The *FGF2* gene in a myopia animal model and human subjects

Jianhong An,1,2 Edward Hsi,3,4 Xiangtian Zhou,1,2 Yijin Tao,1,2 Sub-Hang Hank Juo,3,5 Chung-Ling Liang6

1School of Optometry and Ophthalmology and Eye Hospital, Wenzhou Medical College, Wenzhou, Zhejiang, China; 2State Key Laboratory Cultivation Base and Key Laboratory of Vision Science, Ministry of Health P.R. China and Zhejiang Provincial Key Laboratory of Ophthalmology and Optometry, Wenzhou, Zhejiang, China; 3Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 4Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 5Department of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan; 6Bright-Eyes Clinic, Kaohsiung, Taiwan

**Purpose:** Fibroblast growth factor-2 (*FGF2*) has been implied in the development of myopia according to previous studies investigating *FGF2* in the sclera and retinal pigment epithelium. This study measured retinal *FGF2* gene expression in an animal model and also tested for the association between single nucleotide polymorphisms (SNPs) in *FGF2* and high myopia.

**Methods:** The guinea pigs were assigned to 2 groups: form deprivation myopia (FDM) for two weeks and normal control (free of form deprivation). Biometric measurement was performed and *FGF2* expression levels were compared among the FDM eyes, the fellow eyes of the FDM group and the normal eyes in retina. We also enrolled 1,064 cases (≥-6.0 D) and 1,001 controls (≥-1.5 D) from a Chinese population residing in Taiwan. Six tagging SNPs were genotyped to test for an association between genotypes and high myopia.

**Results:** The FDM eyes had the most prominent changes of refraction and axial length. Compared with the mRNA levels of *FGF2* in the normal eyes, the FDM eyes had the highest levels of mRNA (*p*=0.0004) followed by the fellow eyes (*p*=0.002). The FDM and normal eyes became more myopic compared with the fellow eyes, but the fellow eyes became more hyperopic (*p*=0.004) in the end of the experiment which may be due to its relatively short axial length when compared with normal eyes (*p*=0.05). The SNP genotypes were all in Hardy–Weinberg equilibrium. However, none of the SNPs were significantly associated with high myopia (all *p* values >0.1).

**Conclusions:** We identified a significant change of *FGF2* expression in the FDM eyes but *FGF2* genetic variants are unlikely to influence susceptibility to myopia. There may be a systemic effect to influence gene expression and refraction on the fellow eyes, which may perturb emmetropization in the fellow eyes. Our data also suggest using normal eyes rather than the fellow eyes as the control eyes when study the form deprivation myopia.

Myopia is a common eye condition worldwide, and its prevalence varies widely among populations and ages [1-3]. Myopia is extremely common in Taiwan. When the definition of <-6 D is used, the prevalence of high myopia is 18% among young Taiwanese men and 24% among young Taiwanese women [3]; both of which are even higher than the 13.1% reported among young men in Singapore [2]. Furthermore, the frequency of high myopia (<-6.0 D) has increased in young Taiwanese people: 10.9% in 1983 and 21% in 2000 [4]. While studies have found several environmental risk factors, twin studies have indicated a strong genetic influence on refractive errors with estimates of heritability between 58 and 90% [5-8]. Several studies have also shown that a family history of myopia is a significant risk factor [9-13]. Recently, genetic association studies including genome-wide association studies have reported several susceptibility genes to non-syndromic myopia [14-22]. Genetic association studies are subject to the type I error, especially when the sample size is small. Therefore, replication of the genetic effects in an independent sample and the support from a functional study are important ways to reduce false positive findings.

Scleral remodeling is one of the important mechanisms for the development of myopia. In experimental myopia, eye growth is accompanied by altered proteolytic activities which could serve to remodel the structural components of the scleral extracellular matrix (ECM) [23]. Fibroblast growth factor 2 (*FGF2*) has been shown to be involved in the control of ECM turnover [24]. Studies have shown that exogenous delivery of *FGF2* may prevent the development of myopia in chick [25]. Accordingly, *FGF2* is a potential mediator of the retinoscleral signal to control scleral remodeling and ocular growth.

