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A decade in search of myopia genes

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1. ABSTRACT

Nearly half of visual impairment in the world is caused by uncorrected refractive errors, and myopia constitutes a significant proportion of this problem. Moreover, the prevalence of myopia is increasing, especially in Asian countries. Linkage studies have identified at least 18 possible loci (MYP) in 15 different chromosomes associated with myopia, although some of these remain to be confirmed. However, when studies have been carried out to identify specific candidate genes, it is apparent that these genes are often not part of MYP loci. In studying the expression of specific genes that might be responsible for myopia, we are learning that the involvement of various small leucine-rich repeat proteoglycans and growth factors is not a simple one. The emerging picture is one of complex interaction, in which mutations in several genes likely act in concert. The majority of myopia cases are not likely caused by defects in structural proteins, but in defects involving the control of structural proteins. The future of genetic research in this area will likely rely increasingly on microchip array technology.

2. INTRODUCTION

It is estimated that 153 million individuals worldwide have uncorrected refractive error as a cause of visual impairment, which represents 48.7.% of all visually impaired individuals (1). Myopia (short-sightedness) represents a significant proportion of refractive errors, and its prevalence in some Western and Asian countries is high, varying from 13% to 28% in Western adults (2), but alarmingly high in some Asian countries, notably Hong Kong, Taiwan, and Singapore with estimates from 71% to 96% (3). Most importantly, the prevalence of myopia has continued to increase over the past several decades, particularly in Asia (4). That ethnicity is important was highlighted by a recent study of 12-year-old Australian children, which found that greater decreases in spherical equivalent refraction and increases in axial length were associated with the number of myopic parents in children of East Asian ethnicity compared to children of European White ethnicity (5).

While refractive errors can be corrected, this represents a significant burden in terms of health care,

Table 1. Examples of ocular and systemic syndromes that include myopia. Adapted and reproduced with permission from Birkhäuser Verlag

Systemic Syndromes	Locus	Ocular Syndromes	Locus
Aland Island eye disease	XP11.2.3	Achromatopsia 3	8q21-q22
Congenital spondyloepiphyseal dysplasia	12q13.1.1-q13.2.	Albinism	11q14-q21
De Lange syndrome	5p13.1.	Atrophia gyrata	10q26
Donnai-Barrow syndrome	2q24-q31	Choroideremia	Xq21.2.
Down syndrome	Xp11.2.3; 1q43; 21q22.3.	Coloboma	11p13; 8q22.1.; 7q36
Ehlers-Danlos syndrome (type VI)	1p36.3.-p36.2.	Familial exudative vitreoretinopathy	11q13-23; 11p13-12; Xp11.4.
Fabry disease	Xq22	Fundus flavimaculatus	8q21-q22; 1p21-p13
Homocystinuria	21q22.3.	Microcornea	11q13
Knobloch syndrome (type I)	21q22.3.	Myelinated retinal nerve fibers	Unknown
Marfan syndrome	15q21.1.	Myopia-ophthalmoplegia syndrome	Unknown
Noonan syndrome	12q24.1.	Progressive bifocal chorioretinal atrophy	6q14-q16.2.
Polydactyly-myopia syndrome	Unknown	Retinitis pigmentosa	Many
Sick sinus syndrome	Unknown	Retinopathy of prematurity	11q14-q21
Stickler syndrome	12q13.1.1-q13.2.	Wagner syndrome	5q12-q14
Turner syndrome	12q24.1.	X-chromosomal congenital stationary night blindness	Xp11.4.; Xp11.2.3; 5q35;

especially in developing countries. The causes of myopia are multifactorial, with changes in lifestyle, environment, and heredity all playing a part. Although the exact contribution of heredity to myopia has always been a contentious subject, the introduction of modern genetic and biochemical techniques have started to define which genes and molecular entities are associated with the development of myopia. Moreover this data can contribute to better understanding the many genetically caused syndromes that give rise to apparently non-related organ dysfunctions (Table 1), some of which involve key homeobox genes. For example, anophthalmia-microphthalmia can be caused by frameshift and nonconservative missense mutations in the bone morphogenetic protein-4 (*BMP-4*) gene, which encodes for a multifunctional growth factor and belongs to the transforming growth factor beta (TGF-beta) multigene family (6). Further, *BMP-4* interacts with the hedgehog signaling genes in animals, which demonstrates the complexity of interactions that occur when a mutation arises. Although some of these genetically caused diseases are relatively common, the majority of these ocular and systemic syndromes are extremely rare. In addition, while many genetic mutations involve Mendelian inheritance, some do not.

The purpose of this review is to provide an update of the genetics research involved in myopia, which has taken place since our last review of the subject (7).

3. CONTRIBUTIONS OF THE ENVIRONMENT AND GENETICS

3.1. Environmental factors

There is no doubt that environmental factors play a considerable part in the development of myopia. For example, age, gender, education, and near work (reading or working with computers) have all been linked with the increasing severity of myopia (8-12). Moreover, the frequent appearance of myopia during school and college years or, as well as occupations requiring intense and prolonged near work, all suggest that a near-vision stimulus might be active. Differences between urban and rural populations also indicate that myopia in school-age children tends to be lower in rural settings because the children tend to spend more time outdoors (13), which implies less near work and more distance vision. Theories to explain this near-vision stimulus suggest that either insufficient accommodation shifts the image focus during near vision behind the retina (14), or that excessive accommodation causes axial elongation via induced mechanical pressure on the eye wall (15).

