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Gene Expression Signatures of Contact Lens-Induced Myopia in Guinea Pig Retinal Pigment Epithelium

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Purpose. To identify key retinal pigment epithelium (RPE) genes linked to the induction of myopia in guinea pigs.

Methods. To induce myopia, two-week-old pigmented guinea pigs (New Zealand strain, $n = 5$) wore −10 diopter (D) rigid gas-permeable contact lenses (CLs), for one day; fellow eyes were left without CLs and served as controls. Spherical equivalent refractive errors (SE) and axial length (AL) were measured at baseline and one day after initiation of CL wear. RNA sequencing was applied to RPE collected from both treated and fellow (control) eyes after one day of CL-wear to identify related gene expression changes. Additional RPE-RNA samples from treated and fellow eyes were subjected to quantitative real-time PCR (qRT-PCR) analysis for validation purposes.

Results. The CLs induced myopia. The change from baseline values in SE was significantly different ($P = 0.016$), whereas there was no significant difference in the change in AL ($P = 0.10$). RNA sequencing revealed significant interocular differences in the expression in RPE of 13 genes: eight genes were significantly upregulated in treated eyes relative to their fellows, and five genes, including bone morphogenetic protein 2 (*Bmp2*), were significantly downregulated. The latter result was also confirmed by qRT-PCR. Additional analysis of differentially expressed genes revealed significant enrichment for bone morphogenetic protein (BMP) and TGF-β signaling pathways.

Conclusions. The results of this RPE gene expression study provide further supporting evidence for an important role of BMP2 in eye growth regulation, here from a guinea pig myopia model.

Keywords: myopia, RPE, gene expression, Bmp2

Myopia (near-sightedness) is now the most common refractive error worldwide,[1] with rapidly rising prevalence figures attracting the attention of the World Health Organization,[2] due to the associated risks of irreversible sight-threatening complications, including glaucoma, myopic maculopathy, and retinal detachment, from which even low myopes are not exempt.[3] Although most myopia is the product of excessive ocular elongation, the underlying molecular and cellular mechanisms remain poorly understood apart from the recognized connection with abnormal visual experience. Nonetheless, there is convincing evidence that eye growth is regulated locally, with the signals driving growth originating in the retina.[4–8]

Our research focuses on the role of the retinal pigment epithelium (RPE)—a monolayer of cells separating the retina, the presumed source of growth-modulating signals, from the outer choroidal and scleral structural supporting walls.[9–11] Thus the RPE likely plays a key role in relaying signals from the retina to the choroid and sclera, driving both transient and more permanent structural changes that underlie increases in eye length.[12,13] Although barrier and homeostatic roles for the RPE (e.g., supplying nutrients to and regulating the extracellular fluid levels in the outer retina) are well recognized,[10,12] optical defocus-induced gene expression changes in the isolated chick RPE, reported by Zhang et al.[14–16] represent provocative evidence for this new role.

The purpose of the study described here was to investigate myopia-related RPE gene expression changes in young guinea pigs, which are one of the most commonly used mammalian models of myopia.[17–21] Specifically, we used negative power contact lenses (CLs) to induce monocular myopia, as evidenced by changes in both refractive errors

and axial lengths, after five days of lens wear. We also collected RPE samples from both eyes of animals wearing lenses for only one day and subjected samples to RNA-sequencing (RNA-seq) and real-time reverse transcription quantitative PCR (RT-qPCR), targeting in the latter case, bone morphogenic protein 2 (*Bmp2*), one of the genes identified to show defocus-induced gene expression changes in previous studies of chick RPE.

Methods

Animals

Two-week-old New Zealand strain pigmented guinea pigs were used in this study, with breeders obtained from the University of Auckland (Auckland, New Zealand). Pups were bred on site and weaned at five days of age and reared as single-sex pairs in transparent plastic tubs (41 × 51 × 22 cm). Animals were housed in a temperature-controlled room with a 12-hour light/12-hour dark cycle (on at 9:00 AM, off at 9:00 PM), with a cage floor illuminance of approximately 160 to 180 lux. Animals had free access to water and were fed a high-fiber guinea pig diet (Teklad 2041, Envigo), along with fresh fruit and vegetables three times a week as dietary enrichment. All animal care and treatments used in this study conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental protocols were approved by the Animal Care and Use Committee of the University of California, Berkeley.

