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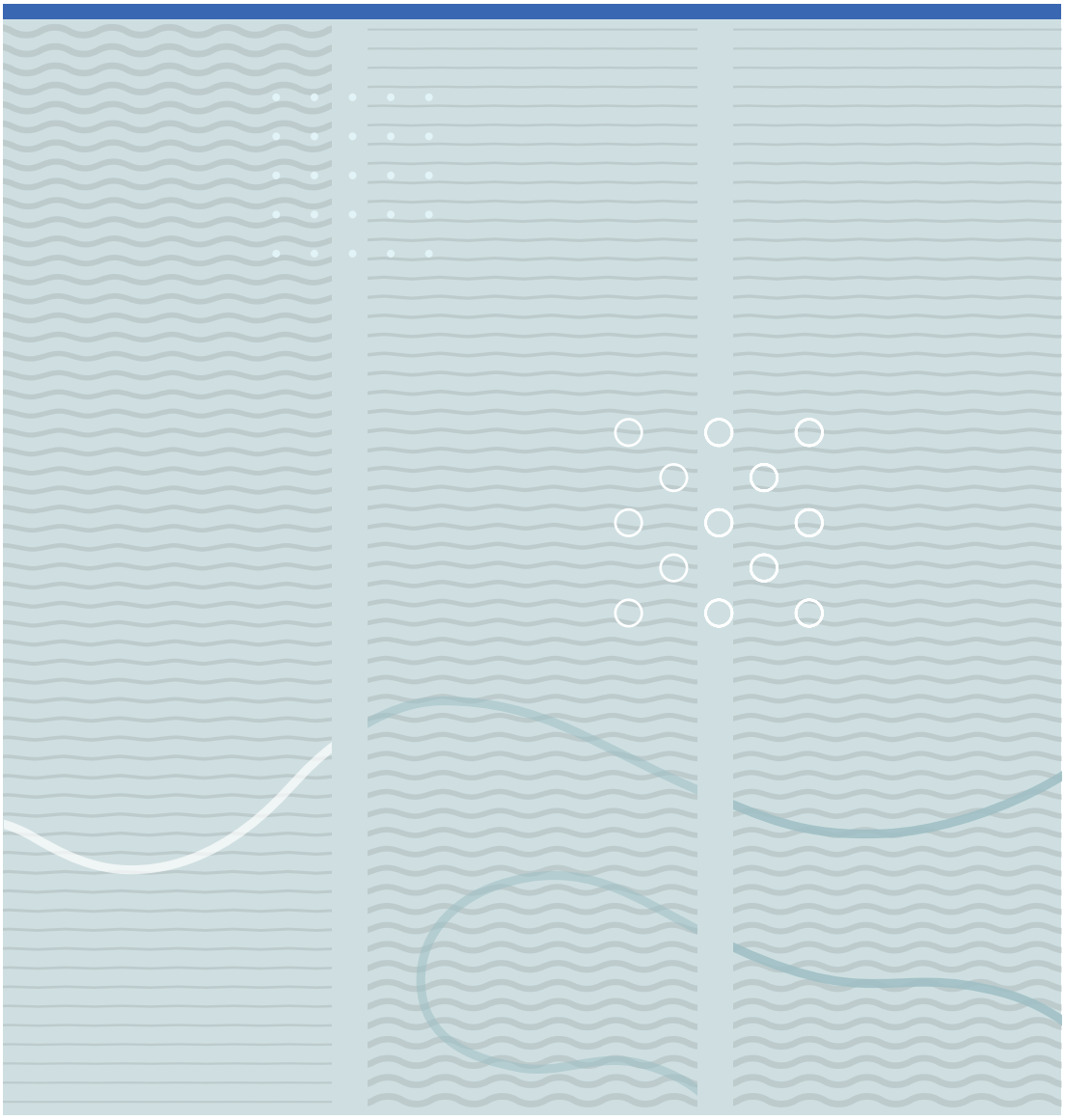
Lene Aarvelta Hagen

Refractive errors, ocular dimensions, and cone opsins in Norwegian adolescents



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To Amalie, Nikolai and Arne.

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Kongsberg, February 2020

Lene A. Hagen

Abstract

The worldwide increase in myopia prevalence is of concern since the ocular elongation raises the risk of secondary ocular pathology. In order to establish effective myopia prevention strategies, a deeper knowledge of the mechanism underlying refractive error development is needed. Refractive errors are the result of a highly complex process of ocular growth; influenced by environmental factors and with a genetic predisposition. The ocular growth is primarily regulated by visual signals, initiated by light absorption in the long (L), middle (M), and short (S) wavelength sensitive cones on the retina. The aim of this thesis was to explore and describe refractive errors, ocular dimensions, and whether myopia was associated with L:M cone ratios and heterozygosity/homozygosity of common L or M cone opsin exon 3 haplotypes in adolescents in Norway.

A cross-sectional study was conducted in a representative sample of Norwegian 16–19-year-olds. Cycloplegic autorefraction and ocular biometry were measured, L and M cone opsin genes were analysed, and individual L:M cone ratios were estimated. After 2 years, cycloplegic autorefraction and ocular biometry measurements were repeated in a subsample. The myopia prevalence was low in the Norwegian adolescents, even though they have few daylight hours available in the autumn-winter period and are in a high-performing education system. Emmetropes/low-hyperopes exhibited coordinated ocular growth at 18 years of age. Myopia was found to be associated with both low L:M cone ratios and heterozygosity of common L cone opsin exon 3 haplotypes in females.

The results indicated a well-adapted emmetropisation mechanism in the Norwegian adolescents and suggested that a low genetic predisposition protected this population from myopia. Individual differences in L:M cone ratios and common L cone opsin polymorphism may be of importance for personalised myopia prevention and management strategies.

Key words: Refractive error, myopia, hyperopia, ocular dimensions, ocular axial length, crystalline lens, cone opsin, cone opsin exon 3 haplotype, L:M cone ratio, person-centred eye-care.

List of papers

Paper I

Hagen, L. A., Gjelle, J. V. B., Arnegard, S., Pedersen, H. R., Gilson, S. J., & Baraas, R. C. (2018). Prevalence and Possible Factors of Myopia in Norwegian Adolescents. *Scientific Reports*, 8(1), 13479. doi:10.1038/s41598-018-31790-y

Paper II

Hagen, L. A., Gilson, S. J., Akram, M. N., & Baraas, R. C. (2019). Emmetropia Is Maintained Despite Continued Eye Growth From 16 to 18 Years of Age. *Investigative Ophthalmology and Visual Science*, 60(13), 4178-4186. doi:10.1167/iovs.19-27289

Paper III

Hagen, L. A., Arnegard, S., Kuchenbecker, J. A., Gilson, S. J., Neitz, M., Neitz, J., & Baraas, R. C. (2019). The association between L:M cone ratio, cone opsin genes and myopia susceptibility. *Vision Research*, 162, 20-28. doi:10.1016/j.visres.2019.06.006

Abbreviations

A	Alanine
AO	Adaptive optics
CREAM	The International Consortium for Refractive Error and Myopia
D	Dioptre
D_3	Cholecalciferol
DC	Dioptre cylinder
DNA	Deoxyribonucleic acid
DTL	Dawson, Trick, and Litzkow
ERG	Electroretinogram
GWAS	Genome-wide association study
HMC	Heidelberg Multi-Color
Hz	Hertz
I	Isoleucine
ID	Identification number
L	Leucine
L cone	Long wavelength sensitive cone
L:M cone ratio	Relative number of long vs middle wavelength sensitive cones
LCR	Locus control region
LED	Light emitting diode
logMAR	The Logarithm of the Minimum Angle of Resolution
M	Methionine
M cone	Middle wavelength sensitive cone
mRNA	Messenger ribonucleic acid
MYP1	The first designated high-myopia gene
no.	Number
OCT	Optical coherence tomography
OPN1LW	Genetic designation for the L cone opsin gene
OPN1MW	Genetic designation for the M cone opsin gene
QQ-plot	Quantile-quantile plot
RT-PCR	Reverse transcription polymerase chain reaction
S	Serine
S cone	Short wavelength sensitive cone
SD	Standard deviation
SER	Spherical equivalent refractive error
SNP	Single nucleotide polymorphism
V	Valine
Xq28	Chromosome band on the long arm of the X-chromosome

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1 Introduction

1.1 Background

Refractive errors are common eye disorders that pose a global public health challenge (Holden et al., 2016; Lou, Yao, Jin, Perez, & Ye, 2016). In 2015, uncorrected refractive errors were the second most common cause of blindness and the leading cause of vision impairment worldwide (Flaxman et al., 2017). Additional negative consequences are loss of productivity (T. S. Smith, Frick, Holden, Fricke, & Naidoo, 2009) and impaired academic performance (Kulp et al., 2016; Narayanasamy, Vincent, Sampson, & Wood, 2015a, 2015b; Orlansky et al., 2015). The worldwide prevalence of myopia is reported to be increasing (Hashemi et al., 2018; Holden et al., 2016), and the increase has been particularly dramatic in certain areas of Southeast Asia where myopia is reported in 80–90% of the adolescents (Jung, Lee, Kakizaki, & Jee, 2012; Lee, Jee, Kwon, & Lee, 2013; J. F. Wu et al., 2013). The increase in myopia prevalence is of concern, since the ocular elongation associated with myopia raises the risk of sight-threatening secondary ocular pathologies, such as macular degeneration, retinal detachment, cataracts, and glaucoma (Flitcroft, 2012; Ohno-Matsui, 2018; Ohno-Matsui, Lai, Lai, & Cheung, 2016; Verhoeven et al., 2015; T. Y. Wong, Ferreira, Hughes, Carter, & Mitchell, 2014). Hence, effective and safe myopia prevention and management strategies that aim to prevent myopia onset and decrease myopia progression are needed. Current optical, pharmacological, and environmental interventions for myopia management show quite variable efficacy (Wildsoet et al., 2019). More knowledge of how individual factors influence myopia susceptibility may be the key to a deeper understanding of the mechanisms of myopia, to invent effective and safe therapeutic interventions for myopia prevention and management, and to identify those who will most likely respond to specific interventions (Wildsoet et al., 2019). These are all important elements to improve and ensure future well-advised, person-centred eye-care.

Refractive errors are the result of a highly complex process of ocular growth; a process that is influenced by environmental factors and has a genetic predisposition (Flitcroft,

2013; Wallman & Winawer, 2004). Visual defocus – initiated by photons of light captured in the photoreceptors and decoded locally in the retina – is assumed to play a crucial role in this process. This is revealed from experimental studies in animals, in which form-deprivation and lens-induced defocus are reported to guide ocular growth and consequently regulate the refractive state of the eye (Chakraborty, Ostrin, Benavente-Perez, & Verkicharla, 2020; Wallman & Winawer, 2004). An improved understanding of how individual differences – in biology, behaviour, and environment – are associated with myopia susceptibility, will provide better opportunities to practice person-centred eye-care in an evidence-based manner in the future (Baraas, Hagen, Pedersen, & Gjelle, 2017; Sacristán, 2013). This thesis focuses on refractive errors and ocular dimensions in adolescents living in Norway and whether individual differences in the cone opsins on the retina may be associated with myopia susceptibility.

1.2 Refractive errors and ocular dimensions

Refractive error, also termed ametropia, is the result of a mismatch between the eye's refractive components and the ocular axial length. Myopia is the result when the eye is too long for its refractive power and hyperopia when the eye is too short. Images of distant objects will be focused in front of the retinal photoreceptors in myopes and behind the retinal photoreceptors in hyperopes, causing blurred retinal images in unaccommodated eyes. Regular refractive astigmatism occurs when the eye has different refractive errors in two meridians, commonly caused by corneal and/or lenticular toricity. Emmetropia, on the other hand, refers to an eye that has no refractive error. The optical power of the refractive components is, in emmetropes, matched with the ocular axial length, such that images of distant objects are sharply focused at the retinal photoreceptors without accommodation.

1.2.1 Worldwide prevalence of refractive errors

The worldwide prevalence of myopia and hyperopia in adults older than 30 years were estimated to be 26.5% and 30.9%, respectively, in a recent systematic review and meta-analysis of 50 studies on myopia and 46 studies on hyperopia (Hashemi et al., 2018). Myopia and hyperopia were here defined as spherical equivalent refractive error (SER) < -0.50 dioptres (D) and > +0.50D, respectively, from non-cycloplegic data. Several studies have reported that the myopia prevalence has increased in the last few decades (Hashemi et al., 2018; Vitale, Sperduto, & Ferris III, 2009; Williams et al., 2015). Holden et al. (2016) estimated from a meta-analysis of 145 studies that the worldwide prevalence of myopia (SER ≤ -0.50D) and high myopia (SER ≤ -5.00D) were predicted to increase from 22.9% and 2.7%, respectively, in 2000, to 49.8% and 9.8%, respectively, by 2050. In contrast to the dramatic increase in myopia prevalence reported in certain regions of Southeast Asia (Jung et al., 2012; Lee et al., 2013; C.-W. Pan, Ramamurthy, & Saw, 2012; J. F. Wu et al., 2013), the myopia prevalence in Danish medical students (Fledelius, 2000) and Danish conscripts (Jacobsen, Jensen, & Goldschmidt, 2007) were suggested to have remained stable over the last century; note that both Danish studies were based on non-cycloplegic refractive errors, which means that the results could have been affected by accommodation. Differences in study design, such as the use of non-cycloplegic data (I. G. Morgan, Iribarren, Fotouhi, & Grzybowski, 2015; Sankaridurg et al., 2017) or different definitions of refractive errors (Cumberland, Bountziouka, & Rahi, 2018), affect prevalence data across studies and may limit the results from meta-analyses.

The prevalence of refractive errors varies with age, sex, ethnicity, and geographical region. Rudnicka et al. (2016) performed a meta-analysis of 143 studies on childhood myopia. Standardised to 2005, the myopia prevalence (SER ≤ -0.50D) was estimated to be 6.3%, 69.0% and 79.6% in East Asian 5-, 15- and 18-year-olds, respectively, and 1.6%, 16.7% and 22.8% in Caucasian 5-, 15- and 18-year-olds, respectively. The odds of myopia were found to be 2.6 times higher in urban versus rural regions, the myopia prevalence was reported to increase more in East Asian than in Caucasian children, and a sex

difference in myopia prevalence was seen from the age of 9 years in both Caucasians and East Asians, with twice as many myopic females than males by the age of 18 years (Rudnicka et al., 2016). A higher myopia prevalence in female children and adolescents are also reported in other studies (Czepita, Czepita, & Safranow, 2019; Guo et al., 2016; Y. Li, Liu, & Qi, 2017; L. J. Wu et al., 2015), but not in all (Hashemi et al., 2014; Maul, Barroso, Munoz, Sperduto, & Ellwein, 2000). It is unclear whether the sex difference in myopia is related to females being more exposed to environmental risk factors of myopia, such as less time spent outdoors (French, Morgan, Mitchell, & Rose, 2013), or whether there are biological factors that make females more susceptible to develop myopia.

Whereas the myopia prevalence usually increases with age in the childhood years, the hyperopia prevalence usually decreases. Data from a meta-analysis of 40 studies on hyperopia prevalence in children (SER ≥ +2.00D; cycloplegic data only) showed a decrease from around 8% at the age of 6 years to around 1% at the age of 15 years, with higher hyperopia prevalence in Caucasian children and in rural regions, but with no clear association with sex (Castagno, Fassa, Carret, Vilela, & Meucci, 2014).

Prevalence data on myopia and hyperopia in adolescents between 15 and 20 years of age are summarised in Table 1, grouped by East Asian and other countries. The myopia prevalence (defined as SER ≤ -0.50D or SER < -0.50D) ranged from 32.5% to 96.5% in East Asian countries and from 0.8% to 18.6% elsewhere, if excluding the myopia prevalence of 59.1% in East Asian adolescents who lived in Australia (French, Morgan, Burlutsky, Mitchell, & Rose, 2013). The hyperopia prevalence (SER ≥ +2.00D) ranged from 0.5% to 4.6% in East Asian countries, and from 0.7% to 17.7% elsewhere (Table 1).

Table 1. Prevalence of myopia and hyperopia in 15–19 years old adolescents

Myopia and hyperopia prevalence (%) in adolescents in the age range 15–19 years in (A) East Asian countries and (B) elsewhere. Myopia was defined as SER ≤ -0.50D except from three studies that defined myopia as SER < -0.50D (Jung et al., 2012; Lee et al., 2013; D. J. Qian et al., 2016). Hyperopia was defined as SER > +2.00D. The results were mainly based on cycloplegic autorefraction, but a few studies used cycloplegic retinoscopy (Dandona et al., 2002; Maul et al., 2000; Murthy et al., 2002; Zhao et al., 2000). Two

studies included male participants only (Jung et al., 2012; Lee et al., 2013) and are here marked with an asterisk (*).

Country	Ethnicity	n	Age (years)	Myopia (%)	Hyperopia (%)
A) EAST ASIAN COUNTRIES					
Urban South Korea (Jung et al., 2012)	Korean; males only	23619	19	96.5*	NA
Rural South Korea (Lee et al., 2013)	Korean; males only	2805	19	83.3*	NA
Urban (48.4%) and rural (51.6%) China (J. F. Wu et al., 2013)	Not given	373	16–18	83.1	1.3
Urban China (He et al., 2004)	Han (Chinese)	376	15	78.4	0.5
Singapore (Dirani et al., 2009)	Chinese, Malay, Indian, and other	1249	11–20	69.5	4.6
Rural China (D. J. Qian et al., 2016)	Han, Dai, Yi, Bai, and other	2069	13–16	52.1	NA
Rural China (He, Huang, Zheng, Huang, & Ellwein, 2007)	Not given	452	16	46.8	1.0
Rural China (Zhao et al., 2000)	Not given	905	14–15	38.8	1.1
Urban Malaysia (Goh, Abqariyah, Pokharel, & Ellwein, 2005)	Malay, Chinese, Indian, and other	321	15	32.5	0.9
B) NON-EAST ASIAN COUNTRIES					
Australia (Sydney) (French, Morgan, Burlutsky, et al., 2013)	East Asian	232	17	59.1	0.9
UK (Northern Ireland) (McCullough, O'Donoghue, & Saunders, 2016)	Caucasian UK children	226	18–20	18.6	17.7
Australia (Sydney) (French, Morgan, Burlutsky, et al., 2013)	European Caucasian	684	17	17.7	2.0
Suburban Chile (Maul et al., 2000)	Not given	395	15	16.7§	8.1§
Urban India (Murthy et al., 2002)	Not given	381	15	10.8	3.9
Semi-urban South Africa (Naidoo et al., 2003)	African, Indian, mixed	326	15	9.6	0.7
Rural India (Dandona et al., 2002)	Not given	258	15	6.7	1.2
Urban (67.3%) and rural (32.7%) Iran (Fotouhi, Hashemi, Khabazkhoob, & Mohammad, 2007)	Not given	120	15	4.9	10.3
Rural Nepal (I. G. Morgan, Rose, & Ellwein, 2010; Pokharel, Negrel, Munoz, & Ellwein, 2000)	Mixed Mongolian, Aryan, and Aboriginal ancestry	386	15	0.79	NA

This table is partly reproduced from Table 5 presented in paper I.

§ The prevalence data presented for Maul et al. (2000) were estimated from the prevalence data reported per group of males and females.

The prevalence of refractive astigmatism is reported to be relatively high at birth, decreasing rapidly the first years of life, and becoming considerably lower in children and adolescents older than four years of age (Gwiazda, Grice, Held, McLellan, & Thorn, 2000; Mutti et al., 2004). A study of American infants reported 41.6% astigmatism [> 1.00 dioptre cylinder (DC)] at 3 months of age decreasing to 4.1% at 3 years of age (Mutti et al., 2004). A three-year longitudinal study of 6–7 and 12–13 years old Caucasian children in Northern Ireland reported the total prevalence of astigmatism (≥ 1.00DC) to be relatively stable with 22.9% in 6–7-year-olds and 17.5% in 15–16-year-olds, although changes in the degree of astigmatism occurred in some individuals (O'Donoghue, Breslin, & Saunders, 2015). Higher prevalence of astigmatism has been reported in certain ethnic groups, such as East Asian, Native American, and Hispanic (Read, Collins, & Carney, 2007), and with higher degree of both myopia and hyperopia (Dobson, Harvey, & Miller, 2007; Heidary, Ying, Maguire, & Young, 2005; O'Donoghue et al., 2011; Read et al., 2007; Rezvan et al., 2011). The worldwide prevalence of astigmatism (> 0.50DC) in adults older than 30 years was reported to be 40.4% in a recent systematic review and meta-analysis (Hashemi et al., 2018).

1.2.2 Prevalence of refractive errors in adolescents in Northern Europe

There is scarcity of refractive error studies performed by cycloplegia in adolescents older than 15 years of age in Northern Europe, except from the report of 18.6% myopia (SER ≤ -0.50D) and 17.7% hyperopia (SER ≥ +2.00D) in Caucasian 18–20-year-olds in Northern Ireland (McCullough et al., 2016), see Table 1. A few studies have reported on refractive errors in adolescents younger than 15 years. In Caucasian 12–13-year-olds in Ireland (Harrington, Stack, Saunders, & O'Dwyer, 2019), Northern Ireland (O'Donoghue et al., 2010), and England (Logan, Shah, Rudnicka, Gilmartin, & Owen, 2011), the myopia prevalence (SER ≤ -0.50D) was reported to be 17.4%, 17.7%, and 18.6%, respectively, and the hyperopia prevalence (SER ≥ +2.00D) was reported to be 9.5%, 14.7%, and 10.4%, respectively. In Denmark, 17.9% myopia (SER ≤ -0.50D) were reported in 14–17-year-olds of unknown ethnicity; note that this study used 2 drops of tropicamide 1% for

accommodation control (Lundberg et al., 2018). In Sweden, 44.9% myopia (SER ≤ -0.50D) and 8.4% hyperopia (SER ≥ +1.00D) have been reported in 12–13-year-olds of unknown ethnicity (Villarreal, Ohlsson, Abrahamsson, Sjostrom, & Sjostrand, 2000). This study used 1–2 drops of tropicamide 0.5% for accommodation control, in combination with retinoscopy.

Two studies have reported on cycloplegic refractive error data in adolescents and young adults in Norway. In 1971, Larsen (1971) reported 13.7% myopia (SER < -1.00D) and 27.4% hyperopia (SER > +1.00D) using cycloplegic retinoscopy in 12–14-year-olds in the Western region of Norway. In 1998, Kinge, Midelfart, and Jacobsen (1998) reported 33% myopia (SER ≤ -0.25D) and 47.3% hyperopia (SER ≥ +0.50D) obtained with subjective refraction under cycloplegia in young adults (mean age: 21.7 years) in mid-Norway. None of the Norwegian studies included adolescents aged 16–19 years, and ethnicity was not given.

1.2.3 Emmetropisation

The distribution of refractive errors changes with age. At birth and in early infancy, the distribution of SER is typically normally distributed with a moderately hyperopic mean refractive error. In the first year of life, the rapid growth of the ocular components leads to a less hyperopic mean refractive error with less variation through the process of emmetropisation (Flitcroft, 2013; Mayer, Hansen, Moore, Kim, & Fulton, 2001; Mutti et al., 2005; Mutti et al., 2018). Emmetropisation is believed to be an active process that is guided by visual experience to regulate the ocular growth to approach emmetropia (Wallman & Winawer, 2004; Wildsoet, 1997), or perhaps low hyperopia (I. G. Morgan et al., 2010). I. G. Morgan et al. (2010) suggested that low hyperopia was the natural endpoint of emmetropisation because, in populations with a low prevalence of myopia, low hyperopia continued to be the most prevalent refractive state from 5 to 15 years of age. A leptokurtic distribution of refractive errors is common after the first phase of emmetropisation (Flitcroft, 2013, 2014). The refractive state is usually maintained over

the following years by coordinated ocular growth, primarily by changes in the crystalline lens power that compensate for the ocular elongation (Mutti et al., 2018). Failure in emmetropisation or disruption of coordinated ocular growth may result in refractive errors (Flitcroft, 2013, 2014). As a consequence of myopia progression, often after the age of six years, a more skewed distribution with less leptokurtosis develops. The negative skew is larger, and the onset is earlier, in populations with high myopia susceptibility (Flitcroft, 2013, 2014).

Experimental models in animals have provided useful insight into the mechanism of emmetropisation, showing that ocular growth is guided by visual signals and controlled locally within the eye (Chakraborty et al., 2020; Schaeffel & Feldkaemper, 2015; E. L. Smith, Hung, & Arumugam, 2014; Troilo et al., 2019; Wallman & Winawer, 2004). Both form-deprived vision and lens-induced defocus are reported to induce abnormal ocular growth and refractive errors in a variety of species – although with some inter-species differences in the ocular responses – and if the visual manipulation is removed, the changes in ocular growth is reported to reverse such that the refractive state of the eye may approach emmetropia (Chakraborty et al., 2020). Form-deprivation by diffusers or eyelid suture does typically lead to choroidal thinning, abnormal ocular axial elongation, and myopia (E. L. Smith et al., 2014; Wallman & Winawer, 2004); indicating that high-contrast visual stimulation is essential for normal ocular growth (Chakraborty et al., 2020). The degree of ocular growth correlates with the magnitude of image degradation, and the effect decreases with age and with increased ambient illumination (Chakraborty et al., 2020; E. L. Smith et al., 2014). In humans, a similar mechanism may explain ocular elongation and myopia that are associated with conditions that deprive form vision early in life, such as congenital cataracts, corneal opacities, and ptosis (Chakraborty et al., 2020; Gee & Tabbara, 1988; Rabin, Van Sluyters, & Malach, 1981; Twomey et al., 1990; von Noorden & Lewis, 1987). Induced myopic and hyperopic defocus are, in animals, reported to guide the ocular growth to compensate for the imposed refractive error (Chakraborty et al., 2020). These experiments indicate that the sign and the magnitude of defocus can be distinguished by the mechanism that regulates ocular growth (E. L. Smith et al., 2014; Wallman & Winawer, 2004). The

effect appears to work best at younger ages, is effective even after section of the optic nerve or ablation of the fovea, and if defocus is restricted to a local region of the retina, the ocular growth changes in the corresponding local region (Chakraborty et al., 2020; E. L. Smith et al., 2014). This implies that the retina not only decodes the visual defocus but also generates growth-modulating signals that are proposed to be transmitted in a signalling cascade from the retina to the retinal pigment epithelium, then to the choroid and finally to the sclera (Wallman & Winawer, 2004). In humans, small compensatory changes in axial length and choroidal thickness have been observed after short periods of induced myopic or hyperopic defocus (Moderiano et al., 2019; D. Wang et al., 2016). Furthermore, in a small group (n = 13) of 11 years old children, slower ocular axial growth was found in eyes with monocular myopic defocus, induced by spectacle lenses, compared with the fully-corrected eyes (Phillips, 2005). It is not clear whether the response to form-deprivation and lens-induced defocus is related to the same biological mechanism (Chakraborty et al., 2020). Moreover, the visual system seems to be more sensitive to myopic than hyperopic defocus, suggesting that different biological mechanisms may underlie the ocular growth in response to myopic versus hyperopic defocus (Chakraborty et al., 2020).

1.2.4 Ocular growth from birth to adolescence

Longitudinal studies have provided data on normal growth of ocular dimensions in humans from birth up to approximately 15 years of age, but there is scarcity of data in older adolescents. The ocular axial length increases rapidly the first year of life, slower up to 6–7 years of age (Mutti et al., 2018), and even slower over the next childhood years (Jones et al., 2005; H. B. Wong, Machin, Tan, Wong, & Saw, 2010). An increase in the vitreous chamber depth is the main contributor to ocular axial growth (Jones et al., 2005; Mutti et al., 2018; H. B. Wong et al., 2010), whereas growth of the anterior segment length, as defined from the front of the cornea to the back of the crystalline lens, is suggested to be more or less complete by the first 1–2 years of age (Iribarren, 2015). In the first year of life, flattening of the cornea leads to a rapid decrease in corneal power,

and the corneal power is reported to be relatively stable after this age (Jones et al., 2005; Mutti et al., 2005; Mutti et al., 2018). The crystalline lens power, however, has been reported to decrease rapidly up to approximately 10 years of age (Mutti et al., 2018; Mutti et al., 1998) and continues to decrease, although at a slower rate, up to around 14 years of age (Jones et al., 2005). The decrease in crystalline lens power is associated with flattening of the lens curvatures, changes in the refractive index, as well as thinning of the lens (Jones et al., 2005; Mutti et al., 2018; Zadnik et al., 2004). In the first 3 months of life in premature infants, a minor thickening is reported in the crystalline lens (Cook, White, Batterbury, & Clark, 2003). Otherwise, a thinning of the lens is reported from 3 months of age up to around 10 years of age, with a thickening of the lens thereafter (Jones et al., 2005; Mutti et al., 2018; H. B. Wong et al., 2010). Thinning of the crystalline lens is proposed to result from compaction of fibres in the lens nucleus defeating a slower addition of new fibres in the lens cortex (Brown, Sparrow, & Bron, 1988; Iribarren, 2015). The observed increase in the anterior chamber depth, up to 10 years of age, is suggested to be a consequence of crystalline lens thinning rather than growth of the anterior segment (Iribarren, 2015; Shih, Chiang, & Lin, 2009).

Sorsby, Benjamin, Sheridan, Stone, and Leary (1961) suggested that ocular axial growth would cease at 13–14 years of age, when distinguished from the ocular axial growth that leads to myopia, based on data from a cross-sectional study of 1432 British children. Fledelius, Christensen, and Fledelius (2014), on the other hand, suggested coordinated ocular growth to continue up to the age of 18 years, based on longitudinal data in 16 Danish emmetropes. Few longitudinal studies have reported on crystalline lens power in adolescents older than 15 years of age, but cross-sectional data in Chinese adolescents indicated that crystalline lens power stabilizes after 14 years of age (Xiong, Zhang, et al., 2017), whereas longitudinal data in young Norwegian adults (aged 20.6 ± 1.2 years at inclusion) indicated that the crystalline lens continued to compensate for ocular axial growth in early adulthood (Iribarren, Midelfart, & Kinge, 2015; Kinge, Midelfart, Jacobsen, & Rystad, 1999). Throughout childhood and adolescence, females are reported to have, on average, shorter ocular axial lengths, steeper corneal curvatures, and more powerful corneas and crystalline lenses than males (Ip, Huynh, et al., 2008; Iribarren, Morgan,

Chan, Lin, & Saw, 2012; S. M. Li et al., 2015; Lu et al., 2016; Mutti et al., 2018; Twelker et al., 2009).

Knowledge about normal ocular growth may be of importance to predict children and adolescents at risk of developing myopia. Longitudinal studies on changes in ocular dimensions before and after myopia onset show increased ocular axial growth the year before onset (Mutti et al., 2007; Rozema, Dankert, Iribarren, Lanca, & Saw, 2019; Xiang, He, & Morgan, 2012), and an acceleration in the crystalline lens power loss up to 1 year before onset (Rozema et al., 2019), which is followed by a deceleration in crystalline lens power loss around the time of onset (Mutti et al., 2012; Rozema et al., 2019). It is suggested that myopia develops when the crystalline lens has reached a physiological limit in the ability to compensate for the ocular axial growth (Iribarren, 2015; Mutti et al., 2012; Rozema et al., 2019; Xiong, Zhang, et al., 2017), and that in the course of changes in crystalline lens thickness during childhood, the minima in crystalline lens thickness may appear at a later age in those with later age of myopia onset (Mutti et al., 2012). In both children and adolescents, the crystalline lens is reported to be thinner and weaker in myopes compared with emmetropes and hyperopes (Iribarren et al., 2012; S. M. Li et al., 2016). The best predictor for juvenile-onset myopia (below 13 years of age) in a study of 6–11 years old non-myopic children was, however, determined to be cycloplegic SER (Zadnik et al., 2015).

