

**518 Factors mediating myopia**

Thursday, May 11, 2017 8:30 AM–10:15 AM

Exhibit/Poster Hall Poster Session

**Program #/Board # Range:** 5460–5479/B0617–B0636

**Organizing Section:** Anatomy and Pathology/Oncology

**Program Number:** 5460 **Poster Board Number:** B0617

**Presentation Time:** 8:30 AM–10:15 AM

**Opposing contributions by D2 receptor activation on form-deprivation myopia development in mice**

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**Purpose:** Many studies have shown that retinal dopamine is a major regulator of postnatal eye growth and myopia in animal models. In the present study, we determined the effects of the dopamine D2 receptor agonist, quinpirole, on form-deprivation myopia development (FDM) and whether quinpirole acts at dopamine D2 receptors (D2R) to exert its effect on myopia development using D2R knockout (KO) mice.

**Methods:** Wild-type C57/BL6 (WT) littermates and corresponding D2R KO mice were subjected to FDM at postnatal 28–56 days of age. Both groups were intraperitoneally injected daily with either quinpirole (high dose: 10 mg/kg/day; low dose: 1 mg/kg/day) or vehicle for 4 weeks (starting from postnatal day 28). Their body weight, refraction, corneal radius of curvature and ocular axial components were measured at the end of 4-weeks of treatment.

**Results:** Consistent with our recent report, D2R KO attenuated FDM development compared to WT littermates ( $-2.29 \pm 0.44$  D in D2R KO versus  $-5.23 \pm 0.44$  D in WT,  $p < 0.05$ ). In the WT mice, the high and low doses of quinpirole had opposite effects on FDM development: high dose promoted myopia development ( $-8.83 \pm 0.72$  D in WT-high dose versus  $-5.23 \pm 0.44$  D in WT-vehicle,  $p < 0.05$ ) while low dose inhibited it ( $-1.79 \pm 0.35$  D in WT-low dose versus  $-5.23 \pm 0.44$  D in WT-vehicle,  $p < 0.05$ ). Importantly, these opposing effects of quinpirole on FDM in WT mice were absent in D2R KO mice (D2R KO-high dose or D2R KO-low dose versus D2R KO-vehicle,  $p > 0.05$ ). In parallel with refraction changes, these opposing quinpirole effects were accompanied by changes in vitreous chamber depth and axial length that were consistent with those on FDM development. Furthermore, in the D2R KO mice, quinpirole or vehicle treatment did not alter FDM-induced elongation of vitreous chamber depth and increases in axial length.

**Conclusions:** Quinpirole has opposing dose dependent effects on FDM stemming from its interaction with D2R. As D2R is a GPCR coupled to various signaling transduction pathways mediating different responses that may oppose one another, it is conceivable that their activation by this D2 partial agonist is dose dependent. An additional possibility is that quinpirole activates presynaptic D2R autoreceptor-linked signaling, which in turn reduces extracellular space dopamine levels and attenuates dopaminergic signaling.

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**Presentation Time:** 8:30 AM–10:15 AM

**The efficacy of the dopamine D2 agonist quinpirole at inhibiting ocular growth in chicks is dependent on time-of-day**

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**Purpose:** Injections of the D2 dopamine agonist quinpirole prevent the development of negative-lens-induced myopia in chicks by inhibiting ocular growth. Because retinal dopamine levels fluctuate in a diurnal rhythm, being highest during the day, and because they depend on light intensity, it is possible that the growth inhibiting effects of quinpirole might differ depending on when it is administered. We tested this hypothesis.

**Methods:** 12-d old chicks wore monocular -10D spectacle lenses (image behind the retina; hyperopic defocus) for 5 days. The lens-wearing eye was injected intravitreally, once daily, with 20  $\mu$ l of quinpirole (10 nmoles) or 20  $\mu$ l saline, at the following times: 7:00 am (drug,  $n=12$ ; saline,  $n=5$ ), 12:00 pm (drug,  $n=6$ ; saline,  $n=6$ ), or 7:00 pm (drug  $n=18$ ; saline  $n=12$ ). Because there were no between-group differences in the saline controls, these data were combined. Ocular dimensions were measured using high frequency A-scan ultrasonography on day 1, and again on day 5. Refractions were measured on day 5 using a Hartinger's refractometer.

**Results:** We found that injections at noon *only* were effective at inhibiting myopia (ANOVA  $p=0.002$ ; drug vs saline:  $-1.1$  D vs  $-4.3$  D; post-hoc Bonferroni  $p=0.03$ ). Injections at 7am and 7pm had no effect ( $-2.6$  and  $-3.3$  vs  $-4.3$ ;  $p > 0.5$ ). The myopia inhibition was the result of inhibiting axial growth (change in axial length: ANOVA  $p=0.003$ ; drug vs saline: 241 vs 485  $\mu$ m; post-hoc Bonferroni  $p=0.007$ ); there was no such inhibition in either the 7 am or 7 pm injection groups (363  $\mu$ m and 483  $\mu$ m vs 485  $\mu$ m;  $p=0.18$ ; 0.99 respectively). The changes in vitreous chamber depth were consistent with that of axial length for all groups. Finally, choroids in the noon injection group, which inhibited eye growth, showed less thinning in response to the negative lens-induced defocus than did those of the 7 am group (ANOVA  $p=0.02$ ;  $-27$  vs  $-127$   $\mu$ m; post-hoc Bonferroni  $p=0.053$ ), but did not differ from the thinning in the 7 pm group ( $-49$   $\mu$ m;  $p > 0.5$ ).

**Conclusions:** Quinpirole is not effective at inhibiting ocular growth when given first thing in the morning, or late in the day, indicating that there is a diurnal rhythm in susceptibility to this drug. This supports the possibility that there are differential susceptibilities for other, more clinically-relevant drugs, such as low-dose atropine.

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**Presentation Time:** 8:30 AM–10:15 AM

**The Effects of Adenosine Antagonist, 7-Methylxanthine, on Emmetropization in Rhesus Monkeys**

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**Purpose:** Laboratory studies and clinical trials suggest that the adenosine receptor antagonist, 7-methylxanthine (7MX), retards myopia progression. The aim of this study was to determine whether 7MX alters the compensating refractive changes produced by optical defocus in rhesus monkeys.

**Methods:** Starting at age 3 weeks, monkeys ( $n=37$ ) were reared with either -3D ( $n=24$ ) or +3D ( $n=13$ ) lenses over their treated eyes and plano lenses over their fellow eyes. Ten and 6 of the monkeys reared with -3D (-3-7MX) and +3D lenses (+3-7MX) were also given 100 mg/kg of 7MX by mouth BID until the end of lens wear (143 $\pm$ 15 days old). The eye's refractive status, corneal power and axial dimensions were assessed periodically throughout the treatment period. Data on normal refractive development were obtained from 35 untreated monkeys.

**Results:** At the end of the treatment period, the control animals reared with monocular +3 and -3 D lenses exhibited significant compensating hyperopic (+1.72±0.56D) and myopic anisometropias (-2.13±1.18D), respectively. In contrast the -3-7MX monkeys exhibited significant hyperopic ametropias in both eyes (median: treated eye, +5.47D, p=0.002; fellow eye, +4.28D, p=0.01), but were on average isometric (+0.34±1.96 D). During the treatment period, the +3-7MX monkeys also manifest significant hyperopic shifts in both eyes, which were larger than those observed in +3 D controls (treated eyes: 2.32±1.06D vs 0.03±0.64D, p=0.002; fellow eyes: 0.66±1.13D vs -1.74±0.68D, p=0.003), but interestingly similar amounts of hyperopic anisometropia (+1.72±0.56D). The relative hyperopic changes in the 7MX-treated monkeys were associated with increases in choroidal thickness and reduced vitreous chamber elongation rates.

