7900HT Fast Real-time PCR System (Applied Biosystems, Foster City, CA). Data were analysed using the 'Comparative C_T method' where the amount of target normalized to an endogenous reference and relative to a calibrator (known individual value) is calculated as $2^{-(\text{deltadeltaCT})}$ (User bulletin #2, ABI Sequence Detection Systems) [deltadeltaC_T is the difference between the normalized C_T (cycle threshold) of an unknown sample and the normalized C_T of the calibrator]. Results were plotted as the number of copies present in each individual with respect to the assigned number of copies in the calibrator. The assay targeted on the opsin genes (Figure 2) revealed that employing real-time custom gene expression assays is a reliable way to study copy number variation. The number of copies expected to be present for the opsin genes were observed in affected males (4 copies), control males (3 copies) and a carrier female (7 copies). [87] There are minimal studies on CXorf2 in the literature, and only 3 copies of CXorf2 have been reported within the opsin gene array.[156,157] Figure 3a shows quantification of opsin gene expression by real-time PCR. Expression pattern follows the number of copies previously reported by Young et al. [84] Figure 3b shows quantification of CXorf2 gene expression by real-time PCR. All affected males show an expression of up to 5 copies of CXorf2. The calibrator was assigned a value of 3 copies based on and relative to the findings of the number of known copies of opsin gene array. Our results with the assay targeted on the CXorf2 gene indicate that the affected male individuals in this pedigree express up to 5 copies of CXorf2 (Figure 3). This finding was also found in the second pedigree from Moorfields, but was not found in the Bornholm Eye Disease pedigree. The copy number variations were confirmed using the NimbleGen array CGH product as well, which has an average SNP probe spacing for chromosome X of 340bp. (Figure 4). Copy number variations have been proposed to play a role in disease inheritance and susceptibility to disease due to their ability to affect genic dosage. [158] Hence, we believe that these CXorf2 gene alterations may be responsible for this phenotype. [159]

MYP2 Locus on Chromosome 18p11.31

We have screened almost all of the known candidate genes within the expanded 7.6cM interval of the MYP2 chromosome 18p11.31, and have found no mutations or associated polymorphisms of significance that travel with the high myopic affection status. None of the sequence variants found co-segregated with the affected phenotype. Specifically, there were no heterozygous or homozygous polymorphisms observed only in affected individuals in any MYP2 pedigree. An interim summary of the screening progress was published in 2004.[160] Since moving to Duke, the CLUL1, MRCL3, MRLC2, YES1, C18ORF2, METTL4, KNTC2, ENOSF1, ADCYAP1, TYMS, CR627458, COLEC12, THOC1, and CETN1 genes were screened in unrelated controls and in representative DNA samples of high myopia affected and unaffected family members from the original 7 MYP2 pedigrees. All candidate genes were screened by direct sequencing. Consistent segregation of a mutation with the disease was not demonstrated in any of the 7 families. Novel SNPs were found and submitted to the dbSNP database.

Despite replication from 2 independent studies for this locus, the results from the international high myopia dataset using the Illumina IVb SNP linkage panel have not supported the presence of a linked interval at chromosome 18p11.31. Rationale for this ranges from limited SNP spacing of the commercial Illumina panel with inadequate interval coverage, the strong likelihood of a monogenic basis for the limited number of families (or single family) used for this initial microsatellite-identified locus, and the possibility of locus race-specific associations (the initial study showed significant linkage in one large Chinese

family alone, and one of the replication studies was a Hong Kong Chinese cohort). The literature is replete with examples of initial microsatellite results not confirmed in another sample set or analysis. [161,162]

MYP5 locus on chromosome 17q21–23

Two genes have been screened-DHX40, and SEPT4. No sequence variants were found to segregate or to be associated with the disease phenotype.

As stated before, significant association (p <0.05) under a model assuming dominance to 2 COL1A1 SNPs (rs2075555 – G allele in intron 11 with p = 0.0071 and OR =1.36, and rs2269336 - G allele in the 5" upstream region with p = 0.014 and OR =1.31) was found in a case/ control Japanese high myopia cohort. [139] We genotyped the same 8 SNPs using the TaqMan protocol on 141 high myopia Duke families with no pedigree or Mendelian error inconsistencies previously screened by the CIDR genotyping described above. The SNPs selected are shown in Figure 5, and genotyping results are shown in Table 3.

The rs1800211 SNP showed moderate significant association in our cohort, but we did not replicate significance of the 2 SNPs more highly associated in the Japanese cohort. This warrants further investigation, as the rs1800211 SNP is exonic, and exhibits potential functional implications for the protein.

