Form-Deprivation Myopia in Chick Induces Limited Changes in Retinal Gene Expression

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Purpose. Evidence has implicated the retina as a principal controller of refractive development. In the present study, the retinal transcriptome was analyzed to identify alterations in gene expression and potential signaling pathways involved in form-deprivation myopia of the chick.

Methods. One-week-old white Leghorn chicks wore a unilateral image-degrading goggle for 6 hours or 3 days ($n = 6$ at each time). Total RNA from the retina/(retinal pigment epithelium) was used for expression profiling with chicken gene microarrays (Chicken GeneChips; Affymetrix, Santa Clara, CA). To identify gene expression level differences between goggled and contralateral nongoggled eyes, normalized microarray signal intensities were analyzed by the significance analysis of microarrays (SAM) approach. Differentially expressed genes were validated by real-time quantitative reverse transcription–polymerase chain reaction (qPCR) in independent biological replicates.

Results. Small changes were detected in differentially expressed genes in form-deprived eyes. In chickens that had 6 hours of goggle wear, downregulation of bone morphogenetic protein 2 and connective tissue growth factor was validated. In those with 3 days of goggle wear, downregulation of bone morphogenetic protein 2, vasoactive intestinal peptide, preopro-urotensin II–related peptide and mitogen-activated protein kinase phosphatase 2 was validated, and upregulation of endothelin receptor type B and interleukin-18 was validated.

Conclusions. Form-deprivation myopia, in its early stages, is associated with only minimal changes in retinal gene expression at the level of the transcriptome. While the list of validated genes is short, each merits further study for potential involvement in the signaling cascade mediating myopia development. (*Invest Ophthalmol Vis Sci.* 2007;48:3430–3436) DOI: 10.1167/iovs.06-1538

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Despite its major public health impact, the etiology of myopia is poorly understood. Persuasive evidence in animals and humans has localized the controlling mechanism(s) for refractive development in large part to the retina.[1–4] Although evidence implicates various specific neurotransmitters or other intercellular signaling molecules, a cohesive understanding of the retinal cells and signaling pathways that account either for normal refractive development or for refractive errors has remained elusive.[4]

Form deprivation has been a useful biological model for assessing mechanisms potentially pertinent to clinical myopia.[1,2] Disturbances of visual input cause myopia in chicks, as well as in tree shrews, primates, and other mammals. Disturbances of visual input also cause myopia in children.[2,4] Although form deprivation induces a robust myopic response in newly hatched chicks, as evaluated in the current study, its effects persist to a more limited extent in chickens at ages that correspond developmentally to human adolescence.[5] As further validations for the use of this model in the study of signaling pathways potentially pertinent to the clinical disorder, an antimyopia drug first identified in form-deprivation myopia in the chick[6] has been shown to be active against human myopia in two multicenter clinical trials,[7,8] and risk factors suggested by the pharmacology of this model have been corroborated indirectly through cross-sectional clinical surveys.[9]

To approach retinal signaling pathways potentially underlying the pathogenesis of experimental myopia, we profiled gene expression patterns in the combined retina and retinal pigment epithelium (RPE) from chicks with form-deprivation myopia, a well-established eye-growth model.[4] The recent availability of chicken genome microarrays permits such a transcriptome-level study.

We specifically assayed the retina/RPE after 6 hours and 3 days of unilateral goggle wear. Although minor contralateral effects are recognized in chick eye-growth models,[10,11] potential spurious differences between birds complicate statistical approaches to interbird comparisons, and our bioinformatics approach stresses the most important experimental-to-contralateral control eye in individual birds. Although choroidal thinning comprises the predominant anatomic change in goggled chick eyes at 6 hours, increased scleral proteoglycan synthesis by that time reveals activation of a retina-to-sclera signaling pathway that will produce later measurable effects on scleral growth.[12] Because of potential roles of diurnal cycling of both retinal dopamine and ocular dimensions in refractive development,[4] sampling at 6 hours thus is a reasonable time to expect activation and transcription of at least initial genes pertinent to signaling a scleral growth response, but it is shorter than a diurnal cycle and may be less likely to induce potential diurnal–circadian confounding than would sampling at a later time. While expression of immediate-early genes is altered at earlier times,[13] such nonspecific genes alone may not reveal specific pathways or mediators of eye growth. With

longer times of goggle wear, it becomes increasingly ambiguous whether altered genes identify a pathway generating myopic growth or instead reflect secondary changes such as those necessary for the retina to conform anatomically to the enlarging scleral–choroidal coat. By day 3, chick eyes manifest the growth and refractive effects of goggle wear,[12,14] and despite the diurnal–circadian issues just discussed, this time permits characterization of established progressing myopia and minimizes more marked secondary effects that may occur with longer-term visual manipulations and still more pronounced anatomic growth.