CL-Based Myopia-Inducing Treatment

To induce myopia, guinea pig subjects wore monocular −10 diopter (D) rigid gas-permeable CLs. Details of the lens design, as well as wearing and monitoring schedules, are described in a previous publication.[22] In brief, the CLs (Valley Contax, Springfield, OR, USA), were made from acrylic fluorosilicone material, which has a high oxygen permeability (65%), and custom designed for young guinea pig eyes, with a 6.00 mm overall diameter, 5.00 mm optic zone diameter, and 3.38 mm base curve. Whereas all treated eyes wore CLs continuously, individual lenses were removed and replaced with a clean lens every morning and otherwise checked three times a day. After their removal, CLs were soaked in a combination of Boston protein remover and Boston Simplus solution (Bausch and Lomb, Rochester, NY, USA), with each lens being rinsed thoroughly with Opti-Free soft contact lens solution (Alcon, Fort Worth, TX, USA) before its next insertion.

In Vivo Study of Effects of CLs on Ocular Parameters

Immediately before the start of CL wear, baseline spherical equivalent refractive error (SE) and axial length (AL) data were collected, with additional data collected after one and five days of CL wear. A total of five guinea pigs participated in this experiment. Measurements were performed on awake animals at approximately the same time of day, starting around 2:00 PM, to avoid the possible confounding effects of circadian rhythms on eye growth.[23] Refractive errors were measured using streak retinoscopy (Welch Allyn, Skaneateles Falls, NY, USA), following cycloplegia with 1% cyclopentolate hydrochloride (Bausch & Lomb), instilled 30 minutes before measurement. Reported SEs represent the averages of results from two researchers (S.G. and Q.Z.). ALs were measured by noncontact biometry using the Lenstar (Haag-Streit Holdings, Köniz, Switzerland). The ALs reported here refer to the distance from the anterior surface of the cornea to the inner retinal surface, with each data point representing the average of at least five readings.

RPE Gene Expression Experiment

Contact Lens Treatment. For this experiment, CL wear was initiated at 2:00 PM. Four guinea pigs (three females and one male) wore a CL in their right eye for one day, with the CLs being removed just before the animals were sacrificed for tissue collection. The decision to collect RPE after just one day of CL wear was based on refractive error and biometric data collected in the in vivo experiment described above, with our interest being in the molecular signals driving "myopic" eye growth changes, as opposed to gene expression changes resulting from ocular dimensional changes (see results). Additional RPE samples were also collected from three untreated animals to evaluate natural interocular differences.

RPE Collection. Guinea pigs were euthanized with an intracardiac injection of sodium pentobarbital (Euthasol; Virbac Animal Health, Ft. Worth, TX, USA) delivered under gaseous anesthesia (5% isoflurane in oxygen), after which eyes were quickly enucleated and immediately immersed in chilled PBS. To collect RPE samples, scissors were used to first open each eye, just behind the limbus; the anterior segment, including the crystalline lens, and retina were then carefully removed, and the remaining posterior eye cup with RPE exposed immersed in RNAlater Stabilization Solution (Invitrogen, Carlsbad, CA, USA) for five minutes. Finally, a 1 mL syringe filled with PBS and attached 30-gauge needle was used to gently flush and so detach the RPE from the choroid. RPE fragments were collected in 1.5 mL tubes, spun down, lysed with RLT buffer from RNeasy Mini kits (Qiagen, Valencia, CA, USA), and immediately stored at −80°C for later use. Choroidal and scleral samples were separated by forceps, cut into small pieces, and lysed with RLT buffer, for use in quality control testing.

RNA Extraction From RPE. Total RNA was purified from RPE samples using RNeasy Mini kits (Qiagen), with on-column DNase digestion, according to the manufacturer's protocol. RNA concentration and A260/A280 optical density ratio were then measured, for quantification and quality control respectively, with a spectrophotometer (NanoDrop 2000; NanoDrop Technologies, Inc., Wilmington, DE, USA). The quality of collected RNA was also evaluated using an Agilent 2100 Bioanalyzer. The quality of the samples, especially the absence of choroidal and scleral contaminants was further assessed through measurement of the expression of *Col1a1*, a representative gene for each of the latter tissues, in RPE samples, and of *Rpe65*, an RPE-specific gene. All data sets were normalized to *β-actin*. The sequences of the forward primer and the reverse primer used for PCR are shown in Supplementary Table S1.