1.3 Factors of myopia

1.3.1 Environmental factors

Environmental factors are believed to contribute to the development of myopia, and time spent outdoors is one of the environmental factors assumed to be of importance (French, Ashby, Morgan, & Rose, 2013; Ho, Wu, & Liou, 2019; Jones et al., 2007; Ramamurthy, Lin Chua, & Saw, 2015; Rose, Morgan, Ip, et al., 2008; P. C. Wu, Tsai, Wu, Yang, & Kuo, 2013). Results from a meta-analysis showed that increased time spent outdoors in childhood

has a preventive effect on myopia onset, which is most effective at younger ages (Xiong, Sankaridurg, et al., 2017). Time spent outdoors was, however, reported to have no measurable effect on myopia progression in those who already were myopes (Xiong, Sankaridurg, et al., 2017). Moreover, decreased myopia incidence is reported in randomized controlled trials with increased outdoor time during the school day as intervention (Deng & Pang, 2019; He et al., 2015; P. C. Wu et al., 2018). The protective factors of spending time outdoors are yet to be determined. Bright light exposure is believed to be a strong candidate, since bright light exposure is shown to prevent form-deprivation myopia in animals, although the effects are more variable on lens-induced myopia (Ashby, 2016; Karouta & Ashby, 2014; Norton, 2016; Norton & Siegwart, 2013). Furthermore, bright light exposure is shown to be associated with slower eye growth in humans (Hua et al., 2015; Read, Collins, & Vincent, 2015; P. C. Wu et al., 2018). The effect may be related to the release of dopamine that inhibits axial elongation, in line with experimental studies in animals (Feldkaemper & Schaeffel, 2013; X. Zhou, Pardue, Iuvone, & Qu, 2017). Low serum vitamin D levels have been associated with higher risk of myopia (C. W. Pan, Qian, & Saw, 2017), but results from a meta-analysis of serum vitamin D level and vitamin D pathway genes indicated that vitamin D levels may be a substitute for outdoor light exposure rather than being a direct causal risk factor of myopia (Tang et al., 2019). The high luminance levels outdoors may lead to constriction of the pupil which further increases the depth of focus and improves the retinal image quality (Blackie & Howland, 1999). Other possible protective factors of spending time outdoors may be the dioptric structure of the environment outdoors compared with indoors (Flitcroft, 2012), the spectral composition of the light (Chakraborty et al., 2020), or the effect of daylight exposure on circadian rhythms and ocular growth (Chakraborty et al., 2018). Seasonal variation in ocular growth and myopia progression is reported, with slower ocular growth and a less negative change in SER in periods with more daylight hours (Cui, Trier, & Munk Ribel-Madsen, 2013; Fulk, Cyert, & Parker, 2002; Gwiazda, Deng, Manny, & Norton, 2014). These results support the protective effect of time outdoors since children are likely to stay outdoors longer in the summer (Deng, Gwiazda, & Thorn, 2010), although less educational demands and less time spent on near work indoors in the summer period

are suggested to be other potential factors. A meta-analysis indicated that the odds of myopia increased by 2% per dioptre-hour extra near work per week (H. M. Huang, Chang, & Wu, 2015), and continuous reading on short reading distances, rather than the total duration of reading, has been suggested as possible risk factors from cross-sectional data (Ip, Saw, et al., 2008). The results on associations between myopia and near work are, however, inconsistent (Ramamurthy et al., 2015), and further studies are needed to establish whether there really is a causal relationship between near work and myopia.

1.3.2 Myopia genetics

Common myopia is considered to be a complex disorder, influenced by environmental factors but with a genetic predisposition (Tedja et al., 2019). The heritability of myopia is expected to be between 60% and 80% (Sanfilippo, Hewitt, Hammond, & Mackey, 2010; Tedja et al., 2019), even though the reports vary widely dependent on the population studied and the methodology used (Dirani et al., 2006). Recent molecular technologies and systematic research have provided new knowledge on the genetic background of refractive error (Cai, Shen, Chen, Zhang, & Jin, 2019). This offers new possibilities, such as the ability to use genetic risk scores to predict children who are at risk of myopia. Current genetic risk scores are, however, no better than the prediction from cycloplegic SER (area under curve = 0.67 versus 0.87) (Ghorbani Mojarrad, Plotnikov, Williams, & Guggenheim, 2020; Zadnik et al., 2015). A recent meta-analysis of two large genome-wide association studies (GWAS) – the International Consortium for Refractive Error and Myopia (CREAM) and 23AndMe – identified a total of 161 loci for refractive error, with a genetic correlation of 0.78 between the European and Asian participants (Tedja et al., 2018). Still, these genetic variants explained only 7.8% of the phenotypic variation in refractive error (Tedja et al., 2018). The results imply the mechanism of myopia to be complex, with many genetic variants of small effect, but do also underline the limitation in current knowledge (Cai et al., 2019; Tedja et al., 2019). It is important to note that the genes on the X-chromosome were excluded from these GWAS studies. Yet, the results confirmed the role of a light-induced retina-to-sclera signalling cascade in refractive error

development, and suggested mechanisms such as light detection and release of glutamate from photoreceptors to bipolar cells to be important factors (Tedja et al., 2018).

Syndromic forms of myopia are often genetic in origin and associated with systemic or ocular abnormalities (Flitcroft, Loughman, Wildsoet, Williams, & Guggenheim, 2018). A recent study that compared genes for syndromic myopia with genes for common myopia, identified 21 novel genes as well as several variants already known from the GWAS studies (CREAM and 23AndMe) (Flitcroft et al., 2018). This implied that genetic variants, within the same genes, may harbour pathogenic mutations as well as benign polymorphisms that have more subtle effects on refractive error (Flitcroft et al., 2018). The study did also identify a number of genes on the X-chromosome to be associated with myopia, not included in the CREAM and 23AndMe analysis since the X-chromosome was excluded from these studies (Flitcroft et al., 2018). The results suggest that genes on the X-chromosome play a role in myopia development, and the first designated high-myopia gene, MYP1, was indeed located at Xq28 on the X-chromosome (Schwartz, Haim, & Skarsholm, 1990; Young et al., 2004). Bornholm Eye Disease, characterized by X-linked high myopia and cone dysfunction, is mapped to MYP1 and reported to be caused by rare polymorphisms in exon 3 of the cone opsin genes localized on Xq28 (McClements et al., 2013). It is well known that cone opsin genes are highly polymorphic (Maureen Neitz, Neitz, & Grishok, 1995; Winderickx, Battisti, Hibiya, Motulsky, & Deeb, 1993), but the role of more benign polymorphisms in the cone opsin genes on refractive error development is still unknown. This is further discussed in section 1.4.1.

1.4 The role of cone opsins in myopia susceptibility

As mentioned in section 1.2.3, the ocular growth and the refractive state are assumed to be guided by visual signals (Wallman & Winawer, 2004), initiated by photons of light captured by the photopigment in the photoreceptors. The cone photoreceptors mediate chromatic and achromatic spatial vision with high spatial and temporal resolution at both

photopic and mesopic light levels. Three classes of cone photoreceptor are present in a normal human retina and form the basis for trichromacy, each class distinguished by a photopigment sensitive to light of long (L), middle (M) or short (S) wavelengths. This section focuses on the L and M cone opsins – the part of the L and M cone photopigment that determines the spectral sensitivity of the L and M cone photoreceptors – and how cone opsin exon 3 haplotypes and L:M cone ratio may be associated with myopia susceptibility.

1.4.1 L and M cone opsin genetics

The cone photopigment consists of the chromophore and the cone opsin. The chromophore absorbs the light quanta captured by the cone photoreceptor and transforms the radiant energy into electrical activity via isomerisation. The cone opsin is a chain of amino acids in the disc membrane in the cone outer segment, of which the amino acid sequence determines the wavelength of peak absorption of the photopigment (Asenjo, Rim, & Oprian, 1994; Nathans, Thomas, & Hogness, 1986). The genes encoding the L and M cone opsins, OPN1LW and OPN1MW, are arranged in a tandem array on the X-chromosome at Xq28 (Vollrath, Nathans, & Davis, 1988), which is the location of MYP1 (see section 1.3.2) (Young et al., 2004). Figure 1 shows illustrations of the opsin gene array at Xq28 on the X-chromosome for two normal trichromats; one female and one male. The opsin gene array typically has one copy of OPN1LW followed by one or more copies of OPN1MW (Macke & Nathans, 1997), with only the first two cone opsin genes in the array commonly expressed on the retina (Bollinger, Sjoberg, Neitz, & Neitz, 2004; T. Hayashi, Motulsky, & Deeb, 1999). Both OPN1LW and OPN1MW have six exons with almost identical nucleotide sequences (Nathans, Thomas, et al., 1986). Exon 5 encodes the amino acid dimorphisms that produce the largest shift in the spectral sensitivity that separates the L and M cone opsins, while exon 2, 3 and 4 encode amino acid dimorphisms that produce smaller spectral shifts (Asenjo et al., 1994; J. Neitz & Neitz, 2011). Note that exon 1 and 6 show typically no variation between or among OPN1LW and OPN1MW (Asenjo et al., 1994; J. Neitz & Neitz, 2011). OPN1LW and

OPN1MW are prone to recombination during meiosis because of their similarity and adjacent arrangement. Recombination may result in intermixed genes and redistributed opsin gene arrays that cause inherited red-green colour vision deficiencies (Nathans, Piantanida, Eddy, Shows, & Hogness, 1986; J. Neitz & Neitz, 2011), in addition to large diversity in the amino acid sequences of the L and M cone opsin, also in normal trichromats (Maureen Neitz et al., 1995; Winderickx et al., 1993). Five dimorphic amino acid positions (L153M, V171I, A174V, I178V, and S180A) are encoded by the single-nucleotide polymorphisms (SNPs) in exon 3. The single letter is an abbreviation for the amino acid at the polymorphic positions encoded by exon 3; A for alanine, I for isoleucine, L for leucine, M for methionine, S for serine, and V for valine, specified at positions 153, 171, 174, 178 and 180. See also Figure 1. The substitution of serine by alanine at position 180 (S180A) is the only amino acid dimorphism encoded by exon 3 that produces a spectral shift (Asenjo et al., 1994; Carroll, Neitz, & Neitz, 2002), and is also known to shift the Rayleigh match midpoint when it occurs in the L cone opsin (J. Neitz & Jacobs, 1986; Winderickx et al., 1992). A green-shifted Rayleigh match has been reported in myopes (Rucker & Kruger, 2006; Wienke, 1960), which may indicate that the myopes in these reports had serine at L position 180.

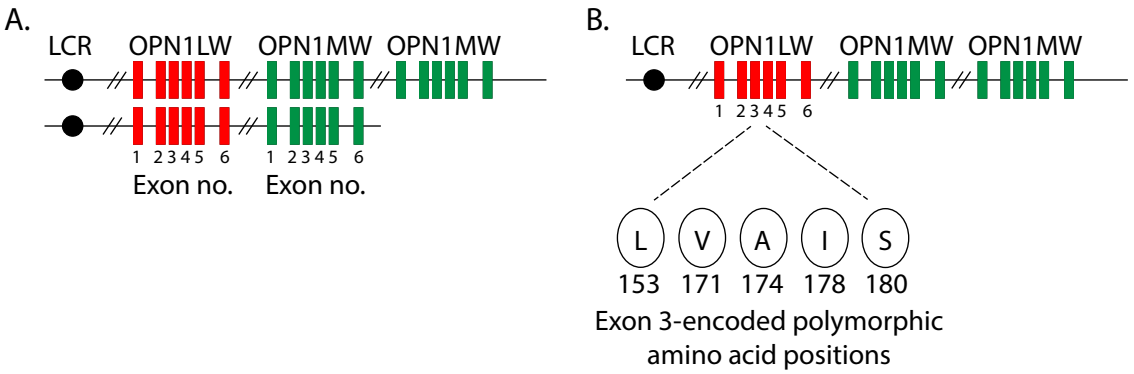

Figure 1. Illustration of cone opsin gene arrays

Cone opsin gene arrays in (A) a female normal trichromat with a single OPN1LW followed by two and one copies of OPN1MW, respectively, in each of her cone opsin arrays at Xq28, and (B) a male normal trichromat with a single OPN1LW followed by two copies of OPN1MW in his cone opsin array. Exon 1–6 are represented by boxes coloured red for OPN1LW and green for OPN1MW, and the locus control region (LCR; see details in 1.4.2)

is represented by a black filled circle. The L exon 3 haplotype is in (B) designated by the amino acid combination LVAIS; the single letter amino acid codes are L for leucine, V for valine, A for alanine, I for isoleucine, and S for serine specified at positions 153, 171, 174, 178 and 180. The exon 3 haplotype LVAIS is associated with ~100% correctly spliced mRNA and a normal amount of photopigment in the cones harbouring the gene. The figure is modified after Buena-Atienza et al. (2016).

Myopia has been associated with rare L/M interchange exon 3 haplotypes (Buena-Atienza et al., 2016; Greenwald, Kuchenbecker, Rowlan, Neitz, & Neitz, 2017; J. Li et al., 2015; Orosz et al., 2017); combinations of single-nucleotide polymorphisms (SNPs) that have arisen as a result of recombination of OPN1LW and OPN1MW. These haplotypes are shown to cause incorrect exon 3 splicing in the messenger RNA (mRNA) and reduced amount of photopigment in the cone photoreceptor (Buena-Atienza et al., 2016; Greenwald et al., 2017; Ueyama et al., 2012). Because of a disrupted splicing code, exon 3 will occasionally be excluded from the mRNA, and the result is a mixture of full-length and exon 3-skipped mRNA (M. Neitz, Patterson, & Neitz, 2019). A full-length mRNA is required to make a functional photopigment, thus the amount of photopigment in the cone is determined by the amount of full-length versus exon 3-skipped mRNA.

Table 2 summarises L/M interchange exon 3 haplotypes that have been associated with myopia and incorrect exon 3 splicing; LIAVA, LVAVA, MIAVA, and LIAVS. The % correctly spliced transcripts presented were estimated from reverse transcription polymerase chain reaction (RT-PCR) products from minigene splicing assays (Buena-Atienza et al., 2016). A severely reduced amount of photopigment is expected in cones harbouring LVAVA (Buena-Atienza et al., 2016). Bornholm Eye Disease, the syndrome mapped to the locus of MYP1 (see section 1.3.2) and characterized by high myopia, dichromacy and visual acuity loss (Haim, Fledelius, & Skarsholm, 1988; Michaelides et al., 2005; Schwartz et al., 1990; Young et al., 2004), was found to be caused by LVAVA (McClements et al., 2013). LVAVA was also found in Chinese families with high myopia but no colour vision deficiency (J. Li et al., 2015). No functional photopigment is expected in cones harbouring

LIAVA (Buena-Atienza et al., 2016). LIAVA was reported in individuals who had high myopia with dichromacy, or even blue cone monochromacy when LIAVA was present in the first two positions of the L/M cone opsin gene array (Gardner et al., 2014; Greenwald et al., 2017; Patterson et al., 2018). In the cases of LIAVA and LVAVA, myopia is suggested to be modulated by the ratio of functional versus less-than-normally functioning cones in the cone mosaic (Greenwald et al., 2017; Patterson et al., 2018). The cone mosaic will have normal functioning cones adjacent to cones with severely reduced amount of functional opsin, or with no functional opsin at all. Neighbouring cones with different levels of opsin expression may stimulate ON bipolar cells even when there is no contrast information in the visual scene, and erroneous contrast signal may appear, suggested to stimulate eye growth (Greenwald et al., 2017; Patterson et al., 2018). The ratio of functional versus less-than-normally functioning cones determines the amount of erroneous signalling and is thus suggested to modulate the myopia (Patterson et al., 2018).

Table 2. L/M interchange exon 3 haplotypes

An overview of L/M interchange exon 3 haplotypes that have been associated with myopia. The estimated % correctly spliced transcripts were estimated from RT-PCR products from minigene splicing assays (Buena-Atienza et al., 2016).

Exon 3 haplotype *	Estimated % correctly spliced transcripts	Associated with myopia in following studies
LIAVA	0	Patterson et al. (2018); Greenwald et al. (2017); Gardner et al. (2014)
LVAVA	< 20	Patterson et al. (2018); Orosz et al. (2017); Greenwald et al. (2017); J. Li et al. (2015); Gardner et al. (2014)
MIAVA	< 20	Gardner et al. (2014)
LIAVS	20–30	Mizrahi-Meissonnier, Merin, Banin, and Sharon (2010)

* Each letter is here an abbreviation for the amino acid at the polymorphic positions encoded by exon 3, see text and Figure 1 for details.

Certain combinations of nucleotide polymorphisms in exon 3 of OPN1LW and OPN1MW may give rise to less severe exon 3 splicing defects (M. Neitz & Neitz, 2018). These variants may reduce the amount of photopigment in the cones, although to a lesser degree than for the rare interchange haplotypes LIAVA and LVAVA, and not necessarily in combination with a shift in the spectral sensitivity of the opsin (Carroll et al., 2002; J. Neitz, Neitz, He, & Shevell, 1999). It is not unlikely that mild exon 3 splicing defects may be associated with myopia susceptibility in common myopia, since there is large diversity in the amino acid sequences of the L and M cone opsin in normal trichromats (Maureen Neitz et al., 1995; Winderickx et al., 1993). This may give rise to a cone mosaic that consists of normal functioning cones adjacent to less-than-normally functioning cones. A cone mosaic with differences in the functioning of the cones, due to variation in the amount of photopigment, may interfere with the process of emmetropisation. A recent Australian study found two OPN1LW variants that greatly reduced the number of spliced and unspliced transcripts, thus expected to reduce the amount of opsin in the L cones, to be only present in myopic participants (Mountford et al., 2019). As for syndromic myopia that is associated with rare L/M exon 3 interchange haplotypes (Greenwald et al., 2017; Patterson et al., 2018), the myopia susceptibility may be modulated by the relative number of functioning versus less-than-normally functioning cones. Whether mild exon 3 splicing defects are associated with myopia susceptibility is unknown.

1.4.2 The cone mosaic and L:M cone ratio

The L, M, and S cone opsins have overlapping spectral sensitivity curves with peak absorption in the range 549–559 nm, 530–536 nm, and 420 nm, respectively (J. Neitz & Neitz, 2011; M. Neitz et al., 2019). The S cones constitute only 5–10% of the cones, are not present in the foveal centre, and have the highest density at approximately 0.5 degrees eccentricity (Calkins, 2001; Curcio et al., 1991). The L and M cones, however, are present throughout the whole retina but are highly concentrated in the fovea centralis (Curcio, Sloan, Kalina, & Hendrickson, 1990). The arrangement of the L and M cones is reported to be random, but with a tendency to have patches consisting of only

L or M cones, which may be an advantage for high-frequency spatial vision (Hofer, Carroll, Neitz, Neitz, & Williams, 2005; Roorda, Metha, Lennie, & Williams, 2001). See illustrations of cone mosaics in Figure 2. Estimates of the relative number of L versus M cones (L:M cone ratio) show large individual variation with, on average, more L than M cones in normal trichromats (Carroll et al., 2002; Hofer et al., 2005). The L:M cone ratio is reported to increase from the fovea to the periphery of the retina (Hagstrom, Neitz, & Neitz, 1998; Kuchenbecker, Sahay, Tait, Neitz, & Neitz, 2008; M. Neitz, Balding, McMahon, Sjoberg, & Neitz, 2006).

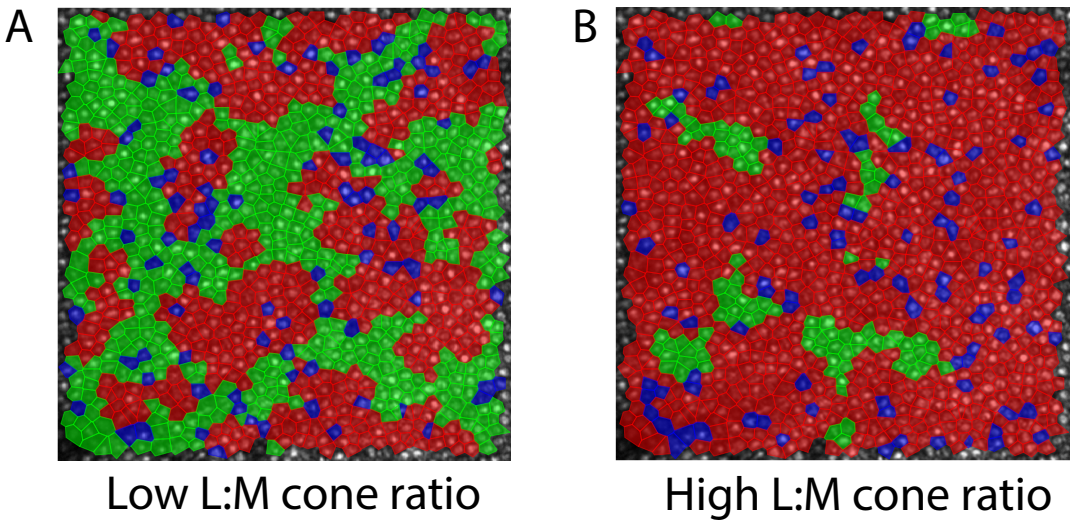

Figure 2. Illustration of L:M cone ratios

Hypothetical cone photoreceptor mosaics with low (A: 50% L cones) and high (B: 90% L cones) L:M cone ratios. L, M and S cones are synthetically labelled red, green and blue, respectively, using a hypothetical clustering algorithm.

The first direct evidence of a large variation in L:M cone ratios came from studies using high-resolution adaptive optics (AO) imaging in combination with retinal densitometry (Hofer et al., 2005; Roorda & Williams, 1999). Hofer et al. (2005) estimated the L:M cone ratio to range from 1.1:1 (~52% L cones) to 16.5:1 (~94% L cones) in 8 colour normal

males and to be 0.37:1 (~27%) in a protan carrier. The males had previously participated in a larger study that reported mean L:M cone ratio of 2.7:1 (~73% L cones) in colour normal American Caucasian males estimated by full field electroretinogram (ERG) flicker photometry (Carroll et al., 2002). When the ERG-derived L:M cone ratios were corrected for a ~1.5-fold larger contribution from the M cones relative to the L cones in the ERG signal, the two estimates were in high agreement (Hofer et al., 2005). Even though a change in chromatic adaptation may occur as the ERG stimulus wavelength is changed (Schmidt, Touch, Neitz, & Neitz, 2016; Stockman, Jagle, Pirzer, & Sharpe, 2008), these results validate ERG flicker photometry to be a reliable method to estimate L:M cone ratios objectively in vivo (Carroll et al., 2002; Hofer et al., 2005). This is given that corrections are made for differences in crystalline lens density and individual variation in the L cone peak sensitivity (Bieber, Kraft, & Werner, 1998; Carroll, McMahon, Neitz, & Neitz, 2000). Over time, a large variety of indirect methods have been used to estimate the L:M cone ratios in vivo, such as psychophysics (de Vries, 1949; Kremers et al., 2000; Nerger & Cicerone, 1992; Rushton & Baker, 1964), ERG (Carroll et al., 2002; Kremers et al., 2000; Kuchenbecker et al., 2008), and VEP (N. Zhou, Atchison, Zele, Brown, & Schmid, 2015), as well as in vitro by analyses of mRNA levels (Hagstrom et al., 1998; Hagstrom, Neitz, & Neitz, 2000; M. Neitz et al., 2006; Yamaguchi, Motulsky, & Deeb, 1997).

The reason for the large individual variation in L:M cone ratio is unclear (McMahon, Neitz, & Neitz, 2004), but transcription of the cone opsin gene requires interaction between the locus control region (LCR) – an enhancer upstream of OPN1LW that is shared by OPN1LW and OPN1MW – and the cone opsin gene promoter for the specific gene (see Figure 1) (Nathans et al., 1989; Smallwood, Wang, & Nathans, 2002; Y. Wang et al., 1992). Epigenetic silencing (Knoblauch, Neitz, & Neitz, 2006; J. Neitz & Neitz, 2011), or the way the chromatin is looped in the nucleus (McMahon, Carroll, Awua, Neitz, & Neitz, 2008), may be factors that determine the probability for LCT to interact with the first or the second gene in the cone opsin array, and hence the L:M cone ratio.

Myopia is suggested to be modulated by the ratio of functioning versus less-than-normally functioning cones in syndromic myopia caused by L/M interchange haplotypes

(Greenwald et al., 2017; Patterson et al., 2018), as mentioned in 1.4.1. Likewise, in common myopia, myopia susceptibility is suggested to be higher in individuals with symmetric L:M cone ratios (near 50% L cones) (J. Neitz & Neitz, 2015; N. Zhou et al., 2015). Figure 2 illustrates cone mosaics with low (symmetric; near 50% L cones) and high (skewed; near 100% L cones) L:M cone ratios. That East Asian individuals have a high myopia susceptibility are indicated from the reports of earlier myopia onset and higher myopia prevalence in East Asian compared with Caucasian populations (Rudnicka et al., 2016). Interestingly, mean L:M cone ratio in East Asian males is reported to be lower (more symmetric) than in American Caucasian males (Carroll et al., 2002; Kuchenbecker, Neitz, & Neitz, 2014; Yamauchi, Yatsu, Kuchenbecker, Neitz, & Neitz, 2013), in line with the theory of an association between myopia susceptibility and L:M cone ratio. L:M cone ratios were measured with ERG flicker photometry in these studies. N. Zhou et al. (2015) estimated L:M cone ratio by measuring L:M amplitude modulation ratio with multifocal visual evoked potentials and a silent substitution paradigm. They reported the L:M amplitude modulation ratio, in a peripheral ring at 13 to 20 degrees, to decrease with a more myopic refractive error. In chicken, Gisbert and Schaeffel (2018) used the red and yellow oil droplets in retinal flat mounts to estimate M:L cone ratio. Both vitreous chamber depth and refractive error in the control eyes were associated with the M:L cone ratio, even though the degree of induced form-deprivation myopia was not.

Red-green colour vision deficient individuals have highly skewed L:M cone ratios, since either the L or the M cone photoreceptors are not expressed in their retinas. Two different studies – one study in 15 to 18 years old Chinese students (Y. S. Qian et al., 2009) and one study in 7 to 12 years old Iranian school children (Ostadimoghaddam et al., 2014) – have both reported a lower myopia prevalence and a less myopic SER in a group of red-green colour vision deficient individuals compared with individuals with normal colour vision. The ocular axial length was measured in the study of Chinese students only, and protan individuals were reported to have shorter ocular axial length than the control group (Y. S. Qian et al., 2009). These reports support the hypothesis of a lower myopia susceptibility in individuals with more skewed L:M cone ratios.

2 Motivation and aim of research

2.1 Motivation

The worldwide increase in myopia prevalence (Holden et al., 2016), and the associated increase in sight-threatening myopia-related ocular complications (Verhoeven et al., 2015), require new and effective myopia management strategies that aim to prevent myopia onset and decrease myopia progression. In-depth knowledge about the mechanism of refractive error development may be the key to reach this aim. One step forward is to understand how the distribution of refractive errors and the ocular growth patterns vary with ethnicity, geographical region, and age. Another step forward is to understand how individual differences may influence the individual's susceptibility to myopia.

There is a scarcity of studies on refractive errors and ocular growth in Northern Europe, as well as in Caucasian adolescents older than 15 years of age. Norway has a large seasonal variation in daylight hours, due to its Northerly latitude, and a high-performing education system (OECD, 2016) with extensive use of near electronic devices (OECD, 2015). The long period with few daylight hours available in the autumn and winter season, in combination with many hours of near work indoors, may make the Norwegians prone to develop myopia. Yet, the myopia prevalence in Norwegian adolescents older than 15 years of age is unknown, and it is unclear whether coordinated ocular growth is still present at that age.

Individual L:M cone ratios are suggested to be associated with myopia susceptibility (J. Neitz & Neitz, 2015; N. Zhou et al., 2015), and a cone mosaic with different levels of functional photopigment is suggested to modulate syndromic myopia associated with rare L/M interchange exon 3 haplotypes (Greenwald et al., 2017; Patterson et al., 2018). The role of common L and M cone opsin gene polymorphisms in myopia susceptibility is, however, unclear. If mild exon 3 splicing defects play a role in susceptibility to common myopia, females who are heterozygous for their L and/or M cone opsin exon 3 haplotypes may have a higher frequency of myopia than males and females who are

homozygous. This is because exon 3 heterozygous females are twice as likely to have a mild exon 3 splicing defect than males and homozygous females, and thus more likely to have cones with different levels of opsin expression on their retina. The myopia susceptibility, however, may be modulated by the organisation and the ratio of L and M cones. New knowledge about refractive errors and ocular growth in Norwegian adolescents older than 15 years of age, and whether myopia is associated with L:M cone ratio and heterozygosity/homozygosity of common L or M cone opsin exon 3 haplotypes, may make a small, yet important, contribution in the effort to reduce the increase in myopia prevalence worldwide.

2.2 Aim and objectives

The aim of this thesis was to explore and describe refractive errors, ocular dimensions, and whether myopia was associated with heterozygosity/homozygosity of common L or M cone opsin exon 3 haplotypes and L:M cone ratio in adolescents in Norway, a country with large seasonal differences in daylight. The study sample was 16–19 years old students, primarily of Caucasian ethnicity, who lived and had grown up in the Southeast Norway. Three research objectives were formulated to achieve the aim of the thesis, with main focus in each paper as specified in the parenthesis below.

i. To estimate the prevalence of refractive errors and to assess whether there was an association between myopia and self-reported time spent on activities outdoors and indoors (paper I)
ii. To examine whether maintenance of emmetropia and low hyperopia was associated with continued coordinated ocular growth from 16 to 18 years of age (paper II)
iii. To estimate individual L:M cone ratios and to assess whether myopia was associated with L:M cone ratio and heterozygosity/homozygosity of common L or M cone opsin exon 3 haplotypes in normal trichromats (paper III)

3 Methods

This section gives a general overview of the methods used in this thesis. Detailed descriptions are deferred to the papers themselves.

3.1 Overview of study design and participants

Table 3 provides a simplified overview of the study design. A cross-sectional study was carried out in 2015–2016 on a representative sample of 16–19 years old Norwegian adolescents. Initial measurement data (see details in 3.2), cycloplegic refractive errors, and ocular dimensions were collected from all participants. In addition, a subsample reported estimates of their time spent on activities indoors and outdoors. These data are presented in paper I. In 2018, follow-up data on cycloplegic refractive errors and ocular dimensions were collected in a subsample of the participants from the data collection in 2016 (referred to as baseline). These data are presented in paper II. A sample of normal trichromats, both males and females, participated in additional measures of L and M cone opsin genetics and L:M cone ratios as part of the first data collection. A control group, consisting of five red-green colour vision deficient males and one protan carrier, was included to validate the estimates of L:M cone ratios. These data are presented in paper III.

The participants were recruited from two upper-secondary schools located in Southeast Norway at 60° latitude north. Students in both academic and vocational studies were invited to participate in the first data collection, and all measurements were performed at the respective schools within normal school hours at a time suitable for the participant. Information about the study and invitation to participate were given in the classes as well as on the schools' webpages. All 16–19 years old students who gave consent were included in the cross-sectional data on refractive errors and ocular dimensions (paper I), and the sample was representative of the schools' catchment area with respect to ethnicity and grade point averages (see details in paper I: Supplementary Information).

Parts of the analyses were restricted to Northern European Caucasian participants who reported to have grown up in Norway.

Table 3. Overview of the study design

A simplified overview of the study design and how the data relate to each paper.

<table>
<tr><th colspan="3">CROSS-SECTIONAL STUDY
2015–2016</th></tr>
<tr><td>Total sample:
439 participants
Age: 16–19 years
90.9% Caucasian</td><td>Baseline data:
♦ Initial measurements
♦ Cycloplegic autorefraction
♦ Cycloplegic ocular biometry</td><td>Questionnaire:
♦ Self-reported time spent indoors and outdoors (n = 269)</td></tr>
<tr><td colspan="3">Paper I</td></tr>
<tr><td colspan="2">2-YEAR FOLLOW-UP STUDY</td><td>ADDITIONAL CROSS-SECTIONAL DATA</td></tr>
<tr><td colspan="2">93 of the original 439 participants
Mean age: 16.7 ± 0.3 years at baseline (2016)

Follow-up data obtained in 2018:
♦ Initial measurements
♦ Cycloplegic autorefraction
♦ Cycloplegic ocular biometry
♦ Serum vitamin D_3</td><td>136 normal trichromats of the original 439 participants
Age: 16–19 years

Control group:
5 colour vision deficient males and 1 protan carrier, recruited outside of the original study

Data:
♦ L and M cone opsin genetics
♦ ERG-derived L:M cone ratio</td></tr>
<tr><td colspan="2">Paper II</td><td>Paper III</td></tr>
</table>

In the follow-up study in 2018 (paper II), all participants who were 16 years at the time of the first data collection in 2016 and still students in upper-secondary school, got a personal invitation to participate for further tests. Information about the follow-up study

and invitation to participate were given in the classes and by personal contact via email or phone. All who gave consent were included in the analyses, except from one female participant who had performed a crosslinking treatment for keratoconus in the period after the baseline measurement.