**Conclusions:** The results demonstrate that, in primates, daily systemic administration of 7-MX increases choroidal thickness, reduces overall axial elongation rates, promotes the development of hyperopia in control eyes, blocks compensating myopia produced by hyperopic defocus, and augments hyperopic shifts in response to imposed myopic defocus. The results suggest that adenosine receptors play a critical role in emmetropizing responses, especially to hyperopic defocus.

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**Presentation Time:** 8:30 AM–10:15 AM

**Adenosine Receptor Immunoreactivity in the Non-Human Primate Ocular Posterior Segment**

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**Purpose:** Adenosine receptor (ADOR) antagonists such as 7-methylxanthine (7-mx) have been shown to slow myopia progression. Adenosine receptors are found throughout the body, and regulate the release of neurotransmitters such as dopamine and glutamate. However, the role of adenosine in eye growth is unclear. Evidence suggests that 7-mx increases scleral collagen fibril diameter, hence, stiffening the sclera and preventing axial elongation. This study used immunohistochemistry (IHC) to examine the distribution of the ADORs in the posterior segment of the normal monkey eye to help elucidate the mechanism of action.

**Methods:** Eyes were enucleated from two male Rhesus monkeys (age 150 days). Anterior segments were removed, and eyecups were fixed in 4% paraformaldehyde (PFA), cryoprotected with successive sucrose infiltrations, dissected into leaflets, and flash-frozen. Tissue was cryosectioned, post-fixed with 4% PFA, washed with water and Hank's Balanced Salt Solution (HBSS), blocked, and incubated overnight with primary antibody (ADORA1 1:100; ADORA2a 1:100; ADORA2b 1:50; and ADORA3 1:100). Sections were washed with HBSS, and secondary antibody (AlexaFluor488 goat anti-rabbit) was applied for 1 hour. Sections were washed, coverslipped with Prolong Diamond with DAPI (Molecular Probes), and imaged.

**Results:** All four anti-ADORA antibodies showed high immunoreactivity in somas in the ganglion cell layer, and moderate immunoreactivity in photoreceptor inner segments. A1 staining was conspicuous in the outer plexiform layer and in the retinal nerve fiber layer (RNFL), but mostly absent in the RPE, choroid and sclera. A2a

staining was high in the RNFL and outer choroid, moderate in scleral fibroblasts and throughout the retina, and mostly absent in the RPE. A2b staining was high in isolated scleral fibroblasts and occasional nuclei in the RPE, moderate in the outer and inner plexiform layers and the RNFL, and mostly absent in the choroid. A3 staining was conspicuous in photoreceptor processes spanning the outer nuclear layer, and was moderate in the RNFL, the choroid, and in scleral fibroblasts.

**Conclusions:** IHC indicated differential patterns of expression of the four adenosine receptors in the retina, choroid, and sclera of the normal non-human primate eye. The presence of ADORs in scleral fibroblasts suggests a mechanism by which ADOR antagonists may increase scleral stiffness to prevent myopia.

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**Presentation Time:** 8:30 AM–10:15 AM

**Subconjunctival injection of gap junction antagonist (18-β-glycyrrhetic acid) induces myopia in guinea pigs**

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**Purpose:** Gap junctions are involved in multiple retinal signaling pathways including visual acuity regulation and light adaptation which were thought to be related to myopia. This study evaluated the effect of gap junction mediated retinal network in refraction development.

**Methods:** Pigmented guinea pigs (3w old) were raised with normal vision (NC groups) or with form deprivation (FDM groups) for 4w. The FD eye was daily subconjunctival injected 18-β-glycyrrhetic acid (18-β-GA, a gap junction antagonist, low dose: 40μg/100μl, high dose: 120μg/100μl), or its vehicle (DMSO), with injection volume of 100μl. The ocular biometry including refraction, vitreous chamber depth (VCD) and axial length (AL) was measured at the onset and on day 14 and 28. Retinal functions were monitored by electroretinography (ERG) on day 21.

**Results:** Though the applied dosages of 18-β-GA did not alter the ERG responding, they were sufficient to affect the refraction development in guinea pigs. The refraction of NC+ DMSO, NC+low dose and NC+ high dose was -0.67±0.65D, -2.18±1.41D and -3.38±2.04D respectively at day 14; and -0.85±0.69D, -3.07±2.42D and -5.44±2.07D respectively at day 28 (p < 0.001, repeated one-way ANOVA). The elongation of VCD and AL was consistent with refractive changes (VCD: p=0.065; AL: p=0.004, repeated one-way ANOVA). The application of 18-β-GA enhanced FDM as well. The refraction of FDM+ DMSO, FDM+low dose and FDM+ high dose was -5.15±1.68D, -6.82±1.89D and -6.47±2.39D respectively at day 14; and -8.94±2.04D, -10.81±2.41D and -11.16±2.68D respectively at day 28 (p=0.16, repeated one-way ANOVA). However, retinal gap junction blocking did not increase VCD and AL elongation in FDM groups (VCD: p=0.498; P=0.230, repeated one-way ANOVA).

**Conclusions:** Retinal gap junction blocking induces myopia and increases FDM in guinea pig with a dosage did not alter retinal functions, indicating the retinal network mediated by gap junction plays important role in refraction development.

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### Myopia-Inhibiting Muscarinic Antagonists Also Block $\alpha_{2A}$ -Adrenoceptor Signaling

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**Purpose:** Atropine is used to prevent myopia, but its target receptor is unknown. It is a potent muscarinic acetylcholine receptor (mAChR) antagonist, but there is significant evidence that it may inhibit myopia through non-mAChR means (McBrien et al. 2012. OPO). Atropine may act at  $\alpha$ -adrenoceptors (Chang et al. 1995. Eur J Pharmacol), and the most potent myopia-inhibiting ligand found to date (MT3) has equally high affinity at mAChR  $M_4$ ,  $\alpha_{1A}$ - and  $\alpha_{2A}$ -adrenoceptors (Näreoja et al. 2011. BJP). We hypothesized that atropine and other myopia-inhibiting mAChRs work through  $\alpha_{2A}$ -adrenoceptors.

**Methods:** Human  $M_4$  ( $M_4$ ), chicken  $M_4$  ( $cM_4$ ), or human  $\alpha_{2A}$ -adrenoceptor (hADRA2A) clones were transiently co-transfected with cAMP response element luciferase vector (CRE-Luc) and constitutively active *Renilla* luciferase vector (RLuc) into HEK293T cells. The ability of increasing concentrations of antagonist (AT: atropine, MT3, HM: himbacine, PZ: pirenzepine, TP: tropicamide, OX: oxyphenonium, QNB; DC: dicyclomine & MP: mepenzolate) to inhibit agonist-induced CRE-Luc expression was measured using the Dual-Glo<sup>®</sup> Luciferase Assay System (Promega). Normalized data were graphed as curve-fitted log dose-responses and  $pIC_{50}$  values were obtained from nonlinear regression analysis.

**Results:** There were no significant functional differences for antagonists at  $M_4$  compared to  $cM_4$  with the exception of MT3 ( $pIC_{50} = 8.08/6.35$ ,  $p < 0.0001$ , Fig. 1); relative potencies were: QNB (9.94/9.51) > AT (9.41/9.15) ≤ OX (9.40/9.25) > MP (8.85/8.45) > HM (7.98/8.25) > DC (7.82/7.39) > PZ (7.63/7.31) > TP (6.81/6.61). At hADRA2A, relative potencies were: MT3 (7.81) > HM (4.78) > AT (4.34) > QNB (3.58) > OX (3.33) > MP (3.10) > PZ (3.062) > TP (2.83) > DC (no effect).