RPE RNA Sequencing and Bioinformatics Analysis. Samples were sequenced at the California Institute for Quantitative Biosciences at UC Berkeley (QB3, Berkeley, CA, USA) using an Illumina HiSeq4000 (Illumina, Inc., San Diego, CA, USA). Reads were trimmed with Cutadapt 3.4 to remove 3′ adapter sequences,[24] and quality control was performed using FastQC 0.11.7.[25] Reads were aligned to the *Cavia porcellus* (cavPor3.0) reference genome using Spliced

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Transcripts Alignment to a Reference aligner 2.7.1a.[26] Count data were generated with TPMCalculator 0.0.4,[27] and differential gene expression analysis was performed using DEseq2 1.32.0.[28]

Differential gene expression analysis was limited to samples from three female animals (n = 3 treated and n = 3 fellow eyes), after principal component analysis revealed that samples separated by sex along PC1 and PC2, as opposed to Condition (treated vs. fellow eyes). Thus, the samples from the one male animal (treated and fellow eyes) were excluded from analysis to remove this source of variation. A paired design was used to account for matched eyes (i.e., from the same animal) when fitting the linear model. Genes with a false discovery rate (FDR) < 0.1 were considered differentially expressed. A gene set enrichment analysis (GSEA) was then performed on the set of differentially expressed genes (13 genes) to determine enriched biological themes. Note that although the sample size is small, similar sample sizes have been previously and successfully used in studies involving RNA-seq analysis of the retinal transcriptome in mice[29] and of myopia-related gene expression changes in the sclera of guinea pigs.[30]

PCR (RT-qPCR) Evaluation of RPE Bmp2 Expression. RNA was reverse transcribed to cDNA (SuperScript III First-Strand Synthesis System for reverse transcription quantitative PCR; Invitrogen). Quanti-Tect SYBR Green PCR Kits (Qiagen) were used for mRNA amplification. Melt curves were examined to verify the yield of single peak products. All real-time PCR reactions were performed in triplicate (StepOnePlus Real-Time PCR System; Applied Biosystems, Foster City, CA, USA) with the following settings: one cycle of 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for one minute.

The choice of *Bmp2* for RT-qPCR validation was based on our RNA-seq results, which revealed significant treatment-related *Bmp2* gene expression changes (see results), and our previous related findings in chicks implicating RPE-*Bmp2* in eye growth regulation. RPE-RNA samples from treated and fellow eyes were used. *Bmp2* expression levels were also measured in RPE samples from three untreated guinea pigs that were matched in age to those subjected to monocular CL wear, i.e. 15 days-old. Details of the tested primers are summarized in Supplementary Table S1. mRNA expression was normalized to both *Gapdh* and *β-actin*, with results reported as the relative expression of (ΔΔCt: control sample = 1).

Statistical Analyses

All in vivo refractive error and biometric data, as well as RT-qPCR data, were analyzed using JMP Pro version 14.3.0 (SAS Institute Inc.). The former results are summarized as mean interocular differences (Treated eye − Fellow eye), ± Standard deviation. Paired Student *t*-tests with Bonferroni correction were used to compare interocular differences at days one and five with baseline data. To verify the high purity of isolated RPE samples, *t*-tests with Bonferroni correction were used to compare the RT-qPCR gene expression data across tissues (i.e., in RPE compared to choroid and sclera). For RNA-seq analysis, the Benjamini-Hochberg procedure (as implemented in DESeq2) was used to control the FDR, and FDR < 0.1 was set as a cutoff to determine differentially expressed genes. Paired Student *t*-tests were used to compare the RT-qPCR gene expression data from lens-treated and fellow control eyes. In all cases, P values < 0.05 were considered to be significant.

Results

In Vivo Contact Lens-Induced Changes

After one day of imposed optical defocus (i.e. with -10 D CLs), treated eyes exhibited significant myopia relative to their untreated fellows (Day 1: −2.24 ± 1.75 vs. 0.59 ± 0.99 D, P = 0.016, Fig. 1A). This interocular difference in refractive error increased further after an additional four days of lens wear (Day 5: −4.56 ± 1.46 vs. 0.48 ± 0.75 D, P = 0.004, Fig. 1A). The CL-wearing eyes also showed a trend toward increased AL after just one day of CL wear (0.094 ± 0.047 vs. 0.056 ± 0.039 mm, P = 0.10), although here, interocular differences in AL did not reach statistical significance until day 5 of CL wear (0.306 ± 0.081 vs. 0.172 ± 0.057 mm, P = 0.004, Fig. 1B). The decision to collect RPE after 1 day of CL wear was based on these data