SUMMARY

No results have implicated more than just a single gene, or have expanded into an analysis of a specific pathway. This may implicate additional genes in a pathway also shown to be risk factors for myopia in validation studies and point to potential haplotype-specific treatments. No candidate genes have been shown to account for even a modest fraction of the familial risk of myopia, and for most the data are conflicting about whether a true association exists. The genes underlying all high-grade myopia peaks except possibly for chromosome Xq28 remain unidentified, suggesting substantial genetic heterogeneity. Consideration is needed to assess the role of environmental factors to genetic influences, such as interactions of early-age near-work and genotype. [10,163] Consideration also needs to be given to the identification of phenotypes indicating etiologically homogeneous subgroups, e.g. early age-of-onset, with/without retinal degenerative changes, or classification by individual response to treatments that reduce accommodation to near objects, such as progressive addition lens use. [164] Candidate gene studies underscore that myopia is very complex, in fact, so complex that single candidate gene studies are unlikely to demonstrate the type of relationships needed to account for the majority of susceptibility genes. There is a need for a genome-wide approach, incorporating candidate genes but not restricted to the study of candidate genes, to explore the relative contributions and interactions between known candidate genes and possibly novel genes in increased myopia susceptibility.

Acknowledgments

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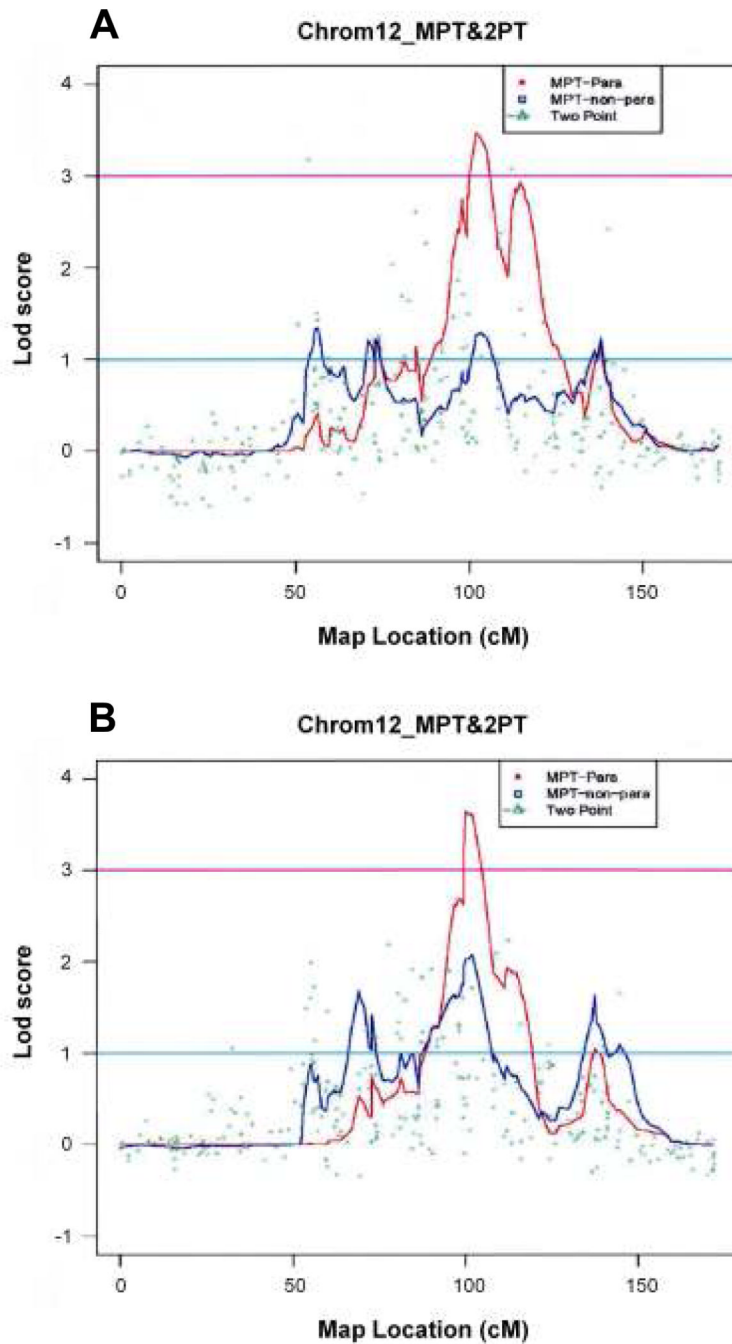
Figure 1.
The Center for Inherited Research (CIDR) International Family High Myopia linkage study analyses show strong confirmation for linkage of one or more high-grade myopia susceptibility genes to the MYP3 locus at chromosome 12q21–23. The major contributors to this locus with two peaks are from the Duke and Hellerup sites. The Melbourne, Toulouse and Cardiff sites did not have significant linkage to this locus. The strongest consensus linkage region is near 101.97cM, which replicates previous MYP3 locus analyses.

