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Research Paper

Violet Light Exposure Can Be a Preventive Strategy Against Myopia Progression

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ABSTRACT

Prevalence of myopia is increasing worldwide. Outdoor activity is one of the most important environmental factors for myopia control. Here we show that violet light (VL, 360–400 nm wavelength) suppresses myopia progression. First, we confirmed that VL suppressed the axial length (AL) elongation in the chick myopia model. Expression microarray analyses revealed that myopia suppressive gene *EGR1* was upregulated by VL exposure. VL exposure induced significantly higher upregulation of *EGR1* in chick chorioretinal tissues than blue light under the same conditions. Next, we conducted clinical research retrospectively to compare the AL elongation among myopic children who wore eyeglasses (VL blocked) and two types of contact lenses (partially VL blocked and VL transmitting). The data showed the VL transmitting contact lenses suppressed myopia progression most. These results suggest that VL is one of the important outdoor environmental factors for myopia control. Since VL is apt to be excluded from our modern society due to the excessive UV protection, VL exposure can be a preventive strategy against myopia progression.

1. Introduction

The global increase of myopia, or short-sightedness, is becoming a serious health hazard in the world (Dolgin, 2015). In the United States and Europe, the incidence of myopia has doubled, compared to 50 years ago (Dolgin, 2015). This phenomenon is especially profound in East Asia where the incidence has increased by about 60% over the past 50 years (Dolgin, 2015), and today >80% of teenagers and young adults are myopic (Lougheed, 2014). Myopia is the most common refractive error of the eye and is basically caused by the elongation of the axial length (AL) of the eyeball. A refractive error is represented by the unit diopter (D), and a negative value indicates myopia. Blindness could occur in high myopic patients, *i.e.*, −6 D or worse. The etiology of myopia remains unknown, but some epidemiological studies have suggested that increased near vision tasks such as reading, using computers and smartphones are possible risk factors (Ip et al., 2008). Recently, the time spent outdoors was proposed as a protective factor (French et al., 2013a, 2013b; Guggenheim et al., 2012; Ip et al., 2008; Jin et al., 2015; Jones-Jordan et al., 2014; Jones et al., 2007; Read et al., 2014; Rose et al., 2008), and the beneficial effect of high ambient light for the protection of myopia has been confirmed in chicks, mice, and monkeys (Karouta and Ashby, 2015; Norton and Siegwart, 2013; Smith et al., 2012; Stone et al., 2013; Tkatchenko et al., 2013). Additionally, some clinical trials indicated that increased outdoor activity of students had an *anti*-myopia effect (He et al., 2015; Jin et al., 2015; Wu et al., 2013). However, the protective mechanism of outdoor light against myopia progression is still unclear.

The spectral composition of outdoor light, *i.e.* sunlight, is characterized by abundant short wavelength visible components such as blue and green rather than red (Thorne et al., 2009). Recently, Foulds et al. (2013) reported that blue light had a suppressive effect against myopia.

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However blue light components are abundant in our LED society, because most of the light sources are blue LED and too much exposure to blue light is a concern (Chang et al., 2015; Czeisler, 2013).

Then, we hypothesized that violet light, which has a shorter wavelength than blue light and is a missing light component in modern society as described later, may play an important role in myopia control. According to the international lighting vocabulary of the Commission Internationale de l'Eclairage (CIE) (CIE, 2006), the bottom limit of the visible light is defined to be between 360 and 400 nm which overlaps the upper end of ultraviolet (UV) A region (Krutmann et al., 2014). This range, in fact, is visible as violet light, but it is also recognized as UV as well. Our society provides many UV protective items such as UV protective eyeglasses, contact lenses (CLs) and window glass for eye care, extending beyond 360 nm to the 400 nm range. In other words, violet light (wavelengths from 360 nm to 400 nm) is excluded from our society due to the policy of UV protection, although the range of violet light is visible light. If violet light is an important outdoor environmental wavelength for myopia control, our society will need to reconsider how to manage this range of light considering the recent "myopia boom" problem.

To confirm the violet light hypothesis, we conducted experiments *in vivo*, *in vitro*, and human clinical studies. Here, we found that myopia progression was suppressed by 360–400 nm violet light in the chick myopia model, which upregulated *EGR1*, the known myopia protective gene. Furthermore, in clinical studies, we demonstrated that the more violet light transmitted through eyeglasses and CLs, the slower the progression of myopia. We propose that violet light exposure can be a preventive strategy for myopia control in modern society.

2. Material and Methods

2.1. Chick Experiments

2.1.1. Animals

Male White Leghorn chicks were obtained from the Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). All the animal experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement, Institutional Guidelines on Animal Experimentation at Keio University, and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines for the use of animals in research. The protocol for this study was approved by the Ethics Committee on Animal Research of the Keio University School of Medicine (Approval number: 12098). Myopia was induced in chicks by covering the right eye with glass or plastic goggles (Figs. S1a, S2) six days after hatching. The left eye was uncovered and served as the control. Chicks wore goggles for 7 days in a 12-hour light and dark cycle. Fluorescent light, violet light (VL), blue light, and UVB light were illuminated for 7 days in 12-hour on and off cycles. Chicks were checked twice a day. If the goggle from the covered eye fell off during the 7 days, the goggle was placed again but those covered eyes were excluded from the analysis. The eyes of chicks were enucleated after euthanasia with CO_2 on day 13. After extra ocular tissue was cleaned, tissue buttons 6 mm in diameter were excised with a surgical trephine from the part of the posterior hemisphere located temporal to the exit site of the optic nerve. Chorioretinal buttons were dissected from the tissue buttons and specimens from each sample were saved as one sample.

2.1.2. Illuminance and Irradiance Measurements, and Adjustment in Chick Experiment

We prepared the three chick groups, VL– group, VL+ group, and Blue Light group. In the VL– group, we used only fluorescent light (Fig. 1a) (TBL14/5N. OHM ELECTRIC INC., Saitama, Japan). In the VL+ (peak: 365 nm, Fig. 1b) group, we used VL fluorescent light (PL-S 9W/08. Philips International B.V., Amsterdam, The Netherlands) plus fluorescent light (TBL14/5N). In the Blue Light (peak: 470 nm, Fig. 1b) group, we used blue LED (LP-B56A5111A. OptoSupply Limited, Hong Kong, China) plus fluorescent light (TBL14/5N). First, we measured illuminance and irradiance in chick cages at every 10 cm square. Next, we adjusted the illuminance (lux) so there were no significant differences among the groups. There were no significant differences in illuminance (lux) between the VL– group and VL+ group; 1262 ± 502 in VL– group, 1349 ± 462 in VL+ group (Fig. 1c–k). There were no significant differences in illuminance (lux) among the three groups; 1230 ± 353 in VL– group, 1116 ± 270 in VL+ group, and 1035 ± 373 in the Blue Light group (Fig. 1l, m). Then we adjusted irradiance (W/m^2) for the entire spectroscopic range, including UV so there were no significant differences between VL+ group and Blue Light group, 11.191 ± 3.449 W/m^2 in VL+ group, and 11.590 ± 3.973 W/m^2 in the Blue Light group (Fig. 1l, m). We measured UV (290–390 nm) irradiance in chick cages at every 10 cm square using UV meter, UV-340A (SATO SHOUJI INC., Kanagawa, Japan). UV (290–390 nm) irradiance (mW/cm^2) in the VL+ group was 0.413 ± 0.238 and 0 in VL– group (Fig. 1c–k).