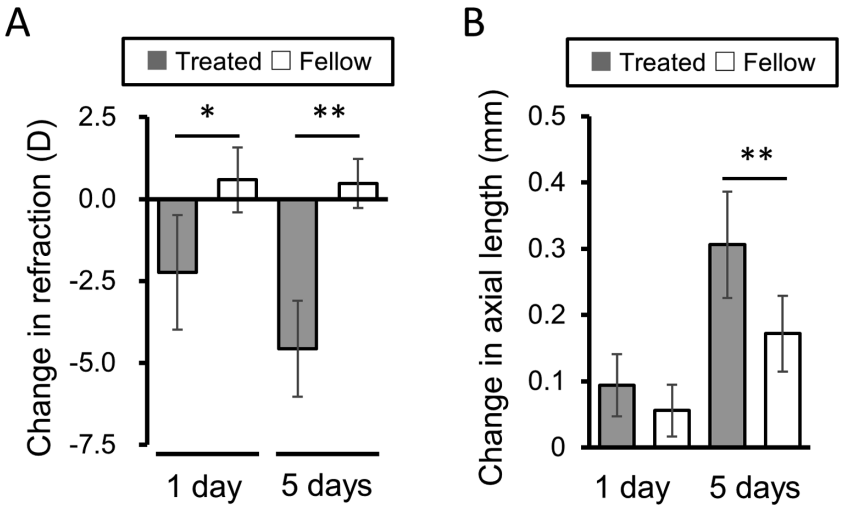

Figure 1. Changes from baseline values in refractive errors (A) and axial lengths (B) after one and five days of monocular imposed hyperopic defocus (−10 D contact lens), initiated at two weeks of age. * P < 0.05, ** P < 0.01.

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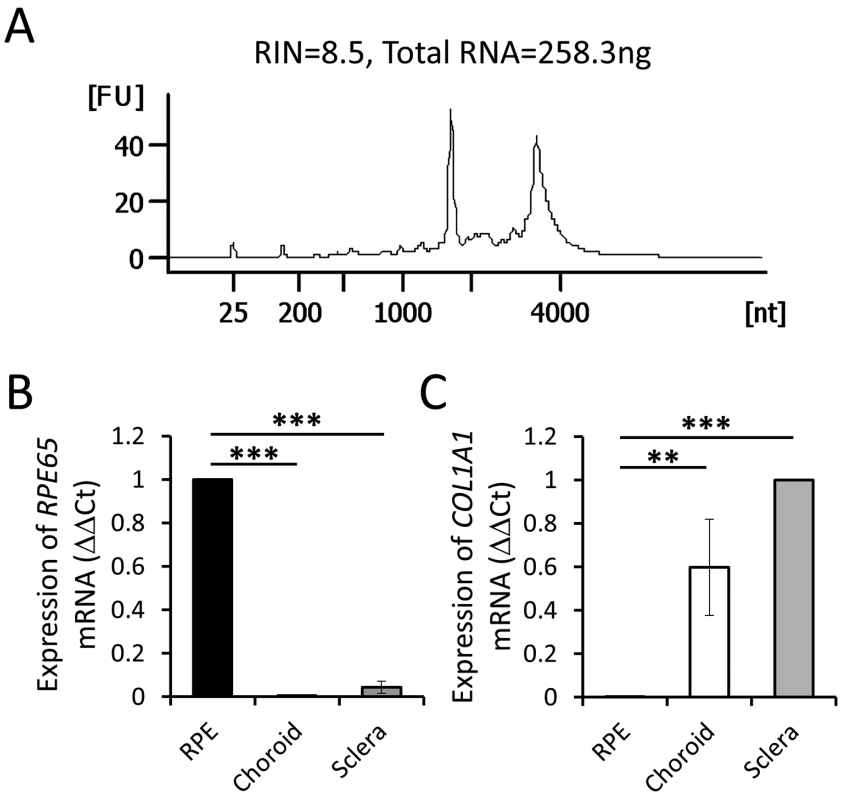

Figure 2. Representative bioanalyzer output for RNA extracted from collected samples (A); and gene expression levels of *RPE65*, an RPE specific gene, and *COL1A1*, a choroid sclera-specific gene, measured by RT-qPCR in RPE, choroid and scleral samples from untreated animals (B and C, respectively), both data sets normalized to β-actin. ** $P < 0.01$; *** $P < 0.001$.

