• Basic Research •

# Vasoactive intestinal peptide, a promising agent for myopia?

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## Abstract

**• AIM: To investigate the role of vasoactive intestinal peptide (VIP) in form-deprivation myopia (FDM).**

**• METHODS: FDM was created in three groups of eight chicks by placing a translucent diffuser on their right eyes. Intravitreal injections of saline and VIP were applied once a day into the occluded eyes of groups 2 and 3, respectively. Retinoscopy and axial length (AL) measurements were performed on the first and 8th days of diffuser wear. The retina mRNA levels of the VIP receptors and the ZENK protein in right eyes of the three groups and left eyes of the first group on day 8 were determined using real time polymerase chain reaction (PCR).**

**• RESULTS: The median final refraction (D) in right eyes were -13.75 (-16.00, -12.00), -11.50 (-12.50, -7.50), and -1.50 (-4.75, -0.75) in groups 1, 2, and 3, respectively (*P*<0.001). The median AL (mm) in right eyes were 10.65 (10.00, 11.10), 9.90 (9.70, 10.00), and 9.20 (9.15, 9.25) in groups 1, 2, and 3, respectively (*P*<0.001). The median delta-delta cycle threshold (CT) values for the VIP2 receptors were 1.07 (0.82, 1.43), 1.22 (0.98, 1.65), 0.29 (0.22, 0.45) in right eyes of groups 1, 2, and 3, and 1.18 (0.90, 1.37) in left eyes of group 1, respectively (*P*=0.001). The median delta-delta CT values for the ZENK protein were 1.07 (0.63, 5.03), 3.55 (2.20, 5.55), undetectable in right eyes of groups 1, 2, and 3 and 1.89 (0.21, 4.73) in left eyes of group 1, respectively (*P*=0.001).**

**• CONCLUSION: VIP has potential inhibitory effects in the development of FDM.**

**• KEYWORDS:** vasoactive intestinal peptide; form-deprivation myopia; myopia; chicks; ZENK protein; vasoactive intestinal peptide receptor

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## INTRODUCTION

Genetic and environmental factors are implicated in the etiology of myopia[1-2]. Studies have shown that a decrease or complete loss of image quality of an eye in the postnatal period results in an overgrowth that causes myopia[3]. This condition in the postnatal period of young animals is called form-deprivation myopia (FDM)[3]. FDM can be induced experimentally by the application of translucent occluders over an animal's eyes[4]. Many candidate molecules, including vasoactive intestinal peptide (VIP), have been proposed to be involved in the patho-physiology of myopia[5-6]. Experimental investigations in animals have indicated that restricted vision affects eye growth and leads to an increase in axial length (AL). The increase in AL was associated with changes in neurotransmitters and growth factors, such as dopamine[7-8], retinoic acid[9], and glucagon[10].

VIP is a peptide hormone from glucagon family. VIP mainly plays a role in the gastrointestinal system as a smooth muscle relaxer, but it is also found in the brain and eyes where it functions as a neuoromodulator[11]. Although a few studies have indicated that VIP, which exists mostly in the choroid tissue of the eye, impacts myopia[5-6], the results of some animal experiments contradict this finding[12]. Publications on the effects of VIP in FDM are lacking and have inconsistent results[5-6,12-15]. The ZENK transcription factor is a protein expressed in glucagon amacrine cells in the retina of the chick. The changes in the ZENK expression affect the ocular growth. Its upregulation has been shown to be related with the suppresion of AL elongation[16-17]. The expressions of VIP receptors (also called $VPAC_1$ and $VPAC_2$) and the ZENK protein have been shown to be associated with FDM in several studies[5,17-19]. Our aim was to investigate the clinical effects of VIP and its effects on the expression of VIP receptors in experimental FDM.

## MATERIALS AND METHODS

The study adhered to the ARVO Statement for the Use of

Animals in Ophthalmic and Vision Research. This study was approved by the Institutional Animal Care and Use Committee of Eskisehir Osmangazi University Medical Faculty (EOUMF) on the 19th of October, 2011, under decision number 169-1. All experimental procedures and genetic analyses were performed at the EOUMF Physiology Department according to the guiding principles for the care and use of animals (Anadolu University Animal Experiments Local Ethics Committee Guidelines 2011; 1.1.2).