3.2. Genetic factors

Investigations to determine the amount of variance in refractive error explained by genetic factors have found that it is highest in twin studies, varying from 75% to 94% (16-19), while in non-twin family studies it is considerably lower, with ranges of 27% to 55% in one modeling study (20). The corollary of course is that the contribution of shared environmental effects is much higher in non-twin family studies. Early epidemiological studies that investigated familial refractive error and adjusted for environmental factors found a heritability for refraction (21, 22) that has since been strengthened by adult twin studies. For example, Hammond *et al* (16) found that if myopia and hyperopia were treated as binary traits, then the heritability was 90% for myopia, and Teikari *et al* (23) estimated myopia heritability at 58% when myopia was assumed to be a dichotomous variable. Similar heritable factors also appear to account for 80% of juvenile myopia (24).

3.3. The Beaver Dam study

Using the Beaver Dam Eye Study baseline data, Klein *et al* recently extended their previous investigation of refractive errors using familial correlation, commingling, and segregation analysis (25). Mostly consistent with their previous study that employed generalized estimating equations (26) they found higher correlations between siblings compared to parent-offspring (0.3.44 vs. 0.1.71), and cousins compared to avuncular pairs (0.1.00 vs. 0.0.83; avuncular relationships are those between nephews/nieces and aunts/uncles). A cohort effect was suggested by the data, which might be a function in part of near-work activity in younger generations. The commingling analysis found that the best fit was obtained using multiple rather than single distributions, which could be driven by genetics, major environmental factors, or a combination of

both. Segregation analysis, however, which attempts to fit genetic and non-genetic models with and without polygenic components, showed that while the best Mendelian models were those that incorporated a major gene effect with additional polygenic affects, such models did not fully explain the data when compared to general transmission models. While many explanations were proposed, including a cohort effect similar to that observed in the familial correlation analysis, the authors favored a model in which several genes exert a modest effect on the etiology of refractive error.

3.4. The GEM study

In the last few years, aspects of the Australian GEM twin study have shed more light on myopia. The study involved 1,224 twins of whom 690 were monozygotic (identical twins) and 534 dizygotic (fraternal twins) with ages from 18 to 86 years. The results showed that adult onset myopia accounted for about a third of all myopia and was present in 8.2.% of all twins. After excluding adult-onset myopia, heritability was estimated at 74% for the spherical equivalence (SE) measure in males and 88% in females, with less unique environmental effects and more additive genetic effects in females (27). To examine the relationship between axial length and refraction, a bivariate Cholesky decomposition model was constructed to determine the extent to which genetic and environmental effects affect axial length as well as refraction (it is assumed that one genetic component underlying the control of one phenotype affects another phenotype as well). While the genetic findings were similar in men and women (for example, 23% and 27% of SE variance were due to additive and non-additive genetic factors, respectively, that influence axial length), women had a far higher unique environment effect (55% versus 17% for men) (19). Thus it can be concluded that axial length and refraction share common genes in terms of etiology. Similar outcomes were reported for corneal astigmatism/curvature, with heritability estimates of 60% and 71%, respectively, higher non-additive genetic factor influence, and again substantial sex differences (28). Other non-ophthalmic traits were investigated and include birth weight (29), current height and weight (30), and personality characteristics (assessed using the International Personality Item Pool inventory) (31). Whereas birth weight and height were not found to be significantly associated with myopia, for females $\geq$ 80 kg, there was a significant risk for myopia (odds ratio, OR, 1.4.8) when lightest and heaviest quartiles were compared. Moreover a significant association between openness, a characteristic of intellect and myopia was found in multivariate analysis.

3.5. Update summary

Although considerably more data has accumulated in the last 5 years to support the effects of genetic factors on myopia and aspects of refractive error, in general populations it is still hard to create models that show anything but a modest effect on their etiologies. Thus we are still left with the impression that the influence of environment exerts a greater effect than does the concerted action of several genes.

4. LINKAGE STUDIES

4.1. Overview

The large spectrum of myopia-associated disorders observed with some 150 genetic syndromes, some of which are systemic, and some specific to the eye, underscore the concept that myopic refractive errors are likely etiologically heterogeneous. In this situation, multiple genetic and epigenetic factors combine at different stages of development.

Mapping genes using classic Mendelian inheritance principles through the technique of linkage analysis, even though the methods are now much more sophisticated, is a time-honored method of identifying possible candidates. When we reviewed the state of linkage studies in 2004/2005 there were 5 potential loci; now as many as 20 have been proposed, although confirmation for some of these locations are still lacking, and so some loci must be regarded as tentative. In addition, it must be iterated that some of these earlier investigations involved study of relatively small families, which may mean that any loci identified only represent a small proportion of the inheritability of myopia. On other hand, larger studies have started to appear, and in time it is hoped that as more refined mapping of genomic regions of interest occurs, the nature of conflicting reports will resolve in favor of more definitive loci, and better linkage to candidate genes.