METHODS

One-day old white Leghorn chicks (CBT Farms, Chestertown, MD) were housed in brooders in a 12-hour light–dark cycle with fluorescent lighting (Chroma 50; General Electric, Fairfield, CT) with irradiance of approximately 50 $\mu W/cm^2$ at chick eye level. They received food (Chick Chow; Purina, Indianapolis, IN) and water ad libitum. At 1 week of age and at the onset of the light phase, the chicks were anesthetized with inhalation ether, and a unilateral translucent white plastic goggle was glued to the periorbital feathers to induce ipsilateral form-deprivation myopia,[1,2] alternating between the left or right eye. After either 6 hours ($n = 8$) or 3 days ($n = 8$) of goggle wear, the chicks were killed by decapitation. Because the ocular effects of goggle wear by white Leghorn chicks are well characterized,[12] ocular refractions and eye measurements were not obtained for the particular eyes profiled, to avoid potential confounding affects of anesthesia and postmortem mRNA degradation. The enucleated eyes were opened at the equator, and the retina/RPE was dissected from the goggled and control eyes. The tissues were individually frozen and stored in liquid nitrogen until processed. The experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RNA Isolation

Each frozen retina/RPE sample was ground under liquid nitrogen with a mortar and pestle. RNA was then isolated from each preparation (TRIzol; Invitrogen, Carlsbad, CA) followed by purification and DNase treatment (RNeasy columns; Qiagen, Inc, Valencia, CA). The samples were measured on a spectrophotometer (ND-1000 UV-Vis; NanoDrop Technologies, Wilmington, DE) for quantification and purity, with 260/280 nm absorbance ratios between 1.8 and 2.1. To evaluate RNA integrity further, an aliquot of each RNA sample was loaded on an RNA chip (RNA 6000 Nano LabChip; Agilent Technologies, Santa Clara, CA) and placed in a bioanalyzer (model 2100; Agilent Technologies). RNA integrity was verified by electropherograms and gel-image analysis to visualize the intact ribosomal bands using the accompanying software (Bioanalyzer; Agilent Technologies). Aliquots of the RNA samples were stored individually at −80°C.

Microarray Target Preparation and Hybridization

Microarray targets were prepared with total RNA from each eye of six chicks from each of the two experimental groups. All protocols were conducted as described in the manufacturer's manual (Affymetrix GeneChip Expression Analysis Technical Manual; Affymetrix, Inc.). Briefly, 3 μg of total RNA from each eye was converted to first-strand cDNA by using reverse transcriptase (Superscript II; Invitrogen) primed by a poly(T) oligomer that incorporates the T7 promoter. Second-strand cDNA synthesis was followed by in vitro transcription for linear amplification of each transcript and incorporation of biotinylated CTP and UTP. The cRNA products were fragmented to 200 nucleotides or less, heated at 99°C for 5 minutes, and hybridized for 16 hours at 45°C to 1 of 24 chicken genome microarrays (GeneChip; Affymetrix). The microarrays were then washed at low (6× SSPE [NaCl, NaH_2PO_4, H_2O, and EDTA]) and high (100 mM MES [2-(*N*-morpholino)ethane sulfonic acid], 0.1 M NaCl) stringency and stained with streptavidin-phycoerythrin. Fluorescence was amplified by adding biotinylated anti-streptavidin and an additional aliquot of streptavidin-phycoerythrin stain. Using a microarray scanner (GeneChip 3000 7G; Affymetrix), the fluorescence signal (570 nm excitation) was recorded at a 2.5-μm resolution. Data were then collected (GCOS software, ver. 1.4; Affymetrix), and probe level intensities were exported as .cel files for subsequent summarization and analysis.

