Original Article

The investigation on the role of mitochondrial fusion protein 1 in the development of myopia

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Purpose: The aim of this study is to preliminarily investigate the expression of mitochondrial fusion protein 1 (*MFN1*) in a lens-induced animal myopia (LIM) model and to explore the relationship between *MFN1* and the visual development. **Materials and Methods:** *MFN1* gene expression in guinea pigs was examined during the development of minus LIM, 15 tri-colored guinea pigs were obtained, and one eye of each pig was randomly selected and treated with −7.00D lenses. Ocular refraction and axial length were collected before intervention and 1, 2, and 3 weeks after intervention. After the refraction and axial length measurements at 1, 2, and 3 weeks of lens intervention, five guinea pigs were randomly selected. *MFN1* expression in the retina of both eyes was tested by immunohistochemistry technique. **Results:** *MFN1*-positive cells could be observed in the retina of both eyes. The positive cells in the LIM eyes were staining deeper, and much more positive cells could be observed. Furthermore, *MFN1*-positive expression could be seen mainly in ganglion cells after 1 week of minus lens intervention, and with time extension, more and more positive cells appeared in the rod-cone cell and bipolar cell layer, and this phenomenon could not be found in the normal control eyes. **Conclusion:** This study suggested that *MFN1* might be correlated to the development of myopia.

Key words: *MFN1*, myopia, guinea pigs

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Myopia is the most common eye disease of visual impairment in the world; for the past few decades, the prevalence and severity of myopia have risen dramatically around the world,[1] and in some countries of East Asia, more than 80% school children now develop myopia and do so at an earlier age.[2] Low to moderate myopia can be corrected with glasses, contact lenses, or refractive surgery; however, high myopia may be associated with a substantial risk of potentially blinding ocular pathologies, such as cataract,[3] retinal detachment,[4] and glaucoma.[5]

Myopia is complex in nature with multifactorial etiology, and it is influenced by numerous genetic and environmental factors. In recent years, many researches have focused on the mechanisms underlying the pathogenesis of myopia.[6,7] However, the mechanism for the development of myopia is still not fully understood. Although environment (in particular, education, urbanization, outdoor activity, and close work) clearly plays a role in myopia, numerous cross-sectional studies suggest that genetic heritability might be as high as about 80%.[7] Moreover, in recent years, some progresses have been made toward the molecular mechanisms of the disease, a number of candidate genes have been identified, and many genes and loci related to the disease have been found through gene linkage and correlation studies.[8,9] However, most of these genes have not been independently validated and no confirmed causal gene has been found for the disease.

MFN1 gene codes for the cell and ganglion cell layer1, which is located on the outer membrane of cell. *MFN1* is a protein-coding gene of 45.5 kb with 18 exons. It plays a pivotal role in mediating mitochondrial fusion in mammalian cells.[10] *MFN1* is the main molecule that regulates mitochondrial fusion and facilitates the binding of mitochondria in the early stage of the fusion. It plays an important role in the movement of mitochondria and acts together with intramembrane protein optic atrophy 1 (*OPA1*), which is widely distributed in retinal ganglion cells as a dynein-related protein and essential for synaptic structure of retinal ganglion cells.[11] Research has shown that the deletion of *OPA1* in the optic nerve atrophy model rats can lead to the structure change of dendrites in retinal neural cells.[12] *MFN1* and *OPA1* may together protect the cell against spontaneous apoptosis[13] and have impact on the adjustment of retinal mitochondria. Because the eye is a high-energy-consuming organ, changes in mitochondrial function may affect the development of myopia, suggesting that pathogenesis of myopia may be associated with the mitochondria.

However, not much has been done about the role of *MFN1* on myopia. Based on our recent human study,[14] which showed that *MFN1* gene might be correlated with myopia, we hope to further explore whether there is any change in the gene expression in animal models and to get a better understanding about the correlation of *MFN1* and myopia.

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Materials and Methods

Fifteen tri-colored guinea pigs were obtained from the Animal Experiments Laboratory of North Sichuan Medical College and were reared in large cages. One eye of the guinea pigs was randomly selected and treated with − 7.00D lenses. The other eye served as an internal control group. The method of cycloplegia was induced with three drops of tropicamide, and the refraction status was measured by means of streak retinoscopy in hand-held, awake animals. Stable refraction was generally obtained after 30 min when no pupillary response was observed. All refractive data were referred to the spherical equivalent refraction. The axial length of the eyes was measured by an A-scan ultrasound while the animals were anesthetized with ketamine (80 mg/kg) by intramuscular injection.

