THE CAUSES AND CONSEQUENCES OF CHILDHOOD MYOPIA

Willem Tideman

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PUBLICATIONS ON WHICH THIS THESIS IS BASED

Part II Consequences

Chapter 2
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Chapter 3
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Part III Ocular biometry development

Chapter 4
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Chapter 6
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Chapter 7
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Chapter 8
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Chapter 9
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Part V Genetic risk of myopia in children

Chapter 10
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Chapter 11
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PART I

INTRODUCTION AND DESIGN

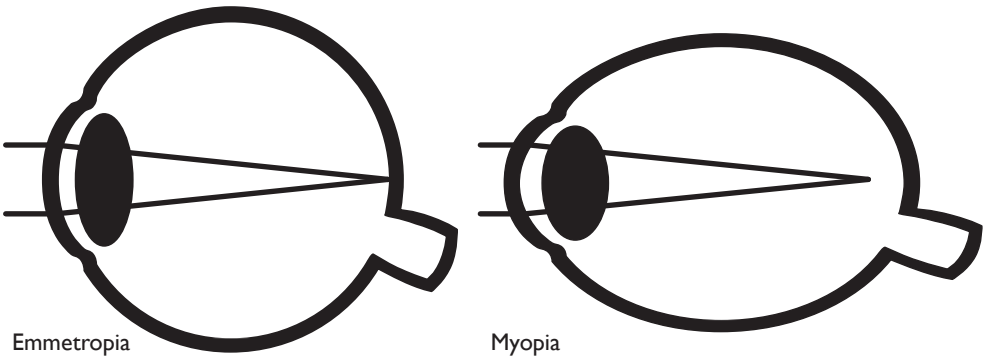
CHAPTER 1

GENERAL INTRODUCTION

The primary function of the eye is to convert visual stimuli to electrical signals to facilitate visual perception. Visual perception is the result of light entering the eye at the cornea, moving through the anterior chamber, crystalline lens, corpus vitreous and ending at the retina, where the light is converted into electrical signals, which are sent to and processed in the brain. Optimal visual acuity at distance without accommodation can only be obtained through a precise match of all refractive components of the eye. This is necessary to create a focal plane on the retina and a sharp image projected on the photoreceptor cells of the retina (figure 1).[1] This ideal refractive state is called emmetropia.

The most important refractive components are the cornea, crystalline lens and the axial length. A mismatch in the refractive power of the components can result in a refractive error. The two most common forms of refractive error are myopia, in which the focal plane is located in front of the retina (figure 1), and hyperopia, in which the focal plane is behind the retina.

Figure 1 Focal point of a normal (emmetropic) eye (left); and a myopic eye (right)



The work in this thesis is focused on myopia and the development of the ocular refractive components. Myopia, also known as nearsightedness, is defined as a refractive error of ≤-0.5 diopter (D). Myopia is derived from the Greek word "muopia", which means 'contracting' or 'to shut eyes'. Squinting is a symptom, resulting in a horizontal stenopeic slit, which you generally observe in children with a low degree of myopia in order to improve visual acuity at a distance. Currently there are various options to correct myopia, such as glasses, contact lenses and refractive surgery. The first person assumed to have myopia correction was Nero. The roman emperor held a curved emerald in front of his eye during gladiator performances.[2] It was only until 1600 years later that the scientific framework behind this observation was established by Kepler.[3] In 1813 the first epidemiologic study described the association between myopia and educational level.[4] It took halfway the 19th century before the relation between myopia and axial length was established.[5]

Eye growth

The refractive components can be measured as ocular biometry. Changes in ocular biometry occur gradually during childhood and teenage years, thereby modulating refractive error. The refractive power of the cornea shows the largest shift in the first year of life; whereas transition of the crystalline lens leading to alteration of the anterior and posterior curvature and the refractive index gradient, occurs in the first 10 years of life.[6-8] These two structures are the most important biometric components in the anterior part of the eye and responsible for establishing the focal point of the incoming image. Growth of the vitreous chamber to the apex of the orbita occurs during the first 25 years under influence of visual stimuli. When the focal point and the fovea are accurately aligned by corpus vitreous elongation, the eye will become emmetropic, i.e., without refractive error. In myopia, this process is dysregulated and the corpus vitreous grows beyond the focal point.

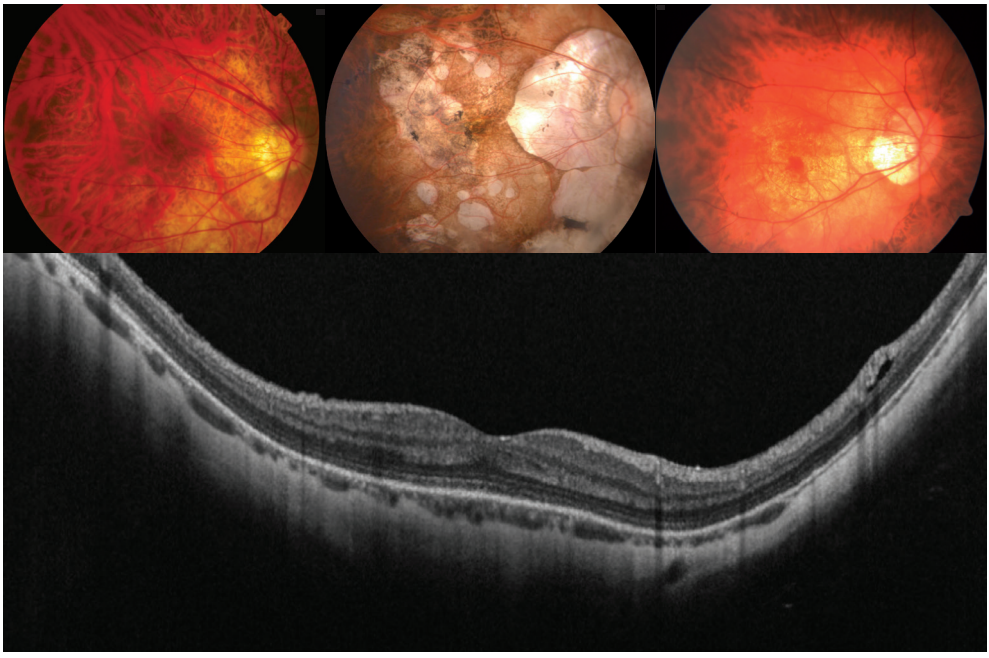
The most important biometric component for myopia is the axial length of the eye. This is measured as the distance between the center of the corneal thickness at the front of the eye to the fovea at the back. Boys have on average a higher axial length, a steeper corneal radius and a thinner lens thickness than girls.[9-11] Previous studies have described the ocular biometry at different ages (table 1).[6,9-15] Measurements are comparable between Asian and European children up to age 6 years, but axial length increases in growth after this age, corresponding to higher myopia prevalence.[9] Unambiguous grounds for this predilection are unknown, but a different lifestyle with more myopia risk factors has been hypothesized to be a major determinant.

Clinical relevance

Myopia is generally considered as a nonthreatening condition which is easy correctable with glasses, contact lenses or refractive surgery. However, high myopia is currently one of the largest contributors to visual impairment and blindness in developed coun-

tries.[16] High myopia is the result of excessive growth of the axial length of the eye, which causes structural changes in the sclera, choroidea, retina and optic nerve (figure 2). The morphological changes in these structures lead to an increased risk of myopic macular degeneration, retinal detachment or glaucoma.[17,18]

Figure 2 Clockwise from top left: peripapillary atrophy, myopic macular degeneration, macular bleeding, OCT of a staphyloma with thin choroid



Risk factors

Myopia is thought to be the result of an interplay between environmental and genetic factors.[19] Since the beginning of the 17th century, environmental factors are suggested to be the primary players. Several theories about the development of myopia have been postulated. Johannes Kepler was the founder of the nearwork hypothesis in 1611.[20] He thought his nearsightedness was caused by a surfeit of studying astronomy tables. Franciscus Donders described a higher prevalence of myopia in patients who belonged to higher socio-economic classes.[21] It was only until the 20th century that the outdoor hypothesis of more time spent outdoors being protective against myopia development was proposed with increasing evidence.[22,23]

What is known about eye growth and changes of biometric measures in children? Limited data is available to study eye growth in European children below 10 years of age, and most Asian studies consisted of only cross-sectional data with a single measurement

(table 1). Normative data in European children in the most important age categories for myopia development are currently unavailable, as are data on the effect of environmental factors on growth of axial length and induction of myopia at a very young age.

Table 1 Average axial length, corneal radius of curvature and AL/CR ratio in population based studies stratified by gender

Study	Mean Age (years)	Mean Axial length (mm)	Mean Corneal radius (mm)	Mean AL/CR ratio
GUSTO, Singapore				
All	3.0	21.71	7.77	2.81
STARS, Singapore				
All (n = 469)	5.1	22.36	7.71	2.90
Boys (n = 239)		22.62	7.76	2.91
Girls (n = 230)		22.08	7.65	2.89
SMS, Australia				
All (n = 1716)	6.7	22.61	7.78	2.91
Boys (n = 872)		22.89	7.85	2.92
Girls (n = 844)		22.32	7.70	2.89
ACES, China				
All (n = 2235)	7.1	22.72	7.80	2.91
Boys (n = 1285)		22.91	7.85	2.92
Girls (n = 912)		22.46	7.72	2.91
SCORM, Singapore				
All (n = 1747)	7.9	23.32	7.74	3.01
Boys (n = 887)		23.62	7.80	3.03
Girls (n = 860)		23.02	7.68	3.00
CHASE, England				
All (n = 1179)	10.9	23.23	7.82	2.97
Boys (n = 561)		23.47	7.87	2.98
Girls (n = 618)		23.01	7.77	2.96
CCC2000, Denmark				
All (n = 1323)	11.7	23.19	–	–
Boys (n = 633)		23.50	–	–
Girls (n = 690)		22.90	–	–
SMS, Australia				
All (n = 2311)	12.7	23.38	7.78	3.01
Boys (n = 1174)		23.58	7.83	3.01
Girls (n =1137)		23.18	7.73	3.00
ACES, China				
All (n = 1875)	13.7	24.39	7.80	3.13
Boys (n = 972)		24.63	7.88	3.13
Girls (n = 903)		24.17	7.73	3.13

Genetics

The genetic component of myopia has already long been recognized. Evidence was derived from family [24] and twin studies,[25,26] and from studies in offspring of myopic parents.[27,28] Linkage (MYP 1-18) and candidate gene studies (*CTNND2*) identified potential genes, but the different studies lacked overlap in results.[29-38]

More recently genome-wide association study (GWAS) analyses were introduced, with the advantage of discovering differences in the genome, single nucleotide polymorphisms (SNPs), associated with refractive error in large populations with different refractive errors. The first European results for refractive error were identified in single studies and were near the genes *GJD2* and *RASGRF1*.[39-41] After these findings, the international Consortium for Refractive Error and Myopia (CREAM) identified a total of 26 loci for spherical equivalent in 45,758 individuals.[42] Concurrently, 23andMe, a direct-to-consumer genetic testing company, published similar findings based on age of wearing first pair of glasses for myopia, with 14 overlapping loci and 13 new loci with equal effect sizes between the studies.[43,44] Age dependent effects were not identified, as all participants were older than 25 years. Whether different pathways play a role at any given age remains unknown.