Bioinformatics Analyses

Probe intensity files (Affymetrix .cel) were exported from the software and processed (ArrayAssist Lite ver. 3.1; Stratagene, La Jolla, CA) to yield .chp files containing robust multichip analysis (RMA) expression values[15] and Affymetrix flags (present, absent, marginal) for at least 2 of the 12 arrays at each time. The normalized microarray signal intensities were then analyzed by the significance analysis of microarrays (SAM) approach,[16] with a ≥1.2-fold change filter and a two-class, paired design in which the sample from each goggled eye was analytically paired with the contralateral control eye. The SAM false-discovery rate was set arbitrarily at 13% for each time.

Real-Time Quantitative RT-PCR

Using real-time quantitative reverse transcription–polymerase chain reaction (qPCR) and RNA preparations from different chicks than those used for the microarray profiling, we performed independent biological validations of expression profiling at the RNA level for the known genes with ≥1.4-fold change. From each eye of the two other chicks at each time point, 3 μg of retina/RPE RNA was converted into cDNA (Superscript II First Strand cDNA Synthesis Kit; Invitrogen). Primers were generated (Primer Express Software; Applied Biosystems, Inc. [ABI]) for each of the following chicken genes, indicated by the *Gallus gallus* gene names followed by the gene symbols, NCBI (National Center for Biotechnology Information, Bethesda, MD) annotation numbers, and primer sequences: bone morphogenetic protein 2 (*BMP2*; NM_204358; 5′: CAGACTTTGGTCAATTCGGTGAA, 3′: AGCATTGAGATAGCACTCAGTTCTGT); connective tissue growth factor (*CTGF*; NM_2204274; 5′: GCACTGGCCGCCTACAGA, 3′: AATTGGCACGCATCATGGT); mitogen-activated protein kinase phosphatase 2 (*MKP-2*; NM_204838; 5′: AGCCCTGCTGAACGTCTCA, 3′: AGGGATGCACTTGTACTGGTAGTG; neurotensin (*NTS*; XM-416126; 5′: GCTGACAGTGTATCAACTCCAAAAA, 3′: TCAAATGCGTCTTGCTGAAGTAA); prepro-urotensin II–related peptide (*LOC404534*; NM_206989; 5′: TGCTGAGCCAGAGCTGTTGT, 3′: CCATCCTCCCCCAAACCTA); and vasoactive intestinal peptide (*VIP*; NM_205366; 5′: CCTTTGATGCAGCCAGTGAA, 3′: GTGTGTTCTGCAAAATGTCTGATTC). Primer sets, optimized for chicken sequences, were purchased (QuantiTect Assays; Qiagen) for endothelin receptor type B (*EDNRB*; XM-417001); interleukin 18 (*IL-18;* NM_204608); neurotensin (*NTS*; XM-416126); mutL homolog 1, colon cancer, nonpolyposis type 2 (*Escherichia coli*) (*MLH1*; XM-418828); and prepro-urotensin II–related peptide (*LOC404534*; NM_206989). For clarity, prepro-urotensin II–related peptide is referred to as prepro-URP.

With a real-time PCR system (model 7500; ABI) and 96-well plates, 30 μL PCR reactions were performed in triplicate for each gene of interest using cDNA, gene-specific primers and an RT-PCR master mix (either Power SybrGreen; ABI; or QuantiFast SYBR Green Master Mix; Qiagen). The PCR reaction consisted of 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Computer analyses were performed (7500 software; ABI) with the comparative ΔΔCt relative quantification method[17] after determining that the efficiencies of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase and the genes of interest were the same.

RESULTS

Expression Profiling of Retina/RPE: Goggled Versus Contralateral Nongoggled Eyes

Using the chicken genome microarrays (Affymetrix Chicken GeneChips), we performed expression profiling of retina/RPE

genes from unilaterally goggled eyes and their contralateral eyes at each of the two time points and identified changes at the level of the transcriptome. Although later releases of the chicken genome are now available,[18] the microarrays used here were fabricated from release 1 of the chicken genome (May 2004) and contain 32,773 chicken transcripts.[19] Considering this breadth of genome representation, we found surprisingly small alterations in expression levels of the sampled genes.

With a false-discovery rate of 13%, SAM generated a list of 15 genes for 6 hours of visual deprivation and a list of 280 genes for 3 days of visual deprivation (see Supplementary Tables S1A and S1B online at http://www.iovs.org/cgi/content/full/48/8/3430/DC1). Comparing the goggled with contralateral eyes showed that the maximum–minimum changes in individual genes were +1.36/−2.16-fold at 6 hours and +1.55/−2.18-fold at 3 days. Of these genes, most were identified as differentially expressed at just one time point (Fig. 1; Supplementary Tables S1C, S1D, S1E). Only seven genes were identified as differentially expressed at both times.