In the cross-sectional study of L:M cone ratio and cone opsin genes (paper III), the inclusion criteria were Caucasian ethnicity, age 16–19 years, normal colour vision, being healthy with no known ocular abnormalities and no medication, stereo acuity ≤ 120'', and normal corrected visual acuity. Students who met these criteria were invited to participate in full-field ERG flicker photometry measurements to estimate the L:M cone ratio. The control group was recruited from the colour vision clinic at the National Centre for Optics, Vision and Eye Care at the University of South-Eastern Norway.

3.2 Initial measurements and questionnaire

A set of initial measurements and a questionnaire were performed on all participants. The measurements included ocular dominance, visual acuity, stereo acuity, body height, and colour vision. The data collection started with a face-to-face interview to gather self-reported information on age, sex, ethnicity, ocular and general health, medication, and family history of colour vision deficiencies. Ocular dominance was determined, and habitual logMAR visual acuities were measured monocularly and binocularly with a Bailey-Lovie acuity chart presented on a calibrated monitor at 4 meters distance. Habitual stereo acuity was measured as retinal disparities ranging from 15 to 480 seconds of arc with the TNO Stereotest (Laméris Ootech, WC Ede, Netherlands) at 40 cm distance. Seca 217 stable stadiometer for mobile height measurement (Seca Deutschland, Hamburg, Germany) was used to measure body height without shoes, to the nearest 0.1 cm. The exact same unit was used throughout the study. Colour vision was tested in all participants with the Ishihara (24 plates edition, 1964; Kanehara Trading INC, Tokyo, Japan) and the Hardy-Rand-Rittler (4^{th} edition 2002, Richmond Products, Albuquerque, NM) pseudo-isochromatic plates under 781 (±67) lux ("True Daylight Illuminator with

Easel", Richmond Products, Albuquerque, NM) according to guidelines. All participants in the control group and a subgroup of the normal trichromats, who were included in paper III, performed the Rayleigh match in the dominant eye with an HMC (Heidelberg Multi-Color) Oculus Anomaloscope MR (Typ 47700, Oculus Optikgeräte GmbH, Germany), as described elsewhere (Pedersen et al., 2018).

All participants were given a modified version of the questionnaire used in the Sydney Myopia study (Ojaimi et al., 2005), translated into Norwegian [see L. A. Hagen, Gilson, and Baraas (2020) for questionnaire]. The questionnaire asked for socio-demographic data such as country of birth, type of current housing, and access to near electronic devices. The participants were also asked to estimate daily time spent on various indoor and outdoor activities in the weekday and weekend, as well as to estimate the ratio of indoor to outdoor time in the school holidays. Compared with the original Sydney Myopia questionnaire, some of the questions were modified for Norwegian conditions, e.g. winter outdoor activities such as skiing or skating were included. Questions related to diet, smoking, and sun exposure/protection were excluded. For a reliable comparison with other studies using the Sydney Myopia questionnaire, the same four categorical response options were given for the estimate of time spent on activities; "Not at all", "Less than 1 hour", "1–2 hours", or "3 hours or more", and the approach for the calculation of mean activity hours per day was the same as used in the studies of comparison (Dirani et al., 2009). The questionnaire was given in the winter (February and March), so self-reported time spent on indoor and outdoor activities was estimated for the wintertime. All participants answered the questionnaire, but only the subgroup that completed all questions about indoor and outdoor activities was included in the analyses of time spent indoors and outdoors.

3.3 Cycloplegic autorefraction and ocular biometry

The use of cycloplegia is essential in studies of refractive errors and ocular dimensions to ensure data with minimal effect of accommodation (F. Huang et al., 2017; I. G. Morgan

et al., 2015; Neri et al., 2015; Sun et al., 2018; Zhu et al., 2016). In this study, topical cyclopentolate hydrochloride 1% (Minims single dose; Bausch & Lomb UK Ltd, England) was administered 15–20 minutes prior to the measurements. One drop was given if the iris was blue to green, whereas two drops were given if the iris was green to brown. If the pupil was not fully dilated after 15–20 minutes, another drop of cyclopentolate was administered. An optometrist confirmed the pupil to be fully dilated before the measurements were taken.

Cycloplegic autorefraction was measured with a Huvitz HRK-8000A Auto-REF Keratometer (Huvitz Co. Ltd., Gyeonggi-do, Korea), and the exact same instrument was used throughout the study. The Huvitz HRK-8000A uses a Hartmann-Shack wavefront sensor to estimate the refractive error from a wavefront reflected from the retina; a technique reported to provide valid and reliable results (Park et al., 2015). The sphere and the cylinder were measured to the nearest 0.01D at a vertex distance of 13.5 mm, and the mean of five automatically performed measurements was used for the analyses. Calibration checks were performed daily according to guidelines before the measurements. One optometrist performed all the autorefractor measurements in 2015 and 2016, whereas two additional optometrists performed the measurements in 2018.

Two IOLMaster instruments (Carl Zeiss Meditec AG, Jena, Germany) were used for cycloplegic ocular biometry; the IOLMaster 500 in 2015, and the IOLMaster 700 in 2016 and 2018. It would have been ideal to use the exact same instrument throughout the study, but the IOLMaster 700 was preferred for measurements from 2016 because this instrument, as opposed to the IOLMaster 500, could provide measurements of crystalline lens thickness and central corneal thickness. Furthermore, the repeatability and reproducibility of IOLMaster 700 were reported to be good, and the agreement with the IOLMaster 500 was reported to be high (Akman, Asena, & Gungor, 2016; Srivannaboon, Chirapapaisan, Chonpimai, & Loket, 2015). The exact same IOLMaster 700 instrument was used for all measurements performed in the follow-up study – both at baseline in 2016 and at follow-up in 2018. Calibration checks of the instruments were performed daily according to guidelines before the data were collected.

The IOLMaster 500 is a non-contact, high-resolution biometry device reported to perform valid measurements with high repeatability (Santodomingo-Rubido, Mallen, Gilmartin, & Wolffsohn, 2002; Sheng, Bottjer, & Bullimore, 2004). The instrument uses partial coherence interferometry to measure ocular axial length to the nearest 0.01 mm. At least five reliable measurements were taken of which the mean was used in the analyses; reliability was defined as signal-to-noise ratios > 2.0. The anterior corneal curvatures in two principal meridians were estimated to the nearest 0.01 mm by automatic keratometry. For this, the IOLMaster 500 uses image analysis of a 2.3 mm diameter hexagonal array of 6 light points reflected from the surface of the tear film (Santodomingo-Rubido et al., 2002). The keratometer measurement consisted of five individual measurements and was repeated at least three times, of which the mean was used in the analyses. One optometrist performed all the IOLMaster 500 measurements.

The IOLMaster 700 uses swept-source optical coherence tomography (OCT) to obtain measurements of anterior chamber depth, crystalline lens thickness, and ocular axial length, all parameters to the nearest 0.01 mm, in addition to central corneal thickness to the nearest 1 μm. An OCT image visualized the anatomical details along a longitudinal section of the entire eye, as illustrated in Figure 3, and a scan of the central 1.0 mm zone of the retina was used to control the fixation. The anterior corneal curvatures in two principal meridians were measured to the nearest 0.01 mm by 18 reference points of light reflected from the tear film, the light points were distributed in three hexagonal patterns with diameters of approximately 1.5, 2.4, and 3.2 mm (Hoffer, Hoffmann, & Savini, 2016; Omoto et al., 2019). One optometrist performed all the IOLMaster 700-measurements in 2016, whereas additional two optometrists performed the measurements in 2018. All parameters used in this study are reported to have high inter-operator reproducibility with intraclass correlation coefficients estimated to be from 0.99 to 1.00 (Srivannaboon et al., 2015). High agreement between IOLMaster 700 and IOLMaster 500 data, as well as with Lenstar LS900 data, was recently confirmed in a new study (Bullimore, Slade, Yoo, & Otani, 2019). In that study, Bullimore et al. (2019) reported repeatability and reproducibility for the IOLMaster 700 to be ±0.014 mm and ±0.023 mm for ocular axial length, ±0.02 mm and ±0.02 mm for anterior chamber depth,

±0.02 mm and ±0.05 mm for crystalline lens thickness, and ±0.26 D and ±0.27 D for corneal power, respectively.

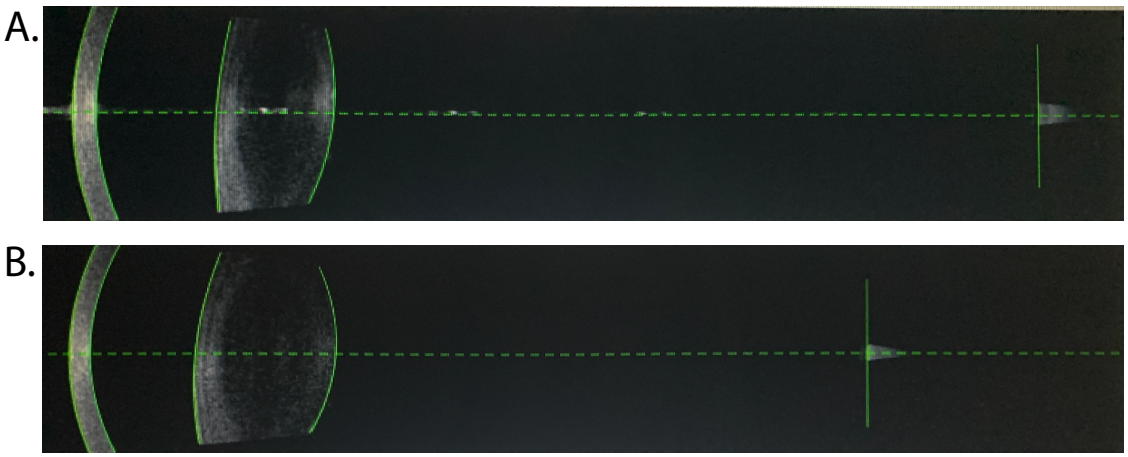

Figure 3. Images from the IOLMaster 700

B-scan images of two eyes. (A) 26.38 mm long eye and 3.09 mm thick crystalline lens. (B) 20.94 mm long eye and 3.67 mm thick crystalline lens.

3.4 Follow-up study with estimates of crystalline lens power

In the follow-up study, baseline measurements were made in March 2016 and repeated in January/February 2018. The follow-up measurements started with a face-to-face interview to update the self-reported information on medication, ocular and general health. Habitual logMAR visual acuity, stereo acuity, body height, cycloplegic autorefraction, and cycloplegic ocular biometry were measured with the exact same instruments and following the same procedures as at baseline. Vitamin D_3 levels were measured at follow-up only. The dried blood spot technique was used to collect blood samples for the measurement of serum vitamin D_3 concentration, and the samples were sent to Vitas AS (Oslo, Norway) for analysis.

Crystalline lens power at baseline and follow-up were determined by individual three-surface biconic (toric) eye models based on the Gullstrand-Emsley model (Emsley, 1979),

see Figure 4. Optic Studio v.14.2 (Zemax LLC, Kirkland, WA, USA) was used to set up a ray tracing model over a 1-mm pupil diameter. Individual eye models were constructed from the measured cycloplegic spherocylindrical refractive error at a 13.5 mm vertex distance, and from the anterior corneal radius of curvatures and axes, anterior chamber depth, crystalline lens thickness, and vitreous chamber depth taken from the cycloplegic ocular biometry data. The biconic cornea was set up with the flattest and steepest anterior curvatures along the corresponding axis. A Zemax merit function was used to optimize the front and back surface crystalline lens curvatures to give a 1-mm diameter wavefront the best focus at the retina, while forcing the ratio of crystalline lens surface powers to the total equivalent power to be the same as in the Gullstrand-Emsley model. These ratios were set to 38.0% and 63.3% for the front and back surface power, respectively, and the crystalline lens power was calculated from the estimated crystalline lens curvatures. The Zemax files used are available online (Lene A. Hagen, Gilson, Akram, & Baraas, 2019).

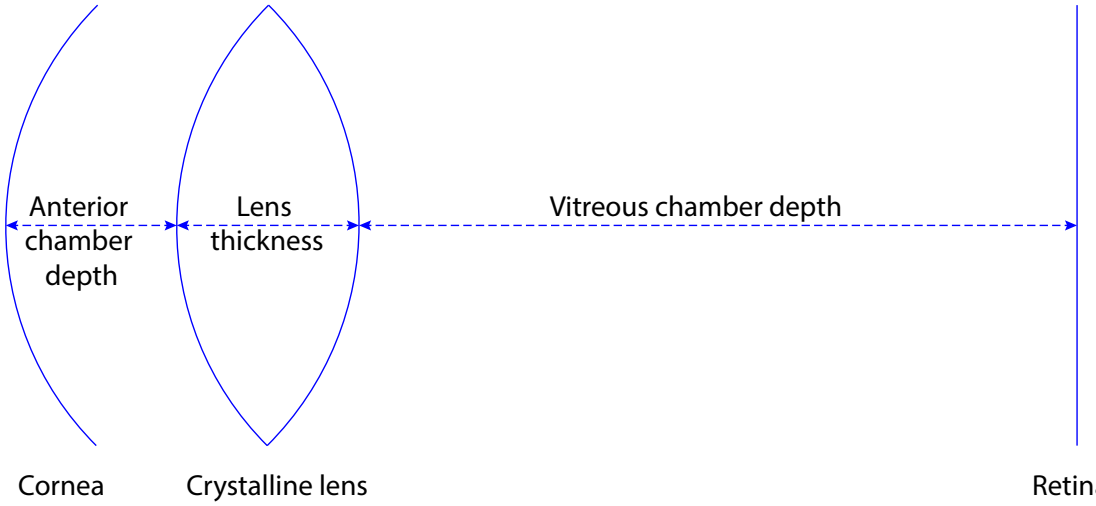

Figure 4. The three-surface biconic eye model

The individual eye models were based on the Gullstrand-Emsley eye model as illustrated here, with the anterior corneal surface to the left, followed by the crystalline lens front and back surface. The refractive indices were set to 1.416 for the crystalline lens and to 1.333 for the anterior and vitreous chambers, as per the Gullstrand-Emsley model (Emsley, 1979).

3.5 L and M cone opsin genetics

Saliva samples were obtained from all participants by Oragene 500 DNA collection kit (DNA Genotek Inc., Ottawa, ON, Canada) and sent to the Neitz Lab at the University of Washington, Seattle, for analyses of cone opsin genetics. DNA was extracted, and single-nucleotide polymorphisms (SNP) genotyping was performed using the MassArray system (Agena Bioscience, Inc., San Diego, CA), as described by Davidoff, Neitz, and Neitz (2016). Five different SNPs were used in the MassArray assay to estimate the percentage of genes in the first position of the cone opsin gene array, the proportion of L cone opsin genes, as well as the spectral sensitivity difference between the L and M cone opsins encoded by the genes (Davidoff et al., 2016). The number of L and M cone opsin genes was estimated as the inverse of the percentage of genes in the first position in the array (Davidoff et al., 2016). The five SNPs used in the MassArray assay were three at codons 116, 180 and 230 that control spectral tuning differences within the L and M cone opsin genes, one at codon 309 that distinguishes the L and M cone opsins, and one in the promoter region that differs between the first and the downstream genes in the array. The MassArray assay was also used to check for known mutations in the L, M and S cone opsin genes. Furthermore, DNA was amplified by polymerase chain reaction, and exons 2, 3, and 4 of the L and M cone opsin genes were sequenced by a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), as described previously (Dees, Gilson, Neitz, & Baraas, 2015). The nucleotide sequences (haplotypes) were determined and designated by the combination of amino acids at the polymorphic positions 65, 111, 116 encoded by exon 2; positions 153, 171, 174, 178, 180 encoded by exon 3; and positions 230, 233, 236 encoded by exon 4. L and M cone peak sensitivities were determined by the amino acids specified at the spectral tuning sites (Asenjo et al., 1994; Davidoff et al., 2016; J. Neitz & Neitz, 2011).

The cone opsin genetics were, in paper III, used to confirm colour vision status of the participants and to determine female normal trichromats who were heterozygous or homozygous for their L and M exon 3 haplotype(s). Genetically determined L and M cone peak sensitivities were used in the estimates of L:M cone ratios (see details in 3.6). All

male and female normal trichromats had a single L cone opsin gene at the first position of the opsin gene array (both arrays in females) followed by one or more M cone opsin genes, except from two females with the arrays “LMM+LML” and “LMMM+LMML”. In these females, the position of the extra L cone opsin gene was not clear from the genetics data, making them possible deutan carriers since the real position of the extra L cone opsin gene could potentially be second in the array. While all female normal trichromats reported to have no known colour vision deficiencies in their family, the sample of normal trichromats in paper III may still include a few unidentified carriers of red-green colour vision deficiency.

3.6 Estimates of L:M cone ratios

Individual L:M cone ratios were estimated from spectral sensitivity data measured with full-field ERG flicker photometry. This is a procedure that is reported to be efficient and reliable when corrections are made for crystalline lens density and individual variation in the L cone opsin spectral sensitivity (Bieber et al., 1998; Carroll et al., 2000; Carroll et al., 2002; Hofer et al., 2005). The ERG measurements were in most cases performed as part of the first data collection, conducted after the test eye was dilated with 1–2 drops of cyclopentolate hydrochloride 1% (Minims single dose; Bausch & Lomb UK Ltd, England) to ensure a fully illuminated retina. The other eye was covered with an eye patch. In the control group of red-green colour vision deficient males, in the protan carrier, and in another few cases, 1–2 drops of tropicamide 0.5% (Minims single dose; Bausch & Lomb UK Ltd, England) were used for dilation, since accommodation control was not needed for the ERG measurement. A fully dilated pupil was confirmed before the measurements started. A corneal Dawson, Trick, and Litzkow (DTL) (Dawson, Trick, & Litzkow, 1979) fibre electrode (DTL ERG Thread, Unimed Electrode Supplies, Surrey, England) was used as the active electrode, and two skin contact electrodes were used as the reference and ground. The reference and ground electrodes were applied just below and above the eye, respectively, after the skin was cleaned by alcohol. To ensure good connection and transmittance of electrical signals throughout the measurements, conductive paste

(Ten20, Weaver and Company, Aurora, CO, USA) was used under the skin electrodes and a sheet of adhesive film was used on top. All electrodes were connected to an amplifier (DP-301 Differential Amplifier, Warner Instrument Corp., Hamden, CT, USA), and adjustment of the electrodes were made if the bio signal was noisy.

The ERG system was a modified version of that used in the study by Carroll et al. (2000). Four different LEDs (3 Watt) created the full-field ERG flicker stimulus that was presented in Maxwellian view by a Meade 30 mm telescope lens (Series 5000 82° Ultra Wide Angle, Meade Instruments Corp., Irvine, CA, USA). The reference light (519 nm) and each of three test lights (465, 634, and 655 nm) were superimposed to illuminate the retina at a temporal frequency of 31.25 Hz; each light was modulated with a 25% duty cycle, and the reference and the test light were presented in antiphase. A total of 115 cycles were presented in one run, and the ERG signal was processed and averaged over the last 100 cycles, as described by Jacobs, Neitz, and Krogh (1996). The wavelength emission profiles of the LEDs were measured with a spectrophotometer (SpectraScan PR650, Photo Research, NY, USA).

Figure 5 shows an image of a person aligned in front of the ERG system. The participants were asked to fixate at a cross in the centre of the test field and to not blink while the ERG stimulus was presented (~3.6 seconds per presentation), to ensure that the retina was fully illuminated by the ERG flicker stimulus during the presentation. The alignment of the ERG system was controlled before and regularly during the period of measurements. The intensity of the test light was adjusted until the ERG signal amplitude from the test and reference light matched. This procedure was independently repeated three times for each of the three different test lights, and the mean values were used as the final spectral sensitivity values. All ERG measurements were performed in a test room with an ambient illumination between 150 and 300 lux. One operator (author of the thesis) performed all the measurements.

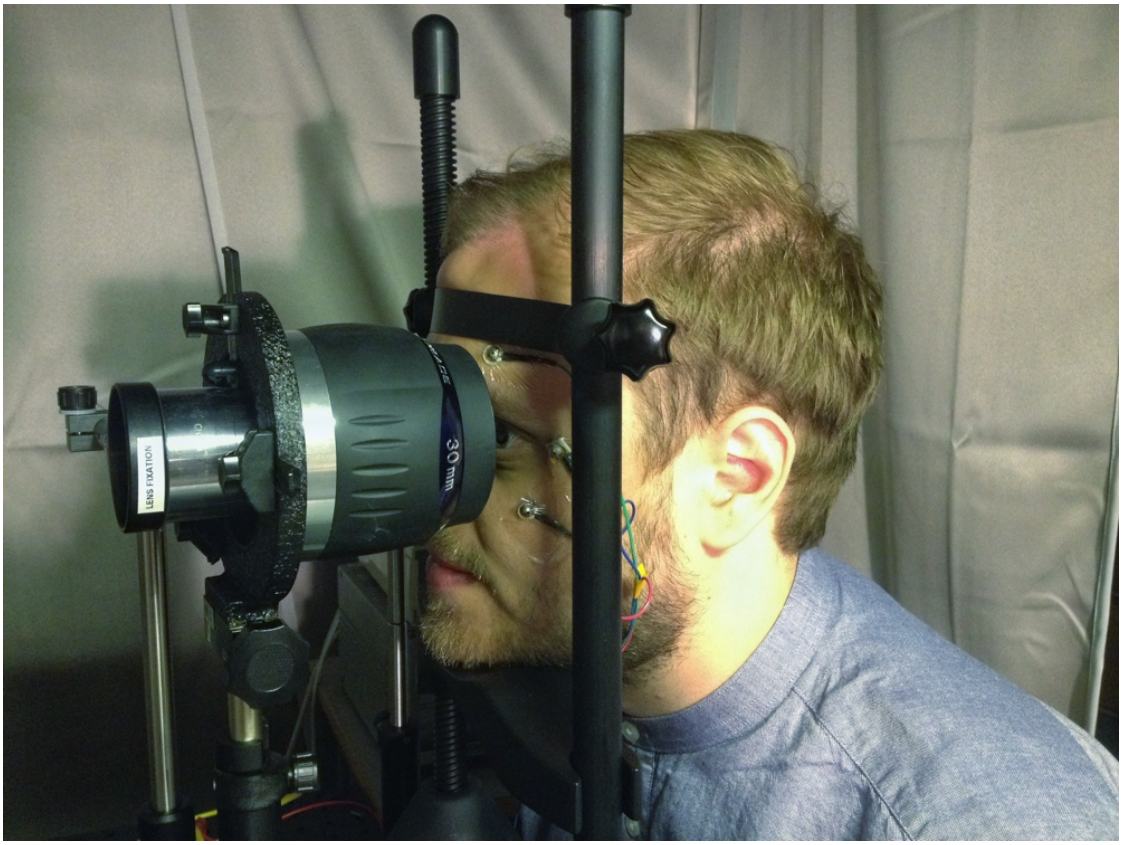

Figure 5. Full-field ERG flicker photometry

Measurement of spectral sensitivity data by full-field ERG flicker photometry. The photo is used with permission granted from the person depicted, who did not participate in this study.

L and M cone spectral sensitivity functions were determined for each individual from the cone opsin genetics, with the photopigment optical density set to 0.35 and 0.22 for the L and M cone opsin, respectively (Carroll et al., 2000; Carroll et al., 2002). The ERG-derived spectral sensitivity data were corrected for optical density of the crystalline lens by an age-dependent lens correction (Pokorny, Smith, & Lutze, 1987), before estimates of the cone contribution ratio were made by obtaining the best fit of a weighted sum of the L and M cone spectral sensitivity functions to the ERG-derived spectral sensitivity data. Figure 6 illustrates the ERG-derived spectral sensitivity data together with the L and M cone spectral sensitivity functions in two individuals. Estimates of the percentage of

L cones (%L) were computed from the L and M weights [100 × L / (L + M)], and the cone ratios were finally adjusted with a factor of 1.5, as previously suggested from the comparison of cone ratio estimates from ERG and AO (Hofer et al., 2005).

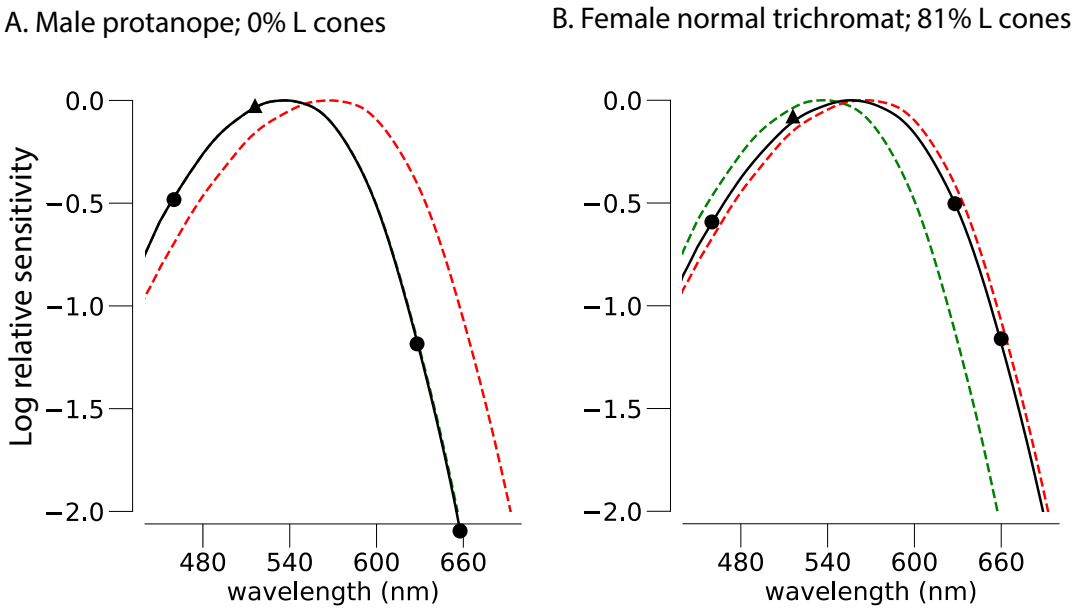

Figure 6. Illustration of ERG-derived spectral sensitivity data

ERG-derived spectral sensitivity data for (A) a male protanope estimated to have 0% L cones and (B) a female normal trichromat estimated to have 81% L cones. The three black circles represent ERG data for the three test lights (465 nm, 634 nm, and 655 nm) and the black triangle represents the reference light (519 nm). The black line represents the best fitted spectral sensitivity function for the ERG data, and the red and the green dashed lines represent the individual's L and M cone spectral sensitivity functions, respectively, determined from genetics.

Since individual variation in L and M cone peak sensitivities is shown to have impact on the estimates of L:M cone ratios (Bieber et al., 1998; Carroll et al., 2000), the estimates were based on genetically determined L and M cone peak sensitivities. In female normal

trichromats who had L cone opsin genes encoding spectrally *distinct* L cone peak sensitivities on the two X-chromosomes, L:M cone ratios were given as a potential range from the estimate based on the lowest L cone peak sensitivity to the estimate based on the highest L cone peak sensitivity; the real L:M cone ratio is determined by the degree of X-chromosome inactivation in each cell in the females (Jorgensen et al., 1992; Lyon, 1961, 1972; Sharp, Robinson, & Jacobs, 2000). In the analyses of an association with refractive error, the L:M cone ratio estimates based on the mean L cone peak sensitivity were used for this group. Estimated L:M cone ratios are less influenced by variation in M than L cone peak sensitivity (Bieber et al., 1998; Carroll et al., 2000), and of this reason, mean M cone peak sensitivity was used to estimate the L:M cone ratio if the individual had two M cone opsin genes encoding spectrally *distinct* M cone peak sensitivities.

Several approaches were implemented to validate the L:M cone ratio. The L:M cone ratio estimates were validated in a control group of five red-green colour vision deficient males – one protanope, two deuteranopes, one protanomalous, and one deuteranomalous – who all had their colour vision status confirmed by cone opsin genetics, as well as by Rayleigh anomaloscopy. One genetically confirmed protan carrier, expected to have a low L:M cone ratio, was included as a female control. Rayleigh anomaloscopy was performed in 34 of the normal trichromats to confirm that Rayleigh match midpoint correlated with the variation in L cone peak sensitivity but not with the estimated %L cones; this would indicate that variation in L cone peak sensitivity was accounted for in the L:M cone ratio estimates. Finally, repeatability measurements of the L:M cone ratio estimates were performed in a group of normal trichromats. See paper III for details.

3.7 Statistical analysis

Data were analysed with the statistical computing software R, version 3.4.0 (R Core Team, 2016), and the significance level was set to 5%. Several statistical analysis methods were applied. The Clopper-Pearson interval method was used for calculation of 95% binomial confidence intervals, and the method of Sison and Glaz was used for calculation

of 95% confidence intervals for multinomial proportion. Pearson's Chi-squared test and Fisher's Exact test were used to assess the association between two categorical variables. The normality of the variables was assessed by QQ-plots, histograms, and the Shapiro-Wilk test, and correlations were assessed using Pearson coefficients or Spearman rho. One-way analysis of variance in addition to Student's or Welch's two independent sample *t*-tests for equal or unequal variances, respectively, were used to examine between-group differences. Mean differences between baseline and follow-up data were examined by paired *t*-test, and Wilcoxon rank-sum test was used for non-normal data. Kruskal-Wallis and Bonferroni corrected pairwise comparison by Wilcoxon were used to assess differences in SER and ocular dimensions between groups. Multiple linear regression analyses and multivariate logistic regression analyses were performed, and likelihood ratio tests were used to compare models.

3.8 Ethical considerations

The study was approved by the Regional Committee for Medical and Health Research Ethics in Southeast Norway [Ref: 2014/1778; see Regional Committees for Medical and Health Research Ethics (2014)] and was carried out in compliance with the principles embodied in the Declaration of Helsinki. All participants were 16 years or older, which means that they were considered fully competent to consent to participate in research according to the Norwegian Health Research Act. Prior to the data collection, written and verbal information about the study were given, and full written consent was obtained. The information included that the participation was voluntary, they were allowed to withdraw the consent at any time, and they were allowed to consent to the whole or parts of the study. Efforts were made to provide clear and easy-to-understand information on storage, export, and use of the biological samples; blood for vitamin D_3 analyses and saliva for cone opsin genetics. The aim was to implement a person-centred approach throughout the study by taking into account the uniqueness of the participants, their individual needs and preferences (Baraas et al., 2017; Epstein, Fiscella, Lesser, & Stange, 2010; Mead & Bower, 2000; S. Morgan & Yoder, 2012). Contact information for

the principal investigator (Rigmor C. Baraas), as well as for the administrative organizer (the author of this thesis), was easily available in case of any questions. All participants were sent additional information in personal text messages to their cell phones, also with the opportunity to ask questions. Text messages were chosen as the form of communication on the advice of a group of adolescents.

Detailed instructions were given before each measurement in the data collection. The use of muscarinic antagonists (cyclopentolate hydrochloride 1% or tropicamide 0.5% eye drops), corneal electrodes for the ERG measurements, and the blood samples for vitamin D_3 measurements could cause some discomfort, and special care was taken in these situations. In case of unexpected reactions to the muscarinic antagonists, such as allergy or acute narrow-angle glaucoma, an EpiPen adrenaline 0.3 mg auto-injector (Meda Pharma GmbH & Co KG, Germany) and pilocarpine nitrate 2% (Minims single dose; Bausch & Lomb UK Ltd, England) were readily available. Individual results were given to each participant after the measurements; provided at a suitable location to ensure confidentiality. If deemed necessary from the results, the participants were referred to an eye or a general health examination. No information was given to the participants from the genetics or vitamin D_3 analyses, as informed to the participants before the consent was given.

All data were stored with personal 4-digit IDs (identification numbers) in a password-protected digital database without personal information such as name, address, e-mail address, or phone number. A paper-based code list, that linked each ID to the participant's names, was stored separately. The blood and saliva samples were stored in a biobank, approved by the Regional Committee for Medical and Health Research Ethics in Southeast Norway [Ref: 2014/1778; see Regional Committees for Medical and Health Research Ethics (2014)], and sent for analyses with no data other than the ID. All biological samples were destroyed after the analyses.

The results of this study were published in peer-reviewed international journals and presented at international conferences. In line with a person-centred philosophy (Barry & Edgman-Levitan, 2012; Mead & Bower, 2000; S. Morgan & Yoder, 2012), results were

also presented to eye-care providers in Norway, teachers and students at upper-secondary school, as well as communicated on webpages and in journals available to the general public.

4 Main results

This section provides a short summary of the main results of the three papers included in this thesis. Further details are to be found in the enclosed papers.