Hypothesis

The primary hypothesis for this thesis is that the fundamentals of ocular biometry and adult refractive error are formulated in early life. In the context of etiology as well as prevention it is necessary to identify genetic and environmental determinants which play a role early in life, and design a framework for eye growth. The studies presented in this thesis are focused on the causes and consequences of early onset myopia.

Objectives

The major aims of this thesis are to assess:

1 The effect of early onset myopia on development of visual impairment later in life (Chapters 2-3)
2 The development of ocular biometry from young childhood to adulthood and the association with prenatal and postnatal growth. (Chapters 4-6)
3 The association of environmental risk factors on ocular biometry and myopia at a young age. The exposures of interest include outdoors exposure, nearwork, computer use, vitamin D and reading habits. (Chapters 7-9)
4 The effect of genetic factors at various ages on the development of ocular biometry and refractive error and the interactions between gene and environment. (Chapters 10-11)

General epidemiologic design

The studies presented in this thesis were embedded in the population-based prospective cohort study Generation R, ALSPAC, the Rotterdam Study, and studies from the CREAM consortium.

The Generation R Study

The Generation R study is a population-based prospective birth cohort study from fetal life until young adulthood from Rotterdam, The Netherlands. The study was designed to identify early life environmental and genetic risk factors for normal and abnormal development or disease during fetal life, early childhood and teenage years.[45,46] A total of 9778 mothers and their children were included between April 2002 and January 2006, ideally during early pregnancy. Assessment of 8879 mothers during pregnancy included physical examinations and ultrasounds. Postnatal measurement from birth to 4 years of age were conducted in the child health care centers. At six years of age all children were invited to the research center to have a detailed examination, including eye measurement of the ocular biometry, visual acuity, and questions about ophthalmic medical history. At 9 years of age a follow up visit was planned, which included the same eye measurements with additionally an optical coherence tomography (OCT) scan, cycloplegic refractive error and a MRI scan. Questionnaires about the development and behavior of the children were filled in by the parents from pregnancy until the current stage.

Avon Longitudinal Study of Parents and Children (ALSPAC)

Pregnant women with an expected date of delivery between 1st April 1991 and 31st December 1992, resident in the former Avon health authority area in Southwest England, were eligible to participate in this population-based birth cohort study.[47] 13,761 women were recruited. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Follow up of the children was between birth and 17 years with questionnaires and clinic visits. Non-cycloplegic refractive error was measured from 6 years onward and ocular biometry measurements were performed at 15 years of age.

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study of middle-aged and elderly subjects (45+ years of age) living in Ommoord, a suburb of Rotterdam, the Netherlands. In brief, the Rotterdam Study consists of three independent cohorts (RS-I (55+ years of age), RS-II (55+ years of age), and RS-III (45+ years of age)); this study examined cardiovascular, endocrine, neurological, respiratory, and ophthalmic out-

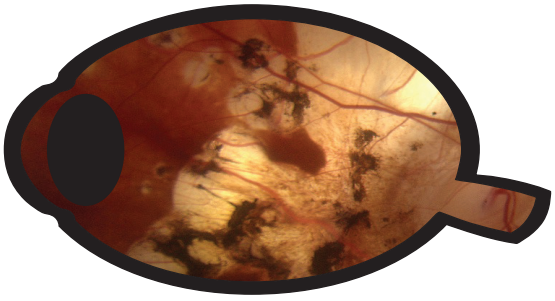
comes. Baseline examinations – including BCVA and refractive error measurements – were performed from 1990 to 1993 (RS-I), 2000 to 2002 (RS-II), and 2006 to 2008 (RS-III). Axial length was measured in a subset of RS-III at baseline and in a subset of the studies during follow-up examinations (RS-I: 2009-2011, RS-II: 2011-2012, RS-III: 2011-2012). In total, 5,686 subjects were eligible for our analysis for axial length (RS-I, N = 1,005; RS-II, N =1,524, RS-III, N = 3,157).

CREAM

The studies mentioned above took part in a collaborative study named CREAM (Consortium of Refractive Error And Myopia). CREAM is a consortium of more than 35 population-based studies with genetic as well as phenotypic myopia data. The overall aim of CREAM is to identify genetic risk factors for myopia and ocular biometry. In the large GWAS studies with adults with age older than 25 years were eligible to participate. Within the consortium adult as well as children studies are present. Currently 8 studies have participants with an age lower than 25 years.

PART II

CONSEQUENCES



CHAPTER 2

BIJZIENDHEID, EEN GROEIEND PROBLEEM

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Polling JR,

van der Schans A,

Verhoeven VJM,

Klaver CCW

ABSTRACT

Myopia is an eye disorder with the most rapid increase in prevalence worldwide. It develops in childhood, with a peak incidence between the ages of 13 to 15 years. Especially high myopia, i.e. a refractive error of -6 diopters or more, increases the risk of permanent visual impairment during adulthood due to structural abnormalities of the retina and optic nerve. The cause of myopia is complex. Lifestyle factors in childhood, such as limited time spent outdoors and close work - such as reading and smartphone usage - are risk factors. Furthermore, genetic studies have revealed more than 100 factors associated with the development of myopia. Pharmacological and optical interventions to inhibit myopia progression are increasingly applied. The use of atropine eye drops in children has been shown to be an effective treatment.

Casus

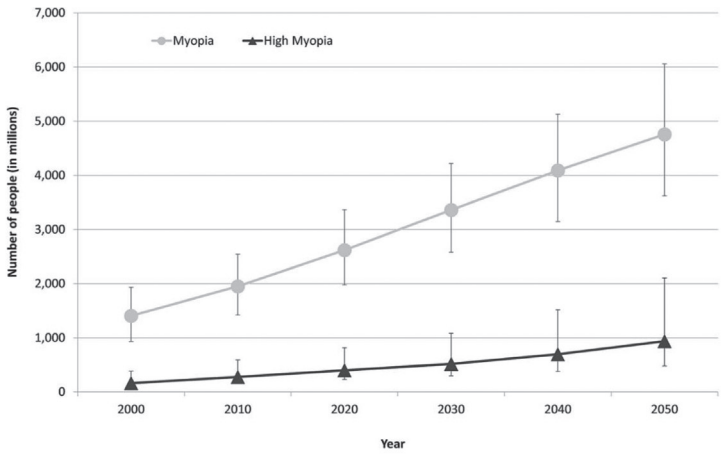
Een 8-jarige meisje komt bij de huisarts met hoofdpijn en moeite met kijken op het schoolbord. De opticien heeft een brilsterkte van -1 dioptrie gemeten. Vader en moeder dragen allebei een bril, moeder heeft brilsterkte -4 dioptrie en vader -6. De leeftijd van hun eerste bril was echter na het 10e jaar. Het meisje zit op ballet en houdt heel erg van lezen en spelen op haar tablet. De ouders vragen of er nog maatregelen nodig zijn en wat de visuele vooruitzichten zijn.

Introductie

Bijziendheid (myopie) lijkt een onschuldige kwaal die met optische hulpmiddelen goed verdragen kan worden, maar het venijn zit, zoals altijd, in de staart. De verdeling van brilsterkten over de algemene bevolking heeft een gemiddelde rondom brilsterkte 0 en een forse uitloop naar de hogere minsterkten. Het zijn juist de minsterkten van -6 dioptrie of meer (hoge myopie) die geassocieerd zijn met oculaire morbiditeit. 1 op de 3 mensen met hoge myopie ontwikkelt slechtziendheid door structurele veranderingen van de retina (netvlies) en de oogzenuw, welke leiden tot myope maculadegeneratie (slijtage van de gele vlek), ablatio retinae (netvlies loslating), en glaucoom (verlies van zenuwvezels met opticoneuropathie).[48]

Het aantal bijzienden in de wereld is de laatste decennia sterk toegenomen. In Azië is het probleem het grootst; in landen zoals Taiwan, Zuid-Korea en Singapore is nu 80-90% van de twintigjarige bevolking bijziend.[49] Vroeger was dit rond de 20%. Ook in Europa is nu gemiddeld 1 op de 3 personen bijziend; dit is bij 60 jarigen 1 op de 4, echter bij de jongere generaties stijgt dit al tot 1 op de 2.[50] Recent onderzoek heeft voorspeld dat 50% van de wereld bevolking bijziend zal zijn in 2050 (Figuur 1).[51] Dit roept een aantal vra-

Figuur 1 Wereldwijde toename van myopie prevalentie



De lijnen representeren 95% betrouwbaarheids intervallen. Uit: *Holden BA; Ophthalmology, 2016.51.*

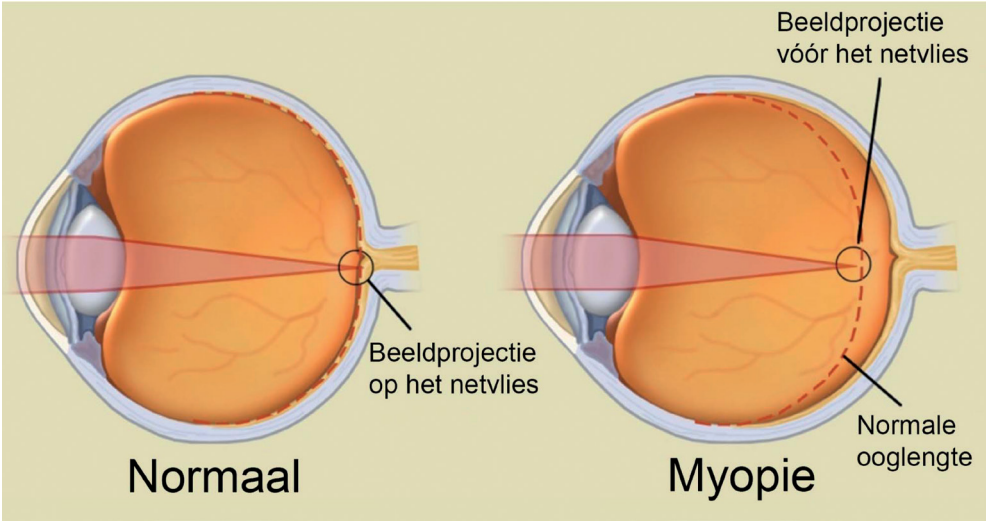
gen op. Wat zijn de gevolgen van de toenemende prevalentie voor de oogzorg? Wat zijn de visuele consequenties voor het individu op lange termijn? Is myopie te voorkomen en kan progressie afgeremd worden?

Wat is myopie? Hoe ontstaat het?