**Animals** One-day-old white Leghorn chicks were obtained from a local farm and reared in a temperature-controlled environment (26℃) under a 12-hour light/dark cycle with a light level of 250 lx on the floor of the cage provided by standard fluorescent tubes. Twenty-seven chicks were included in this study. The animals were divided into three groups: control (group 1; *n*=9), saline (group 2; *n*=9) and VIP (group 3; *n*=9).

**Induction of Myopia** On posthatching day 6 (P6), all chicks were monocularly deprived of pattern vision by attachment of a translucent diffuser to the feathers surrounding the right eye with contact cement 2 to 3h after the beginning of the light phase. The diffusers were hand-made, hemispherical, thin plastic shells with frosted surfaces. Their rims were approximately 1 mm wide and were attached to the feathers around the right eye with cyanoacrylic glue under light ether anesthesia. The rims of the diffusers were placed far enough from the eyelids to ensure that they did not interfere with their function. In all experiments, the right eyes were covered with the diffusers for various lengths of time, whereas the left eyes remained uncovered and served as internal, genetically identical and functionally independent controls.

***In Vivo* Injections** All intravitreal injections were performed after exposure to diethyl ether by inhalation. Proparacaine drops (Alcaine, Alcon, Turkey) were administered prior to the attachment of a translucent diffuser and all intravitreal injections and measurements. Topical antibiotic eye drops (gentamicin 0.3%) were applied four times/day in all animals after the intravitreal injections. One ophthalmologist (Gursoy H) applied intravitreal injections to the right eyes of the chicks in groups 2 and 3 between posthatching day 6 (P6) and P13 using a Hamilton syringe with a 26 gauge needle. The chicks received a total of seven injections, once every 24h for 7d, and ocular measurements were taken on P13. The right eyes of group 2 received daily injections of 10 μL of saline (0.9% NaCl), while the right eyes of group 3 received daily injections of 10 μL of VIP (Sigma-Aldrich, Steinheim, Germany) (0.5 ng/μL). The ophthalmologists didn't know which chicks had intravitreal injections of saline or VIP. All injections were applied 2 to 3h after the beginning of the light phase. The initial injections were applied before the attachment of a translucent diffuser.

**Assessment of Myopia** Ocular measurements were taken on P6 and P13. Two consecutive measurements per eye were performed by 2 ophthalmologists (Gursoy H and Cakmak AI), and the mean values were recorded. Cycloplegia was achieved 30min after instilling two drops of 1% cyclopentolate 5min apart. The refractive error measurements of both open and closed eyes were performed by streak retinoscopy under cycloplegia at a distance of 30 cm. Spherical equivalent (SEq) was calculated by adding the spherical value and half of the cylindrical value. SEq was used for analysis. The AL of the eyes was obtained transpalpebrally with B-scan ultrasonography (Advent AB, Accutome, Inc., Malvern, PA, USA).

**Animals Sacrifice** All animals were sacrificed by an overdose of diethylether and cervical dislocation at the end of the deprivation period, P13. The left eyes of group 1 and the right eyes of all three groups were enucleated and immediately placed into a petri dish filled with ringer solution for immediate preparation. The eyes were perforated using a cannula and were opened by cutting around the iris with scissors. The anterior segment of the eye was discarded, and the vitreous gel was removed. Fixation was performed by immersion in 4% paraformaldehyde plus 3% sucrose in 0.1 mol/L phosphate buffer (pH 7.4) for 24h at room temperature. The eyes were hemisected equatorially with a razor blade, and the anterior portions were discarded along with the vitreous gel.

**RNA Extraction and Real Time Polymerase Chain Reaction** The retinal tissues of left eyes of group 1 and the right eyes of all three groups were analyzed.

The mRNA levels of VIP1, VIP2 and ZENK in relation to the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were determined using real time polymerase chain reaction (RT-PCR) with Taqman prob. Total RNA was extracted from the retinal tissue using the RNA stabilization reagent (Qiagen, Germany) according to the manufacturer's instructions and was quantified by measuring its absorbance at 260 nm (Nanodrop1000; Thermo, Wilmington, DE, USA). Aliquots of 20 μL of RNA from each group were used to synthesize complementary DNA (cDNA). The newly synthesized cDNA, which was stored at -20˚C, was used for the mRNA assay of the VIP1 receptors, VIP2 receptors, and ZENK protein. cDNA (5 μL) from each group was amplified in 20 μL of reaction mixture. RT-PCR was performed by real-time monitoring of the increase in the amount of Taqman prob using Rotor-Gene 6000 RT-PCR (Qiagen, Germany). The oligonucleotide sequences of the cDNA primers were designed at Gene Research Laboratories, UK. The following primers were used: rat VIP1, 5′-GAGAGGAAAGACAGCGTTGG-3′ (sense) and 5′-CAGAAGGACCTGGGTGTTGT-3′ (antisense); VIP2, 5′ GCAAGCTCAGCCTGGTATTC-3′ (sense) and 5′-AGGTAGGCCAGGAAACACCT-3′ (antisense); and