2.1.3. Refraction and Biometry of Chick

Before covering the eye on day 6 and euthanizing on day 13, both eyes were measured for refraction and biometry. All measurements were performed with the chicks awake and under natural viewing conditions, with no use of cycloplegic agent or lid retractors as described previously (Garcia de la Cera et al., 2006). We compared changes in refractive value, anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL) which sum of the ACD and LT and VCD among chick groups under various light conditions. Refraction of chick was measured using autorefractometer ARK-700A (NIDEK, Aichi, Japan). Biometry (ACD, LT, VCD, and AL) of chick eye was measured using B-scan ultrasonography (Fig. 1d), US-4000 (NIDEK). The average velocity of sound in the ocular media was set to 1550 m/s to calculate intraocular distances as previously described (Zhu et al., 2013). We confirmed significant correlations between AL measured by B-scan ultrasonography and AL measured by full-eye-length swept-source optical coherence tomography (SS-OCT) scan biometry IOLMaster® 700 (Carl Zeiss Meditec, Jena, Germany) (Fig. S1b, c) in the preliminary experiment. The examiners were masked through all measurements to avoid group identification.

2.1.4. Spectral Transmission of Glass and Plastic Goggles of Chick Experiment

The UV–visible absorption spectra of these samples were recorded with a U-4100 spectrophotometer (Hitachi, Ltd., Tokyo, Japan) (Figs. S1a, S2).

2.1.5. Transmittance of Violet Light Through Plastic Goggles of Chick Experiment

We measured the transmittance of violet light using UV meter UV-340A (SATO SHOUJI INC.) and transmitted illuminance using illuminometer LX-1108 (KENIS LIMITED, Osaka, Japan) as follows. First, we measured UV irradiance and the illuminance through a translucent plastic goggle (Fig. S2) by itself inside a chick cage. These served as the control data (= a) of UV irradiance and illuminance, and their transmittance was set as 100%. Next, we measured UV irradiance and the illuminance (= b) through opacified plastic goggles which were used to cover the right eye for 1 week in the chick experiment. The transmittance of violet light and transmitted illuminance by percentage were calculated as b/a × 100 (Fig. 1i).

2.1.6. 25-OH Vitamin D Concentration Assay

Immediately after euthanasia, blood was taken from the chick heart. 25-OH vitamin D concentration (Fig. 1j, k, p) was measured using radioimmunoassay as described previously (Hollis et al., 1993).