**Conclusions:** Here, we confirm reports that MT3 is a high affinity antagonist at hADRA2A (Fig. 2) and mAChR  $M_4$ , and show that other mAChR antagonists have functional effects at hADRA2A.  $pIC_{50}$  data for these ligands at hADRA2A, but not  $M_4/cM_4$ , correlate well with their reported abilities to inhibit myopia in the chick (Luft et al. 2003. IOVS). Doses ≥ 10 mM are required for myopia inhibition by mAChR antagonists; thus, low potency of most mAChR antagonists at hADRA2A corroborates the hypothesis that hADRA2A may contribute to regulation of eye growth. While there are no species-differences in the affinities of orthosteric ligands, there is a significant difference in the affinity of MT3 at  $M_4$  compared to  $cM_4$ .

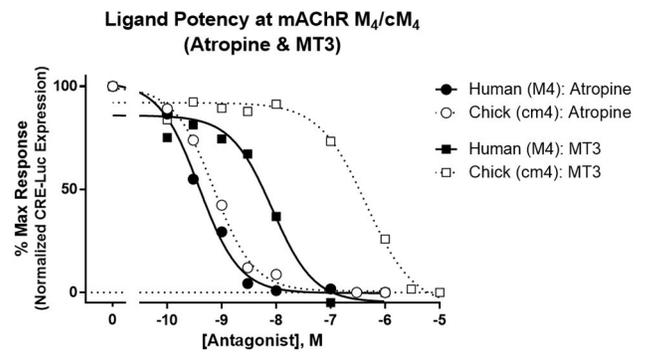


Figure 1. Potency of atropine (circle) and MT3 (square) at human (black) and chicken (white) mAChR  $M_4$ .  $pIC_{50}$  values  $\pm$  SEM were calculated for atropine:  $M_4 = 9.41 \pm 0.05$  versus  $cM_4 = 9.15 \pm 0.07$  and MT3:  $M_4 = 8.08 \pm 0.08$  versus  $cM_4 = 6.35 \pm 0.12$ ,  $p < 0.0001$  unpaired t-test, two-tailed.

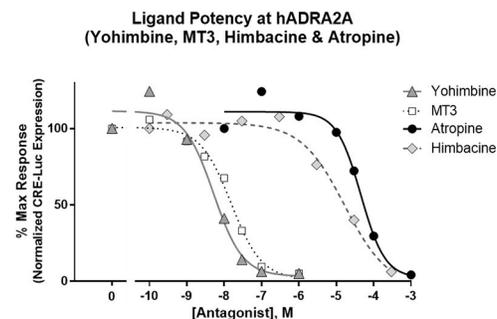


Figure 2. Potency of yohimbine ( $\blacktriangle$ ), MT3 ( $\square$ ), himbacine ( $\diamond$ ) and atropine ( $\bullet$ ) at human  $\alpha_{2A}$ -adrenoceptor (hADRA2A).  $pIC_{50}$  values  $\pm$  SEM for yohimbine ( $8.28 \pm 0.12$ ) and MT3 ( $7.81 \pm 0.11$ ) are consistent with high affinity at hADRA2A. After MT3, himbacine ( $pIC_{50} = 4.78 \pm 0.23$ ) and atropine ( $pIC_{50} = 4.34 \pm 0.11$ ) are the most potent blockers of hADRA2A-mediated signalling. Their affinity for hADRA2A is low, but falls within the range of the concentration of drug expected to reach the retina after intravitreal injection of in the chick.

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Presentation Time: 8:30 AM–10:15 AM

### The Effects of Atropine on the Development of Binocular and Monocular Lens-Induced Myopia in Marmosets

Alexandra Benavente-Perez<sup>1</sup>, Ann Nour<sup>1</sup>, Tobin Ansell<sup>1</sup>, Xiaoying Zhu<sup>1</sup>, Rita Nieu<sup>1</sup>, Harrison Feng<sup>1</sup>, Xu Cheng<sup>2</sup>, Noel A. Brennan<sup>2</sup>, David Troilo<sup>1</sup>. <sup>1</sup>Biological Sciences, SUNY College of Optometry, New York, NY; <sup>2</sup>Johnson and Johnson Vision Care, Inc., Jacksonville, FL.

**Purpose:** Topical low-dose atropine has been identified as a possible drug treatment to reduce myopia progression in children, although there remain questions about the dosing and mechanisms of action. In this study we present preliminary evidence of atropine effects on the development of experimental myopia in marmosets.

**Methods:** Marmosets wore -5D single vision soft contact lenses either binocularly (n=15) or monocularly (OD -5D, OS plano, n=7) from 70 days of age for 10 weeks while being treated monocularly on the right eye with 1%, 0.1%, or 0.005% atropine (vehicle drops on left eyes). On-axis vitreous chamber depth (VC) and refractive state (Rx) were measured and compared interocularly, and also to monocularly treated -5D marmosets without atropine (n=8) and untreated marmosets (n=14).

#### Results:

Rearing marmosets with binocular -5D contact lenses induced significantly greater Rx and VC compensatory changes than treating

them monocularly (overall Rx and VC change on treated eyes at the end of treatment, mean±SE: binocular group  $-6.76\pm 1.50D$ ,  $0.85\pm 0.13mm$ ; monocular group  $-2.93\pm 1.56D$ ,  $0.65\pm 0.10mm$ ,  $p<0.001$ ). Atropine 1% effectively eliminated the response to monocular -5D lenses seen in non-atropine treated marmosets. Unlike the significant induced axial myopia seen in non-atropine eyes (interocular VC difference:  $0.13\pm 0.05mm$ , Rx difference:  $-2.81\pm 0.74D$ ,  $p<0.05$ ), there were no interocular differences in VC or Rx at any ages tested in atropine treated marmosets (Repeated ANOVA Rx,  $p=0.23$ ; VC  $p=0.35$ ). However, low dose atropine (0.005% and 0.01%) was ineffective in reducing axial myopia in the binocular -5D lens reared marmosets (overall VC change at the end of treatment for 0.01% atropine; OD:  $0.85\pm 0.05mm$ , OS:  $0.87\pm 0.04mm$ ,  $p=0.68$ ; 0.005% atropine; OD:  $0.84\pm 0.05mm$ , OS:  $0.81\pm 0.04mm$ ,  $p=0.30$ )

**Conclusions:** The compensatory response to imposed hyperopic defocus is greater in binocularly treated marmosets suggesting interocular interactions in the visual control of emmetropization. Atropine at higher doses blocked lens imposed myopia by reducing axial growth in monocular treatments where less myopia was produced. Lower doses were not effective in reducing myopia at higher rates of development in binocularly treated animals.

**Commercial Relationships:** Alexandra Benavente-Perez, Johnson and Johnson Vision Care, Inc. (F); Ann Nour, Johnson and Johnson Vision Care, Inc. (F); Tobin Ansel, None; Xiaoying Zhu, None; Rita Nieu, None; Harrison Feng, None; Xu Cheng, Johnson and Johnson Vision Care, Inc (E); Noel A. Brennan, Johnson and Johnson Vision Care, Inc (E); David Troilo, Johnson and Johnson Vision Care, Inc (F)

**Support:** Johnson and Johnson Vision Care, Inc.

**Program Number:** 5467 **Poster Board Number:** B0624

**Presentation Time:** 8:30 AM–10:15 AM

#### **The Effect of Atropine on Lens Induced Myopia in the Guinea Pig**

*Sally A. McFadden.* Faculty of Science and IT, University of Newcastle, Callaghan, NSW, Australia.

