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Research Paper

Role of Chronic Inflammation in Myopia Progression: Clinical Evidence and Experimental Validation

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ABSTRACT

Prevention and treatment of myopia is an important public problem worldwide. We found a higher incidence of myopia among patients with inflammatory diseases such as type 1 diabetes mellitus (7.9%), uveitis (3.7%), or systemic lupus erythematosus (3.5%) compared to those without inflammatory diseases ($p < 0.001$) using data from children (<18 years old) in the National Health Insurance Research database. We then examined the inhibition of myopia by atropine in Syrian hamsters with monocular form deprivation (MFD), an experimental myopia model. We found atropine downregulated inflammation in MFD eyes. The expression levels of c-Fos, nuclear factor κB (NFκB), interleukin (IL)-6, and tumor necrosis factor (TNF)-α were upregulated in myopic eyes and downregulated upon treatment with atropine. The relationship between the inflammatory response and myopia was investigated by treating MFD hamsters with the immunosuppressive agent cyclosporine A (CSA) or the inflammatory stimulators lipopolysaccharide (LPS) or peptidoglycan (PGN). Myopia progression was slowed by CSA application but was enhanced by LPS and PGN administration. The levels of c-Fos, NF-κB, IL-6, and TNF-α were upregulated in LPS- and PGN-treated eyes and downregulated by CSA treatment. These findings provide clinical and experimental evidence that inflammation plays a crucial role in the development of myopia.

1. Introduction

The prevalence of myopia has rapidly increased in recent decades, resulting in a significant global public health concern (Morgan et al., 2012; Pan et al., 2012). Globally, there are approximately 153 million people over the age of 5 years who suffer from visual defects (8 million of whom suffer from blindness) caused by uncorrected myopia and other refractive errors (Resnikoff et al., 2008). Myopia is an important and often undertreated eye disease. Although most cases of myopia can be corrected with glasses, contact lenses, or refractive surgery, uncorrected refractive errors still account for ~33% of visual impairments (Dandona and Dandona, 2001; McCarty, 2006). High-degree myopia is

Abbreviations: Systemic lupus erythematosus, SLE; type 1 diabetes mellitus, T1DM; monocular form deprivation, MFD; nuclear factor κB, NFκB; interleukin, IL; tumor necrosis factor α, TNF-α; cyclosporine A, CSA; lipopolysaccharide, LPS; peptidoglycan, PGN; collagen I, COL1; muscarinic acetylcholine receptor, mAChR; transforming growth factor β, TGF-β; matrix metalloproteinase 2, MMP2; juvenile chronic arthritis, JCA; refractive error, RE; National Health Insurance Research database, NHIRD; International Classification of Diseases, 9th Revision, Clinical Modification, ICD-9-CM; phosphate-buffered saline, PBS; Dulbecco's Modified Eagle Medium, DMEM; fetal bovine serum, FBS; Tris-buffered saline, TBS; 4',6-diamidino-2-phenylindole, DAPI; extracellular signal-regulated kinase, ERK; protein kinase B, AKT; phosphoinositide 3-kinase, PI3K; hazard ratio, HR; confidence interval, CI; retinal pigment epithelial cell, RPE.

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an especially important visual affliction because of the higher risks of macular and retinal complications. Myopia results primarily from abnormal elongation of the vitreous chamber of the eye (Curtin, 1985). This condition is recapitulated in the monocular form deprivation (MFD) animal model, which has been used to study myopia pathogenesis (McKanna and Casagrande, 1981). Eye elongation is associated with remodeling of the sclera (Marzani and Wallman, 1997; McKanna and Casagrande, 1981), loss of scleral tissue via reduced connective tissue synthesis, and increased collagen I (COL1) degradation, resulting in changes in the composition and ductility of the sclera (McBrien et al., 2000; Rada et al., 2002). Recent studies in monkeys showed that the retina—specifically, photoreceptors and retinal pigment epithelium—plays an important role in modulating eye growth and axial length (Smith et al., 2009; Smith et al., 2005; Smith et al., 2007) by producing activating signals that promote scleral tissue remodeling (Chen et al., 2012).

Animal studies of myopia have shown that atropine—a non-selective muscarinic acetylcholine receptor (mAChR) antagonist—effectively prevents the axial elongation, leading to myopia (Fan et al., 2007; Rada et al., 2000; Shih et al., 1999). Atropine inhibits myopia progression in the tree shrew, monkey, chicken, guinea pig, rat, mice, and Syrian hamster, and its effectiveness has also been demonstrated in human clinical trials (Shih et al., 1999; Shih et al., 2001). However, the mechanistic basis for this effect is still unclear (McBrien et al., 2013).

Various molecules have been implicated in myopia progression. In myopic eyes, transforming growth factor (TGF)-β and matrix metalloproteinase (MMP)2 expression is elevated, whereas COL1 expression is downregulated (Lin et al., 2006). TGF-β regulates cellular functions such as cell growth, differentiation, inflammation, and wound healing, while MMP family members play major roles in the breakdown of the extracellular matrix, tissue reconstruction (Wojciechowski et al., 2010; Wojciechowski et al., 2013), and tissue vascularization during the inflammatory response. The dysregulation of MMPs has also been proposed as a mechanism of pathogenesis in myopic eyes (Guggenheim and McBrien, 1996; Yang et al., 2010); MMP2 expression is upregulated in the sclera of chicks and tree shrews in which myopia has been induced by form deprivation (Guggenheim and McBrien, 1996; Jones et al., 1996; Rada and Brenza, 1995; Rada et al., 1999). TGF-β regulates the level of MMP2 via activation of nuclear factor (NF)-κB, a transcription factor that modulates the expression of various inflammatory cytokines in fibroblasts (Wang et al., 2011b).

Several reports have proposed a role for inflammation in myopia progression. A 26-year follow-up of patients with juvenile chronic arthritis (JCA) revealed myopic refractive errors (REs) in a greater fraction of these patients than in age-matched control subjects, suggesting a correlation between JCA and myopia. The study also suggested that the higher incidence of myopia was due to the weakening of scleral connective tissue as a result of chronic inflammation (Fledelius et al., 2001; Herbort et al., 2011). In addition, acute onset myopia may be a presenting feature of systemic lupus erythematosus (SLE) (Ayazi et al., 1982; Kamath et al., 2013; Shu et al., 1992). In this study, we provide clinical and experimental evidence to support an association between inflammation and myopia progression.

2. Materials and Methods

2.1. Nationwide Population-based Retrospective Cohort Study

2.1.1. Data Source

The National Health Insurance Research database (NHIRD), maintained by the National Health Research Institutes, is population-based and derived from the claims data of the National Health Insurance program, a mandatory-enrollment, single-payment system created in 1995, covering over 99% of Taiwan's population until the end of 2014. This file contained all medical claims and the information of insurants and provided a valuable resource, unique opportunity, and sufficiently large sample size to pursue the objectives addressed by this study. The high validity of the diagnostic data from the NHIRD has been previously reported (Cheng et al., 2011; Kang et al., 2010). Files for children (age <18 years) included 50% of those randomly selected from the Children's Registries from 1996 to 2008. The index of inflammatory diseases, including SLE, type 1 diabetes mellitus (T1DM), and uveitis, was coded based on the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM). Urbanization level was divided into seven categories based on a previous report (Liu et al., 2006), with levels 1 and 7 defined as highest and lowest, respectively. Because there were few children in levels 5–7, these were combined with level 4. Because of the personal electronic data privacy regulation, insurants' identities were encrypted before data were sent to the researcher; nonetheless, the study was approved the Institutional Review Board in China Medical University Hospital (CRREC-103-048).

2.1.2. Study Sample

Children newly diagnosed with SLE between 2000 and 2004 were identified from a database based on the ICD-9CM code (ICD-9-CM code 373.34, 695.4, and 710.0) as SLE cohort, and the date of SLE diagnosis was considered as the index date. We excluded patients with myopia (ICD-9-CM code 367.1), cataract (ICD-9-CM code: 366 and 743.3), glaucoma (ICD-9-CM code: 365), and uveitis (ICD-9-CM code: 360.11, 360.12, 362.18, 363.00, 363.01, 363.03, 363.05–363.08, 363.1×, 363.20, 363.21, 363.4×, 364.00–364.02, 364.04, and 364.1×–364.3×) before the index date. For each patients with SLE, four insured children were randomly selected from children without SLE, frequency-matched on sex, age (per 1 years), and index year using the same inclusion criteria as that of the SLE cohort, as control cohort. The index date for non-SLE group was randomly assigned a day and month and the index year was assigned the same year as the SLE group. We retrieved 1188 patients with SLE and 4752 children without SLE. The SLE and non-SLE cohorts were followed from index date to myopia diagnosis, withdrawal from the NHI program, or the end of 2008, whichever occurred first. Similar analyses to investigate the occurrence of myopia were also performed in T1DM (ICD-9-CM code: 250.X1 and 250.X3) with an appropriate non-T1DM cohort. The T1DM were determined through record linkage with the catastrophic illness registry of NHIRD. For uveitis (ICD-9-CM code: 360.11, 360.12, 362.18, 363.00, 363.01, 363.03, 363.05–363.08, 363.1×, 363.20, 363.21, 363.4×, 364.00–364.02, 364.04, and 364.1×–364.3×) cohort, we include the uveitis cohort from 2000 to 2005 and we exclude those patients with myopia before the index date. The non-uveitis cohort for uveitis was frequency-matched in sex, age (per 1 years), parents' occupational status, urbanization and index year.