Table. Differentially Expressed Genes in RPE From Treated (−10 D CL Wear) Versus Fellow Eyes

	Gene ID	Gene Symbol	Gene Name	FDR
1	ENSCPOG00000038307	*Id3*	Inhibitor of DNA binding 3, HLH protein	5.96E-04
2	ENSCPOG00000000887	*Glrx5*	Glutaredoxin 5	2.21E-02
3	ENSCPOG00000006702	*Aplp1*	Amyloid beta precursor like protein 1	3.02E-02
4	ENSCPOG00000006643	*Gtf3c3*	General transcription factor IIIC subunit 3	5.75E-02
5	ENSCPOG00000032374	*Ankrd46*	Ankyrin repeat domain 46	5.75E-02
6	ENSCPOG00000039740	*Col8a2*	Collagen type VIII alpha 2 chain	5.75E-02
7	ENSCPOG00000004932	*Nog*	Noggin	6.27E-02
8	ENSCPOG00000007879	*Asah1*	N-acylsphingosine amidohydrolase 1	6.27E-02
9	ENSCPOG00000015694	*Sema4a*	Semaphorin 4A	6.27E-02
10	ENSCPOG00000031437	*Bmp2*	Bone morphogenetic protein 2	6.27E-02
11	ENSCPOG00000010894	*Hsd11b2*	Hydroxysteroid 11-beta dehydrogenase 2	7.07E-02
12	ENSCPOG00000010027	*Prss12*	Serine protease 12	9.44E-02
13	ENSCPOG00000026695	*Mroh1*	Maestro heat like repeat family member 1	9.44E-02

and our interest in examining the molecular signals driving "myopic" eye growth changes, as opposed to gene expression changes linked to structural changes in one or more tissues of enlarged (myopic) eyes.

Myopia-Related RPE Gene Expression Changes

Quality of Isolated RPE. Analysis of collected RPE samples showed well preserved RNA (RNA integrity number (RIN) > 8), with approximately 250 ng collected from each eye (Fig. 2A). As further indicators of the high quality of the RPE samples, they showed significantly higher expression of *Rpe65* (an RPE-specific gene) compared with expression levels in choroid and sclera (Fig. 2B), and on the other hand, showed minimal expression of *Col1a1*, the selected choroid-sclera-specific gene (Fig. 2C).

The −10 D Defocus-Induced Differential RPE Gene Expression

RNA-Seq Analysis. To examine the transcriptional changes triggered by a known myopia-inducing defocus stimulus, RNA-seq was undertaken on RPE samples collected

