· Original article ·

Lack of association between the TIGR gene mutation and the high myopia in Chinese children

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Foundation item: Hunan Education Department Funded Project, China (No. 07C155)

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Received: 2010-12-13 Accepted: 2011-01-11

Abstract

• AIM: To screen TIGR/myocilin gene (*MYOC*) mutation in high myopic Chinese children with family history.

• METHODS: Gene sequencing was performed in exon 3 of the TIGR gene in high myopic Chinese Children. The coding sequence of TIGR exon 3 was screened by capillary electrophoresis sequencing. The sequence alterations were analyzed by bioinformatics.

• RESULTS: TIGR gene mutation was not found in high myopic patients and normal controls group.

• CONCLUSION: No identified gene mutation is found in TIGR gene in high myopic Chinese children.

• KEYWORDS: high myopia; open angle glaucoma; mutation; genes; gene sequence

DOI: 10.3969/j.issn.1672-5123.2011.02.04

Wang P, Ye ZC, Tao LJ, Gao XR, Xie LH, Yang HL, Wang XL. Lack of association between the TIGR gene mutation and the high myopia in Chinese children. *Guoji Yanke Zazhi (Int J Ophthalmol)* 2011;11(2):210-213

INTRODUCTION

High myopia, classified as pathological myopia or progressive myopia or degenerative myopia, is a world-wide prevalent and sight-threatening disease. An epidemiological investigation showed that the incidence of high myopia in general population in China was 6.98%. The recent study[1] in China mainland reported the prevalence of myopia in primary school was 13.7%, in junior high school was 42.9%, in high school was 69.7%.

High myopia has been identified to have close relationship with POAG[2,3], there is an increased frequency of open angle glaucoma in myopia as well as an increased prevalence of myopia in patients with glaucoma or ocular hypertension. In the recent 20 years, great achievements have been made in glaucoma genetic mechanism, especially in trabecular meshwork induced glucocorticoid response (TIGR) gene, which had been identified as a glaucoma-causative gene and candidate-gene of POAG. The subsequent studies, analyzing the prevalence of the mutation on GLC1-A, repeated the initial results and identified additional mutations[4], almost all located in exon 3 of the TIGR/*MYOC*. Most of these mutations were missense, located in the exon 3 that encodes the olfatomedin homology domain.

Based on the previous researches, we hypothesize that high myopia is also associated with the mutations of TIGR, TIGR/*MYOC* gene may play a role in myopia susceptibility. So in this study, we aimed to identify mutations in TIGR exon 3 in high myopic Chinese children.

MATERIALS AND METHODS

Patients Subjects were recruited from Hunan Children's Hospital Ophthalmology Department from 2006 to 2009. Informed consent was obtained from the parents to collect blood samples from their children for the genetic analysis. Each subject received a comprehensive eye examination, including visual acuity, refraction, slit lamp, and dilated fundus examination. Central corneal curvature was measured using autokeratometry (Canon RK-5 Auto Ref-keratometer, Canon, Inc., Tokyo, Japan). Axial length was measured using A-Scan ultrasound (BME-100 A System; Chinese Academy of Medical Sciences Bio-medical engineering Research institute, Beijing, China) (Table 1).

The subjects were enrolled according to the following criteria: (1) High myopia group (n = 100) with children who had been diagnosed with high myopia by cycloplegic refraction under retinoscope examination, and also at least one of the parents was with high myopia, the spherical equivalent refractive error in both eyes >-6.00D. (2) Control group (n = 80) with spherical equivalent refractive error in both eyes > -1.00D and < +1.00D. Subjective refraction details were obtained from the subjects' optometrists. All subjects were healthy with no clinical evidence of syndromic disease or other

Table 1 Summary of ocular data of myopic subjects (D = diopters)

Ocular parameter (unit)	Right eye	Left eye
Spherical power(D)	-8.57±2.10	-8.63±2.07
Equivalent spherical power(D)	-9.33±2.12	-9.38±2.09
Astigmatism(D)	-1.42±0.69	-1.51±0.73
Corneal cylindrical power(D)	-1.68±0.83	-1.73±0.99
Axial length(mm)	26.43±1.22	26.32±1.30

ocular abnormality. This study is approved by Human Subjects Ethics Subcommittee of Pharmacologic Research Center of Central Southern University.

Genotyping Venous blood samples were collected, and DNA was extracted from the leukocytes using a modified salt precipitation method. A fragment corresponding to exon 3 of the TIGR/*MYOC* gene was amplified by PCR and capillary electrophoresis sequencing.

