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病理性近視之基因體篩選(1/3)

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Introduction

High Prevalence Rate of Myopia in Taiwan

Myopia is common in the Taiwan, and its cost to society is high. A nationwide survey which is performed in 2000 to determine the prevalence and severity of myopia among schoolchildren in Taiwan and to compare these findings with the results of the last survey performed in 1995 showed that the myopia rate increased from 20% at 7 years, to 61% at 12 years, and 81% at 15 years. A myopic rate of 84% is found for schoolchildren aged 16 years through 18 years. The mean refractive index reached myopic status at the age of 8, and increased to -4.12 D in girls and -3.15 D in boys at the age of 18 years. The prevalence of high myopia (> -6.0 D) at the age of 18 years is 24% in girls and 18% in boys. The increase in axial length corresponded with the progression of myopia. The prevalence and severity of myopia in schoolchildren in Taiwan in 2000 increased compared to 1995, with the most severe increases occurring in younger age groups.(1)

Pathologic Myopia

A variety of mechanisms of simple myopia have been postulated before, but most investigators are now convinced that myopia is multifactorial in nature, and that excessive near-work or prolonged reading is the main cause of myopia. It is believed that excessive or sustained accommodation produces tension in the ciliary muscle and with it, a tension in the extraocular muscles of convergence(2;3). But in so-called pathologic myopia, the situation is totally different. Pathologic myopia is caused by excessive axial elongation that primarily involves the ora-equatorial area and the posterior pole. Peripheral fundus changes and posterior staphyloma formation are ophthalmoscopic evidences of this process. Heredity is the basic determinant of ocular refraction, but numerous agents produce both temporary and permanent myopias.(4)

Severe myopia of -6.00 diopters (D), described as "pathologic" myopia, is associated with glaucoma, macular degeneration, cataracts, and retinal detachment, and it contributes significantly to loss of vision in adults.(5;6) In the study from western country, the frequency of myopia at or above this level in myopic populations has been reported to range from 27% to 33.2%, which corresponds to rates of 1.7% to 2.1% for the general population.(4) Pathologic myopia occurs primarily because of increased axial length of the eye rather than corneal or lenticular conical changes. Population and family studies in

Chinese have provided evidence for a genetic component to pathologic myopia.(7) The incidence of myopia among school children in Taiwan approaches 70%, and the prevalence of high myopia (> -6.0 D) at the age of 18 years is 24% in girls and 18% in boys, which suggest that this population is genetically susceptible to myopia.(1;8;9) Children of myopic parents are more likely to have myopia than are children of nonmyopic parents.(10;11) The ocular components (axial length, anterior chamber depth, and corneal curvature) and refractive errors of MZ twins are more closely aligned than are those of DZ twins.(12-14)

Chromosomal Study of Pathologic Myopia –Chromosome 18p

Autosomal recessive and autosomal dominant modes of inheritance of pathologic myopia have been suggested. Recessive inheritance has been the recent paradigm and may represent the most common mode of inheritance.(15;16) Analysis of selected pedigrees, however, suggests that pathologic myopia may also be inherited as a dominant gene with variable penetrance. In Naiglin's study, they showed that the autosomal dominant transmission mode showed a much greater likelihood than the autosomal recessive mode, which therefore is rejected. No evidence of linkage is found with any of the studied markers. In addition, the absence of linkage with chromosome 18p11.31 markers, a locus linked to familial high myopia in 6 North American families and 1 family of Chinese descent, demonstrated the genetic heterogeneity of the disease.(17) DelBono et al. recently described 52 two- and three-generation families with two or more individuals affected by juvenile-onset myopia (-0.75 D by age 15 years) and are autosomal dominant.(11) Young et al. conducted a genomewide screen to map the gene(s) associated with high, early-onset, autosomal dominant pathologic myopia. Eight families that each included two or more individuals with >=-6.00 diopters (D) myopia, in two or more successive generations, are identified. After a genomewide search, evidence of significant linkage is found on chromosome 18p. The maximum LOD score is 9.59, with marker D18S481, at a recombination fraction of .0010. Haplotype analysis further refined this myopia locus to a 7.6-cM interval between markers D18S59 and D18S1138 on 18p11.31.(18) They also showed that pathologic myopia is likely correlated with autosomal dominant form.