2.2. Animals

Golden Syrian hamsters aged 3 weeks and weighing 80–90 g were used for the experiments. In total, 160 hamsters were used in this study. Albino and pigmented guinea pigs aged three weeks were also used in this study. The animals were kept under a 12-hour light/12-hour dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of China Medical University and were in accordance with the guidelines for the Use of Animals in Ophthalmic and Vision Research. Hamsters were raised with right eyelid fusion for 21 days. MFD was induced in the right eye (with the left eye serving as a control) of animals randomly assigned to treatment or control groups ($n = 10$ animals each) receiving daily applications of drug or phosphate-buffered

saline (PBS), respectively, to both eyes. MFD was induced in guinea pigs by covering the right eye with a cloth attached to the skin at a distance of at least 1 cm from the eye for 21 days. Guinea pigs were randomly assigned to treatment or control groups ($n = 10$ animals each) receiving daily applications of drug or PBS, respectively, to both eyes.

2.3. Cell Culture

ARPE-19 human retinal pigment epithelial cells were obtained from Bioresource Collection and Research Center, HsinChu, Taiwan (BCRC; BCRC-60,383). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2, with medium replacement every 3–4 days. Sclera were placed in a 60-mm culture dish in DMEM supplemented with 10% FBS to isolate primary scleral fibroblasts; those from fewer than three passages were used in experiments. Cells were seeded in 6-well plates (1×10^5 cells/well) and treated with lipopolysaccharide (LPS) at 100 ng/ml or left untreated for 4 h, followed by 100 μM atropine for 24 h. Cell lysates were collected for quantitative (q)PCR to determine gene expression levels.

2.4. cDNA Microarray Analysis

Sclera tissues were obtained from eyes with or without MFD. Total RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA integrity and purity were determined using an Agilent Bioanalyzer. Five different total RNA samples were pooled together (equal amounts) for cDNA microarray analysis. cDNA microarray analysis was performed using Affymetrix GeneChip Human Genome U133 Plus 2.0 and the procedures followed the manufacturer's guidance. cDNA microarrays were scanned using a GeneArray Scanner. The image files (.cel format) were analyzed by DNA Chip Analyzer software. Genes that were differentially expressed with a difference >1.2 fold between control eyes and myopic eyes were selected for ingenuity pathway analysis.

2.5. Physiological Measurements and Tissue Preparation

RE, i.e., the spherical component RE, which is defined as the mean RE in horizontal and vertical meridians, was measured using a handheld streak retinoscope. Animals were anesthetized with 10% ether in O_2. Ocular refraction was evaluated at the start and end of the experiment. At the end of the study, animals were sacrificed by CO_2 asphyxiation according to the guidelines of the Public Health Service, Office of Laboratory Animal Welfare, National Institutes of Health, and American Association of Veterinary Medicine. Eyes were enucleated using a razor blade on an ice plate under a surgical microscope (Topcon, Tokyo, Japan) by making a cut perpendicular to the anterior-posterior axis approximately 1 mm posterior to the ora serrata. The iris and ciliary body of the anterior segment of the eye were separated. Posterior sclera were excised using a 7-mm diameter trephine. The axial lengths were determined by A-scan ultrasonography (PacScan 300 Plus, New York, USA). The average of 10 different measurements was used.

2.6. Gene Expression Profiling by PCR Array

Total RNA of sclera tissues was isolated using an RNeasy Mini kit (Qiagen) and used for PCR array analysis. RNA integrity and purity were determined using an Agilent Bioanalyzer. Total RNA (1 μg) in a 20-μl final volume was reverse-transcribed using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Expression of genes involved in myopia progression was determined by using 96-well RT2 Profiler PCR Arrays-Human Autophagy (Qiagen, Frederick, MD, USA) in a LightCycler 480 PCR system (Roche, Germany).

2.7. Immunofluorescence Staining

Primary sclera fibroblast cells plated on cover slides were washed with Tris-buffered saline (TBS) and subsequently fixed with 4% paraformaldehyde and washed twice with TBS before blocking with 1% bovine serum albumin, 0.1% Triton X-100 for 1 h. Cells were incubated with anti-MMP2 or anti-collagen I antibodies for 1 h. Cells were washed three times with TBS and incubated with the appropriate secondary antibody and DAPI (4′,6-diamidino-2-phenylindole) DNA stain. After three washes with TBS, cells were imaged using fluorescence microscopy. All experiments were performed at least in triplicate.

2.8. Analysis of MMP2 and MMP9 Activity

Here, 1×10^6 cells were seeded in 24-well plates for at least 12 h. Cells were washed with PBS three times and incubated with culture medium without FBS in the presence or absence of 1 μg/ml LPS or 1 μg/ml LPS + 100 μM atropine or 1 μg/ml LPS + 50 μM diacerein. Culture supernatants were collected after 48 h and mixed with an equal volume of loading buffer (125 mM Tris–HCl, pH 6.8, 3% SDS, 40% glycerol, 0.02% bromophenol blue). To measure MMP-2/MMP-9 activity, samples were separated by 8% SDS-PAGE containing 0.1% gelatin.

2.9. Western Blot Analysis

ARPE-19 cells were treated with PBS (control), 100 ng/ml lipopolysaccharide (LPS; Sigma), or LPS + 100 μM atropine for 30 min. After treatment, 30 μg of the total cell lysate was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblot analysis. The primary antibodies used included extracellular signal-regulated kinase (ERK) (Thr202/Tyr204), protein kinase B (AKT) (Ser473), phosphoinositide 3-kinase (PI3K) (p85(Tyr458)/p55(Tyr199)), NF-κB (p65, Ser536), and c-Fos (Ser32) (Cell Signaling, Beverly, MA). Anti-rabbit and anti-mouse secondary antibodies conjugated with horseradish peroxidase were also used. Immunoreactive protein bands were detected using an enhanced chemiluminescence kit (ECL, Pierce, Thermo Fisher Scientific, Pittsburgh, PA, USA). Equal loading was confirmed by probing the blots with an anti-β-actin antibody (Abcam, Cambridge, MA, USA) as well as antibodies against anti-ERK, AKT, PI3K, NF-κB, and c-Fos.

2.10. qPCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and 5 μg RNA was reverse-transcribed to cDNA using the Superscript First Strand Synthesis system (Invitrogen, Carlsbad, CA). Primers and probes used for qPCR were selected from the Universal Probes Library (Roche, West Sussex, UK). Transcript levels were normalized to that of glyceraldehyde 3-phosphate dehydrogenase in each sample.

2.11. Immunohistochemistry

Eyes were collected from atropine-treated and control animals, embedded in paraffin, and cut at a thickness of 20 μm; sections were collected on glass slides. Antigen retrieval was performed by boiling the slides in citrate buffer (pH 6.0); sections were then stained with antibodies against interleukin (IL)-6, tumor necrosis factor (TNF)-α, TGF-β, MMP2, c-Fos, NF-κB, and mAChR 1 and 3.

Table 1
Demographic factors and comorbidity of study participants according to type I diabetes mellitus, uveitis, and systemic lupus erythromatosus.

	T1DM control ($n = 2196$)		T1DM ($n = 549$)		*p*-Value	Uveitis control ($n = 3120$)		Uveitis ($n = 780$)		*p*-Value	SLE control ($n = 4752$)		SLE ($n = 1188$)		*p*-Value
Variable	n	%	n	%		n	%	n	%		n	%	n	%	
Gender					0.99					0.99					0.99
Girl	1180	53.73	295	53.73		1272	40.77	318	40.77		3736	78.62	934	78.62	
Boy	1016	46.27	254	46.27		1848	59.23	462	59.23		1016	21.38	254	21.38	
Age, years					0.99					0.99					0.99
1–6	636	28.96	159	28.96		692	22.18	173	22.18		560	11.78	140	11.78	
7–12	904	41.17	226	41.17		944	30.26	236	30.26		1480	31.14	370	31.14	
13–18	656	29.87	164	29.87		1484	47.56	371	47.56		2712	57.07	678	57.07	
Means (SD)	9.93	(4.62)	9.91	(4.61)	0.93	11.56	(4.74)	11.59	(4.74)	0.99	12.77	(4.07)	12.79	(4.07)	0.91
Urbanization					0.46					0.99					0.74
Level 1 (highest)	614	27.96	141	25.68		927	29.62	231	29.62		1331	28.01	333	28.03	
Level 2	703	32.01	170	30.97		1020	32.69	255	32.69		1486	31.27	378	31.82	
Level 3	405	18.44	115	20.95		632	20.26	158	20.26		903	19	210	17.68	
Level 4 (lowest)	474	21.58	123	22.4		544	17.44	136	17.44		1032	21.72	267	22.47	
Parents' occupational status					0.05					0.99					0.46
White collar	1250	56.92	289	52.64		1780	57.05	445	57.05		2629	55.32	665	55.98	
Blue collar	709	32.29	182	33.15		992	31.79	248	31.79		1659	34.91	421	35.44	
Other	237	10.79	78	14.21		348	11.15	87	11.15		464	9.76	102	8.59	

Abbreviations: T1DM, type 1 diabetes mellitus; SLE, systemic lupus erythromatosus; SD, standard deviation.