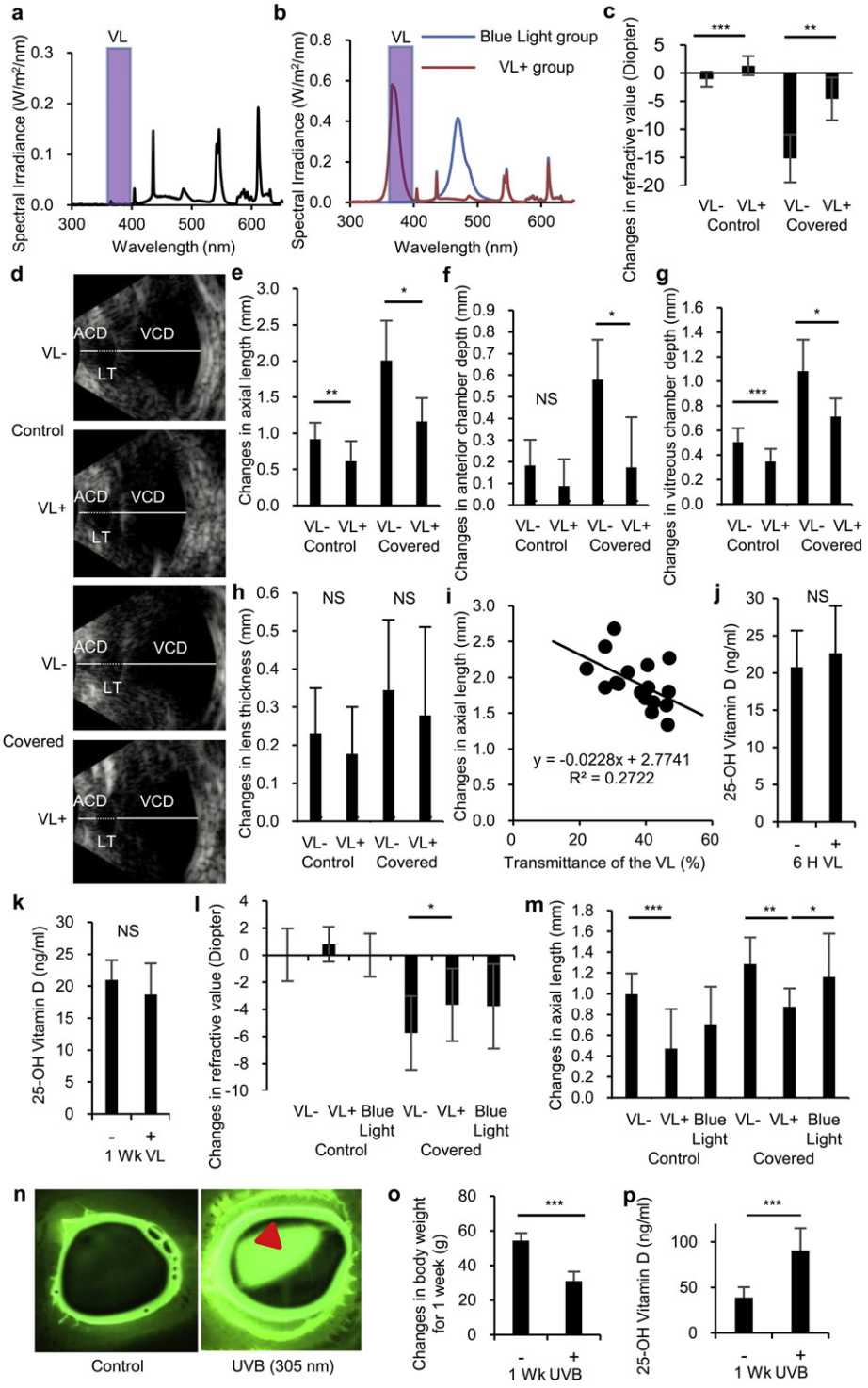

Fig. 1. Inhibiting the progression of myopia by irradiation of violet light (VL) in chick. (a) Spectral irradiance of fluorescent light in VL–, VL+, Blue Light groups. (b) Spectral irradiance of VL fluorescent light (peak: 365 nm) plus fluorescent light in VL+ group and blue LED plus fluorescent light in Blue Light group. (c) Changes in refractive value (spherical equivalent [SE]) for 1 week (from day 6 to 13). Changes in SE are significantly more hyperopic in VL+ group than those in VL– group in control eyes (n = 15 per group) and covered eyes (n = 4 in VL– group, n = 5 in VL+ group). (d) Typical B-scan ultrasonography. Axial length (AL), the sum of the anterior chamber depth (ACD) and lens thickness (LT) and vitreous chamber depth (VCD), in VL+ group is shorter than that in VL– group in both control and covered eyes. (e) Changes in AL for 1 week. Changes in AL in VL+ group was significantly smaller than that in VL– group in both control and covered eyes. (f) Changes in ACD for 1 week. (g) Changes in VCD for 1 week. Changes in VCD in VL+ group was significantly smaller than that in the VL– group in both control and covered eyes. (h) Changes in LT for 1 week. (i) Significant correlation between changes in AL (n = 17) for 1 week and transmittance of the VL (Spearman's rank correlation coefficient = −0.513 [$P = 0.035$]). (j) 25-OH vitamin D concentration (Vit D) in chick after 6 h VL irradiation (n = 8 per group). H, hours. (k) Vit D in chick after 1 week VL irradiation (n = 14 in VL– group, n = 16 in VL+ group). (l) Changes in SE for 1 week. Changes in SE are significantly less myopic in VL+ group than those in VL– group in covered eyes (n = 14 per group). (m) Changes in AL for 1 week. Changes in AL in VL+ group was significantly smaller than that in VL– group in both control and covered eyes. Changes in AL in VL+ group was significantly smaller than that in Blue Light group in covered eyes. (n) Fluorescein corneal staining photograph. Though there is no staining in control group (left), there is corneal erosion (red arrow) from ultraviolet B (UVB)+ group (right). (o) Changes in chick body weight for 1 week. Changes in body weight in the UVB+ group was significantly smaller than those in the UVB– group (n = 18 in UVB– group, n = 21 in UVB+ group). (p) Vit D in chick after 1 week UVB irradiation (n = 18 in UVB– group, n = 21 in UVB+ group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, *t*-test for (c), (e), (f) and (g), Mann-Whitney U test for (l), (o) and (p), oneway ANOVA which is followed by a *post hoc* Tukey HSD test for (m). Data are shown as mean ± standard deviation (SD). VL, violet light. NS, not significant. Wk, week.

2.2. Microarray Analysis

Chicken (V2 026441) Gene Expression Microarray 4 × 44 K (Agilent Technologies, California, USA) was used for microarray analysis. The arrays were in a 4-plex format (4 arrays per slide), with 43,803 *Gallus gallus* probes represented. The experiment was processed according to standard operating procedures. Briefly, sixteen eyeballs of eight chicks from the two experiment groups were divided into four subgroups: VL−/control, VL+/control, VL−/covered, VL+/covered. Total RNA of the four chorioretinal tissues in each subgroup were extracted and mixed (300 ng for each eyeball and 1.2 μg in total/subgroup). RNA purifying, hybridization, washing, staining, imaging, and signal extraction were performed according to Agilent-recommended procedures. The microarray principal component analysis (Fig. 2a–c) was conducted as described previously (Sharov et al., 2015). We deposited our microarray data in a MIAME-compliant database; the accession number is GSE90118.

2.3. Real-time RT-PCR Analysis

Total RNA was extracted from chorioretinal buttons dissected from chick tissue or cultured cells using RNeasy Plus Mini kit (Qiagen, Venlo, Nederland), and reverse-transcribed using PrimeScript II first strand cDNA Synthesis Kit (Takara Bio, Otsu, Japan) or ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan). Quantitative PCR assays were performed on a StepOnePlus Real-Time PCR System using TaqMan Universal PCR master mix (Life Technologies, Carlsbad, USA) with TaqMan Gene Expression Assay Mix of *Bmp2* (Mm01340178_m1), *Ednrb* (Mm00432989_m1), *Egr1* (Mm00656724_m1), *Fgf2* (Mm00433287_m1), *Igf1* (Mm00439560_m1), *Il18* (Mm00434225_m1), *Irbp* (Mm00450076_m1), *Lumican* (Mm01248292_m1), *Sfrp1* (Mm00489161_m1), *Tgfb1* (Mm01178820_m1), *Vegfa* (Mm01281449_m1), *Vip* (Mm00660234_m1), and *Wnt2b* (Mm00437330_m1) or Platinum SYBR Green qPCR SuperMix-UDG (Life Technologies, Carlsbad, USA) with a chick *EGR1* primer (Forward: ACTAACTCGTCACATTCGCA, Reverse: TGCTGAGACCGAAGCTGCCT) (Ashby et al., 2010). Eukaryotic 18S rRNA TaqMan MGB probe or chick *ACTB* primer for SYBR green assay (Forward: GCGCTCGTTGTTGACAAT, Reverse: CATCACCAACGTAGCTGTCTTT) (Tomonari et al., 2005) was used as endogenous control. Data were analyzed with StepOne Software version 2.3 (Applied Biosystems, Waltham, USA).

2.4. Irradiation of Violet Light to a Photoreceptor Cell Line 661w

1.5×10^4 per well of the murine photoreceptor 661 W cells (a kind gift from Dr. Muayyad Al-Ubaidi, University of Oklahoma Health Sciences) were plated in 24-well plates in a Dulbecco's Modified Eagle's Medium (DMEM) (08456-65, Nacalai Tesque, Kyoto, Japan) with 10% of fetal bovine serum (FBS) and 1% of Penicillin-Streptomycin at 37 °C with 5% CO_2 24 h prior to the light exposure. The cells were maintained in the dark with tin foil and treated under dim light except the intended light irradiation. 600 mJ/cm^2 of 360, 370, 380, 390, and 400 nm light in violet light (equivalent to the amount of the sun light from north direction at 10:00 AM for 100 min; Fig. 2h) were exposed to the cells after changing the medium to transparent phosphate buffered saline (PBS). The spectral irradiance was measured by the spectrometer UVNb-50 (StellarNet Inc., Tampa, USA), and the temperature during the light exposure was maintained by fan. The light exposure was performed at 7:00 AM and the cells were maintained in the dark until the cells were harvested after 30 min from the start of irradiation.

2.5. Detection of Apoptosis in the Eye Balls of Chick by TUNEL Assay

We confirmed the safety to ocular tissue using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay to detect apoptotic cells. After the eye balls were enucleated and fixed with 4% paraformaldehyde for 12 h. Specimens were then dehydrated in a graded series of sucrose and cut into 8-μm thickness sections with optimal cutting temperature (OCT) compound in −20°C. Cell apoptosis was detected by TUNEL using ApopTag *In Situ* Apoptosis Detection Kits (Chemicon International, Darmstadt, Germany; cat. #S7165) according to the manufacturer's instructions. Nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI). The images of the sections were captured immediately with a fluorescence microscope using a 40x objective (Axio Observer. D1; Leica, Wetzlar, Germany).

2.6. Clinical Study: Violet Light Through the Eye Suppressed AL Elongation in Children: Retrospective Comparison in AL Elongation Among Patients Who Wore Non-Violet Light Transmitting Eyeglasses, Partially Violet Light-blocking Contact Lenses (CL) and Violet Light Transmitting CL

The Keio University School of Medicine Ethics Committee approved the clinical studies. All patients who wore non-violet light transmitting eyeglasses, partially violet light-blocking CL and violet light transmitting CL were followed at Kato Eye Center. All procedures involving human subjects were performed in accordance with the tenets of the Declaration of Helsinki.