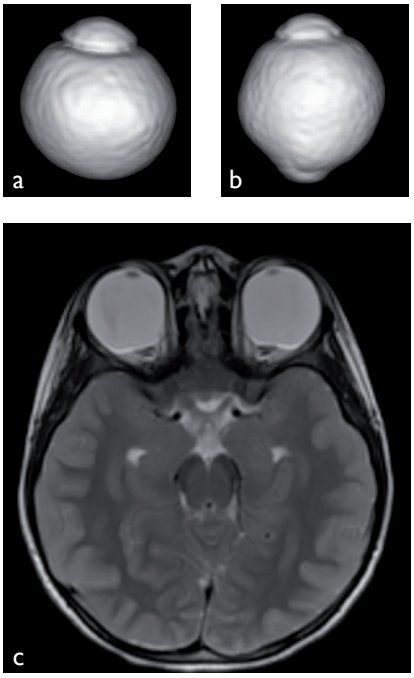
Myopie wordt gekenmerkt doordat het brandpunt van de invallende lichtstralen in het oog geprojecteerd wordt vóór het netvlies i.p.v. erop (Figuur 2). Het resultaat is een onscherp beeld. Of het brandpunt op de retina valt wordt bepaald door de lens, de cornea en de lengte van het oog.[6] De eerste twee groeien met name gedurende de eerste vijf levensjaren. Echter, de grootste oorzaak van myopie is groei van de achterste oogkamer richting de apex van de orbita (Figuur 3). Enige groei hiervan is normaal bij kinderen en noemen we emmetropisatie. Hierbij zorgen visuele stimuli ervoor dat het focuspunt op de fovea (centrum van de gele vlek) van de retina valt. Bij myopie wordt deze regulatie niet goed afgestemd en groeit het oog te ver door.

De groei van het oog is te kwantificeren door het meten van de aslengte van het oog, d.w.z. de afstand van het centrum van de cornea (hoornvlies) tot aan de fovea. De gemiddelde aslengte is bij de geboorte 17,5 mm en groeit tot gemiddeld 23,5 mm op volwassen leeftijd.[52] Een hoog bijziend oog groeit door tot tenminste 26 mm lengte, maar dit kan oplopen tot >30 mm. De eerste levensjaren is de groei het snelst en deze stopt doorgaans rond 13-jarige leeftijd, maar bij myopie kan de groei doorgaan tot zelfs 25 jarige leeftijd. Hoe vroeger de myopie ontstaat, hoe groter de kans op hoge myopie op latere leeftijd.[53]

Figuur 2 Normaal oog zonder refractieafwijking (emmetropie; links) en met myopie (rechts)



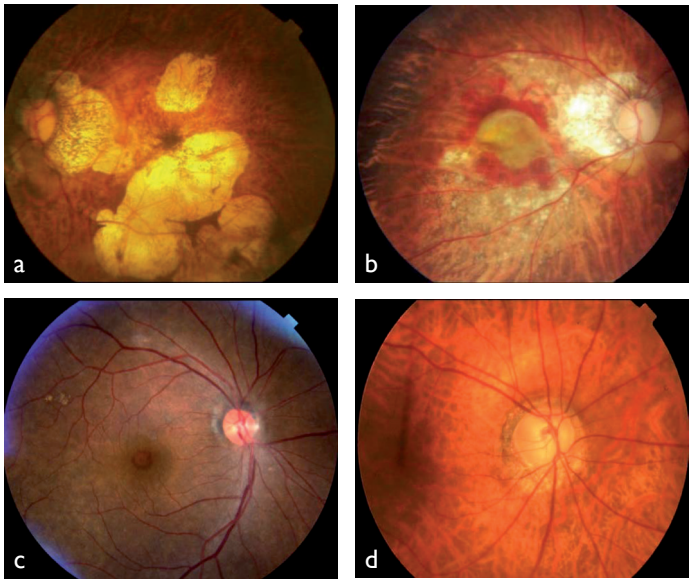
Figuur 3 MRI beelden van het oog: A. 3D beeld van emmetroop oog; B. hoog myoop oog met stafyloom; C. bijna complete vulling van de orbita door het oog bij patiëntje met brilsterkte -34 Dioptrie bij het syndroom van Donnai-Barrow. Figuur 3a en b uit: *Moriyama et al. Ophthalmology, 2011.*[69]



Retinale gevolgen van myopie

Een aslengte van >26 mm leidt vaak tot retinale verdunning en toenemende tractie van het glasvocht aan de retina; dit veroorzaakt moeilijk te behandelen retinale afwijkingen. Een van de gevolgen is het vormen van stafylomen (uitbochting van de achterkant van het oog) met myope maculadegeneratie, gekenmerkt door lacquer cracks (scheuren in het membraan van Bruch), retinale atrofie, choroidale neovascularisaties, maculagaten of schisis (splijting) van de retina (Figuur 4).[54] Vooral als deze afwijkingen de fovea aantasten ontstaat er ernstige slechtziendheid. Het dunner worden van het choroid (vaatvlies) kan retinale atrofie veroorzaken. Dit is als eerste te zien rondom de oogzenuw waar het choroid het dunst is en dit uit zich in peri-papillaire atrofie. Choroidale neovascularisaties zijn vaatnieuwvormingen vanuit het choroid naar de fovea en kunnen daar een fibrovasculaire, gepigmenteerde laesie veroorzaken, de zogenaamde Fuchs vlek. Een andere complicatie is ablatio retinae, een netvlies loslating. Personen met een myopie van -3D of meer hebben een 10x verhoogde kans hierop en ook hierbij geldt dat dit op jongere leeftijd gebeurt naarmate de aslengte hoger is.[55] Prodromen van een ablatio zijn lichtflitsen door tractie aan de retina en deze kunnen worden gevolgd door een scheur of een gat in de perifere retina. Als er dan door de opening vocht onder de retina komt, gaat deze

Figuur 4 Complicaties bij hoge myopie: chorioretinale atrofie (a); subretinale neovascularisatie (b); macula gat (c); peripapillaire atrofie (d)



afliggen, en ontstaat er een zogenaamde rhegmatogene ablatio. Tenslotte komt glaucoom ook vaker voor bij myopie. De oorzaak hiervan is grotendeels onbekend en de relatie met hoge oogdruk niet eenduidig. Het herkennen van glaucoom bij myopie is niet gemakkelijk doordat de oogzenuw vaak een schuine implant in de retina heeft; de glaucomateuze gezichtsvelduitval kan bij myopie veel sneller optreden.[56]

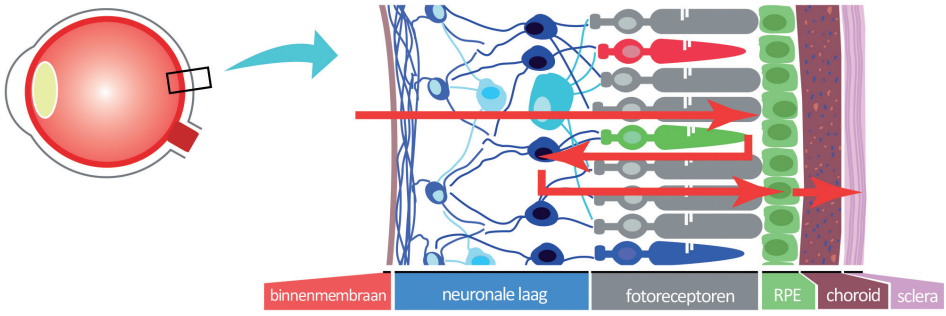
Voor slechts enkele retinale complicaties bestaat er een behandeling.Neovascularisaties kunnen in een beginstadium worden behandeld met maandelijkse anti-VEGF injecties in het oog, vergelijkbaar met de behandeling voor natte leeftijdsgebonden macula degeneratie (LMD). In tegenstelling tot LMD is een serie van 3 injecties vaak al voldoende om de bloeding te stoppen. Rhegmatogene ablatio's worden tegenwoordig meestal geopereerd door middel van een trans pars plana vitrectomie, met de visuele uitkomst sterk afhankelijk van aan- of afliggen van de macula. Glaucoom bij myopie kent geen andere behandeling dan glaucoom zonder myopie, d.w.z. behandeling met oogdruppels die de druk verlagen en laserbehandeling. Glaucoomchirurgie wordt meestal afgeraden wegens de kans op perforaties door de dunne sclera.

De hierboven genoemde complicaties vragen om oplettendheid van zorgverleners bij visusklachten geuit door een hoog-myope patiënt. Myope fundusafwijkingen kunnen al vanaf jonge leeftijd te zien zijn, maar veroorzaken meestal pas een visusdaling boven de 45 jaar.

Signaal cascade is oorzaak

Om inzicht te krijgen in de pathologie, wordt er de laatste jaren veel wetenschappelijk onderzoek verricht om meer mogelijkheden voor preventie en therapie te verkrijgen. De huidige inzichten over de ontstaanswijze van myopie wijzen erop dat in reactie op de projectie van licht een signaalcascade ontstaat in de retina die via het pigmentepitheel en de choroidea uitmondt in de sclera (Figuur 5). Daar vindt vervolgens remodelering van collageen structuren plaats die het oog langer maken. Deze hypothese wordt onderbouwd door grote studies waarbij gezocht werd naar genen die geassocieerd zijn met refractie afwijkingen. De gevonden genen spelen een rol in o.a. neurotransmissie, ionkanalen en de vitamine A cyclus, alle drie belangrijke onderdelen van signaaltransductie.[43,57] Tevens zijn er genen gevonden die een functie hebben in extracellulaire matrix of betrokken zijn bij oogontwikkeling.[58] Tezamen vormen de genen de eerste stap in het ontrafelen van myopisatie. De genen die nu bekend zijn verklaren echter nog maar een kleine proportie (~12%) van de variantie van refractie.[57] Er zullen meer genen zijn en de variantie zal voor een groot deel verklaard worden door interactie van genen met omgevingsfactoren.

Figuur 5 Schematische weergave van de myopie signaalcascade



Visuele stimuli die de retina bereiken initiëren een signaal cascade die start in de fotoreceptoren, daarna via de amacriene en bipolaire cellen door het retinale pigment epitheel en choroidea (vaatvlies) gaat, en eindigt in de sclera al waar het aanzet tot remodellering van de extracellulaire matrix en groei van het oog.