The EnVision System peroxidase kit (DAKO, Carpentaria, CA, USA) was used to visualize immunoreactivity.

2.12. Statistical Analysis

Patients and control groups were compared in terms of the distribution of demographic factors, including sex, age, urbanization, and parent's occupation with the χ^2 test. The incidence rates of myopia were calculated by using the number of developed myopia dividing by person-years at risk in both cohorts. We estimated the cumulative incidence of myopia using the Kaplan–Meier method, and the significance of these curves were assessed by the log-rank test. The Cox proportional hazards model was used to calculate the hazard ratio (HR) and 95% confidence interval (CI) of myopia in patients with inflammatory diseases in comparison to those of controls. All analyses were performed with SAS statistical software (version 9.4 for Windows; SAS Institute, Inc., Cary, NC, USA). Statistical significance was determined as $p < 0.05$.

Table 2
Cox model measured hazard ratio and 95% confidence intervals of myopia associated with inflammatory diseases and covariates.

Variables	Crude	Adjusted▲	Crude	Adjusted▼	Crude	Adjusted△
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Disease	T1DM		Uveitis		SLE	
No	1	1	1	1	1	1
Yes	1.55 (1.27–1.90)***	1.57 (1.29–1.92)***	1.46 (1.16–1.85)**	1.47 (1.16–1.86)**	1.44 (1.21–1.72)***	1.47 (1.23–1.74)***
Gender						
Girl	1	1	1.20 (0.98–1.48)	1.14 (0.93–1.41)	1	1
Boy	0.81 (0.67–0.96)*	0.79 (0.66–0.95)*	1	1	1.08 (0.91–1.29)	0.88 (0.74–1.04)
Age, years						
1–6	4.87 (3.24–7.33)***	4.86 (3.23–7.31)***	7.49 (4.94–11.35)***	7.83 (5.15–11.89)***	7.16 (5.55–9.24)***	7.4 (5.72–9.56)***
7–12	3.79 (2.53–5.70)***	3.78 (2.52–5.69)***	4.68 (3.07–7.13)***	4.82 (3.16–7.34)***	3.72 (2.91–4.76)***	3.74 (2.92–4.78)***
13–18	1	1	1	1	1	1
Urbanization						
Level 1 (highest)	1	1	1	1	1	1
Level 2	0.88 (0.70–1.10)	0.89 (0.71–1.12)	0.88 (0.68–1.13)	0.78 (0.60–1.01)	0.90 (0.74–1.09)	0.89 (0.73–1.08)
Level 3	0.94 (0.73–1.21)	0.92 (0.71–1.19)	0.78 (0.58–1.05)	0.68 (0.50–0.92)*	0.95 (0.76–1.19)	0.94 (0.75–1.18)
Level 4 (lowest)	0.80 (0.62–1.04)	0.86 (0.66–1.12)	0.79 (0.57–1.10)	0.87 (0.63–1.22)	0.89 (0.72–1.10)	0.90 (0.72–1.13)
Parents' occupational status						
White collar	1	1	1	1	1	1
Blue collar	0.7 (0.57–0.86)***	0.75 (0.61–0.93)**	0.75 (0.59–0.95)*	0.78 (0.61–1.00)	0.82 (0.70–0.97)*	0.88 (0.74–1.05)
Other	0.72 (0.53–0.98)*	0.71 (0.52–0.96)*	0.70 (0.49–1.00)	0.69 (0.48–0.99)*	0.7 (0.52–0.93)*	0.7 (0.52–0.94)*

Abbreviation: T1DM, type 1 diabetes mellitus; SLE, systemic lupus erythematosus; HR, hazard ratio; CI, confidence interval.
▲: Multivariable analysis including T1DM, gender, age (categorical), urbanization, and parents' occupational status.
▼: Multivariable analysis including uveitis, gender, age (categorical), urbanization, and parent' occupational status.
△: Multivariable analysis including SLE, gender, age (categorical), urbanization, and parent' occupational status.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Incidence density rates and hazard ratios of myopia stratified by the severity of inflammatory diseases.

Diseases	Average frequency for medical visit, per years	N	Event no.	Person-years	IR	Adjusted HR (95% CI)★	*p* for trend
T1DM control		2196	366	10,083.49	36.3	1.00	<0.001
T1DM cohort	<13	187	31	836.09	37.08	1.09 (0.75–1.57)	
	13–17	212	59	935.54	63.06	1.71 (1.30–2.25)***	
	>17	150	43	574.27	74.88	1.93 (1.41–2.65)***	
Uveitis control		3120	267	11,873.05	22.49	1.00	<0.001
Uveitis cohort	≤1	263	19	1411.26	13.46	0.52 (0.32–0.82)**	
	2–3	260	30	939.56	31.93	1.48 (1.01–2.16)*	
	≥4	257	45	507.37	88.69	5.76 (4.15–8.00)***	
SLE control		4752	508	17,320.17	29.33	1.00	<0.001
SLE cohort	≤1	581	75	2573.09	29.15	0.90 (0.70–1.14)	
	2–3	137	24	336.57	101.02	3.62 (2.55–5.13)***	
	≥4	470	62	1124.71	55.13	2.59 (1.98–3.40)***	

Abbreviation: T1DM, type 1 diabetes mellitus; SLE, systemic lupus erythematosus; IR, incidence density rates, per 1000 person-years; HR, hazard ratio; CI, confidence interval.
★: Adjusted for gender, age (continuous), urbanization, and parents' occupational status in Cox proportional hazards regression.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3. Results

3.1. Association Between Inflammatory Disorders and Subsequent Myopia Risk

A retrospective cohort study was conducted using data from children (<18 years old) in the NHIRD to determine whether the inflammatory diseases T1DM, uveitis, and SLE are associated with the incidence of myopia. Al total of 549 T1DM, 780 uveitis, and 1188 SLE patients were newly diagnosed and randomly matched with regard to age, sex, and index year with subjects without T1DM, uveitis, or SLE from the general population in a 1:4 ratio (Table 1). Cohorts were followed until the end of 2008, when the incidence of myopia was assessed. The risks of myopia was higher by 1.57 fold (95% CI = 1.27–1.92) in T1DM, 1.47 fold (95% CI = 1.16–1.86) in uveitis, and 1.47 fold (95% CI = 1.23–1.74) in SLE cohorts than in controls (Table 2). The data in Table 3 show that patients with T1DM who visited clinics >17 times per year exhibited a 1.93-fold increased risk of myopia after we adjusted for age, sex, and urbanization (95% CI, 1.41–2.65) compared with the control cohort. The risk of myopia development increased from 1.09 (95% CI, 0.75–1.57) in patients with <13 medical visits to 1.71 (95% CI, 1.30–2.25) in patients with 13–17 medical visits and further increased to 1.93 (95% CI, 1.41–2.65) in patients with ≥17 medical visits compared with the control cohort (trend test, $p < 0.001$). For uveitis patients, the risk of myopia development increased from 0.52 (95% CI, 0.32–0.82) in patients with <1 medical visit to 1.48 (95% CI, 1.01–2.16) in patients with 2–3 medical visits and further increased to 5.76 (95% CI, 4.15–8) in patients with ≥4 medical visits compared with the control cohort (trend test, $p < 0.001$). For SLE patients, the risk of myopia development increased from 0.90 (95% CI, 0.70–1.14) in patients with <1 medical visit to 3.62 (95% CI, 2.55–5.13) in patients with 2–3 medical visits; however, it was reduced to 2.59 (95% CI, 1.98–3.40) in patients with ≥4 medical visits compared with the control cohort (trend test, $p < 0.001$). The cumulative incidence of myopia by the end of the follow-up period was 7.9%, 3.7%, and 3.5% higher in the T1DM, uveitis, and SLE groups, respectively (Fig. 1a–c) than in control subjects. These findings provide a clinical correlation for the association between inflammatory diseases and the occurrence of myopia.