Ocular refraction and axial length were performed before the experiment and 1, 2, and 3 weeks after minus lens intervention. The measurement was repeated at least three times for each eye, and the refraction and axial length of each guinea pig at every measurement were averaged. ANOVA, with repeated measures design, was used to investigate the influence of the intervention on the axial length and refraction. A one-way ANOVA, followed by Student's unpaired *t*-test, with Bonferroni correction for multiple testing, was used to analyze ocular parameters between groups.

This study was complied with the tenets of the Association for Research in Vision and Ophthalmology statement for the use of Animal in Ophthalmic and Vision Research. After the refraction and axial length measurements at 1, 2, and 3 weeks of minus lens intervention, five guinea pigs were randomly selected and were deep anesthetized with 0.03 ml/kg chloral hydrate by intraperitoneal injection. With the animals under deep anesthesia, both eyes were enucleated at a similar time point (between 4:00 and 6:00 PM) to minimize the effect of diurnal variation on gene expression. 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 neutral buffer formaldehyde solution. Twenty-four hours later, the retina was embedded in paraffin to detect expression of *MFN1* with immunohistochemistry (Streptavidin-Perosidase (SP) three steps). Finally, guinea pigs were sacrificed by an overdose of 10% chloral hydrate. The results of immunohistochemical detection were described and analyzed qualitatively.

Results

Before the intervention, there were no statistically significant differences between the two groups in terms of the refraction (P = 0.860) and axial length data (P = 0.115). However, repeated measures ANOVA [Tables 1 and 2] revealed a statistically significant effect of minus lens intervention and a significant minus lens intervention by time interaction for the axial length and refraction from baseline (P = 0.000). The lens-induced myopia (LIM) eyes became more myopic by 4.70D and had an increase of axial length by 0.46 mm after lens induction for 3 weeks [Table 3]. The average increase of axial length was 0.46 mm in lens-induced eyes and 0.18 mm in the normal control eyes [Table 4].

The results of protein expression [Figs 1-3] represented the control and lens-induced eyes, respectively. *MFN1*-positive cells could be observed in the retina of both eyes, mainly in the cytoplasm and cell membrane of the ganglion cells; *MFN1*-positive cells appeared brownish or yellow. In the LIM eyes, the immunopositive cells were staining deep, and more positive cells could be observed; however, *MFN1*-positive cells scattered expressed in the ganglion cell layer in the control eyes. Furthermore, *MFN1*-positive expression could be seen mainly in ganglion cells at the 1st week of treatment; with the extension of lens induction time, many *MFN1*-positive cells also appeared in the bipolar cell layer and the rod-cone cell layer, and this phenomenon could not be found in normal control eyes.

Discussion

To the best of our knowledge, this is the first study that describes the *MFN1* gene expression and myopia. In a previous British study,[15] five single nucleotide polymorphism (SNP) loci around the *MFN1* gene were found strongly associated with myopia (including high, medium, and low myopia). Especially, the SNP locus of rs6794192 and rs7618348 showed lower *P* values. In our recent study,[14] we genotyped rs3976523

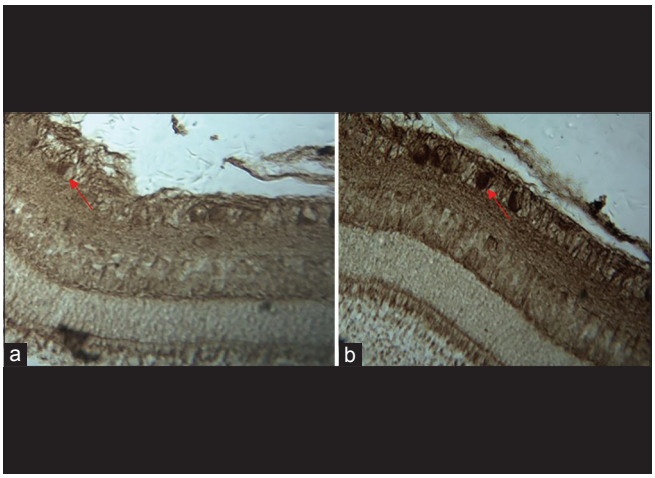

Figure 1: (a and b) One week after treatment – Mitochondrial fusion protein 1-positive cells were observed in the retina of both eyes, mainly in the cytoplasm and cell membrane of the ganglion cells, and the positive cells seem staining deep in the lens-induced eyes