**Table 1 The comparisons of the spherical equivalent refractive error and the axial length (AL) of the right eyes on P6 and P13 among the three groups** *n*=8

| Measurements | Group 1 | Group 2 | Group 3 | *P* |
|---|---|---|---|---|
| Spherical equivalent refractive error (D) | | | | |
| P6 | 2.75 (2.25, 3.50) | 1.25 (-0.25, 2.50) | 2.25 (1.75, 2.75) | 0.035[a] |
| P13 | -13.75 (-16.00, -12.00) | -11.50 (-12.50, -7.50) | -1.50 (-4.75, -0.75) | <0.001[a] |
| *P* | 0.012[a] | 0.012[a] | 0.012[a] | |
| Axial length (mm) | | | | |
| P6 | 9.00 (9.00, 9.05) | 9.00 (8.95, 9.10) | 9.00 (8.95, 9.00) | 0.762 |
| P13 | 10.65 (10.00, 11.10) | 9.90 (9.70, 10.00) | 9.20 (9.15, 9.25) | <0.001[a] |
| *P* | 0.012[a] | 0.011[a] | 0.027[a] | |

The 25th and 75th percentile values are given in parentheses along with the medians. [a]*P*<0.05.

ZENK 5'- ACTAACTCGTCACATTCGCA -3' (sense) and 5'- TGCTGAGACCGAAGCTGCCT-3′ (antisense). For the housekeeping gene GAPDH the primer sequences were as follows 5′-CCTGGACCACCCAGCCCAGCA-3′ (sense) and 5′-TGTTATGGGGTCTGGGATGGA-3′ (antisense). The RT-PCR thermal cycling conditions were as follows: 15min at 42℃ and 10min at 4℃ for cDNA synthesis followed by 10min at 95℃ and then 50 cycles of 20s at 95℃, 30s at 55℃, and 20s at 72℃. RT-PCR data were collected using the Rotor-Gene 6000 detection system. Cycle threshold (Ct) values were determined by automated threshold analysis. Primer quality (lack of primer-dimer amplification) was confirmed by melting curve analysis. Relative quantification of gene expression was performed using the standard curve method, and the standard curves were constructed using serial dilutions of control mRNA or RT-PCR amplicons. All experiments were standardized with GAPDH (ratios of VIP1, VIP2 and ZENK to GAPDH) to account for loading differences. Gene expression levels (mRNA) were reported using the median as a point estimator and the range of values.

**Statistical Analysis** The data failed the Shapiro-Wilk test for normality. Therefore, the SEq refractive error and the AL values of the right and left eyes on P6 and P13 were compared among the three groups using the non-parametric Kruskal-Wallis one-way analysis of variance by the ranks test and Tukey's HSD multiple comparisons test. The initial measurements of the right and left eyes for each group on P6 were compared to those on P13 using the Wilcoxon signed-rank test. Delta-delta Ct ($\Delta\Delta C_t$) assumes that the primers for the unknown and reference genes have very similar efficiency (*i.e.* reference gene amplification is nearly the same level as that of the unknown gene). The $\Delta\Delta C_t$ values for the VIP receptors and the ZENK protein were compared among the right eyes of groups 1, 2, and 3 and the left eyes of group 1 using non-parametric Kruska-Wallis one-way analysis of variance by the ranks test and Tukey's HSD multiple comparisons test. *P*-values <0.05 were considered to indicate statistical significance. The statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

One chick in each group was excluded from the statistical analysis because of endophthalmitis development after the third injection. Therefore, eight chicks in each group were analyzed.

The comparisons of the SEq refractive errors and the AL values of the right eyes on P6 and P13 among the three groups are presented in Table 1. And the comparisons of the initial SEq refractive errors and the AL values on P13 for the right eyes of each group are shown in Table 1.