For selecting retina/RPE genes to validate by qPCR, we required that the change exceed 1.4-fold in either the up- or downregulated direction to ensure adequate qPCR sensitivity. Only two genes after 6 hours of goggle wear and 15 genes after 3 days of goggle wear met this criterion (Table 1). Of these genes, one (*BMP2*) was downregulated at both times. Of the altered genes detected just after 3 days of goggle wear, one gene (*PHLDA2*) was detected by two distinct probe sets in the microarrays.

All primary data have been deposited in the Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/ NCBI) database (accession number GSE6543; GEO platform number GPL3213).

Independent Biological Validations

Genes for each time point were validated by qPCR with retina/RPE RNA from two independent chicks not used for microarray analysis. We assayed only genes for known gene products (Table 1). For the two chicks with 6 hours of monocular form deprivation, qPCR confirmed downregulation in the retina/RPE of goggled eyes of *BMP2* and *CTGF*. For the two chicks with 3 days of deprivation, qPCR confirmed downregulation of *BMP2*, *VIP*, prepro-URP (*LOC404534*), and *MKP-2* and upregulation of *EDNRB* and *IL-18*. The changes suggested for altered expression of *MLH1*, *PHLDA2*, and *NTS*, however, were not confirmed by qPCR (data not shown). Whereas the sensitivity for detecting low-abundance RNAs is higher for qPCR than with microarrays, we are aware of no studies ascertaining whether microarrays or qPCR perform better in discriminating low-magnitude differences in abundance.

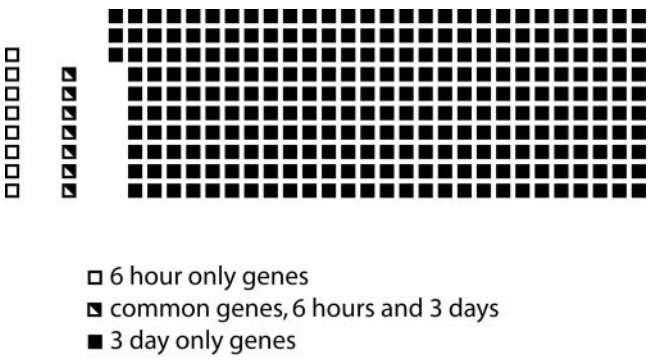

FIGURE 1. Differentially expressed genes at the two times of goggle wear. The differentially expressed genes are indicated for 6 hours only (n = 8 genes, *left*), 3 days only (n = 273 genes, *right*), and the overlap of the two times (n = 7 genes, *middle*). For specific gene lists, see Supplementary Table S1, http://www.iovs.org/cgi/content/full/48/8/3430/DC1.

DISCUSSION

Using gene microarrays (GeneChips; Affymetrix) and an avian myopia model with at least some established relevance to human myopia, we identified differentially expressed genes in the retina/RPE, but the changes in their expression levels were quite small. Given the extensive evidence implicating the retina in the regulation of eye growth and the robustness of the myopic response in chicks to deprivation of form vision, we did not predict these minimal changes in retinal gene expression. Induction of form-deprivation myopia thus does not require major alterations in the expression of retinal genes, at least not those genes represented in the extensive list of the microarrays used here (Affymetrix Chicken GeneChips).

Affymetrix GeneChip platforms are designed to identify RNA abundance changes rather than to explore altered posttranscriptional pathways (e.g., posttranslational protein modification), which may be detected only indirectly if there is an effect on downstream RNA expression. Thus, posttranslational regulatory mechanisms may be essential for the pronounced developmental effects in form-deprivation myopia. For instance, posttranslational mechanisms regulate the activity of the rate-limiting enzyme in the biosynthesis of dopamine,[20] a retinal neurotransmitter previously implicated in refractive development.[4]

The two time points selected for gene profiling of the chick retina, 6 hours and 3 days, should reflect gene changes at myopia onset and after myopia is well established, respectively. The shorter deprivation period, 6 hours, is sufficient to induce acute changes in choroidal thickness and longer-term changes in scleral proteoglycan synthesis. After 3 days of visual deprivation, changes are evident in refraction and axial length.[12] Presumably, eye-growth signaling from the retina is active at each of these time points. Only a proportion of differentially expressed genes were common to both times (Fig. 1; Supplementary Table S1E http://www.iovs.org/cgi/content/full/48/8/3430/DC1). The difference in the profiles at these two times raises the question of whether the signaling mechanisms that initiate myopia differ from those that maintain its progression.