Genomic DNA was extracted from collected blood samples. Buccal cells in 5mL sterile saline were centrifuged for 10 minutes at 4200 rpm. The supernatant was removed, and the pellet resuspended in 100μL 50mmol/L sodium hydroxide. The suspension was heated to 95°C for 10 minutes before neutralization with 15μL 1 mol/L Tris/HCl (pH 8.0). After mixing and centrifugation the supernatant was used neat or diluted 1:5 in PCR reactions.

The exon 3-5 polymerase chain reaction (PCR) mixture contained reactions (50μL) were performed 32 cycles containing gDNA 1μL, 10* PCR Buffer 5μL, dNTP (2.5mmol/L each) 1μL, F primer (10μmol/L) 0.5μL, R primer (10μmol/L) 0.5μL, rTaq (TaKaRa) 0.5μL, ddH20. DNA was stored at -40°C.

The investigated DNA sequences were amplified using the following primers:

The exon 3-5: forward 5-ggA TTA AgT ggT gCT TCg TT-3_ and reverse 5- TTC TTC TCC Agg ggg TTg TAg TC-3_.

Sequence primer:SF:5-AgT ATg ACC TCA TCA gCC AgT T-3; SR:5-CAg CCT TCA CTg TCT Cgg TA-3

The exon 3-3: forward 5-ACA ggC ACA ggT ATC AgC AAg-3_ and reverse 5- AgC AAA gAT TCC CAC AAA gTT C-3_.

Sequence primer:SF:5- ggg AgC Agg CTg AAg ggA g-3; SR: 5- gAA ATT gTC TAC gCC CTC Ag-3

Statistical Analysis Sequence analysis was done by the genotyper software. DNAssist Version 1.0 compared nucleotide sequences with the published DNA sequence of *MYOC* (GenBank NM_000261).

RESULTS

No alterations or mutations were identified in exon 3 of the TIGR/*MYOC* gene in the group of high myopic patients and control subjects.

DISCUSSION

In the early 20 century, Stilling and Elshing predicted that high myopia was actually a kind of glaucomatous disease, which progressed slowly and occult. Many clinical features in high myopia are similar to POAG, both hypersensitive responsed to corticosteroids with intraocular pressure elevation compared to emmetropic population. It is hypothesized that *MYOC* gene mutation or polymorphism may play a role in myopia susceptibility.

It was estimated that IOP elevated in more than 27.8% high myopia population. It was a major predisposes risk factor of high myopia and contributed to myopic aggravation. The clinical data and epidemic investigations indicated that incidence of high myopia in POAG population had reached to 1.20%[4], which was significantly higher than in general population[1].

Glaucoma occurs and develops occult, especially in early stage, glaucomatous diagnosis for the patients with high myopia is very difficult using routine examinations. Usually there is no significant IOP elevation in high myopia -POAG patients. The glaucomatous excavation of optic nerve head can not be easily distinguished due to the tilt of the optical nerve. Vision decreasing and visual field loss are often considered due to the progress of myopia, so many high myopia-POAG patients are usually misdiagnosed by ophthalmologists. In the recent ten years, large number of clinic data[5-8], especially the morphology of optic nerve head and the optic nerve fiber defects by OCT, contribute to the advanced diagnosis technique and skills.

It was discovered that both high myopia and POAG were hypersensitive response to corticosteroids, only 4%-5% in general population while 90% in POAG. Steven found that IOP increased in about 88% high myopia patients after corticosteroids administration, of which more than 31% IOP was up to 31mmHg. The mechanisms were presumed to relate with the gene-regulating steroid receptors.

In 1997 Nguyen and Polansky first identified TIGR protein / glycoprotein inducing pattern on human cultured trabecular meshwork cells after long-term exposure to corticosteroids. *MYOC* was the first disease-causing gene identified for POAG and almost 80 mutations have been reported[9-13]. Most mutations locate in the third exon which encodes a 250-amino acid domain with homology to olfactomedin. Mutations in *MYOC* are racial/ethnic specific and some of them have been found only in a specific region. So we selected the third exon as our testing sequence for the TIGR gene mutation.

So far, 11 *MYOC* mutations have been identified in Chinese patients or pedigrees and seven of them were Chinese specific[14-17]. Ge *et al*[16] screened a large Chinese POAG family which pedigree has four generations with 53 individuals, and 13 patients for TIGR gene mutation and found out that all subjects in this research had the same mutation is a "C-to-T" transition, changing at position 370 and predicting a Pro370Leu. Another investigation[17] was done recently from central China in a four-generation family affected with POAG, the results suggest that the *MYOC* c.1084G >- may contribute to a genetic predisposition to POAG.