4.1 Paper I

Hagen, L. A., Gjelle, J. V. B., Arnegard, S., Pedersen, H. R., Gilson, S. J., & Baraas, R. C. (2018). Prevalence and Possible Factors of Myopia in Norwegian Adolescents. Scientific Reports, 8(1), 13479.

This paper presents the first study on refractive errors and ocular dimensions in 16–19 years old adolescents living in Southeast Norway. The aim was to estimate the prevalence of refractive errors and to assess whether there was an association between myopia and self-reported time spent on activities outdoors and indoors. The results summarised here are limited to the participants who reported to have grown up in Norway and were of Northern European Caucasian ethnicity (n = 393, 41.2% males).

The prevalence of myopia (SER ≤ -0.50D) and hyperopia (SER ≥ +0.50D) were 12.7% and 56.7%, respectively. High myopia (SER ≤ −6.00D) was found in 0.5%. Females had higher myopia prevalence than males (15.6% vs. 8.6%, p = 0.046) and on average shorter ocular axial length (mean ± SD: 23.28 ± 0.83 vs. 23.66 ± 0.86 mm, p < 0.001) and steeper corneal curvatures (7.78 ± 0.25 vs. 7.87 ± 0.30 mm, p = 0.003). The frequency of refractive astigmatism (≥ 1.00DC) and anisometropia (≥ 1.00D) were 8.9% and 3.6%, respectively.

Time spent outdoors and indoors were estimated from the questionnaires (n = 269). More myopes than non-myopes (14% vs. 4%, p = 0.007) reported to spend most of their time indoors during the summer holidays, but no difference was found for the other holidays. Total self-reported time spent outdoors was not associated with myopia, even though myopes reported to spend less time doing outdoor sport than non-myopes

(0.9 ± 0.8 vs. 1.3 ± 1.0 hours per day, $p = 0.03$). There were no associations between myopia and self-reported time spent on near work.

4.2 Paper II

Hagen, L. A., Gilson, S. J., Akram, M. N., & Baraas, R. C. (2019). Emmetropia Is Maintained Despite Continued Eye Growth From 16 to 18 Years of Age. Investigative Ophthalmology and Visual Science, 60(13), 4178-4186.

The aim of this paper was to examine whether emmetropia and low hyperopia were maintained from 16 to 18 years of age, and if so, whether it was associated with continued coordinated ocular growth. Cycloplegic autorefraction and ocular dimensions were measured in 93 Norwegian adolescents (mean ± *SD* age: 16.7 ± 0.3 years, 36.6% males) and repeated after two years.

The prevalence of emmetropia and low hyperopia (-0.50D < SER < +2.00D) were found to be relatively stable, present in 91.4% at baseline and 89.2% at follow-up. Emmetropes and low hyperopes who maintained their refractive error, experienced continued ocular axial growth (+0.059 ± 0.070 mm) as well as a decrease in crystalline lens power (−0.064 ± 0.291D) and increased anterior chamber depth (+0.028 ± 0.040 mm); 24% experienced a thinning of the crystalline lens. The crystalline lens thickened more than 0.02 mm in 45% of the persistent emmetropes/low-hyperopes and 86% of the myopes.

Those with a more negative refractive error at baseline experienced a larger negative change in refractive error over the 2-year study period ($R^2 = 0.178$, $p < 0.001$), and the negative change was associated with excessive elongation of vitreous chamber depth and increase in crystalline lens power ($R^2 = 0.752$, $p < 0.001$); both statistical models were adjusted for sex. Annual incidence of myopia (SER ≤ -0.50D) was 1.2%, and annual decline of hyperopia (SER ≥ +0.50D) was 4.7%. There was no difference in vitamin D_3 level between those who experienced negative versus positive changes in refractive error.

4.3 Paper III

Hagen, L. A., Arnegard, S., Kuchenbecker, J. A., Gilson, S. J., Neitz, M., Neitz, J., & Baraas, R. C. (2019). The association between L:M cone ratio, cone opsin genes and myopia susceptibility. Vision Research, 162, 20-28.

This paper presents for the first time L:M cone ratios, L and M cone opsin genes, and their associations with myopia in 16–19 years old Norwegian Caucasian normal trichromats (*n* = 136, mean ± *SD* age: 16.9 ± 1.0 years, 44.1% males). The aim was to assess whether myopia was associated with estimated L:M cone ratios and heterozygosity/homozygosity of common L or M cone opsin exon 3 haplotypes. In the analyses, the estimates of %L cones in females with *distinct* L cone peak sensitivities (*n* = 43) were based on the mean L cone peak sensitivity, under the assumption that each X-chromosome was silenced in half of the cells by X-chromosome inactivation (Jorgensen et al., 1992; Lyon, 1961, 1972; Sharp et al., 2000).

The frequency of myopia (SER ≤ -0.50D) was 8.3% in the males and 19.7% in the females. Myopia was more frequent in females who were heterozygous for their specific L cone opsin exon 3 haplotype(s) (*n* = 54; 24.1% myopia) than in females who were homozygous (*n* = 22; 9.1% myopia) and in males (*n* = 60; 8.3% myopia).

Estimated %L cones (mean ± *SD*) in males and females were 79.8 ± 11.8% and 83.9 ± 9.6%, respectively, which is higher than previously reported in males in other populations (Carroll et al., 2002; Hofer et al., 2005; Kuchenbecker et al., 2014; McMahon et al., 2008; Yamauchi et al., 2013). Females with low %L cones were on average more myopic than females with high %L cones (mean ± *SD* SER: -0.03 ± 1.2D vs. +0.58 ± 0.8D, *p* = 0.01), but no direct associations were found between estimated %L cones and myopia in the males. The frequency of alanine at L cone opsin position 180 in the male normal trichromats (55%) was higher than reported in East Asian males (~20%) (Deeb, Alvarez, Malkki, & Motulsky, 1995; S. Hayashi, Ueyama, Tanabe, Yamade, & Kani, 2001).

5 Discussion

This thesis has investigated refractive errors, ocular dimensions, and whether myopia was associated with L:M cone ratios and heterozygosity/homozygosity of common cone opsin exon 3 haplotypes in adolescents in Southeast Norway. The results showed a low myopia prevalence in Norwegian adolescents, despite high educational pressure and few daylight hours available in the autumn-winter period each year. Hyperopia was the most common type of refractive error, and persistent emmetropes/low-hyperopes were found to still exhibit coordinated ocular growth at 18 years of age. This indicated a well-adapted emmetropisation mechanism and suggested that a low genetic predisposition protected this population from myopia. Differences in the L:M cone ratios on the retina were associated with myopia, and the myopia frequency was higher in females who were heterozygous for their L cone opsin exon 3 haplotypes than males and homozygous females. These findings offer new insight into the reported sex difference in myopia onset (Rudnicka et al., 2016). The results emphasise that individual differences in the cone opsins may be of importance for personalised myopia prevention and management strategies; a requirement for person-centred eye-care in the future.

5.1 Environmental risk factors of myopia

Increased exposure to environmental risk factors of myopia – such as less time spent outdoors and more intensive education – is suggested to explain the rapid increase in myopia prevalence in the East Asian countries the last few decades (I. G. Morgan et al., 2018; I. G. Morgan & Rose, 2019; Rose, French, & Morgan, 2016; Rose, Morgan, Smith, et al., 2008). It is not clear why spending time outdoors prevents myopia, but high levels of daylight exposure are assumed to be of great importance since bright light is shown to prevent form-deprivation myopia in animals (Ashby, 2016; Karouta & Ashby, 2014; Norton, 2016; Norton & Siegwart, 2013) and to slow ocular growth in humans (Hua et al., 2015; Read et al., 2015; P. C. Wu et al., 2018).

The data in this thesis showed a low myopia prevalence in adolescents in Norway (~13%; paper I). This was an unexpected result because Norwegian adolescents have few daylight hours available in the long autumn and winter period (see Figure 1 in paper I), extensive use of near electronic devices (OECD, 2015), and a high-performing education system (OECD, 2016). A comparison with data on 12–14 years old Norwegian adolescents, published in 1971 (Larsen, 1971), implied that the myopia prevalence in Norway may have been stable for the last 40 years. This is in contrast to the reports of increased myopia prevalence worldwide (Hashemi et al., 2018; Holden et al., 2016).

Increased time spent outdoors is reported to prevent myopia onset and to possibly slow myopia progression (Xiong, Sankaridurg, et al., 2017). In the Norwegian adolescents, more myopes than non-myopes reported to spend most of the time indoors in the summer holiday, and the myopes reported to spend less time on outdoor sport than the non-myopes. There were, however, no differences between myopes and non-myopes in the self-reported time spent outdoors in the other holidays and no differences in total self-reported time spent outdoors in the wintertime (paper I). Moreover, Norwegian adolescents reported to spend a similar amount of time outdoors as Singaporean adolescents who had a much higher prevalence of myopia (~70% myopia in 11–20-year-olds) (Dirani et al., 2009). This raises the question of whether time spent outdoors *per se* can explain the low myopia prevalence seen in Norwegian adolescents. A greater release of dopamine and higher levels of vitamin D_3 are hypothesised to play a role in myopia protection related to outdoor time (C. W. Pan et al., 2017). In the Norwegian adolescents, no associations were found between changes in the refractive error and the level of vitamin D_3 (paper II), as also confirmed in other recent studies (Cuellar-Partida et al., 2017; Tang et al., 2019). Longitudinal studies have reported the protective effect of outdoor time on myopia onset to be more efficient around 6 years of age compared with 11–12 years of age (Xiong, Sankaridurg, et al., 2017), implying that a younger eye is more sensitive to environmental influences on ocular growth, as also suggested from animal studies (Chakraborty et al., 2020). Because spending time outdoors every day is a common practice in Norway both in kindergarten and at primary school (up to 12 years

of age), it is possible that time spent outdoors in early childhood may have delayed the myopia onset in Norwegian adolescents.

No associations were found between myopia and the self-reported time spent on near work in the Norwegian adolescents (paper I). Furthermore, despite being in a high-performing education system with high levels of indoor activity and near work, the longitudinal data showed a stable refractive error in emmetropes and low hyperopes from 16 to 18 years of age (paper II). The findings suggest a well-adapted emmetropisation mechanism in this population. Emmetropisation does ideally lead to emmetropia, or perhaps low hyperopia (I. G. Morgan et al., 2010), that is maintained throughout childhood and adolescence by coordinated ocular growth (Mutti et al., 2018). The low myopia prevalence in the Norwegian adolescents and the leptokurtic distribution of SER around a low hyperopic mean SER (mean SER: +0.55D; see Figure 2 in paper I) were consistent with this (I. G. Morgan et al., 2010). The longitudinal data showed that persistent emmetropes/low-hyperopes exhibited coordinated ocular growth up to at least 18 years of age (paper II), although at a slower rate than in younger children (Zadnik et al., 2004).

If high levels of daylight exposure are crucial to protect against myopia, the results raise questions whether there could be other environmental or biological factors, perhaps a low genetic predisposition, that protected the Norwegian adolescents from myopia. Identifying these factors may be of importance to further understand the mechanism of myopia. As a first step, the results in this thesis showed that individual differences in the L and M cone opsins may play a role in myopia susceptibility (see 5.2 and paper III).

5.2 Biological factors of myopia

The data in this thesis showed that individual differences in the L:M cone ratio and in the L cone opsin genes were associated with susceptibility to common myopia (paper III). That susceptibility to common myopia may be modulated by the individual L:M cone ratio has been proposed earlier (J. Neitz & Neitz, 2015; N. Zhou et al., 2015), has been reported

in animal experiments (Gisbert & Schaeffel, 2018), and is supported by the reports of low myopia prevalence in red-green colour vision deficient individuals (Ostadimoghaddam et al., 2014; Y. S. Qian et al., 2009). The visual signals that guide ocular growth in the process of emmetropisation, as well as to maintain emmetropia (Chakraborty et al., 2020; Wallman & Winawer, 2004), are initiated by light absorption in the cone photoreceptors on the retina. Erroneous contrast signals may stimulate increased ocular growth, as implied in myopia associated with rare L/M interchange exon 3 haplotypes (Greenwald et al., 2017; Patterson et al., 2018). Patches of the same cone type on the retina are suggested to be beneficial for achromatic high spatial frequency vision (Hofer et al., 2005; Roorda et al., 2001), and as illustrated in figure 7, the size of these patches will likely increase in retinas with higher L:M cone ratios, possibly contributing to protection from common myopia. In accordance with this hypothesis, the data in this thesis showed a less myopic refractive error in females with high compared to low L:M cone ratios (paper III).

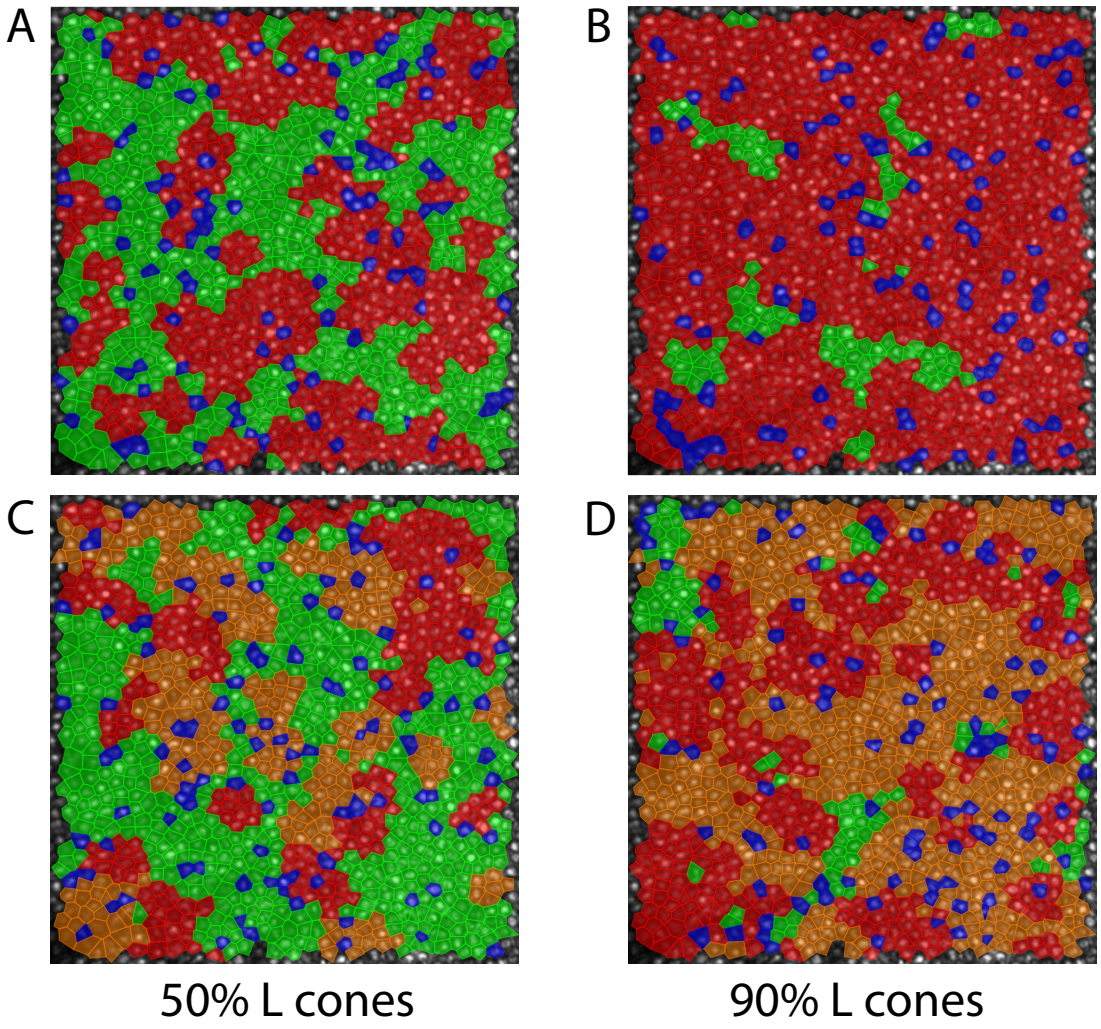

Figure 7. Illustration of hypothetical cone photoreceptor mosaics

The top row illustrates cone mosaics in an L exon 3 homozygous female, or a male normal trichromat, with (A) 50% L cones and (B) 90% L cones. L, M and S cones are synthetically labelled red, green and blue, respectively. The bottom row illustrates cone mosaics in a female, who is heterozygous for an L exon 3 haplotype that causes a mild splicing defect, with (C) 50% L+L' cones and (D) 90% L+L' cones. L' cones are here defined as L cones with a reduced amount of L cone opsin – caused by the splicing defect – and are labelled orange.

There is a general agreement that genetic factors contribute to the development of myopia, however, there is a problem of "missing heritability". This means that there is a gap between the heritability estimates from twin studies on refractive errors (60–80%) and the variation in refractive error (<10%) explained so far by the currently identified gene variants known from the recent meta-analysis of two large GWAS studies (Tedja et al., 2018). Genes on the X-chromosome were, however, not included in these GWAS studies (see section 1.3.2). The data in this thesis, as well as in other recent studies (Mountford et al., 2019; M. Neitz & Neitz, 2019), show that polymorphisms of the L cone opsin genes, located on the X-chromosome, may be implicated in susceptibility to common myopia. Rare L/M interchange exon 3 haplotypes, with severe splicing defects that cause greatly reduced amount of photopigment in the cone photoreceptors, have previously been associated with myopia (Buena-Atienza et al., 2016; Greenwald et al., 2017; Ueyama et al., 2012). In this thesis, a higher myopia prevalence was found in L exon 3 heterozygous females compared to males and homozygous females. Exon 3 heterozygous females are twice as likely to carry a common opsin gene exon 3 haplotype with a mild splicing defect – that causes slightly reduced level of opsin expression in the cones that harbour the gene (M. Neitz & Neitz, 2018) – than males and homozygous females. This supports the hypothesis that cone opsin gene polymorphism and exon 3 haplotypes with mild splicing defects may interfere with the development of common myopia. Figure 7C illustrates a hypothetical cone mosaic in a female who is heterozygous for an L exon 3 haplotype with a mild splicing defect, and therefore harbours two sets of L cones with slightly different levels of opsin expression. This cone mosaic may degrade the achromatic high spatial frequency signal, and possibly increase the susceptibility to common myopia, compared to the cone mosaic in normal trichromatic males and exon 3 homozygous females, who only have one set of L and M cones (see Figure 7A). Furthermore, the myopia susceptibility may be modulated by the relative number of functioning versus less-than-normally functioning cones, as for syndromic myopia that is associated with rare L/M exon 3 interchange haplotypes (Greenwald et al., 2017; Patterson et al., 2018). The results underscore the need for more research into the role of cone opsin genes in the development of refractive error.

The distribution of refractive errors varies with ethnicity and geographical region, and earlier myopia onset and higher myopia prevalence are reported in East Asian compared with Caucasian populations (Rudnicka et al., 2016); see also Table 1. In accordance with the theory that high L:M cone ratios are associated with low myopia susceptibility (J. Neitz & Neitz, 2015; N. Zhou et al., 2015), mean L:M cone ratio in Norwegian Caucasian colour normal males and females was found to be considerably higher than reported for East Asian (Kuchenbecker et al., 2014; Yamauchi et al., 2013) and African colour normal males (McMahon et al., 2008), as well as slightly higher than reported for American Caucasian colour normal males (Carroll et al., 2002). In all the studies of comparison, the L:M cone ratios were estimated in-vivo, using the objective method of full-field ERG flicker photometry, adjusted for the optical density of the crystalline lens and individual differences in genetically determined L cone peak sensitivity (Bieber et al., 1998; Carroll et al., 2000). The frequency of alanine at position 180 on the L cone opsin in Norwegian Caucasian males was higher than in Japanese males (Deeb et al., 1995; S. Hayashi et al., 2001) (see details in Table 5, paper III) and may be another factor that protected the Norwegian Caucasian males against myopia. Alanine at L position 180 shifts the peak sensitivity of the L cones closer to the peak sensitivity of the M cones, as compared with serine at L position 180 (Asenjo et al., 1994; Carroll et al., 2002). This may be an advantage in low light levels because a narrower spectral separation of the L and M cone opsins is advantageous for achromatic spatial vision (Osorio, Ruderman, & Cronin, 1998) and reduces the amount of dark noise (Lewis & Zhaoping, 2006). This indicates that the high L:M cone ratio, and perhaps the high frequency of alanine in Norwegian Caucasian males, may be factors that protected the Norwegian Caucasian adolescents against myopia, even when exposed to environmental risk factors of myopia. A low number of myopes in the sample of males, presumably as a consequence of the low myopia susceptibility in Norwegian adolescents, may explain why no direct associations were found between the estimated L:M cone ratios and the refractive errors in the males in this study. Further studies would be needed to support this interpretation.

In order to provide the best person-centred eye-care, it will be helpful to predict children and adolescents at risk of myopia. A less hyperopic cycloplegic SER is reported to be the

best predictor of future myopia in children aged 6–11 years (Zadnik et al., 2015). In accordance with this, the Norwegian adolescents with the most negative SER at baseline showed the greatest negative change in SER from 16 to 18 years (paper II). Accelerated ocular growth is reported to precede myopia onset in children (Mutti et al., 2007; Rozema et al., 2019), and a deceleration in the crystalline lens power loss is reported around the time of onset (Mutti et al., 2012; Rozema et al., 2019). Myopia occurs when the ocular axial length increases more than compensated for by crystalline lens power loss, maybe because the crystalline lens has reached a limit in power loss (Iribarren, 2015; Mutti et al., 2012; Rozema et al., 2019; Xiong, Zhang, et al., 2017). In this thesis, this was indicated from the lack of correlation between the ocular axial length and the crystalline lens power in the myopes, in contrast to the negative correlation in emmetropes and hyperopes (paper II). Furthermore, an association between the age at minimum crystalline lens thickness and the age at myopia onset is proposed (Mutti et al., 2012). This theory was also supported by the results in this thesis; almost 25% of the persistent emmetropes/low-hyperopes exhibited a thinning of the crystalline lens at 18 years of age (paper II), even though the crystalline lens is commonly reported to increase in thickness from around 10 years of age (Jones et al., 2005; H. B. Wong et al., 2010). Indeed, crystalline lens development is confirmed to be one of several genetic pathways associated with refractive errors (Flitcroft et al., 2018). Further studies to elucidate the role of the crystalline lens in myopia development are of interest.

5.3 A sex difference in myopia onset

A sex difference in myopia onset is suggested from a meta-analysis by Rudnicka et al. (2016) that reported the frequency of myopia in both Caucasian and East Asian females to be twice that of males in late adolescence. A higher myopia prevalence in females than males is confirmed in several recent studies (Czepita et al., 2019; Guo et al., 2016; Y. Li et al., 2017; Lu et al., 2016), and consistent with this, the myopia prevalence in Norwegian adolescents in this thesis was found to be 16% in females and 9% in males (paper I). If heterozygosity of mild exon 3 haplotypes increases myopia susceptibility, this may, at

least partially, explain the earlier myopia onset and the higher myopia frequency in females compared with males. Note that the frequency of myopia was approximately equal in the groups of males (~8%) and L exon 3 homozygous females (~9%) but much higher in the group of L exon 3 heterozygous females (~24%) (paper III). The sex difference in myopia onset could be related to females being more exposed to environmental risk factors, such as less time spent outdoors (French, Morgan, Mitchell, et al., 2013), however, in this thesis, Norwegian males and females reported to spend a similar amount of time outdoors and indoors. In a cross-sectional study of Korean adult females, females with a younger age at menarche were found to have a higher degree of myopia (Lyu et al., 2015), whereas in a longitudinal study of Singaporean males and females from 6 to 14 years of age, myopia was found to be associated with early peak height velocity but not with age at puberty (Yip et al., 2012). Puberty and peak height velocity usually occur at a younger age in females than males (Khan, 2019; Liu, Wikland, & Karlberg, 2000; Yip et al., 2012), suggesting that these factors could be related to the sex difference in age at myopia onset. Puberty data were not obtained in this thesis, but in the longitudinal data of Norwegian adolescents from 16 to 18 years of age, no correlations were found between changes in SER and body height. Earlier myopia onset leads to a higher risk of developing high-grade myopia and secondary ocular pathology (Willis et al., 2016; T. Y. Wong et al., 2014), emphasising the importance of understanding the mechanism of earlier myopia onset in females.

5.4 Strengths and limitations of the work

The data in this thesis make an important contribution to the scarcity of reports on refractive errors and ocular growth patterns in the Northern European countries, as well as in Caucasian adolescents older than 15 years of age worldwide. Even though participation was voluntary, the study sample was shown to be representative of the schools' catchment area in terms of ethnicity and grade-point averages, and the catchment area was representative of the Norwegian population with respect to socio-demographic status (see Supplementary Information for paper I). Strengths in the

longitudinal study on refractive errors and ocular growth were that both cycloplegic autorefraction and ocular biometry were performed, by the same instruments, at baseline and follow-up. The inclusion of a large number of participants, in particular females, was a strength in the estimates of L:M cone ratios. Other strengths were the use of an objective method to measure the spectral sensitivity data in a fully dilated eye, and that individual cone opsin genetics were implemented in the estimates of L:M cone ratio to correct for individual variation in the L and M cone opsin peak sensitivities.

Norway stretches from 58° to 71° latitudes north, and the results in this thesis were estimated in adolescents living at 60° latitude north. If daylight exposure is an important component of refractive error development, the refractive error results may not be representative for adolescents living in the far north of Norway, where the seasonal variation in daylight is even more extreme. Since there were slightly more females than males in the study sample, and the frequency of myopia was found to be higher in the females, the overall myopia prevalence reported may be overestimated. Nevertheless, if the real myopia prevalence is even lower than reported here, the conclusions made in this thesis are still supported. Objective measures of light exposures and working distances, in addition to the use of questionnaires, could have given more accurate and reliable estimates of time spent on activities indoors and outdoors (Alvarez & Wildsoet, 2013; Dharani et al., 2012). Affordable equipment to make these measurements in such a large number of participants was, however, not available at the time of the study. Individual values, rather than fixed values for photopigment optical density and age-dependent values for lens correction, could have made the L:M cone ratio estimates more accurate (Bieber et al., 1998). The associations between myopia susceptibility and heterozygosity of common L cone opsin exon 3 haplotypes and L:M cone ratio need to be confirmed in a population with high myopia prevalence, preferably in a longitudinal study. Each of the enclosed papers contains further details on the strengths and limitations of this thesis.

5.5 Future perspectives

In order to provide the best person-centred eye-care and to plan for the best myopia prevention and management strategy for each individual, it is valuable to predict children at risk of myopia before the myopia onset. Further longitudinal studies of ocular growth patterns – including the crystalline lens power – from different ages, ethnicities, and geographical locations may provide better myopia prediction models. Knowledge of typical ocular growth patterns *before* and *at* the onset of myopia, as compared to normal coordinated ocular growth at the respective age, may be helpful. The coordinated ocular growth at 18 years of age in this thesis (paper II) imply that normative data of ocular growth patterns are of importance, not only in children but also in older adolescents and young adults. The aim of developing better myopia prediction models is to reduce the increase in myopia prevalence, by implementing myopia prevention strategies in patients at risk – *before* the myopia onset.

The results in this thesis do also suggest milder versions of L cone opsin gene polymorphisms, in combination with the L:M cone ratio, to be implicated in myopia susceptibility (paper III). The findings raise questions about what level of reduced amount of cone photopigment that interferes with normal emmetropisation. Additionally, it would be of interest to identify specific exon 3 haplotypes that may increase the susceptibility to common myopia. Further research is needed, and if substantiated, effective and reliable methods for L:M cone ratio estimates and cone opsin genetics analyses that are easy to use in both large-scale research studies and clinical practice, need to be developed. This could take us to the ultimate goal to offer personalised myopia prevention and management strategies.

6 Conclusion

The increase in myopia prevalence worldwide is of concern since the associated ocular elongation raises the risk of secondary ocular pathology. Thus, interventions are important to prevent the onset of myopia – or at least decrease the myopia progression – with the aim to cease excessive ocular axial growth and reduce the risk of myopia-related ocular complications.

This thesis showed a low and stable myopia prevalence in Caucasian adolescents in Southeast Norway – despite high educational pressure, extensive use of near electronic devices, and few daylight hours in the long autumn-winter period. Persistent emmetropes exhibited coordinated ocular growth at 18 years of age; the ocular axial elongation was mainly compensated for by a decrease in crystalline lens power. Furthermore, individual differences in L:M cone ratios and common cone opsin polymorphism were found to be associated with myopia susceptibility, implying that individual differences in the cone opsins may influence the effect of environmental risk factors on the refractive error development.

The results in this thesis emphasise the need for a person-centred approach to the prevention and management of refractive errors, in which the treatment plan should be personalised for each individual. First, this requires the regular performance of a comprehensive eye examination that includes the use of cycloplegic refraction and ocular biometry measurements, as well as taking into account the patient's age, sex, ethnicity, behaviour, environment, and other personal preferences. Second, individual variations in biology, such as genetic predisposition, should be taken into account. The eye-care provider needs to be able to identify those patients who are at risk of myopia – preferably before myopia onset – as well as those who most likely will benefit from a specific treatment. In that way, the choice of individual treatment – single vision spectacles or any optical, pharmacological, or environmental interventions for myopia prevention and management – can be personalised to the individual patient. Moreover, the choice of treatment must ensure that the individuals are given optimal visual conditions, and the

various alternatives should be presented to the patient in a well-informed way, both to empower the patient and to make room for shared decision-making.

While extensive research on myopia genetics has made promising progress in the development of genetic risk scores, there is still a need for further research on genetics and other biological variations in refractive error development. This may form the basis of improved risk calculators and prediction models for refractive errors, as well as the development of new, effective, and safe interventions for the prevention and management of myopia. These are important elements in order to reduce the increase in myopia prevalence worldwide. The results in this thesis infer that also individual differences in the L:M cone ratio, as well as common cone opsin polymorphism encoded on the X-chromosome, are of importance in this work.

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Paper I

Hagen, L. A., Gjelle, J. V. B., Arnegard, S., Pedersen, H. R., Gilson, S. J., & Baraas, R. C. (2018). Prevalence and Possible Factors of Myopia in Norwegian Adolescents. *Scientific Reports*, 8(1), 13479. doi:10.1038/s41598-018-31790-y

SCIENTIFIC REPORTS

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Prevalence and Possible Factors of Myopia in Norwegian Adolescents

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East Asia has experienced an excessive increase in myopia in the past decades with more than 80% of the younger generation now affected. Environmental and genetic factors are both assumed to contribute in the development of refractive errors, but the etiology is unknown. The environmental factor argued to be of greatest importance in preventing myopia is high levels of daylight exposure. If true, myopia prevalence would be higher in adolescents living in high latitude countries with fewer daylight hours in the autumn-winter. We examined the prevalence of refractive errors in a representative sample of 16–19-year-old Norwegian Caucasians (n = 393, 41.2% males) in a representative region of Norway (60° latitude North). At this latitude, autumn-winter is 50 days longer than summer. Using gold-standard methods of cycloplegic autorefraction and ocular biometry, the overall prevalence of myopia [spherical equivalent refraction (SER) ≤ -0.50 D] was 13%, considerably lower than in East Asians. Hyperopia (SER $\geq +0.50$ D), astigmatism ($\geq$1.00 DC) and anisometropia ($\geq$1.00 D) were found in 57%, 9% and 4%. Norwegian adolescents seem to defy the world-wide trend of increasing myopia. This suggests that there is a need to explore why daylight exposure during a relatively short summer outweighs that of the longer autumn-winter.

East and Southeast Asia have experienced an excessive increase in myopia in the past few decades, with more than 80% of the younger generation now affected[1,2]. Myopia is a major health concern[3–5], as myopia, and in particular high myopia, may lead to potentially sight-threatening secondary ocular pathology[6]. The "epidemic" scale of myopia is most commonly observed in highly economically developed countries, where children complete secondary education and many undertake upper- and post-secondary studies, combined with limited time spent outdoors[7,8].

Environmental and genetic factors are both assumed to contribute in the development of refractive errors[9,10], although there is no general agreement on the etiology of myopia. The environmental factor argued to be of greatest importance in preventing myopia is time spent outdoors prior to myopia *onset*[11–13] (it is debated whether time outdoors has an effect on myopia *progression*[14–19]). A dose-response relationship between daylight (outdoor) exposure and ocular axial elongation (associated with developing myopia) has been inferred[17]. Reported seasonal variation in axial length growth and myopia progression (with decreased eye growth and decreased myopia progression in periods with increased number of daylight hours[20,21]) is often cited in support of the protective effect of outdoors. Such an explanation warrants further examination and calls for refractive error data from different parts of the world[3,22], in particular countries with high performing education systems and differing levels of seasonal variation in daylight.