Omgevingsfactoren

Veel buiten spelen op de kinderleeftijd is de sterkste (beschermende) risicofactor die we nu kennen.[22,59] In een Chinese gerandomiseerde trial bleken kinderen die 3 jaar verplicht 40 minuten buiten moesten spelen 30% minder myopie te ontwikkelen dan hun leeftijdsgenoten die niet extra buiten speelden.[60] Het beschermende effect van buiten spelen wordt gewijd aan lichtintensiteit: binnenshuis is de intensiteit ongeveer 500 lux en buitenshuis is dit overdag 15000-40000 lux. Het mechanisme van de lichtbescherm-

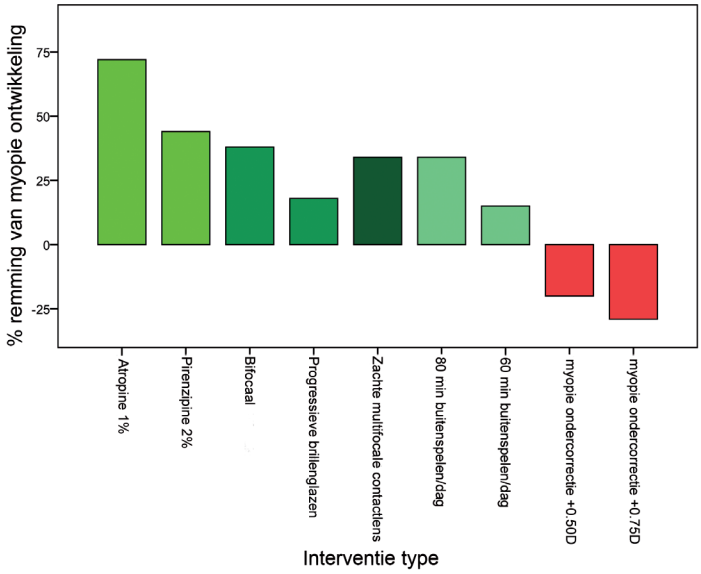
ing wordt toegeschreven aan de uitstoot van dopamine door de amacrine cellen van het netvlies, die een remmende werking heeft op de signaal cascade.[61,62]

De associatie tussen het verrichten van veel dichtbijwerk en myopie is minder duidelijk. Dichtbijwerk (o.a. aantal leesuren, gebruik van tablets, mobiele telefoons etc.) is lastig te kwantificeren, hetgeen leidt tot inconsistente bevindingen. Vooral het uren achtereen verrichten van dichtbijwerk of te werken op een korte afstand lijken het risico te vergroten.[63] De hypothese voor de rol van dichtbijwerk en ooglengtegroei is een toename van onscherpte in het perifere deel van het netvlies bij dichtbij kijken. Daar ligt het brandpunt dan achter het netvlies en is er sprake van hypermetropie. Uit dierexperimentele studies blijkt dat deze *perifere hypermetrope defocus* een trigger is voor verdere ooggroei naar achteren. De prolate (ei) vorm van het oog die een myoop toch al heeft wordt door het verrichten van veel dichtbijwerk versterkt.[64]

Behandeling

Het belangrijkste doel van de behandeling bij kinderen is het voorkomen van hoge myopie, of de sterkte zoveel mogelijk beperken indien er al sprake is van hoge myopie om de kans op complicaties later in het leven zo laag mogelijk te houden. Complete stilstand van de groei voor het 15e jaar wordt helaas nog niet vaak bereikt ondanks de huidig beschikbare medicamenteuze en optische interventies (figuur 6). Ondercorrectie van myopie

Figuur 6 Effect van de verschillende behandelingen op het verminderen van myopie progressie



De rode balkjes laten zien dat de groei van het oog toeneemt met behandeling; de groene balkjes laten een remmend effect op de groei zien. Uit: *Sankaridurg & Holden, Eye, 2014.*[64]

door brillenglazen of contactlenzen met onvoldoende sterkte werkt myopie progressie in de hand en moet derhalve sterk afgeraden worden.

Medicamenteuze behandeling met atropine, een niet-selectieve anti-muscarine antagonist, bereikt in de hoogste doseringen (0,5%-1,0%) een reductie van progressie tot 70%.[65] Deze hoge concentraties zorgen echter voor veel bijwerkingen zoals lichtschuwheid door de gedilateerde pupil en wazig zien van dichtbij door de volledige accommodatie verlamming.[66] De klachten kunnen echter goed bestreden worden door een bril met multifocale, meekleurende glazen en zijn maar in een klein deel van de kinderen een reden om te stoppen met behandeling. Het werkingsmechanisme van atropine is onduidelijk. Muscarine receptoren zijn aanwezig in de retina en sclera en aangezien deze beide structuren betrokken zijn bij de signaalcascade van myopisatie lijkt het aannemelijk dat atropine deze cascade onderbreekt. Permanente schade als gevolg van atropinegebruik is niet beschreven; belangrijk is dat er geen retinale schade door verhoogde lichtexpositie optreedt.[67] In Nederland wordt atropine steeds meer voorgeschreven voor myopie controle bij kinderen die kans hebben op hoge myopie. Een studie uitgevoerd bij progressief hoog myope kinderen in het ErasmusMC laat zien dat de 0.5% concentratie ook door kinderen van Europese afkomst goed verdragen wordt en dat het vergelijkbare groeiremming teweeg brengt als bij Aziatische kinderen.[66] Desondanks is de nieuwe trend uit Azië om kinderen tussen de 3 en 10 jaar bij beginnende myopie reeds te behandelen met lagere concentraties atropine (tot 0,01%), welke minder bijwerkingen en minder 'rebound' groei geven. Nadeel van deze concentraties is dat zij minder effectief zijn; zij bereiken een maximale remming van slechts 25-50% (figuur 6). Bij kinderen die nog niet lang myoop zijn en die nog geen hoge brilsterkten hebben is het echter een goed alternatief.[65]

Optische interventies zijn ook in zwang. Zij werken via een ander mechanisme dan atropine en beogen de perifere hypermetrope defocus te verminderen. Zowel de nachtlenzen (ortho-keratologie) als de speciaal vormgegeven mulifocale zachte contactlenzen zorgen voor een 25-50% reductie van de progressie. De veiligheid van ortho-K wordt vaak betwist naar aanleiding van publicaties over complicaties zoals micro bacteriële keratitis. Hoewel deze complicaties zeldzaam zijn en passen bij contactlensgebruik, is het een reden om voorzichtigheid te betrachten, vooral bij jonge kinderen.[68] In de Verenigde Staten is deze therapie voor myopie controle echter zeer populair.

Het toepassen van deze interventies vormt het begin van de strijd tegen slechtziendheid door myopie. Belangrijk is de behandelstrategie op de individuele patiënt af te stemmen en rekening te houden met de refractie en aslengte aan het begin van de behandeling, de snelheid van progressie, familiair voorkomen en de aanwezigheid van omgevingsfactoren. Juist de combinatie van leefstijladviezen tezamen met een interventie zal het grootste effect geven.

Wat doet u in uw rol als huisarts bij dit 8-jarige meisje en wat vertelt u de ouders? Op de leeftijd van 8 jaar zal het sterke accommodatieve vermogen van de lens interfereren met een brilmeting indien geen druppels gebruikt worden. Een verwijzing naar oogarts of orthoptist voor een cycloplegische brilmeting is dan ook noodzakelijk. Hoewel nog niet heel gebruikelijk in deze leeftijdsgroep, is het verstandig dat deze naast de refractie ook een oculaire biometrie verricht om de aslengte van het oog en de kromming van het hoornvlies op te meten. Daaruit zal blijken of patiëntje inderdaad myoop is door een te

lange aslengte voor haar leeftijd. Als huisarts kunt u de ouders vertellen dat het raadzaam is de brilsterkte beter dan -6 dioptrie te houden en de aslengte onder de 26 mm om later in het leven de kans op ernstige slechtziendheid zo klein mogelijk te houden. Een aanpassing in de leefstijl met meer dan 2 uur per dag buitenspelen en het inperken van langdurig (>45 minuten) achter elkaar dichtbij kijken is een noodzakelijke eerste stap. Mocht het kind daarna toch verdere progressie van de aslengte groei doormaken, dan zal een interventie met atropine of vormvaste contactlenzen geïnitieerd door de oogarts of orthoptist op zijn plaats zijn (www.myopie.nl).

Conclusie

Myopie is veel meer dan een alledaagse refractieafwijking. Het kan leiden tot slechtziendheid met name door myope maculadegeneratie. Vooral een hogere aslengte, met het ontstaan van myopie voor 10 jaar, hebben een verhoogd risico op complicaties op relatief jonge leeftijd. Atropine is op dit moment de meest effectieve interventie, maar heeft aanzienlijke bijwerkingen. De stijgende prevalentie, het grote effect van leefstijl en de risico's van hoge myopie maken erkenning van het probleem en een multidisciplinaire aanpak door oogartsen, orthoptisten, optometristen, opticiens, huisartsen, maar ook door jeugdartsen, maatschappelijke gezondheidszorg, scholen en wetenschappers noodzakelijk.

CHAPTER 3

ASSOCIATION OF AXIAL LENGTH WITH RISK OF UNCORRECTABLE VISUAL IMPAIRMENT FOR EUROPEANS WITH MYOPIA

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ABSTRACT

Importance: Myopia (nearsightedness) is becoming the most common blinding eye disorder in younger persons in many parts of the world. The visual impairment is associated with structural changes of the retina and the globe due to elongation of the eye axis. How axial length and myopia relates to the development of visual impairment over time is unknown.
Objectives: To study the relationship between axial length, spherical equivalent and visual impairment, and to make projections of visual impairment for regions with high prevalence rates.
Design: Population-based and case-control cohorts.
Setting: Rotterdam Study I-III, Erasmus Rucphen Family Study (ERF), and MYopia STudy (MYST) from the Netherlands.
Participants: 15,404 individuals with spherical equivalent and 9,074 individuals with axial length; right eyes were used for analyses.
Main outcomes and measures: Visual impairment and blindness (defined according to the WHO criteria as visual acuity <0.3), and predicted rates of visual impairment specifically for myopes.
Results: Of the 15693 individuals in this study, the mean (SD) age was 61.3 (11.4) years and 8962 (57.1) were female. Axial length ranged from 15.3 to 37.8 mm; 819 individuals had an axial length ≥26 mm. Spherical equivalent ranged from -25 to +14; 796 persons had high myopia (≤-6D). The prevalence of visual impairment varied from 1% - 4.1% in the population-based studies and was 5.4% and 0.3% in MYST cases and controls, respectively. The prevalence of visual impairment rose with increasing axial length and spherical equivalent with a cumulative incidence of visual impairment at age 75 years of 3.8% (se 1.3) for axial length 24-26 mm, increasing to more than 90% (se 8.1) for axial length ≥30 mm. The cumulative risk of visual impairment was 5.7% (se 1.3) at age 60, and 39% (se 4.9) at age 75 for high myopia. Projections of these data suggest that visual impairment will increase 7-13-fold by 2055 in high-risk areas.
Conclusions and relevance: This study demonstrated that visual impairment correlates with axial length and spherical equivalent, and may be unavoidable at the most extreme values in this population. Preventative strategies for myopia development and its complications could avoid an increase of visual impairment in the working age population.