Multiple comparisons between groups were performed by the Tukey's HSD test. On P6, the right eyes in groups 1 and 3 were significantly more hyperopic than the right eyes in group 2. On P13, the eyes in groups 1 and 2 were significantly more myopic than the VIP injected eyes (group 3). The eyes in groups 1 and 2 had significantly higher AL values than the eyes in group 3. The AL was significantly higher at the final visit in all groups, so the eyes were significantly more myopic at the final visit than at baseline.

The comparisons of the SEq refractive errors and the AL values of the left eyes on P6 and P13 among the three groups are presented in Table 2. And the comparisons of the initial SEq refractive errors and AL values on P13 for the left eyes of each group are shown in Table 2.

On P6, the left eyes in groups 1 and 3 were significantly more hyperopic than the left eyes in group 2. The final SEq refraction on P13 was similar among the three groups; however, Tukey's HSD multiple comparisons test showed that the AL values on P13 were significantly higher in group 1 than in groups 2 and 3. All eyes were hyperopic, although the decrease in hyperopia from baseline to P13 was significant in groups 1 and 3.

The mRNA levels of the VIP1 receptor were undetectable in all groups. The comparisons of the $\Delta\Delta C_t$ values for VIP2 receptor and the ZENK protein among the right eyes of groups 1, 2, and 3 and the left eyes of group 1 are presented in Table 3.

Multiple comparisons between groups were performed by Tukey's HSD test. The $\Delta\Delta C_t$ values for VIP2 receptor were significantly different between the right eyes in groups 1 and 3 (*P*=0.013), between the right eyes in groups 2 and 3 (*P*=0.002),

**Table 2 The comparisons of the spherical equivalent refractive error and the axial length (AL) of the left eyes on posthatching day 6 (P6) and posthatchig day 13 (P13) among the three groups** *n*=8

| Measurements | Group 1 | Group 2 | Group 3 | *P* |
|---|---|---|---|---|
| Spherical equivalent refractive error (D) | | | | |
| P6 | 3.25 (2.75, 3.75) | 1.00 (0.50, 2.00) | 2.25 (1.75, 2.50) | 0.002[a] |
| P13 | 1.00 (0.50, 1.50) | 1.25 (-1.75, 1.75) | 1.25 (1.00, 1.75) | 0.303 |
| *P* | 0.017[a] | 0.574 | 0.017[a] | |
| Axial length (mm) | | | | |
| P6 | 8.95 (8.90, 9.10) | 9.00 (8.95, 9.10) | 9.00 (8.95, 9.00) | 0.827 |
| P13 | 9.50 (9.20, 9.60) | 9.20 (9.15, 9.35) | 9.20 (9.15, 9.35) | 0.005[a] |
| *P* | 0.011[a] | 0.011[a] | 0.059 | |

The 25th and 75th percentile values are given in parentheses along with the medians. [a]*P*<0.05.

**Table 3 The comparisons of the delta delta cycle threshold ($\Delta\Delta C_t$) for VIP2 receptors and ZENK protein among the right eyes of group 1, 2, 3 and the left eyes of group 1 using the Kruskal–Wallis one-way analysis of variance by ranks test** *n*=8

| $\Delta\Delta C_t$ | Right eyes | | | Left eyes | *P* |
|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 1 | |
| VIP2 receptors | 1.07 (0.82, 1.43) | 1.22 (0.98, 1.65) | 0.29 (0.22, 0.45) | 1.18 (0.90, 1.37) | 0.001[a] |
| ZENK protein | 1.07 (0.63, 5.03) | 3.55 (2.20, 5.55) | 0 (Undetectable) | 1.89 (0.21, 4.73) | 0.001[a] |

The 25th and 75th percentile values are given in parentheses along with the medians. [a]*P*<0.05.

and between the right eyes in group 3 and the left eyes in group 1 (*P*=0.025). The $\Delta\Delta C_t$ values for the ZENK protein were significantly different between the right eyes in groups 2 and 3 (*P*<0.001) and between the right eyes in group 3 and the left eyes in group 1 (*P*=0.045).