Generally speaking we follow the convention that in chromosome linkage studies a significant LOD (logarithm of odds) score has to be 3.0. except in the case of X-linked studies, in which 2.5. is sufficient. However, Lander and Kruglyak have proposed that a LOD score of 1.9. or a nonparametric sib-pair *p* value of 0.0.0074 constitutes evidence of genome-wide suggestive linkage (32). Moreover, the same authors have suggested that a *p* value of 0.0.1 is adequate for replication of a previously published genome-wide significant result. This is not a universally accepted criterion, which is why we may refer to such replication results as suggestive of confirmation (i.e., tentative) rather than definitively confirming linkages.

4.2. X-linked loci

MYP1 (Table 2) was the first identified locus on the X chromosome mapped to Xq28 in 1990 (33), and although the high myopia is associated with non-progressive cone dysfunction (34), the color vision deficiency also appears to vary according to pedigree. Until recently, it remained unclear which gene was responsible for these defects, but work conducted by Metlapally *et al* has suggested one gene candidate: TEX28 (35). To better understand the role of the proposed gene, one has to first appreciate that the visual cone pigment (opsin) genes comprise a contiguous array in which a red (L) gene is followed by one or more copies of the green (M) gene. Changes to the array with hybrid opsin genes in the first or second positions result in color vision defects (36). The array structure is also unusual in that is an example of segmental duplications—a region of repetitive DNA segments. TEX28 is a nested gene within the array that is expressed by the testis, kidney, blood, and 5 ocular tissues,

Table 2. Loci of chromosomes associated with high-grade and low-grade myopia

Locus (MYP)	Chromosome Region	LOD Score[1]	REFERENCES	Candidate Genes
1 (H)[1]	Xq28	4.8.	33	TEX28
2 (H)	18p11.3.1	8.3.	42	*TGIF* (doubtful)
3 (H)	12q23-24; 12q14.3.-21.3.1	3.9.; 3.9.	43, 49	*SYT1*?; probably not lumican or decorin genes
4 (H)	7q36	2.8.[2]	57	
5 (H)	17q21-22	3.2.	110	*COL1A1*
6 (L)[2]	22q12	3.5.	45	
7 (L)	11p13	6.1.	50	*PAX6*
8 (L)	3q26	3.7.	50	
9 (L)	4q12	3.3.	50	
10 (L)	8p23	4.1.	50	
11 (H)	4q22-27	3.6.	111	
12 (H)	2q37.1.	5.7.	54	
13 (H)	Xq23-q25	2.8.	38	
14 (L)	1p36	9.5.	112	
15 (H)	10q21.1.	3.2.	62	*PCDH15*, *ZWINT*
16 (H)	5p15.3.3-p15.2.	4.8.	61	*IRX1*, *IRX2*, *POLs*, *CCT5*, *CTNND2*
17 (L)	7p15.2.	5.9.	58	
18 (H)	21q.22.3.		63	*UMODL1*

Abbreviations: high myopia (H), low myopia (L). [1]Where several markers were identified, the maximum (peak) LOD score is given. LOD scores ≥ 3.0. are considered significant linkages, except in the case of X-linked loci, when it is ≥ 2.5.; [2]not a significant linkage. [3]Reference is the one originally identifying the locus.

and comprises 5 exons that span almost the entire distance between the protein-encoding regions of the opsin genes and *TKTL1*, a transketolase gene. Normally, there are 3 repetitive copies of TEX28 that are intercalated within and translated in the opposite sense to the opsin gene array (37). What Metlapally *et al* demonstrated is that in 5 high myopic pedigrees there were one fewer, or 4 or 5 copies of TEX28 (35). The phenomenon of copy number variants (CNV) has been proposed as a factor in disease inheritance and susceptibility as it affects "gene dosage," and thus could be the cause of high myopia in this X-linked locus.

Zhang *et al* identified a locus for high myopia at Xq23-q25, which has been termed MYP13, based on a family of Han ethnicity (6 males affected, 4 generations; 5 affected, 11 unaffected participated in the study) (38). The same investigative group also identified another locus at Xq25-q27.2., based upon study of another slightly larger Han ethnicity family, which is outside of MYP1, but overlaps MYP13 (39). However, it is unclear from this data whether this locus is distinct from MYP13. The results of the first large-scale study of high-grade myopia (254 families from 5 independent sites, 1411 subjects) tentatively confirmed the Xq28 locus although the peak LOD score was only 1.6.9, and these results appeared to only be confined to the Duke subset (Duke University Medical Center, Durham, NC, USA) using the DOM model (40). A much higher although non-significant LOD score of 2.4.0 was obtained for the MYP13 locus (Xq24), which again suggests confirmation, but was confined to the Cardiff (Wales) subset. Both these latter results indicate that X-linked loci for myopia are probably quite population or ethnic-specific and not present in all populations.

4.3. MYP 2 and 3

A large United Kingdom (UK) study (51 families comprising 306 individuals, with phenotypic information available for 254) (41) found no correspondence with the MYP2 locus, first identified by Young *et al* (42), nor MYP5. However, the 12q locus (MYP3) also originally mapped by Young *et al* was tentatively confirmed, although this result depended on corrections in statistical analysis (43). The MYP3 locus in this instance was estimated to be responsible for high myopia in more than 25% with autosomal dominant transmission (41). Nevertheless, it should be pointed out that methodological considerations in this study (no whole genome analysis; only 3 small and selected loci tested; non-significant data; and cumulative data from several, but rather small families) might not make it statistically comparable to other studies.