Furthermore, transmission disequilibrium tests (TDT) with both the Statistical Analysis for Genetic Epidemiology (SAGE) 3.1 TDTEX and GENEHUNTER 2 (GH2) programs were performed using chromosome 18p

marker alleles for this interval. The markers in marker order in this region were D18S1146 (p = 0.227), D18S481 (p = 0.001), D18S63 (p = 0.062), D18S1138 (p = 0.0004), D18S52 (p = 1.79 x 10(-6)), and D18S62 (p = 0.141). GH2 TDT analysis revealed the p values for the best allele for the markers: D18S1146 (p = 0.083), D18S481 (p = 0.108), D18S63 (p = 0.034), D18S1138 (p = 0.011), D18S52 (p = 0.007), and D18S62 (p = 0.479). These data suggest that the gene for 18p11.31-linked high myopia is most proximal to marker D18S52, with a likely interval of 0.8 cM between markers D18S63 and D18S52. Due to the contraction of the interval size by TDT, these results provide a basis for further focused positional cloning and candidate gene analysis at the MYP2 locus.(19)

A search for genes and/or expressed sequence tags physically mapped between markers D18S63 and D18S59 revealed 49 unidentified transcripts, 2 mRNAs for an open reading frame (KIAA0249 and KIAA0211), and 13 mRNAs and 25 sequences for regulatory or structural genes (National Center for Biotechnology Information database). Among these are adenylate cyclaseactivating polypeptide, thymidylate synthase, protein tyrosine phosphatase receptor, the subunit of guamine nucleotide-binding protein, protein tyrosine phosphatase, Niemann-Pick C disease protein, and the subunit of laminin (LAMA). Of these, LAMA is a biologically relevant candidate gene for this newly identified myopia locus, since it is a component of a structural glycoprotein found in the ocular scleral wall.

Laminin is present in the eye as a constituent of the elastic system in the trabecular meshwork(20) and zonular (oxytalan) fibers of the lens.(20) It has also been identified in the astrocytic and vascular endothelial-cell basement membranes of the laminar-beam margins of the rodent lamina cribosa.(21) More recently, Marshall has localized laminin to the oxytalan and elaunin microfibrils of human sclera by immunoelectron microscopy.(22) These microfibrils comprise two of the three components of the elastic-fiber system that make elastic tissue more stretchable than collagen, a fibrous protein that provides tensile strength.(23;24) Marshall suggests that laminin may bind these microfibrils to collagen fibrils, since laminin has been shown to have binding sites for several extracellular matrix components, including collagen.(22) These properties of laminin and its localization in the sclera make LAMA an attractive candidate gene.

Chromosomal Study of Pathologic Myopia –Chromosome 12q

A second locus for familial high myopia mapping to chromosome 12q was

also found in another study.(25) In this study, markers flanking or intragenic to the genes for the 18p locus, Stickler syndromes type I and II (12q13.1-q13.3 and 6p21.3), Marfan syndrome (15q21.1), and juvenile glaucoma (chromosome 1q21-q31) showed no linkage to the myopia in this family. The maximum LOD score with two-point linkage analysis in this pedigree was 3.85 at a recombination fraction of .0010, for markers D12S1706 and D12S327. Recombination events identified markers D12S1684 and D12S1605 as flanking markers that define a 30.1-cM interval on chromosome 12q21-23, for this second myopia gene. A search for genes and/or expressed sequence tags physically mapped between the two markers D12S1684 and D12S317 reveals 119 unidentified transcripts, 12 mRNAs, and 30 sequences for regulatory or structural genes (National Center for Biotechnology Information database). Selected genes among these are those for PAH, lumican, decorin, and dermatan sulfate proteoglycan (DSPG3). Although an intragenic polymorphic marker for PAH shows significant linkage to the myopia in this pedigree, it is unclear how mutations in PAH may be associated with high myopia. Of 328 different mutations by state that have been collected by the PAH Mutation Analysis Consortium Database, the majority are rare mutations causing hyperphenlyalaninemia, and the remainder are polymorphic variants without apparent effect on phenotype (Nowacki et al. 1998).