First, we retrospectively compared the AL elongation for 1 year between the non-violet light transmitting [VL (−)] eyeglass group and the violet light transmitting [VL (+)] CL group. All VL (−) eyeglasses, 14 types (Table S1c), are blocking violet light (Fig. 3a), whereas VL (+) CL, 6 types (Table S1d), are transmitting violet light (Fig. 3b). This portion of the study included 310 right eyes of 310 Japanese students with myopia, equal or worse than −1.00 D who were followed for over 1 year after wearing VL (−) eyeglasses or VL (+) CL at Kato Eye Center. They were divided into two groups: the VL (−) eyeglass group, comprising 211 eyes of 211 patients (age range, 10–15 years; mean age, 12.2 ± 1.7 [standard deviation] years) who wore VL (−) eyeglasses (Table S1c); and the VL (+) CL group, comprising 99 eyes of 99 patients (age range, 10–15 years; mean age, 13.9 ± 1.1 years) who wore VL (+) CL (Table S1d). The main outcome measures were the differences in the AL elongation for 1 year after wearing eyeglasses or CLs between the two groups. The patient background data are shown in Table S1a. The inclusion criteria for both groups were: age range 10–15 years with myopia equal or worse than −1.00 D. The exclusion criteria for both groups were strabismus, amblyopia, uveitis, cataracts, corneal diseases, glaucoma, and active ocular or systemic diseases. Although there was a difference in the number of samples between the two groups, all cases satisfied the inclusion criteria and were included in the study.

Next we retrospectively compared the AL elongation for 1 year between the partially VL-blocking CL group and the VL (+) CL group. All partial VL-blocking CL, 10 types (Table S2b), are partially blocking violet light (Fig. 3c), whereas VL (+) CL, 7 types (Table S2c), are transmitting violet light (Fig. 3b). This portion of the study included 147 right eyes of 147 Japanese students with myopia equal or worse than −1.00 D who were followed for over 1 year after wearing partially VL-blocking CL or VL (+) CL at Kato Eye Center. They were divided into two groups: the partially VL-blocking CL group, comprising 31 eyes of 31 patients (age range, 13–18 years; mean age, 14.7 ± 1.3 years) who wore partially VL-blocking CL (Table S2b); and the VL (+) CL group, comprising 116 eyes of 116 patients (age range, 13–18 years; mean age, 15.1 ± 1.4 years) who wore VL (+) CLs (Table S2c). There was a difference in the number of samples between the two groups, but all cases satisfied the inclusion criteria and were included. The main outcome measures were the differences in the AL elongation for 1 year after wearing CL between the two groups. The patient background data are shown in Table S2a. The inclusion criteria for both groups were: age range was 13–18 years with myopia equal or worse than −1.00 D. The exclusion criteria for both groups were strabismus, amblyopia, uveitis, cataracts, corneal diseases, glaucoma, and active ocular or systemic diseases. The