Identity of Validated Differentially Expressed Genes

Although we cannot exclude potential roles in myopia for the genes that did not meet our criteria for validation, we assumed that those genes with the greater changes in expression were reasonable choices for qPCR validation. Only a limited number of altered genes fulfilled our validation criteria (Table 1). As both perspective and hypotheses for further study, the differentially expressed gene at only 6 hours (*CTGF*) may play a role in the onset of myopia; those at 3 days only (*VIP*, prepro-URP, *MKP-2*, *EDNRB*, and *IL-18*), in established myopia, and the one common to both times (*BMP2*), in both myopia onset and in established myopia (Table 1).

In comparing the retina/RPE of eyes with altered visual input with that of unimpaired contralateral control eyes, differential mRNA expression could reflect genes involved in eye-growth regulation, but it also could represent genes involved in visual processing, genes concerned with the endogenous diurnal ocular rhythms known to be disrupted in form-deprivation myopia, and/or genes mediating retinal expansion to fill the enlarging area of the choroid and sclera in myopia.

TABLE 1. Comparison of Selected Genes: Goggled versus Contralateral Nongoggled Eyes

Probe ID	Gene Description	Fold-Change by Microarray*	Fold-Change by qPCR†
6 Hours of unilateral goggle wear			
Gga.3950.1.S1_at	Bone morphogenetic protein 2 (*BMP2*)	−2.16	Confirmed (−2.34)
Gga.4051.1.S1_at	Connective tissue growth factor (*CTGF*)	−2.02	Confirmed (−1.43)
3 Days of unilateral goggle wear			
Gga.3950.1.S1_at	Bone morphogenetic protein 2 (*BMP2*)	−2.18	Confirmed (−1.48)
Gga.666.1.S1_a_at	Vasoactive intestinal peptide (*VIP*)	−1.99	Confirmed (−1.54)
Gga.9482.1.S1_at	Prepro-urotensin II-related peptide (*LOC404534*)	−1.67	Confirmed (−3.35)
Gga.385.1.S1_at	Mitogen-activated protein kinase phosphatase 2 (*MKP-2*)	−1.46	Confirmed (−3.14)
Gga.3306.1.S1_s_at	Endothelin receptor type B (*EDNRB*)	+1.52	Confirmed (+2.23)
Gga.19049.1.S1_at	Interleukin 18 (*IL-18*)	+1.45	Confirmed (+1.48)
GgaAffx.23773.1.S1_s_at	mutL homolog 1, colon cancer, Nonpolyposis type 2 (*E. coli*) (*MLH1*)	+1.47	Not confirmed by qPCR
Gga.6448.1.S1_s_at GgaAffx.21949.1.S1_at	Pleckstrin homology-like domain, Family A, member 2 (*PHLDA2*)	+1.44 and +1.54 (two sequences on microarrays)	Not confirmed by qPCR
Gga.10167.1.S1_at	Neurotensin (*NTS*)	+1.44	Not confirmed by qPCR
Gga.19378.1.S1_at	Finished cDNA, clone ChEST761o6	+1.46	Not tested
Gga.13134.1.S1_at	Finished cDNA, clone ChEST663o7	+1.46	Not tested
GgaAffx.12815.1.S1_at	Similar to RIKEN cDNA 2010011I20 (*RCJMB04 23o22*)	+1.45	Not tested
Gga.14902.1.S1_at	Finished cDNA, clone ChEST485k2	+1.44	Not tested
GgaAffx.2577.2.S1_s_at	Similar to hypothetical protein FLJ35961 (*LOC419690*)	+1.41	Not tested

* Change of gene expression by microarray for goggled versus nongoggled contralateral eye.

† Number in parentheses is the mean change in confirmed genes from the qPCR (real-time quantitative RT-PCR) of two independent biological replicates, goggled versus nongoggled contralateral eye.

Although the present results connect each of these peptide messengers to form-deprivation myopia, the profiling experiments do not distinguish whether these peptides act locally within the retina or instead are secreted from the retina to interact with scleral cells. The peptide products of the validated genes, besides potential involvement in intraretinal signaling, however, each have known actions on morphogenesis, cell proliferation, and/or extracellular matrix proliferation that may be pertinent to myopia pathogenesis, and each merits further investigation to establish any potential role(s) in refractive development.