Yang *et al* mapped a consanguineous Chinese family with autosomal recessive high myopia and provisionally confirmed a locus at 14q22.1.-24.2. (D14S984-999) with a maximum LOD score of 2.1.9 (44). Although this score is not significant, the study is interesting because it was the first study of a family with autosomal recessive high myopia rather than the more commonly reported autosomal dominant form. Two other investigations into genetically isolated Amish and Jewish Ashkenazi populations with mild or moderate myopia observed no linkage with loci on chromosomes 12 (MYP3) and 18 (MYP2) (44, 46), but in one instance a linkage to MYP6 was established (45). Finally, two other investigations into the MYP2 linkage, the first in Hong Kong, the other in France, also failed to establish a connection of high myopia with locus 18p (47, 48), although the selection of families in both these studies does not rule out the possibility. Summarizing, it does appear that the MYP2 locus is very heterogeneous and more confirmation studies of this locus would be welcome.

More recently, refinement of the general MYP3 locus has been obtained in a German 6-generation kindred (49) using whole-genome scans employing modern single nucleotide polymorphism (SNP) chip technology, and the minimum consensus region refined to a location between SNP_A-1507739 (alias rs1373877) at position 63,662,789 bp and SNP_A-1509586 (alias rs717996) at position 82,636,288 bp, with a new interval of 6.8.cM. Li *et al* also identified a 9.9.7 cM region in the MYP3 locus (12q21) using the SNP approach rather than microsatellite analysis

(40). Total HLOD scores for this region were 3.4.8 with strongest contributions from the Duke subset. Thus, it would appear that this locus is one of the most homogenous markers of high-grade myopia identified to date, and with perhaps 25 database-indexed genes in the region, it is hoped that several of these will be identified as candidates for further investigation of the pathophysiology of myopia and eye development.

4.4. Other MYP loci

In an attempt to verify the loci of MYP7-10, which were initially identified by twin studies (50), an independent yet ethnically and phenotypically similar twin cohort was investigated in Australia but negative linkage outcomes were reported (51). Li *et al* also reported replication of the MYP6 locus, as well as MYP11, MYP12, and MYP14 (40). However, maximum LOD scores were not significant and only linked to 1 of the 5 sites in each instance: 1.6.6 (DOM) and 2.0. (non-parametric linkage analysis; NPL) (MYP6, 22q12.3.); 2.2.6 (DOM) and 2.8.1 (NPL) (MYP11, 4q24); 1.5.2 (DOM) (MYP12, 2q37.1.); and 1.8.0 (DOM) (MYP14, 1p36.3.2). On the other hand, Klein *et al* conducted nonparametric genome-wide linkage analyses of 834 sibling pairs in 486 extended pedigrees (subjects from the Beaver Dam Eye Study) and tentatively confirmed the MYP6 locus at 22q11 (52) originally reported as 22q12 by Stambolian *et al* (45).

Collectively, these reports suggest that at the very least, high-myopia loci are heterogeneous. On the other hand, recent evidence suggests that low or moderate myopia may also map coincident with or close to these high myopia loci: three large families drawn from the GEM study, who had low to moderate myopia, were mapped and the strongest linkage signal was localized to 2q37.1. (53), which is within the MYP12 region, although the location of interest was 5 cM distal to the area associated with the maximum LOD score reported originally by Paluru *et al* (54). Using microsatellite markers, Schäche *et al* also refined their earlier mapping work (51) and discovered a new locus at 2q37 (1.8.3 cM) that is distinct from the MYP12 locus (55). Peak LOD scores were 3.9.7 (DOM) and 3.4.8 (NPL). Thus it would seem that chromosome 2 might harbor at least two separate locations that influence types of myopia.

In the last few years, more novel loci have been discovered in high myopia families. One French linkage study of 26 families (233 subjects) that employed microsatellite analysis discovered a 7.8.1 cM interval that mapped to 7p15 in the entire population with a maximum LOD score of 4.0.7 obtained using nonparametric multipoint linkage analysis (56). However, using parametric models this segment was nonsignificant. These data are interesting because the authors had previously found a linkage to 7q36 (MYP4) but were unable to replicate their previous findings, perhaps due to dilution of genetic markers as extra families were added to the study (57).

While the findings of Klein *et al* were only suggestive of a linkage at marker D7S3051 (7p21) based on multipoint analysis (52), Ciner *et al* also conducted a quantitative trait locus linkage analysis in 96 African American families (493 individuals; the Myopia Family Study) and discovered a significant linkage to chromosome 7 in regard to SE refractive error (58). Maximum LOD score was 5.8.7 in the 7p15.2. region although there were several significant scores for the region 45 to 53 cM. Adding 36 White families (260 individuals) to the African American families and analyzing the genetic data as a binary trait of myopia, the same authors also found a suggestive linkage in the same genomic area (D7S817; NPL score 2.5.9 (p = 0.0.05)) (59). Finally, the same group carried out a quantitative trait locus linkage analysis in 61 Old Order Amish families (411 individuals) and 49 Orthodox Ashkenazi Jewish families (542 individuals) and then performed a meta-analysis of these results combined with previous evaluations of White and African American families (60). The combined analysis demonstrated a meta-P_{PW} value of 0.0.0134 at 46 cM on 7p14, which supports an area associated with myopia but which is most likely distinct from that reported by Klein *et al* (52). Taken together, this body of data indicates that there are at least 2 locations on the 7p chromosome region that are associated with myopia, although so far the regions of interest have not been refined sufficiently to identify gene candidates.

