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# Evaluation of *EGR1* as a candidate gene for high myopia

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**Purpose:** *EGR1* (OMIM 128990) is an early growth response gene that has been shown to be related to myopia by upregulation and down-regulation of axial eye growth in experimentally induced chicken myopia and knockout mice. The purpose of this study was to test whether variations in the human *EGR1* gene are related to high myopia.
**Methods:** Genomic DNA was prepared from leukocytes of peripheral blood. Cycle sequencing was used to detect sequence variations in *EGR1*.
**Results:** No pathological mutations were detected upon sequencing of the coding regions and the adjacent intronic regions of *EGR1* in 96 unrelated Chinese subjects with high myopia. Only one silent variation, c.1026G>A (p.V342V), was detected in one patients with high myopia, which would not affect the encoded protein. For all 96 subjects, only one allele was detected in each of the three known single nucleotide polymorphisms (SNPs) in *EGR1*.
**Conclusions:** We found no evidence that *EGR1* is responsible for high myopia in these patients.

Myopia is a leading visual problem that affects an average of one-third of the world's population with the highest prevalence among East Asians [1-4]. The cost involved in correcting myopia is about one-fourth of the entire expenditure in ophthalmology and optometry [2,5]. Its extreme form, high myopia, is the fourth most common cause of irreversible blindness [6,7]. Evidence has demonstrated that genetic factors play an important role in the development of high myopia [1,8-11]. Molecular genetic investigations of high myopia have become a hot topic in recent years. However, the genes responsible for most high myopia are, as yet, unknown.

Expression of *ZENK* (the chicken and mouse ortholog of mammalian *EGR1*, OMIM 128990) has been shown to be involved in ocular growth and refraction. Experiments in animal models have shown that upregulation of *ZENK* in retinal glucagon amacrine cells is assumed to create a STOP signal to inhibit axial eye growth whereas down-regulation of *ZENK* is associated with axial eye growth [12-16]. Therefore, loss/reduction-of-function mutation of *ZENK* might theoretically be associated with myopia. Indeed, *EGR1* knockout mice had longer eyes and a relative myopic shift in refraction [17]. Therefore, it would be interesting to know if there are mutations in *EGR1* of human individuals with myopia especially high myopia where excessive axial elongation of the eye is a common prominent feature.

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Here, we analyzed *EGR1* in 96 Chinese patients with high myopia. No mutation was identified in *EGR1*. The results indicate that *EGR1* is unlikely to be responsible for high myopia in these patients.

## METHODS

*Subjects:* The procedure for collecting subjects and obtaining informed consent was the same as previously described [18]. This study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Ophthalmological examinations were performed by ophthalmologists (Q.Z. and X.G.). A total of 96 Chinese subjects with high myopia were recruited who met the following criteria, 1) bilateral refraction of –6.00 D or lower (spherical equivalent) and 2) did not have any other known ocular or systemic disease. The refractive error was measured with cycloplegic autorefraction after mydriasis (Mydrin®-P, a compound tropicamide; Santen Pharmaceutical Co. Ltd., Osaka, Japan) for all eyes. Genomic DNA was prepared from venous blood.

*Mutation detection:* Five pairs of primers (Table 1) were used to amplify the two coding exons and the adjacent intronic sequence of the *EGR1* gene (NCBI human genome build 36.3, NC_000005.8 for genomic DNA, NM_001964.2 for mRNA, and NP_001955.1 for protein). DNA sequences of the amplicons were identified with ABI BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA) on an ABI 3100 Genetic Analyzer (Applied Biosystems). Sequencing results from patients as well as from *EGR1* consensus sequences from the NCBI Human Genome Database (NC_000005.8) were imported into the SeqManII program of the Lasergene package (DNAStar Inc., Madison,

Table 1. Primers used for polymerase chain reaction amplification and sequencing of *EGR1*.

| Exon | Primer sequence (5′-3′) | Size of PCR product (bp) | Annealing temp (°C) | Note |
|---|---|---|---|---|
| 1 | F-GTCGCCGCCTGCACGCTTCT<br>R-AAACCCGGCTCTCATTCTAA | 550 | 64 | GC Buffer |
| 2a | F-CCTCGGGAGTCAATGGTAG<br>R-GTGGGTGCCGCTGAGTAAATG | 430 | 63 | GC Buffer |
| 2b | F-ACCGGCCTCCTCGTCCTCA<br>R-ACTCCACTGGGCAAGCGTAAG | 530 | 63 | |
| 2c | F-CTTCGCTAACCCCTCTGTCTA<br>R-GGTGGCCGGGGATGGATAAG | 539 | 63 | |
| 2d | F-TGAACGCAAGAGGCATACCAA<br>R-GGCCATCTCCTCCTCCTGTCC | 481 | 64 | GC Buffer |

GC buffer was provided by Takara Biotechnology (Dalian) Co. Ltd (Liaoning, China) and is designed for amplification of templates having complex secondary structure or high GC content. In the "Primer sequence" column, "F" indicates the forward sequence and "R" indicates the reverse sequence.