3.2. Genes Involved in Inflammation are Upregulated in Myopia

To study the role of inflammation in myopia progression, a PCR array for inflammatory cytokines and receptors was used to determine the differential expression of genes in a MFD animal model. After 21 days, transcript levels for the transcription factors *c-Fos* and *NF-κB* were 1.25 and 1.52 fold higher, respectively, in sclera of MFD eyes compared to that of non-MFD eyes ($p < 0.05$) (Table 4). The same pattern was observed for various inflammatory cytokines including *IL-6* (2.05 fold), *TNF-α* (1.54 fold), *TGF-β* (1.49 fold), and *IL-1β* (1.87 fold) ($p < 0.05$). Conversely, the expression of the anti-inflammatory cytokine IL-10 was 0.58 fold lower in MFD than in non-MFD eyes ($p < 0.05$). Because atropine affects both the sclera and retina, the differential expression of inflammatory genes was examined in human retinal pigment epithelial cells (ARPE-19) and scleral fibroblasts. The expression of *mAChR 1* and *3*, *c-Fos*, *IL-6*, *IL-1β*, *TGF-β*, *TNF-α*, and *NF-κB* was upregulated by LPS treatment, but this effect was suppressed in both cell types in the presence of atropine ($p < 0.05$). In contrast, *IL-10* expression was suppressed by LPS and enhanced by atropine ($p < 0.05$). These results suggest that the inflammatory response is linked to myopia progression.

To study the molecular mechanism of how atropine inhibited inflammation, ARPE-19 cells were treated with LPS or LPS/atropine for 4 h. The activation of ERK and its downstream signaling molecule c-FOS by LPS was inhibited by atropine treatment. Atropine treatment also inhibited the LPS activation of PI3K, AKT, and NF-κB (Fig. 2a). Similar results were found in primary RPE cells isolated from Syrian hamsters (Fig. 2a). These results indicate that atropine inhibits inflammation through downregulation of the ERK-c-FOS and PI3K-AKT-NF-κB pathways. Moreover, we found that atropine decreased the expression levels of MMP2 and increased COL1 expression in primary sclera fibroblasts isolated from Syrian hamster (Fig. 2b). MMP2 activity was increased in both RPE cells and sclera fibroblasts activated by LPS, and decreased by atropine and diacerein (an anti-inflammatory compound) (Fig. 2c, d).

After MFD, we found a time-dependent increase in the expression of MMP2 and TGFβ and a time-dependent decrease of collagen I levels in the retina and sclera from day 2 to day 21 by immunohistochemistry (Fig. 3a). The same trend was also observed for the expression levels of NF-κB, c-FOS, IL-6, and TNF-α (Fig. 3b).

3.3. Myopia Progression is Inhibited by Atropine

The change in RE for the PBS-treated MFD group was −7.22 ± 0.22 for MFD eyes. The change in RE decreased as a function of atropine concentration: at 0.125%, 0.5%, and 0.1% atropine, the changes in RE values were −5.45 ± 0.07, −4.89 ± 0.18, and −4.74 ± 0.39 D, respectively, for the MFD eye (ANOVA $p < 0.001$; Fig. 4a). The changes in axial length in the control and 0.125%, 0.5%, and 0.1% atropine groups were 1.04 ± 0.12, 0.80 ± 0.09, 0.75 ± 0.26,

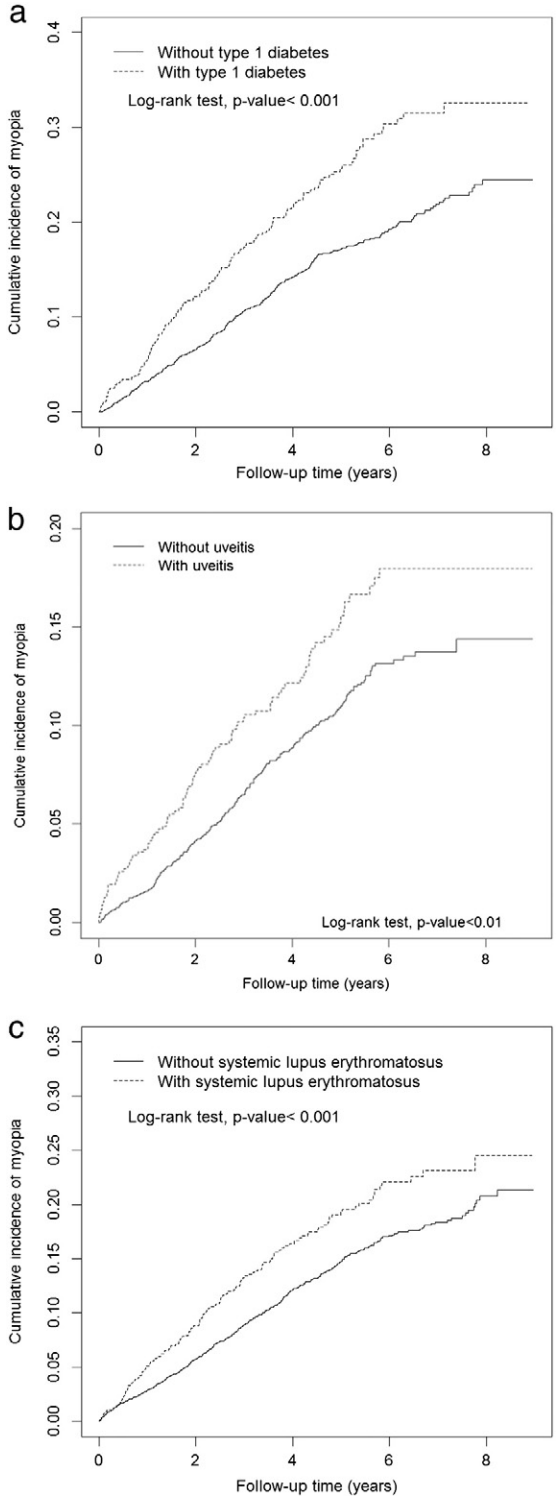

Fig. 1. Association between systemic inflammatory diseases and myopia incidence. Cumulative incidence of myopia is shown for control subjects and patients with (a) type I diabetes mellitus, (b) uveitis, or (c) systemic lupus erythromatosus.

and 0.68 ± 0.31, respectively (ANOVA $p < 0.05$; Fig. 4b). These data suggest that atropine administration inhibits myopia progression.

To confirm the induction of myopia in our animal model, we determined the expression levels of *TGF-β* and *MMP2* in the sclera by quantitative real-time PCR. Expression levels of *TGF-β* and *MMP2* were higher by 1.49 and 1.59 fold, respectively, in MFD eyes ($p < 0.05$) (Table 4). Although the expression of *mAChR2*, *4*, and *5* was similar between groups, *mAChR1* and *3* levels were 1.54 and 1.68 fold higher, respectively, in MFD than in non-MFD eyes ($p < 0.05$) (Table 4).

mAChR1 and 3 expression levels were higher in the sclera of the MFD than in the non-MFD eye; mAChR1 and 3 were downregulated in MFD eyes after atropine treatment as compared to the levels in PBS-treated MFD eyes (Fig. 4c). Compared to PBS treatment, treatment with 1% atropine decreased MMP2 expression and increased COL1 expression in the sclera of MFD eyes, as determined by immunohistochemistry (Fig. 4d). The TGF-β level was also upregulated in both the retina and sclera by MFD, but this effect was suppressed by atropine (Fig. 4e). These results indicate that the expression of genes that promote myopia progression via tissue remodeling is altered by MFD but corrected by atropine treatment. The expression of c-Fos and NF-κB in the retinas of MFD eyes was higher than that in non-MFD eyes, but was suppressed by treatment with 1% atropine (Fig. 4f; Supplementary Figs. 1, 2). IL-6 and TNF-α immunoreactivity in the retina was elevated in MFD eyes, but the expression of these factors was reduced by application of 1% atropine. Conversely, IL-10 expression was elevated following atropine treatment (Fig. 4g; Supplementary Figs. 1, 2). These results indicate that atropine inhibits myopia progression through downregulation of inflammation in the eye.

3.4. Suppression of Inflammation Inhibits the Progression of Myopia

To determine whether decreased inflammation inhibits myopia progression, the immunosuppressive agent cyclosporine A (CSA) was applied to the eyes of hamsters and the RE was measured on day 21. The changes in RE for the PBS and CSA-treated groups were −3.77 ± 0.48 and −2.29 ± 0.50 D, respectively ($p < 0.0001$; Fig. 5a), indicating that the progression of myopia was blocked. This finding was supported by the observation of concomitant decreases in MMP2 and TGF-β expression (Fig. 5b).