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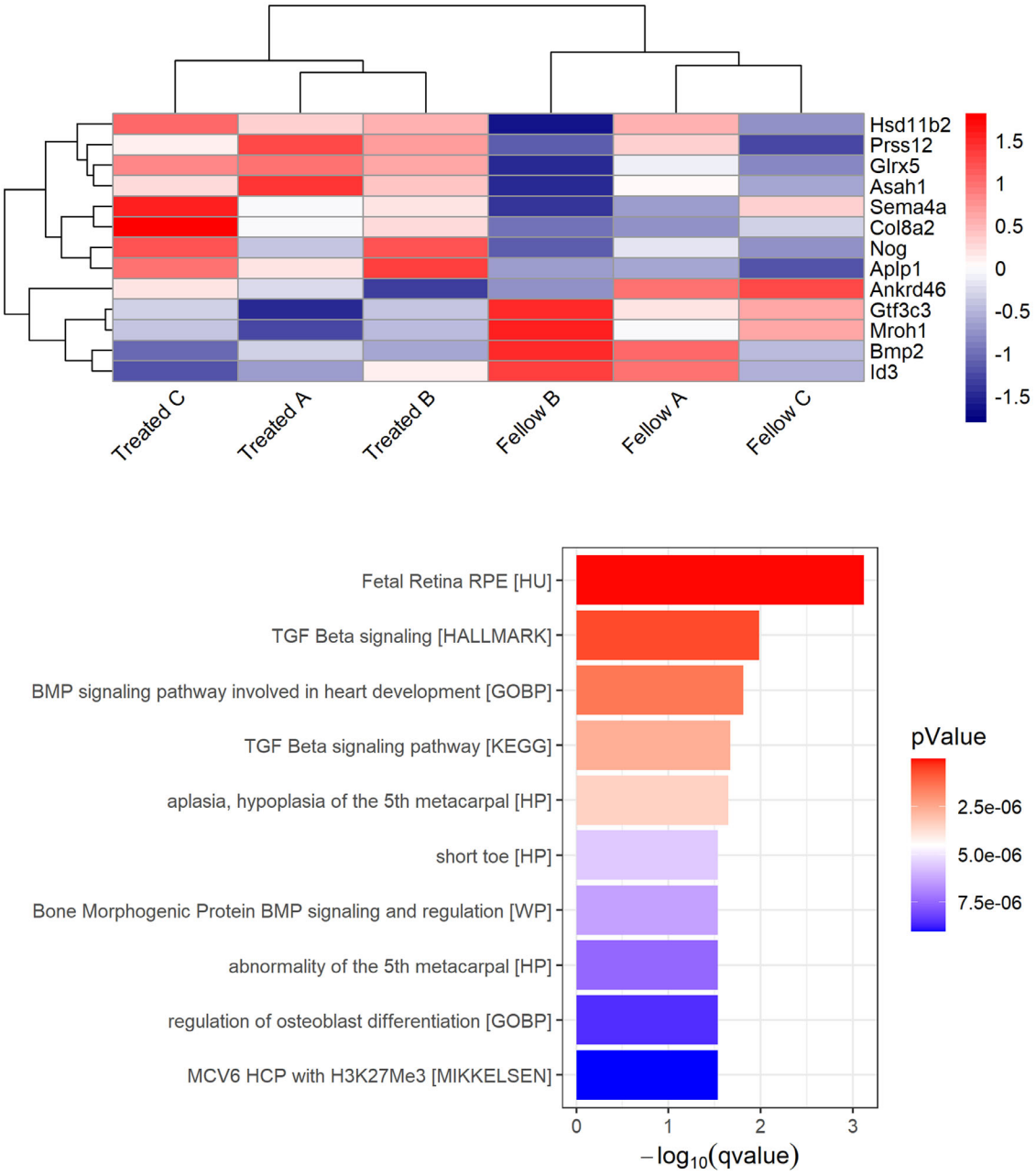

Figure 3. Effects of 1 day of exposure to hyperopic defocus (−10 D CL wear) on BMP and TGF-β signaling pathways in RPE; heatmap showing relative expression levels (z-scores) of genes found to be significantly upregulated (top 8 rows) and downregulated (bottom 5 rows) in RNA-seq analysis of treated versus fellow eyes. FDR < 0.1 (*Top panel*). Top 10 GSEA categories for the 13 differentially expressed genes include enriched biological pathways related to BMP and TGF-β signaling, and cell type enrichment for fetal RPE (*Bottom panel*). Categories are ranked by significance (decreasing $-\log_{10}$ [q value]) and color represents *P* values. The GSEA gene set for each category is listed in brackets. $n = 3$ for treated and fellow eyes (from 3 female animals), respectively. A, B, and C denote data from individual guinea pigs.

from treated and fellow eyes after just one day (24 hours) of −10 D CL wear. We found 13 differentially expressed genes (DEGs) at an FDR < 0.1 (eight upregulated genes in treated vs. fellow eyes: *Hsd11b2, Prss12, Glrx5, Asah1, Sema4a, Col8a2, Nog, Aplp1*; and five downregulated genes in treated vs. fellow eyes: *Ankrd46, Gtf3c3, Mroh1, Bmp2, Id3*) (Table and Fig. 3A). Log-fold changes in gene expression were modest (<1.3-fold) for all DEGs, although perhaps not unexpected, given the short (24-hour) treatment duration. GSEA analysis on the 13 differentially expressed genes revealed enriched biological process categories related to BMP signaling ("BMP signaling pathway involved in heart development," "bone morphogenic protein [BMP] signaling and regulation") and TGF-β signaling. Additionally, we found significant cell type enrichment for RPE ("fetal retina RPE") (Fig. 3B).