The first aim of the present study was to measure *FGF2* gene expression during the development of myopia in the mammals. The guinea pig model of ocular growth was used and retinal *FGF2* was measured in the myopic eyes, the fellow eyes of the same animals, and the normal eyes from control animals. Given that a change of retinal *FGF2* expression was associated with myopia development in the animal study, we then tested whether genetic variants of *FGF2* were associated with high myopia in human subjects. The second aim was to test for any association between single nucleotide
polymorphisms (SNPs) of FGF2 and high myopia in a Chinese population residing in Taiwan.

**METHODS**

Animal model and biometric measurement: Guinea pigs have been increasingly used as an alternative to other species in the study of myopic development [26]. All animals underwent biometric measurement before the experiment. The animal care guidelines comparable those published by the Institute for Laboratory Animal Research. The pigmented guinea pigs (three weeks old) were randomly assigned to the form deprivation myopia (FDM) group (n=14) and normal control (free of form deprivation, n=13). Animals in the FDM group wore a facemask that covered the right eye for two weeks [26]. The facemask was then removed from the animals and biometric measurement was performed in both eyes of each animal immediately. In addition, we also had the time points of biometric measurement for the normal control group matched the FDM group. Notably, we used a different inbred line of guinea pigs from what we used in the previous study [26], which led to a more efficient induction of FDM.

Biometric measures include refraction, anterior chamber depth (AC), lens thickness (LT), vitreous chamber depth (VC), and axial length (AL). The detailed measurements can be found elsewhere [26]. In brief, refraction was measured in the vertical pupil meridian by an eccentric infrared photorefactor. Since it is easy to handle guinea pigs, we could align their heads by hand until the pupil was clearly visible in the video frame. Three readings of the refractive error in the vertical meridian were recorded for each eye, and averaged data were used for further analyses. The A-scan ultrasound (AVISO Echograph class I-Type Bat; Quantel Medical, Clermont-Ferrand, France) was used to measure axial dimensions on the same day as refractions were measured. The cornea was topically anesthetized and velocities of sound were assumed as previously described [26]. Each eye was measured at least eight times, and the averages of these parameters measurements were used for analysis.

Tissue preparation: All animal were terminated by an overdose of sodium pentobarbitone at a similar time point (between 1:00 and 3:00 PM) to minimize the effect of diurnal overdose of sodium pentobarbitone at a similar time point (between 1:00 and 3:00 PM) to minimize the effect of diurnal variation on gene expression. The eyes were enucleated and placed onto a filter paper in a Petri dish containing chilled Ringer’s solution. A circumferential incision was made along the limbus, followed by removal of the cornea, crystalline lens and vitreous body. The entire retina was separated from the choroid while the sample was soaked in iced Ringer’s solution. The retina was placed immediately into Trizol reagent (Invitrogen, Carlsbad, CA) and homogenized using the Mixer Mill MM400 (Retsch, Haan, Germany), and then moved to −80 °C before total RNA was isolated.

The animal research in this study was approved by the Animal Care and Ethics Committee at Wenzhou Medical College (Wenzhou, China). The treatment and care of animals were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Gene expression: FGF2 mRNA from 14 FDM eyes, 14 fellow eyes, and 26 normal eyes were measured by real-time PCR. For the normal eyes, we used the averaged expression data from both eyes of a same animal. Therefore, the sample size for normal controls was 13. Real-time PCR was run to detect the mRNA levels of FGF2. The PCRs were performed in an Applied Biosystems 7500 Real-Time PCR System using 2× SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA). Total RNA was extracted from the retina with Trizol reagent (Invitrogen, Grand Island, NY) and confirmed using spectrophotometry and formaldehyde/agarose gel electrophoresis. To remove contaminating genomic DNA, 1 μg of total RNA was treated with 1 U RNase free DNase I (Promega, Madison, WI) at 37 °C for 30 min and then heated with 1 μl stop solution (Promega) at 65 °C for 10 min. Subsequently, 0.5 μg of total RNA in each sample was reversely transcribed (M-MLV reverse transcriptase; Promega) using 0.04 μg random primers (Promega) in a total volume of 20 μl according to manufacturer’s instructions. The expression level of FGF2 mRNA was normalized to that of an internal control actin by using the equation of log_{10} (2^−ΔΔCt), where ΔΔCt=(CT_{FGF2}−CT_{actin}). The median and mean of log_{10} (2^−ΔCt) and its standard deviation (SD) was calculated. Another internal control, 18s RNA, was used in a subset of samples to test whether different internal controls would lead to different conclusions. We used the relative expression level to indicate the fold change between different types of eyes by using the equation of 2^−ΔΔCt. The paired t-test was used to compare the difference of FGF2 expression between FDM and fellow eyes, and unpaired t-test was used for the data from different groups of animals.