Of even greater interest are candidate genes relevant to ocular structures that map to this region. Decorin and lumican are members of the small interstitial proteoglycan family of proteins that are expressed in the extracellular matrix of various tissues. Both interact with collagen and limit the growth of fibril diameter.(26-28) Decorin and lumican are present in corneal stroma and in the interstitial matrices of the heart, aorta, skeletal muscle, skin, and intervertebral disks.(29) DSPG3, another small interstitial proteoglycan, is expressed in cartilage, as well as in ligament and placental tissues.(30) Whereas the presence of lumican or DSPG3 has not been demonstrated in sclera, other members of the proteoglycan family, such as decorin, biglycan, and aggrecan, have been found to be present in this tissue.(31) Fibrillogenesis of the sclera may be affected by mutations in these candidate proteins, as has been demonstrated in other connective-tissue disorders that manifest with myopia, such as Sticker syndrome and Marfan syndrome.

Significant changes in proteoglycan synthesis have been shown to correlate with changes in the rate of axial elongation during post natal ocular growth and during the development of myopia in a variety of animal models suggesting that proteoglycans play a critical role in determining the

biomechanical properties of the sclera.(32-48) Moreover, two previous genetic loci associated with familial high myopia (MYP1 and MYP3) have been mapped to Xq28 and 12q21-23, respectively 14,15, which include or are near the loci for biglycan (Xq27ter) 16 decorin (12q21-q22) 17 and lumican (12q21.3- q22) 18,19 genes, suggesting that mutations in these extracellular matrix components may be involved in some forms of human myopia.

The defects observed in sclera collagen fibril diameter and organization in lumican deficient mice will be expected to result in significant changes in the biomechanical properties of the sclera and lead to severe defects in ocular shape and size.(49;50) Volumetric estimations of eye size in wild type and lumican deficient mice suggest that eyes are larger in lumican deficient mice. Interestingly, 12q21-23 (Myp 3) that includes several SLRP genes including DSPG-3, decorin and lumican has been identified with autosomal dominant high myopia.(25) Although heteroduplex and sequence analysis excluded lumican as the causative gene involved in the family with 12q21-23 linked high myopia, a mutation in one or both alleles of the lumican gene will lead to significant defects in scleral extracellular matrix which, in turn, could result in alterations in ocular shape and size and severely affect vision.

Chromosomal Study of Pathologic Myopia –Chromosome 11

In contrast, nanophthalmos is an uncommon developmental ocular disorder characterized by a small eye, as indicated by short axial length, high hyperopia (severe farsightedness), high lens/eye volume ratio, and a high incidence of angle-closure glaucoma. Clinical and genetic evaluations of members of a large family in which nanophthalmos is transmitted in an autosomal dominant manner. Ocular examinations of 22 affected family members revealed high hyperopia (range +7.25 to +13.00 diopters; mean +9.88 diopters) and short axial length (range 17.5519.28 mm; mean 18.13 mm). Twelve affected family members had angle-closure glaucoma or occludable anterior-chamber angles. Linkage analysis of a genome scan demonstrated highly significant evidence that nanophthalmos in this family is the result of a defect in a previously unidentified locus (NNO1) on chromosome 11. The gene is localized to a 14.7-cM interval between D11S905 and D11S987, with a maximum LOD score of 5.92 at a recombination fraction of .00 for marker D11S903 and a multipoint maximum LOD score of 6.31 for marker D11S1313. Therefore, NNO1 is associated with nanophthalmos or with an angle-closure glaucoma phenotype.(51) These phenotypes are the reverse of the phenotype of pathologic myopia. It also can provide a clue to study the

genomic study of pathologic myopia in the opposite direction.

Nanophthalmos is thought to result from the arrested development of the eye during the embryonic stage (Duke-Elder 1964), but the specific underlying genetic and biochemical causes are unknown. Studies of animal models and hereditary human ocular disorders indicate that multiple transcriptional activators play important roles in eye development in animal model systems, but the factors that determine globe size in humans are not well understood.(52;53) Thickened sclera is a common manifestation of nanophthalmos, and some studies have reported unusual collagen bundles and aberrant glycosaminoglycan metabolism in some, but not all, cases.(54-56) However, the relationship of these aberrant structural and metabolic properties to the causes of human nanophthalmos remains poorly defined.