Table 1: The results of axial length analysis by ANOVA (mm)

	Week 0 (time)	Week 1 (time)	Week 2 (time)	Week 3 (time)	*P*		
					Time	Group	Group by time
LIM eyes	8.06±0.14	8.17±0.15	8.35±0.11	8.52±0.05	0.000 (*F*=320)	0.001 (*F*=30)	0.000 (*F*=77)
Control eyes	7.97±0.16	8.01±0.16	8.04±0.13	8.15±0.11			

Week 0: Before intervention; Week 1, Week 2 and Week 3: 1 week, 2 weeks, and 3 weeks after the lens induction, respectively. LIM eyes: The group of lens-induced eyes, Control: The control group, LIM: Lens-induced myopia

Table 2: The results of refraction analysis by ANOVA

	Week 0 (time)	Week 1 (time)	Week 2 (time)	Week 3 (time)	*P*		
					Time	Group	Group by time
LIM eyes	2.60±0.52	2.17±0.41	−0.38±0.64	−2.10±0.45	0.000 (*F*=422)	0.000 (*F*=126)	0.006 (*F*=167)
Control eyes	2.57±0.51	2.32±0.52	1.08±0.50	0.95±0.33			

Week 0: Before intervention; Week 1, Week 2 and Week 3: 1 week, 2 weeks, and 3 weeks after the lens induction, respectively. LIM eyes: The group of lens-induced eyes, Control: The control group, LIM: Lens-induced myopia

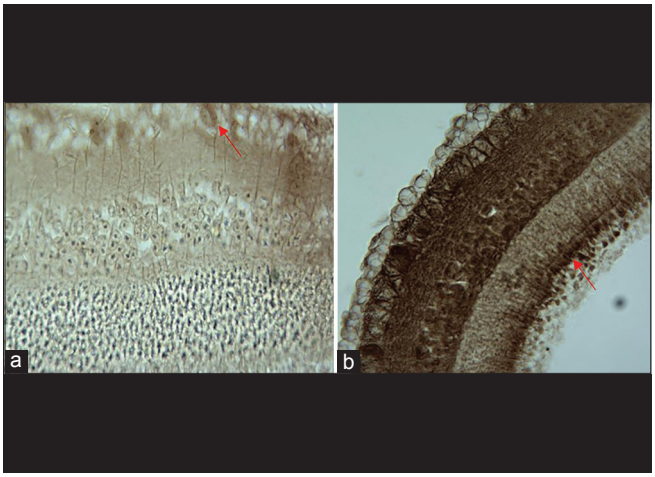

Figure 2: (a and b) Two weeks after treatment – The positive cells in the lens-induced eyes were staining deep, abundant-positive cells could be observed in the ganglion cell layer, and many mitochondrial fusion protein 1-positive cells could also be seen in the bipolar cell layer and rod-cone cell layer; however, positive cells only scattered in the ganglion cell layer in the control eyes

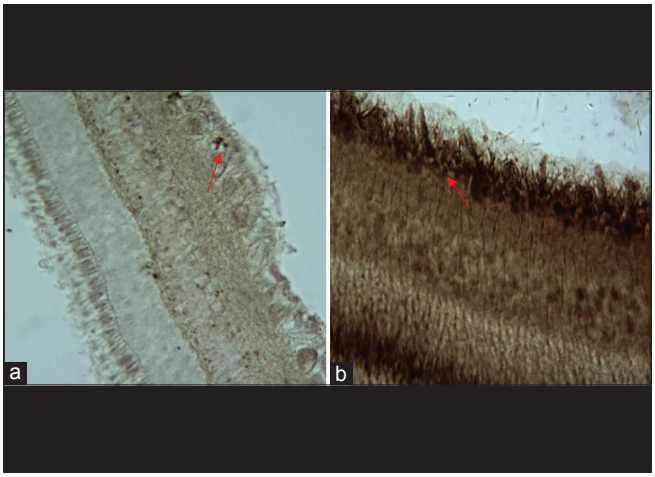

Figure 3: (a and b) Three weeks after treatment – Mitochondrial fusion protein 1-positive cells scattered expressed in the ganglion cell layer and the bipolar cell layer of the control eyes, while many positive cells appeared in the rod-cone cell, the bipolar cell and ganglion cell layer in the lens-induced eyes