A genome-wide scan of 94 high myopia cases drawn from a Chinese population and the same number of control subjects using microsatellite markers refined by genotyping SNP markers revealed a location on chromosome 5p that had not been previously reported (61). A maximum LOD score of 4.8.1 was associated with the 5p15.3.3-p15.2. region (an interval of 17.4.5 cM). There are 25 known genes in this region and although no definitive candidates have been identified, the authors of the study suggest 5 potential genes associated with transcriptional, ATP-binding or protein-binding activities.

The major findings of the study conducted by Klein *et al* included the reporting of 2 novel gene loci on chromosome 1q (52). Exact multipoint p values for 1q24 and 1q41 were 0.0.27 and 0.0.0019, respectively, which suggests that there is evidence for at least one locus associated with refractive error. Another new locus was also reported by Nallasamy *et al* in a Hutterite population in South Dakota (United States of America, USA) that had high-grade myopia (62). With a peak multipoint LOD score of 3.2.2 at marker D10S1643, the 2.6.7 cM region maps to chromosome 10q21.1., with the possibility of at least 2 gene candidates: *PCDH15*, which is a member of the cadherin superfamily of calcium-dependent cell-cell adhesion molecules, and *ZWINT*, which is involved in mitotic checkpoint signaling. Finally, Nishizaki *et al* reported a new locus at chromosome 21q22.3. based an analysis of SNPs associated with marker D21S0083i) in a population of 520 highly myopic Japanese individuals (63). After full Bonferroni correction, one SNP remained statistically significant and mapped to the *UMODL1* gene.

4.5. Thoughts on linkage approaches

Many of the linkage studies conducted have helped define loci on different chromosomes that are

associated with high-grade or low-grade myopia but only in a few instances have candidate genes been proposed, and in those cases we have still to associate a refractive error with an SNP or CNV issue, or a specific deletion or translocation of genetic material. One should therefore ask the question, how successful are linkage studies likely to be in this regard?

The first issue is narrowing down the region of interest to a small enough area that gene candidates can be reasonably proposed. Refined mapping has improved recently, but as this occurs it does appear that many more loci are being defined, associated with specific populations. This is not surprising; the involvement of such genes in refractive errors appears to be heterogeneous with regard to more general populations and it is possible that only a small number of genes may turn out to be important. Another aspect is that our phenotypic definition may be too broad. If phenotypes were more narrowly defined in terms of myopia, which is difficult because such parameters as axial length or corneal curvature do not lend themselves so easily to definition in populations compared to SE, perhaps more specific gene involvement could be found. The alternative is to pursue the other approach: select gene candidates based upon biochemical and physiological development of eye structures, and the hunt for them in myopic populations (64-67).

5. CANDIDATE GENES

5.1. Overview

Although the prevalence of high-grade myopia is relatively low compared with low to moderate myopia, and there are difficulties associated with defining high myopia as a discrete trait, the search for such myoepigenic genes is necessary to identify candidates that in combination of various allelic forms might explain the heritability of the more common forms of myopia. In addition, once identified, such candidate genes can be explored in relation to scleral tissue remodeling, or other processes involved in the development of juvenile or adult-onset myopia.

5.2. Small leucine-rich repeat proteoglycans

Several candidate genes that encode for members of structural protein families called small leucine-rich repeat proteoglycans (SLRPs) have been proposed in association with high myopia (68-74). Many of these genes are involved with extracellular matrices, such as tendons, cartilage, skin, and sclera, and are required in several organ systems, although some are more specific to the eye. Some mutations in these genes, therefore, are likely to have serious consequences, but less serious mutations in several SLRPs might have an aggregate effect that can cause myopia. Underexpression or overexpression of such genes may also constitute another cause of disease. For example, it is likely that the expression of several SLRPs in scleral tissue may be fundamental to regulation of the biochemical properties of the scleral extracellular matrix (ECM). Expression of the *PRELP* gene (proline arginine-rich end leucine-rich repeat protein), in particular, has been found at a high level in human sclera (75). One could, therefore, imagine a situation in which defective control of the *PRELP* or other genes might lead to incorrect ratios of protein being expressed that result in faulty ECM, incorrect shaping of the eyeball, and hence the development of myopia. It is also noteworthy that thus far, research in connection with scleral cDNA libraries has established that the most redundant connective tissue-related genes code for alpha-A-crystalline, X-alpha-1 collagen, and beta-5 integrin, but that ECM matches include genes that express biglycan, syndecan, decorin, fibromodulin, PRELP, transgelin, TIMP-1, and fibulin 1 (64).