One of the genes located on 11 is the PAX6 gene (MIM 106210), a homeodomain gene that produces a protein known to play a critical role in ocular development in mice and humans.(57;58) The mouse Pax6 gene has been implicated in the phenotype "small eye" in mice, but reduced globe size is not a feature of aniridia (MIM 106210; Mouse Genome Informatics) or of the several other ocular phenotypes reported for PAX6 defects in humans.(52;53;59-62) In the Unigene Human Sequence Collection database (National Center for Biotechnology Information), the PAX6 UniGene cluster, Hs.89506, is located in the 3842-cM region of chromosome 11 between markers D11S1324 and D11S914. The proximal marker for this bin, D11S914, is 12 cM telomeric to D11S905, the distal flanking marker for the NNO1 interval.(63) Glaser and colleagues used somatic-cell hybrids containing deletions in human chromosome 11 to place PAX6 distal to D11S907,(59) which is 8.1 cM distal to D11S905.(63) Thus, a defect in PAX6 appears to be ruled out as the cause of nanophthalmos, on the basis of the placement of PAX6 outside of the NNO1 genetic inclusion interval. Therefore, it is worthy continuing evaluation of additional genes in the region as potential NNO1 candidates, including more precise localization of genes for which previous placements are approximate.

The Importance of Localization of Pathologic Myopia Gene in Taiwan

As aforementioned, Chinese have provided evidence for a genetic component to pathologic myopia.(7) The incidence of myopia among school children in Taiwan approaches 70%, and the prevalence of high myopia (> -6.0 D) at the age of 18 years is 24% in girls and 18% in boys, which suggest that this population is genetically susceptible to myopia.(1;8;9) **The prevalence of**

high myopia in Chinese is different from the situation in Caucasian. But in previous Study, Children of myopic parents are more likely to have myopia than are children of nonmyopic parents.(10;11) The ocular components (axial length, anterior chamber depth, and corneal curvature) and refractive errors of MZ twins are more closely aligned than are those of DZ twins.(12-14) These studies provides a strong clue for us to study the possible candidate gene for pathologic myopia, which itself can be used to trace the gene for myopia. Pathologic myopia does not relate to other ocular and systemic disease,(64) it can be a good candidate to approach the myopia gene. Because it maybe have some important relationships between the genes (lumican、decorin、laminin、DSPG3、PAH and Pax6) to the pathological changes of myopia. So, we compare the allele frequency of sequence alterations between patients and control subject at the beginning. Then, we will further sequence the cDNA for lumican, decorin, laminin, pax6, PAH and DSPG3 to find out the possible mutation in the family with well-defined pedigree.(30;65;66)

Subjects and Methods

Subjects

This project will comprise 300 sibship pedigrees ascertained, during 2002-2005, through Department of Ophthalmology, National Taiwan University Hospital, according to a unified ascertainment protocol. All ascertained families include, at minimum, a sib pair with myopia, together with parents and additional siblings when available. Validation of the diagnosis of myopia in the index sib pair is based on either retinoscopy, and/or autorefractor. Age at diagnosis (AAD), of both members of the index sib pair, is initially restricted to the age of 6 years.

These families consented to participate in the study. Criteria for selection included a history of onset of myopia at age <12 years in all affected subjects (parents and offspring), myopia of -6.00 D, and two or more generations affected. The diagnosis of myopia is determined by the refractive error. Anisometropic individuals, with a refractive error of <-6.00 D for one eye and >-6.00 D for the other eye, with at least a 2-D difference between the two eyes, are considered unaffected. Individuals are excluded if there is known ocular disease or insult that could predispose to myopia, such as retinopathy of prematurity or early-age media opacification, or if they had a known genetic disease associated with myopia, such as Stickler or Marfan syndrome.

Affection status is difficult to determine for a common complex quantitative trait such as myopia. Despite the fact that refraction depends on corneal curvature, lens power, and axial length (measurements difficult to obtain in most of our participants), historically in the literature pathologic myopia has been defined as a refractive error of -6.00 D. The degree and progression of myopia has environmental influences as well. An affection status of -6.00 D degrees of myopia is chosen to select for the more severe phenotypic form of this disorder, under the assumption that this will disproportionately represent a genetic etiology. This is further supported by the low frequency of pathologic myopia represented in the general population.(64)

Anisometropic individuals are classified as "unaffected" to insure that the phenotype/genotype is as specific as possible. Anisometropia may be secondary to a nongenetic event, such as inadvertent occlusion of one eye or unilateral ptosis (67) or retinal hemorrhage during early childhood (68). There may not be residual structural changes observable during a routine screening of an adult.