**Purpose:** Atropine has long been proposed as a treatment for myopia: early studies show that atropine can slow myopia progression in children. However, rebound occurs and unacceptable side effects include blurred vision and photophobia (Tong et al, 2009). These side effects are not observed with lower doses of atropine (0.5% and 0.1%) which still inhibit myopic progression, although with reduced treatment impact (Chia et al, 2012). We studied the effect of atropine eye drops on eye growth and on the initial response to lens-induced myopia in young guinea pigs to determine if atropine can inhibit defocus-induced myopia.

**Methods:** To induce myopia, guinea pigs wore a -6D lens on one eye from 7-14 days of age. Simultaneously, animals received either Atropine (0.1%, N=19) or PBS (1mM, N=16) on the lens-wearing eye. To test the effect on normal growth, another group (N=7) that did not wear lenses, received 1% Atropine on one eye and PBS on the fellow eye from 9-24 days of age. Drugs were topically applied to the cornea twice/day. After 1 week of eye drops (at 15 or 17 days of age), both eyes were cyclopleged and refractive error measured. Animals were anaesthetised with isoflurane and axial length measured with high frequency ultrasound. Intraocular pressure was measured in the normal growth animals after 2 weeks of eye drops. The difference between the two eyes (diff) was assessed with matched-pair t-tests.

**Results:** Both groups wearing a lens and treated with either saline or 0.1% atropine developed significant relative myopia (diff:  $-2.6\pm 0.7D$ ,  $p=.014$  and  $-2.7\pm 0.8D$ ,  $p=.013$  respectively) and ocular expansion ( $30\pm 13\mu m$ ,  $p=.032$ ;  $34\pm 16\mu m$ ,  $p=.047$  respectively). The degree

of myopia and ocular increase was similar in both groups ( $p=.93$  and  $p=.85$ ). However, atropine treated eyes developed a thinner crystalline lens than saline-treated animals ( $-16\mu m$  Vs.  $+4\mu m$ ) and increased anterior chamber depths ( $+29\mu m$  Vs  $+1\mu m$ ). Similarly, 6/7 animals not wearing a lens but treated with 1% atropine also had thinner lenses (mean diff to saline eye:  $-27\pm 7\mu m$ ,  $p=.01$ ). However, 1% atropine did not change normal refractive development over 1 week (diff of .99D,  $p=.4$ ) or intraocular pressure after 2 weeks.

**Conclusions:** Low dose Atropine fails to inhibit the early induction of myopia and ocular elongation from minus lens wear in young guinea pigs, and instead affects the anterior of the eye, suggesting it does not directly target myopia initiation from defocus signals.

**Commercial Relationships:** Sally A. McFadden, Newcastle Innovation (P)

**Support:** HMRI/NI G1501382

**Program Number:** 5468 **Poster Board Number:** B0625

**Presentation Time:** 8:30 AM–10:15 AM

#### **Attenuation of myopia progression by aripiprazole is dependent on form deprivation**

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**Purpose:** Although there is suggestive pharmacological evidence that dopamine D2 receptor activation promotes form-deprivation myopia (FDM) development, this notion requires further validation. This uncertainty exists because the drugs used for this purpose are only proved to be effective on development of FDM, but the exact cause and mechanism of myopia is unknown. To address this question, we evaluated the effects of a dopamine D2 receptor partial agonist, aripiprazole, to assess D2 receptor involvement in controlling FDM development.

**Methods:** C57BL/6 mice were raised either in a visually normal environment or wore sight compromising goggles inducing FDM development from a postnatal age of 28 to 56 days. Both groups were divided into 4 sub-groups including control, vehicle and two different aripiprazole dosage (1 mg/kg and 10 mg/kg) treatments groups who were injected in the peritoneum with this drug. Their body weight, refraction, corneal radius of curvature, and ocular axial components were measured prior to and after the experiment. Monocular photopic flash electroretinograms were recorded from the form-deprived eyes.

**Results:** In normal mice, daily injection of aripiprazole did not affect normal postnatal refraction development. However, in the FDM group, the higher aripiprazole dose attenuated form deprived myopia development ( $5.98\pm 0.92D$  in FDM - aripiprazole versus  $10.38\pm 0.88D$  in FDM - vehicle,  $p<0.05$ ), with a shortened vitreous chamber depth ( $0.015\pm 0.006mm$  in FDM - Aripiprazole versus  $0.035\pm 0.003mm$  in FDM - vehicle,  $p<0.05$ ). Neither of the drug treatments altered corneal radius of curvature and other axial components from those in the vehicle-treated and control groups. The aripiprazole dose did not affect FDM development at low dose, but decreased the ERG b-wave amplitudes of the form-deprived eyes in a dose-dependent manner.

**Conclusions:** Aripiprazole only attenuated FDM without altering refraction development under normal vision environment or in vehicle injected mice. This dependence on form deprivation suggests that vision obstruction may somehow alter the response patterns controlled by D2R-linked signaling. Additional studies are warranted to gain insight into the underlying molecular mechanisms accounting for why aripiprazole suppresses myopia development.

**Commercial Relationships:** Furong Huang; Fen Wan, None; Yanan Yang, None; Jia Qu, None; Xiangtian Zhou, None

**Support:** Natural Science Foundation of Zhejiang (LQ16H120005, <http://www.zjnsf.gov.cn/>)

**Program Number:** 5469 **Poster Board Number:** B0626

**Presentation Time:** 8:30 AM–10:15 AM

**Effect of Topical Latanoprost on Myopia Progression in Guinea Pigs**

*Nevin El-Nimri, Christine F. Wildsoet.* Vision Science, UC Berkeley, Berkeley, CA.

**Purpose:** To determine, using the guinea pig form deprivation myopia model, whether ocular hypotensive prostaglandin analogs, which are very effective in reducing intraocular pressure (IOP) in humans, can also slow myopia progression.

**Methods:** Young guinea pigs underwent monocular form deprivation (FD; white plastic diffusers) from 14-days of age for 12-weeks. After the first week, FD eyes also received daily topical A) latanoprost (Lat, 0.005%, n=4) or B) vehicle (Veh; artificial tears; n=4). Weekly tonometry (iCare), retinoscopy and high frequency A-scan ultrasonography were undertaken to monitor IOP as well as refractive error and ocular axial dimensions (recorded as spherical equivalent values and optical axial lengths respectively). Additional IOPs were recorded at 6 h intervals, both at baseline and monthly, to examine diurnal IOP variations.

**Results:** Lat both reduced IOP and slowed myopia progression relative to the vehicle treatment. Mean interocular IOP differences ( $\pm$  SD) changes from baseline values of  $-0.58 \pm 0.94$  mmHg (Veh) and  $-1.25 \pm 1.1$  mmHg (Lat) to  $0.56 \pm 1.34$  mmHg (Veh) and  $-7.11 \pm 3.66$  mmHg (Lat) at 12 weeks. Interocular optical axial length differences changed from  $-0.01 \pm 0.04$  to  $0.22 \pm 0.13$  mm (Veh) and  $-0.05 \pm 0.06$  to  $-0.01 \pm 0.05$  mm (Lat), and interocular refractive error differences changed from  $0.81 \pm 1.46$  to  $-8.5 \pm 0.70$  D (Veh) and  $-0.44 \pm 1.1$  to  $0.5 \pm 1.06$  D (Lat). IOP fluctuations (maximum-minimum) appeared to be lower and less variable in FD Lat eyes compared to FD Veh eyes ( $8.78 \pm 1.21$  mmHg (Lat) vs.  $11.92 \pm 4.50$  mmHg (veh), although not significantly so ( $p=0.26$ ).

**Conclusions:** The results demonstrate that daily topical latanoprost is effective in both lowering IOP and slowing myopia progression in FD eyes of young guinea pigs. This result contrasts with those with the ocular hypotensive drug, timolol, a beta-blocker, which was shown in previous studies to be relatively ineffective in slowing myopia progression in chicks and humans.