INTRODUCTION

Myopia (nearsightedness) is a common refractive error, and generally considered as a nonthreatening condition which can be corrected with glasses, contact lenses, or refractive surgery. Nonetheless, myopia has increased rapidly during the past 30 years, predominantly in East Asia.[50,70-72] The trait results from excessive growth of the eyes' axial length, which is a sum of the anterior chamber depth, lens thickness, and vitreous chamber depth.[26,73,74] High myopia is defined as a spherical equivalent of ≤-6 diopters (D) with an axial length generally exceeding 26 mm.[75] The frequency of high myopia in the general population is estimated to be 3-20%.[16,72,76,77]

High myopia is currently one of the leading causes of legal blindness in developed countries due to complications occurring in adulthood, such as myopic macular degeneration, early cataract, retinal detachment, and/or glaucoma.[16] The rapid increase combined with the sight-threatening complications represents a significant public health burden.[78,79] Studies addressing the relationship between myopia and ocular pathology found that only few eyes with mild-to-moderate myopia develop ocular pathology in contrast to many eyes with high myopia.[48,80-83] From this, it seems a logical assumption that a longer axial length is associated with higher risks of visual impairment.[82,84,85] Nevertheless, precise risk estimates of the association between axial length and lifetime visual function are currently lacking.

In this study, we investigated the relationship between axial length, spherical equivalent and visual impairment as a function of age. We combined epidemiologic studies from the same research center to maximize the number of persons with very long axial length and high spherical equivalent, and to achieve sufficient statistical power for lifetime analyses. Next, we extrapolated our risk estimates to make a prediction of the rise in visual impairment in regions which have recently experienced a high increase in myopia prevalence. The goal of our study was to provide insights into the potential visual morbidity of the myopic shift that is occurring all over the world.

PATIENTS AND METHODS

Study populations

This study included cross-sectional data from 15,693 subjects of European descent (age 25+ years) from the population-based cohort studies Rotterdam Study I-III (RSI-III), the genetic isolated population Erasmus Rucphen Family Study (ERF), and the high-myopia case-control MYopia Study (MYST), all of which were conducted in or near Rotterdam, the Netherlands. All subjects with available data on best-corrected visual acuity and axial length or spherical equivalent were included. The rationale and study design of the studies have been described elsewhere.[86,87] A short description per study can be found in the eMethods. Measurements in all studies were collected after receiving approval from the medical ethics committee of the Erasmus University Medical Center, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Ophthalmic examination

Participants in the RS, ERF and MYST studies received an extensive ophthalmological examination as described previously.[86] This examination included a non-cycloplegic measurement of refractive error for both eyes using a Topcon RM-A2000 auto-refractor (Topcon Optical Company, Tokyo, Japan). After additional subjective refraction, best-corrected visual acuity was measured using the Lighthouse Distance Visual Acuity Test, a modified version of Early Treatment Diabetic Retinopathy Study (ETDRS) chart.[88] Axial length was measured using a Lenstar LS900 (Laméris Ootech, Haag-Streit, UK; in RSI-III) or an A-scan ultrasound device (Pacscan, Sonomed Escalon, Germany; in ERF and RS-III). Measurements of axial length were introduced in a later phase of RSI-III; therefore measurements of axial length were available in 5686 study participants of these studies. MYST subjects with an axial length >30 mm underwent an A-scan.

Statistical analysis

All subsequent analyses were performed on right eyes; left eyes were used if measurements on right eyes were not available. Spherical equivalent was calculated using the standard formula: spherical equivalent = sphere + (½ cylinder). In the analyses regarding spherical equivalent, subjects with a history of cataract surgery or refractive surgery were excluded unless data on spherical equivalent prior to surgery was available. Visual impairment was defined as (best-corrected visual acuity <0.3 and ≥0.05) or blindness (best-corrected visual acuity <0.05) according to the World Health Organization criteria.[89] We investigated the association between axial length and spherical equivalent, and axial length or spherical equivalent with birth year using ordinary least squares linear regression models with restricted cubic splines with three knots (10^{th}, 50^{th}, and 90^{th} percentiles) for axial length and birth year, and five (5^{th}, 27.5^{th}, 50^{th}, 72.5^{th}, and 95^{th} percentiles) for spherical equivalent and birth year, and the association between axial length and spherical equivalent. In the associations of axial length and spherical equivalent with birth year the MYST case-control study was excluded due to the study design. Prevalence estimates were calculated in percentages (($N_{visual\ impaired}/N_{total\ group}$)*100). Logistic regression was used to calculate odds ratios (OR) for visual impairment per axial length or spherical equivalent category. Axial length (<24, 24- 26, 26 -28, 28-30 and ≥30 mm) and spherical equivalent (>-0.5, -0.5 - -6 -, -6 - -10, -10 - -15 and ≤-15D) were categorized. High myopia was defined as ≤-6D. Quadratic terms were used to test for non-linearity of visual impairment risk. Analyses were stratified for age (<60 and ≥60 years), and adjusted for gender, age and cohort. Analyses on axial length were additionally adjusted for height.[90] Cumulative risk of visual impairment (i.e., VA <0.3) was estimated per axial length and spherical equivalent category using Kaplan-Meier product limit analysis. All participants ≥75 years of age were censored at 75 years of age in order to ensure unbiased estimates.

Projections of future visual impairment

In order to demonstrate the potential burden of visual impairment with increasing prevalences of myopia, we extrapolated the risk estimates from the current study to published reports on high myopia prevalences.[49] We considered five studies from Singapore,[91-95] four studies from the republic of Korea,[96-99] and one European consortium study,[50] as they were all population-based, using auto refraction or subjective refraction, and had reported age-specific myopia prevalences. Prevalence per birth decade was calculated by extracting age of participants from start year of the study. Weighted prevalence was calculated per birth decade per region. The projected increase in prevalence of visual impairment was calculated using the reported myopia prevalences and this study's cumulative risk of visual impairment. Ordinary least squares linear regression models were performed in R. Other statistical analyses were performed using the SPSS software package version 21.0 (IBM, Armonk, NY).

RESULTS

General characteristics

The selection of participants eligible for the current analysis is shown in Figure 1; the distribution of general characteristics is summarized in Table 1. Data on axial length was available in 9063 participants; data on spherical equivalent was available in 15406 participants. The studies comprised 819 persons with axial length ≥26 mm, and 806 persons had high myopia (≤-6D). In the population studies, the weighted mean axial length was 23.51 mm (SD: 1.23); in MYST, the mean axial length was 27.47 mm (SD: 1.82) in cases and 23.53 mm (SD: 0.83) in controls. The population-based studies showed a slight gender difference: males had a longer axial length (23.73 mm) than females (23.16 mm; P <0.001), and were more likely to have an axial length ≥26 mm (4.9% of males vs 2.3% of females; P <0.001). Visual impairment ranged from 1% - 4.1% in the population-based studies; and was 5.4% in cases and 0.3% in controls in MYST. Visual impairment was not associated with gender in any study (overall 1.3% of males vs 1.2% of females; P 0.69). The correlation between axial length and spherical equivalent (adjusted for age, gender, height) is shown in Figure 2 (r^2 quadratic 0.71).

Figure 1 Flowchart participants in analysis of axial length and spherical equivalent and visual impairment

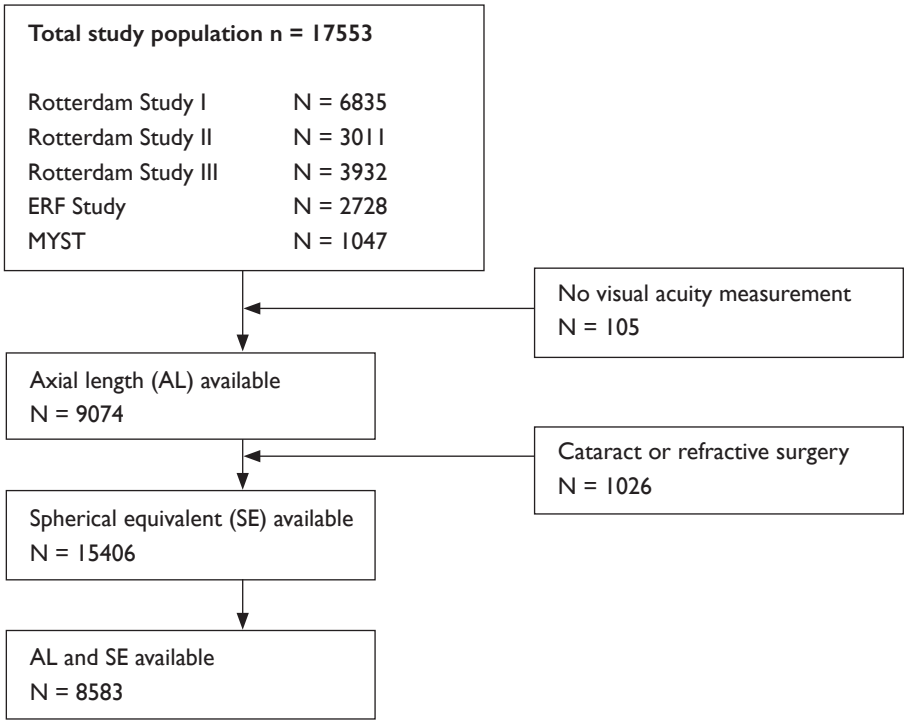


Figure 2 The correlations between spherical equivalent and axial length (N = 8583)

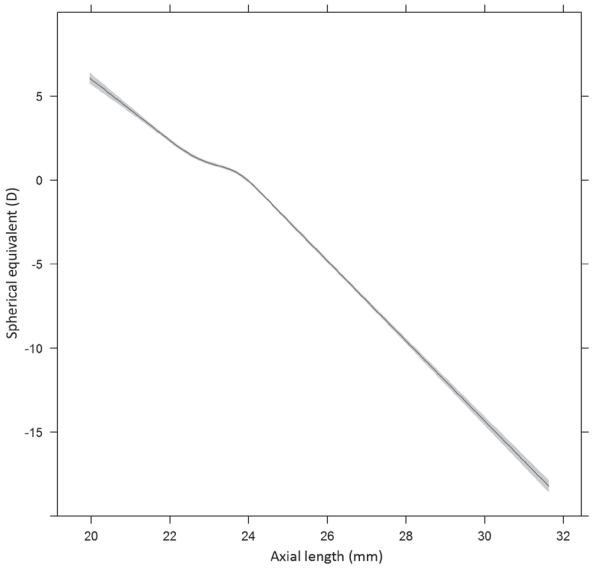


Table 1 General characteristics of the study participants for axial length and spherical equivalent