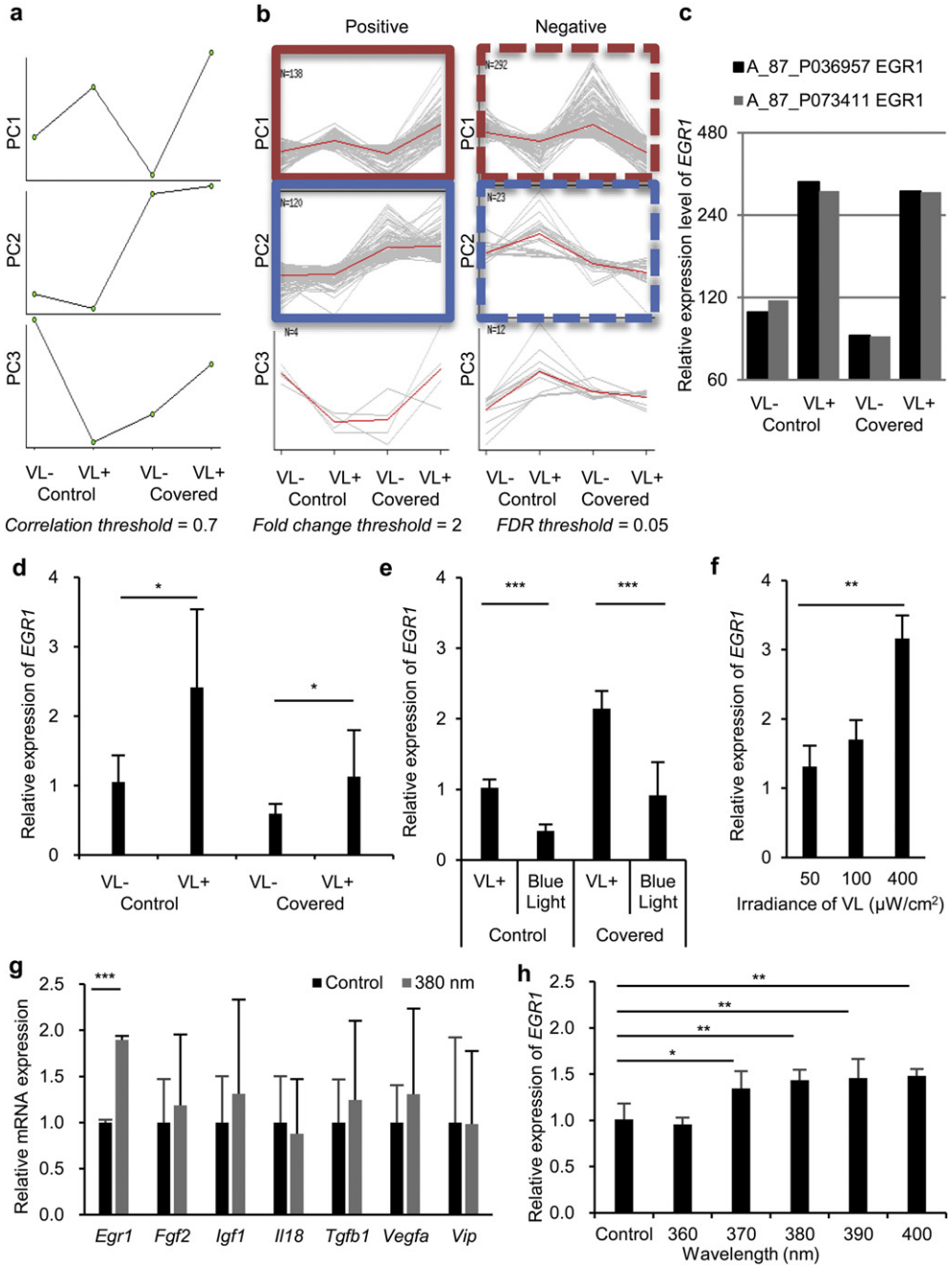

Fig. 2. Upregulation of a myopia protective gene *EGR1* by violet light (VL) exposure. (a) A result of principal component analysis (PCA) (Sharov et al., 2015) in 4 groups. And they were further divided into positive and negative groups. (b) Genes in PC1 group were responsive to VL. FDR, false discovery rate. (c) *EGR1*, myopia protective gene, was only clustered gene to PC1 group among previously reported myopia related genes. The previously reported myopia promoting genes such as *TGF1*, *IGF1* were not found in PC1 group *in vivo*, which means they did not respond to VL. (d) Relative mRNA expression of *EGR1* in chick chorioretinal tissue after VL exposure (n = 5). Note that VL exposure induces significant upregulation of *EGR1*. (e) Relative mRNA expression of *EGR1* in chick chorioretinal tissue after VL and blue light exposure (n = 4, 5). Note that VL exposure induced significantly higher upregulation of *EGR1* than blue light. (f) Relative mRNA expression of *EGR1* in chick chorioretinal tissue after 50, 100, and 400 μW/cm^2 of VL exposure (n = 5). There were significant differences in mRNA expression of *EGR1* between 50 and 400 μW/cm^2 groups. (g) Relative mRNA expression of known myopia-related genes after 380 nm light exposure to the murine photoreceptor 661 W cells detected by real-time RT-PCR (n = 4). Note that *EGR1* is significantly upregulated among those genes. (h) Relative mRNA expression of *EGR1* after from 360 nm to 400 nm light exposure to 661 W cells (n = 4). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Mann-Whitney *U* test for (d), t-test for (e, g), oneway ANOVA which is followed by a *post hoc* Tukey HSD test for (f, h). Data are shown as mean ± SD. VL, violet light.

objective refraction was measured by autorefractometry (TONOREF II, NIDEK and KR-8100PA, TOPCON, Tokyo, Japan). The AL was measured by the phakic mode of the IOLMaster® (Carl Zeiss Meditec).

To identify the factors affecting AL elongation after wearing non-VL transmitting eyeglasses and VL transmitting CLs, we performed a stepwise multiple regression analysis. The outcome was the AL elongation for 1 year. The covariates were age, sex, type of lens (non-VL transmitting eyeglasses or VL transmitting CLs), and initial AL. Multicollinearity was not a factor.

The UV–visible absorption spectra of eyeglasses, CLs, and intraocular lenses (IOLs) were recorded with a UV-2600 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) (Figs. 3a–c, 4i).

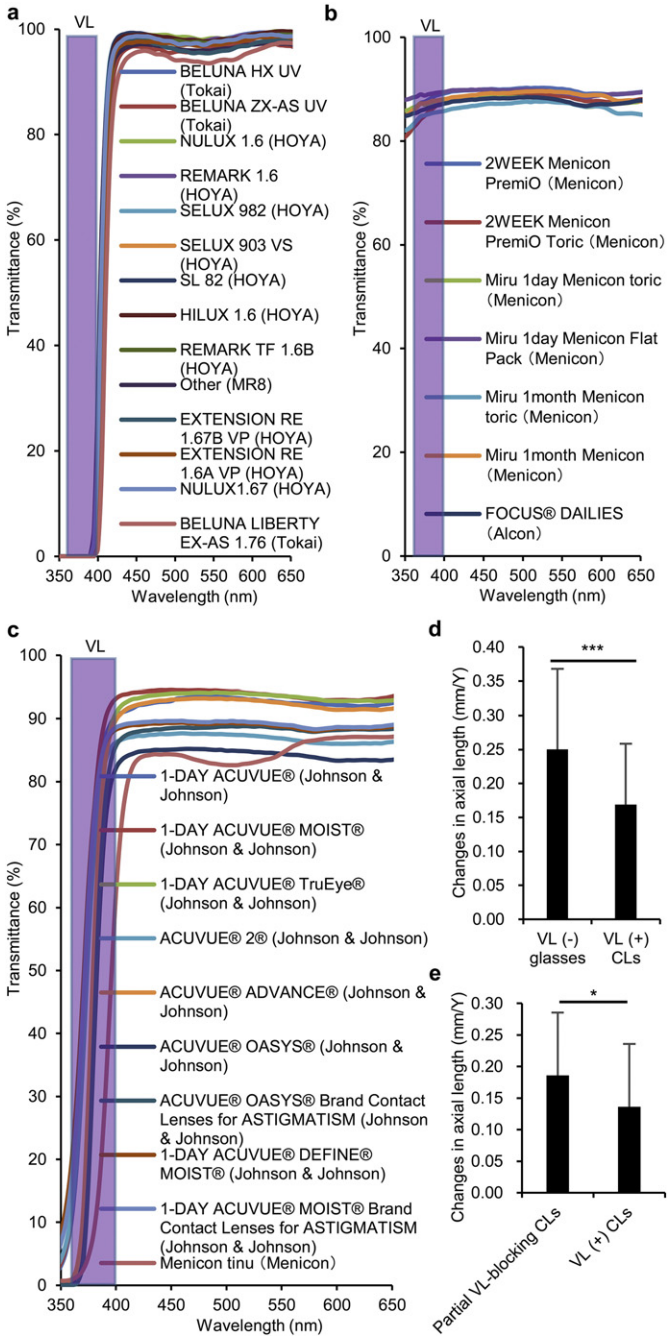

Fig. 3. Comparison of axial length (AL) elongation among children who wore non-violet light (VL) transmitting eyeglasses, partially VL-blocking contact lenses (CL), and VL transmitting CL. (a) Spectral transmission of non-VL transmitting (VL [−]) glasses (14 lenses at−3 D). (b) Spectral transmission of VL transmitting (VL [+]) CLs (7 lenses at−3 D). (c) Spectral transmission of partially VL-blocking CLs (10 lenses at−3 D). (d) Changes in AL for 1 year. Changes in AL in the VL (+) CL group (n = 99) were significantly lower than those in the VL (−) glasses group (n = 211). (e) Changes in AL for 1 year. Changes in AL in the VL (+) CL group (n = 116) were significantly lower than the partially VL-blocking CL group (n = 31). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Mann-Whitney *U* test. Data are shown as mean ± SD. VL, violet light.

2.7. Spectral Irradiance of the Sunlight Penetrated Through the UV Blocking Glass Window at an Office, Automobile and Hospital

These spectra were measured by the Blue-Wave spectrometer UVNb-50 (StellerNet Inc.) (Fig. 4e). Office: The spectral irradiance data in an office were collected at a medical office of Keio University Hospital, Tokyo, Japan at approximately 12:00 local time on June 4, 2015. Automobile: The spectral irradiance data in automobile were collected in a taxi randomly selected on a Tokyo street in Japan at approximately 10:30 local time on June 11, 2015. Hospital: The spectral irradiance data in hospital were collected in the entrance hall of Keio University Hospital, Tokyo, Japan at approximately 13:40 local time on June 11, 2015. All the data were collected on early-summer sunny days in Tokyo, Japan (latitude: 35°, longitude: 139°).

2.8. Total Spectral Radiant Flux of LED Light, Fluorescent Light, Incandescent Light

These were measured using with SLMS-1021 (Labsphere, Inc., New Hampshire, USA) (Fig. 4f–h).