To test whether increased inflammation enhances the progression of myopia, LPS and peptidoglycan (PGN) (500 ng/ml), which are inducers of inflammation originating from Gram-negative and -positive bacterial cell walls, respectively, were applied to the eyes of MFD mice every second day for 21 days. The changes of RE for PBS, LPS, and PGN-treated animals were −4.27 ± 0.49, −5.90 ± 0.54, and −4.90 ± 0.83 D for occluded eyes, respectively (Fig. 5c; $p < 0.0001$). We also found an increase in RE in unblocked eyes. The changes of RE for PBS, LPS and PGN-treated animals were −2.58 ± 0.36, −4.13 ± 0.9 and −5.03 ± 0.43 D for unblocked eyes, respectively, suggesting a direct link between inflammation and myopia progression (Fig. 5c; $p < 0.01$). The decline in RE was accompanied by upregulation of MMP2 and TGF-β (Fig. 5d). To further evaluate this mechanism, LPS and PGN were applied to the eyes of hamsters without MFD. After 21 days, the changes of RE for the PBS-treated group were −0.88 ± 0.18 and −0.18 ± 0.57 D for the right and left eyes, respectively. These values declined to −3.69 ± 0.57 and −3.95 ± 0.97 D, respectively, upon LPS treatment, and −3.03 ± 1.04 and −3.55 ± 0.35, respectively, in PGN-treated eyes, and these differences were statistically significant with respect to the PBS-treated group ($p < 0.001$; Fig. 5e). These changes occurred concurrently with the upregulation of TGF-β and MMP2 expression (Fig. 5f).

CSA reduced c-Fos and NF-κB expression (Fig. 6a) that was stimulated by LPS or PGN (Supplementary Fig. 3). IL-6 and TNF-α levels, which were increased by LPS or PGN treatment (Supplementary Fig. 4), were decreased by CSA (Fig. 6b); IL-10 immunoreactivity was reduced by LPS or PGN (Supplementary Fig. 4) but increased after CSA treatment (Fig. 6b). Taken together, these results indicate that induction of inflammation causes acceleration of myopia, which can be reversed by application of anti-inflammatory agents.

Table 4
Gene expression levels in scleral tissue and fibroblasts and retinal piement epithelial cells as determined by real-time PCR.

Gene symbol[a]	Accession No.	Sclera tissue		Retinal pigment epithelial cell		Sclera fibroblast	
		CL[b]	CR[b]	LPS[c]	LPS-atropine[d]	LPS[c]	LPS-atropine[d]
Chrm 1	NM_080773.1	1 ± 0.17	1.54 ± 0.12[#]	1.54 ± 0.21	0.65 ± 0.16[#]	1.49 ± 0.18	0.73 ± 0.11[#]
Chrm 2	NM_031016.1	1 ± 0.14	0.98 ± 0.15[#]	0.55 ± 0.23	1.96 ± 0.15[#]	1.48 ± 0.17	1.24 ± 0.14
Chrm 3	NM_012527.1	1 ± 0.15	1.68 ± 0.21[#]	1.27 ± 0.18	0.75 ± 0.21[#]	1.58 ± 0.15	0.70 ± 0.23[#]
Chrm 4	NM_031547.1	1 ± 0.2	0.89 ± 0.3	0.84 ± 0.07	0.91 ± 0.25	1.07 ± 0.23	1.11 ± 0.17
Chrm5	NM_017362.4	1 ± 0.22	0.84 ± 0.14	0.74 ± 0.11	0.83 ± 0.15	0.93 ± 0.15	0.98 ± 0.12
Fos	DQ089699.1	1 ± 0.1	1.25 ± 0.18[#]	2.39 ± 0.18	0.69 ± 0.17[#]	1.00 ± 0.11	0.93 ± 0.17
IL10	NM_012854.2	1 ± 0.13	0.58 ± 0.1[#]	0.46 ± 0.17	1.92 ± 0.16[#]	0.56 ± 0.19	1.05 ± 0.21[#]
IL-6	NM_012589.1	1 ± 0.18	2.05 ± 0.26[#]	3.28 ± 0.17	0.89 ± 0.26[#]	3.02 ± 0.16	0.79 ± 0.18[#]
IL1b	NM_031512.2	1 ± 0.12	1.87 ± 0.14[#]	2.63 ± 0.15	0.57 ± 0.19[#]	2.84 ± 0.21	0.75 ± 0.13[#]
Tgfb1	NM_021578.2	1 ± 0.13	1.49 ± 0.12[#]	1.48 ± 0.14	0.76 ± 0.13[#]	2.15 ± 0.23	0.48 ± 0.24[#]
Tnfa	AF269159.1	1 ± 0.15	1.54 ± 0.15[#]	4.3 ± 0.25	0.82 ± 0.11[#]	1.49 ± 0.15	0.80 ± 0.2[#]
NFkB	AF079314.1	1 ± 0.1	1.52 ± 0.14[#]	1.99 ± 0.12	0.76 ± 0.17[#]	4.50 ± 0.27	0.36 ± 0.3[#]
MMP2	NM_031054.2	1 ± 0.19	1.59 ± 0.08[#]	1.73 ± 0.14	0.78 ± 0.24[#]	1.79 ± 0.17	0.74 ± 0.19[#]

[#] Statistically significant ($p < 0.05$) by the Student's *t*-test for paired comparisons between LPS and LPS + atropine.
[a] Genes that were differentially expressed between CR and CL in the cDNA microarray.
[b] CR, form-deprived right eye (occluded eye); CL, left eye.
[c] Cells were treated with 100 ng/ml LPS for 4 h.
[d] Cells were treated with 100 ng/ml LPS and 100 μM atropine for 4 h.

4. Discussion

The inflammatory response attracts cytokines, prostaglandins, blood cells, and growth and cytotoxic factors to the site of infection or injury and also redirects blood flow to these areas (Kawai and Akira, 2006); this response induces local biochemical reactions, resulting in tissue remodeling (Kim et al., 2012). In myopia, similar structural modifications occur, and it was therefore hypothesized that inflammation could play a role in myopia progression.

Myopia is more common among diabetic patients than in the general population (38% vs. 27%) (Fledelius, 1983), and type I and II diabetes mellitus are risk factors for myopia (Chen et al., 2008; Cordain et al., 2002; Jacobsen et al., 2008; Tarczy-Hornoch et al., 2006; Wu et al., 2005). Type 2 diabetes mellitus is an inflammatory disease and is associated with elevated serum levels of IL-1β and -6, TGF-β, and TNF-α (Donath and Shoelson, 2011). Similarly, visual system dysfunction, such as myopia, has been reported in up to 30% of SLE patients. Moreover, the inflammatory condition dry eye

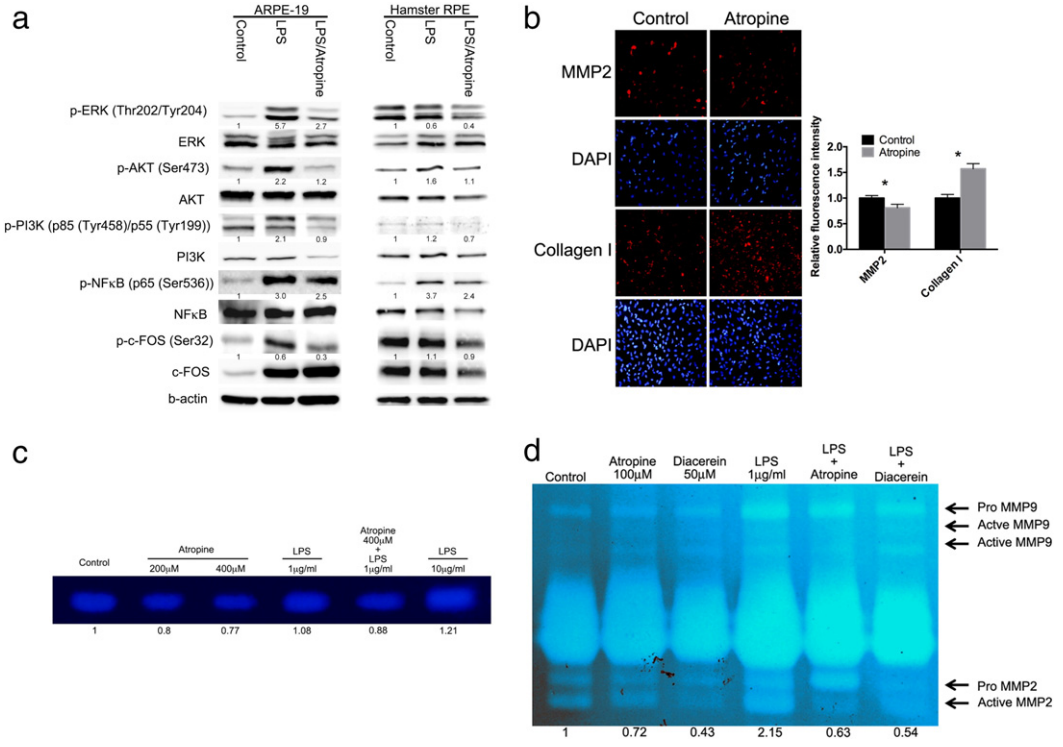

Fig. 2. Effect of atropine on the expression of genes and signaling pathways involved in tissue remodeling. (a) Effect of atropine on LPS-induced activation of NF-κB and AP1 via inhibition of the PI3K–AKT and MAPK pathways. ARPE-19 and primary retinal pigmented epithelial cells were treated with PBS (control), 100 ng/ml LPS, or LPS + 100 μM atropine for 30 min and then harvested for western blot analysis to determine the phosphorylation status of ERK (Thr202/Tyr204), AKT (Ser473), PI3K (p85(Tyr458)/p55(Tyr199)), NF-κB (p65, Ser536), and c-Fos (Ser32). Fold changes of phosphorylated signaling molecules were normalized to levels of non-phosphorylated proteins. The intensities of each band were determined by ImageJ software. (b) Primary scleral fibroblast cells were treated with or without 100 μM atropine for 24 h. Immunofluorescence analysis of MMP2 and COL1 expression in primary scleral fibroblasts; nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). (c, d) Gelatin zymograph of pro- and active MMP2 in ARPE-19 retinal pigment epithelial cells (c) or scleral fibroblasts (d) treated with 1 μg/ml LPS with or without atropine. The MMP2 inhibitor diacerein was used as the control.