RPE Bmp2 Expression Changes. As expected, *Bmp2* expression levels, normalized to *Gapdh* and *β-actin*, in RPE samples from the right and left eyes of untreated guinea pigs were not significantly different ($P = 0.61$ and 0.71; Figs. 4A, 4C, respectively). In contrast, *Bmp2* gene expression in RPE from eyes subjected to 1 day of −10 D

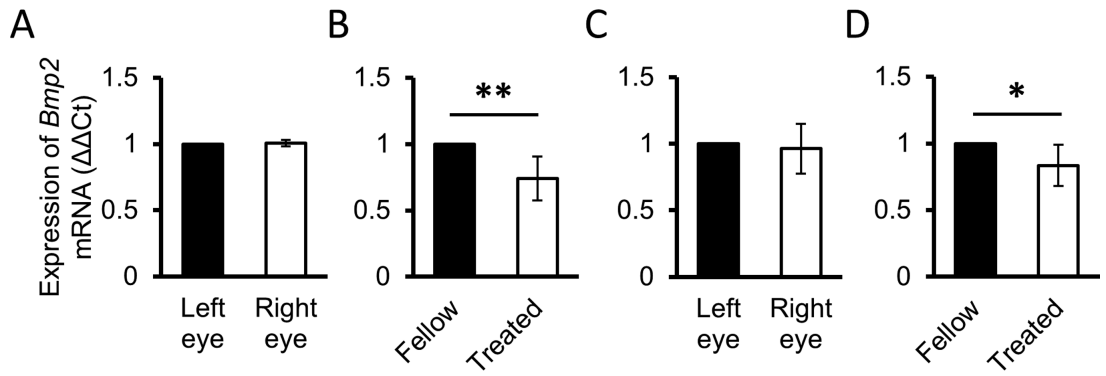

Figure 4. mRNA expression levels in RPE normalized to *Gapdh* (A and B) and *β-actin* (C and D) for *Bmp2* from 15-day-old animals. (A) *Bmp2* gene expression in RPE from untreated animals; there is no difference between right and left eyes ($n = 3$ animals) normalized to *Gapdh*. (B) *Bmp2* gene expression after 1 day of monocular −10 D CL wear is significantly downregulated ($n = 7$ animals) normalized to *Gapdh*. (C) *Bmp2* gene expression in RPE from untreated animals; there is no difference between right and left eyes ($n = 3$) normalized to *β-actin*. (D) *Bmp2* gene expression after one day of monocular −10 D CL wear is significantly downregulated ($n = 7$ animals) normalized to *β-actin*. * $P < 0.05$, ** $P < 0.01$.

CL wear showed significant downregulation, to 74 ± 13 % and 84 ± 15 % relative to expression levels in the RPE of their fellows ($P = 0.002$ and 0.029; Figs. 4B, 4D, respectively).

Discussion

The data presented here provide new insights into the role of the guinea pig RPE for encoding myopia-inducing altered visual experiences via changes in gene expression. The main contributions of this study can be summarized as follows: First, we successfully isolated the RPE in guinea pigs, which is the first report, to the best of our knowledge. Second, biological enrichment analysis of genes that were differentially expressed in RPE across treated and fellow (control) eyes identified pathways relating to TGF-*β* and BMP signaling and regulation. Third, we showed that *Bmp2* gene expression is downregulated in guinea pig RPE after one day of exposure to myopia-inducing optical defocus (imposed with negative CLs). Finally, enrichment analysis identified cell type enrichment for fetal RPE, which supports our claim of successful RPE isolation from guinea pig eyes and complements the results presented in Figure 2.

Based on the result of the changes in refraction and AL, we found significant differences in both refraction and AL compared to control eyes after five days of CL wear. In contrast, there was a significant difference in refraction but not AL after one day of CL wear. Therefore, the one-day post-use period was selected to examine the early stage of myopia progression in the current study.

A link between TGF-*β* signaling, as indicated by gene expression changes, and eye growth regulation, is not a new observation. As one example involving chicks, short-term exposure to +10 D lenses (imposed myopic defocus), which slows eye elongation, resulted in selective upregulation of TGF-*β*2 in RPE, by up to 3.5- and 7.5-fold after two and 48 hours of exposure, respectively. However, there was no significant change in TGF-*β*2 expression in RPE in eyes wearing −10 D lenses.[31] On the other hand, in the tree shrew, 24 hours of −5 D lens wear resulted in a 1.4-fold downregulation of TGF-*β*1 gene expression in RPE, although this treatment was without effect on TGF-*β*2 gene expression.[32] Although these various reports hint at roles for one or more RPE-derived TGF-*β*s in eye growth regulation (specifically, ocular growth inhibition), direct supporting evidence is still lacking.