**Commercial Relationships:** Nevin El-Nimri, None; Christine F. Wildsoet, None

**Program Number:** 5470 **Poster Board Number:** B0627

**Presentation Time:** 8:30 AM–10:15 AM

**Amphiregulin Antibody and Reduction of Axial Elongation in Experimental Myopia**

*Wen Jun Jiang<sup>1</sup>, Jian Feng Wu<sup>1</sup>, Dadong Guo<sup>1</sup>, Hongsheng Bi<sup>1</sup>, Jost B. Jonas<sup>2</sup>.* <sup>1</sup>Ophthalmology, Eye Institute of Shandong University of Traditional Chinese Medicine, Jinan, China; <sup>2</sup>Medical Faculty Mannheim of the 16 Ruprecht-Karls-University Heidelberg, Mannheim, Germany.

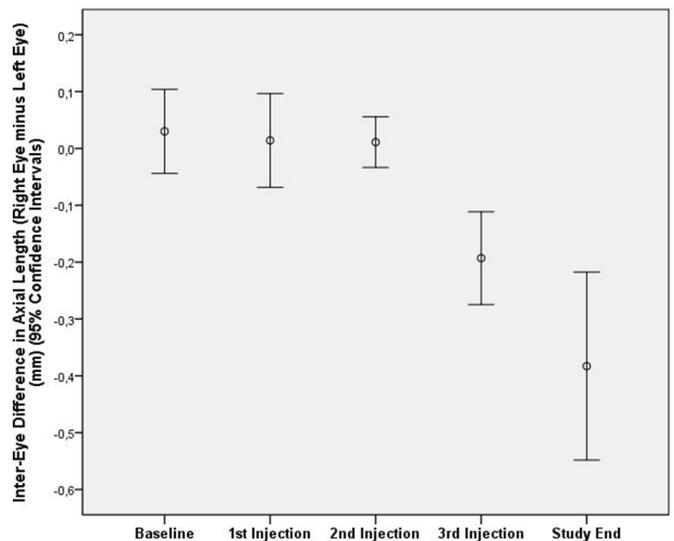
**Purpose:** To examine the influence of an intraocularly applied antibody to amphiregulin, a bifunctional growth modulator interacting with the epithelial growth factor/tissue growth factor- $\alpha$  receptor, on ocular axial elongation.

**Methods:** Guinea pigs (age:2-3 weeks) undergoing unilateral or bilateral lens-induced myopization (group 1), and guinea pigs which were primarily myopic at baseline without additional lens-induced myopization (group 2), received unilateral intraocular injections of amphiregulin antibody (doses:5,10, or 15 $\mu$ g) three times in intervals of 9 days, while the contralateral eyes received intraocular injections

of Ringers solution. A third group of guinea pigs with normal refractive error at baseline and without lens-induced myopization received intraocular unilateral injections of amphiregulin (doses:0.25,0.50 or 1.00ng, respectively), and injections of Ringers solution into the contralateral eyes.

**Results:** In intra-animal inter-eye comparison and intra-eye follow-up comparison in groups 1 and 2, the study eyes as compared to the contralateral eyes showed a dose-dependent reduction in axial elongation. In group 3, study eyes and control eyes did not differ significantly in axial elongation. Immunohistochemistry revealed amphiregulin labelling at the retinal pigment epithelium in eyes with lens-induced myopization and Ringers solution injection, but not in eyes with amphiregulin antibody injection.

**Conclusions:** Repeated intraocular injection of amphiregulin-antibody was associated with a reduction of lens-induced axial myopic elongation and with reduction of the physiological eye enlargement in young guinea pigs. In contrast, intraocularly injected amphiregulin in a dose of  $\leq 1$ ng did not show a significant effect. Amphiregulin may be one of several essential molecular factors for axial elongation in young guinea pigs.



**Commercial Relationships:** Wen Jun Jiang, None; Jian Feng Wu, None; Dadong Guo, None; Hongsheng Bi, None; Jost B. Jonas, Biocompatible UK Ltd. (F)

**Program Number:** 5471 **Poster Board Number:** B0628

**Presentation Time:** 8:30 AM–10:15 AM

**Transient exposure of high concentration oxygen induces sustained myopia in adult mice**

*Kiwako Mori<sup>1,2</sup>, Toshihide Kurihara<sup>1,2</sup>, Xiaoyan Jiang<sup>1,2</sup>, Erisa Yotsukura<sup>1,2</sup>, YASUHISA TANAKA<sup>1,2</sup>, Shin-ichi Ikeda<sup>1,2</sup>, Maki Miyauchi<sup>1,2</sup>, Hidemasa Torii<sup>1,2</sup>, Kazuo Tsubota<sup>1</sup>.*

<sup>1</sup>Ophthalmology, Keio University, Shinjuku, Japan; <sup>2</sup>Laboratory of Photobiology, Keio University, Tokyo, Japan.

**Purpose:** Progression of myopia can be observed in adult human even after 20 years old, though myopia generally develops in children of school age. Particular population in adult progressive myopia may develop pathologic myopia from high myopia; however, its mechanism and therapeutics have still not been established. Several literatures reported that myopia may be induced under the circumstances of hyperbaric oxygenation in human. In this study, we examined effects of high concentration oxygen for the refraction in adult mice.

**Methods:** Ocular components of C57BL6/J mice including refraction, corneal radius (CR), and axial length (AL) were measured by an infrared-light refractometer (Steinbeis Transfer Center, Germany) or an optical coherence tomography device (Envisu R4310, Leica) at the postnatal 5 weeks old (P5W) as a baseline analysis. Mice were randomly divided into two groups, Oxygen group and Control group. The mice in the Oxygen group were put into a chamber with 85% oxygen concentration for 78 hours at P6W, and then put back under a normal oxygen environment. In the Control group, mice were put under normal air during the all experimental period (n=6). The ocular components were examined again 1 week and 6 weeks after oxygen exposure (at P7W and P12W, n=6). The mice were kept under a 12/12-hour light-dark cycle. Statistical analysis was performed using Student's t-test.

**Results:** Compared to the Control group, the Oxygen group showed a significant myopic change at P7W and P12W. The mean values of diopter change from the baseline in each group were +10.71 vs -6.78 (at P7W, p<0.01) and +7.52 vs -7.20 (at P12W, p<0.01). The AL between the groups did not show significant difference; however, the CR (mm) in the Oxygen group was significantly short (1.50 vs 1.44 at P12W, p<0.05), and the AL/CR ratio was significantly increased (2.29 vs 2.40 at P12W, p<0.01).

**Conclusions:** Myopia was induced by high concentration oxygen exposure and sustained at least for 6 weeks in adult mice. The phenotype depends on changes in the corneal structure rather than the AL. These data suggested that high concentration oxygen may affect anterior components of the eye to induce a myopic refraction shift.

**Commercial Relationships:** **Kiwako Mori**, **Toshihide Kurihara**, None; **Xiaoyan Jiang**, None; **Erisa Yotsukura**, None; **YASUHISA TANAKA**, None; **Shin-ichi Ikeda**, None; **Maki Miyauchi**, None; **Hidemasa Torii**, None; **Kazuo Tsubota**, None

**Program Number:** 5472 **Poster Board Number:** B0629

**Presentation Time:** 8:30 AM–10:15 AM

**BMP2 Protein Increases the Expression of Genes for Inhibitor of DNA Binding Proteins in Cultured Chick Scleral Fibroblasts**

*Yan Zhang, Wendy Yang, Abraham Hang, Emilia Zin, Mariana Garcia, Mei Li, Christine F. Wildsoet.* School of Optometry, Univ of California, Berkeley, Berkeley, CA.