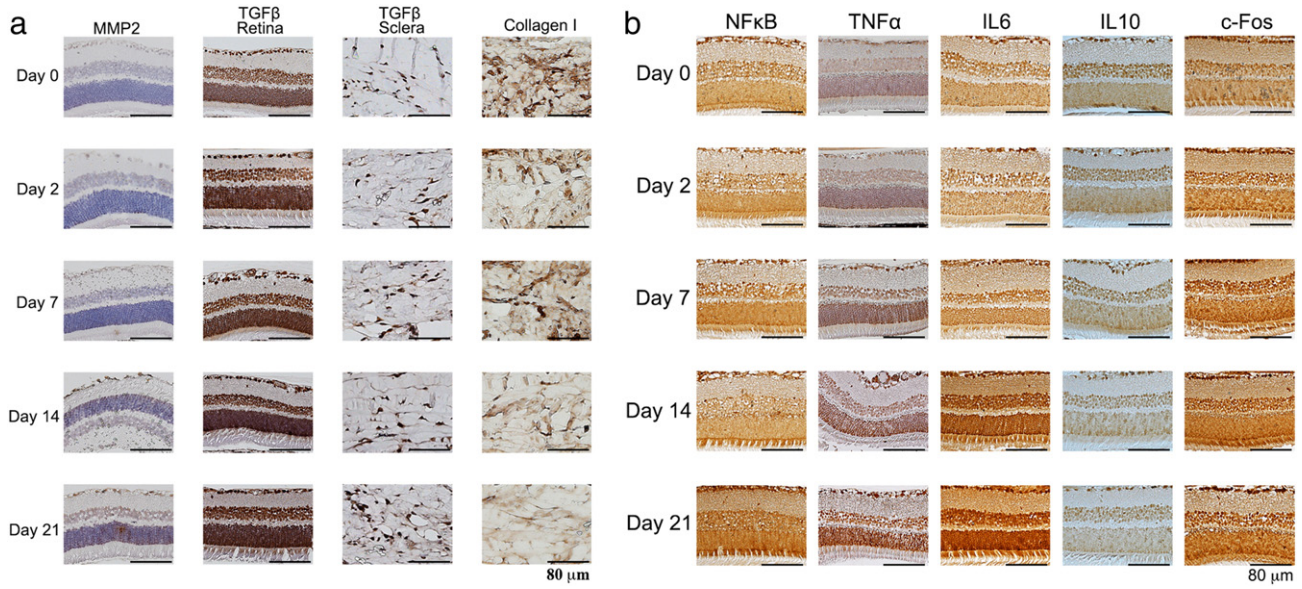

Fig. 3. Levels of tissue remodeling proteins and inflammatory molecules increase over time in occluded eyes. MFD was induced in Syrian hamsters, and the occluded eyes were collected at days 0, 2, 7, 14 and 21. (a) Immunohistochemical analysis of TGF-β, MMP2, and collagen I expression in occluded eyes. (b) Immunohistochemical analysis of NF-κB, c-FOS, TNF-α, IL-6, and IL-10 levels in occluded eyes.

syndrome as well as episcleritis and scleritis are presenting features of SLE (Palejwala et al., 2012; Sivaraj et al., 2007). Uveitis can induce acute or constitutive myopia and myopic shift, whereas acute myopia is observed in patients with acute scleritis (Gross et al., 1993). Among all uveitis cases, approximately 6% are found in individuals under 18 years of age (Nguyen and Foster, 1998), and the most common cause of intraocular inflammation is JCA-associated uveitis (Paivonsalo-Hietanen et al., 2000). Among all JCA patients, approximately 10–20% suffer from chronic uveitis (Boone et al., 1998; Kesen et al., 2008; Kotaniemi et al., 1999; Malleson, 1998). Chronic uveitis is generally asymptomatic in children with JCA, but the disease progresses gradually, possibly resulting in blindness. The high incidence of uveitis among JCA patients may increase the progression of myopia. Higher expression levels of the inflammatory cytokines IL-6 and TNF-α were found in the aqueous humor of uveitis patients (Chen et al., 2015). These increased levels of IL-6 and TNF-α in the eye support the progression of myopia. The systemic acute or chronic inflammatory state associated with these diseases likely increases the occurrence of myopia.

Myopia has been linked to urbanization; however, in this study, no differences were noted between T1D, uveitis, and SLE patients and control subjects in terms of urbanization (Tables 1 and 2). The results indicate that the inflammatory reactions initiated by T1D, uveitis, and SLE are major contributors to the incidence of myopia. Significantly, in the T1D, uveitis and SLE groups, a higher incidence of myopia was observed among younger patients (under 12 years of age) than in patients aged 13–18 years, which suggests that the younger one affected by inflammation, the higher risk of myopia.

In MFD hamsters, inflammatory genes were differentially expressed in the sclera and retina. Furthermore, consistent with previous reports (Guggenheim and McBrien, 1996; Honda et al., 1996; Lind et al., 1998; Seko et al., 1995), *TGF-β* and *MMP2* transcript levels were elevated in myopic as compared to non-myopic eyes; in contrast, *COL1* expression was downregulated. These changes were reversed by application of atropine in cultured primary sclera fibroblasts and retinal pigment epithelial cells as well as in vivo. The increase in RE by CSA treatment and decrease in RE by LPS or PGN demonstrate that inflammation plays a key role in myopia progression. In addition to hamsters, we also found a similar increase in the inflammatory response in both albino guinea pigs and pigmented guinea pigs. MFD was induced in guinea pigs by covering the right eye with a cloth attached to the skin at a distance of at least 1 cm from the eye (Supplementary Fig. 5). Atropine (1%) was applied to the eyes of the guinea pigs, and the RE and axial lengths were measured on day 21. The change in RE for the PBS-treated group was -9.22 ± 0.93 D for MFD eyes. Upon treatment with atropine, these values changed to -6.79 ± 1.00. The change in axial length for the PBS-treated group was 1.17 ± 0.01 mm for MFD eyes. Upon treatment with atropine, these values changed to 1.14 ± 0.01 mm (Supplementary Table 1). Both RE and axial length showed statistically significant differences between PBS- and atropine-treated MFD eyes (all $p < 0.005$). The expression levels of MMP2, TGF-β, and c-Fos increased in myopic eyes, whereas that of COLI decreased. Atropine treatment decreased MMP2, TGF-β, and c-Fos expression and increased COL1 expression in the sclera and retina of MFD eyes compared to eyes treated with PBS (Supplementary Figs. 6, 7). IL-10 was downregulated by MFD, and this effect was suppressed by atropine (Supplementary Fig. 7). These results reveal consistent outcomes for a different MFD method as well as in a different species of animal.

Studies in various cell lines have shown that activation of mAChRs is associated with airway inflammation. Activation of mAChR stimulates the release of the pro-inflammatory chemotactic factor leukotriene B4 from macrophages, which can be inhibited by the mAChR M3 inhibitor tiotropium (Buhling et al., 2007). In guinea pigs, atropine inhibited the pro-inflammatory vagovagal reflex, eosinophil infiltration into the lung, and lung neutrophilia induced by diesel soot (McQueen et al., 2007). Application of an M3 mAChR inhibitor reduced airway inflammation and lowered IL-4, -5, and -13 and TGF-β1 expression in the bronchoalveolar lavage fluid in acute or chronic asthma (Ohta et al., 2010). LPS-induced acute lung injury was inhibited by atropine and mAChR M3 antagonist treatment, as indicated by a decrease in neutrophil infiltration and downregulation of TNF-α and IL-6 expression (Xu et al., 2012). Taken together, these results indicate that the inflammatory response is increased by mAChR activation, and is suppressed by mAChR antagonism.