## DISCUSSION

In the present experimental study, we observed a significant decrease in myopia after the intravitreal VIP injections. FDM was produced in all groups, but the intravitreal VIP injections significantly reduced the myopia observed at the final measurement. The final AL value was lowest in the group 3, and this finding was consistent with the fact that we observed the lowest myopia in that group. The expression of VIP receptors and the ZENK protein were analyzed to support our clinical findings. We showed that the intravitreal injections of VIP down-regulated the expressions of VIP2 receptor and the ZENK protein. However, we could not show a clear association between these mRNA findings and the possible mechanisms underlying the partial blockage of FDM by intravitreal injections of VIP.

The results of studies of the role of VIP in FDM have been inconsistent. It is claimed that VIP is involved both in the development of the refractive status and in the development of FDM in chicks[20]. Wiesel and Raviola[13] found that VIP level decreased in FDM, while Seltner and Stell[5] found that both VIP agonists and antagonists blocked FDM. Contrary to the report by Wiesel and Raviola[13], Stone *et al*[15] showed increased expression of VIP in FDM in monkeys. Wang *et al*[21] investigated the role of VIP antagonist in FDM and concluded that they decrease the development of FDM in chicks. In 1997, Basmak and Tuncel[22] obtained results consistent with those of the current study by injecting VIP into occluded chick eyes, but it was just a clinical trial without any PCR analysis. Dopamine is another neuro-peptide investigated for its role in myopia. In many studies, dopamine agonists blocked FDM[7-8]. It should be noted that VIP and dopamine appear to exert synergistic effects on retinal cAMP level[14]. This may be a common pathway through which dopamine agonists and VIP block FDM. In the present study, we tried to prevent FDM by injecting VIP based on previous reports by Wiesel and Raviola[13], Basmak and Tuncel[22] .

Several reports have shown that the expression of VIP2 receptor was up-regulated in high myopia[16-17]. We found that the mRNA level of VIP1 receptor was undetectable in all groups, so we concluded that VIP1 receptor was not involved in ocular growth. On the other hand, the mRNA level of VIP2 receptor was significantly reduced in the occluded chick eyes after the intravitreal VIP injections (group 3), but the expression of VIP2 receptor was similar among the other three groups. Based on our findings, we conclude that FDM is not associated with any changes in VIP2 receptor expression but that exogenous intravitreal VIP injections can down-regulate the expression of this receptor. Contrary to our findings, Liu *et al*[18] found that VIP2 receptor was up-regulated in the eyes with FDM compared to the unoccluded eyes. We hypothesized that the partial suppression of FDM in the current study could be due to VIP agonistic and antagonistic effects. The antagonistic effect of intravitreal VIP injections on VIP receptor levels could be produced by the VIP fragments formed by hydrolysis in the chick's eye[5,23]. The observed down-regulation of VIP2 receptor in the eyes of group 3 in our study supported our hypothesis because reduced receptor expression causes decreased agonistic activity of VIP. Similar to dopamine, VIP agonistic activitiy could prevent FDM through cAMP-dependent mechanisms[14].

The ZENK transcription factor (ZENK protein) regulates ocular growth. Schippert *et al*[17] showed that the ZENK knockout mice had more myopia than the mice in whom the ZENK gene was expressed. This protein induces the expression of many growth factors, such as fibroblast growth factor and platelet-derived growth factor[17]. These factors suppress ocular growth, so a lower expression level of the ZENK protein was expected in the eyes with high FDM compared to the VIP injected eyes (group 3), as shown in several previous experimental studies[16-17]. However, the most outstanding finding regarding the expression of the ZENK protein was the undetectable mRNA levels in the eyes of group 3 after the VIP injections. This could have led to an increased level of myopia in the third group. However, the partial blockage of FDM obtained after the intravitreal VIP injections was *via* other mechanisms that outweighed the possible consequences of a decreased ZENK mRNA level. The mRNA level of ZENK protein in the right and left eyes of group 1 was consistent with the fact that a decreased ZENK protein level is associated with FDM[17]. Although the difference failed to reach statistical significance, the median $\Delta\Delta C_t$ for the ZENK protein was 1.07 in the occluded eyes in group 1, while it was 1.89 in the unoccluded eyes in group 1. In the right eyes of the second group, the mRNA level of the ZENK protein was surprisingly up-regulated. This could have been associated with the prevention of FDM, but the second group received intravitreal saline injection, which is not thought to be involved in the prevention of FDM. The increase in the ZENK mRNA level could be a response to the simple trauma of intravitreal injection[24].