Decorin and lumican SLRPs mapped to the 12q21-22 and 12q21.3.-q22 regions respectively, may play a role in scleral collagen fibril formation and organization of the ECM through the inhibition of spontaneous collagen assembly (74). They were considered candidates on the basis of several animal experiments, although the evidence was more consistent for lumican (76, 77) than decorin (78, 79). However, genetic sequencing of both the *LUM* and *FMOD* (fibromodulin) genes from the same family used to initially codify the MYP3 locus established that neither gene was associated with high-grade myopia (80). Nevertheless, the positive results of Wang *et al* (81), who investigated SNPs in the 5'-regulatory region of the *LUM* gene in 120 patients with high myopia versus 137 controls, suggested that the promoter region of the *LUM* gene might be involved with high myopia, and that this was worthy of further research. Majava *et al* also explored the sequence of the *LUM*, *FMOD*, *PRELP*, and *OPTC* (opticin) genes in 85 English and 40 Finnish patients with high myopia (82). Four SNP changes in the *OPTC* gene were identified in both high-grade myopia and 3 SNPs occurred in family members with low myopia or emmetropia. Although the evidence is suggestive rather than compelling in regard to *OPTC* gene involvement, larger studies may help define which SNPs are more critical to myopia development. No differences between myopic individuals and controls were found for the *PRELP* gene, but one SNP change for the *LUM* gene and 3 SNPs in the *FMOD* gene were concluded to be good candidates for high myopic involvement. More recent research conducted by Wang *et al* however has cast more doubt on *LUM* involvement, and such involvement has been characterized as “premature” (83). In this study of 288 patients with high myopia and 208 control subjects, no association was found between the SNP reported by the Taiwanese authors (rs3759223) (81) and high myopia. It had been noted that strong deviation from the Hardy-Weinberg equilibrium was present in the Taiwanese study and that the difference between the observed and expected genotype frequency might have been due to genotyping errors or population admixture errors (83). Preliminary research reported by Pang also indicates that no sequence alterations in the lumican gene were found in 94 Hong Kong myopia patients (the study also had 94 control subjects) (65). Thus, the evidence is mounting against the *LUM* gene having significant involvement with myopia.

5.3. Nyctalopin

Research has been conducted to examine the possibility that mutations in the nyctalopin gene (*NYX*), located at Xp11.4., which normally result in the complete form of congenital stationary night blindness (CSNB1), can

cause high myopia without CSNB. Screening of 52 probands who had high myopia not inherited as an autosomal trait resulted in the identification of 2 different missense mutations in 2 individuals who did not have night blindness (84). What is potentially most interesting is that abnormal forms of the NYX protein might cause aberrant cone signaling via the ON pathway akin to retinal blur in their effects, thus stimulating myopia.

In one study of nob mice, which share the same mutation in the *NYX* gene as found in humans, when form deprivation was applied to both wild type and nob mice, the largest myopic shift was observed in the nob mice, suggesting involvement of nyctalopin in the development of myopia (85).

5.4. Transforming growth beta-induced factor (TGIF)

Another approach has been to screen candidate genes that have been implicated in animal experiments as involved in the biochemical processes of scleral change associated with axial elongation. Transforming growth beta-induced factor (TGIF) is a transcriptional repressor that is thought to either bind directly to DNA or interact with TGF-beta-activated intracellular signaling receptors (Smads), thus effecting repression of TGF-beta-responsive gene expression. Because the gene maps to the MYP2 locus it was considered to be a promising candidate, and early work in a Chinese population appeared to confirm this possibility (86). However, two studies in Asian and Caucasian populations did not find sequence alterations in the *TGIF* gene that were associated with disease phenotype (87, 88). Moreover a recent study in which refractive and ocular biometric measurements were undertaken in subjects from a Caucasian population in connection with SNP analysis did not find any significant associations (89). In their Chinese study, Wang *et al* also found no association of high myopia with *TGIF* as well as *TGFB1*, a gene encoding for TGF-beta1, a member of the TGF-beta superfamily (83). This latter result was surprising because it is well known that TGF-beta entities participate in extracellular matrix remodeling associated with myopia.

5.5. Hepatocyte growth factor (HGF) and cMET

Hepatocyte growth factor (HGF) is another cytokine that is broadly expressed in the eye, is involved in ocular physiological and pathological process, but does not map to any known MYP locus. Nevertheless, there was evidence of an association between 3 SNPs in the 5' region of the *HGF* gene and early-onset high myopia in a Han Chinese population (90). This study highlights in particular the concept that many genes may have small or non-detectable individual effects but significant aggregate consequences in regard to the development of myopia.

The receptor for HGF, cMET, a member of the tyrosine kinase receptor family, has also been studied in regard to myopia. Children from an ongoing Singapore study into myopia (the Singapore Cohort Study of the Risk Factors for Myopia) at one primary school were used in the discovery genotyping set (including only children of Chinese descent) while children from two other schools were used as the replication set (91). Out of 12 SNPs, one particular *cMET* SNP (rs2073560) was significantly associated with refractive error, but most interestingly children who had at least 1 copy of this variant allele had a more pronounced change in SE over a 3-year period regardless of their initial refractive status. Using 146 White families (649 subjects) drawn from the USA, Yanovitch explored the degree of myopia (high, mild to moderate, any, and extreme) versus emmetropia in relation to SNPs for *HGF* and *cMET* (92). While no association of myopia with c-MET polymorphisms was found, significant association of several SNPs with myopia was found with mild to moderate or any myopia showing the most consistent associations for SE and SP (sphere) phenotypes. Two of the SNPs (rs12536657 and rs2286194) appeared to act in concert. Wang *et al*, however did not confirm any associations of myopia with *HGF* in their study of Chinese subjects (83).

What should be made of these apparent contradictory results? One explanation is that both genes are probably heterogeneous in regard to population subtypes, although the discrepancies between individual studies may also be partly due to study design. In addition, it is possible that the influence of both these genes is relatively small in regard to myopia development, and more confined to low rather than high myopia.