	RS-I	RS-II	RS-III	ERF	MYST	
Axial length					*cases*	*controls*
N	1005	1524	3157	2353	672	363
Male (%)	443 (44.1)	697 (45.7)	1376 (43.6)	1058 (45.0)	249 (37.1)	174 (47.9)
Age, years (SD)	62 (5)	62 (5)	57 (7)	50 (13)	47 (13)	50 (13)
Age, range	55 – 80	55 – 88	46 – 89	25 – 87	25 – 80	25 – 89
< 60 years	443 (44.1)	659 (43.2)	2237 (70.9)	1785 (75.9)	555 (82.6)	284 (78.2)
≥ 60 years	562 (55.9)	865 (56.8)	920 (29.1)	568 (24.1)	117 (17.4)	79 (21.8)
Axial length (mm)						
Mean (SD)	23.5 (1.3)	23.6 (1.2)	23.7 (1.3)	23.3 (1.1)	27.5 (1.8)	23.5 (0.8)
< 24	706 (70.2)	1076 (70.6)	2031 (64.3)	1871 (79.5)	2 (0.3)	259 (71.3)
24 – 26	269 (26.8)	396 (26.0)	976 (30.9)	441 (18.7)	126 (18.8)	102 (28.1)
26 – 28	26 (2.6)	46 (3.0)	134 (4.2)	39 (1.7)	340 (50.6)	2 (0.6)
28 – 30	1 (0.1)	3 (0.2)	15 (0.5)	2 (0.1)	132 (19.6)	0
≥ 30	3 (0.3)	3 (0.2)	1 (0.0)	0	72 (10.7)	0
Visual acuity						
> 0.5	980 (97.5)	1467 (96.3)	3030 (96.0)	2270 (96.5)	582 (86.6)	360 (99.1)
0.3 - 0.5	19 (1.9)	27 (1.8)	94 (3.0)	51 (2.2)	48 (7.2)	2 (0.6)
0.05 - 0.3	6 (0.6)	16 (1.0)	23 (0.7)	24 (1.0)	23 (3.4)	0
< 0.05	0 (0)	14 (0.9)	10 (0.3)	(0.3)	19 (2.8)	1 (0.3)
Spherical equivalent						
N	6382	2465	3405	2261	538	353
Male (%)	2605 (40.8)	1127 (46)	1487 (44)	1017 (45)	198 (37)	170 (48)
Age, years (SD)	70 (9)	64 (7)	57 (6)	50 (13)	46 (13)	49 (13)
Age, range	55 – 106	55 – 95	46 – 87	25 – 80	25 – 80	25 – 79
< 60 years	1155 (18.1)	878 (36)	2472 (73)	1738 (77)	455 (85)	279 (79)
≥ 60 years	5227 (81.9)	1587 (64)	933 (27)	523 (23)	83 (15)	74 (21)
Spherical equivalent, D						
Mean (SD)	0.87 (2.5)	0.49 (2.5)	-0.30 (2.6)	0.12 (2.1)	-10.0 (3.6)	0.03 (1.0)
> -0.5	5158 (80.8)	1863 (75.6)	2131 (62.6)	1636 (72.4)	0	261 (74.0)
-0.5 – -3.0	769 (12.1)	379 (15.4)	774 (22.7)	479 (21.2)	0	88 (24.9)
-3.0 – -6.0	346 (5.4)	179 (7.3)	390 (11.5)	112 (5.0)	39 (7.2)	4 (1.1)
-6.0 – -10.0	81 (1.3)	34 (1.3)	100 (2.9)	30 (1.3)	263 (48.9)	0
-10.0 – >-15.0	19 (0.3)	7 (0.3)	8 (0.2)	3 (0.1)	187 (34.8)	0
≤-15.0	9 (0.1)	3 (0.1)	2 (0.1)	1 (0.0)	49 (9.1)	0
Visual acuity						
> 0.5	5562 (87.2)	2323 (94.2)	3270 (96.0)	2185 (96.6)	474 (88.1)	350 (99.1)
0.3 – 0.5	557 (8.7)	82 (3.3)	102 (3.0)	45 (2.0)	35 (6.5)	2 (0.6)
0.05 – 0.3	186 (2.9)	36 (1.5)	23 (0.7)	23 (1.0)	15 (2.8)	0
< 0.05	77 (1.2)	24 (1.0)	10 (0.3)	8 (0.4)	14 (2.6)	1 (0.3)

Values are the absolute numbers (%) or mean (SD).

Cohort effect

As the cohorts had different starting points in time, we considered a potential cohort effect. We observed a linear increase in axial length with birth year (Figure 3a), and estimated an axial length increase of 0.008 mm/year (se 0.003; *P* 0.007) adjusted for height, gender, and cohort. Similarly, we found a shift from hyperopia to myopia with more recent birth years, in particular from 1920 onwards (Figure 3b) and a higher overall myopia-prevalence in the younger cohorts (Table 1).

Visual impairment in the case-control versus population-based cohorts

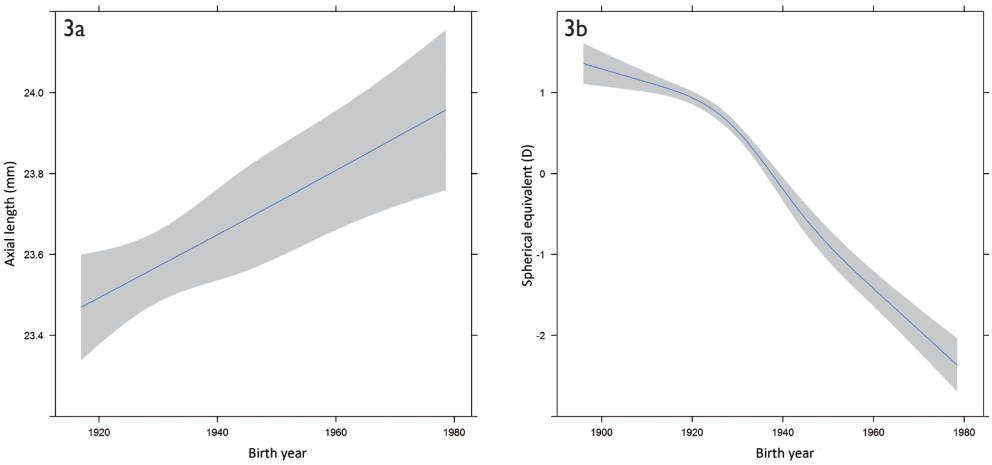
To investigate potential selection bias on visual impairment in the case-control study MYST, we compared the proportion of eyes with visual impairment as a function of axial length between the studies. We observed similar frequencies of visual impairment in two axial length strata in the population studies and the case-control study (<26 mm 0.8% vs 1.2% $P = 0.66$; ≥26 mm 7.1% vs 4.0% $P = 0.09$). As the population-based studies comprised more 60+ participants, the proportion of persons with visual impairment was higher in all refractive error strata. However, after adjustment for age there was no difference in prevalence of visual impairment between the population-based and the case-control studies (high myopia $P = 0.56$; non-high myopia $P = 0.19$), indicating that selection of particularly visual impaired persons in MYST was unlikely and combining study data is valid. Refractive and cataract surgery was applied more often in participants with higher axial length (population-based studies 23.92 vs 23.50 mm; $P = 0.007$, case-control study 27.94 vs 25.81 mm; $P < 0.001$) and participants with visual impairment (population-based 11% (75/686) vs 3% (387/14514) $P < 0.001$; and case-control study 10% (13/128) vs 3% (30/893) $P < 0.001$).

In subjects with axial length ≥26 mm, the frequency of visual impairment was 6.1%, which increased exponentially with age (age^2 $P < 0.001$). The groups were stratified in the age groups <60 and ≥60 years of age. In the age group <60 years in eyes with axial length ≥26 mm and <26 mm, the prevalence of visual impairment was 4.1% versus 0.9%. In the age group ≥60 years these prevalences were 13.0% versus 1.6% respectively. With respect to refractive error, the prevalence of visual impairment was 5.3% in myopes vs 3.7% in non-myopes in those aged ≥60 years, and 1.5% vs 0.9% in those <60 years.

Risk of visual impairment as a function of axial length and spherical equivalent

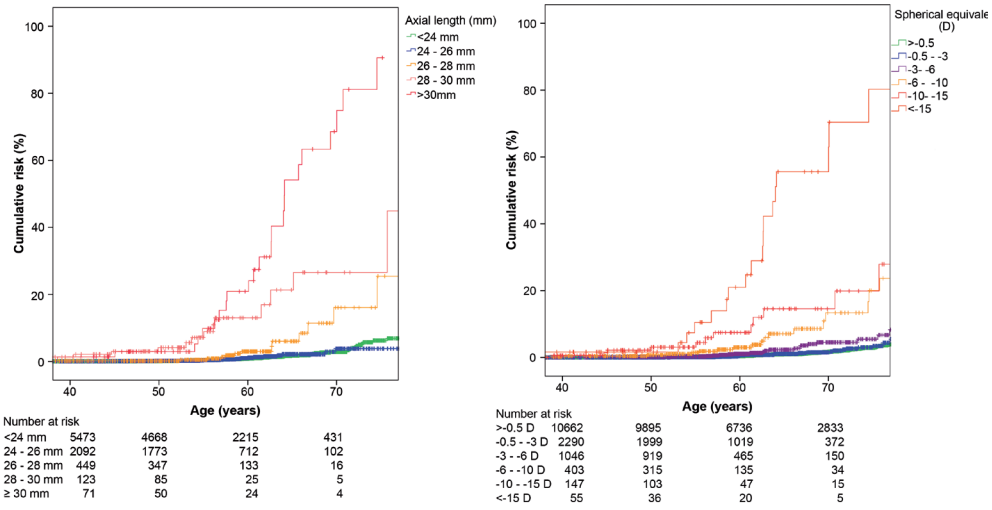
Subsequently, we combined data from all cohorts, maximizing statistical power. First, we performed logistic regression analysis to estimate the odds ratio (OR) of visual impairment with increased axial length and spherical equivalent in two age strata. In the age group <60 years, eyes with axial length ≥28 had 11 – 24 times higher risk for visual impairment than eyes <24 mm. In the age group ≥60 years, all categories ≥26 mm had

Figure 3 a. Axial length (N=8039) and b. Refractive error (N=14513) in the 20th century*



*Only Rotterdam Study I-III and ERF are used for these figures.

Figure 4 Cumulative risk of visual impairment as a function of axial length and spherical equivalent



higher risk (OR 3 – 94; table 2) than eyes <24 mm. For spherical equivalent, trends were similar with the highest risks for high myopia (table 2). When axial length as well as spherical equivalent were both added to the model, axial length still had a significant association with visual impairment (OR 1.46 (95% CI 1.09 – 1.97) per mm), but spherical equivalent did not (OR 0.98 (95%CI 0.86 – 1.10) per diopter).

Next, we examined the cumulative risk of visual impairment in relation to axial length and spherical equivalent (Figure 4). By age 75, the cumulative risk of visual impairment

was 6.9% (standard error (se): 1.3) for axial length <24 mm, 3.8% (se 1.3) for 24-26 mm, 25.4% (se 10.3) for 26-28 mm, 26.6% (se 8.1) for 28 - 30 mm, and 90.6% (se 8.1) for ≥30 mm. The cumulative risk of visual impairment for eyes with axial length 26-28 mm increased gradually from 60 years onwards, whereas eyes with axial length ≥28mm were increasingly visually impaired from approximately 45 years. Spherical equivalent showed similar trends, although cumulative risks were slightly lower than for axial length. By age 75, the cumulative risk of visual impairment was 2.9% (se 0.3) for spherical equivalent >-0.5D, 3.8% (se 0.7) for -0.5 to -6D, 20.0% (se 5.9) for -6 to -10D, 19.9% (se 6.8) for -10 to -15D and 80.3% (se 11.0) for ≤-15D.