Genetic association study: The present study participants were enrolled from the general population with ages between 16 and 45 years. The enrollment was conducted in southern Taiwan between 2003 and 2009. All the participants were of Chinese descents. All the cases had myopia in both eyes and had a spherical refraction ≤−6.0 D in at least one eye. A subject with a spherical refraction ≥−1.5 D in the more myopic eye was defined as a control. We used negative cylindrical powers in all subjects. In addition, none of the controls had received any previous refractive surgery. The refractive error was measured without cycloplegia for subjects with ages ≥18 years and with cycloplegia with ages <18 years. The refractive error was measured using autorefractometers (Topcon KR-8100 or RM-8800; Topcon, Tokyo, Japan) for all eyes. A written informed consent was given by each subject or custodian (if the age of the participant was less than 18 years old). The study was approved by the Institutional Review Board at the Kaohsiung Medical University Hospital, Taiwan. The research followed the tenets of the Declaration of Helsinki.
SNP selection and genotyping: We first selected the tagging single nucleotide polymorphisms (tSNPs) at the FGF2 gene from the release 3.0 Phase II data of the HapMap Project using the Tagger Pairwise method [27]. tSNPs were chosen according to the following criteria: \( r^2 \geq 0.8 \) and the minor allele frequency (MAF) \( \geq 0.10 \% \) in the Han Chinese population. A total of six tSNPs met the selection criteria, which were rs308442 (intron 1), rs17473132 (intron 1), rs308379 (intron 1), rs1048201 (intron 1), rs308379 (3′UTR), and rs308381 (3′UTR). Genotyping was performed by using TaqMan technology. Briefly, PCR primers (Table 1) and TaqMan minor groove binder (MGB) probes were designed and reactions were performed in 96-well microplates with ABI 9700 thermal cyclers (Applied Biosystems, Foster City, CA). The condition to run real-time PCR was as follows: 50 °C for 2 min; 95 °C for 10 min; 95 °C for 15 s; 60 °C for 1 min; the last 2 steps repeated for 45 cycles. Fluorescence was measured with the ABI 7900 Real Time PCR System (Applied Biosystems) and analyzed with its System SDS software version 1.2.3.

Statistical analysis for genetic polymorphism studies: The allele frequency was obtained by direct gene counting. Hardy–Weinberg equilibrium (HWE) was tested in controls [28] by using the \( \chi^2 \) test for each SNP. According to the myopic status and three genotypes of each SNP, the \( \chi^2 \) test for a 2×3 contingency table or Fisher exact test was performed. The genotype specific odds ratio (OR) was first checked to test for the allele dominance. If one allele is dominant over the other allele, the two genotypes containing the dominant allele would be combined to increase the statistical power. When there is no evidence of dominance, we prefer to not collapse heterozygotes with minor homozygotes unless the number of minor homozygotes is too small.

RESULTS

Confirmation of phenotypic changes induced by form deprivation: The refractions of the guinea pig eyes in the two groups indicated hyperopia before the experiment (3 weeks of age). No significant differences of refraction, or AL, AC, LT, or VC (by the paired \( t \)-test) between the two eyes of a same animal (Table 2 and Table 3) at the beginning of the experiments. The FDM eyes became more myopic by 4.32 D and had an increase of axial length by 0.37 mm after form deprivation for two weeks (Table 2). The refraction data showed the normal eyes also had a myopic shift in two weeks, which may reflect physiologic emmetropization. Unexpectedly, the fellow eyes became more hyperopic when compared with the refraction shift in the normal control eyes. The average increase of AL was 0.25 mm in the fellow eyes 0.21 mm in the fellow eyes and 0.25 mm in the normal eyes (Table 2). The largest increase of eye component in the FDM eyes was in VC (Table 3). On the contrary, the average VC was not increased in the fellow eyes of the FDM group in the end of the experiments, while the normal eyes had increased VC (Table 3). Other parameters such as AC and LT had no significant differences between the two eyes of a same animal after form deprivation for two weeks.