**Purpose:** The Bone Morphogenetic Protein (BMP) signaling pathway has previously been linked to defocus-driven eye growth regulation in chick. This study used cultured chick scleral fibroblasts as an *in vitro* model system to investigate the effects of recombinant

human BMP2 (rhBMP2) protein on elements of this signaling pathway.

**Methods:** Chick scleral fibroblasts (CSFs) were cultured in DMEM/F12 medium with 10% FBS. The CSFs were treated with 0, 1, 10, 100, or 1000 ng/ml BMP2 in DMEM/F12 medium for two hours, after which cells were collected and RNA extracted, purified and transcribed into cDNA. Gene expression of BMP2, BMP4, BMP7, BMP1A, BMP1B, BMP2, SMAD1, SMAD5, SMAD9, ID1-4 was quantified with qPCR and protein expression of the same signaling molecules examined with Western blot and immunocytochemistry.

**Results:** Genes for all of the above signaling molecules were detected, as were the SMAD1, p-SMAD1, and p-SMAD1/5 proteins. With all applied concentrations of rhBMP2, the gene expression of Inhibitor of DNA Binding proteins (ID1-4), were significantly increased (Table 1).

**Conclusions:** The BMP signaling pathway is very likely involved in chick scleral remodeling, as the exposure of cultured CSF to rhBMP2 resulted in significant changes in gene expression of down-stream transcription factors, i.e., Inhibitor of DNA Binding proteins.

Table 1. ID gene expression changes (up-regulation) in CSFs in response to applied rhBMP2.

	1 ng/ml	10 ng/ml	100 ng/ml	1000 ng/ml
ID1	86-fold	127-fold	168-fold	174-fold
ID2	26-fold	30-fold	35-fold	32-fold
ID3	13-fold	13-fold	12-fold	11-fold
ID4	3-fold	4-fold	3-fold	3-fold

**Commercial Relationships:** **Yan Zhang**, None; **Wendy Yang**, None; **Abraham Hang**, None; **Emilia Zin**, None; **Mariana Garcia**, None; **Mei Li**, None; **Christine F. Wildsoet**, None  
**Support:** NIH Grant K08 EY023609 (YZ), R01 EY012392 (CFW)

**Program Number:** 5473 **Poster Board Number:** B0630

**Presentation Time:** 8:30 AM–10:15 AM

**Myopia progression in adult *Lrp2/Bugeye* fish**

*Tiffany Tran<sup>1</sup>, Karla Villafan<sup>1</sup>, Kevin Z. Kwan<sup>1</sup>, D Joshua Cameron<sup>2</sup>.*

<sup>1</sup>Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona, CA; <sup>2</sup>College of Optometry, Western University of Health Sciences, Pomona, CA.

**Purpose:** Myopia occurs when light is focused prematurely on the neural retina. Zebrafish are a reliable model in terms of human vision research because they mimic much of the human visual system physiologically. By using the Optokinetic Response (OKR), many variables can be determined for both binocular and monocular visual functions. We used the OKR to calculate the visual acuity for the Low-Density Lipoprotein Receptor-Related 2 (*Lrp2*)/Bugeye fish binocularly and monocularly and tracked visual changes throughout adulthood. We hypothesized that visual acuity would diminish over time; an indication of myopia increase; the visual acuity as well as eye/head ratio will be used to verify if this hypothesis is proven or not.

**Methods:** Zebrafish were raised under normal conditions. Zebrafish binocular and monocular visual acuity was measured using the optokinetic response in *Lrp2/Bugeye* and quantified in cycles/degree (c/d). Visual acuity was correlated to eye and head size due to the asymmetric buphthalmia present in these fish. Initial visual acuities were taken at about 4 months of age before any of the fish showed noticeable buphthalmia. Subsequent measurements tracked myopia progression for each eye independently and also correlated the corresponding binocular vision. Statistical analysis was done using SPSS.

**Results:** As we had seen previously with similarly aged adult zebrafish, *Lrp2/Bugeye* fish had initial average visual acuities at < 0.60 c/d but were quite variable. With each progressing month, the visual acuities increased, though as an eye became larger the

visual acuities quickly diminished. Visual acuity and eye/head ratios determined at several weeks and then subsequent months and compared to baseline values. As with wild-type fish their VA continued to improve with age. However, fish whose eye(s) developed buphthalmia had a significant reduction in their VA. Many fish exhibited binocular summation that enabled them to see better with both eyes despite one eye being poorer than the other. While others, had binocular inhibition or showed partial summation.

**Conclusions:** Myopia is a condition that is caused by a plethora of factors. In this experiment, visual acuity measurements were determined using the OKR in adult zebrafish. Specifically, the *Lrp2*/Bugeye mutant fish displayed a progressive myopia development that can be tracked longitudinally using functional studies. This model may prove beneficial for future myopia studies.

**Commercial Relationships:** Tiffany Tran, None; Karla Villafan, None; Kevin Z. Kwan, None; D Joshua Cameron, None

**Program Number:** 5474 **Poster Board Number:** B0631

**Presentation Time:** 8:30 AM–10:15 AM

**LRP2 mediates retinoid homeostasis to regulate eye growth**

Ross F. Collery<sup>2,1</sup>, Kerry N. Veth<sup>1</sup>, Brian A. Link<sup>1</sup>. <sup>1</sup>Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI; <sup>2</sup>Ophthalmology and Visual Sciences, Medical College of Wisconsin, Milwaukee, WI.

**Purpose:** Mutations in *Lrp2*, a large transmembrane protein involved in receptor-mediated endocytosis and transcellular trafficking, are associated with pathological myopia in humans and zebrafish. In the eye, *Lrp2* is expressed exclusively in the retinal pigment epithelium and ciliary epithelia. *Lrp2* has many identified ligands, including the plasma retinol carrier, Rbp4. Since altered retinoid signaling has been implicated in experimental myopia, we explored the role of this pathway in the *lrp2* mutant phenotype.

**Methods:** To test the role of *Lrp2* in retinol trafficking, we constructed transgenic zebrafish in which Rbp4-GFP was secreted into the serum from liver hepatocytes, the endogenous source of Rbp4. Using CRISPRs to delete *stra6*, we tested the significance of upregulated *Stra6* for the large eye phenotype of *lrp2*<sup>-/-</sup> mutants.

**Results:** RNA transcript analysis showed altered levels of retinoic acid (RA) target genes and factors associated with retinoid homeostasis in *lrp2*<sup>-/-</sup> mutant eyes including *rlbp1*, *crabp2*, and *stra6*. Serum retinol levels were also significantly reduced compared to wild-type fish. *lrp2*<sup>-/-</sup> fish exposed to RA from 1-2 months of age were more sensitive to its effects than wild-type sibling controls. Importantly, *lrp2*<sup>-/-</sup> fish exposed to the lowest dose of RA showed ocular enlargement greater than DMSO-treated mutants, as well as RA- or DMSO-treated wild-type fish. Furthermore, *lrp2*<sup>-/-</sup> mutants showed genetic interaction with mutants for *cyp26a1*, a cytochrome P450-type enzyme that degrades RA. In humans, *cyp26a1* variants have been shown to be associated with myopia. Our genetic experiments showed that *lrp2*<sup>-/-</sup>;*cyp26a1*<sup>+/-</sup> animals exhibited exacerbated myopia compared to *lrp2*<sup>-/-</sup> mutant fish alone. Rbp4-GFP accumulated in the sclera and choroid layers of *lrp2*<sup>-/-</sup> mutant eyes, but not in those of wild-type sibling fish. In vivo fluorescent recovery after photobleaching analysis indicated that loss of *Lrp2* prevented normal Rbp4-GFP uptake into RPE cells. *lrp2*<sup>-/-</sup> *stra6*<sup>-/-</sup> double mutant fish were partially rescued for the myopic phenotype compared to *lrp2*<sup>-/-</sup> single mutants.