Figure 2.
Schematic representation of the arrangement of Opsin gene array and CXorf2 genes on chromosome Xq28 locus source - UCSC genome browser. OPN1LW= opsin 1 low wavelength –red cone pigment gene, OPN1MW =opsin 1 medium wavelength –green cone pigment gene, TKTL1= transketolase-related gene. Vertical bars are exons, horizontal bars are introns, and arrow heads indicate the direction of transcription. A color version of this figure is available online at www.optvissci.com.
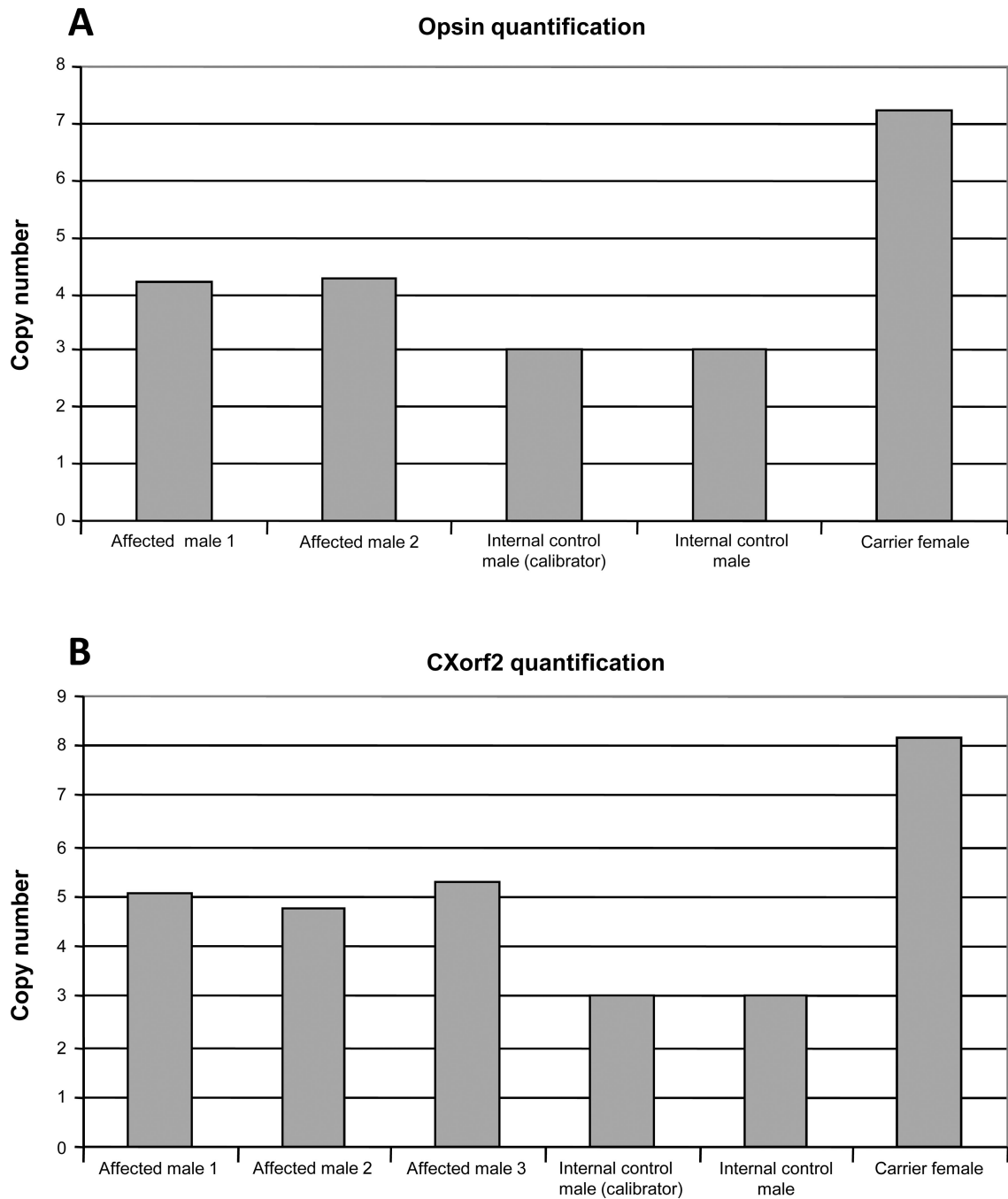
A