Gene expression: The mRNA levels of FGF2 were highest in the FDM eyes, followed by the fellow eyes of the FDM group and lowest in the eyes of the normal controls that were free of form deprivation. This pattern was present no matter actin or 18s RNA was used as the internal control. The differences of
### Table 3. The length of each eye component. The data are presented as mean±SD.

| Type of eye | AC (mm) | LT (mm) | VC (mm) |
|-------------|---------|---------|---------|
|             | FD group (n=14) | Normal (n=13) | FD group (n=14) | Normal (n=13) | FD group (n=14) | Normal (n=13) |
|             | FDM | Fellow | L | R | FDM | Fellow | L | R | FDM | Fellow | L | R |
| Wk 0        | 1.01±0.04 | 1.02±0.04 | 1.04±0.04 | 1.03±0.04 | 3.71±0.08 | 3.69±0.07 | 3.72±0.07 | 3.72±0.08 | 3.10±0.07 | 3.10±0.06 | 3.12±0.08 | 3.12±0.08 |
| p value     | 0.10 | 0.15 | 0.07 | 0.46 | 0.84 | 0.13 | 0.22 | 0.22 | 3.88±0.07 | 3.89±0.07 | 3.90±0.10 | 3.89±0.10 |
| Wk 2        | 1.05±0.03 | 1.04±0.04 | 1.05±0.04 | 1.05±0.04 | 3.23±0.13 | 3.16±0.11 | 3.16±0.09 | 3.16±0.09 | 3.10±0.13 | 3.09±0.06 | 3.16±0.11 | 3.16±0.09 |
| p value     | 0.22 | 0.37 | 0.37 | 0.37 | 0.80 | 0.01 | 0.01 | 0.01 | 3.23±0.13 | 3.09±0.06 | 3.16±0.11 | 3.16±0.09 |

FD: form deprivation; FDM: form deprivation myopia. P values are the comparison between the two eyes of a same animal using the paired t-test.
the mRNA levels between any two groups are shown in Figure 1. Using the data in the normal eyes as the reference, mRNA levels in the fellow eyes of the FDM group was increased by 1.7 fold \( (p=0.0027) \), and by 2.7 fold \( (p=0.0004) \) in the FDM eyes. The difference between FDM and fellow eyes of a same animal was also significant (by 1.6 fold, \( p=0.033 \)).

**Genetic association study:** A total of 1,064 cases and 1,001 controls were included in the present study. The mean age was 21.8 years for cases and 21.6 years for controls. The spherical refractions ranged from \(-6.0\) D to \(-23.5\) D with a mean of \(-8.0\) D and SD of 1.8 D for cases. For the controls, the spherical refractions ranged from 0.75 D to 1.5 D, and the mean\(\pm\)SD was \(-0.4\pm0.6\) D. The call rate for the 6 SNPs ranged from 93% to 97%.

The frequencies of the genotypes and the associations between high myopia (\(\leq-6.0\) D) and the six tSNPs are shown in Table 4. All the SNPs were in HWE in the controls. There was no significant difference of either allele frequency (data not shown) or genotype frequency (Table 4) between cases and controls. We further performed exploratory analysis to compare extreme myopia (\(\leq-10\) D) versus more stringent and controls. We further performed exploratory analysis to compare extreme myopia (\(\leq-10\) D) versus more stringent controls. This approach also did not reveal any significant associations when a correction of multiple testing correction by either the Bonferroni method \( (p=0.32\) after the Bonferroni correction) or permutation test \( (p=0.23) \). Accordingly, we failed to show any association between \( FGF2 \) SNPs and high myopia or extreme myopia.