Taken together, all high myopia (≤-6D) had a cumulative risk of visual impairment of 5.7% (se 1.3) at age 60 years, and of 39% (se 4.9) at age 75 years. For spherical equivalent between ≤-0.5 and >-6D, these risks were 0.8% (se 0.2) and 3.8% (se 0.7), respectively. These estimates were used for comparison with other areas in the world (see below).

Table 2 Risk of visual impairment (visual acuity <0.3) per axial length and spherical equivalent category <60 years and ≥60 years of age

	<60 years OR (95% CI)	**≥60 years OR (95% CI)**		**<60 years OR (95% CI)**	**≥60 years OR (95% CI)**
Axial length (mm)			*Spherical equivalent (D)*		
<24	1 [Reference]	1 [Reference]	>-0.5	1 [Reference]	1 [Reference]
24 – 26	0.95	0.65	-0.5 – -3.0	0.69	0.92
	(0.51 – 1.80)	(0.29 – 1.48)		(0.34 – 1.43)	(0.62 – 1.35)
26 – 28	2.01	3.07	-3.0 – -6.0	1.42	1.71
	(0.88 – 4.62)	(1.26 – 7.49)		(0.66 – 3.05)	(1.07 – 2.74)
28 – 30	11.01	9.69	-6.0 – -10	2.95	5.54
	(5.23 – 23.10)	(3.06 – 30.71)		(1.35 – 6.42)	(3.12 – 9.85)
≥ 30	24.69	93.62	-10 – -15	6.79	7.77
	(11.02 – 55.31)	(38.35 – 228.55)		(2.87 – 16.06)	(3.36 – 17.99)
			≤-15	27.85	87.63
				(11.34 – 68.37)	(34.50 – 222.58)

Models are adjusted for age and gender. Abbreviations: D, diopter; OR, odds ratio.

Projection of visual impairment to regions with increasing myopia prevalence

Reported prevalence estimates of myopia in three geographic areas (Singapore, Republic of Korea and Western-Europe) were used to estimate increase in prevalence of visual impairment as a function of birth year. Prevalence rates of visual impairment will rise in all areas, most prominently for the ages beyond 75 years (Table 3). By the year 2055, visual impairment will have increased two to threefold in Europe, thee to fivefold in

Table 3 Prevalence of myopia per birth year decade and related increase in prevalence of visual impairment (VI) at 60 and 75 years of age

		Myopia prevalence (%)		Surplus of VI (%, 95% CI)	
Region	**Birth year**	**Myopia**	**High myopia**	**60 years of age**	**75 years of age**
Europe, No.					
683	1920 – 1930	122 (17.9)	9 (1.4)	0.21 (0.11 – 0.31)	1.17 (0.81 – 1.54)
6280	1930 – 1940	1036 (16.5)	94 (1.5)	0.21 (0.11 – 0.30)	1.16 (0.81 – 1.51)
17119	1940 – 1950	2568 (15.0)	205 (1.2)	0.18 (0.09 – 0.26)	1.00 (0.69 – 1.31)
18888	1950 – 1960	4552 (24.1)	416 (2.2)	0.30 (0.16 – 0.44)	1.70 (1.18 – 2.21)
9792	1960 – 1970	3437 (35.1)	274 (2.8)	0.42 (0.22 – 0.61)	2.31 (1.60 – 3.03)
7906	1970 – 1980	3178 (40.2)	269 (3.4)	0.49 (0.26 – 0.72)	2.73 (1.90 – 3.57)
808	After 1980	342 (42.3)	33 (4.1)	0.54 (0.28 – 0.79)	3.04 (2.13 – 3.96)
Singapore, No.					
141	before 1920	46 (32.6)	4 (3.1)	0.41 (0.22 – 0.61)	2.33 (1.63 – 3.04)
1395	1920 – 1930	324 (23.2)	39 (2.8)	0.32 (0.17 – 0.48)	1.88 (1.33 – 2.43)
3236	1930 – 1940	880 (27.2)	126 (3.9)	0.41 (0.22 – 0.60)	2.40 (1.71 – 3.10)
3389	1940 – 1950	847 (25.0)	142 (4.2)	0.40 (0.22 – 0.59)	2.41 (1.73 – 2.10)
4094	1950 – 1960	1388 (33.9)	270 (6.6)	0.59 (0.32 – 0.87)	3.61 (2.60 – 4.62)
2437	1960 – 1970	1155 (47.4)	280 (11.5)	0.94 (0.51 – 1.38)	5.85 (4.25 – 7.45)
15086	After 1970	11963 (79.3)	1976 (13.1)	1.28 (0.68 – 1.87)	7.62 (5.46 – 9.80)
Republic of Korea , No.					
63	1920 – 1930	22 (34.9)	0	0.28 (0.14 – 0.42)	1.33 (0.85 – 1.81)
2768	1930 – 1940	498 (18.0)	28 (1.0)	0.19 (0.10 – 0.29)	1.04 (0.71 – 1.37)
3809	1940 – 1950	602 (15.8)	46 (1.2)	0.19 (0.10 – 0.27)	1.03 (0.71 – 1.35)
4344	1950 – 1960	1381 (31.8)	65 (1.5)	0.33 (0.17 – 0.49)	1.74 (1.18 – 2.31)
4516	1960 – 1970	2692 (59.6)	181 (4.0)	0.67 (0.35 – 0.99)	3.68 (2.53 – 4.83)
4381	1970 – 1980	3189 (72.8)	250 (5.7)	0.86 (0.45 – 1.27)	4.77 (3.30 – 6.25)
28642	After 1980	26866 (93.8)	1078 (19.4)	1.70 (0.92 – 2.49)	10.39 (7.51 – 13.29)

VI = Visual impairment (visual acuity <0.3).

VI at 60 years was calculated using the formula (% myopia - % high myopia) * 0.008 + % high myopia * 0.057.

VI at 75 years was calculated using the formula (% myopia - % high myopia) * 0.038 + % high myopia * 0.39.

95% CI were calculated using 1.96 * standard error of the cumulative risk.

Proportions are cumulative risks derived from the Rotterdam Studies, ERF and MYST.

Singapore and even three to six fold in the Republic of Korea. In the latter country, more than 10% (95%CI 8 – 13) of the population will suffer from visual impairment due to myopia at the age of 75 years.

DISCUSSION

In this study, which included several cohorts sequentially executed at the same research center and which covered a large range of axial length and spherical equivalent, we found increasing prevalence rates of myopia with birth year. Axial length was highly correlated with spherical equivalent, and both showed a close relationship with visual impairment. Of all high myopes, 39% developed visual impairment at age 75 years. In particular those at the more extreme ends of the axial length spectrum were at great risk of visual impairment: risks increased from 3.8% in eyes with axial length <26mm, to 25% in eyes with axial length ≥26mm and to >90% in eyes with axial length ≥30mm. Projections of these risks to areas with a high incidence of myopia indicate that visual impairment will be rising considerably as the population ages, and one in ten persons will develop visual impairment in the most endemic regions.

Strength and limitations

A strength of this study is the large study sample of all Rotterdam cohorts to maximize statistical power and the numbers of persons at the extreme ends of the phenotype. The Rotterdam study assessment of refractive error and visual impairment over 25 years. MYST is the only high myopia case-control study in Europe to date. All studies used identical study protocols, and were carried out at the same research center and examiners. This increased homogeneity across studies, validating a pooled analysis of outcomes. A potential source of limitation is selective non-participation of disabled persons in the population-based studies, as well as selective participation of visually disabled in the case-control study. These biases did not appear to play an important role, as visual impairment per se was not differentially distributed in any of the studies. For projection of our findings to high risk regions, we exploited data from local prevalence studies. These studies used different methodology for biometry and refractive error, however, given the small differences of outcome parameters between machines, we do not think this distorted our prediction estimates.[100,101] The cumulative risk in the extreme high myopia group (≤-15 D) may have been overestimated as a result of the relatively low number at the higher ages. Nevertheless, the strong rise of visual impairment at a relatively early age underscored the lifetime visual morbidity in this category. Another limitation may be projection of data from a European study population to Asian ethnicity, although there is no evidence that ocular morbidity resulting from myopia varies among ethnicities.

Interpretation of results

These results suggest that more persons will become visually impaired in the following decades. The current myopia figures as well as the expected increase in myopia prevalence are comparable between Europe and the United States,[72] and hence, we expect a similar rise of visual impairment.[102] The current myopia epidemic in countries as Korea, Taiwan and Singapore will cause an exponential rise in visual impairment to a frequency of 5-10% in the 75+ population after 2040. Our estimates imply that the current lack of intervention will continue. When health and ophthalmic care, and future preventative and therapeutic means to interfere with development of myopia improve, these estimates will be overstated.

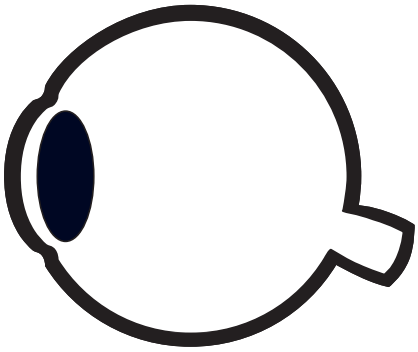
The relatively young age of onset of visual impairment in myopia contributes to its increased morbidity. The impact on personal lives and public health can be more devastating for myopia than for eye diseases with an older onset like age-related macular degeneration or open angle glaucoma.[103] An early age-related penetrance of myopic complications was also noted by other studies.[104-108] The increasing prevalence and relatively early-onset of visual impairment necessitate implementation of effective preventive and therapeutic measures. Currently, there is little one can do to counteract the morbidity. Studies have shown that a 40 minutes/day Increase in outdoor time in schoolchildren will reduce myopia incidence by 10%.[60] Pharmacologically, atropine was shown to be the most effective treatment to reduce myopia progression, but has serious side effects and shows a rebound effect when medication is stopped.[65,66] Medical treatments of myopia-related complications are increasing, but still do not always improve visual outcome.[109] Anti-VEGF therapy is available for subretinal neovascularization, surgery for detachments and epiretinal membranes, and laser for retinal holes with traction. However, no treatment options are available for the most frequently occurring complication: myopic staphyloma with subsequent retinal atrophy or macular schisis.[48] It is likely that the public and scientific awareness for myopia and myopic complications will increase when the current population of high myopes ages and will be more at risk of visual impairment.