**Conclusions:** These and other results suggest that RPE cells modulate emmetropization in part by controlling homeostasis of periocular retinoids, and dysregulation contributes high myopia. This process is primarily mediated through *Lrp2*, and its loss results in accumulation of periocular retinol-RBP4 (the precursor for RA) and enhanced import (via upregulated *Stra6*).

**Commercial Relationships:** Ross F. Collery, None; Kerry N. Veth, None; Brian A. Link, None  
**Support:** NIH/NEI R01EY016060; NIH/NEI P30EY001931

**Program Number:** 5475 **Poster Board Number:** B0632

**Presentation Time:** 8:30 AM–10:15 AM

**Inhibition of choroidal retinoic acid synthesis by dichloro-all-trans-retinone (DAR)**

Angelica Harper<sup>1</sup>, Tim Mather<sup>2</sup>, Jody A. Summers Rada<sup>1</sup>. <sup>1</sup>Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; <sup>2</sup>Oklahoma Medical Research Foundation, Oklahoma City, OK.

**Purpose:** Choroidal retinaldehyde dehydrogenase 2 (RALDH2) has been identified as a potential pharmaceutical target for the control of postnatal ocular growth. Our lab has recently developed a novel RALDH2 selective inhibitor, dichloro-all-trans-retinone (DAR) to examine the role of RALDH2 in the control of visually guided eye growth. The objective of the current study was to evaluate the selectivity and potency of DAR, *in vitro* and *ex vivo* as a foundation for future *in vivo* experiments on DAR's efficacy as an ocular growth modulator.

**Methods:** Enzyme specificity and mechanism of inhibition was determined *in vitro* using NADH assays with recombinant chick and human RALDH1, RALDH2, RALDH3, and ALDH2. *In vitro* and *ex vivo* experiments utilized a cell line with doxycycline-inducible RALDH2 [(<sup>Dox</sup>)RALDH2-eGFP 293], as well as choroidal lysates isolated from chick eyes following 4 days of recovery from form deprivation myopia. Retinoic acid (RA) synthesis by choroidal lysates with and without DAR (0–10 μM) was quantified by HPLC/MS. DAR toxicity was evaluated on chick sclera utilizing an *in vitro* proteoglycan synthesis assay.

**Results:** *In vitro* assays using recombinant protein indicated that DAR inhibits both chick and human RALDH2 with an IC<sub>50</sub>=52.2 nM and 173 nM, respectively. DAR also inhibited RALDH1 and RALDH3 at higher concentrations (RALDH1, IC<sub>50</sub>=670 nM; RALDH3, IC<sub>50</sub>=340 nM), but had no inhibitory effect on human mitochondrial ALDH2. Inhibition of all RALDH enzymes was concentration and time dependent, suggesting irreversible inhibition. Pretreatment of (<sup>Dox</sup>)RALDH2-eGFP 293 cell lines with DAR (0–2 μM; in the presence of 10 μM retinaldehyde) resulted in significant inhibition of RALDH activity in cell lysates (↓96%, p<0.001, ANOVA) and significant inhibition of RA synthesis *in vitro* (↓42%, p<0.001, ANOVA). Similarly, following incubation of choroids isolated from control and recovering eyes with DAR (0–5 μM; 18 hrs at 37°C) significant inhibition of RALDH activity in tissue lysates was also observed (↓38%, p<0.05, ANOVA), with an IC<sub>50</sub>=53.6 nM. Incubation of sclera with DAR resulted in significant inhibition of scleral proteoglycan synthesis at concentrations >10 μM (p<0.001, ANOVA) (IC<sub>50</sub>=15.5 μM).

**Conclusions:** Dichloro-all-trans-retinone effectively inhibits RALDH activity and RA synthesis in cultured cells and in isolated choroids.

**Commercial Relationships:** Angelica Harper, OUHSC (P); Tim Mather, OUHSC (P); Jody A. Summers Rada, OUHSC (P)  
**Support:** F31EY025168 (ARH), R01 EY09391 (JAS)

**Program Number:** 5476 **Poster Board Number:** B0633

**Presentation Time:** 8:30 AM–10:15 AM

**MicroRNA profiling reveals the differentially expressed microRNAs are associated with metabolic imbalance in myopic guinea pigs**

*Dadong Guo, Wenjun Jiang, Hongsheng Bi.* Cell Biology, Eye Institute of Shandong University of Traditional Chinese Medicine, Ji'nan, China.

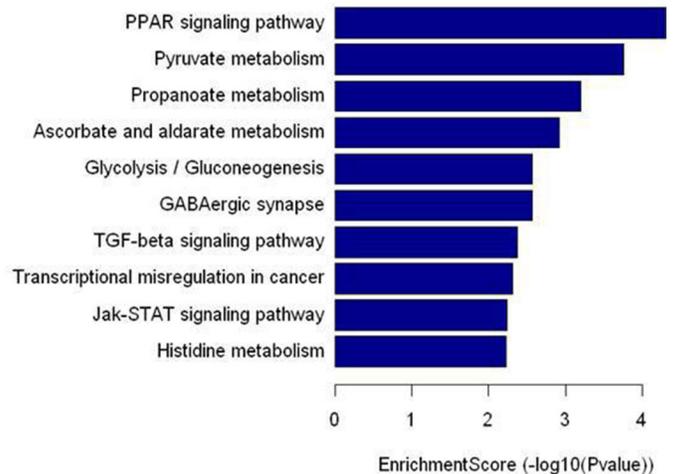
**Purpose:** Myopia is the leading cause of visual impairment in the world. The sclera is critical in determining the absolute size of the eye, and thus plays a role in the development of myopia. MicroRNAs (miRNAs) involve in many physiological and pathological processes in the disease progression. The present study aimed to investigate the role of miRNAs in the development of myopia in guinea pig sclera.

**Methods:** We induced a myopic guinea pig model with -10D negative lens on the right eye using 10 guinea pigs (3-week-old). Meanwhile, the left eyes were covered with plano lens and were as self-control. Before and after induction of myopia for 2 weeks, we assessed the alterations of ocular axial length and refractive error, identified the differentially expressed microRNAs in lens-induced myopic (LIM) guinea pig sclera versus fellow subject based on sequencing data, and performed the bioinformatic analysis for the differentially expressed miRNAs. Moreover, we also validated the differentially expressed miRNAs, and measured the expression of peroxisome proliferator-activated receptor  $\alpha$  regulated by differentially expressed miRNAs at the both mRNA and protein levels.

**Results:** Our results indicated that compared with LIM fellow eyes, axial length were increased whereas the refraction decreased, accompanied by statistically differences in LIM eyes. There were 27 differentially expressed miRNAs, 10 upregulated and 17 downregulated miRNAs in LIM eyes versus LIM fellow eyes. Bioinformatics analysis indicated that the miRNA-targeted genes were classified into 1108 categories, including cellular process, single-organism process, single-organism cellular process, biological regulation, metabolic process and so on. KEGG function annotation showed the signaling pathways regulated by differentially expressed miRNAs were mainly related to PPAR signaling pathway. Our Q-PCR and western blot assays also validated downregulated expressions of PPAR- $\alpha$  in LIM eyes.

**Conclusions:** The occurrence of myopia is closely linked to the activation of multiple signaling pathways, including PPAR signaling pathway, pyruvate metabolism, propanoate metabolism, GABAergic synapse, TGF- $\beta$  signaling pathway. These signaling pathways can be regulated by differentially expressed miRNAs. Our results indicate that the occurrence of myopia may be associated with metabolic imbalance.

**Signaling pathways of differentially expressed genes**



KEGG signaling pathway analysis.