2.9. Statistical Analysis

T-test and Mann-Whitney *U* Test was used to compare data between the two groups for the chick and *in vitro* experiments, and for human clinical studies. Oneway ANOVA test was used to compare the 3 or more groups for the chick and *in vitro* experiments. This was followed by a *post hoc* Tukey HSD test which takes multiple testing into account. A P value < 0.05 was considered significant. All statistical analyses including multiple regression analysis were performed using IBM SPSS Statistics version 21.0 (IBM Corp, New York, USA).

3. Results

3.1. Protective Effect of Violet Light Against Myopia in Chick Models

In order to confirm the protective effect of violet light against the progression of myopia, we performed studies combining two well-established chick myopia models, the form-deprivation and lens-induced models, with or without violet light (Fig. 1a, b). The study eyes were covered with glass goggles (−9 D) from days 6 to 13 for seven days (Fig. S1a), which was expected to produce myopia (Guo et al., 1995). The contralateral eye remained uncovered to provide the control. The covered eye without violet light exposure (n = 4) developed −15.18 D (mean value) of myopia, whereas the one with violet light (n = 5) developed only −4.59 D of myopia ($P = 0.005$). Interestingly, the control eye without violet light exposure (n = 15) developed −1.08 D of myopia, but the one with violet light (n = 15) developed hyperopia of +1.31 D ($P < 0.001$) (Fig. 1c). These results suggest that violet light is important for not only the prevention of myopia progression but also the onset of myopia. The size parameters of the eyeball showed that AL, anterior chamber depth and vitreous chamber depth elongated in the covered eye without violet light, compared to the one with violet light (Fig. 1d–g). There were no differences in lens thickness (Fig. 1h). We used the latest clinical devices to increase the accuracy of measurement of chick ocular biometry (Fig. S1b) and found significant correlations between the AL measured by B-scan ultrasonography and AL measured by full-eye-length swept-source optical coherence tomography (SS-OCT) scan biometry IOLMaster® 700 (Carl Zeiss Meditec) (Fig. S1c) in a preliminary experiment (n = 37). The suppressed elongation of the AL and vitreous chamber depth was also observed even in the control eyes under violet light exposure (Fig. 1d, e, and g). During the experiment we noticed that the glass goggles with 100% transmittance of violet light showed the maximal protective effect for myopia progression. So we adjusted the transmittance of violet light by using translucent plastic goggles with variable transparencies (Fig. S2). The AL elongation depended on the transmittance of violet light through the plastic goggles covering the eye (n = 17) (Fig. 1i). We found a statistically significant correlation (Spearman's rank correlation coefficient = −0.513 [$P = 0.035$]), showing the more violet light provided, the better the myopic control.

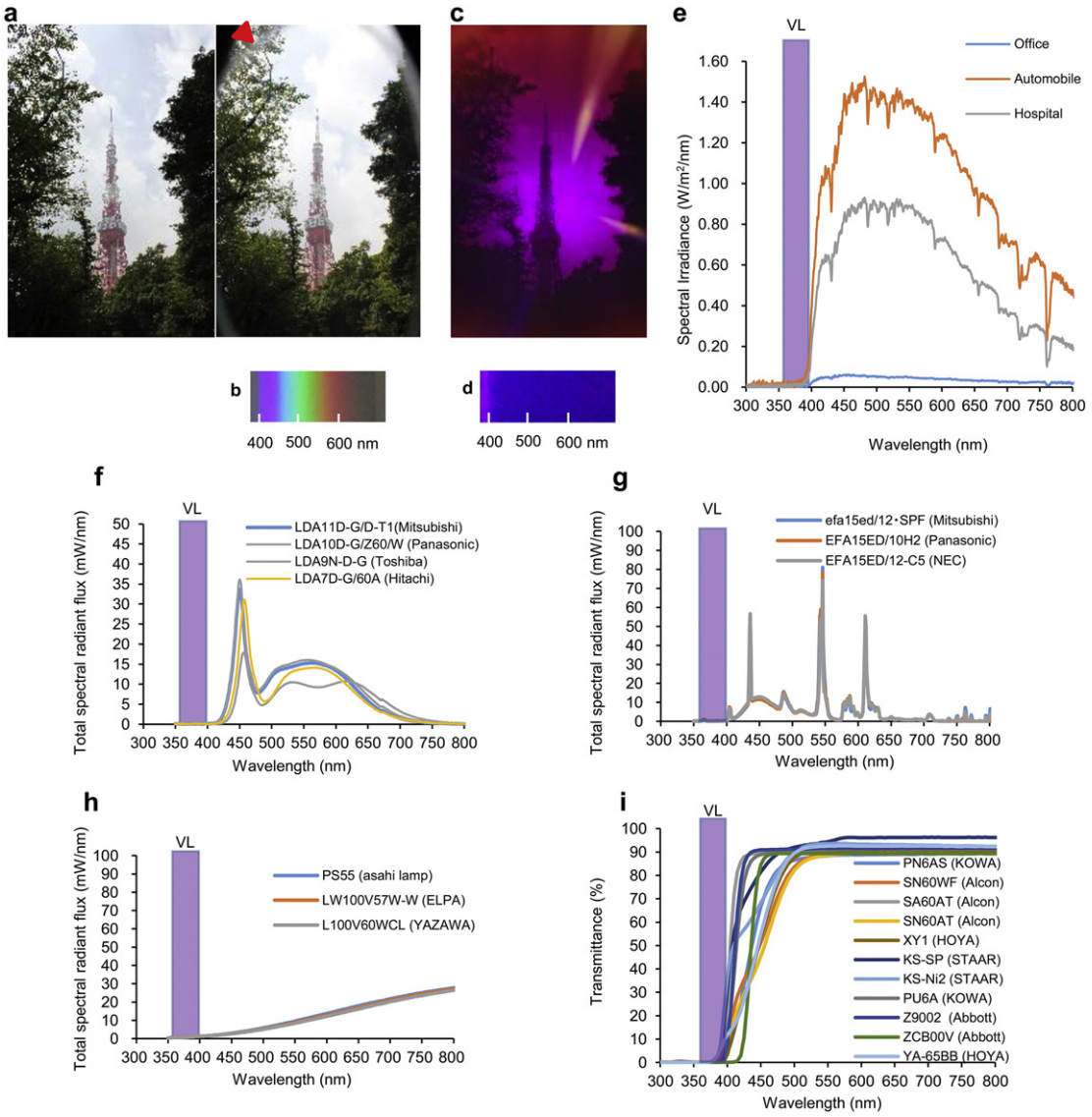

Fig. 4. Deficiency of violet light (VL) in modern society. (a) An outdoor scene photographed with (right) and without (left) a 400 nm-high-pass ordinary eyeglass lens. A part of the rim (red arrow) is seen. (b) The color-band spectrum of the scene from Fig. 4a that clearly shows the absence of light below 400 nm. (c) An outdoor scene photographed with a 400 nm-high-cut filter. A few lens flares are seen. (d) The color-band spectrum of the scene from Fig. 4c. The line situated below 400 nm is VL that is deficient in modern society. Brightness and contrast were adjusted to +60% and +30%, respectively, to visualize the originally dim violet line more clearly. Figures 4a and 4c were taken by a digital single-lens reflex (DSLR) camera, EOS-550D (Canon Inc., Tokyo, Japan) near TOKYO TOWER around noon on July 14, 2016. Figures 4b and 4d were taken by the same camera through a homemade spectrometer made of cardboard and a diffraction grating sheet that has 500 gratings per 1 mm. (e) Typical spectrum patterns of sunlight transmitted through a UV-protected glass windows at an office, automobile and hospital. No light below 400 nm wavelength penetrated the UV-protected glass. (f) Typical light spectrum emitted from light emitting diode (LED) light. Note the VL spectrum is completely missed. (g) Typical light spectrum emitted from fluorescent light. Note the VL spectrum is very low. (h) Typical light spectrum emitted from incandescent light. Note the VL spectrum is very low. (i) Intraocular lenses (11 lenses at +20 D). Intraocular lenses mostly block VL. VL, violet light.