An ophthalmology examination is performed by one of the authors (Y.F.Shih) at the time of sample collection, for most participants, and for at

least one member of each family. For family members who are not locally available, examination records are obtained from the individual's ophthalmologist. The ophthalmologic evaluation included retinoscopy, a slit-lamp evaluation of the anterior segment, measurement of intraocular pressure, and a fundus examination, with special notation on the health and degree of cupping of the optic nerve head. Cycloplegic retinoscopy is performed in children age 10 years. In most instances, at least one affected adult from each family also underwent axial-length measurements of their eyes and keratometry measurements of their corneas. Most participants declined to have these measurements taken. Venous blood is collected after informed consent is obtained. This study is approved by National Taiwan University Hospital

Mutation Aanalysis

Genomic DNA was extracted from 3 mL of whole blood with a kit (PureGene, Gentra systems, Minneapolis, MN). Sequence alterations were detected by PCR, followed by direct sequencing. Sequencing was performed with an automated DNA sequencener (ABIPRISM 3100 Genetic Analyzer).

Result

We compared the sequence alternation among four genes in chromosome 12q (decorin、lumican、DSPG3、PAH) which maybe have some important relationships with pathological changes of myopia.

For decorin, we screened four single nucleotide polymorphisms (rs1803344，rs3138268，rs2070985，rs2070984) and the results were:

(1) rs1803344(C→G)：the nucleotides in rs1803344 were “C” in all 71 blood samples.
(2) rs3138268(G→A)：the nucleotides in rs3138268 were “G” in all 71 blood samples.
(3) rs2070985(C→G)：the rs2070985 in 66 blood samples were “C”, in 1 blood sample was “G”, in 3 blood samples were “heterozygous” and 1 blood sample was unknown.
(4) rs2070984(C→T)：the nucleotides in rs2070984 were “C” in all 71 blood samples.

For lumican, we screened seven single nucleotide polymorphisms (rs1802763, rs1802743, rs2300588, rs3741835, rs3741834, rs3759223, rs3759222) and the results were:

(1) rs1802763(T→C)：the nucleotides in rs1802763 were “T” in all 71

blood samples.

(2) rs1802743(C→A)：the nucleotides in rs1802743 were “C” in all 71 blood samples.

(3) rs2300588(C→A)：the rs2300588 in 21 blood samples were “C”, in 49 blood sample were “A”, and in 47 blood samples were “heterozygous”.

(4) rs3741835(A→G)：the rs3741835 in 87 blood samples were “A”, in 9 blood samples were “G”, and in 19 blood samples were “heterozygous”.

(5) rs3741834(G→A)：the rs3741834 in 64 blood samples were “G”, in 15 blood samples were “A”, and in 36 blood samples were “unknown”.

(6) rs3759223(A→G)：the rs3759223 in 64 blood samples were “A”, in 46 blood sample were “G”, and in 7 blood samples were “heterozygous”.

(7) rs3759222(T→G)：the rs3759222 in 17 blood samples were “T”, in 55 blood samples were “G”, and in 45 blood samples were “heterozygous”.

For DSPG3, we screened two single nucleotide polymorphisms (rs1135866 and rs1920748) and the results were:

(1) rs1135866(A→G)：the nucleotides in rs1135866 were “A” in all 71 blood samples.

(2) rs1920748(C→T)：the rs19207483 in 2 blood samples were “T”, in 112 blood sample were “T”, and in 3 blood samples were “heterozygous”.

For PAH, we screen two single nucleotide polymorphisms (rs1042503 and rs3828474) and the results were:

(8) rs1042503(C→T)：the nucleotides in rs1042503 were “C” in all 117 blood samples.

(9) rs3828474(C→T)：the rs3828474 in 6 blood samples were “C”, in 70 blood samples were “T”, and in 41 blood samples were “heterozygous”.

Discussion

1. According the results of single nucleotide polymorphism we selected, sequence alternation really exists in blood samples of pathological myopia. However, the blood samples are not enough for us to calculate the odd ratio between the patient and control subjects. The collection of enough

number of blood samples is the most important goal of the project at the second year.

2. rs2070985 and rs3741834 are unknown in parts of blood samples. In these cases, we suspect that it might be due to the insertion or deletion mutations of chromosomal DNA for these two loci.
3. SNPs rs3828474 (from C to T) was found in our study, but it is not found in the database of N.C.B.I. (C→G). This is a very interesting finding in our study. We suspect the SNP may be special in Taiwanese people. In order to prove that, we need to collect more samples and compare all the information between different countries.

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