Opsin quantification

B

CXorf2 quantification

Figure 3.
(A) Quantification of opsin gene expression by real-time polymerase chain reaction. Expression pattern follows the number of copies previously reported by Young et al 2004. [87] **(B)** Quantification of CXorf2 gene expression by real-time polymerase chain reaction. All affected males show an expression of up to 5 copies of CXorf2. The calibrator was assigned a value of 3 copies based on and relative to the findings of the number of known copies of opsin gene array.
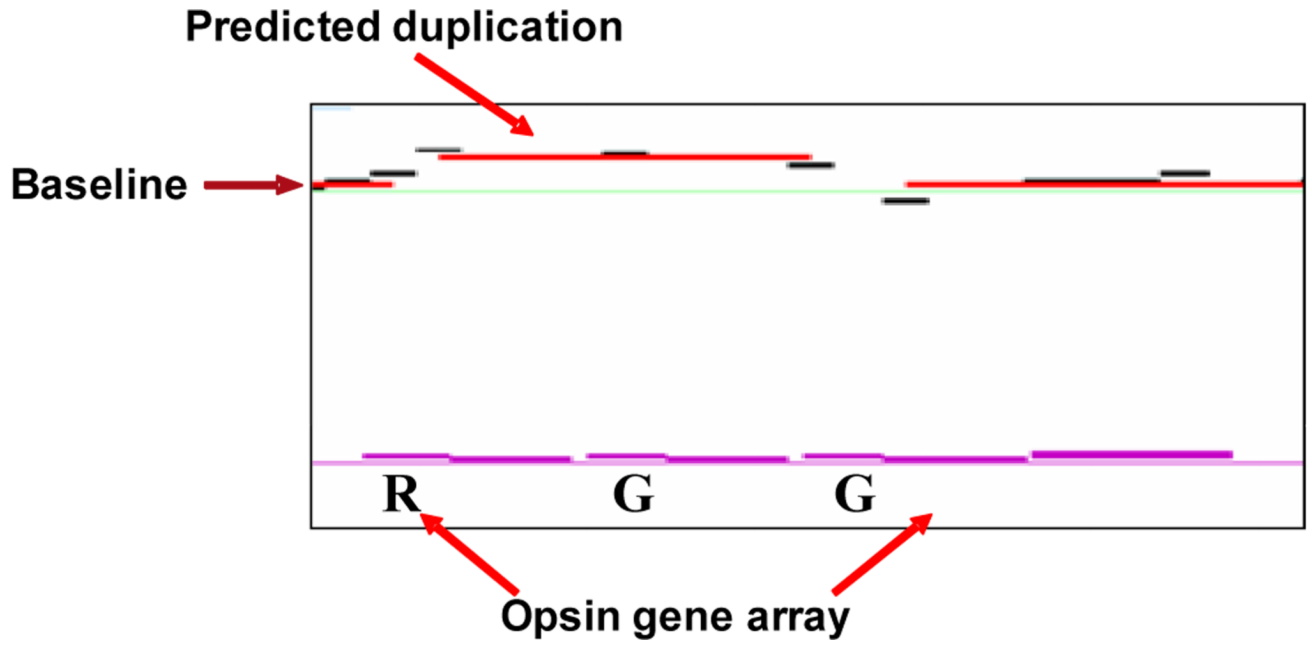
Figure 4.
Array comparative genomic hybridization (CGH) y-axis represents log2 ratio calculated by subtracting the log value of the reference sample intensity from the log of the test sample intensity. R= red opsin gene, G= green opsin gene. A color version of this figure is available online at www.optvissci.com.
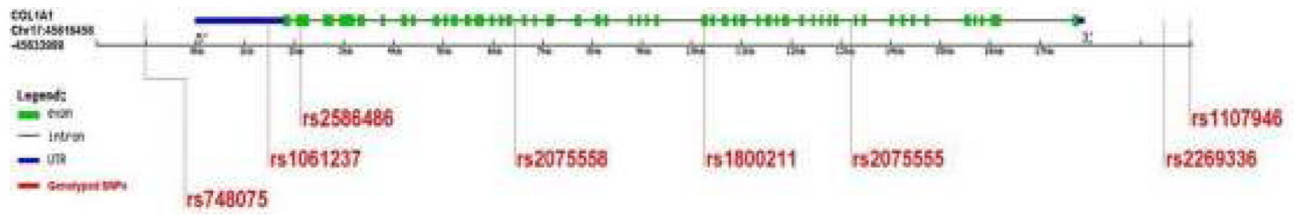
Figure 5.
Human COL1A1 gene structure. The size of each exon and intron is not to scale. Vertical lines show 8 single nucleotide polymorphisms (SNPs) with their respective public reference SNP rs number from the dbSNP database.