These results were different from the three mutation sites described initially by other investigations in POAG patients from Western countries. All these finding identified that the TIGR gene mutation and polymorphism are discrepant from populations of different ethnic origins

Possible high myopia gene loci have been mapped up on high myopia now[18-25]. Several highly penetrant genetic loci for non-syndromic myopia have been mapped. However, none of the causative mutations has yet been found. Candidate gene association studies have led to the identification of several high myopia susceptibility genes (Table 2) including TIGR/*MYOC* gene. But the knowledge on TIGR gene of high myopia still remains limited.

Table 2 High myopia susceptibility genes

Gene	Locus	Reference
Myocilin (*MYOC*)	1q23	[18]
Hepatocyte growth factor (*HGF*)	7q21	[19]
Paired box gene 6 (*PAX6*)	11p13	[20]
Collagen, Type II alpha 1 (*COL2A1*)	12q13	[21]
Lumican (*LUM*)	12q21	[22]
Collagen, Type I alpha 1 (*COL1A1*)	17q21	[23]
Transforming growth induced factor (*TGIF*)	18p11	[24]
Transforming growth factor beta 1 (*TGFβ1*)	19q13	[25]

The latest researches on TIGR/*MYOC* gene and High myopia results some conflicted conclusions[16,17]. Three previous studies[26,27,28] have tested for an association between high myopia and polymorphisms in *MYOC* in Chinese ethnicity. In two of the studies, a significant association was found while in the third, there was no association. The report[28] from Hong Kong showed that TIGR/*MYOC* proximal promoter GT-repeat polymorphism is not associated with myopia. The recent study [28] aimed to clarify the relationship between the *MYOC* microsatellites and high myopia using a large number of Chinese families living in Hong Kong. The relationship was further delineated by investigating additional tag single nucleotide polymorphisms (SNPs) spreading across the *MYOC* gene. A family-based association study approach was used to avoid false positive results due to population stratification. In this study, two microsatellites and two SNPs were found by FBAT analysis to show linkage and association with high myopia in the Hong Kong Chinese population. A stepwise CLR analysis of case-pseudocontrol dataset indicated that the two SNPs could each account for the association results of the two microsatellites and that these two SNPs seemed to exhibit separate main effects on high myopia. The results of Zayats[29] suggested that TIGR/*MYOC* polymorphisms have a very low, or possibly negligible, influence on high myopia susceptibility in subjects of European ethnicity.

The ethnic difference of the respective study populations is an appealing explanation for these discrepant findings. Different populations may exhibit differences in allele frequencies and linkage disequilibrium patterns at specific loci Thus, the role of *MYOC* in high myopia in Chinese subjects may be dissimilar to that in Caucasians.

Although no TIGR mutation in high myopic children was found in our study, it predicted that the incident of TIGR mutation is very low in these subjects, which indicated that there will be another mechanism contribute to the ocular pressure elevation in high myopic patients. It is recommended that larger studies and multicenter clinical trials for further researches in the future. It is also noteworthy that some factors that stimulate myocilin expression in TMC have also been implicated in the regulation of postnatal eye growth and myopia through bFGF, TGF β, and oxidative mitochondrial pathways[30].

Our results suggest that *MYOC* mutation possibly has negligible influence on high myopia susceptibility in subjects of south central Chinese ethnicity. Our understanding of the contributions made by TIGR gene to the risk of high myopia is incomplete. It is possible that the combined effects of gene mutation or polymorphism may differ in different racial groups, accounting for some of the observed differences in myopic susceptibility. Also the large group of family historic high myopia should be included in our test in the future, and the final conclusion should be done with large number cases testing and multiple center studies.

Acknowledgements: We are grateful to the members in Pediatric Research Laboratory who participated in this study.

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高度近视儿童中未检测到 TIGR 基因突变

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基金项目:中国湖南省教育厅资助项目(No. 07C155)

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摘要

目的:在具有家族史的高度近视儿童中进行 TIGR 基因突变筛查。

方法:对高度近视儿童中 TIGR 基因第三外显子进行毛细血管电泳测序检测,并对结果进行序列分析。

结果:在近视组和对照组中均未发现 TIGR 基因突变。

结论:未能发现 TIGR 基因突变与高度近视相关证据。

关键词:高度近视;开角型青光眼;突变;基因;基因测序