The BMPs, a large and diverse group of signaling proteins, belong to the TGF-β superfamily of structurally related proteins. Although originally identified by their actions on bone, BMPs influence embryonic morphogenesis and postnatal processes in many tissues.[21,22] They are expressed in ocular tissues, such as retina, cornea and conjunctiva, where they may participate in embryogenesis and postnatal proliferation, differentiation, and repair.[23–27] BMP6 and BMP7 alter proteoglycan synthesis in cultured scleral cells (Shelton L et al. *IOVS* 2006;47:ARVO E-Abstract 1804). The mRNA for BMP2, the specific gene identified in this study, has been noted, in a candidate gene approach, to be downregulated in myopic chick eyes. Available only in abstract form, this report does not specify the tissue(s) studied (Escaño MFT et al. *IOVS* 1999;40: ARVO Abstract 2393).

CTGF (also called CCN2), a member of the cysteine-rich CCN matricellular regulatory protein family, interacts with integrins, heparin sulfate–containing proteoglycans, and the low-density lipoprotein receptor-related protein at the cell surface.[28–30] Its varied activities include roles in development, cell adhesion, cell proliferation, and extracellular matrix deposition/remodeling.[29,31] CTGF seems to be induced by, bind to, and mediate at least some of the effects of transforming growth factor (TGF)-β,[30,31] itself independently implicated in refractive development in the chick.[32] Although not previously studied in experimental myopia, CTGF has been associated with corneal wound healing, corneal scarring, conjunctival fibrosis, the responsiveness of trabecular meshwork cells and vitreoretinal fibrosis.[30,33,34]

Widely recognized as a neuropeptide used for signaling by mature central and peripheral neurons and expressed by a subset of retinal amacrine cells,[35] VIP has been implicated in experimental myopia in chicks (McGlinn AM et al. *IOVS* 1998; 39:ARVO Abstract 3293)[36,37] and monkeys.[38,39] VIP also is a regulator of the coordinated growth of the brain and nonneuronal tissues during embryogenesis, with system-dependent stimulatory or inhibitory effects.[40] These developmental effects include, for instance, the stimulation of cell division and differentiation of RPE cells of chick.[41]

Urotentsin II–related peptide (URP), the proteolytic product of the precursor peptide translated from the gene identified here (*LOC404534*), is a recently identified biologically active peptide with functions that are just beginning to be reported.[42–44] Closely related biochemically to urotensin II, URP activates the urotensin II receptor with high affinity.[42,43,45] We are aware of no prior reports implicating URP or urotensin II in the eye. Among many effects, activation of the urotensin receptor by urotensin II stimulates growth signaling pathways,[46,47] and thus URP merits future investigation as a potential retinally derived regulator of refractive development.

EDNRB, one of two endothelin receptors and the receptor subtype found to be differentially expressed, not only mediates vasoconstriction but also influences cell proliferation, collagen biosynthesis, and other potential developmental effects.[48–50] Because the chicken retina is avascular, differential expression of EDNRB in myopia suggests a potential nonvascular role for endothelins after form deprivation. As a specific example of a nonvascular role in the retina, the EDNRB in Müller cells seems involved in the response to light damage in the mouse.[51] Because *EDNRB* and not one of the endothelin precursor proteins[48,49] is differentially expressed, the endothelin system

may be involved in intraretinal signaling in form-deprivation myopia rather than in signaling to the sclera.

IL-18, a member of the IL-1 cytokine superfamily, is a major regulator of immune responses[52] but also influences vascular development, neurodegeneration and the function of a variety of other cell types.[53–55] Previously identified in retina and other ocular cells, IL-18 is not necessary for the manifestations of experimental autoimmune uveitis[56] but seems essential for normal retinal vascular development in mice.[57] How or whether altered expression of IL-18 modulates refractive development and/or pertinent retinal signaling requires future investigation.

MKP-2, a dual-specificity protein phosphatase,[58,59] is a component of the mitogen activated protein kinase (MAPK) pathways—sequential intracellular activation steps that influence many physiological processes, including proliferation and differentiation. Responding to many messengers, the MAPK pathways are regulated by scaffold proteins, compartmentalization and the phosphorylation state of the various intermediaries.[60–62] Altered expression of MKP-2 implicates intraretinal activity of MAPK pathways in response to any of the implicated peptides and/or other potential messengers mediating the myopia response.