which was linked with rs6794192 (r^2 = 0.942) in the myopia population, and no correlation was found between rs3976523 and myopia. For rs7618348, we found that the *P* values in the allele difference between controls and myopia patients were more than 0.05. However, we also genotyped another two other loci in the *MFN1* gene (rs6762399 and rs13098637) in our previous study.[14] Interestingly, we found that rs13098637 locus located in an intron at the center of the *MFN1* gene was significantly correlated to myopia.

Although human studies have revealed *MFN1* as a candidate gene of myopia, the relationship between the *MFN1* expression and myopia has not been investigated. In this study, we further investigated the expression of *MFN1* in the retina, so as to get a better understanding about its possible involvement in the occurrence or development in the mammalian myopia.

Guinea pig is a kind of mammals, the eyeball structure is similar to the human's, and myopia animal model of guinea pig has successfully been established.[16] All guinea pigs were raised with a monocular −7.00D lens, and the fellow eye was untreated and served as the self-control group. During the 1st week, both axial length (0.04 mm in the control eyes and 0.11 mm in the experimental eyes) and refraction data changed in both groups (0.25D in the control eyes and 0.43D in the experimental eyes), but the refraction data did not show any statistically significant difference (*P* = 0.388) whereas the axial length showed a statistically significant difference (*P* = 0.008). The axial length in the lens-induced eyes increased rapidly when compared to control group, owing to the minus lens intervention. The refraction data are the combined effect of axial length and the refractive components of the eye, but we suspect that the refractive components did not change to compensate for the axial length change during the 1st week in this research. This could be the reason for the refraction data did not show a significant difference between the two groups during the 1st week while the axial length showed a significant difference. Over the time of intervention, about 2 weeks later, the refraction data and axial length were both significantly different between the two groups. Three weeks after the intervention, the lens-induced eyes became more myopic, and the mean refraction was −1.78D in LIM eyes whereas it was +1.05D in control eyes, which indicated that myopia animal model had been successfully established by a −7.00D minus lens.

In the current study, *MFN1*-positive cells could be observed in the retina of both eyes. In the LIM eyes, the immunopositive cells were staining deeper and more positive cells could be observed while *MFN1*-positive cells only scattered expressed in the ganglion cell layer of the control eyes. In addition, in the LIM eyes, 1 week after minus lens intervention, we could find that *MFN1* expression positive cells mainly located in the ganglion cell layer, with the intervention time extension, more and more positive cells occurred in the bipolar cell layer and the cone-rod cell layer, especially in the cone-rod cell layer, abundant-positive cells could be seen and the dyeing color was very deep. However, these phenomena could not be found in the control eyes. These results indicated that *MFN1* overexpression in the retina of the

Table 3: The refraction data measured in the two eyes

	Week 0	Week 1	Week 2	Week 3
LIM eyes	2.60±0.52	2.17±0.41	−0.38±0.64	−2.10±0.45
Control	2.57±0.51	2.32±0.52	1.08±0.50	0.95±0.33
t	0.177	−0.877	−6.054	−12.200
P	0.860	0.388	0.000	0.000

Week 0: Before intervention; Week 1, Week 2 and Week 3: 1 week, 2 weeks, and 3 weeks after the lens induction, respectively. LIM eyes: The group of lens-induced eyes, Control: The control group, LIM: Lens-induced myopia

Table 4: The results of axial length in both eyes by *t*-test

	Week 0	Week 1	Week 2	Week 3
LIM eyes	8.06±0.14	8.17±0.15	8.35±0.11	8.52±0.05
Control	7.97±0.16	8.01±0.16	8.04±0.13	8.15±0.11
t	1.625	2.850	5.819	7.163
P	0.115	0.008	0.000	0.000