Since several previous studies suggested the possible involvement of active vitamin D for the prevention of myopia (Choi et al., 2014; Yazar et al., 2014), we checked the vitamin D level 6 h (n = 8 per group) and 1 week (n = 14 in VL– group, n = 16 in VL+ group) after violet light exposure. However, we found no differences between the VL+ and VL– groups for both time points, with an average level around 20 ng/ml in each group (Fig. 1j, k). The optimum wavelength for vitamin D production is around 300 nm light (Lehmann et al., 2007); vitamin D did not seem to be involved in the mechanism of violet light effect.

3.2. The Effect of Blue Light and UV Light Wavelength for Myopia Control in Chick

To further confirm that violet light is the significant myopia protective wavelength, we performed a similar lens-induced myopia chick study with or without blue light (blue LED maximal wavelength around 470 nm). Our experiment with blue LED light (1035 ± 373 lux) showed a weak suppression of refractive progression (n = 14 per group) (Fig. 1l), but the effect of violet light for axial elongation was significantly ($P < 0.05$) stronger than blue LED light in the covered eye (Fig. 1m). This experiment was performed with the same irradiance for the entire spectroscopic range, including UV showing the stronger protective effect of violet light than blue light (Fig. 1m). Since LED light with high intensity (20,000–40,000 lux) has been reported to be protective (Karouta and Ashby, 2015), the blue light wavelength itself may have its own function. In a similar, but separate study using the shorter wavelength at 305 nm light (UVB), chicks kept both eyes closed all day because the UVB exposure caused severe corneal keratitis (Fig. 1n) due to corneal epithelial cell apoptosis (Fig. S3) with weight loss (n = 18 in UVB– group, n = 21 in UVB+ group) (Fig. 1o). Although we found

high serum vitamin D levels 1 week after UVB exposure (Fig. 1p), we abandoned this study, concluding that UVB light is not the therapeutic wavelength target.

3.3. Microarray Analysis of Chick Retina and Principle Components Analysis

To find the possible molecular mechanism of myopia protection by violet light, we performed expression microarray analysis of chick retina. The mRNA were obtained at day 13 from the following four groups: control eyes with or without violet light exposure and covered eyes with or without violet light exposure, and then the gene expression pattern was compared among them. Principle component analysis, which is to find major patterns of variability in gene expression, was performed and we found that the largest gene population (PC1, positive: n = 138, negative: n = 292) was affected by violet light treatment (Fig. 2a). On the other hand, the second largest gene population (PC2, positive: n = 120, negative: n = 23) was affected in the eyes covered with a plastic lens (Fig. 2a, b). The previously reported myopia-related genes (Ashby et al., 2014; Hawthorne and Young, 2013; Ma et al., 2014; Mathis et al., 2014; McGlinn et al., 2007; Ritchey et al., 2012; Wisard et al., 2011; Yoshikawa et al., 2014) such as *Bmp2, Ednrb, Fgf2, Igf1, Il18, Irbp, Lumican, Sfrp1, Tgfb1, Vegfa, Vip, and Wnt2b* were not found in the PC1 group *in vivo*, which indicates that they responded less to violet light. In the PC1 group, only one myopia protective gene (Pardue et al., 2013), *EGR1* (ZENK, zif268), was found among the previously reported myopia related genes (Fig. 2c).

3.4. EGR1 Expression by Real-time PCR in the Chick Chorioretina Tissues

We examined *EGR1* expression by real-time PCR in the chick model. As expected, *EGR1* was confirmed to be upregulated ($P < 0.05$) by violet light exposure (n = 5 per group) (Fig. 2d). After we matched for irradiance in the entire spectroscopic range including UV, we observed that violet light exposure induced significantly higher upregulation of *EGR1* than blue light (n = 4 in VL+ group, n = 5 in blue light group) (Fig. 2e). *EGR1* mRNA varied according to the strength of violet light (n = 5 per group) (Fig. 2f). These results suggest a particular wavelength characteristic for the suppression of myopia progression, and *EGR1* may be one of the responsible genes for the myopia protection phenotype by violet light.

3.5. Myopia-Related Gene Expression Change by Violet Light in vitro

The 380 nm LED light (unit-area dose amount 600 mJ/cm^2) was exposed to cone photoreceptor cell line 661 W and those genes were analyzed by real time PCR 30 min after the start of irradiation (n = 4). Interestingly, *EGR1* was the only gene which was significantly changed (upregulated; $P < 0.05$), while all other known myopia-related genes were not changed (*Fgf2, Igf1, Il18, Tgfb1, Vegfa, and Vip*, Fig. 2g) or were undetermined (*Bmp2, Ednrb, Irbp, Lumican, Sfrp1, and Wnt2b*). In order to confirm the wavelength specificity for *in vitro* cell culture model, we used different wavelengths of light at 360 nm, 370 nm, 380 nm, 390 nm, and 400 nm, and found that all violet light wavelengths increased *EGR1* expression except 360 nm (n = 4) (Fig. 2h).

3.6. Child Myopia Progression With Eyeglasses and CLs

We have speculated that currently available UV protective eyeglasses may not transmit violet light due to the over protection of UV. Fig. 3a shows the transmittance light spectrum of the most commonly used eyeglasses in Japan. No violet light transmittance could be detected in all lenses. In contrast, there are two types of CLs, one with complete violet light transmittance (Fig. 3b) and the other with partial transmittance (Fig. 3c). We compared the AL changes of patients wearing eyeglasses and CLs at Kato Eye Center where the AL of the eyes had been recorded over several years. First, we observed that the AL elongation for 1 year in myopic children corrected by non-violet light transmitting (VL [−]) eyeglasses (n = 211) (Fig. 3a) was 0.25 mm (mean value), whereas those wearing violet light transmitting (VL [+]) CLs (n = 99) (Fig. 3b) resulted in 0.17 mm (mean value) ($P < 0.001$) (Fig. 3d). Due to the retrospective study design, there were significant differences in patient age, initial objective refraction, and initial AL between these two groups (Table S1). Since the age, gender and level of myopia are known to relate to myopia progression, we performed a stepwise multiple regression analysis to examine the factors affecting AL elongation (Table S1b). It demonstrated that the AL elongation was significantly associated with age, sex and type of lens (non-violet light transmitting eyeglasses or violet light transmitting CL). These negative coefficients for age, sex, and type of lens indicated that AL elongation was greater among younger ages, wearing UV protective eyeglasses, and male, respectively.