TGF-β has been implicated in many human diseases and disorders, including myopia, although it is not known whether TGF-β is produced

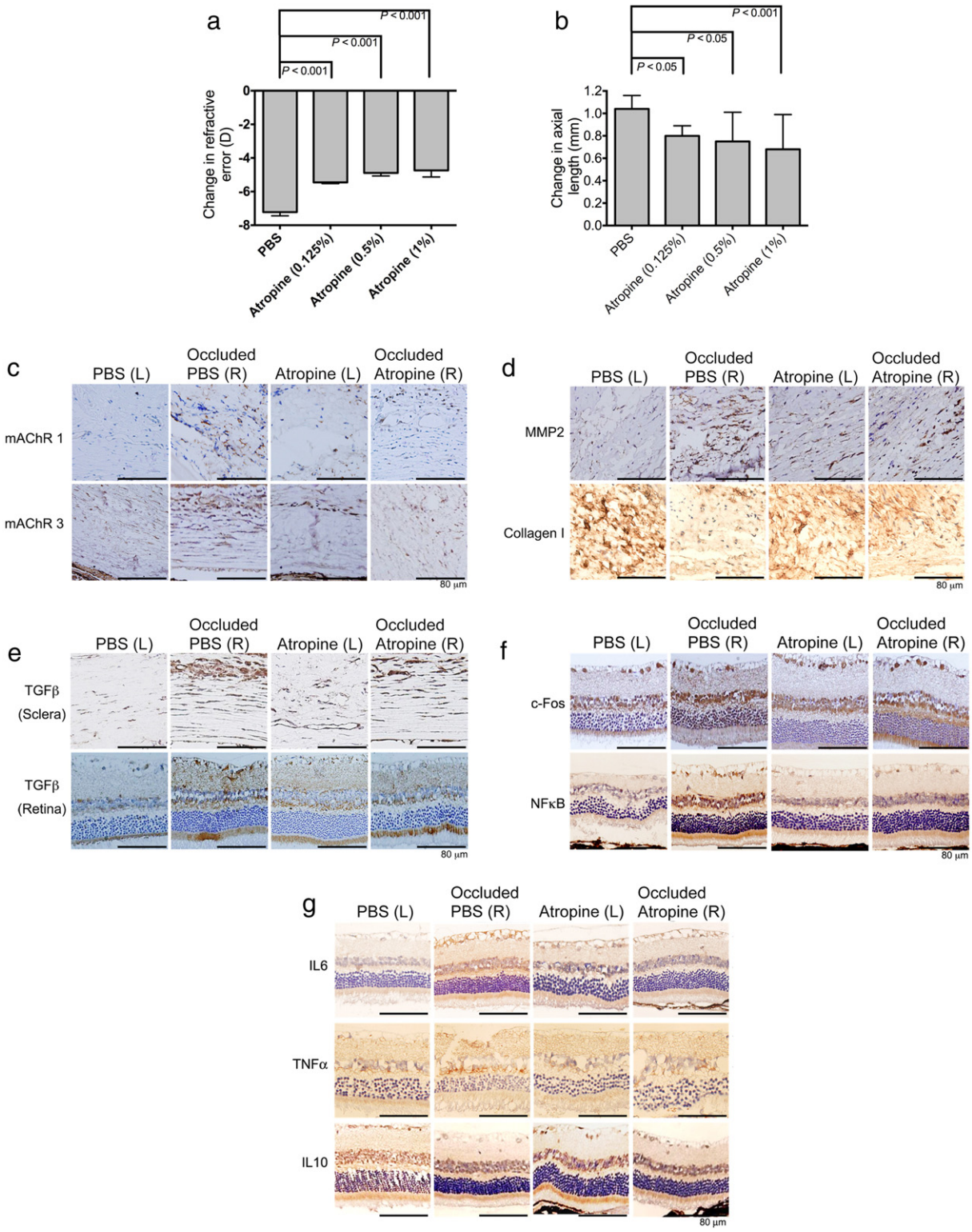

Fig. 4. Effect of atropine on myopia progression. (a) The RE was determined as the difference in diopter measurements taken after and before MFD. The ANOVA test was used to determine significant differences ($p < 0.0001$), and Dunnett's multiple comparisons test was used for paired comparisons between PBS- and atropine-treated eyes. $p < 0.05$ was considered statistically significant. (b) The axial length was determined as the difference in diopter measurements taken after and before MFD. ANOVA was used to determine significant differences ($p = 0.0042$) and Dunnett's multiple comparisons test was used for paired comparisons between PBS- and atropine-treated eyes. $p < 0.05$ was considered statistically significant. (c–g) Immunohistochemical analysis of mAChR1 and 3 (c), MMP2 and COL1 (d), TGF-β (e), c-FOS and NF-κB (f), and IL-6, TNF-α, and IL-10 (g) in control eyes (control [L]), occluded eyes (control [R]), 1% atropine-treated control eyes (atropine [L]), and 1% atropine-treated occluded eyes (atropine [R]).

by scleral fibroblasts or by other cells such as microglia that activate its expression in scleral tissue (Li et al., 1999). TGF-β, IL-6, and TNF-α activate NF-κB, an important transcription factor in the modulation of the inflammatory response. One target of TGF-β signaling via NF-κB is MMP2 (Wang et al., 2011b), which can cleave collagens including COL1, thereby promoting myopia progression

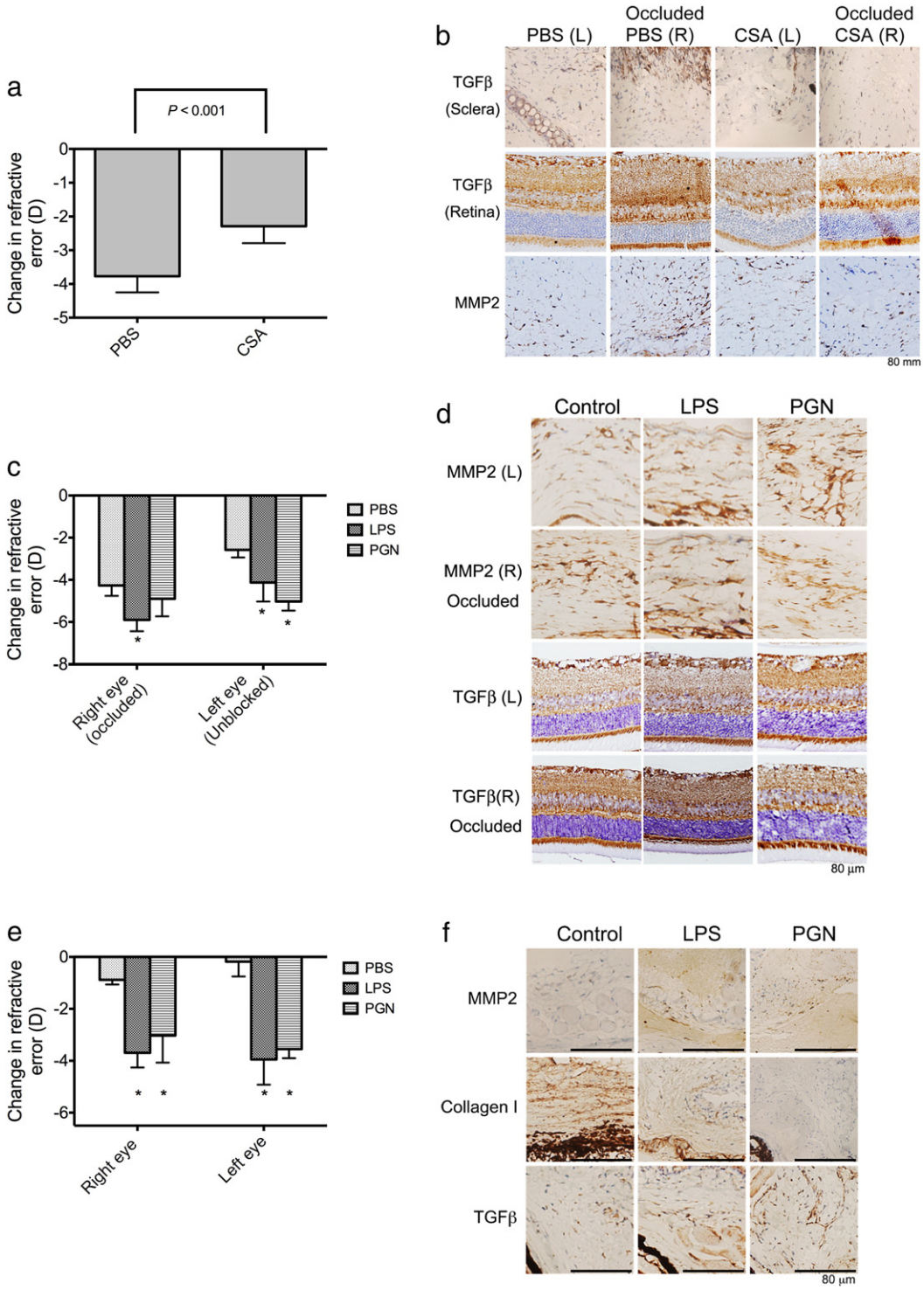

Fig. 5. Effect of suppressing or increasing inflammation in the eye on myopia progression. (a) The RE was determined as the difference in diopter measurements taken after and before MFD in hamsters treated for 21 days with 3% CSA. (b) Immunohistochemical analysis of TGF-β and MMP2 expression in control eyes (control [L]), occluded eyes (control [R]), 3% CSA-treated control eyes (CSA [L]), and 3% CSA-treated occluded eyes (CSA [R]). (c) The refractive error was determined as the difference in diopter measurements taken after and before MFD in hamsters treated for 21 days with LPS or PGN. *Indicates statistically significant compared with the untreated control. (d) Immunohistochemical analysis of TGF-β and MMP2 expression in control eyes ([L]) and form-deprived occluded eyes ([R]) with or without LPS or PGN. (e) The refractive error was determined as the difference in diopter measurements in hamsters treated for 21 days with LPS or PGN. *Indicates statistically significant compared with PBS-treated control. (f) Immunohistochemical analysis of TGF-β, MMP2, and collagen I expression in eyes treated with or without LPS or PGN.