Week 0: Before intervention; Week 1, Week 2 and Week 3: 1 week, 2 weeks, and 3 weeks after the lens induction, respectively. LIM eyes: The group of lens-induced eyes, Control: The control group, LIM: Lens-induced myopia

experimental eyes might be associated with the development of LIM. From both the *MFN1* expression results and refraction data, we also speculated that minus lens intervention may interrupt the emmetropization in experimental eyes of animals and also the physiological control of gene expression. However, the specific mechanism was not clear. At present, the investigation of *MFN1* expression in the retina had not been extensively studied; in addition, the sample size in this experiment was a little small, and so this investigation could only be looked as a preliminary exploratory research. Unfortunately, in the present study, only according to the images, we only could qualitatively describe this interesting phenomenon on *MFN1* expression. Hence, *MFN1* expression in the retina should be quantitatively studied and analyzed in future research.

Conclusion

We found some rough alterations of *MFN1* expression in the LIM eyes of the guinea pigs. By combining our previous study which found that rs13098637 locus within the *MFN1* gene was related to myopia, we speculated that *MFN1* genetic variants might be likely to influence the development of myopia and that the relationship between myopia and *MFN1* should be worthy of further investigation.

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Conflicts of interest

There are no conflicts of interest.

References

1. Pärssinen O. The increased prevalence of myopia in Finland. Acta Ophthalmol 2012;90:497-502.
2. Liu HH, Xu L, Wang YX, Wang S, You QS, Jonas JB. Prevalence and progression of myopic retinopathy in Chinese adults: The Beijing eye study. Ophthalmology 2010;117:1763-8.
3. Kanthan GL, Mitchell P, Rochtchina E, Cumming RG, Wang JJ. Myopia and the long-term incidence of cataract and cataract surgery: The Blue Mountains eye study. Clin Experiment Ophthalmol 2014;42:347-53.
4. Mitry D, Charteris DG, Fleck BW, Campbell H, Singh J. The epidemiology of rhegmatogenous retinal detachment: Geographical variation and clinical associations. Br J Ophthalmol 2010;94:678-84.
5. Marcus MW, de Vries MM, Junoy Montolio FG, Jansonius NM. Myopia as a risk factor for open-angle glaucoma: A systematic review and meta-analysis. Ophthalmology 2011;118:1989-94.e2.
6. Dirani M, Chamberlain M, Shekar SN, Islam AF, Garoufalis P, Chen CY, *et al*. Heritability of refractive error and ocular biometrics: The genes in myopia (GEM) twin study. Invest Ophthalmol Vis Sci 2006;47:4756-61.
7. Lopes MC, Andrew T, Carbonaro F, Spector TD, Hammond CJ. Estimating heritability and shared environmental effects for refractive error in twin and family studies. Invest Ophthalmol Vis Sci 2009;50:126-31.
8. Hysi PG, Young TL, Mackey DA, Andrew T, Fernández-Medarde A, Solouki AM, *et al*. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. Nat Genet 2010;42:902-5.
9. Solouki AM, Verhoeven VJ, van Duijn CM, Verkerk AJ, Ikram MK, Hysi PG, *et al*. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. Nat Genet 2010;42:897-901.
10. Santel A, Frank S, Gaume B, Herrler M, Youle RJ, Fuller MT. Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in mammalian cells. J Cell Sci 2003;116(Pt 13):2763-74.
11. Williams PA, Piechota M, von Ruhland C, Taylor E, Morgan JE, Votruba M. Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. Brain 2012;135(Pt 2):493-505.
12. Williams PA, Morgan JE, Votruba M. Opa1 deficiency in a mouse model of dominant optic atrophy leads to retinal ganglion cell dendropathy. Brain 2010;133:2942-51.
13. Davies V, Votruba M. Focus on molecules: The OPA1 protein. Exp Eye Res 2006;83:1003-4.
14. Zou YC, Lei JH, Wang Y, Xu S. Correlation between polymorphisms in the MFN1 gene and myopia in Chinese population. Int J Ophthalmol 2015;8:1126-30.
15. Andrew T, Maniatis N, Carbonaro F, Liew SH, Lau W, Spector TD, *et al*. Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. PLoS Genet 2008;4:e1000220.
16. Zhi-Jie S, Jin-Song Z, Kai-Di W, Xiao-Fang Z, Xiu-Juan L. Inhibitory of pirenzepine on form deprivation myopia and its mechanism in the guinea pig. Rec Adv Ophthalmol 2006;26:904-90.