**Commercial Relationships:** Dadong Guo, Wenjun Jiang, None; Hongsheng Bi, None

**Support:** NSFC Grant 81303081, 81173440

**Program Number:** 5477 **Poster Board Number:** B0634

**Presentation Time:** 8:30 AM–10:15 AM

**Quantitative proteomics analysis of chick retina in response to nicotinic acid (NA) oral intake and lens-induced myopia (LIM)**

*Hu XIAO, Sze Wan Shan, Thomas Lam, Rachel Ka-man Chun, Chi-ho To.* School of Optometry, The Hong Kong Polytechnic University, Hong Kong, China.

**Purpose:** Protective effect of NA on LIM eye growth in chicks was reported previously. To understand the underlying biological mechanism, protein profiling of the myopic chick retinas in response to oral intake of NA was studied by label free SWATH-MS.

**Methods:** White Leghorn chicks at 4 days old (PN4) were randomly allocated into 2 groups (treatment: n=8; control: n=8). A single dose of 1ml NA (150mg) was orally fed daily in treatment group, while the control chicks received saline orally for 10 days (PN4 to PN14). At PN10, -10D lenses were attached to both eyes of all chicks. The refractive errors and ocular parameters were examined on PN10 and PN14. The changes of refractive errors and ocular parameters before and after the treatment were analyzed by t-test (SPSS 23.0). The retinal samples were collected on PN14. Differentially expressed proteins between 2 groups were identified and quantified by LC-MS/MS.

**Results:** After 4 days of lens wear, chicks in the treatment group became significantly less myopic than the controls, the changes of refractive error (treatment vs. control;  $-5.563 \pm 0.513D$  vs.  $-8.875 \pm 0.463D$ ;  $p < 0.001$ ), vitreous chamber depth ( $0.548 \pm 0.146mm$  vs.  $0.713 \pm 0.084mm$ ;  $p = 0.015$ ), and axial length ( $0.801 \pm 0.172mm$  vs.  $0.983 \pm 0.093mm$ ;  $p = 0.024$ ) were all significant. A total of 4832 proteins were identified (at 1% FDR) which formed a chick retinal proteome library for SWATH data analysis. Thirty differentially expressed proteins were found ( $p < 0.05$ ; fold changes  $> 1.3$  folds with not less than 2 peptides). According to bioinformatics analysis, the top 5 identified pathways were insulin resistance, regulation of actin cytoskeleton, insulin signaling, endocytosis and phototransduction.

**Conclusions:** Oral intake of NA retarded myopic development in LIM chicks. Proteomic approach help identify differentially expressed proteins in response of NA intake, and the potential pathways that involved. For examples, GYS2 was differentially

expressed which is involved in both insulin resistance and insulin signaling pathways, and these 2 pathways were previously reported in modulating eye growth. ARPC1A and GRK1 which involved in endocytosis pathway were found significantly changed. Our results suggested possible underlying pathways which may be involved in the protective effect of NA on myopic eye growth. New candidate proteins were identified for further confirmation.

**Commercial Relationships:** Hu XIAO, None; Sze Wan Shan, None; Thomas Lam, None; Rachel Ka-man Chun, None; Chi-ho To, None

**Program Number:** 5478 **Poster Board Number:** B0635

**Presentation Time:** 8:30 AM–10:15 AM

**Ion and Proteome Expression in Early Development of Refractive Errors**

Sheila G. Crewther<sup>1</sup>, Nina Riddell<sup>1</sup>, Alan Marshall<sup>2</sup>. <sup>1</sup>Psychological Science, La Trobe University, Melbourne, VIC, Australia; <sup>2</sup>Department of Ecology, Evolution and Environment, La Trobe University, Melbourne, VIC, Australia.

**Purpose:** Although much is known is about the ultrastructural, physiological, and genomic changes associated with development of optically induced refractive errors, little research exists about concurrent early changes in biometrics, proteins and ion distribution patterns across the retina/RPE.

**Methods:** Chicks were fitted with +10D lenses, -10D lenses or No Lens on post-hatch day 5. Biometrics were performed on 5 chicks per lens group following a further 6 or 48hrs. After anesthesia, eyes were enucleated and the posterior retina/RPE was divided and concurrently prepared for elemental microanalysis on a scanning electron microscope and for proteomics by LC-ESI-MS/MS on a LTQ-orbitrap Elite (Thermo-Scientific). Proteomic raw data was processed using MaxQuant. Differentially abundant proteins were identified using t-test (FDR<0.05) in Perseus and pathway expression changes were identified using Gene Set Enrichment Analysis (GSEA).

**Results:** Biometrics showed 3.9D and 7.7D of refractive compensation (RC) to +10D defocus after 6 and 48 hours respectively, while RC to -10D was -2.6D and -9.7D at the same times. X-Ray microanalysis demonstrated that 6hrs of RC to optical defocus is accompanied by increased hyperosmolarity for K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> ions across the retina/RPE compared to No Lens eye. This hyperosmolarity persisted through to 48hrs of defocus. In line with this, expression of proteins in the Solute-Carrier (SLC) mediated transmembrane transport pathway for ions and water was significantly down-regulated following 6hrs of myopia induction.

**Conclusions:** Chicks show substantial refractive compensation and associated hyperosmotic shifts and protein expression changes after just 6 hours of refractive compensation to defocusing lenses. These findings highlight the importance of the acute period post-lensing for understanding the aetiology of optically-induced growth changes.

**Commercial Relationships:** Sheila G. Crewther, None; Nina Riddell, None; Alan Marshall, None

**Program Number:** 5479 **Poster Board Number:** B0636

**Presentation Time:** 8:30 AM–10:15 AM

**Retina/RPE proteome profiles in the chick model of optically-induced refractive error**

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**Purpose:** Several exploratory transcriptome studies have profiled gene expression in the widely used chick model of refractive error, however, relatively little is known about corresponding proteome changes. Thus, this study used an exploratory label-free relative quantification approach to profile protein expression in the chick retina/RPE following 6 and 48hrs of defocus-induced myopia and hyperopia.

**Methods:** Chicks were raised from post-hatch day 5 with monocular +10D or -10D lenses, or No Lens. Following 6 and 48hrs, five chicks per lens-group were anesthetized and biometric measures were collected. Retina/RPE sections were then removed from the posterior eyecup and prepared for analysis by LC-ESI-MS/MS on a LTQ-orbitrap Elite (Thermo-Scientific). Raw data was processed using MaxQuant. Differentially-expressed proteins were identified using t-test (FDR<0.05) in Perseus, and Reactome pathway expression changes were identified using Gene Set Enrichment Analysis (GSEA; FDR<0.05).

**Results:** A large number of proteins were differentially-expressed in both negative (327 at 6hrs & 252 at 48hrs) and positive (162 at 6hrs & 542 at 48hrs) lens groups relative to age-matched controls. These single protein expression changes were highly similar across the lens groups, with only two proteins (E1BZE1/AHSG & R4GFD0/PSME4) differentially-expressed in a sign-of-defocus dependent manner. Pathways related to ribosomal protein expression were down-regulated in both lens groups at 6hrs. Further pathways related to energy metabolism, SLC-mediated transmembrane transport, insulin secretion, GABA synthesis/release/re-uptake, and hemostasis were down-regulated in the 6hr negative lens group only. The same pathways were down-regulated over time in normally developing animals.

**Conclusions:** Similarities across negative and positive lens groups suggest that most of the measured expression changes were responses to non-specific factors (e.g. blur or physiological stress). Although GSEA confirmed the early involvement of several pathways previously hypothesized to play a role in myopia induction (e.g. insulin, GABA, & energy metabolism), the results also suggest that expression changes in these pathways may reflect an acceleration of the normal development program. Further research investigating this interplay with normal development may help to elucidate the mechanisms driving these expression changes and clarify cross-study disparities.

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