(Frost and Norton, 2007; Lin et al., 2008; Seko et al., 2008; Wang et al., 2011a). Signals mediated by TGF-β, IL-6 and TNF-α predominantly culminate in the activation of NF-κB, a transcription factor that is important for mediating inflammation. In this pathway, the signal from pro-inflammatory mediators leads to the activation of IκB kinase α/β, which then phosphorylates IκB; its subsequent degradation activates NF-κB, which drives the production of numerous pro-inflammatory cytokines, including TNF-α and IL-6. Another

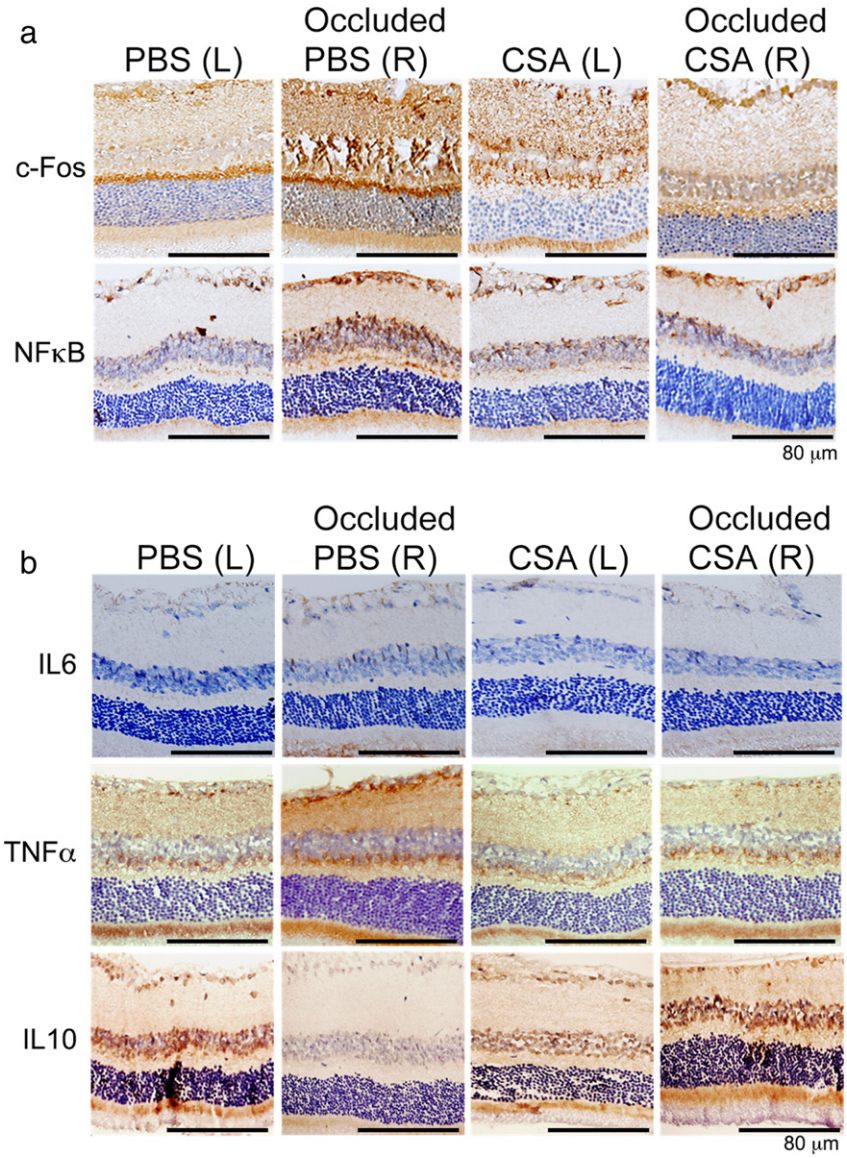

Fig. 6. Expression levels of inflammation-related transcription factors and cytokines in cyclosporine A-treated myopic eyes. (a) Immunohistochemical analysis of c-Fos and NF-κB expression in control eyes (control [L]), occluded eyes (control [R]), 3% CSA-treated control eyes (CSA [L]), and 1% atropine-treated occluded eyes (CSA [R]). (b) Immunohistochemical analysis of IL-6, TNF-α, and IL-10 expression in control eyes (control [L]), occluded eyes (control [R]), 3% CSA-treated control eyes (CSA [L]), and 3% CSA-treated occluded eyes (CSA [R]).

important transcription factor involved in pro-inflammatory cytokine expression is activator protein 1 (AP1), the activation of which is mediated by the phosphorylation and activation of mitogen-associated protein kinases (MAPKs) such as Janus kinase (JNK), p38, or extracellular signal-regulated kinase (ERK), which then activate c-Jun. and/or c-Fos to promote inflammatory cytokine expression. NF-κB and AP1 also stimulate the production of pro-inflammatory cytokines, and there is considerable overlap in the target genes activated by these two factors (Huang et al., 2009; Nguyen et al., 2007; Ogawa et al., 2004). TNF-α may act in a paracrine feedback loop in the retina or sclera to activate NF-κB during myopia progression. It was shown that their activation by LPS was suppressed by atropine in retinal cells (Fig. 7).

In this study, we found a link between inflammatory reactions and the progression of myopia. Atropine has side effects such as photophobia and cycloplegia, which is why it is rarely used as a treatment for myopia in Western countries. This discovery is important for ophthalmologists because it provides them with a potential treatment alternative to atropine that they can offer to their patients. In conclusion, these findings provide evidence for the involvement of inflammatory responses in myopia and suggest that treatment with atropine or anti-inflammatory agents can effectively inhibit myopia progression.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.07.021.

Author Contribution

Conceived and designed the experiments: LW and HJL. NHRID data retrieval and analysis: HJC. Performed the experiments: CYC, THC, YAH, and YCH. Analyzed the data: CYC, THC, LW, and HJL. Interpreted the data: CCW, LW and HJL. Wrote the paper: HJC, CCW, and LW.

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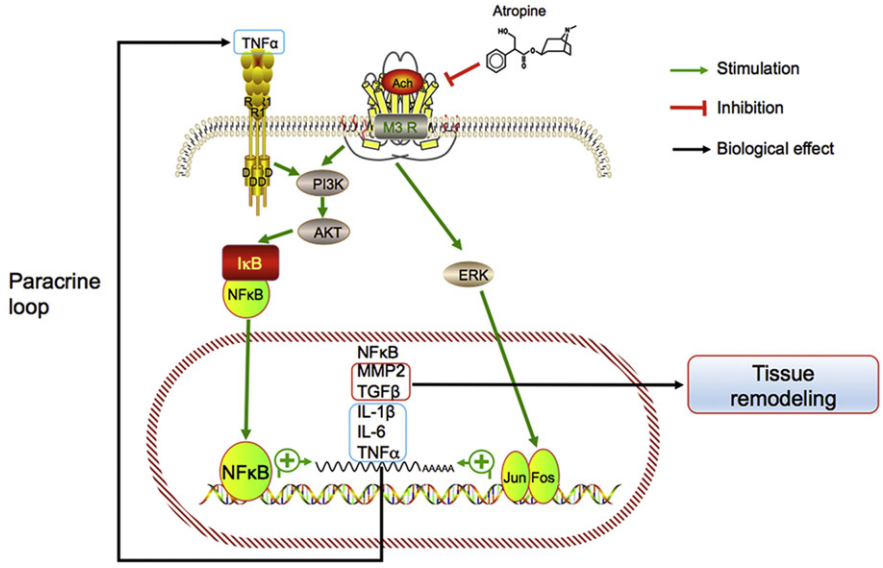

Fig. 7. Model of the association between inflammation and myopia progression. Activated mAChR3 (M3R) activates phosphoinositide 3-kinase (PI3K)–AKT and mitogen-associated protein kinase (MAPK) signaling pathways, in turn activating NF-κB and AP1 (i.e., the Jun.-Fos heterodimer) and stimulating the expression of the target genes NF-κB, MMP2, TGFβ, IL-1β and -6, and TNF-α. MMP2 and TGF-β promote tissue remodeling and TNF-α may act in a paracrine feedback loop in the retina or sclera to activate NF-κB during myopia progression.

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