We hypothesized three possible explanations for the partial blockage of FDM after intravitreal VIP injection. We thought both agonistic and antagonistic activities played roles in the prevention of FDM. First, VIP could act like dopamine on a retinal cAMP level[14]. This agonistic effect could partially block FDM in the same manner as dopamine agonists[25]. Second, the antagonistic effects of the VIP fragments formed by hydrolysis could partially block FDM through unknown pathways. Some studies concluded that both VIP antagonists and down-regulation of VIP2 receptor expression prevent FDM[18-19,21]. Several reports have shown that the expression of VIP is positively correlated with AL elongation[20]. Finally, the down-regulation of the VIP2 receptor level augments the antagonistic effects of VIP fragments.

The strengths of the current study include its comparative design and that it was an experimental trial of the use of intravitreal VIP to block FDM. Our efforts to support the current clinical findings by performing PCR analysis of retinal tissues provide some clues into the mechanisms underlying the role of VIP in FDM. However, there are some limitations of this study, including limitations associated with the injection technique, the methodology, and the size of the study group. These limitations were unavoidable, except for the number of chicks studied. This study would be statistically stronger if we used a larger study group. We tried to inject the same doses of VIP in all chicks by using the same syringe at the same time of the day. However, it was not possible to obtain completely uniform VIP doses in all groups. We had to rely on our retinoscopy and ultrasonography measurements. However, the transpalpebral measurement of AL using contact B-scan ultrasonography was particularly subjective. We obtained a higher final AL in the first and second groups compared to the final AL in the third group in which intravitreal VIP injections were applied. These findings support our clinical findings by retinoscopy. However, the median AL was 9.20 mm in both the right and left eyes of group 3 despite the differences in refraction (-1.5 D myopia in the right eyes versus 1.5 D hyperopia in the left eyes). We concluded that this technique for measuring AL was not sufficiently sensitive to detect small differences in the AL values in the chick eyes. We measured refraction using retinoscopy under cycloplegia, but the accommodation of the examiner and the residual accommodation in the chick eyes could have affected the values we obtained[26]. In the present study we analyzed the VIP1 and VIP2 receptors in the retinal tissues, but it has been reported that VIP also acts at $PAC_1$ receptors[27]. We could have gained more information regarding the mechanism of VIP in blocking FDM, if gene expression levels for $PAC_1$ were also investigated.

In conclusion, in our study, intravitreal VIP injections partially blocked FDM in chicks. Both the agonistic and antagonistic effects of VIP could play a role in this prevention of FDM because the expressions of the ZENK protein and VIP2 receptor were down-regulated in the VIP-treated eyes. There is no single peptide, which has been shown previously to have definitive control of AL elongation and emmetropization. Several neuropeptides normally localized in the retina have been reported to be involved in the development of FDM[5]. VIP is a neuropepide, which seems to play an important role in the emmetropization process[20]. VIP signaling pathway may be a promising target for myopia prevention. Further studies are required to clarify the mechanisms underlying the action of VIP in FDM.

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**Conflicts of Interest**: **Cakmak AI**, None; **Basmak H**, None; **Gursoy H**, None; **Ozkurt M**, None; **Yildirim N**, None; **Erkasap N**, None; **Bilgec MD**, None; **Tuncel N**, None; **Colak E**, None.

**REFERENCES**

1 Morgan IG, Ohno-Matsui K, Saw SM. Myopia. *Lancet* 2012;379(9827):1739-1748.

2 Wojciechowski R. Nature and nurture: the complex genetics of myopia and refractive error. *Clin Genet* 2011;79(4):301-320.

3 Flitcroft DI. Is myopia a failure of homeostasis? *Exp Eye Res* 2013;114:16-24.

4 Pardue MT, Stone RA, Iuvone PM. Investigating mechanisms of myopia in mice. *Exp Eye Res* 2013;114:96-105.

5 Seltner RL, Stell WK. The effect of vasoactive intestinal peptide on development of form deprivation myopia in the chick: a pharmacological and immunocytochemical study. *Vision Research* 1995;35(9):1265-1270.

6 Rymer J, Wildsoet F. The role of retinal pigment epithelium in eye growth regulation and myopia: a review. *Vis Neurosci* 2005;22(3):251-261.