Norway's northern latitude stretches from 58° to 71° North, with even those living in Southeast Norway (60° North) experiencing large seasonal variation in daylight exposure, from less than 6 hours in December to around 19 hours in June (Fig. 1)[23]. Norway is a highly economically developed country, ranked as number 1 in the Human Development report 2016, with high gender equality[24]. Norwegian children start primary school at age 6 years and complete 10 years of compulsory schooling before reaching upper secondary school, at age 16 years. Most of today's adolescents will also have attended kindergartens from age 1–5 years (76.2% in 2005)[25]. The Norwegian education system is high-performing, as classified by the Organisation for Economic Co-operation and Development (OECD) Programme for International Student Assessment (PISA), with both mean performance and the proportion of top performers above the OECD average in science, reading and mathematics[26]. Near work includes high usage of near electronic devices (NED) at school and at home, with the use of NED reported to be above the OECD average[27].

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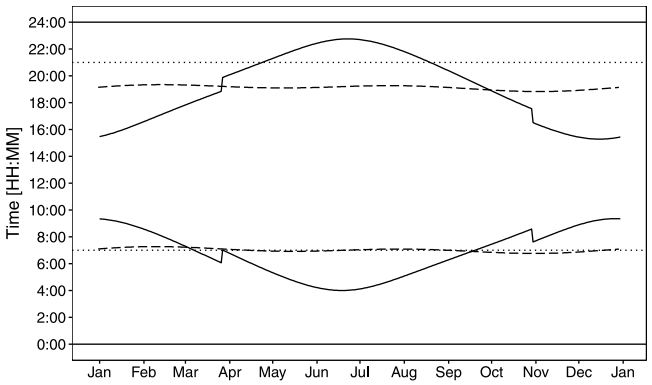

Figure 1. Seasonal variation in sunrise and sunset time. The solid line shows the seasonal variation in sunrise and sunset time in Southeast Norway (60° North, 9° East; range of daylight hours: 5 h 59 min – 18 h 44 min). The sudden change in late March and October is due to daylight saving time. For a comparison, the dashed line shows the sunrise and sunset time in Singapore (1° North, 103° East; range of daylight hours: 12 h 3 min – 12 h 12 min)[23]. The dotted lines show the amount of daylight available for a child sleeping 10 hours each night.

If high levels of daylight exposure are necessary to protect against myopia, it is reasonable to hypothesize that myopia onset will occur earlier, progression will be faster, and prevalence will be higher in adolescents living in countries with relatively few daylight hours across an extended (5–6 months of autumn-winter) period[28], particularly so, if combined with a high level of near work[29,30]. The current study tested this hypothesis. Its aim, therefore, was to examine the prevalence of refractive errors in adolescents in Southeast Norway and assess the relationship between refractive errors, ocular biometry, sex and environmental factors such as self-reported time spent on activities outdoors and indoors.

Methods

Study Population and Recruitment. A cross-sectional study was carried out on students from the only two upper secondary schools within a catchment area comprising five municipalities in Southeast Norway during 2015–2016. The catchment area is representative of the Norway population in terms of socio-demographic status (details are given in Supplementary Tables S1–S4), with 70.7% living in urban settlements and an average population densities of 4–36 persons/km^2 [31]. The total population of the region was 49,293 in 2016, with 1,737 of these aged 16–19 years[32,33]. The total student population of the two schools was 1,970 (age 16–24 years), 676 and 1,294 in the first and second schools respectively. The students attend school 5 days a week for 5–8 hours per day, with the school day beginning no earlier than 8 am; in addition, students undertake homework in the evenings and on weekends. By agreement with school administrators, we were given access to 898 students (45.6%) who were all invited to participate; all students in all three years in the first school and those in their first year (typical age 16–17 years) in the second school. The sample was representative of the school's catchment area with respect to ethnicity and grade point averages (see Supplementary Tables S2 and S5). The study was carried out at the schools during normal school hours.

Verbal and written information about the study was given, and possible consequences of the study were explained to all participants before written informed consent was obtained. The research was approved by the Regional Committee for Medical Research Ethics for the Southern Norway Regional Health Authority and carried out in accordance with the principles embodied in the Declaration of Helsinki. A person aged 16 years or older is considered an adult and fully competent to consent to participate in research according to the Norwegian Health Research Act.

Participants. Of those invited, a sample of 439 (48.9%) students aged 16–19 years [mean age (*SD*): 16.7 (±0.9) years, 41.9% males] agreed to participate in the study. Self-reported ethnicity was mainly European Caucasians (90.9%); other ethnicities were Asian (5.5%), African (1.4%), South American (0.9%), or mixed (defined as having parents of two different ethnicities, 1.4%).

Analysis beyond calculation of prevalence of hyperopia and myopia was limited to the participants who reported to have both grown up in Norway and who were of Northern European (Caucasian) ethnicity [n = 393, mean age 16.7 (±0.9) years, 41.2% males], hereafter termed Norwegians. This group included participants born in Norway (98.7%) and five participants born in a different Northern European country (1.3%; born in Denmark, Iceland, Germany and Holland), all of whom reported to have moved to Norway during their childhood. Removal of these five participants from the group had no overall effect on the results. The Norwegian participants were grouped according to sex and age for the purpose of analysis (16-years-olds: n = 224, 42.4% males; 17–19-years-olds: n = 169, 39.6% males).

Cycloplegic Autorefraction and Other Measurements. Cycloplegic autorefractions were obtained with a Huvitz HRK-8000A Auto-REF Keratometer (Huvitz Co. Ltd., Gyeonggi-do, Korea), 15–20 minutes after instillation of topical 1% cyclopentolate hydrochloride (Minims single dose; Bausch & Lomb UK Ltd, England). One drop of cyclopentolate was used for blue- and green-eyed participants, and two drops for brown-eyed

participants. The mean of five measurements automatically performed by the instrument (Huvitz HRK-8000A) were used for further analyses. One qualified optometrist (author JVBG) performed all autorefraction and biometry measurements.

Ocular axial lengths (AL) and corneal radii (CR) were measured with Zeiss IOLMaster (Carl Zeiss Meditec AG, Jena, Germany). Body height was measured with the Seca 217 stable stadiometer for mobile height measurement (Seca Deutschland, Hamburg, Germany).

Questionnaire. Participants completed an online questionnaire, an adapted version of the one used in the Sydney Myopia study[34], to obtain demographic data and to quantify the amount of time spent on various indoor and outdoor activities. Demographic data included place of birth, number of years lived in Southeast Norway, house type and distance to school. Information about access to, and use of, near electronic devices (NED; smart phones, tablets, computers) was also collected.

The reported mean hours per day spent on outdoor- and indoor- activities were calculated for those participants who completed all questions related to time spent on various activities [68.4%, n = 269, 40.1% males, mean age 16.7 (±0.9) years]. Indoor activities included mean time spent on reading and writing on paper (books, newspapers, magazines), use of NED, indoor sport (gymnastics, dance, ball games, etc) and other indoor activities (watching television, playing video games, hobbies, cooking, etc). Outdoor activities included mean time spent on outdoor sport (cycling, skiing, running, etc) and other outdoor activities (walking to school, hiking, fishing, hunting, spending time in the garden etc). The participants were asked to estimate the daily time usually spent on these activities for both weekdays and weekends and about what they do in the school's recess time. They were given four categorical response options for the estimate of activity hours per day; "Not at all", "Less than 1 hour", "1–2 hours", or "3 hours or more". The mean numbers of activity hours per day were calculated using "0 hour", "1 hour", "2 hours" or "3 hours" for each option, respectively, as follows:

$$Mean\ hours\ per\ day = \frac{(hours\ spent\ on\ weekdays \times 5) + (hours\ spent\ on\ weekends \times 2)}{7} \quad (1)$$

Finally, the participants were asked to estimate the ratio of indoor to outdoor activities during their school holidays. Data were collected during February and March at both schools.

Analysis. Spherical equivalent refractive errors (SER = sphere + ½ cylinder), specified in terms of a 13.5 mm vertex distance, were used to classify refractive errors. Myopia was defined as SER ≤ −0.50 D, emmetropia as −0.50 D < SER < + 0.50 D, and hyperopia as SER ≥ + 0.50 D. The most positive meridian of the autorefractor measurement was defined as the sphere, and the prevalence of refractive astigmatism is reported as negative cylinder refraction ≥1.00 DC. SER, sphere and refractive astigmatism were all well correlated between the right and left eyes (SER: Spearman rho (ρ) = 0.94; sphere: ρ = 0.92; refractive astigmatism: ρ = 0.59; all $p < 0.001$), and thus only data from the right eye are presented. A SER-difference ≥1.00 D between right and left eye was defined as anisometropia. CR data represent the mean of the corneal radii measured in the flattest and steepest meridians. AL/CR-ratios were also calculated.

The Clopper-Pearson interval method and the method of Sison and Glaz were used for calculation of 95% binomial and multinomial proportion confidence intervals (CI), respectively. QQ-plots, histograms and the Shapiro-Wilk test were used to assess the normality of the variables. Means (±*SD*) are reported, in addition to the median (50th percentile) for non-normal data. The chi-square test, Fisher's exact test, and independent sample *t*-test were used to assess differences in prevalence and mean values between groups. Maximum likelihood estimate was used to fit a suitable distribution to the data for SER[35].

Linear regression analyses were performed with SER, AL, AL/CR-ratio and cylinder as the dependent outcome variables. Multivariate logistic regression analyses were performed, with the presence of myopia as the dependent outcome variable. Likelihood ratio tests were performed to compare models. Odds ratios (*OR*) and 95% CI are presented, with the significance level set at 0.05. All statistical analyses were performed using R statistical software, version 3.4.0[36] including the packages MASS[35] and gmodels[37].

Results

Refractive Errors. Table 1 shows an overview of the prevalence of refractive errors by age and sex, independent of ethnicity (a) and for those defined as Norwegians (b). The overall prevalence of hyperopia and myopia was 55.4% and 13.4%, respectively. All results are from here on related to those defined as Norwegians.

The prevalence of hyperopia and myopia in Norwegians was 56.7% and 12.7%, respectively. Figure 2 shows the leptokurtic distribution of SER [D] for 16–19-year-old Norwegians. The SER mean (±SD) was +0.55 (±1.29) D and median was +0.61 D (range: −6.45–7.71 D). Myopia was more prevalent among females than males [15.6% versus 8.6%, Fisher's exact test, $p = 0.046$]. The prevalence of hyperopia decreased with age, with the prevalence of myopia increasing in parallel (Table 1b, column 6 and 8). However, the prevalence of high myopia, defined as SER ≤ −6.00 D, was very low, at 0.5% (CI: 0.1–1.8%). In contrast, the prevalence of moderate to high hyperopia, defined as SER ≥ + 2.00 D, was higher, at 6.4% (CI: 4.2–9.2%). Refractive astigmatism (≥1.00 DC) was found in 8.9% (CI: 6.3–12.2%) and anisometropia (≥1.00 D) in 3.6% (CI: 2.0–5.9%) of participants.

Ocular Biometry and Body Height. Table 2 shows mean AL, CR and AL/CR categorized by age, sex, and refractive error. Mean AL was significantly longer (23.66 vs. 23.28 mm, $t(391) = -4.46$, $p < 0.001$) and mean corneal curvature (CR) was significantly flatter (7.87 vs. 7.78 mm, $t(305) = -3.00$, $p = 0.003$) in males compared with females. Overall, AL and CR were highly correlated (Pearson; $r = 0.53$ in females, $r = 0.69$ in males, $p < 0.001$), and both AL and AL/CR were significantly negatively correlated with SER in both males and females (AL: $r = -0.62$, (females), $r = -0.47$ (males), $p < 0.001$; AL/CR: $r = -0.84$ (females), $r = -0.77$ (males), $p < 0.001$).

	Age (years)	Group	*n*	Mean (*SD*) SER [D]	Myopia % (CI)	Emmetropia % (CI)	Hyperopia % (CI)
(a) ALL ETHNICITIES	16–19	All	439	+0.51 (1.29)	13.4 (8.7–18.3)	31.2 (26.4–36.1)	55.4 (50.6–60.2)
		Females	255	+0.39 (1.30)	16.9 (10.6–23.1)	27.5 (21.2–33.7)	55.7 (49.4–62.0)
		Males	184	+0.67 (1.25)	8.7 (1.6–16.4)	36.4 (29.3–44.1)	54.9 (47.8–62.6)
	16	All	246	+0.59 (1.23)	11.0 (4.9–17.5)	31.3 (25.2–37.8)	57.7 (51.6–64.3)
		Females	139	+0.50 (1.10)	14.4 (6.5–22.9)	25.9 (18.0–34.4)	59.7 (51.8–68.2)
		Males	107	+0.72 (1.37)	6.5 (0.0–16.3)	38.3 (29.0–48.1)	55.1 (45.8–64.9)
	17–19	All	193	+0.40 (1.35)	16.6 (9.3–23.9)	31.1 (23.8–38.5)	52.3 (45.1–59.7)
		Females	116	+0.26 (1.50)	19.8 (11.2–30.0)	29.3 (20.7–39.5)	50.9 (42.2–61.1)
		Males	77	+0.60 (1.06)	11.7 (1.3–23.8)	33.8 (23.4–45.8)	54.5 (44.2–66.6)
(b) NORWEGIANS	16–19	All	393	+0.55 (1.29)	12.7 (7.9–18.0)	30.5 (25.7–35.8)	56.7 (51.9–62.0)
		Females	231	+0.45 (1.27)	15.6 (9.1–22.2)	28.1 (21.6–34.8)	56.3 (49.8–62.9)
		Males	162	+0.70 (1.30)	8.6 (1.2–16.7)	34.0 (26.5–42.1)	57.4 (50.0–65.5)
	16	All	224	+0.63 (1.23)	10.3 (4.0–17.1)	30.8 (24.6–37.7)	58.9 (52.7–65.8)
		Females	129	+0.56 (1.05)	13.2 (5.4–22.2)	25.6 (17.8–34.6)	61.2 (53.5–70.3)
		Males	95	+0.74 (1.43)	6.3 (0.0–17.0)	37.9 (28.4–48.6)	55.8 (46.3–66.5)
	17–19	All	169	+0.44 (1.37)	16.0 (8.3–23.7)	30.2 (22.5–37.9)	53.8 (46.2–61.6)
		Females	102	+0.31 (1.50)	18.6 (8.8–28.9)	31.4 (21.6–41.7)	50.0 (40.2–60.3)
		Males	67	+0.65 (1.12)	11.9 (1.5–24.7)	28.4 (17.9–41.1)	59.7 (49.3–72.5)

Table 1. Mean spherical equivalent error SER (standard deviation, *SD*) in diopters [D] and the prevalence of refractive error type (%) for the right eyes categorized by age and sex of (a) all 16–19-year-olds, independent of ethnicity ($n = 439$), and (b) 16–19-year-old Norwegians ($n = 393$). Prevalence is given with 95% confidence intervals (CI). Myopia was defined as SER ≤ -0.50 D, emmetropia as -0.50 D $<$ SER $< +0.50$ D, and hyperopia as SER $\geq +0.50$ D.

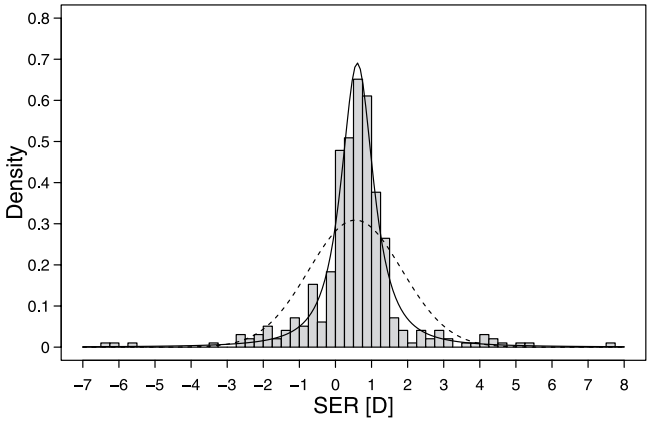

Figure 2. Distribution of SER. The leptokurtic distribution of cycloplegic SER [D] for the right eyes of 16–19-year-old Norwegians ($n = 393$; skewness $= -0.24$, kurtosis $= 11.3$). The dashed curve shows a normal distribution with the same mean and standard deviation as the data, and the solid curve shows a *t*-distribution fitted to the data by maximum likelihood [degrees of freedom (df) $= 1.63$, location (m) $= 0.61$, scale (s) $= 0.50$][35].

The mean height of participants was 172.2 (±8.7) cm, with males being on average taller than females [179.2 (±7.1) cm vs. 167.3 (±6.0) cm, $t(309) = 17.3$, $p < 0.001$]. Height correlated with AL overall (Pearson; $r = 0.28$, $p < 0.001$) and in females (Pearson; $r = 0.23$, $p < 0.001$), but not in males (Pearson; $r = 0.14$, $p = 0.08$). Height did not correlate with SER.

Outdoor and Indoor Activity Time. Times spent doing outdoor and indoor activities were calculated for the subset of Norwegian participants who answered all questions related to time spent on various activities. Although this subgroup represented only 68% of the total group, there were no differences between this smaller sample ($n = 269$) and the whole sample of Norwegian participants ($n = 393$) in prevalence of myopia (12.3% vs. 12.7%), emmetropia (30.9% vs. 30.5%) or hyperopia [56.9% vs. 56.9%; $\chi^2(2) = 0.03$, $p = 0.984$]. These participants reported to spend, on average, 3.8 (±1.8) and 10.5 (±2.4) hours per day outdoors and indoors, respectively. Most of the participants (93%) reported staying indoors in their school recess time. Myopes spent, on average, less time doing outdoor sport per day [0.93 (±0.8) h] than non-myopes [emmetropes and hyperopes combined: 1.32 (±1.0) h; $t(267) = -2.24$, $p = 0.03$], but total time spent outdoors was not associated with myopia [myopes:

Age		*n*	SER [D] Mean (*SD*)	AL [mm] Mean (*SD*)	CR [mm] Mean (*SD*)	AL/CR Mean (*SD*)
16–19	All	393	+0.55 (1.29)	23.44 (0.86)	7.82 (0.27)	3.00 (0.09)
	Females	231	+0.45 (1.27)	23.28 (0.83)	7.78 (0.25)	2.99 (0.10)
	Males	162	+0.70 (1.30)	23.66 (0.86)	7.87 (0.30)	3.01 (0.09)
	Myopes	50	−1.60 (1.34)	24.22 (0.79)	7.74 (0.25)	3.13 (0.09)
	Emmetropes	120	+0.18 (0.23)	23.51 (0.75)	7.77 (0.27)	3.03 (0.07)
	Hyperopes	223	+1.23 (0.97)	23.22 (0.83)	7.86 (0.27)	2.95 (0.07)
16	All	224	+0.63 (1.23)	23.38 (0.82)	7.81 (0.28)	3.00 (0.09)
	Females	129	+0.56 (1.05)	23.21 (0.76)	7.78 (0.26)	2.99 (0.09)
	Males	95	+0.74 (1.43)	23.62 (0.85)	7.85 (0.31)	3.01 (0.10)
17–19	All	169	+0.44 (1.37)	23.51 (0.91)	7.83 (0.26)	3.00 (0.10)
	Females	102	+0.31 (1.50)	23.36 (0.91)	7.79 (0.24)	3.00 (0.11)
	Males	67	+0.65 (1.12)	23.73 (0.88)	7.89 (0.28)	3.01 (0.08)

Table 2. Mean (*SD*) axial length (AL), corneal radius (CR) and AL/CR-ratio for the right eye of 16–19-year-old Norwegians ($n = 393$) categorized by age, sex, and refractive error.

	Model A			Model B		
	β	OR (95% CI)	*p*	β	OR (95% CI)	*p*
Intercept	−2.150	0.12 (0.02–0.75)	0.026	−2.041	0.13 (0.05–0.33)	<0.001
Sex, male	−0.625	0.54 (0.21–1.25)	0.164	−0.636	0.53 (0.21–1.21)	0.146
Sport outdoors	**−0.754**	**0.47 (0.27–0.78)**	**0.005**	**−0.678**	**0.51 (0.30–0.82)**	**0.007**
Other outdoors	**0.438**	**1.55 (1.07–2.28)**	**0.022**	**0.400**	**1.49 (1.04–2.15)**	**0.030**
Read paper	0.260	1.30 (0.75–2.23)	0.344			
NED	0.013	1.01 (0.78–1.31)	0.922			
Other indoors	−0.176	0.84 (0.59–1.18)	0.311			
Sport indoors	0.099	1.10 (0.72–1.72)	0.654			

Table 3. Multivariate logistic regression models with myopia as the outcome variable. (Model A) mean hour of activity [h/day] as predictors and sex as a potential confounder. AIC = 201.0. (Model B) mean hours of sport and other outdoor activities as the predictors, adjusted for sex. AIC = 195.1. Odds ratios (OR) and confidence intervals (CI) are presented.

3.65 (±1.5) h; non-myopes: 3.81 (±1.9) h; $t(267) = 0.47$, $p = 0.64$], neither was time spent on other activities. The hours spent on various indoor or outdoor activities also showed no significant correlations with either SER, astigmatism, AL or AL/CR-ratio.

Females and males spent, on average, the same amount of time outdoors [females: 3.71 (±1.7) h; males: 3.91 (±2.0) h] and indoors [females: 10.68 (±2.3) h; males: 10.26 (±2.4) h]. More than 97% of the students had both their own smart phone and laptop for use at school and for homework. The time spent using NED each day was the same for females and males [females: 5.01 (±1.5) h; males: 4.97 (±1.5) h].

Table 3 shows the models from the multivariate logistic regression, with myopia as the outcome variable, sex as potential confounder, and mean hours of different indoor and outdoor activities as the predictors (Model A). Likelihood ratio tests were used for manual backward selection (Model B). Model B confirmed a lack of significant association of myopia with indoor activities, but showed myopia to be associated with less time spent on outdoor sport ($OR = 0.51$, CI: 0.30–0.82, $p = 0.007$) and more time spent on other outdoor activities ($OR = 1.49$, CI: 1.04–2.15, $p = 0.030$), after adjustment for sex.

Table 4 shows that 94% and 64% reported to spend half or more of the day outdoors in the summer and Easter holidays, respectively. More myopes (14%) than non-myopes (4%) reported to spend most of their time indoors during the summer holidays (Fisher's exact test, $p = 0.01$), with no difference for the other holidays.

Discussion

This is the first report on refractive errors in a representative sample of adolescents in Southeast Norway, with hyperopia found to be the most common type of refractive error. How does the refractive error profile of this adolescent population compare with other adolescent populations? The prevalence of moderate to high hyperopia (SER ≥ +2.00 D) in this sample (6.4%) is higher than that reported for adolescents in both Asia (0.5–4.0%)[38–40] and Australian European Caucasians (2.0%)[5], but lower than among white adolescents in the UK (17.7%)[41]. Comparative data from other published studies on myopia prevalence are summarized in Table 5, with matched myopia definition. The prevalence of myopia is comparable with, albeit slightly lower than for Australian European Caucasians in Sydney[5] and white adolescents in the UK[41]. It was lower than the 27.4% point estimate for myopia in the 15–19-year age group across Europe, calculated by random-effect meta-analysis and age-standardization by Williams *et al.*[42] (mean SER for the two eyes ≤ −0.75 D). The prevalence of myopia was also lower than that reported in a study of Swedish 12–13-year-olds[43], though that study's use of tropicamide 0.5%

	Duration of holiday (time of the year)	Mean # daylight hours in the period	Proportion (%)		Proportions (%) who spend most time indoors		
			Spend half of the day outdoors	Spend more than half of the day outdoors	Myopes	Non-myopes	*p*-value
Summer	8 weeks (mid June–mid August)	17 h 35 min	45	49	14	4	**0.007***
Autumn	1 week (October)	11 h 5 min	38	9	47	53	0.447
Winter	1 week (February)	10 h 36 min	35	8	67	56	0.164
Spring (Easter)	1.4 weeks (March–April)	14 h 21 min	52	12	41	35	0.428

Table 4. Overview of duration, time and mean number of daylight hours for the school holidays in Norwegian upper secondary school, including proportion of students who reported to spend half of the day or more than half of the day outdoors in these periods. Proportions of the students who spend most time indoors are categorized as myopes and non-myopes (*p*-values were calculated using Fisher's exact test for count data).

for accommodation control may have resulted in an artificially high myopia prevalence. The prevalence of myopia observed in the Southeast-Norwegian 16-year-olds is only slightly higher than that reported for 1-year-younger adolescents in rural Nepal, Iran and rural India[44–46] (all considerably lower HDI than Norway). Noteworthy, the prevalence of myopia is considerably lower than that generally reported for adolescents in rural and urban parts of Asia[12,38–40,47–49] [with comparable or lower human development index (HDI) than Norway][24], and Chile[50] (considerably lower HDI than Norway). The ocular biometry data are consistent with the low myopia prevalence, with shorter axial lengths and lower average AL/CR than groups with higher myopia prevalence [cf. Table 2 with Lu *et al.*[51] and Li *et al.*[52]].

While the prevalence of myopia is reported to have been rising around the world, a similar trend in Southeast Norway appears to be absent. Specifically, a 1971 study of 12–14-year-old Norwegian children in West Norway (latitude 60.4°) reported similar cycloplegic SERs to that found here (at latitude 59.7–60.0°), and similarly low myopia prevalence (SER ≤ -1.0) of 13.7% (Table 5)[53]. Interestingly, Fledelius reported stability in the myopia prevalence of Danish medical students over the period 1968–1998[54]. Moreover, the low rate of high myopia (0.5%; SER ≤ -6 D) observed here and the reported higher myopia prevalence in 21-year-olds in mid-Norway [myopia prevalence (SER ≤ -0.25) was 33% in the general population, latitude 63.4°][55] suggest that myopia onset is significantly delayed in Norwegians compared with East-Asians and some other Europe based populations[12,38,39,41,43,47]. The narrow range in refractive errors, higher prevalence of emmetropia with a hyperopic mean SER, coupled with a low prevalence of anisometropia and astigmatism lend support to this suggestion[56–58]. A further increase in myopia prevalence may be expected when the adolescents enter higher education[55].

The education system in Norway is classified as high-performing[26]. The adolescents in this study spent >10 hours per day indoors doing near work including working on NED for >5 hours per day, which was comparable with the amount of time spent on NED reported in a study of sleep in 16–19-year-olds in West Norway (latitude 60.4°, n = 9,846)[59]. But, time spent on near work was not associated with myopia, as reported by others[60,61], neither was total time spent on outdoor activities in the winter — the multivariate analyses showed that the association for other activities outdoors outweighed that of doing sports outdoors. There was, however, an association between myopia and less time spent outdoors in the summer holiday. Interestingly, the mean time spent outdoors in the winter [3.79 (±1.8) hours per day; data collection was February–March] was similar to that reported for East-Asian adolescents [$n = 267$; mean 3.79 (±1.9) hours per day][12] in Singapore, where there is no difference in daylight hours (12 hours per day) between seasons (Fig. 1). This parallel raises the question for Norwegian adolescents, as to why the potential negative consequences of limited daylight exposure during the long autumn-winter period, when there are fewer than 12 hours daylight per day (174 days, including 82 days in November–January with only 6–8 hours daylight per day), do not override the potential positive benefits of the long days during the shorter summer period (124 days with 15–19 hours daylight per day). Note that there is a ceiling effect to the benefits of long summer days, since several hours of the daylight are in the late evening or early hours of the morning when children and adolescents sleep[62,63]. Norwegian children most likely only have access to about 12 hours of the daylight available to them in the spring-summer period (Fig. 1), which is comparable to what the children in Singapore have access to every day of the year. Can the difference in myopia prevalence between Norwegian and for example Singaporean adolescents (12.7% versus 69.5%[12]) be down to the increased time Norwegian adolescents spend outdoors in the 8-week summer holiday only? Considering the effect on myopia progression reported from the outdoor activity clinical trials in East Asia[18], it seems unlikely that this can be the case. This raises the further question in relation to whether exposure to daylight *per se* is the most important factor in the protective effect of outdoor activity [cf. Guggenheim *et al.*[64]]. Could the state of being well adapted to seasonal variations (circannual rhythms) be as important for coordinated eye growth as it is for general health[65]? Is this to a larger degree preserved in Norwegian adolescents, because of more outdoor time since early childhood?

Being outdoors is a part of the Norwegian culture and a major part of growing up. For example, children in Norwegian kindergartens are reported to spend 2 hours per day outdoors in the winter and at least 4 hours in the summer[66]. Furthermore, children are required to stay outdoors during school recess (three to five breaks that accumulates to at least 1 hour per day) all the way through primary school (6–12 years of age), and all year long[67]. Pre-adolescent children spend on average an additional 2 hours outdoors per day after school[68]. These exposure patterns are quite different from those of children attending East-Asian schools where recess time usually is spent indoors[13,17,18]. It has been suggested that 2 hours spent outdoors per day is needed to prevent onset of myopia[17], with outdoor activities having a stronger protective effect in younger children (age 6 years vs. age 11–12 years)[19,69].

Age (years)	n	Myopia definition (SER)	Myopia prevalence (%) matched on myopia definition		Age (years)	n	Country	Ethnicity	HDI 2015[24]	Mean score in PISA 2015[82]	Average scale score TIMSS 2015[83]	Latitude
			Present study	Other studies					(HDI rank)	Science/ Reading/ Mathematics	Mathematics 8th grade	
16	224	<0.00	17.4	**27.5**	**12–14**	**102**	**Norway**[53]	**Not given**	0.949 (1)	498/513/502	512	60.4° N
		<−1.00	5.8	**13.7**	**12–14**	**102**	**Norway**[53]	**Not given**	0.949 (1)	498/513/502	512	60.4° N
		≤−0.50	10.3	**44.9**	**12–13**	**1045**	**Sweden**[43]	**Not given**	0.913 (14)	493/500/494	501	57.7° N
		<−0.50	10.3	**52.1**	**13–16**	**2069**	**Rural China**[47]	**Han, Dai, Yi, Bai and other**	0.738 (90)	518/494/531	N/A	24.5° N
		≤−0.50	10.3	**38.8**	**14–15**	**905**	**Rural China**[38]	**Not given**	0.738 (90)	518/494/531	N/A	40.1° N
		≤−0.50	10.3	**16.7**	**15**	**395**	**Suburban Chile**[50]	**Not given**	0.847 (38)	447/459/423	427	33.5° S
		≤−0.50	10.3	**0.79**	**15**	**386**	**Rural Nepal**[44,84]	**Mixed Mongoloid, Aryan, and Aboriginal ancestry**	0.558 (144)	N/A	N/A	26.6° N
		≤−0.50	10.3	**6.72**	**15**	**258**	**Rural India**[46]	**Not given**	0.624 (131)	N/A	N/A	16.4° N
		≤−0.50	10.3	**10.8**	**15**	**381**	**Urban India**[40]	**Not given**	0.624 (131)	N/A	N/A	28.6° N
		≤−0.50	10.3	**9.6**	**15**	**326**	**Semi-urban South Africa**[85]	**African, Indian, mixed**	0.666 (119)	N/A	372	29.9° S
		≤−0.50	10.3	**78.4**	**15**	**376**	**Urban China**[48]	**Han (Chinese)**	0.738 (90)	518/494/531	N/A	23.1° N
		≤−0.50	10.3	**32.5**	**15**	**321**	**Urban Malaysia**[49]	**Malay, Chinese, Indian and other**	0.789 (59)	N/A	465	3.3° N
		≤−0.50	10.3	**4.9**	**15**	**120**	**Iran**[45]	**Not given**	0.774 (69)	N/A	436	32.4° N
		≤−0.50	10.3	**46.8**	**16**	**452**	**Rural China**[39]	**Not given**	0.738 (90)	518/494/531	N/A	21.8° N
16–19	393	≤−0.50	12.7	**69.5**	**11–20**	**1249**	**Singapore**[12]	**Chinese, Malay, Indian and others**	0.925 (5)	556/535/564	631	1.4° N
17	80	≤−0.50	15.0	**17.7**	**17**	**<1202**	**Australia**[5]	**European Caucasian**	0.939 (2)	510/503/494	505	33.9° S
18–19	89	≤−0.50	16.9	**18.6**	**18–20**	**226**	**UK**[41]	**White UK children**	0.909 (16)	500/497/493	N/A	54.8° N
[18.2 (±0.4)]	89	≤−0.25	18.0	**33.0**	**21.7 (±0.3)**	**112**	**Norway**[55]	**Not given**	0.949 (1)	498/513/502	512	63.4° N

Table 5. Summary of myopia prevalence (%) from this study (four leftmost columns) and from other studies (rows, bold), matched on myopia definition and best matched on age. All results are based on cycloplegic autorefraction measurement, except for a few studies that used cycloplegic retinoscopy[38,40,46,50,53], retinoscopy with tropicamide[43] or cycloplegic subjective refraction[55]. Human Development Index (HDI) 2015[24], mean score in Programme for International Student Assessment (PISA) 2015[82], and average scale score for Trends in International Mathematics and Science Study (TIMSS) 2015[83] for each country are listed (results for Norway in top row). N/A = Not participated (except from Malaysia which participated in PISA 2015, but did not meet the PISA response-rate standards). PISA results given for China are from the area Beijing-Shanghai-Jiangsu-Guangdong, and PISA results for UK are from Northern Ireland. The PISA 2015 OECD average in science/reading/mathematics = 493/493/490[82], and TIMSS 2015 Scale Centerpoint for Mathematics 8th grade = 500[83]. Highest score is best. Latitude for each study region is given in the rightmost column (latitude for present study is 59.7–60.0° N).