**DISCUSSION**

We used the form deprivation to induce myopia in the guinea pigs and compared retinal \( FGF2 \) expression levels among the FDM eyes, fellow eyes of the experimental animals and normal eyes of the control animals. The covered eyes had the highest expression level, followed by the uncovered fellow eyes of the same animals, and eyes of control animals had the lowest expression levels. This result indicates that \( FGF2 \) overexpression in the retina may be associated with the development of form deprivation myopia. We noticed that the fellow eyes had a tendency of hyperopia while the normal eyes in the control group had a myopic shift. From both the \( FGF2 \) expression data and the change of refraction, we speculated that form deprivation may interrupt emmetropization in both eyes of animals and also the physiologic control of gene expression. We also tested for the association between six tSNPs at the \( FGF2 \) gene and high myopia in a Chinese population residing in Taiwan. However, the genetic association study failed to show any significant results. To ensure we would not miss the genetic effect that only exists for extremely high myopia as we saw before [20], we also performed analysis for extreme myopia in comparison with stringently defined controls. This approach also did not reveal any significant associations when a correction of multiple testing was taken into account. Therefore, even though \( FGF2 \) may play a role in the myopia development, its genetic polymorphisms are unlikely to influence inter-individual susceptibility to high myopia.

It is generally accepted that the development of myopia is based on the local control as hemi-retinal occlusion leads to only hemiocular elongation [29]. Therefore, the contralateral fellow eye of an animal that receives experimentally induced myopia has been widely used as a control for myopia studies. In the present study, we showed that \( FGF2 \) expression was also influenced in the fellow eyes as well as biometric data (especially VC). Since all fellow eyes in the 14 guinea pigs had increased refractive powers \( (p=0.004) \) rather than emmetropic shift, we hypothesized that the physiologic emmetropization in the fellow eyes was disturbed. This hyperopic change is probably due to failure of an increase of VC. An increase of \( FGF2 \) expression along with an increase of refractive power in the fellow eyes further
indicates the possibility of perturbed eye development when their contra-lateral eyes are under form deprivation. Previous studies using either three shrews [30] or guinea pigs [31] also demonstrated a relatively hyperopic shift in the fellow eyes of the induced-myopia animals. The above data suggest that independent control animals without any vision manipulations are necessary for a better experimental animal study.

The relationship between the FGF2 expression pattern and myopia is inconsistent among previous studies. The scleral and retinal levels of FGF2 have been reported as no difference between the myopic and fellow eyes in the tree shrew receiving induced myopia [32]. However, their data showed a decrease of mean FGF2 expression in the anterior sclera of myopic eyes than in the normal control eyes although the difference of FGF2 levels did not reach a significant level. Furthermore, the same study [32] demonstrated an upregulation of the FGF2 receptor in the myopic eyes. A recent study using the human scleral tissue showed that atropine increased FGF2 activation in a dose-dependent manner [33], while the authors also reported atropine reduced cell proliferation of scleral fibroblasts. Since atropine has been demonstrated to retard myopia progression in humans [34, 35], their FGF2 expression pattern is unexpected and hard to explain (personal communication with the correspondent author of the study [33]). Similar to our finding, FGF2 was significantly upregulated in the choroid/RPE of minus lens-treated eyes (i.e., eyes of induced myopia) of primate marmoset monkeys as compared with plus lens-treated fellow eyes (i.e., eyes of induced hyperopia) [36].

Previous studies also reported that the refraction and gene expression of fellow eyes can be influenced by the treated eyes [37]. Consistent with the previous study [37], our data showed that the major difference of eye component between fellow