5.6. Myocilin

The myocilin gene, which maps to 1q24-q25 and is thus not linked to any MYP locus, encodes a structural protein that is secreted in many ocular tissues, and mutations in the *MYOC* gene have been proposed as the cause of primary open-angle glaucoma. However, when a genetic analysis of 162 Chinese nuclear families who had at least 2 parents and 1 offspring with high myopia was undertaken in regard to the *MYOC* gene, 2 SNPs were found to be significantly linked and associated with the disease phenotypes (93). Interestingly, none of the SNPs were in the 3 exons, suggested that the effect is one of control over gene expression rather than structural defect of the protein. A study of White subjects drawn from 2 locations (86 families comprising 358 individuals with high myopia plus 56 highly myopic unrelated individuals in the USA; 164 families comprising 604 individuals with high myopia plus 112 highly myopic subjects and 114 emmetropic controls in Wales) found no significant association of the *MYOC* gene with high myopia (94). While heterogeneity between these different populations plausibly accounts for the difference in findings, study differences and possible errors cannot be ruled out at this stage in regard to consideration of the *MYOC* gene having as role in the development of high myopia.

5.7. Collagen genes

The first investigation into the effect of the *COLIA1* gene, which maps to a region that overlaps with the MYP5 locus and encodes collagen type 1 alpha, produced positive results. Using 330 Japanese subjects with high myopia who were matched to the same number of controls, it was discovered that 2 out of 10 SNPs had significantly different frequencies, with 1 SNP in 1 intron and the other upstream of the *COLIA1* gene (95). Again,

this preliminary evidence suggested the possibility of interference of gene expression similar to the *MYOC* gene results. However, 2 years later these findings were challenged with the results of a study also conducted in a high myopia Japanese population by Nakanishi *et al* (96). In these 427 unrelated highly myopic cases and 420 controls, 8 SNPs were selected for genotyping but replication of the previous results failed, and no significant associations were determined. One possible explanation for the dichotomy is that the previous study defined high myopia by refraction (SE) whereas Nakanishi *et al* (96) used axial length. However, a subset analysis conducted on binocular phakic cases by these latter authors using refraction < – 9.2.5 D in both eyes did not alter the results.

In a large cohort-case control study of Taiwanese subjects (471 cases with high myopia; 623 controls) performed by Liang *et al* (97), no association of the identified *COL1A1* SNPs with myopia was found either. Furthermore, analysis of subjects in 2 of 5 centers (family-based datasets from Duke and Cardiff) in which polymorphisms of the *COL1A1* and *COL2A1* (collagen type II) genes were examined demonstrated that while high-grade and any myopia groups had a significant association with *COL2A1*, no significant association with *COL1A1* was detected (98).

Again, we are presented with seemingly disparate results in which initial association with one gene (*COL1A1*) has not been replicated. It can be argued that in some instances this could be due to heterogeneity within populations, but in the case of the Japanese studies this is unlikely. Whether this is due to differing phenotypic definitions used in the studies remains to be seen.

5.8. *PAX6*

Another gene, *PAX6* that is composed of 14 exons, maps to 11p13, the MYP7 locus. It encodes for a protein that is a transcription factor with a paired domain, paired-type homeodomain, and a C-terminal transactivation region (99, 100). In humans, its mutations have been associated with a wide spectrum of disorders, including aniridia, keratitis, cataract, foveal hypoplasia, and morning glory disc anomaly. A study of Australian patients from 4 pedigrees known to have different mutations in the gene (100) agreed with a previous finding (99) that some coding mutations, especially the predicted premature truncated cases are associated with high myopia. A previous finding that truncating mutations are absent in the last half of exon 12 suggested that nonsense-mediated decay acts on mutant *PAX6* alleles (101), which would mean that *PAX6* haploinsufficiency is predicted in high myopia cases. Thus, it appears that *PAX6* haploinsufficiency induces extreme refractive error, perhaps due to form deprivation, elevated intraocular pressure, or corneal remodeling because of aberrant limbal stem cells. In a study of 164 Han Chinese families with 170 highly myopic offspring located in the Beijing area, 4 SNPs in the *PAX6* locus were selected to study the association with high myopia, and 2 of these single markers were found to be significant (102). It is noteworthy that haplotypes carrying the T allele of SNP6 (rs3026393) always demonstrated significantly increased transmission under additive or dominant models whereas haplotypes carrying the G allele showed reduced transmission under the recessive model. Combined with family-based association test data, this indicates that increased transmission of the T allele to highly myopic siblings consistently occurs. Because changes in *PAX6* itself or expression dosage and splicing variations usually have calamitous consequences, in nonsyndromic high myopia, involvement of *PAX6* is likely to be restricted to changes in the regulatory region that have a relatively small effect. This gene complex may also turn out to be one of the first that is exclusively associated with the development of high myopia at a relatively early age as studies that have investigated lower grades of myopia have found no associations with it (103, 104).