Our data for Norwegian adolescents represent further supportive evidence from a real-life experiment. Nonetheless, it is also possible that the early onset of myopia as observed in many East Asian populations may be driven by genetic predisposition more than by environmental factors[10,30].

Sex differences in myopia prevalence have been reported previously[70–72]. As in past studies, females were found to have a higher prevalence of myopia than males. There was a significant correlation between AL and height in females, but not males, which may be related to the age of onset of the childhood growth spurt. Specifically, girls usually show an earlier growth spurt, starting approximately two years ahead of boys[73–75]. There is a parallel here with myopia onset for females, which has been reported to be two years ahead of males[54,75]. The implication of the earlier onset of myopia in females is that they have a higher risk for developing larger myopic errors and secondary ocular pathology — indeed, as reported for older age groups[76–78].

Our study had several limitations. The sample size could have been larger with an even higher response rate, but this is comparable to other studies when considering the narrow age range (Table 5). The population studied may be biased in its representation, although we have shown our sample to be representative for the region of Norway from which it was drawn (see Supplementary Material). It was not representative in terms of sex, with a slightly higher number of females, but considering that more females were myopic this, if anything, might suggest that the true overall prevalence of myopia may be lower. The use of questionnaires for quantifying time outdoors is common in studies of refractive errors[11,69,79], even though there are inherent limitations associated with such an instrument compared with objective measures, for example wearable light meters[80]. This includes analytical problems arising from the use of categorical responses to a continuous event. Nonetheless, the comparisons made above were limited to studies that also made use of questionnaires for quantifying time in the same way.

In summary, this cross-sectional study of adolescents in Southeast Norway revealed hyperopia to be the most common refractive error, with the prevalence of myopia being quite low, despite the few daylight hours in the autumn-winter period and high levels of indoor activity and near work. While the origin of refractive errors is likely multifactorial[56], a dose-response relationship between daylight (outdoor exposure) and ocular axial elongation alone cannot explain the low prevalence in myopia, anisometropia and astigmatism in this population. Genetic and environmental risk factors may impact how refractive errors develop differently[81], and our results may point to a lower genetic predisposition to myopia in this population. Alternatively, perhaps there is a particular combination of genetic predisposition, circannual adaptation, timing and pattern of exposure to myopia-generating environmental triggers that are effective in protecting the population at this latitude against myopia.

Data Availability

Supplementary data on the community profile and demographics, a more detailed summary of refractive errors, time spent on indoor and outdoor activities, and refractive errors of non-Norwegians (n = 46) are available at usn.figshare.com [https://doi.org/10.23642/usn.6022790].

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Author Contributions

R.C.B. and L.A.H. conceived and designed the study, analyzed/interpreted the data, wrote the manuscript, and prepared the figures and tables. R.C.B., L.A.H., J.V.B.G., S.A., H.R.P., S.J.G. conducted the experiments, interpreted data and reviewed the manuscript.

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Supplementary Information

Prevalence and Possible Factors of Myopia in Norwegian adolescents

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Supplementary information about representativeness in the data

The catchment area of the two upper secondary schools included in this study consists of five municipalities with a total area of 2,906 m^2 (Flesberg, Kongsberg, Krødsherad, Modum, and Sigdal), and these are the only two upper secondary schools in the region. The Supplementary Tables S1–S4 show that the catchment area is a representative region of Norway in terms of distribution of age, sex, ethnicity and socio-demographic status as level of education and gross income. Supplementary Tables S2 and S5 show that our study sample is representative of the total population in the region and of the total population in Norway, with respect to distribution of ethnicity and grade point averages.

Table S 1. Distribution of age and sex in the schools' catchment area and total in Norway.

Distribution of age and sex given as number (*n*) and proportions (%) in the two schools' catchment area and total for the population in Norway in 2016.[1]

	The catchment area 2016 (*n* = 49,293)						Norway 2016 (*n* = 5,213,985)					
Age	Males		Females		Total		Males		Females		Total	
(yrs)	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
0–14	4,405	17.7	4,163	17.1	8,568	17.4	478,349	18.2	455,606	17.6	933,955	17.9
15–24	2,975	11.9	2,693	11.1	5,668	11.5	345,180	13.1	324,478	12.5	669,658	12.8
25–49	8,479	34.0	7,775	31.9	16,254	33.0	926,613	35.3	875,848	33.8	1,802,461	34.6
50–64	4,914	19.7	4,776	19.6	9,690	19.7	484,964	18.5	467,847	18.1	952,811	18.3
65–79	3,316	13.3	3,491	14.3	6,807	13.8	307,971	11.7	327,104	12.6	635,075	12.2
≥ 80	851	3.4	1,455	6.0	2,306	4.7	82,034	3.1	137,991	5.3	220,025	4.2

Table S 2. Distribution of ethnicities in our sample, in the schools' catchment area, and total in Norway.

Table showing distribution of ethnicities in our total sample (*n* = 439; age 16–19 yrs), for the two schools' catchment area population, and total for Norway.[2-4] Mixed ethnicity is defined as having parents of two different ethnicities.

Ethnicity	Our sample (*n* = 439)		Catchment area population 2016 (*n* = 49,293)[2-5]		Norway's population 2016 (*n* = 5,213,985)[4-6]	
	n	%	*n*	%	*n*	%
Norwegian	388	88.4	42,733	86.7	4,365,778	83.7
Total other	51	11.7	6,560	13.3	848,207	16.3
European *	11	2.5	3,699	7.5	430,671	8.3
Asian	24	5.5	1,710	3.5	265,721	5.1
African	6	1.4	745	1.5	114,304	2.2
South-American	4	0.9	286	0.6	24,256	0.5
North-American	0	0.0	107	0.2	11,072	0.2
Oceanian	0	0.0	13	0.0	2,183	0.0
Mixed	6	1.4	0	0.0	0	0.0

* European is defined here as originating from European countries other than Norway

Table S 3. Level of education in the schools' catchment area and total in Norway.
Level of education[7] for 16 years and older males and females in the two schools' catchment area and total in Norway in 2015, given as numbers (*n*) and proportions (%).

Education	The catchment area October 2015		Norway October 2015					
	Total (*n* = 40,296)		Males (*n* = 2,117,010)		Females (*n* = 2,104,975)		Total (*n* = 4,221,985)	
	n	%	*n*	%	*n*	%	*n*	%
Below upper secondary education	11,049	27.4	570,495	26.9	557,697	26.5	1,128,192	26.7
Upper secondary education *	17,140	42.5	927,460	43.8	787,750	37.4	1,715,210	40.6
Higher education, short †	11,876	29.5	394,123	18.6	570,821	27.1	964,944	22.9
Higher education, long ‡			211,317	10.0	173,438	8.2	384,755	9.1
Unknown or no completed education §	231	0.6	13,615	0.6	15,269	0.7	28,884	0.7

* Includes intermediate level courses based on completed upper secondary level, but which are not accredited as tertiary education
† Comprises higher education up to 4 years in duration.
‡ Comprises higher education more than 4 years in duration.
§ For many immigrants Statistics Norway[7] has no information about their level of education.

Table S 4. Distribution of gross income in the schools' catchment area and total in Norway.

Gross income[8] for 17 years and older males and females in the catchment area and total in Norway, given as number (*n*) and proportions (%).

Gross income (NOK)	The catchment area 2015 (*n* = 39,532)		Norway 2015 (*n* = 4,150,990)	
	n	%	*n*	%
0–99,999	4,239	10.7	494,000	11.9
100,000–199,999	3,799	9.6	409,366	9.9
200,000 –299,999	6,617	16.7	657,469	15.8
300,000–399,999	6,856	17.3	701,412	16.9
400,000–499,999	5,850	14.8	640,716	15.4
≥500,000	12,171	30.8	1,248,027	30.1

Table S 5. Grade point average in our sample and total for the two schools enrolled in the study.

Grade point average (GPA) was calculated as the average of all grades at the end of lower secondary school for each student, with 60.0 as the best GPA possible. This table is showing the number (*n*) and the proportions (%) of GPA for our sample compared with all students who were in their 1st year of the two upper secondary schools in the same time period. Mean (SD) GPA was 40.8 (± 7.8) for our sample and 38.9 (± 8.5) for the 1st year population at the two schools. The majority of our participants finished lower secondary school in 2014 and 2015, and the average GPA for Norway was 40.4 in 2014[9] and 40.8 in 2015.[10]

GPA	Our sample (*n* = 455) *		1st year school population (*n* = 743)	
	n	%	*n*	%
NA	18	4.0	60	8.1
0.0 – 10.0	0	0.0	3	0.4
10.1 – 20.0	2	0.4	10	1.3
20.1 – 30.0	37	8.1	92	12.4
30.1 – 40.0	169	37.1	276	37.1
40.1 – 50.0	168	36.9	234	31.5
50.1 – 60.0	61	13.4	68	9.2

* Age: 16 – 19 yrs (*n* = 439), age: 20 – 24 yrs: (*n* = 16)

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Paper II

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Emmetropia Is Maintained Despite Continued Eye Growth From 16 to 18 Years of Age

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Purpose. To examine, in Norwegian adolescents, to what degree emmetropia and low hyperopia were maintained from 16 to 18 years of age, and if this was the case, whether it was associated with continued coordinated ocular growth.

Methods. Cycloplegic autorefraction and ocular biometry, including crystalline lens thickness, were measured in 93 Norwegian adolescents (mean age: 16.7 ± 0.3 years; 63.4% females) and repeated after 2 years. Crystalline lens power was determined by ray tracing over a 1-mm pupil, based on the Gullstrand-Emsley model. Serum vitamin D_3 concentration was measured at follow-up.

Results. Emmetropia and low hyperopia (−0.50 diopters [D] < spherical equivalent refractive error [SER] < +2.00 D) were present in 91.4% at baseline and 89.2% at follow-up. The emmetropes and low hyperopes who maintained their refractive error exhibited continued ocular axial growth (+0.059 ± 0.070 mm) together with a decrease in crystalline lens power (−0.064 ± 0.291 D) and a deepening of the anterior chamber (+0.028 ± 0.040 mm). Thinning of the crystalline lens was found in 24%. Overall, the negative change in SER was larger in those with the most negative SER at baseline ($R^2 = 0.178$, $P < 0.001$), and was associated with increases in vitreous chamber depth and in crystalline lens power ($R^2 = 0.752$, $P < 0.001$), when adjusted for sex. There was no difference in vitamin D_3 level between those who exhibited negative versus positive changes in refractive error.

Conclusions. The results show that emmetropic and low hyperopic eyes were still growing in late adolescence, with refractive errors being maintained through a coordinated decrease in crystalline lens power.

Keywords: emmetropia, refractive errors, ocular growth, ocular biometry, crystalline lens

Children, who are usually moderately hyperopic at birth, become gradually less hyperopic, through coordinated eye growth (emmetropization) during infancy and young childhood.[1,2] The natural endpoint of emmetropization may be emmetropia or perhaps low hyperopia,[3] as low hyperopia offers better protection against myopia. Maintaining emmetropia and low hyperopia through continued eye growth requires coordinated changes in the refractive power of the eye. Sorsby et al.[4] suggested coordinated axial growth to cease by 13 years of age, when distinguished from axial growth associated with myopia, and emmetropic growth curves of axial length and refractive components have been reported for children up to 12 to 14 years of age.[5,6] Two studies have reported longitudinal changes in cycloplegic ocular components in young nonmyopic university students 18 to 23 years of age ($n = 25$; contains no data on crystalline lens power)[7] and 20 to 23 years of age ($n = 76$).[8,9] A cross-sectional study in China indicated changes in crystalline lens power to reach a plateau after 14 years of age, independent of refractive error,[10] while others have suggested crystalline lens changes to compensate for axial growth also in young emmetropic adults.[8] There appear to be no longitudinal studies on ocular growth and crystalline lens power in 16- to 18-year-old emmetropic and low hyperopic adolescents who are in a high-performing school system (as defined by OECD[11]).

The aim of this study was to examine to what degree emmetropia and low hyperopia were maintained from 16 to 18 years of age in a group of students in a high-performing school system in Norway with high usage of near electronic devices (see Ref. 12 for more details). The hypothesis was that emmetropia and low hyperopia would be maintained by coordinated growth of the ocular components and, contrary to the suggestion of Sorsby et al.,[4] this process continues throughout adolescence. Furthermore, larger negative changes in refractive error may be found in those who, at baseline, had a more negative refractive error, as reported for younger age groups.[13]

Methods

Participants

A predominantly low hyperopic refractive error (mean spherical equivalent refractive error [SER] = +0.59 ± 1.23 diopters [D]) was found in 16-year-olds in a cross-sectional study performed in a representative region of Norway in 2015

and 2016 ($n = 246$),[12] and all of those who were still students at upper-secondary school in 2018 were invited to participate in a follow-up study ($n = 120$). Of these 120 participants, 93 (77.5%; 16.7 ± 0.3 years; 63.4% females) gave consent for further participation. There was no difference between this sample ($n = 93$) and the original sample of 16-year-olds ($n = 246$) when comparing the frequency of refractive errors [7.5% vs. 11.0% myopia, 57.0% vs. 57.7% hyperopia, $\chi^2(2) = 1.171$, $P = 0.56$], mean SER [+0.49 ± 0.94 D vs. +0.59 ± 1.23 D, Welch $t(216.2) = -0.780$, $P = 0.44$], mean ocular axial length [AL; 23.6 ± 0.7 mm vs. 23.4 ± 0.8 mm, $t(337) = 1.663$, $P = 0.10$], ethnicity [87.1% vs. 91.1% Norwegian Caucasian, $\chi^2(1) = 1.173$, $P = 0.28$], or sex [63.4% vs. 56.5% females, $\chi^2(1) = 1.337$, $P = 0.25$]. Baseline and follow-up data were collected in March 2016 and over 16 days from January 24 to February 9, 2018, respectively. The majority of participants were Norwegian Caucasians who had grown up in Norway (87.1%). Other ethnicities were Asian (6.5%), African (2.2%), South American (1.1%), and mixed (3.2%; defined as having parents of two different ethnicities). The sample included three participants (3.2%) with a known history of strabismus and five participants (5.4%) who reported having one of the following conditions: factor V Leiden thrombophilia, anti-NMDA (N-methyl-D-aspartate) receptor encephalitis, diabetes, scoliosis, and thalassemia. Removing these from the sample did not affect the results.

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics in Southeast Norway. All participants gave written informed consent after explanation of the nature and possible consequences of the study.

Data Collection

Identical protocols were followed at baseline and follow-up with respect to measurement of cycloplegic ocular biometry and autorefraction, as presented previously.[12] For completeness with regard to methods, cycloplegic ocular biometry and autorefraction data were obtained with Zeiss IOLMaster 700 (Carl Zeiss Meditec AG, Jena, Germany; keratometer refractive index 1.3375) and Huvitz autorefractor (HRK-8000A Auto-REF Keratometer; Huvitz Co. Ltd., Gyeonggi-do, Korea) at 13.5-mm vertex distance 15 to 20 minutes after administering 1% cyclopentolate hydrochloride (Minims single dose; Bausch & Lomb UK Ltd., Kingston, England). One drop of cyclopentolate was used if the participant's irides were blue to green and two drops if they were green to brown. The IOLMaster 700 is known to have high measurement repeatability.[14–16] Body height was measured to the nearest 0.1 cm, in a standing position without shoes, with the Seca 217 stable stadiometer for mobile height measurement (Seca Deutschland, Hamburg, Germany).

Individual Eye Models

Crystalline lens power (LP; also commonly abbreviated as P_L) was calculated from individual three-surface biconic eye models based on the Gullstrand-Emsley model constructed by ray tracing in Optic Studio v.14.2 (Zemax LLC, Kirkland, WA, USA). The set of models were calculated using a biconic (toric) cornea, with measured corneal curvature in the steepest and flattest meridians along with the corresponding axis, and the measured cycloplegic spherocylindrical refractive error (sphere, cylinder, and axis) at a 13.5-mm vertex distance. The parameters anterior corneal radius of curvatures and axis (CR_1, CR_2, Axis), anterior chamber depth (ACD), crystalline lens thickness (LT), and AL were taken from the measured biometry data. Per the Gullstrand-Emsley model,[17] the refractive index was set to 1.416 for the crystalline lens and 1.333 for the cornea, aqueous chamber, and vitreous chamber. Front- and back-surface crystalline lens curvatures were optimized through a Zemax merit function utilizing the built-in Damped Least Squares algorithm[18] to minimize the root-mean-square wavefront error (ray tracing of a bundle of rays over a 1-mm pupil diameter) and give the best focus at the retina, while forcing the same ratio of crystalline lens surface powers to that of its total equivalent power (38.0% for the front surface, 63.3% for the back surface) as in the Gullstrand-Emsley model (per Bennett[19] and done in the same way as in Li et al.[20]). Zemax files are available online.[21] LP was calculated from the optimized front- and back-surface crystalline lens curvatures, and corneal power was derived from mean anterior corneal radius of curvature [$CR = (CR_1 + CR_2) / 2$]. Central corneal thickness (CCT) was measured by IOLMaster, whereas vitreous chamber depths (VCD = AL − ACD − LT) were calculated from the measured data.

Vitamin D_3

Serum vitamin D_3 concentration was measured at follow-up by collecting blood samples by the dried blood spot technique (DBS). The samples were analyzed by Vitas AS (Oslo, Norway), where serum concentrations of 25-hydroxycholecalciferol [s-25(OH)D_3] were estimated by the LC-MS/MS DBS method with a detection limit of 5 nM, as described elsewhere.[22]

Statistical Analysis

Data were analyzed with the statistical computing software R, version 3.4.0,[23] and data for the right eyes were used in the analysis. SER was estimated as sphere + ½ cylinder. Moderate/high hyperopia was defined as SER ≥ +2.00 D, low hyperopia as +0.50 D ≤ SER < +2.00 D, emmetropia as −0.50 D < SER < +0.50 D, and myopia as SER ≤ −0.50 D. Persistent emmetropes/low hyperopes were defined as those with astigmatism lower than 1.00 diopter cylinder (DC) who maintained emmetropia or low hyperopia throughout the study period. Decline of hyperopia was calculated as the proportion of participants who were not hyperopic at follow-up but were at baseline. Incidence of myopia was calculated as the proportion of myopic participants at follow-up who were not myopic at baseline. Annual decline and incidence rates were found by dividing the proportions by the study period in years.

The χ^2 test was used to assess differences in prevalence between groups. Mean differences between baseline and follow-up data were examined by paired *t*-test; between-group differences were examined using 1-way analysis of variance and Student's or Welch's two independent sample *t*-tests for equal or unequal variances, respectively, and Wilcoxon rank sum test for nonnormal data. Kruskal-Wallis and Bonferroni corrected pairwise comparison by Wilcoxon were used to assess differences in SER and ocular biometric parameters between the refractive error groups. Pearson correlation (r_P) and multiple linear regressions were used to assess associations between SER and ocular biometric parameters. Significance level was set at $\alpha = 0.05$.

Results

Changes in Refractive Error

As summarized in Table 1, our sample population of Norwegian adolescents showed relatively stable refractive errors over the study period, with emmetropia and low hyperopia representing 91.4% of participants at baseline (16 years of age) and 89.2% at follow-up (18 years of age). The

Table 1. Frequency of Refractive Errors (%) at Baseline and Follow-Up, Grouped by Sex

	All, $n = 93$		Females, $n = 59$		Males, $n = 34$	
Refractive Error	Baseline	Follow-Up	Baseline	Follow-Up	Baseline	Follow-Up
Moderate/high hyperopia	1.1	1.1	1.7	1.7	0.0	0.0
Low hyperopia	55.9	54.8	59.3	57.6	50.0	50.0
Emmetropia	35.5	34.4	30.5	28.8	44.1	44.1
Myopia	7.5	9.7	8.5	11.9	5.9	5.9

annual decline of hyperopia was 4.7%, and the annual incidence of myopia was 1.2%.

Figure 1 shows the SERs at baseline and follow-up. The changes in SER were, in general, minor (mean ± SD: –0.089 ± 0.206 D; range, –0.67 to +0.40), and only 19% exhibited a negative change larger than 0.25 D. Most (77%) participants maintained their baseline refractive error, as defined by change in SER between –0.25 and +0.25 D over 2 years.

The multiple linear regression models in Table 2 show that those with the most negative SER at baseline had the largest negative changes in SER when adjusted for sex (model A: $R^2 = 0.178$, $P < 0.001$; see also the unadjusted regression line in Fig. 1), and the change in SER was also predicted by baseline AL and CR (model B: $R^2 = 0.148$, $P = 0.003$) and by baseline AL/CR (model C: $R^2 = 0.129$, $P = 0.002$). In line with this, change in SER, change in AL, and change in VCD correlated with baseline SER (change in SER: $r_P = 0.375$, $P < 0.001$; change in AL: $r_P = -0.355$, $P < 0.001$; change in VCD: $r_P = -0.404$, $P < 0.001$).

Changes in Ocular Biometric Parameters in Persistent Emmetropes/Low Hyperopes

Throughout the study period, 83 participants (89.2%) maintained emmetropia or low hyperopia, and 75 of these (80.6% of all) had astigmatism lower than 1.00 DC at both baseline and follow-up. The latter group is, from here onward, termed persistent emmetropes/low hyperopes.

Table 3 summarizes the 2-year changes in SER and ocular biometric parameters in the persistent emmetropes/low hyperopes. This group exhibited an increase in mean LT, ACD, VCD, AL, and AL/CR ($P \leq 0.05$), a decrease in mean LP ($P = 0.06$), and a negative change in mean SER ($P = 0.01$). There was a slight thinning of mean CCT ($P < 0.001$), but no change in mean CR ($P = 0.36$). The continued elongation of mean AL (+0.059 ± 0.070 mm) equals a –0.149 D change in SER (it is assumed that 1-mm increase in AL equals –3.05 D change in SER as estimated from baseline data), mainly compensated for by a decrease in mean LP (–0.064 ± 0.291 D) and deepening of mean ACD (+0.028 ± 0.040 mm). The negative correlation between changes in AL and LP was significant ($r_P = -0.314$, $P = 0.006$). When compared with the myopes (Table 3), the group of persistent emmetropes/low hyperopes had a smaller increase in mean VCD, AL, and AL/CR ($P \leq 0.002$), and had a smaller negative change in mean SER ($P = 0.001$), but did not differ in change of mean CCT, CR, ACD, LT, or LP (all $P > 0.05$). Table 4 summarizes the 2-year changes in SER and ocular biometric parameters for all participants grouped by refractive error at baseline.

LT increased for 76% of the persistent emmetropes/low hyperopes (79% of all emmetropes and hyperopes; range, +0.003 to +0.058 mm), with the remainder exhibiting a decrease in LT (range, –0.001 to –0.065 mm). LT increased for all myopes (range, +0.012 to +0.048 mm). The increase in LT was larger than 0.02 mm for 45% of the persistent emmetropes/low hyperopes and 86% of the myopes.

Associations Between Changes in Refractive Error and Ocular Biometric Parameters

As shown in Table 5, a negative change in SER was associated with increased AL and increased LP in a multiple linear

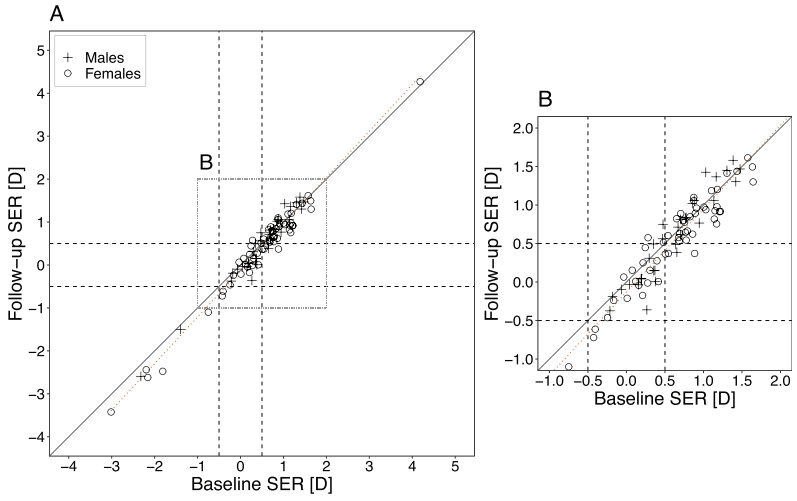

Figure 1. Scatterplots of SER at baseline versus 2-year follow-up for (**A**) all participants and (**B**) the subgroup of participants who had –1.00 D ≤ SER ≤ +2.00 D (for better visualization of the individual data points within the squared area marked by *dotted gray lines* in [**A**]). *Crosses* and *circles* represent males and females, respectively. The *dashed black lines* show the defined limits of myopia (SER ≤ –0.50 D) and hyperopia (SER ≥ +0.50 D), and the *solid black line* and the *dotted red line* indicate a 1:1 relationship and the linear regression, respectively, between SER at baseline and follow-up. All below the *solid black line* exhibited a negative change in SER.

Table 2. Multiple Linear Regression Models Predicting Changes in SER, Adjusted for Sex

	Model A, $R^2 = 0.178$, adj $R^2 = 0.160$				Model B, $R^2 = 0.148$, adj $R^2 = 0.119$				Model C, $R^2 = 0.129$, adj $R^2 = 0.110$			
Variables	Estimate	2.5%–97.5% CI	P	Variables	Estimate	2.5%–97.5% CI	P	Variables	Estimate	2.5%–97.5% CI	P
Intercept	−0.079	−0.15 to −0.01	**0.02**	(Intercept)	1.013	−0.46 to 2.49	0.18	(Intercept)	2.367	0.84 to 3.89	**0.003**
Sex, females	−0.082	−0.16 to 0.00	**0.05**	Sex, females	−0.118	−0.20 to −0.03	**0.008**	Sex, females	−0.107	−0.19 to −0.02	**0.01**
SER baseline	0.084	0.04 to 0.13	**<0.001**	AL baseline	−0.127	−0.20 to −0.05	**0.001**	AL/CR baseline	−0.796	−1.30 to −0.29	**0.002**
				CR baseline	0.251	0.04 to 0.46	**0.02**				

Significant P values (<0.05) in bold. adj, adjusted; CI, confidence interval.

regression model in the group of persistent emmetropes/low hyperopes ($R^2 = 0.705$, $P < 0.001$) and overall ($R^2 = 0.727$, $P < 0.001$), adjusted for sex. To elucidate the differences in each of the refractive error groups, changes in SER were plotted as a function of changes in AL (Fig. 2A) and changes in LP (Fig. 2B). There was a clear negative correlation between changes in SER and AL regardless of refractive error (Fig. 2A: hyperopes: $r_P = -0.408$, $P = 0.002$, emmetropes: $r_P = -0.777$, $P < 0.001$, myopes: $r_P = -0.604$, $P = 0.15$). There was also a clear negative correlation between changes in SER and LP in hyperopes (Fig. 2B: $r_P = -0.479$, $P < 0.001$), but not in emmetropes ($r_P = -0.165$, $P > 0.05$) or myopes ($r_P = -0.099$, $P > 0.05$). The dotted lines in Figure 2 show the calculated change in SER per 1-mm increase in AL (−3.05 D/mm; Fig. 2A), and the change in SER per diopter increase in LP (−1 D per diopter; Fig. 2B). The increase in AL associated with a negative change in SER in hyperopes (open diamonds, dashed line in Fig. 2A) was small compared with myopes and emmetropes. Hyperopes who had a negative change in SER (i.e., a change that brings them nearer to emmetropia) had an increase in LP (dashed line in Fig. 2B nearly overlaps with dotted line). Change in SER did not correlate with change in ACD, change in CR, or change in LT (all $P > 0.05$).

Figure 3 shows change in LP as a function of change in AL for those who had a positive (Fig. 3A) or a negative (Fig. 3B) change in SER. Overall, changes in LP and AL were negatively correlated ($r_P = -0.408$, $P < 0.001$). However, those with a positive change in SER (change > 0.002 D; Fig. 3A, $n = 35$) had a significantly smaller increase in mean AL (+0.04 ± 0.06 mm vs. +0.09 ± 0.08 mm, $P < 0.001$) and larger decrease in mean LP (−0.20 ± 0.23 D vs. +0.00 ± 0.30 D, $P = 0.001$) than those with a negative change in SER (change < −0.002 D; Fig. 3B, $n = 58$). Those who had a positive change in SER larger than 0.25 D (Fig. 3A, filled symbols, $n = 3$; −0.03 ± 0.05 mm) had a decrease in mean AL, which was opposite and significantly different from those who had a negative change in SER larger than 0.25 D (Fig. 3B, filled symbols, $n = 18$), in which mean AL increased (+0.16 ± 0.08 mm, Wilcoxon $P = 0.002$).

Nine participants (9.7% of all; seven persistent emmetropes/low hyperopes) decreased more than 0.02 mm in AL (≥95% limits of reproducibility of the IOLMaster 700[16]); seven of these had positive changes in SER (two with positive changes in SER larger than 0.25 D).

Associations of Crystalline Lens Power With Lens Thickness and Ocular Axial Length

The multiple linear regression presented in Table 6 shows that having a strong LP was associated with having a short AL and a thick LT, when adjusted for sex [model A (baseline): $R^2 = 0.645$, $P < 0.001$; model B (follow-up): $R^2 = 0.646$, $P < 0.001$]. Figure 4 shows that LP and AL were negatively correlated for emmetropes (baseline $r_P = -0.763$, $P < 0.001$; follow-up $r_P = -0.759$, $P < 0.001$) and hyperopes (baseline $r_P = -0.625$, $P < 0.001$; follow-up $r_P = -0.638$, $P < 0.001$), but not for myopes (baseline and follow-up; $P > 0.05$). LP correlated with LT (baseline $r_P = 0.589$, $P < 0.001$; follow-up $r_P = 0.577$, $P < 0.001$), but there was no correlation between changes in LP and LT ($P > 0.05$). There was neither any correlation between SER and LP or between change in SER and baseline LT or LP.

Body Height

From baseline to follow-up, body height increased 1.7 ± 1.3 cm (range, −0.7 to 4.1 cm) in males and 0.9 ± 0.8 cm (range, −0.6 to 3.3 cm) in females. Change in height did not correlate

Table 3. Change in SER and Ocular Biometric Parameters for Persistent Emmetropes/Low Hyperopes and for Baseline Myopes. Data Are Presented as Mean ± SD. The Mean Change From Baseline to Follow-Up for the Group of Persistent Emmetropes/Low Hyperopes Was Assessed by Paired Sample t-Test and Presented as P_{change}. P_W Indicates the Difference Between the Group of Persistent Emmetropes/Low Hyperopes and the Group of Myopes, Assessed by Wilcoxon Rank Sum Test

Parameters	Persistent Emmetropes/ Low Hyperopes, $n = 75$	P_{change}	Myopes, $n = 7$	P_W
SER, D	−0.058 ± 0.195	**0.01**	−0.358 ± 0.180	**0.001**
Sphere, D	−0.040 ± 0.201	0.09	−0.460 ± 0.230	**<0.001**
Cyl, DC	−0.035 ± 0.200	0.13	+0.203 ± 0.275	**0.02**
CR, mm	+0.002 ± 0.022	0.36	+0.000 ± 0.022	0.95
CCT, mm	−0.004 ± 0.004	**<0.001**	−0.003 ± 0.004	0.42
ACD, mm	+0.028 ± 0.040	**<0.001**	+0.004 ± 0.021	0.10
LT, mm	+0.015 ± 0.023	**<0.001**	+0.029 ± 0.011	0.07
LP, D	−0.064 ± 0.291	0.06	−0.154 ± 0.264	0.51
VCD, mm	+0.017 ± 0.071	**0.05**	+0.145 ± 0.068	**<0.001**
AL, mm	+0.059 ± 0.070	**<0.001**	+0.178 ± 0.079	**<0.001**
AL/CR	+0.007 ± 0.012	**<0.001**	+0.023 ± 0.014	**0.002**

Significant P values (<0.05) in bold. Cyl, cylinder power.