| SNP (major/minor) | Status | Major homozygote | Heterozygote | Minor homozygote | MAF | p value |
|-------------------|--------|-----------------|--------------|-----------------|-----|---------|
| rs308442 (T/A)    | Case   | 642 (64.8%)     | 317 (32.0%)  | 31 (3.1%)       | 19.1% | 0.2293 |
| Control           | 620 (63.7%) | 308 (31.7%)     | 45 (4.6%)    | 20.5% |
| rs17473132 (G/A)  | Case   | 930 (88.6%)     | 118 (11.2%)  | 2 (0.2%)        | 5.8%  | 0.9119* |
| Control           | 877 (88.5%) | 111 (11.2%)     | 3 (0.3%)     | 5.9% |
| rs308379 (A/T)    | Case   | 348 (33.2%)     | 516 (49.1%)  | 186 (17.7%)     | 42.3% | 0.6037 |
| Control           | 335 (33.8%) | 467 (47.1%)     | 189 (19.1%)  | 42.6% |
| rs308381 (T/C)    | Case   | 991 (92.3%)     | 81 (7.6%)    | 1 (0.1%)        | 3.9%  | 0.4931* |
| Control           | 940 (93.3%) | 65 (6.5%)       | 2 (0.2%)     | 3.4% |
| rs1048201 (C/T)   | Case   | 260 (24.4%)     | 532 (50.0%)  | 272 (25.6%)     | 50.6% | 0.2736 |
| Control           | 245 (24.5%) | 529 (52.8%)     | 227 (22.7%)  | 49.1% |
| rs3804158 (G/A)   | Case   | 366 (34.8%)     | 497 (47.3%)  | 188 (17.9%)     | 41.5% | 0.5546 |
| Control           | 360 (36.1%) | 477 (47.8%)     | 161 (16.1%)  | 40.0% |

P values were from 2×3 Tables (3 genotypes versus 2 phenotypes). *p-value is adjusted by fisher exact test; MAF: minor allele frequency.

**Table 5. Six tSNPs and their relationships to extreme myopia (refraction in case ≤-10 D; in control ≥-0.5D).**

| SNP (major/minor) | Status | Major homozygote | Heterozygote | Minor homozygote | MAF | p value |
|-------------------|--------|-----------------|--------------|-----------------|-----|---------|
| rs308442 (T/A)    | Case   | 76 (66.1%)      | 35 (30.4%)   | 4 (3.5%)        | 18.7% | 0.7511* |
| Control           | 363 (62.7%) | 187 (32.3%)     | 29 (5.0%)    | 21.2% |
| rs17473132 (G/A)  | Case   | 118 (92.2%)     | 10 (7.8%)    | 0 (0.0%)        | 3.9%  | 0.4028* |
| Control           | 520 (88.3%) | 68 (11.5%)      | 1 (0.2%)     | 5.9% |
| rs308379 (A/T)    | Case   | 35 (26.7%)      | 77 (58.8%)   | 19 (14.5%)      | 43.9% | 0.0276 |
| Control           | 209 (35.4%) | 271 (45.9%)     | 111 (18.8%)  | 41.7% |
| rs308381 (T/C)    | Case   | 123 (93.9%)     | 8 (6.1%)     | 0 (0.0%)        | 3.1%  | 0.8645 |
| Control           | 560 (93.5%) | 39 (6.5%)       | 0 (0.0%)     | 3.3% |
| rs1048201 (C/T)   | Case   | 28 (21.4%)      | 72 (55.0%)   | 31 (23.7%)      | 51.1% | 0.784 |
| Control           | 144 (24.2%) | 314 (52.8%)     | 137 (23.0%)  | 49.4% |
| rs3804158 (G/A)   | Case   | 47 (36.2%)      | 64 (49.2%)   | 19 (14.6%)      | 39.2% | 0.9697 |
| Control           | 216 (36.4%) | 286 (48.2%)     | 91 (15.4%)   | 39.5% |

*p-value is adjusted by fisher exact test.
eyes and FDM eyes was VC. The hyperopic shift in our data may be due to the lack of growth of VC in the fellow eyes (Table 3). The amount of increased AL was largest in the FDM eyes, followed by normal eyes and then the fellow eyes. However, Frost et al. [37] reported the order of increased AL as treated eyes, fellow eyes and normal eyes in their lens-induced myopia in tree shrews. Accordingly, the “cross-over” effect on the fellow eyes may be species specific or model specific.