5.9. Other gene candidates

Matrix metalloproteinases (MMPs), which are undoubtedly involved in the remodeling of the scleral extracellular matrix have been suggested as candidates that might be involved in myopia. Hall *et al* studied 366 elderly subjects in the United Kingdom and investigated the association between their refractive error and 3 SNPs in *MMP-1*, *MMP-3*, and *MMP-9* (105). After developing a logistic regression analysis to predict the risk of myopia, several statistically significant results were obtained with *MMP-3* and *MMP-9*, but the most fascinating findings occurred when the subjects were grouped according to presence of significantly associated alleles so that each group had a progressively higher dosage of the involved SNPs. Whereas a "zero" dosage resulted in a prevalence of 7% in regard to myopia and 4 affected alleles resulted in a prevalence of 15%, 5 and 6 dosages doubled and tripled the prevalence of myopia (28% and 50% respectively). While we should remain cautious about the significance of this data, one possible interpretation is that in order to exert an effect in relation to mild or moderate myopia, there appears to be a threshold number of interacting genes required. However, beyond this threshold, the effects as more gene changes are added are almost multiplicative.

Another MYP locus has been mapped to chromosome 3q26, and in a follow-up study Andrew *et al* confirmed the linkage but also determined from a more refined map that there were 3 loci associated with refractive error: *MFN1*, *SOX2OT*, and *PSARL*, with genome-wide significance for the first 2 gene complexes (106). Mitofusin-1, the expressed protein from *MFN1* is a mitochondrial outer membrane protein that is found widely in human tissues, and appears to be involved with *OPA1* in a regulatory sense with mutations of the latter gene associated with autosomal dominant optic atrophy. *SOX2* is a small fundamental homeobox gene that interacts with *PAX6* in lens development and lies within the intron of the much larger non-coding RNA gene *SOX2OT*. Although its exact function is not known—*SOX2OT* expresses several proteins—it is believed to regulate *SOX2* in a complex fashion. Perhaps what is surprising is the implication that myopia is linked to the expression of 2 mitochondrial genes, *MFN1* and *PSARL*, suggesting that mitochondrial molecular pathways may play a role in the development of myopia, a most unexpected result.

Very recently, Nishizaki *et al* also published data about a novel high myopia locus in which 1 SNP appears to be involved with the *UMODL1* gene, which spans approximately 80 kb and consists of 23 exons and encodes 2 major transcripts generated by alternative splicing (63). UMODL1 proteins are thought to be secreted and associated with ECM proteins involved with cell-to-cell and cell-to-extracellular matrix adhesion, and in cell migration. Thus, while these results might have been expected, they do demonstrate that many ancillary factors are also likely to play a role in the development of high myopia.

The use of microarray technology in the search for candidate genes is also becoming more common and has been used to study the changes in retinal mRNA expression in mice as a result of visual deprivation. Although differences in retinal images were restricted to special features, egr-1 (early growth response protein) expression was reduced regardless of the duration of time period, cFos (a member of the activator protein transcription factor family) mRNA levels changed in synchrony with degradation of the spatial frequency spectrum, and akt2 (a protein kinase), was unregulated after 30 minutes of deprivation (107). Such analyses can thus point the way to other candidate genes that should be examined more closely. In this instance, evaluation of *EGR1* as a candidate gene for high myopia looks unlikely as a study of 96 unrelated Chinese subjects with high myopia found no pathological mutations within the gene (108).

6. SUMMARY AND PERSPECTIVE

Many master developmental genes, as well as specific genes are likely involved in the development or progression of myopia. Although linkage analysis has suggested a number of specific chromosomal regions from which likely candidates can be proposed, it is also clear that such analysis may only address a small proportion of the myopia errors in various populations. While deleterious mutations appear to cause severe ocular disease, it is also likely that SNPs in promoter or other regulatory regions may contribute to the normal variation in refractive error observed in more general populations (109). Moreover, we are beginning to see examples of additive effects of SNPs in gene families that might be classified as nearly neutral mutations that affect the development of low or moderate myopia, whereas specific alleles that are autosomal dominant, highly penetrant, and associated with gene regulation may be more involved with the development of non-syndromal high myopia. The elegant work being performed on Xq28 suggests that changes in CNVs and other mechanisms may be at work in these kinds of situations.

The sometimes frustrating nature of both linkage analysis and the candidate gene approach is evident in the contradictory reports published for specific loci and genes. Coming to a consensus in such situations is difficult. First identifications are often greeted with enthusiasm and then disappointment as follow-on studies fail to replicate results. In this regard, it is incumbent upon study groups to minimize common errors in designs to avoid the possibility of false positive results, as well as recognizing that the genes responsible for development of myopia are heterogeneous and so might be sparsely located in some ethnic and racial groups. Finally, it may be more profitable to develop more specific phenotype definitions of myopia to narrow down the search for potential genes. Our search should also be tempered by the fact that less obvious gene candidates, such as those associated with mitochondrial functions, might be just as important as the obvious genes identified from biochemical studies, particularly when gene interaction is involved.

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Abbreviations: CNV: copy number variant; CSNB: congenital stationary night blindness; ECM: extracellular matrix; GEM: Genes in Myopia; HLOD: heterogenetic logarithm of odds; HPF: hepatocyte growth factor; LOD: logarithm of odds; MMP: matrix metalloproteinase; NPL: non-parametric linkage; OR: odds ratio; PRELP: proline arginine-rich end leucine-rich repeat protein; SE: spherical error; SLRP: small leucine-rich repeat proteoglycans; SNP: single nucleotide polymorphism; TGIF: transforming growth beta-induced factor; UK: United Kingdom; USA: United States of America

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