BMPs are multifunctional growth factors that belong to the TGF-*β* superfamily, with important roles in embryogenesis and osteogenesis.[33–37] Of this family, BMP2 has already been linked to ocular development and growth regulation.[37–40] For example, *Bmp2* gene expression was found to be downregulated in RPE in a chick model of lens-induced myopia.[14,16,41] The finding in the current study of significant downregulation of *Bmp2* gene expression in RPE after just one day of −10 D CL wear is consistent with this finding in chicks.[14,16,41] *BMP2* has also been reported to inhibit adult human RPE cell proliferation,[42] consistent with the profile of a negative growth regulator, although *BMP2* is reported to stimulate the proliferation and differentiation of human scleral fibroblasts in vitro.[43,44] These opposing patterns of behavior may reflect differences in the roles of various ocular tissues in homeostasis and eye growth modulation.

In the context of eye growth modulation, the retinal neurotransmitter, dopamine, appears to play a key role.[45–47] For example, decreases in retinal levels and turnover of dopamine have been linked to experimentally-induced myopia in chicks,[46,47] and intravitreal injection of a dopamine D2 agonist was found to inhibit optical defocus (negative lens)-induced myopia and attenuate the choroidal thinning component of this response.[48] Yet another study in chicks reported increased retinal dopamine release with intravitreal injection of atropine, which also inhibits lens-induced myopia.[49] That retinal dopamine might directly modulate gene expression of *BMP2* in RPE is suggested by results of yet another in vitro study in which dopamine was observed to upregulate *BMP2* expression in human RPE cells.[50] Together, the results of these various studies support a model of local eye growth modulation in which *BMP2* gene expression in the RPE is controlled by a retinal dopamine signaling pathway.

What role does BMP2 play in the changes to the choroid and sclera, which together determine eye size and thus the eye's refractive error? While the expression of *Bmp2* is reported to be decreased in the retinas of myopic guinea

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pigs, no change was found in the choroids, in both cases measured after three weeks of induction with negative lenses.[51] Furthermore, in chicks fitted with −10 D lenses, neither the retina and choroid showed significant differences in gene expression between treated and fellow eyes after either 2 hours or 2 days of treatment.[15] Finally, in a tree shrew study involving a −5 D lens treatment, *Bmp2* expression was downregulated in the retina but no difference was found in the RPE after six hours or one day of treatment.[32] Together, these data hint at possible species differences in the signal pathways regulating eye growth, with BMP2 serving as a negative growth regulator. Evaluation of changes in BMP2 protein levels in key ocular tissues, specifically the choroid and sclera, are also needed to clarify its role in observed structural changes linked to accelerated or inhibited growth and therefore its role in myopia development or inhibition of myopia progression.[15,43,51,52] That BMP signaling has been implicated in blood vessel formation and maintenance of vascular integrity in nonocular tissues,[53,54] provides additional argument for such studies.

Noggin, also known as NOG, is a secreted glycoprotein that is involved in the development of many body tissues, including bone, nerve tissue, muscle, and eye.[55,56] Of potential relevance to the current study, NOG is also known to inhibit several BMPs such as BMP2, BMP4, BMP5, BMP6, BMP7, BMP13, and BMP14,[57] likely through direct binding BMPs, thereby preventing their interactions with receptors. The RNA sequencing data reported here are consistent with a role of NOG in myopia progression, at least in the guinea pig, although roles of other inhibitors of BMPs, such as chordin and ventroptin, cannot be ruled out.[58] Further investigation is warranted to distinguish between these possibilities.

In conclusion, RNA-seq analysis of RPE from eyes exposed to myopia-inducing optical defocus revealed *Bmp2* downregulation, as observed previously with the same treatment in chicks, and confirmed by qRT-PCR. Although this study used just one strain of pigmented guinea pig, these findings add to the growing body of evidence from other animal models and human genetic studies implicating BMPs in eye growth regulation and myopia. Further investigations of the retina-RPE-choroid-sclera signaling pathway underlying myopia progression, both downstream and upstream, are needed to complete this picture. Confirmation of a role for BMP2 as an inhibitory growth regulator and identification of other key molecules have the potential to open the way for novel ophthalmic antimyopia therapeutic interventions directed at these targets.

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