WI) and then aligned to identify variations. Each variation was confirmed by bidirectional sequencing. Mutation description followed the recommendation of the Human Genomic Variation Society (HGVS) [19].

## RESULTS

*Clinical data:* A total of 96 unrelated subjects (54 males and 42 females; mean age 17.07±17.01 years) participated in this study. Refraction measures were −12.11±-4.55 D for the right eye and −12.31±-4.81 D for the left eye.

*Mutation analysis:* Upon complete sequencing analysis of the coding regions and the adjacent intronic regions, no mutation was identified in the 192 chromosomes of *EGR1* in the 96 subjects with high myopia. One silent variation, c.1026G>A (p.V342V), was detected in one patients with high myopia. This variation would not affect the encoded amino acid.

*Single nucleotide polymorphism analysis:* There are three single nucleotide polymorphisms (SNPs) in *EGR1* including rs13181973, rs11953917 (also named rs60458721), and rs1042088. For the 96 subjects with high myopia in this study, only one allele was detected at each of the three SNPs, the C allele for rs13181973, the G allele for rs11953917, and the C allele for rs1042088. This is compatible with the HapMap information for East Asians.

## DISCUSSION

*EGR1* is an early growth response gene that encodes a transcription factor, Egr-1 protein, or ZENK. *ZENK* expression can be upregulated or down-regulated by experimental interference that affects axial eye growth toward hyperopia or myopia in chicken and monkey [12-16]. A knockout of both *EGR1* alleles in C57/BL6 mice leads to longer eyes and a relative myopic shift [17]. However, in human beings, sequence analysis of *EGR1* did not detect any mutation in the 96 patients with high myopia. The results suggest that *EGR1* is unlikely to be the responsible gene for high myopia in these patients.

Apart from *EGR1*, myopia-like changes has been observed in lumican-fibromodulin double null mice [20]. Similarly, no mutation in the *lumican* and *fibromodulin* genes has been identified in families with high myopia [21], although a report claimed an association of myopia with SNPs of *lumican* [22]. The situation in myopia knockout models is different from other disease models such as Leber congenital amaurosis and congenital stationary night blindness where information obtained from animal models is compatible to that from human diseases. In addition, human myopia may be influenced by visual behavior and the visual environment, which may differ greatly between animals and human beings. This may raise the question to what extent does an animal myopia model represent human myopia.

Identification of genes responsible for non-syndromic high myopia is very important but will be very difficult, although several loci for high myopia have been mapped [6, 23-32]. Variations in several genes were reported to associate with non-syndromic high myopia [22,33-37]. However, none of these reports have been confirmed by replication study [21,38-42], which is definitely the first priority in association studies [43-47]. Two genes, TGFB-induced factor homeobox 1 (*TGIF)* and *lumican*, have been excluded as candidate genes for high myopia, but they have still been treated as potential candidate genes in several subsequent studies. False-positive results in association studies have been frequently mistreated as useful clues for subsequent studies in recent years, without any replication study. Many researchers may have not realized that most positive associations published (as high as 95%) are false positives [43,45-49]. The most striking problem is the criteria ($p<5x10^{-2}$ or $1x10^{-2}$) that are inappropriately used to claim an association. The false positive is still rather high even using a more stringent criteria of about $10^{-5}$ suggested by experts in the field [43,44,49-51]. Genetic association study for complex traits is completely different from mapping a gene for a Mendelian trait where, for the latter, a LOD score of 3 or more (2 or more for X-linked trait) is an indication of

statistically significant linkage with a 5% chance of error [52] (this has been well approved by identification of genes responsible for human Mendelian traits in the last two decades). In addition, proper classification of high myopia into two groups, Mendelian trait (familial, congenital, or early onset, moderate to high grade before school age, and myopic fundus change) or complex trait (acquired, late-onset during primary school, slow progress, and minimal or no fundus change), might be helpful in the identification of genes contributing to high myopia [53,54]. It would be very unusual to conduct an association study for Mendelian traits with a prominent heterogeneity such as retinitis pigmentosa, so why is it acceptable to do the same thing for Mendelian high myopia?

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