Table 4. Change in SER and Ocular Biometric Parameters for All Participants Grouped by Refractive Status at Baseline. Data Are Presented as Mean ± SD. P_K Indicates the Difference Between the Groups, Assessed by Kruskal-Wallis

Parameters	Hyperopes, $n = 53$	Emmetropes, $n = 33$	Myopes, $n = 7$	P_K
SER, D	−0.042 ± 0.184*	−0.109 ± 0.203*	−0.358 ± 0.180	**0.001**
Sphere, D	−0.017 ± 0.191*	−0.071 ± 0.220*	−0.460 ± 0.230	**<0.001**
Cyl, DC	−0.050 ± 0.205	−0.077 ± 0.263*	+0.203 ± 0.275	**0.04**
CR, mm	+0.005 ± 0.023	−0.004 ± 0.021	+0.000 ± 0.022	0.23
CCT, mm	−0.004 ± 0.004	−0.003 ± 0.005	−0.003 ± 0.004	0.38
ACD, mm	+0.028 ± 0.041	+0.025 ± 0.036	+0.004 ± 0.021	0.26
LT, mm	+0.017 ± 0.021	+0.013 ± 0.025	+0.029 ± 0.011	0.14
LP, D	−0.003 ± 0.295	−0.171 ± 0.275†	−0.154 ± 0.264	**0.03**
VCD, mm	−0.000 ± 0.060*	+0.051 ± 0.081*†	+0.145 ± 0.068	**<0.001**
AL, mm	+0.045 ± 0.061*	+0.089 ± 0.081*†	+0.178 ± 0.079	**<0.001**
AL/CR	+0.004 ± 0.011*	+0.013 ± 0.013†	+0.023 ± 0.014	**<0.001**

Significant P values (<0.05) in bold.
* Data that are significantly different from the myopes ($P < 0.05$; Bonferroni corrected pairwise comparison by Wilcoxon).
† Significant difference between emmetropes and hyperopes ($P < 0.05$; Bonferroni corrected pairwise comparison by Wilcoxon).

with change in AL, SER, or AL/CR. Mean body height at baseline and follow-up, respectively, were 177.4 ± 5.5 and 179.1 ± 5.8 cm in males and 167.9 ± 5.0 and 168.8 ± 5.0 cm in females.

Serum Vitamin D_3 Concentration

Mean s-25(OH)D_3 concentration was 50.9 ± 19.2 nM (range, 16.9–107.3 nM), as measured in 89 of 93 participants at follow-up. Overall, 41.6% had sufficient levels (≥50.0 nM), 55.1% were mildly deficient (25.0–49.9 nM), 3.4% were moderately deficient (12.5–24.9 nM), and none were severely deficient (<12.5 nM).[22] Those with a negative change in SER larger than 0.25 D did not differ in mean s-25(OH)D_3 concentration from the others ($n = 18$ vs. 71; 51.2 ± 17.9 nM vs. 50.8 ± 19.7 nM, $P = 0.89$), and there was no difference between those with positive versus negative changes in SER ($n = 33$ vs. 56; 50.7 ± 19.6 nM vs. 51.0 ± 19.2 nM, $P = 0.93$). Median s-25(OH)D_3 concentration was significantly higher in nonmyopes than myopes ($n = 80$ vs. 9; median 47.7 nM vs. 33.0 nM; $W = 525$, $P = 0.03$).

Discussion

This is the first report on longitudinal changes in refractive errors and ocular biometric parameters in a population of adolescents who had low myopia prevalence and were students in a high-performing education system. The main result was that emmetropic and low hyperopic eyes were still exhibiting coordinated ocular growth at 18 years of age. A stable refractive error was maintained through continued ocular axial growth and coordinated decrease in mean LP. Although the majority showed crystalline lens thickening, lens thinning appeared to still take place in some of the 18-year-old emmetropes and low hyperopes. Those with a more negative refractive error at baseline exhibited larger negative changes in refractive error over the 2-year period, and the negative changes were associated with excessive elongation of VCD and increase in LP.

The annual axial length growth in persistent emmetropes/low hyperopes at 18 years of age (median: +0.03 mm, range, −0.05 to +0.16) contradicts the report of Sorsby et al.,[4] who suggested ocular axial growth to cease at 13 years of age, and confirms findings reported for a small sample of Danish emmetropes aged 16 to 20 years ($n = 16$; median: +0.05 mm).[24] It is important to note that most participants appeared to have reached full adult height at 18 years of age,[25,26] and no association was found between increases in height and changes in AL from 16 to 18 years of age. The annual percentage change in axial length (0.13%) was lower than reported from 11 to 14 years of age in a study of emmetropic schoolchildren (range, 0.24–0.28% per year).[27] Thus, continued coordinated eye growth in late adolescents is at a slower

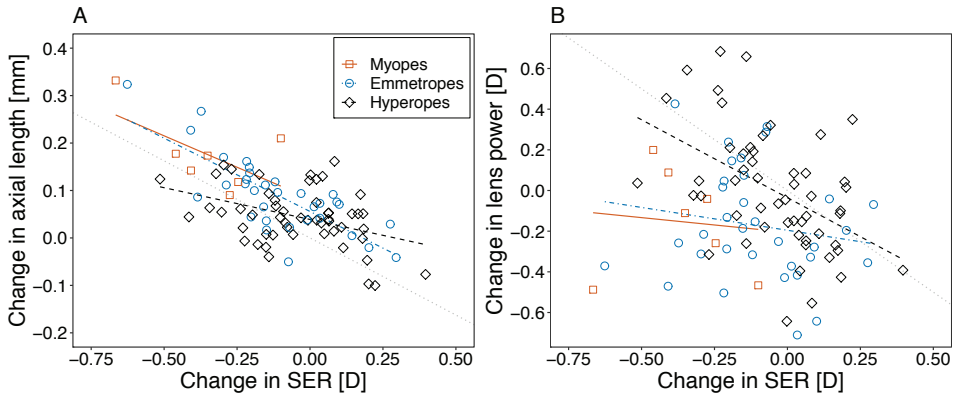

Figure 2. Relationship between change in SER (D) and (**A**) change in AL (mm) and (**B**) change in LP (D). The *gray dotted lines* show the calculated change in SER per 1-mm increase in AL (−3.05 D/mm as calculated from the baseline data) in (**A**), and the change in SER per diopter increase in LP (−1 D per diopter) in (**B**). The symbols represent the refractive status at baseline, and the lines represent the linear regression per refractive error group (*red square/solid line*: myopes, *blue circle/dot dashed line*: emmetropes, *black diamond/dashed line*: hyperopes).

Table 5. Multiple Linear Regression Showing the Association of Changes in SER With Changes in AL and LP, Adjusted for Sex. Model A Is Based on Data From Persistent Emmetropes/Low Hyperopes ($n = 75$), and Model B Is Based on All Participants ($n = 93$)

	Model A, $R^2 = 0.705$, adj $R^2 = 0.693$			Model B, $R^2 = 0.727$, adj $R^2 = 0.717$		
Variables	**Estimate**	**2.5%–97.5% CI**	***P***	**Estimate**	**2.5%–97.5% CI**	***P***
Intercept	0.048	0.00 to 0.09	**0.03**	0.050	0.01 to 0.09	**0.02**
Sex, females	−0.002	−0.05 to 0.05	0.95	−0.007	−0.05 to 0.04	0.79
AL change	−2.216	−2.60 to −1.84	**<0.001**	−2.361	−2.68 to −2.04	**<0.001**
LP change	−0.413	−0.51 to (−0.32)	**<0.001**	−0.416	−0.50 to (−0.33)	**<0.001**

Significant *P* values (<0.05) in bold.

Table 6. Multiple Linear Regression Showing the Association of LP With AL and LT at Baseline (Model A) and at Follow-Up (Model B), Adjusted for Sex

	Model A, Baseline, $R^2 = 0.645$, adj $R^2 = 0.633$			Model B, Follow-Up, $R^2 = 0.646$, adj $R^2 = 0.634$		
Variables	**Estimate**	**2.5%–97.5% CI**	***P***	**Estimate**	**2.5%–97.5% CI**	***P***
Intercept	32.562	24.76 to 40.36	**<0.001**	32.867	25.00 to 40.74	**<0.001**
Sex, females	0.491	0.10 to 0.88	**0.01**	0.591	0.19 to 0.99	**0.004**
AL	−0.989	−1.25 to −0.72	**<0.001**	−1.001	−1.27 to −0.74	**<0.001**
LT	3.881	2.83 to 4.93	**<0.001**	3.838	2.77 to 4.91	**<0.001**

Significant *P* values (<0.05) in bold.

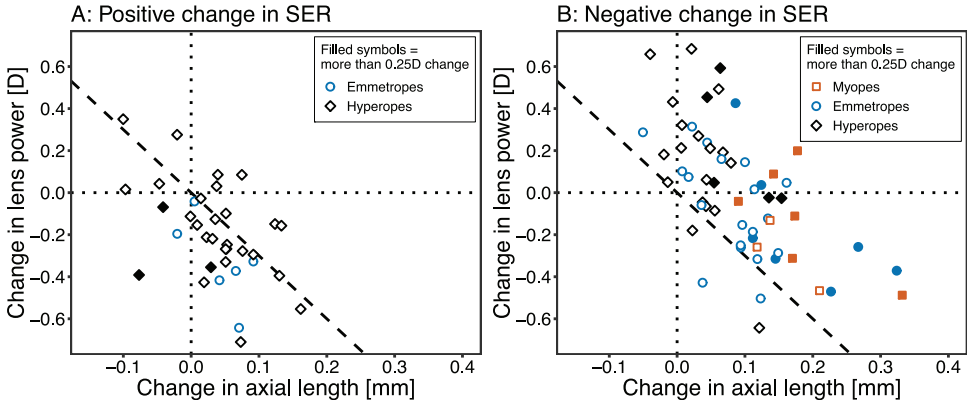

Figure 3. Relationship between change in LP (D) and change in AL (mm) for those who had a (**A**) positive change in SER (>0.002 D; $n = 35$) and (**B**) negative change in SER (< −0.002 D; $n = 58$). The *black dashed lines* show the change in LP per 1-mm increase in AL required to maintain no change in SER (as in Fig. 2), assuming no changes in the other ocular components. Symbols represent the refractive status at follow-up (*square*: myopes, *circle*: emmetropes, *diamond*: hyperopes), and filled symbols represent those with a positive change of more than 0.25 D (in **A**) and negative change of more than 0.25 D (in **B**).

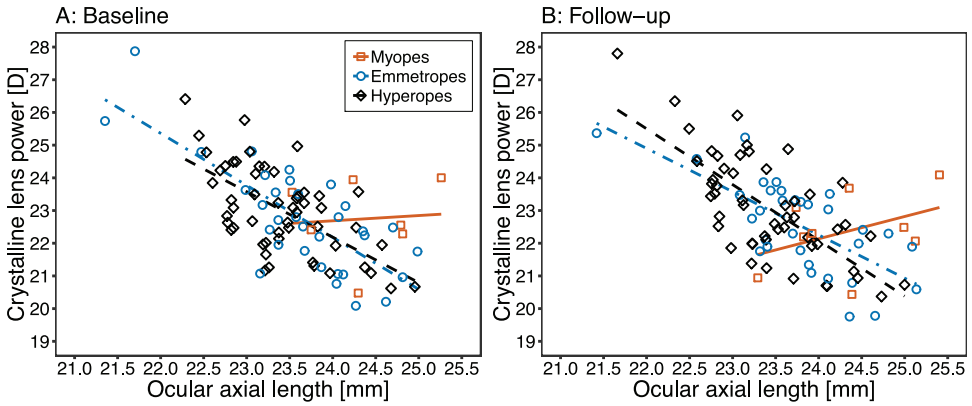

Figure 4. Relationship between LP (D) and AL (mm) at (**A**) baseline and (**B**) follow-up. The symbols represent the refractive error status, and the lines represent the linear regression per refractive error group (*red square/solid line*: myopes, *blue circle/dotdashed line*: emmetropes, *black diamond/dashed line*: hyperopes).

Table 7. Annual Incidence of Myopia, Mean Change in SER (Autorefraction), and Myopia Prevalence for Present Study Compared With Others. In All Studies, Cyclopentolate 1%[35,38] or a Combination of Cyclopentolate 1% and Tropicamide 1%[36] Was Used for Accommodation Control. Myopia Was Defined as SER ≤ −0.50 D and Hyperopia as SER ≥ +0.50 D, If Not Otherwise Noted

Country	Ethnicity	Age, Years	*n*	Annual Incidence of Myopia, %	Mean Annual Change in SER, D			Myopia Prevalence, %	At Age, Years
					Overall	Myopes	Hyperopes		
Norway, present study	87.1% Caucasian	16–18	93	1.2	−0.04	−0.18	−0.02	9.7	18
Australia[36]	Caucasian	12–17	684	2.9	−0.11	−0.3	NA	17.7	17
Northern Ireland[38]	Caucasian	12–13 to 18–20	226	0.7	NA	−0.09	+0.02*	18.6	18–20
Australia[36]	East Asian	12–17	232	7.3	−0.21	−0.3	NA	59.1	17
China[35]	Chinese	18.3–20.3†	2053	13.0‡	−0.16	−0.18	−0.11	78.5	18.3 ± 1.8

* Hyperopia defined as SER ≥ +2.00 D. By this definition in current study, only one was hyperope at baseline and at follow-up. Annual change in SER was +0.04 D.

† Medical university students.[35]

‡ Calculated as the proportion of myopes at follow-up who were not myopes at baseline, divided by 2-year study period.

rate than that observed for children. The continued elongation of AL was mainly compensated for by a decrease in LP and a deepening of the anterior chamber. The latter is known to reduce the effect of LP.[28] The annual loss in mean LP (–0.037 ± 0.15 D per year) was the same as that inferred in a cross-sectional study of mainly myopic Chinese adolescents from 14 to 18 years of age (annual decrease –0.038 D).[10] The crystalline lens thickened for all myopes; however, 24% of the persistent emmetropes/low hyperopes exhibited up to –0.07 mm thinning. Assuming that the repeatability limit for the IOLMaster measurement of LT is ±0.02 mm,[16] 86% of the myopes increased more than 0.02 mm in lens thickness compared with 45% of the persistent emmetropes/low hyperopes. The lens is known to become thinner throughout childhood, reaching a minimum before reversing direction to become thicker, with earlier reversal time being associated with earlier myopia onset.[29] The data presented here confirm that a delay in minimum lens thickness offers protection against myopia, not only in children up to the age 14 years,[29] but also in adolescents up to age 18 years. This adds support to the theory that the balance between correlated developmental changes of the crystalline lens and AL is paramount for maintaining emmetropia.[29,30] Crystalline lens development has indeed been reported to be one of several genetic pathways implicated in myopia pathogenesis.[29,31] That emmetropia is maintained by loss of LP as the eye grows is also indicated from the correlation between the increase in AL and decrease in LP (see Fig. 3). This is further supported by the negative correlation between AL and LP in emmetropes and hyperopes reported here (see Fig. 4) and as reported for Chinese eyes with axial lengths less than 25 mm (age 6–18 years).[10] The weaker association between AL and LP in myopes in this study and in Chinese eyes that were longer than 25 mm[10] may be related to restricted equatorial growth of the crystalline lens in myopes with long eyes, perhaps because of abnormally thicker and longer ciliary muscles,[10,32,33] or to the idea that myopes with long eyes have reached a limit in LP loss because of the internal structure of the lens (e.g., the gradient refractive index profile has reached a maximal rate of increase).[10,34]

The results support the theory that low hyperopia may be the preferred endpoint of refractive development.[3] Only 19% of participants exhibited negative changes in SER larger than 0.25 D over 2 years. The negative changes in SER were larger in those with a more negative refractive error at baseline, as reported for other populations,[9,35,36] and in line with the CLEERE study that reported a less hyperopic (more myopic) refractive error in a child to be the best predictor of future myopia.[13] Negative changes in SER were also associated with having a longer eye at baseline, but there were no associations with baseline crystalline LT or LP, contrary to studies in younger children that have reported both a long eye and a thin and weak crystalline lens as risk factors for developing myopia.[13,37] The annual incidence of myopia and the negative change in SER (see Table 7) were comparable with reports of Caucasians in Northern Ireland from 12 to 13 through to 18 to 20 years of age,[38] but lower than reported for Caucasians and East Asians in Australia from 12 to 17 years of age[36] and for slightly older university students in China.[35]

More than 55% of the participants had lower serum vitamin D_3 concentration than the recommended level of ≥50 nM,[22] and there was no difference in serum vitamin D_3 concentration between those with positive versus negative changes in refractive error. The absence of any apparent correlation with serum vitamin D_3, combined with the low myopia frequency seen in this population,[12] questions the suggested association between low vitamin D_3 levels and increased risk of myopia development.[39,40] That myopes had lower vitamin D_3 concentration than nonmyopes may be related to the myopes preferring indoor activities, as a consequence of their refractive error. This is in line with Norwegian 16- to 19-year old myopes who reported spending more time indoors in the summer holiday and spending less time doing sports outdoors than nonmyopes.[12]

A small number of participants ($n = 9$) decreased in AL beyond the 95% limits of reproducibility of the IOLMaster 700 (±0.02 mm),[16] as has been reported for some Northern Irish adolescents.[41] This may partly be related to thickening or diurnal variations of the choroid.[42] However, shrinkage of AL in response to myopia defocus, beyond what could be explained by measurement errors and thickening of the choroid, has been reported in animals of various species,[43] and following atropine treatment in children.[44,45]

Strengths of current study were cycloplegic measures of both biometry and autorefraction, ensuring minimal influence from accommodation, and use of the exact same instruments to measure cycloplegic refractive errors and ocular biometric parameters at both baseline and follow-up, avoiding a possible source of measurement errors. Limitations were related to implementing the Gullstrand-Emsley lens model with a fixed relationship between the crystalline lens surface powers and a fixed equivalent refractive index for the lens rather than gradient indices.[46,47] Any possible inaccuracies due to these simplifications are, however, similar to those in research using Bennett's method for LP calculation,[19] and are expected to affect baseline and follow-up data equally. Ocular biometry measures may have been taken at a different time point during the day at follow-up versus baseline, but any diurnal variation throughout the time interval of measurements (8 AM to 4 PM)

is estimated to be considerably smaller than the changes reported here.[42] The incidence and reduction rates of myopia and hyperopia, respectively, would need to be confirmed in a larger sample. The reliability and repeatability of Huvitz HRK-8000A is not reported; however, measurements from the Huvitz HRK-7000A are shown to have sufficient test–retest reliability and are in agreement with other autorefractors.[48]

The results from this longitudinal study of changes in refractive errors and ocular biometric parameters from 16 to 18 years of age in students in a high-performing education system[12] show continued ocular axial growth in persistent emmetropes/low hyperopes. A stable refractive error was maintained by a coordinated decrease in LP, confirming that lens development may play a pivotal role in protecting against myopia.

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Investigative Ophthalmology & Visual Science

Paper III

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The association between L:M cone ratio, cone opsin genes and myopia susceptibility

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ABSTRACT

In syndromic forms of myopia caused by long (L) to middle (M) wavelength (L/M) interchange mutations, erroneous contrast signals from ON-bipolar cells activated by cones with different levels of opsin expression are suggested to make the eye susceptible to increased growth. This susceptibility is modulated by the L:M cone ratio. Here, we examined L and M opsin genes, L:M cone ratios and their association with common refractive errors in a population with low myopia prevalence. Cycloplegic autorefraction and ocular biometry were obtained for Norwegian genetically-confirmed normal trichromats. L:M cone ratios were estimated from spectral sensitivity functions measured with full-field ERG, after adjusting for individual differences in the wavelength of peak absorption deduced from cone opsin genetics. Mean L:M cone ratios and the frequency of alanine at L opsin position 180 were higher in males than what has been reported in males in populations with high myopia prevalence. High L:M cone ratios in females were associated with lower degree of myopia, and myopia was more frequent in females who were heterozygous for L opsin exon 3 haplotypes than in those who were homozygous. The results suggest that the L:M cone ratio, combined with milder versions of L opsin gene polymorphisms, may play a role in common myopia. This may in part explain the low myopia prevalence in Norwegian adolescents and why myopia prevalence was higher in females who were heterozygous for the L opsin exon 3 haplotype, since females are twice as likely to have genetic polymorphisms carried on the X-chromosome.

1. Introduction

The prevalence of myopia is increasing around the world, including an associated increased risk of myopia-related complications (Holden et al., 2016). Ethnic and regional differences in myopia prevalence are reported, with East Asians (Pan, Ramamurthy, & Saw, 2012; Rudnicka et al., 2016) having a considerably higher prevalence than Caucasians (Hagen et al., 2018; McCullough, O'Donoghue, & Saunders, 2016). There is no general agreement on the etiology of myopia, but eye growth is primarily regulated by visual signals – processed locally – in the retina (Wallman & Winawer, 2004). The cone photoreceptors are the first step in the signalling cascade and, consequently, are likely to play a role in susceptibility to myopia development.

The human retina contains three classes of cone photoreceptor that are sensitive to light of long (L), middle (M) or short (S) wavelengths. The relative number of L and M cones (L:M cone ratio) varies between individuals, and the mean ratio differs between ethnic groups. A mean L:M cone ratio of 2.7:1 (~73% L cones) has been reported in colour normal American Caucasian males (Carroll, Neitz, & Neitz, 2002; Hofer, Carroll, Neitz, Neitz, & Williams, 2005). In East Asians with reported earlier myopia onset and higher myopia prevalence, the mean L:M cone ratio in colour normal males has been reported to be considerably lower than in American Caucasians (Kuchenbecker, Neitz, & Neitz, 2014; Yamauchi, Yatsu, Kuchenbecker, Neitz, & Neitz, 2013). Refraction and vitreous chamber depth are found to be associated with cone ratio in chickens (Gisbert & Schaeffel, 2018). In humans, an association between symmetric L:M cone ratios (near 1:1) and high susceptibility to myopia development has been proposed (Neitz & Neitz, 2015; Zhou, Atchison, Zele, Brown, & Schmid, 2015). There is evidence for this in the fact that there is a lower prevalence of myopia in red-green colour vision deficient students, who have highly skewed L:M cone ratios as a consequence of lacking L or M cones (Ostadimoghaddam et al., 2014; Qian et al., 2009). Further evidence is the association between myopia and rare interchange haplotypes in exon 3 of the L and M cone opsin

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genes at chromosome location Xq28 (designated OPN1LW and OPN1MW, respectively) (Carroll et al., 2012; Greenwald, Kuchenbecker, Rowlan, Neitz, & Neitz, 2017; McClements et al., 2013; Orosz et al., 2017). The highly variable nucleotide sequences in humans control the spectral tuning of the opsin and affect other aspects of protein structure and function, such as proper splicing of exon 3 in the precursor messenger RNA (pre-mRNA) (Greenwald et al., 2017). In syndromic forms of myopia caused by L/M interchange mutations, incorrect exon 3 splicing leads to greatly reduced amount of functional opsin – or no functional opsin at all – in the affected cones. In such a cone mosaic, neighbouring cones will have different levels of opsin expression. A normally functioning cone that is adjacent to a less-than-normally functioning cone will activate ON-bipolar cells even when there is no spatial contrast information in the visual scene/stimulus. Eye growth is, in these cases, suggested to be modulated by erroneous contrast signals produced by a mosaic of cones with different levels of opsin expression. The degree of erroneous signalling and myopia susceptibility depend on how many cones express the mutant opsin (Greenwald et al., 2017; Patterson et al., 2018). We are hypothesizing that it is not unlikely that other opsin gene exon 3 haplotypes with less severe splicing defects could play a role in common myopia (Neitz & Neitz, 2018). If so, heterozygosity of exon 3 haplotypes could increase myopia susceptibility in females resulting in earlier myopia onset (Rudnicka et al., 2016), because females are twice as likely to carry a cone opsin polymorphism on one of their two X-chromosomes. Heterozygosity of opsin haplotypes in females would translate into a retina where there will be patches with two sets of L and/or M cones expressing different haplotypes, and in the case of an exon 3 splicing defect, one set may give rise to less-than-normally functioning opsin.

If the L:M cone ratio and exon 3 haplotypes play a role in susceptibility to myopia (Greenwald et al., 2017; Neitz & Neitz, 2015; Zhou et al., 2015), it follows that L:M cone ratios, on average, may be higher in a population with low myopia prevalence. Furthermore, differences in exon 3 haplotypes may be observed between myopes and non-myopes, as would difference in myopia prevalence between heterozygous and homozygous females. The current study tested these hypotheses. Its aim, therefore, was to examine L and M opsin genes, L:M cone ratios and their association with refractive errors in Norwegian adolescent males and females. This is a population with low myopia prevalence, despite few daylight hours in the autumn-winter period, large amount of time spent indoors doing near work, and having one of the highest performing education systems in the world according to the Organisation for Economic Co-operation and Development (OECD) (Hagen et al., 2018). L:M cone ratios were estimated with full-field ERG flicker photometry. This is an efficient and reliable procedure for measuring L:M cone ratio when corrections are made for individual differences in the wavelength of peak absorption (λ_{max}) of the L cone opsin and the optical density of the lens (Carroll, McMahon, Neitz, & Neitz, 2000; Carroll et al., 2002).

2. Methods

2.1. Participants

One hundred and thirty-six genetically-confirmed normal trichromats [mean (± SD) age: 16.9 (± 1.0) yrs; 60 males] were included in the study. The participants were recruited by invitation, and the inclusion criteria were: Caucasian ethnicity, age 16–19 years, normal colour vision, being healthy with no known ocular abnormalities and no medication, stereo acuity ≤ 120" (TNO-test), and normal corrected visual acuity. This is a representative subsample of the participants who were included in a larger study of refractive errors in 16–19 year old Norwegian upper secondary school students [n = 393, 16.7 (± 0.9) yrs] (Hagen et al., 2018) in terms of sex [44.1% vs 41.2% males; $\chi^2(1) = 0.24$, $p = 0.62$] and proportion of refractive errors within the groups of males [8.3% vs 8.6% myopia; 58.3% vs 57.4% hyperopia; $\chi^2(2) = 0.02$, $p = 0.99$] and females [19.7% vs 15.6% myopia; 51.3% vs 56.3% hyperopia; $\chi^2(2) = 0.86$, $p = 0.65$]. All were Norwegian Caucasians living in Southeast Norway with normal habitual visual acuity both in the right and left eye [mean logMAR −0.01 (SD: 0.12; range: −0.26–0.62) and mean logMAR −0.02 (SD: 0.13; range: −0.24–0.54), respectively]. Huvitz HRK-8000A Auto-REF Keratometer (Huvitz Co. Ltd., Gyeonggi-do, Korea) and Zeiss IOLMaster (Carl Zeiss Meditec AG, Jena, Germany) were used to measure cycloplegic autorefraction and ocular biometry, described in detail elsewhere (Hagen et al., 2018). For validation of ERG measurements and estimates of L:M cone ratios, five red-green colour vision deficient males (13–66 yrs; 3 single gene dichromats) and one protan carrier (27 yrs) were included.

Colour vision status was confirmed in all participants by genetics, as well as by Ishihara (24 pl. ed., Kanehara Trading INC, Tokyo, Japan, 2005) and Hardy-Rand-Rittler pseudo-isochromatic plates (HRR; 4th edition 2002, Richmond Products, Albuquerque, NM). Rayleigh anomaloscopy was performed, as described elsewhere (Dees & Baraas, 2014; Pedersen et al., 2018), in the dominant eye of all red-green colour vision deficient males, the protan carrier and 34 of the normal trichromats (15 males) (HMC Oculus Anomaloscope MR, Typ 47700, Oculus Optikgeräte GmbH, Wetzlar, Germany).

Informed consent was obtained from all participants after explanation of possible consequences of the study and prior to the experiments. The research was approved by the Regional Committee for Medical Research Ethics for the Southern Norway Regional Health Authority and was conducted in accordance with the principles embodied in the Declaration of Helsinki.

2.2. Genetics

All participants gave saliva samples (Oragene-DNA, OG-500, DNA Self-Collection Kit, DNA Genotek Inc., Ottawa, ON, Canada) for genetic analysis of their cone opsin genes. DNA was extracted, the L and M cone opsin genes were amplified by polymerase chain reaction (PCR), and exon 2, 3 and 4 were sequenced by a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), as described previously (Dees, Gilson, Neitz, & Baraas, 2015). Single-nucleotide polymorphisms (SNP) genotyping by Sequenome MassArray (Sequenome Inc., San Diego, CA, USA) was used to analyse the opsin array composition (Davidoff, Neitz, & Neitz, 2016). The amino acids specified at spectral tuning sites were used to determine the peak sensitivities for the L and M cone opsins (Asenjo, Rim, & Oprian, 1994; Neitz & Neitz, 2011). The genetic analyses were performed in the Neitz Lab at University of Washington, Seattle.

2.3. ERG flicker photometry for estimating L:M cone ratios

Spectral sensitivity functions were measured in the dominant eye with full-field ERG flicker photometry at a temporal frequency of 31.25 Hz, using a method described elsewhere (Carroll et al., 2000; Jacobs, Neitz, & Krogh, 1996; McMahon, Carroll, Awua, Neitz, & Neitz, 2008), with a modified version of the instrument described by Carroll et al. (2000). The ERG signals were created by 4 LEDs (Swanson, Ueno, Smith, & Pokorny, 1987) and presented in Maxwellian view through a Meade 30 mm telescope lens. The ERG system was calibrated by measuring the LED wavelength emission profiles with a spectrophotometer (SpectraScan PR650, Photo Research, NY, USA). The intensity of a monochromatic test light was consecutively adjusted until the ERG signal exactly matched that produced by a fixed-intensity reference light (519 nm). The mean intensity from at least three independent measures for each of three test wavelengths (465 nm, 634 nm and 655 nm) was used for further analyses. Photopigment optical density (OD_L and OD_M) was set to 0.35 and 0.22 for the L and M cone opsin, respectively (Carroll et al., 2000), and the data were corrected for lens absorption by an age-dependent lens correction (Pokorny, Smith, & Lutze, 1987). The spectral sensitivity data were then fitted with a

Table 1
Repeatability measurements of the ERG's %L estimates. Individual estimate of %L from three independent ERG measurements on three different days for four normal trichromats; two males (both with single L genes) and two females (one with *identical* L λ_{max} and one with *distinct* L cone opsin λ_{max}). Individual %L estimates were compared with the mean for the three measurements (|%L – mean|). Mean difference of individual estimates from the mean was 2.3%.

Measure no.	Male A: Single L gene		Male B: Single L gene		Female A: *Identical* L λ_{max}		Female B: *Distinct* L λ_{max}	
	%L	|%L – mean|	%L	|%L – mean|	%L	|%L – mean|	%L	|%L – mean|
1	52.9	2.1	55.7	2.1	62.9	0.8	57.7	4.0
2	50.0	0.8	58.9	1.2	61.1	2.6	59.5	2.1
3	49.6	1.2	58.7	0.9	67.0	3.3	67.8	6.1
Mean	50.9	1.4	57.8	1.4	63.7	2.2	61.7	4.1

weighted sum of individualized L and M cone spectral sensitivity functions, based on the genetically confirmed λ_{max} values for L and M, and estimated %L cones was calculated from the L and M weights [100 × L/(L + M)] (Carroll et al., 2000). The root mean squared error of the fit, on a scale from 0 to 1, was less than 0.05 in all participants included. The estimated cone ratios were adjusted by a factor of 1.5, as suggested by Hofer et al. (2005), to correct for the reported larger contribution of each M cone to the ERG signal when comparing with adaptive-optics imaging combined with retinal densitometry. One operator (author LAH) performed all ERG measurements. The test-retest reliability of the ERG system was measured by three independent measures of the L:M cone ratios performed on different days in two male and two female normal trichromats; see results in Table 1. The individual estimate of %L was never more than 6.1% difference from the mean for the three measurements and showed a repeatability variation within ± 2.3% L cones. Cyclopentolate 1% or Tropicamide 0.5% was administered to dilate the test eye prior to measurements. All ERG measurements were made in an illuminated room between 150 and 300 lux.