Table 1

Mean, standard deviation, and sample size (in parentheses) for each ocular measurement from right (OD) and left (OS) eye by ascertainment site. Duke= DUK, Cardiff=CARD, Toulouse= TOU, Hellerup=HEL, Melbourne=MEL.

Center		DUK	CARD	TOU	HEL	MEL
Sample size		656	252	315	163	15
Sphere (Diopters)	OD	−4.95± 6.0 (627)	−6.48±4.62 (234)	−5.12±5.89 (181)	−4.73± 5.43 (144)	−4.03± 2.54 (10)
	OS	−4.96± 5.93 (627)	−6.39±4.66 (235)	−4.83±5.66 (178)	−5.17± 5.22 (144)	−3.53± 4.39 (9)
Cylinder (Diopters)	OD	0.8± 0.94 (614)	1.09± 1.05 (221)	0.84±0.97 (153)	1.12± 0.35 (121)	2.53± 2.28 (10)
	OS	0.77± 0.9 (610)	1.05± 0.91 (220)	0.92±1.03 (147)	1.12± 0.99 (124)	2.08± 2.14 (9)
Axial Length (millimeters)	OD	25.48± 2.41 (197)	--	25.10± 2.58 (152)	--	24.76± 0.25 (3)
	OS	25.76± 2.45 (193)	--	25.11± 2.68 (151)	--	24.32± 2.17 (3)
Keratometry * (Diopters)	OD	43.71± 3.57 (244)	--	43.30±2.22 (114)	43.18± 1.73 (130)	43.67± 2.23 (5)
	OS	43.63± 3.74 (249)	--	43.30± 2.22 (113)	43.26± 1.63 (132)	43.11± 1.02 (5)

*
The mean and standard deviation of the keratometry measurements was based on the average of the keratometry 1 (K1) and keratometry 2 (K2) readings of each participant

Table 2

Summary of mean, standard deviation and heritability for each quantitative phenotype.

Phenotype	No. of Samples	Mean	Std Dev	Residual heritability (h^2_r)	P-value of h^2_r
Spherical Equivalent (right eye)	1,258	−4.57	5.54	**0.24**	*3.09×10⁻⁹*
Spherical Equivalent (left eye)	1,258	−4.6	5.51	**0.23**	*2.76×10⁻⁸*
Sphere (right eye)	1,224	−5.08	5.65	**0.25**	*3.03×10⁻⁹*
Sphere (left eye)	1,221	−5.09	5.62	**0.25**	*1.67×10⁻⁸*
Cylinder (right eye)	827	1.11	1.33	0.09	0.175
Cylinder (left eye)	822	1.05	0.99	**0.19**	*0.002*
Axis (right eye)	826	85.46	58.36	0.07	0.118
Axis (left eye)	823	86.94	59.15	**0.19**	*0.003*

Table 3

Association analysis of 8 single nucleotide polymorphisms (SNPs) of COL1A1 gene with high myopia for a dataset of 120 Duke families. Those SNPs in **bold** had significant association in a Japanese cohort. (Inamori 2007) [138]

SNP rs	Public Position (bp)	Function	Amino Acid Change	Allele	P-value PDT	P-value GenoPDT	APL
rs748075	45615428	3'Downstream		C/G	0.32	0.62	0.99
rs1061237	45617774	3' UTR		A/G	0.35	0.44	0.87
rs2586486	45618215	Exon	Lys [K]-->Gln [Q]	G/T	1	1	0.29
rs2075558	45622584	Intron		A/C	0.59	0.45	0.21
rs1800211	45626379	Exon	His [H]-->Arg [R]	C/T	1	1	0.02
rs2075555	45629290	Intron		G/T	0.30	0.15	0.65
rs2269336	45635355	5' Upstream		C/G	0.28	0.38	0.32
rs1107946	45635989	5' Upstream		A/C	0.41	0.44	0.9

Single Nucleotide Polymorphism (SNP) rs- public reference # from the dbSNP database. Genotype Pedigree Disequilibrium Test (Geno-PDT), Association in the Presence of Linkage (APL). 163–164