7 Stone RA, Lin T, Laties AM, Iuvone PM. Retinal dopamine and form-deprivation myopia. *Proc Natl Acad Sci U S A* 1989;86(2):704-706.

8 Iuvone PM, Tigges M, Stone RA, Lambert S, Laties AM. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. *Invest Ophthalmol Vis Sci* 1991;32(5):1674-1677.

9 Seko Y, Shimizu M, Tokoro T. Retinoic acid increases in the retina of the chick with form deprivation myopia. *Ophthalmic Res* 1998;30(6):361-367.

10 Feldkaemper MP, Schaeffel F. Evidence for a potential role of glucagon during eye growth regulation in chicks. *Vis Neurosci* 2002;19(6):755-766.

11 Troger J, Kieselbach G, Teuchner B, Kralinger M, Nguyen QA, Haas G, Yayan J, Göttinger W, Schmid E. Peptidergic nerves in the eye, their source and potential pathophysiological relevance. *Brain Res Rev* 2007;53(1):39-62.

12 Mathis U, Schaeffel F. Glucagon-related peptides in the mouse retina and the effects of deprivation of form vision. *Graefes Arch Clin Exp Ophthalmol* 2007;245(2):267-275.

13 Wiesel TN, Raviola E. Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature* 1977;266(5597):66-68.

14 Makman MH, Brown JH, Mishra RK. Cyclic AMP in retina and caudate nucleus: influence of dopamine and other agents. *Adv Cyclic Nucleotide Res* 1975;5:661-679.

15 Stone RA, Laties AM, Raviola E, Wiesel TN. Increase in retinal vasoactive intestinal polypeptide after eyelid fusion in primates. *Proc Natl Acad Sci U S A* 1988;85(1):257-260.

16 Ashby R, Kozulin P, Megaw PL, Morgan IG. Alterations in ZENK and glucagon RNA transcript expression during increased ocular growth in chickens. *Mol Vis* 2010;16:639-649.

17 Schippert R, Burkhardt E, Feldkaemper M, Schaffel F. Relative axial myopia in Egr 1 (ZENK) knockout mice. *Invest Ophthalmol Vis Sci* 2007;48(1):11-17.

18 Liu SZ, Wang H, Jiang JJ Wang PB, Wu XY, Tan XP, Xia ZH. Dynamic expression of VIPR2 in form deprivation myopia. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2005;30(4):456-459.

19 Yiu WC, Yap MK, Fung WY, Ng PW, Yip SP. Genetic susceptibility to refractive error: association of vasoactive intestinal peptide receptor 2 (VIPR2) with high myopia in Chinese. *PLoS One* 2013;8(4):e61805.

20 Shi Y, Gong B, Chen L, *et al*. A genome-wide meta-analysis identifies two novel loci associated with high myopia in the Han Chinese population. *Hum Mol Genet* 2013;22(11):2325-2333.

21 Wang PB, Wang H, Liu SZ, Jiang JJ. Effect of vasoactive intestinal peptide receptor antagonist VIPhybrid on the development of form deprivation myopia in chicks. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2008;33(8):669-675.

22 Başmak H, Tuncel N. The effects of vasoactive intest inal peptide on development of form deprivation myopia in the chick. *XI Congress of the European-Society-of Ophthalmology* 1997 June 1-5; Budapest, Hungary. 1997:1561-1566.

23 Paul S, Volle DJ, Beach CM, Johnson DR, Powell MJ, Massey RJ. Catalytic hydrolysis of vasoactive intestinal peptide by human autoantibody. *Science* 1989;244(4909):1158-1162.

24 Bhattacharyya S, Fang F, Tourtellotte W, Varga J. Egr-1: new conductor for the tissue repair orchestra directs harmony (regeneration) or cacophony (fibrosis). *J Pathol* 2013;229(2):286-297.

25 Yan T, Xiong W, Huang F, Zheng F, Ying H, Chen JF, Qu J, Zhou X. Daily injection but not continuous infusion of apomorphine inhibits form-deprivation myopia in mice. *Invest Ophthalmol Vis Sci* 2015;56(4):2475-2485.

26 Krishnacharya PS. Study on accommodation by autorefraction and dynamic refraction in children. *J Optom* 2014;7(4):193-202.

27 Shen S, Gehlert DR, Collier DA. PACAP and PAC1 receptor in brain development and behavior. *Neuropeptides* 2013;47(6):421-430.