In the estimate of the individual L:M cone ratio, the genetically confirmed L cone λ_{max} was used for all normal trichromatic males (n = 60; all had single L genes) and for all normal trichromatic females who had L cone opsin genes encoding spectrally *identical* L cone opsins in the two X-chromosomes (n = 33). A group of normal trichromatic females had L cone opsin genes in the two X-chromosomes encoding two L cone opsins with *distinct* λ_{max} (n = 43). Individual L:M cone ratios were estimated in three ways for these females: (1) based on mean λ_{max} for the two L cone opsins; (2) based on the L cone opsin with the highest λ_{max}; and (3) based on the L cone opsin with the lowest λ_{max}. These estimates define a range of potential L:M cone ratios for females with *distinct* L λ_{max}, which is determined by the degree of X-chromosome inactivation in each cell (Jorgensen et al., 1992; Lyon, 1972; Sharp, Robinson, & Jacobs, 2000). Variation in M λ_{max} has been shown to have minimal impact on the estimated L:M cone ratio (Bieber, Kraft, & Werner, 1998). For the participants who had M cone opsin genes encoding spectrally *distinct* M cone opsins, mean M λ_{max} was used in the estimate of the individual L:M cone ratio.

2.4. Data analysis

Spherical equivalent refraction (SER) was calculated as sphere + ½ cylinder, wherein the sphere was defined as the most positive meridian of the autorefractor measurement in terms of a 13.5 mm vertex distance. Myopia was defined as SER ≤ −0.50D and hyperopia as SER ≥ +0.50D. Mean corneal radius (CR) was estimated as the mean of the corneal radii measured in the flattest and steepest meridians, and axial length (AL) was used to estimate AL/CR-ratios for each participant.

The analysis was performed by the statistical computing software R, version 3.4.0 (R Core Team, 2016). Correlations were assessed using Pearson (r_P) coefficients, and linear regression analyses were performed with %L cones as the dependent outcome variable. Between-group differences were examined using one-way analysis of variance, and Student's or Welch's two independent sample t tests for equal or unequal variances, respectively. Pearson's Chi-squared test and Fisher's Exact test for count data were used to assess relationship between two categorical variables. Differences were considered significant when $p \leq 0.05$. Datasets of all normal trichromats are available online (Hagen & Baraas, 2019).

3. Results

3.1. Estimated %L cones

Fig. 1 shows the distribution of estimated %L cones for male (Fig. 1A: n = 60) and female normal trichromats who had L opsin genes encoding *identical* L cone λ_{max} (Fig. 1B: n = 33). The estimated %L cones varied from 49.9% to 100.3% for the males and from 64.3% to 99.5% for females with *identical* L cone λ_{max} (all were within 100% L cones when considering a repeatability variation of ± 2.3% L cones). Females had a significantly higher mean (± SD) %L cones than males [86.0 (± 8.6)% vs. 79.8 (± 11.8)%; $t(91) = -2.66$, p = 0.01]. Fig. 2 shows the distribution of estimated %L cones for the females who had L opsin genes encoding two *distinct* L cone λ_{max} (n = 43) based on the highest L λ_{max} for the two L opsins (Fig. 2A), the mean L λ_{max} (Fig. 2B)

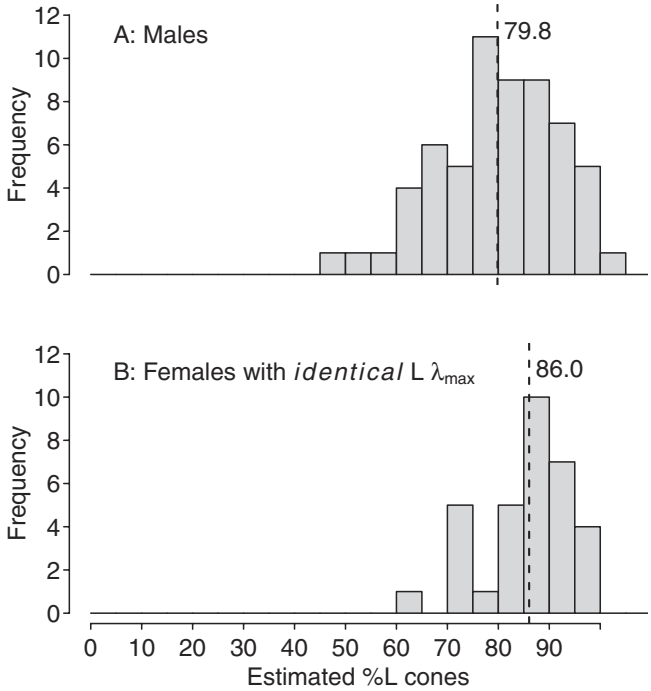

Fig. 1. Distribution of estimated %L cones for (A) male normal trichromats (n = 60) and (B) female normal trichromats who had L opsin genes encoding *identical* L cone λ_{max} (n = 33). The dashed lines illustrate mean %L cones, which was significantly different between males and females [Mean (SD) 79.8 (± 11.8)% vs. 86.0 (± 8.6)%; $t(91) = -2.66$, p = 0.01]. Repeatability variation was estimated to ± 2.3% L cones.

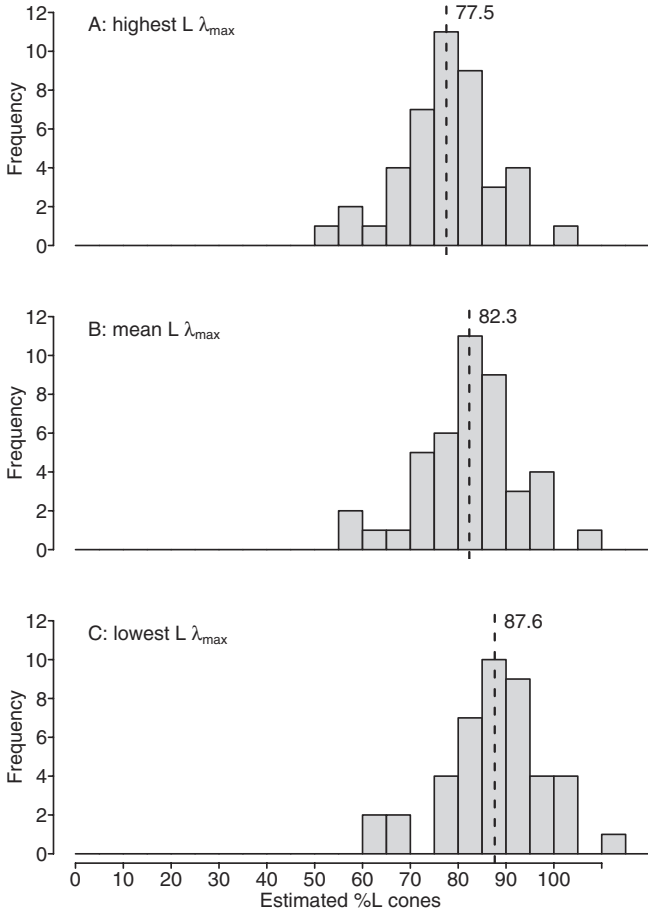

Fig. 2. Distribution of estimated %L cones for female normal trichromats who had L opsin genes encoding *distinct* L cone λ_{max} (n = 43) based on (A) the individual L cone opsin with the highest λ_{max}; (B) mean λ_{max} for their two L cone opsins; and (C) the individual L cone opsin with the lowest λ_{max}. These estimates define a range of potential L:M cone ratios for females with *distinct* L λ_{max}, which is determined by the degree of X-chromosome inactivation in each individual (Jorgensen et al., 1992; Sharp et al., 2000). The dashed lines illustrate mean %L cones.

and the lowest L λ_{max} (Fig. 2C). The mean %L cones was 82.3 (± 10.0)%, with a possible range from mean 77.5% to mean 87.6%. Mean %L cones was also significantly higher for *all* females (n = 76; 83.9 (± 9.6)%) compared with males [n = 60; 79.8 (± 11.8)%; t (1 3 4) = −2.24, p = 0.03], when the mean L cone λ_{max} was used for those with *distinct* L λ_{max} under the assumption that each X-chromosome was silenced in half of the cells by X-chromosome inactivation for estimating mean %L cones.

3.2. Validation of ERG measurements and estimates of %L cones

Rayleigh match midpoint (MMP) correlated significantly with L λ_{max} for 15 male and 9 female normal trichromats with *identical* L λ_{max} (r_P = −0.825, p < 0.001), as expected from previous studies (Winderickx et al., 1992), but not with estimated %L cones (r_P = 0.167, p = 0.44). Thus, the variation in L λ_{max} is removed as a source of error in the estimate of %L cones (Carroll et al., 2002). The results were the same when 10 females with *distinct* L λ_{max} were included in the analyses, with the estimate of %L cones based on their mean L cone λ_{max} (data for 34 normal trichromats: Rayleigh MMP versus mean L λ_{max}: r_P = −0.825, p < 0.001; Rayleigh MMP versus %L cones: r_P = 0.068, p = 0.70). Mean (± SD) Rayleigh MMP and matching range (MR) for normal trichromats were MMP = 42.4 (± 2.1) and MR = 2.8 (± 1.3) for 15 males, MMP = 41.1 (± 2.0) and MR = 2.5 (± 1.9) for 9 females with identical L λ_{max}, and MMP = 41.4 (± 1.2) and MR = 2.5 (± 1.4) for 10 females with distinct L λ_{max}.

Table 2 shows the Rayleigh match results and estimated %L cones for the red-green colour vision deficient male controls. The protan controls were estimated to have approximately 0% L cones. The estimated %L cones for the 13- and 66-year old deuteranope controls were 98% and 88%, respectively. The discrepancy from 100% L cones in the 66-years-old deuteranope, may be due to over-compensation for changes in ocular media with age. The clarity of his crystalline lenses was evaluated using the Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993) and nuclear opalescence was graded and found to be lower than NO2. Nucleus staging was measured to grade 2 with Pentacam HR (Oculus, Typ 70900, Wetzlar, Germany), which is directly comparable with LOCS III NO grade (Pei, Bao, Chen, & Li, 2008). This implies that his lens density was more akin to someone aged 38 years (Pesudovs, Marsack, Donnelly, Thibos, & Applegate, 2004). Choosing a lens density for a 38-year-old gives an estimate of 101.2% L cones for the 66-year-old deuteranope. The deuteranomalous male control had a Rayleigh MMP as low as 16.2, which has been associated with a high OD_L (Thomas & Mollon, 2004). An increase of the OD_L in the estimate of %L from the fixed value of 0.35 to 0.55, results in a decrease in the estimate of %L cones from mean 107.2% (range: 102–112%) to mean 100.6% (96–105%) for the deuteranomalous male (given that he has two different L cone opsins with λ_{max} 553.0 and 555.5 nm). The genetically-confirmed protan carrier control had an estimate of 39% L cones, which was lower than any of the female normal trichromats. She was also heterozygous for the S-opsin mutation T190I, which causes abnormal S-cone function (Baraas, Hagen, Dees, & Neitz, 2012).

3.3. Estimated %L cones related to S180A and photopigment optical density

Table 3 shows the frequency of haplotypes encoded by exon 3 on the L cone opsin gene and the associated expected % correctly spliced transcripts (Buena-Atienza et al., 2016; Greenwald et al., 2017; Neitz &

Table 2
Rayleigh match results and estimated %L cones for the five males who served as controls for validation of ERG measurements and estimates of %L cones, who had known cone opsin genes conferring red-green colour vision deficiency.

Color vision deficiency	Age [years]	Opsin array	λ_{max} [nm]	Rayleigh match		Estimated %L cones	Adjusted %L cones*
				MMP	MR		
Protanope	22	M	530	36.5	73.0	−0.8	–
Protanomalous	21	MMM	533/530	68.0	3.8	−0.3	–
Deuteranope	66	L	559	36.4	72.7	87.6	101.2 (age: 38)
Deuteranope	13	L	559	36.2	72.4	98.4	–
Deuteranomalous	16	LL	555.5/553	16.2	10.0	102.4–111.9 (OD_L = 0.35)	96.1–105.1 (OD_L = 0.55)

* Adjusted %L cones are for the 66-year-old deuteranope when lens density was set to estimated age based on measured nuclear opalescence grade, and for the deuteranomalous based on a higher OD_L. See main text for details.

Table 3
Frequency (%) of haplotypes encoded by exon 3 on the L cone opsin gene and the associated expected % correctly spliced transcripts (Buena-Atienza et al., 2016; Greenwald et al., 2017; Neitz & Neitz, 2018) for male normal trichromats ($n = 60$) with one L cone opsin gene and females ($n = 33$) who had L opsin genes encoding *identical* L cone λ_{max} and had *identical* L exon 3 haplotypes in their two L cone opsin genes.

Exon 3 L cone opsin gene*	Expected % correctly spliced transcripts	Males ($n = 60$)	Females *Identical* L λ_{max} ($n = 33$)
LVAIA	> 75	26.7	15.2
MVAIA	> 75	21.7	18.2
MVVVA	> 75	3.3	0.0
LVVIA	> 75	1.7	0.0
MVVIA	> 75	1.7	0.0
LVAIS	100	28.3	21.2
LIAIS	100	6.7	0.0
MVAIS	100	5.0	6.0
MVVIS	100	3.3	0.0
LVVIS	100	1.7	0.0
Multiple		0.0	39.4

* Five dimorphic amino acid positions are specified by exon 3; L153M, V171I, A174V, I178V, and S180A. The single letter amino acid code used here is as follows: L = leucine, M = methionine, V = valine, I = isoleucine, A = alanine, S = serine.

Neitz, 2018). Five dimorphic amino acid positions are specified by exon 3; L153M, V171I, A174V, I178V, and S180A (single letter amino acid codes are: L = leucine, M = methionine, V = valine, I = isoleucine, A = alanine, S = serine). Serine versus alanine at position 180 (S180A) is the only amino acid substitution encoded by exon 3 that shifts the spectral tuning of the opsin (Neitz & Neitz, 2011). Having serine versus alanine at L position 180 was not significant predictor for %L cones ($\beta = -4.0$, $p = 0.07$) when adjusted for sex in a linear regression [$F(2, 90) = 5.30$, $p = 0.007$, $R^2 = 0.11$] in the group of males and females with *identical* L λ_{max}. See Table 4 for frequency of L and M cone λ_{max}.

Amino acid substitutions encoded by exon 2 have been suggested to regulate the optical density of the M cone opsin (Neitz, Neitz, He, & Shevell, 1999), whether this applies to the L cone opsin is not known. Increasing the OD_L from the fixed value decreases the estimated %L cones, while a change in OD_M has minimal effect on the estimated %L cones. Here, 131 of the normal trichromats (96.3%) had the exact same haplotypes encoded by exon 2 on the L cone opsin gene (TIS). Five females had a different L exon 2 haplotype that may be related to a different optical density of the L cone [one female with *identical* L cone λ_{max} (98.0% L), and 4 females with *distinct* L cone λ_{max} (73%, 78%, 81% and 96% L based on mean L cone λ_{max})]. Removing these females from the group had no effect on the mean %L cones.

3.4. Comparison with other studies

Table 5 gives an overview of mean %L cones and the proportion of S180A from present and other studies. The mean %L cones for the Norwegian male normal trichromats [79.8 (± 11.8)% L] was significantly higher than that reported for African and African American (McMahon et al., 2008) [65.1 (± 10.7)% L; $t(85) = 5.53$, $p < 0.001$], American Caucasian (Carroll et al., 2002; Hofer et al., 2005) [73.1 (± 11.1)% L; $t(120) = 3.24$, $p = 0.002$], and East Asian (Kuchenbecker et al., 2014; Yamauchi et al., 2013) male normal trichromats. L:M cone ratios in all studies were measured by ERG flicker photometry. Table 5 shows that Caucasians (Deeb, Alvarez, Malkki, & Motulsky, 1995; Winderickx, Battisti, Hibiya, Motulsky, & Deeb, 1993) are reported to have a higher proportion of alanine at L position 180 than African (Deeb & Motulsky, 1996) and Japanese (55% versus 20%) (Deeb et al., 1995; Hayashi, Ueyama, Tanabe, Yamade, & Kani, 2001).

3.5. Refractive error

SER, astigmatism and axial length correlated between the right and the left eye ($n = 136$; SER: $r_p = 0.94$; refractive astigmatism: $r_p = 0.43$; axial length: $r_p = 0.92$; all $p < 0.001$). Thus, in further analysis, data from the right eye were used. Table 6 shows mean SER, ocular axial length (AL), corneal curvature (CR), and the frequency of refractive errors for the right eye of normal trichromats along with estimated %L cones for the groups.

Fig. 3 shows the proportion of myopic females with two *distinct* L cone λ_{max} who had low (%L ≤ median) or high %L cones (%L > median). When comparing myopes with non-myopes (emmetropes and hyperopes) in this group, myopia was found to be significantly more frequent in those with low vs. high %L cones ($n = 22$ vs. 21; 31.8% vs. 4.8% myopia; Fisher's exact test $p = 0.046$). Those with low %L cones were also more myopic than those with high %L cones [$n = 22$ vs. 21; mean (SD) SER −0.07 (± 1.2)D vs. 0.81 (± 0.7)D; Welch $t(33.1) = -2.91$, $p = 0.006$]. Likewise, in the group of *all* females ($n = 76$), mean SER was more myopic in those with low %L cones than in those with high %L cones [n = 39 vs. 37; −0.03 (± 1.2)D vs. 0.58 (± 0.8)D; Welch $t(67.8) = -0.52$, $p = 0.01$]. There were no associations between estimated %L cones or L and M cone opsin genetics and refractive error or ocular biometry for the males, but the number of male myopes was low ($n = 5$).

Table 7 shows that there was a significant association between the frequency of refractive error in females and whether a female was homozygous or heterozygous for their specific L exon 3 haplotype(s) (Pearson Chi-Squared test based on 9999 Monte-Carlo resamplings, $p = 0.008$), with less ametropia and more emmetropia among the females who were homozygous for their specific L exon 3 haplotype. Males are, by definition, never heterozygous.

4. Discussion

The results presented here are consistent with the hypothesis that the L:M cone ratio, combined with opsin gene polymorphism and exon 3 haplotypes with less severe splicing defects are implicated in susceptibility to myopia-generating environmental triggers (Neitz & Neitz, 2015; Zhou et al., 2015). The high myopia prevalence in East Asians (Lin, Shih, Hsiao, & Chen, 2004; Pan, Dirani, Cheng, Wong, & Saw, 2015) is not observed in Norwegians despite high educational pressure

Table 4
Frequency (%) of L and M cone λ_{max} [nm] in Caucasian normal trichromats, grouped by sex and whether females have L opsin genes encoding *identical* or *distinct* L cone λ_{max}. For those who have opsin genes encoding *distinct* L or M cone λ_{max}, two values for λ_{max} are given.

		Males ($n = 60$)			Females *Identical* L λ_{max} ($n = 33$)		Females *Distinct* L λ_{max} ($n = 43$)			
M λ_{max}		530	533	530/533	530	530/533	M λ_{max}		530	530/533
L λ_{max}	559	33.3	8.3	1.7	42.4	9.1	L λ_{max}	555.5/559	74.4	9.3
	555.5	53.3	0.0	1.7	45.5	0.0		555/559	4.7	2.3
	555	1.7	0.0	0.0	0.0	0.0		556.5/559	2.3	4.7
	553	0.0	0.0	0.0	3.0	0.0		553/555.5	0.0	2.3

Table 5
An overview of the proportion of S180A in present and previous studies. Mean %L cones for all, and grouped by S180A, are presented for studies that have reported L:M cone ratios. N/A = not available.

		All	Serine at L opsin position 180		Alanine at L opsin position 180		Mean (SD) % L cones		
Ethnicity	Study	*n*	*n*	%	*n*	%	All	$Serine_{180}$	$Alanine_{180}$
Caucasian Norwegian colour normal males	Present study for males whom we have measured %L	60	27	45.0	33	55.0	79.8 (11.8)	77.3 (12.2)	81.9 (11.2)
Caucasian American colour normal males	Carroll et al. (2002) & Hofer et al. (2005)	62	35	56.5	27	43.5	73.1 (11.1)	75.4 (10.8)	70.2 (11.0)
Caucasian colour normal males	Winderickx et al. (1993)	75	46	62.2	28	37.8	N/A	N/A	N/A
African and African American colour normal males	McMahon et al. (2008)	27	26*	96.3*	1*	3.7*	65.1 (10.7)	64.4 (10.2)	84.5
Japanese males	Deeb et al. (1995)	49	41	83.7	8	16.3	N/A	N/A	N/A
Japanese colour normal males	Hayashi et al. (2001)	119	94	79.0	25	21.0	N/A	N/A	N/A

* Frequency are based on L λ_{max} (559 nm for serine and 555.5 nm for alanine).

Table 6
Mean (SD) SER, ocular axial length (AL), corneal curvature (CR), proportion of refractive errors, and mean (SD) estimated %L cones for the 136 normal trichromatic participants grouped by refractive error (MYO = myopia, EMM = emmetropia, HYP = hyperopia).

							Refractive error (%)			Estimated %L			
	n	Age	SER [D]	Range SER [D]	AL [mm]	CR [mm]	MYO	EMM	HYP	All	MYO	EMM	HYP
Males	60	16.8 (0.9)	+0.60 (0.9)	−2.32 to +4.23	23.7 (0.7)	7.9 (0.3)	8.3	33.3	58.3	79.8 (11.8) *n* = 60	84.9 (7.7) *n* = 5	78.6 (13.4) *n* = 20	79.8 (11.4) *n* = 35
Females with *identical* L λ_{max}	33	16.7 (0.9)	+0.15 (1.1)	−3.36 to +1.83	23.4 (0.8)	7.8 (0.2)	21.2	39.4	39.4	86.0 (8.6) *n* = 33	84.9 (9.1) *n* = 7	88.0 (7.3) *n* = 13	84.7 (9.8) *n* = 13
Females with *distinct* L λ_{max}	43	17.1 (1.0)	+0.36 (1.1)	−2.56 to +2.97	23.3 (0.7)	7.7 (0.2)	18.6	20.9	60.5	82.3 (10.0) *n* = 43	77.4 (8.1) *n* = 8	83.8 (14.4) *n* = 9	83.3 (8.6) *n* = 26
All females	76	16.9 (1.0)	+0.27 (1.1)	−3.36 to +2.97	23.3 (0.8)	7.8 (0.2)	19.7	28.9	51.3	83.9 (9.6) *n* = 76	80.9 (9.1) *n* = 15	86.3 (10.7) *n* = 22	83.8 (8.9) *n* = 39

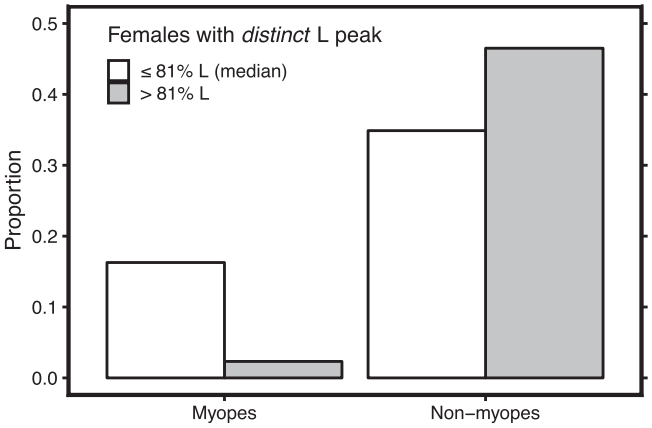

Fig. 3. Association between %L cones and myopia in females with *distinct* L λ_{max} (n = 43; median ≤ 81% L; Fisher's exact test p = 0.05). The participants were grouped by %L (white bars: %L ≤ median; grey bars: %L > median).

Table 7
Frequency (%) of refractive errors in males (n = 60), *all* females (n = 76), and in all females grouped according to being homozygous (n = 22) or heterozygous (n = 54) for their specific L exon 3 haplotype(s). There was a significant association between the refractive error and homozygosity versus heterozygosity for the females (Pearson Chi-Squared test based on 9999 Monte-Carlo resamplings, p = 0.008).

			All females grouped by their L exon 3 haplotype(s)	
	Males	*All* females	Homozygous females	Heterozygous females
n	60	76	22	54
Myopia (%)	8.3	19.7	9.1	24.1
Emmetropia (%)	33.3	28.9	54.5	18.5
Hyperopia (%)	58.3	51.3	36.4	57.4

and low daily light exposure due to few daylight hours in the autumn-winter period (Hagen et al., 2018), but they have a significantly higher mean L:M cone ratio than that previously reported for East Asians (Kuchenbecker et al., 2014; Yamauchi et al., 2013). Furthermore, females with low %L cones (symmetric L:M cone ratios) were on average more myopic than females with high %L cones (skewed L:M cone ratios). Myopia prevalence was higher in females who were heterozygous for the L opsin exon 3 haplotype than in the homozygous females.

4.1. The association between cone opsin and myopia

It is well known that high-grade myopia is associated with rare interchange exon 3 haplotypes, such as LVAVA and LIAVA, of the L or M opsin genes (Carroll et al., 2012; Greenwald et al., 2017; McClements et al., 2013; Orosz et al., 2017) (none in our sample; Table 3), resulting in incorrect splicing of exon 3 and greatly reduced amount of functional opsin in the cones harbouring the mutation. Eye growth associated with rare interchange haplotypes is suggested to be caused by erroneous contrast signals produced by mosaics with both normal cones and cones with mutant opsins (Greenwald et al., 2017; Patterson et al., 2018). It is not unlikely that this mechanism also plays a role in common myopia, because there is large between-individual variation in the amino acid sequences of the L and M opsin genes. Amino acid substitutions can have a less deleterious effect on the cone opsin function than for example LVAVA and LIAVA, without altering the spectral sensitivity or λ_{max} (Carroll et al., 2002; Neitz et al., 1999). How effectively cone photoreceptors signal contrast and spatial frequency information depends on the gene code of opsins expressed in the L and M cones (Greenwald et al., 2017) as well as the organization and the ratio of L

and M cones. The L/M gene array of colour normal males and homozygote females will give a cone mosaic expressing one type of L and one type of M opsin. The number of cones harbouring a less than normal functioning opsin depends on whether the opsin is L or M, whether the amino acid substitution resides on the first or second gene on the array, and the L:M cone ratio, resulting in differences in the ratio of less-than-normal to normal functioning cones. If less-than-normally functioning cones causes ON bipolar cells to signal more contrast than is actually present, then a high contrast spatial-frequency pattern, that moves across the retina due to eye movements, will give rise to less synchronized signals from the ON bipolar cells to ganglion cells and poorer signal fidelity (Ala-Laurila, Greschner, Chichilnisky, & Rieke, 2011). These errors in signalling of spatial contrast information could be the step that sets off the signalling cascade that stimulates eye growth (Wallman & Winawer, 2004). Another factor that may play a role is the organization of L and M cones in patches of the same cone type (Hofer et al., 2005). This patchiness is advantageous for signalling of achromatic spatial information of high spatial frequency (high contrast fine details), as neighbouring cones of different types will give rise to chromatic noise (undesired differences in spectral information) which degrades the achromatic spatial signal (Osorio & Vorobyev, 2008; Roorda, Metha, Lennie, & Williams, 2001; Williams, Sekiguchi, Haake, Brainard, & Packer, 1991). Skewed L:M cone ratio (near 0% or near 100% L cones) makes it more likely that neighbouring cones are of the same type, improving signalling of spatial information (if all cones are normally functioning with the same level of opsin expression). The most skewed L:M cone ratios are found in red-green colour vision deficient individuals, as they have only L *or* M cones in the retina (in addition to the more sparsely distributed S cones) leading to high resolution, low noise signalling. Common forms of congenital red-green colour vision deficiency are indeed associated with low myopia susceptibility and prevalence (Ostadimoghaddam et al., 2014; Qian et al., 2009). In Norway, 8% of males are red-green colour vision deficient, and about 15% females are assumed to be deutan or protan carriers (Baraas, 2008; Waaler, 1968). Higher L:M cone ratios are expected in females when samples of normal females include carriers of deutan colour vision deficiency who have higher L:M ratios than normal males and non-carrier females. The females with highly skewed cone ratios provide a sample within the Norwegian population in which the hypothesis that biased cones ratios protect against myopia can be tested.

4.2. Heterozygosity of common L opsin exon 3 haplotypes

That the L:M cone ratio combined with L opsin exon 3 haplotypes that give rise to mild splicing defects play a role in myopia susceptibility could also explain why myopia prevalence was the same in females who were homozygous for the L opsin exon 3 haplotype as in the males (9% and 8% respectively; Table 7), but much higher in females who were heterozygous for the L opsin exon 3 haplotype (24% myopia). Because females have two X chromosomes, L and/or M opsin exon 3 haplotype heterozygosity translates into a retina where there will be patches with two sets of L and/or M cones expressing different haplotypes, and these haplotypes could give rise to less-than-normally functioning opsin and/or altered spectral sensitivity. It has been shown that females with heterozygote mosaics will vary greatly in chromatic contrast sensitivity, depending on opsin haplotype (Dees et al., 2015) and their L:M ratio (Gunther & Dobkins, 2002). Those with haplotypes that code for more than two different L and/or M cones with large spectral separation and have a low, symmetrical L:M cone ratio, will have improved chromatic sensitivity (Osorio & Vorobyev, 1996), but increased chromatic noise degrading signalling of high-spatial frequency information (Barlow, 1982; Osorio, Ruderman, & Cronin, 1998). This suggests that the sex difference in myopia prevalence could be a consequence of heterozygosity of common L opsin exon 3 haplotypes.

4.3. Serine versus alanine at L opsin position 180 (S180A)

A common polymorphism on exon 3 of the L opsin that affects spectral separation between L and M cones is serine versus alanine at position 180 (S180A). Serine shifts the L cone λ_{max} 3–4 nm (Asenjo et al., 1994; Neitz, Neitz, & Jacobs, 1991), and is known to result in higher sensitivity to red than alanine (Winderickx et al., 1992). A significant green shift has been reported in myopes compared with emmetropes and hyperopes (Rucker & Kruger, 2006), as well as an association between a green shifted Rayleigh match and increased myopia (Wienke, 1960). It is plausible to assume that the myopes in these reports likely had serine, since green shifted (lower) Rayleigh match midpoints are a signature of serine at position 180 (Winderickx et al., 1992). The proportion of S180 was significantly lower in the Norwegian male normal trichromats (45% have serine) than that reported for East Asians (80% have serine) and other more southerly located populations with almost no seasonal variation in daylight (Deeb et al., 1995; Hayashi et al., 2001) (Table 5). But why would a population living at northern latitudes evolve an eye that also is protective against developing myopia? Studies of cone opsin genes in primates indicate that having alanine at position 180 in the L opsin may be an evolutionary result of adaptation to long periods of low light levels (Jacobs, 2008; Jacobs et al., 2017). X-linked cone opsin variations across a lemur clade shows that the most strictly diurnal lemur has serine at position 180, whereas the lemur that is generally diurnal, but also is active at dusk/dawn, has alanine at position 180 (Jacobs et al., 2017). The platyrrhine Aotus, the only anthropoid (monkey) considered to be nocturnal, is reported to only have alanine (Jacobs, 2008). The decrease of L cone λ_{max} and narrowing of the separation between L and M cone λ_{max}, as a consequence of alanine at position 180, not only improves signal-to-noise ratio when the light is bright, as mentioned in 4.1. above, but also when the light is dim, as it reduces dark noise (Lewis & Zhaoping, 2006). This may be an advantage if you spend many hours indoors in low light levels doing near work (Mountjoy et al., 2018; Wu et al., 2018).

4.4. Possible limitations

A larger sample size could have strengthened the results. The low number of myopes reflects the low myopia susceptibility in this population. Further work is needed to see if these findings can be duplicated in a population with high myopia susceptibility.

4.5. Conclusions

High L:M cone ratios are previously suggested to protect against myopia development (Neitz & Neitz, 2015; Zhou et al., 2015), and the results here, showing that Norwegians have higher mean %L cones than East Asians, and that Norwegian females with high %L cones were less myopic, support this theory. Any advantage associated with photoreceptor function during dim light will necessarily also be related to the role circadian clocks play in modulating photoreceptor electrical coupling during day and night and in anticipation of changing light levels (Felder-Schmittbuhl et al., 2018).

Declaration of Competing Interest

Maureen Neitz, Waveshift LLC Code I (Personal Financial Interest), University of Washington Code P (Patent), Jay Neitz, Waveshift LLC Code I (Personal Financial Interest), University of Washington Code P (Patent).

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