Although animal studies have revealed FGFR2 as a candidate gene, the relationship between genetic polymorphisms of FGFR2 and myopia has not been extensively investigated. Tsai et al. [38] examined two SNPs (one is in the promoter and the other in the 3′ UTR) at the FGFR2 gene and reported no association with high myopia in a Chinese population. Mutti et al. [39] also reported no linkage disequilibrium between FGFR2 and myopia using the TDT statistical program in a family data. It needs to be noticed that the failure to identify significant SNPs at the FGFR2 gene does not exclude the importance of this gene in the pathogenesis of myopia. Instead, the results of genetic association study should be interpreted as the genetic variants at the FGFR2 gene may not influence individual susceptibility to high myopia. Namely, the genetic variants of this gene can not serve as a biomarker to predict a high myopia risk.

In conclusion, we identified a significant change of FGFR2 expression levels in the FDM eyes of the guinea pigs. The refractive data indicated that a disturbance of emmetropization of the fellow eyes resulted in their hyperopic shift. Therefore, we suggest using normal eyes rather than the treated eyes, fellow eyes and normal eyes in their lens-induced myopia in tree shrews.

ACKNOWLEDGMENTS

This study was supported by the grant from the Taiwan National Science Council (NSC95–3112-B037–003), the National Basic Research Program of China (973 project) NO: 2011CB504602, National Natural Science Foundation of China (30973278) and Zhejiang Provincial Natural Science Foundation of China (Z2100065). Dr. Chung-Ling Liang and Dr. Suh-Hang Hank Juo contributed equally to the conduct of the this research and are to be considered as co-corresponding authors.