Furthermore, we compared the AL elongation in myopic children corrected by two types of CLs. Since the age of the CL wearer is older than the eyeglass wearer, the patient groups were different from the first study described above. Myopic patients with partial violet light blocking CLs (n = 31) (Fig. 3c) had AL elongation of 0.19 mm (mean value) at 1 year, whereas those wearing violet light transmitting (VL [+]) CLs (n = 116) (Fig. 3b) had 0.14 mm (mean value) ($P < 0.05$) (Fig. 3e). There was no difference in patient background including age, objective refraction, initial AL, and period of observation between these two groups (Table S2). The results indicated that non-violet light transmittance is the contributing factor for myopia progression in children.

3.7. Violet Light Is Deficient in Our Modern Society

Violet light outdoors is abundant (Fig. S4a) but it is excluded indoors (Fig. S4b, c). However, it is difficult to recognize the deficiency of violet color (Fig. 4a–d) to the naked eye, but this wavelength is definitely deficient in our indoor modern lifestyle (Fig. 4e) due to the lack of violet light from light sources such as fluorescent, incandescent, and LED lights (Fig. 4f–h) and also due to the UV protected windows. The missing violet light to the eyes is due to the lack of violet light transmittance through eyeglasses (Fig. 3a), CLs (Fig. 3c) and IOLs (Fig. 4i) in addition to the lack of violet light from light sources (Fig. 4f–h).

4. Discussion

First, we performed experiments on chick myopia models and confirmed our hypothesis that violet light had the suppressive effect against myopia progression. Next, we also performed retrospective human clinical studies and showed violet light had the suppressive effect against myopia in humans, too.

Another key finding of our study is the molecular basis of the link between violet light and myopia. We showed that violet light upregulated *EGR1*, a well established myopia protective gene (Pardue et al., 2013). *EGR1* was first found to be involved in the suppression of elongation in the chick eye (Fischer et al., 1999), and the eye elongated when *EGR1* was knocked down in mouse (Schippert et al., 2007), suggesting this gene is one of the most important genes related to myopia progression. Ashby et al. (2014) reported that *Egr-1* mRNA levels were elevated to almost 2x the control after 3 days recovering from −5 D lens removal in a guinea pig model. In the current study, *EGR1* mRNA levels in VL+ group were elevated to almost 2x the VL– group in both control and covered eyes (Fig. 2d). These results connoted that VL exposure may have a potential myopia suppressive effect up to 5 D depending on the animal species and conditions, although the current study in chick and human showed smaller effects. These data indicated that *EGR1* may be one of the responsible genes for the myopia protection by violet light. The molecular involvement of *EGR1* gene for the suppression of myopia by violet light may open a new opportunity for more sophisticated light environment and new drug development by using it as a molecular

target. However, the chick experiment and the clinical studies have potential dissociation due to the difference of ocular transmittance and photoreceptor spectrum. There is a need to investigate this point in the future.

The light environment of our modern lifestyle has reached a hazardous level for our general health over the past 50 years. People tend to spend more time indoors and the exposure to sunlight has decreased. Several studies have linked increased sleep disorders with too low blue light exposure during daytime and too much blue light at nighttime due to LED lighting (Chang et al., 2015; Czeisler, 2013). Furthermore, the connection between myopia and long wavelength light over 550 nm is controversial. Significant development of myopia was reported by Long et al. (2009) and Liu et al. (2014), respectively, for guinea pigs that were raised in long wavelength light illumination (760 nm) and rhesus monkeys that were raised in a slightly different long wavelength light illumination (610 nm). Long wavelengths of light may facilitate myopia progression. On the other hand, Smith et al. (2015) reported the reduction of myopia progression for rhesus monkeys by green long wavelength lighting (approximately 570 nm). The violet light hypothesis is supported by the animal study with possible mechanism insight and the human clinical observations, which provided valuable practical information for myopia control.

UV light with short wavelengths like UVB in sunlight is a well-established risk factor for skin cancer, pterygium, and cataracts (Yam and Kwok, 2014). Thus, people in modern society tend to avoid UV light. However, overprotection from UV light has also excluded the violet 360–400 nm wavelength in our indoor modern lifestyle (Figs. 4e, S4b,c) due to the non-transparency of the UV protected windows, eyeglasses, CLs and IOLs, as well as the lack of violet light emission from light sources such as fluorescent, incandescent and LED lights (Fig. 4f–h).

Although more research is necessary to provide definitive proof of the protective effects of violet light against myopia progression at the molecular level, we propose reconsideration of the potential health benefit of violet wavelength, especially as a protective factor against myopia. Relatively longer wavelengths such as blue light has less efficacy (Fig. 1m) and can cause retinal damage (Algvere et al., 2006). Light with shorter UV wavelengths than 360 nm cannot penetrate the cornea and lens. Thus, violet light, 360 nm to 400 nm, is the most ideal light for myopia control not only from the point of efficacy but also safety. More violet light exposure in society and personally may stop, at least to some extent, the pandemic of myopia in Asia and other parts of the world.

There are some limitations in the current study. First, there were small sample groups in the chick study especially in the covered eyes. When the goggle from the covered eye fell off during the experiment for 7 days, the goggle was placed again but those covered eyes were excluded from the analysis for data accuracy. Second, chick eyes were measured using ultrasonography, and the ultrasonography measurement is not as accurate as SS-OCT scan biometry. Third, water droplets accumulating inside the covering goggles were difficult to be removed. Therefore, our model was not a pure lens-induced myopia model but contained some “form-deprivation” effects. Fourth, we compared the axial length changes between patients wearing non-violet light-transmitting eyeglasses and violet light-transmitting contact lenses. This comparison was examined between different materials as well as different spectral transmissions.

In summary, the lack of violet light in our modern lifestyle has led to the suppression of the important myopia protective gene such as *EGR1*. Until now no one has anticipated that the deficiency of violet light would have a deleterious health effect on myopia control. The introduction of violet light exposure to our indoor lifestyle is a crucial issue for preventing progression of myopia, but no major side effects are anticipated because violet light is an inherent part of natural sunlight sustaining humans for many centuries. Of course, the meticulous safety evaluation of violet light is mandatory. We hope to reverse the pandemic trend of myopia by the regaining violet light to modern society.

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Conflict of Interest

The authors (HT, TK, KN, KaT) are in the process of applying for a patent (PCT/JP2015/65997) for potential products for myopia suppression.

Author Contributions

HT, TK, YS, and KaT designed the study. HT, TK, YS, XJ, MM, SK, YM, YK, KM, MK, TI, and KiT performed animal and *in vitro* experiments. YS, KN, HG, and MH edited the manuscript. HT, TK, XJ, MM, KK, and SK analyzed the data. MO analyzed microarray data. HT, TK, KO, XJ, SK, and KaT wrote the manuscript.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.12.007.

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