Comparisons to Other Profiling Reports of Chick Form-Deprivation Myopia

In contrast to our use of a chicken genome array, investigators in prior studies seeking altered retinal gene expression in form-deprived chicks have used different approaches, specifically differential display,[63–65] suppressive subtractive hybridization,[66] candidate gene approaches (Escaño MFT et al. *IOVS* 1999;40:ARVO Abstract 2393; Ohngemach S et al. *IOVS* 2003; 44:ARVO E-Abstract 4337),[67–69] and a chicken immune system array (Rada JA et al. *IOVS* 2004;45:ARVO E-Abstract 1160). Many of these researchers explored a limited number of genes rather than analyzing the transcriptome per se, as is possible with microarrays. Several of these groups assayed retina/RPE[65,68,69] similar to our study; other studies included the choroid as well (Rada JA et al. *IOVS* 2004;45:ARVO E-Abstract 1160)[63,66,67]; one group apparently evaluated retina separated from RPE[64]; and another, available only in abstract form, does not specify the assayed eye tissues (Escaño MFT et al. *IOVS* 1999;40:ARVO Abstract 2393). In some of these other studies, gene expression was evaluated at or before 24 hours of visual deprivation (Ohngemach S et al. *IOVS* 2003;44:ARVO E-Abstract 4337),[64,65] but in the rest, gene expression was evaluated in the eyes after deprivation periods of 3 days or longer (Escaño MFT et al. *IOVS* 1999;40:ARVO Abstract 2393; Rada JA et al. *IOVS* 2004;45:ARVO E-Abstract 1160).[63,67–69] Even those prior investigations with broad sampling methods[63–66] identified few differentially expressed retinal genes, despite the rapid and robust eye-growth response to visual deprivation. Except for *BMP2* and *VIP*, we found no statistically significant differential expression or even suggestive changes of the genes identified in these prior studies, most of which are represented in the chicken microarrays used here. Variations in tissues, sampling methods, statistical criteria or other methodologic differences may account for the differences in results between studies. Deprivation time also is likely to be an important parameter because, in the absence of direct interventions that modify growth, it becomes increasingly ambiguous with time whether altered genes potentially represent a pathway producing myopia or instead reflect secondary changes such as those necessary for the retina to accommodate to the expanding vitreous cavity.

Similar to the mRNA profiling, a recent proteomics analysis revealed very few differentially expressed proteins in chick eye models of eye growth,[70] and our statistical criteria did not identify altered mRNA expression for any of these particular proteins. Not optimized for smaller peptides, the proteomics analysis did not identify the products of the genes validated in this study.[70]

Potential Clinical Implications

Much clinical literature supports gene–environment interactions in the pathogenesis of myopia. Human myopia has been linked to a variety of chromosomal locations, but identifying specific genes within these intervals responsible for common clinical myopias has not yet been accomplished.[71] The modest changes and relatively small number of altered genes, both here and in other approaches to profiling retinal gene expression in both chicks (Rada et al. *IOVS* 2004;45:ARVO E-Abstract 1160)[63–66] and monkeys (Lambert et al. *IOVS* 2004;45:ARVO E-Abstract 1161),[39] strikingly contrast with the robust myopic response that follows form deprivation in young animals. Within the limits of the gene representation in the chicken genome arrays used in this study, major changes in retinal gene expression levels may not be necessary for myopia to develop after environmental insults. The genes validated in the current study could suggest directions for clinical genetic studies. The VIP receptor 2 and the BMP type IB receptor map to the regions of the *MYP4* and *MYP11* genes, respectively, and each could be considered a candidate gene and/or a component of pathways for future research based on our profiling results.

The differentially expressed genes in chick form-deprivation myopia suggest potential mechanistic differences between the onset of myopia and the progression of established myopia. Similarly, clinical data have suggested potential mechanistic differences because negative refractive shifts seem to occur more rapidly after than before the onset of myopia.[72] If clinically useful anti-myopia therapies are ever to be introduced, it seems essential to establish whether the mechanisms responsible for myopia's onset differ from those responsible for its progression.

These results demonstrate the potential utility of applying genome-wide profiling to study experimental myopia. It is likely to be informative to extend this strategy to other perturbations that alter postnatal eye growth, such as spectacle lens wear, and to identify gene expression changes in the RPE specifically, in the choroid and in the sclera.

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E R R A T U M

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