REFERENCES

1. Katz J, Tielsch JM, Sommer A. Prevalence and risk factors for refractive errors in an adult inner city population. Invest Ophthalmol Vis Sci 1997; 38:334-40. [PMID: 9040465]
2. Wu HM, Seet B, Yap EP, Saw SM, Lim TH, Chia KS. Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore. Optom Vis Sci 2001; 78:234-9. [PMID: 11349931]
3. Lin LL, Shih YF, Hsiao CK, Chen CJ, Lee LA, Hung PT. Epidemiologic study of the prevalence and severity of myopia among schoolchildren in Taiwan in 2000. J Formos Med Assoc 2001; 100:684-91. [PMID: 11760374]
4. Lin LL, Shih YF, Hsiao CK, Chen CJ. Prevalence of myopia in Taiwanese schoolchildren: 1983 to 2000. Ann Acad Med Singapore 2004; 33:27-33. [PMID: 15008558]
5. Hammond CJ, Snieder H, Gilbert CE, Spector TD. Genes and environment in refractive error: the twin eye study. Invest Ophthalmol Vis Sci 2001; 42:1232-6. [PMID: 11328732]
6. Teikari JM, Kaprio J, Koskenvuo MK, Vannas A. Heritability estimate for refractive errors—a population-based sample of adult twins. Genet Epidemiol 1988; 5:171-81. [PMID: 3049225]
7. Teikari JM, O’Donnell J, Kaprio J, Koskenvuo M. Impact of heredity in myopia. Hum Hered 1991; 41:151-6. [PMID: 1937488]
8. Lyhne N, Sjolie AK, Kyvik KO, Green A. The importance of genes and environment for ocular refraction and its determiners: a population based study among 20–45 year old twins. Br J Ophthalmol 2001; 85:1470-6. [PMID: 11734523]
9. Liang CL, Yen E, Su YJ, Liu C, Chang TY, Park N, Wu MJ, Lee S, Flynn JT, Joo SH. The impact of the family history of high myopia on level and onset of myopia. Invest Ophthalmol Vis Sci 2004; 45:3446-52. [PMID: 15452048]
10. Goss DA, Jackson TW. Clinical findings before the onset of myopia in youth: 4. Parental history of myopia. Optom Vis Sci 1996; 73:279-82. [PMID: 8728496]
11. Zadnik K, Satariano WA, Mutti DO, Sholtz RJ, Adams AJ. The effect of parental history of myopia on children's eye size. JAMA 1994; 271:1323-7. [PMID: 8158816]
12. Mutti DO, Mitchell GL, Moebscherler ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. Invest Ophthalmol Vis Sci 2002; 43:3633-40. [PMID: 12454029]
13. Wu MM, Edwards MH. The effect of having myopic parents: an analysis of myopia in three generations. Optom Vis Sci 1999; 76:387-92. [PMID: 10416933]
14. Hammond CJ, Andrew T, Mak YT, Spector TD. A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of dizygotic twins. Am J Hum Genet 2004; 75:294-304. [PMID: 15307048]
15. Han W, Leung KH, Fung WY, Mak JY, Li YM, Yap MK, Yip SP. Association of PAX6 polymorphisms with high myopia in Han Chinese nuclear families. Invest Ophthalmol Vis Sci 2009; 50:47-56. [PMID: 19124844]
16. Inamori Y, Ota M, Inoko H, Okada E, Nishizaki R, Shiota T, Mok J, Oka A, Ohno S, Mizuki N. The COL1A1 gene and high myopia susceptibility in Japanese. Hum Genet 2007; 122:151-7. [PMID: 17557158]
17. Liang CL, Hung KS, Tsai YY, Chang W, Wang HS, Juo SH. Systematic assessment of the tagging polymorphisms of the COL1A1 gene for high myopia. J Hum Genet 2007; 52:374-7. [PMID: 17273809]
18. Hall NF, Gale CR, Ye S, Martyn CN. Myopia and polymorphisms in genes for matrix metalloproteinases. Invest Ophthalm Vis Sci 2009; 50:2632-6. [PMID: 19279308]
19. Liang CL, Wang HS, Hung KS, Hsi E, Sun A, Kuo YH, Juo SH. Evaluation of MMP3 and TIMP1 as candidate genes for high myopia in young Taiwanese men. Am J Ophthalmol 2006; 142:518-20. [PMID: 16935611]
20. Liang CL, Hsi E, Chen K, Pan Y, Wang Y, Juo SH. Functional polymorphism at 3’ UTR of the PAX6 gene may confer risk for extreme myopia in Chinese. Invest Ophthalmol Vis Sci 2011;52:3505. [PubMed: 21421876]
21. Solouki AM, Verhoeven VJ, van Duijn CM, Verkerk AJ, Ikram MK, Hysi PG, Despriet DD, van Koolwijk LM, Ho L, Ramdas WD, Czudowska M, Kuijpers RW, Amin N, Struchalin M, Aulchenko YS, van Rij G, Riemslag FC, Young TL, Mackey DA, Spector TD, Gorgels TG, Willemsse-Assink JJ, Isaacs A, Kramer R, Swagemakers SM, Bergen AA, van Oosterhout AA, Oostra BA, Rivadeneira F, Uitterlinden AG, Hofman A, de Jong PT, Hammond CJ, Vingerling JR, Klaver CC. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. Nat Genet 2010; 42:897-901. [PMID: 20835239]
22. Hysi PG, Young TL, Mackay DA, Andrew T, Fernandez-Medarde A, Solouki AM, Hewitt AW, Macgregor S, Vingerling JR, Li YJ, Ikram MK, Fai LY, Sham PC, Manyes L, Porteros A, Lopes MC, Carbonaro F, Fahy SJ, Martin NG, van Duijn CM, Spector TD, Rahi JS, Santos E, Klaver CC, Hammond CJ. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. Nat Genet 2010; 42:902-5. [PMID: 20835240]
23. Jones BE, Thompson EW, Hodos W, Waldhiller RJ, Chader GJ. Scleral matrix metalloproteinases, serine proteinase activity and hydralatic capacity are increased in myopia induced by retinal image degradation. Exp Eye Res 1996; 63:369-81. [PMID: 8944544]
24. Pickering JG, Ford CM, Tang B, Chow LH. Coordinated effects of fibroblast growth factor-2 on expression of fibrillar collagens, matrix metalloproteinases, and tissue inhibitors of matrix metalloproteinases by human vascular smooth muscle cells. Evidence for repressed collagen production and activated degradative capacity. Arterioscler Thromb Vasc Biol 1997; 17:475-82. [PMID: 9102165]
25. Rohrer B, Stell WK. Basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-beta) act as stop and go signals to modulate postnatal ocular growth in the chick. Exp Eye Res 1994; 58:553-61. [PMID: 7925692]