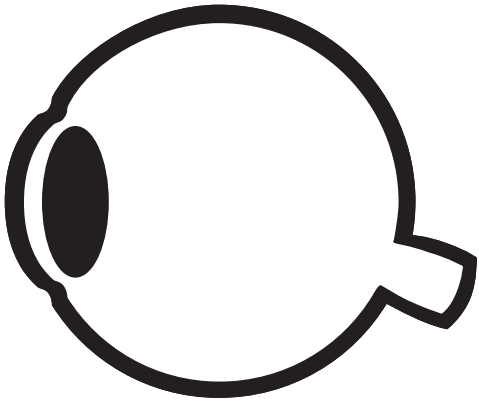
CONCLUSION

We examined the risk of visual impairment in categories of axial length and spherical equivalent using a very large data set of Europeans. The risk of visual impairment was correlated with axial length and spherical equivalent, and reached the highest values for high myopia (≤-15D), in particular for axial length ≥30 mm. Our projections show that myopia with its increasing axial length will bring major threats to the visual health of the public in many societies. Given the global increase of myopia and rise in high myopia, the development of strategies to prevent and overcome its visually impairing complications asks for large scale interventions. This requires increased awareness among policy makers and medical experts regarding the myopia-related risks.

PART III

OCULAR BIOMETRY DEVELOPMENT





CHAPTER 4

GROWTH IN FETAL LIFE, INFANCY, AND EARLY CHILDHOOD AND THE ASSOCIATION WITH OCULAR BIOMETRY

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ABSTRACT

Purpose: To study the effect of fetal and infant growth on ocular biometry, determine the most important period for this association, and to examine genetic overlap with height and birth weight.
Methods: 5,931 children (50.1% girls) from a population-based prospective birth cohort study underwent intra-uterine and infant growth measurements at second and third trimester, and from birth to 72 months. At age 6.2 (SD 0.5) years, a stepwise ophthalmic examination including axial length (AL(mm)) and corneal radius of curvature (CR(mm)) was performed. The associations between prenatal and postnatal growth variables and AL and CR were assessed with conditional linear regression analyses. Weighted genetic risk scores for birthweight and height were calculated and causality was tested with Mendelian randomization.
Results: Weight and head circumference from mid-pregnancy onward were most important prognostic factors for AL and CR. For weight (SDS), the association with AL was greatest for the measurement at 24 months (β 0.152 P <0.001); association with CR was greatest for the measurement at 12 months (β 0.065 P <0.001). The genetic height and birthweight risk scores were both significantly associated with ocular biometry.
Conclusions: Pre- and perinatal growth parameters are associated with ocular biometry in early childhood. Body growth may have a shared genetic background with AL and CR at a young age.

INTRODUCTION

Refractive errors, in particular myopia, are the most common eye disorders worldwide.[50,72,94] These conditions are caused by a failure of emmetropisation, a complex coordinated scaling of the eye's refractive components to place the focal plane on the retina.[1,110,111] Two of the key components in emmetropisation are axial length (AL) and corneal radius of curvature (CR). The ratio of AL/CR strongly correlates with refractive error (RE);[10,112,113] and high values of AL are associated with an increased risk of visual impairment,[114] retinal pathology,[115] and glaucoma.[17]

Currently, several pharmacologic and optical treatments can significantly reduce the progression of myopia in childhood.[64] In particular AL is increasingly being used to monitor the effect of these treatments. A caveat is that these biometric measures show large variation even in subjects with the same refractive error.[111] This asks for a better understanding of their determinants.

Growth trajectories and birth parameters such as height and weight have been associated with ocular biometry.[116,117] Genetic overlap between these traits has also been shown: a higher genetic risk score of height was associated with a higher CR in 15 year old children.[117] Approximately 75% of normal ocular growth occurs intra uterine.[52] Yet, the effect of prenatal growth trajectories on ocular biometry and myopia is unknown.

The aim of this study was to determine the effect of intra uterine growth on ocular biometry in school children, and to investigate potential genetic commonalities with height and birth weight.

MATERIAL AND METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands. A total of 9,778 pregnant women were included in the study with children born between April 2002 and January 2006 and 6,690 participated with their children for physical examination in the research centre at 6 years of age.[46] The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20). Written informed consent was obtained from all participants.

Prenatal measurements

Fetal ultrasound examinations were carried out in early (<18 weeks), mid (18-25 weeks) and late (≥25 weeks) pregnancy. Gestational age was determined using questionnaire and the fetal ultrasound in the first trimester. Head circumference (HC), abdominal circumference (AC) and femur length (FL) were measured using the standardized pro-

cedures to the nearest millimetre in the second and third trimester.[118] Estimated fetal weight was calculated using the Hadlock formula, an estimate based on HC, FL and AC.[119] The data obtained were used to calculate gestational age adjusted standardized deviation score (SDS) for each growth outcome.[118]

Birth parameters and postnatal measurements birth parameters, gestational age, birth weight, and HC were obtained using medical records and hospital registries. SDS for weight for gestational age were calculated according to Northern European growth Standards.[120] Postnatal growth characteristics were measured using standardized schedules and procedures at 6, 12, 24, 36, 48 months in community health centres. SDS for the growth characteristics postnatal were calculated based on Dutch growth reference charts (Growth analyzer 3.0, Dutch Growth Research Foundation). Prenatal growth and postnatal growth patterns, decelerated/normal/accelerated growth, were defined as weight change (in SDS) between second trimester and birth, and birth and 6 months with a decrease or increase with 0.67 SDS or for normal growth within this range. Gestational age at birth categorized in before and after 37 weeks of gestation and birthweight into below and above 2500 grams according to preterm birth and low birth weight standards.

AL and CR

Ocular biometry (AL, CR) was obtained with a Zeiss IOL-master 500 (Carl Zeiss MEDITEC IOL-master, Jena, Germany). Data were collected from right and left eyes. Five measurements of AL were taken of the right and the left eye and averaged. Three measurement of K1 and K2 were taken of the right and left eye, and were averaged. AL/CR ratio was calculated by dividing the mean AL (mm) by the mean CR (mm).

Genetics

Samples were genotyped using Illumina Infinium II HumanHap610 Quad Arrays following standard manufacturer's protocols. Intensity files were analyzed using the Beadstudio Genotyping Module software v.3.2.32, and genotype calling based on default cluster files. Any sample displaying call rates below 97.5%, excess of autosomal heterozygosity (F<mean-4SD) and mismatch between called and phenotypic gender (0.2%) were excluded. Genotypes were imputed for all polymorphic SNPs from phased haplotypes in autosomal chromosomes using the 1000 Genomes GIANTv3 panel.

Covariates

Age, parity, smoking and alcohol use during pregnancy, pre pregnancy weight of the mother, educational level and ethnicity were obtained using questionnaires. Educational level was categorized in primary and secondary or higher education. Ethnicity was classified according to the Dutch standard Classification criteria of Statistics Netherlands,[121]

and grouped into European and non-European. The height of the mother was measured without shoes. Child height and weight were measured at 6 years of age, BMI (kg/m^2) of children was calculated. Twins were excluded for analysis due to their known relation with prenatal growth.

Statistical analysis

Outcomes were AL and CR at age 6 as continuous variables. Linear regression models were used to test for associations between intra-uterine growth parameters and AL, CR of AL/CR ratio. Models were adjusted for potential confounding effects of age, gender, and ethnicity. Covariates were only added to the model if they were significantly ($P < 0.05$) associated with the outcome and determinant independent of age, gender, ethnicity and weight for gestational age. Nonlinearity was tested using quadratic terms and third degree polynomials. We investigated the shape of the association between non-linear associations using ordinary least squares linear regression models with restricted cubic splines with three knots at the 10th, 50th, and 90th percentile. Growth trajectories were tested using restricted growth (<-0.67 SDS difference), normal growth (>-0.67 and <0.67 difference) and accelerated growth (>0.67 SDS difference) in weight during two time spans (from second trimester to birth, and from birth to 6 months postnatal). Conditional analyses were applied to identify the most important time period for the association between pre and postnatal growth with ocular biometry.[122] The conditional analyses were performed using standardized residuals from linear regression models adjusted for prior growth measurements, resulting in statistically independent growth measurement which can be all together added to the multiple regression models.[122] Association between pre- and postnatal growth variables were assessed using standardized residuals with conditional linear regression for AL, CR, and AL/CR to study the effect of all measurements in one model. A genetic risk score was calculated as the sum of beta*allele dosage of each top SNPs per independent locus associated with height (687/695 SNPs available) and birth weight (60/60 SNPs available).[123,124] Effect of the genetic risk scores was tested using linear regression with AL and CR as outcome. To test for causality, the genetic risk scores were used as an instrumental variable in the two-stage least square method for the association between age, sex, and ethnicity standardized residuals of AL and CR and height or birthweight. Ordinary least squares linear regression models and two-stage least square models were performed using the statistical program R (version 3.2.2). All other analyses were performed in SPSS (version 21.0.0.1).

RESULTS

Ocular biometry and covariates were available for 5,931 children. Supplementary figure 1 shows the flow diagram for inclusion of participants. Table 1 shows the general characteristics of the participating children. The average age of the children at the eye examina-

tion was 6.2 years (SD ±0.5 range 4.9 – 9.0 years), and 68.8% of the children were from European descent. Environmental factors or pregnancy related factors such as maternal education season of birth, parity, alcohol or smoking during pregnancy were not associated with AL and CR (supplemental table 1), and were therefore not used as covariates in the models.

Table 1 General and ocular characteristics of the children (n = 5,931)

	All
Child characteristics	
Age child at ocular measurements (years)	6.2 (0.5)
Female sex (%)	50.1 (2,970)
Birth weight (grams)	3427 (552)
Gestational age (weeks)	39.8 (1.8)
Height at 6 years (m)	1.20 (6.0)
Weight at 6 years (kg)	23.3 (4.3)
Head circumference at 6 years (cm)	51.4 (1.6)
Axial length (mm)	22.36 (0.75)
Average corneal radius (mm)	7.77 (0.26)
Average AL/CR ratio	2.88 (0.08)
European ethnicity (%)	66.8 (3,963)

Values are means (SD) or percentages (absolute numbers).

Intra-uterine growth and ocular biometry

Table 2 shows the association of early-, mid- and late pregnancy growth parameters with ocular biometry at age 6. At mid pregnancy, HC showed the highest association with AL and CR, but was not associated with AL/CR. All associations were stronger for late pregnancy. Estimated fetal weight showed the highest association with AL in this trimester; only HC was associated with AL/CR ratio. There was no evidence for non-linearity in any of the associations with prenatal parameters.

Table 3 shows the results of the analyses for growth periods. All children with a fetal growth restriction had smaller AL and CR compared to children with normal fetal growth. Children with fetal accelerated growth had higher AL and CR, but no significant difference in AL/CR. Children with fetal restricted and infant restricted growth had a more myopic AL/CR ratio than the children with normal growth.

Table 2 Fetal and infant growth characteristics and the association with ocular biometry

	Axial length (mm)	Corneal radius of curvature (mm)	AL/CR ratio
Prenatal			
Early pregnancy			
Crown-to-Rump Length (n=1,118)	0.017 (-0.032 – 0.067)	0.007 (-0.012 – 0.025)	0.000 (-0.006 – 0.005)
Mid pregnancy			
Head circumference (n=5,103)	**0.042 (0.022 – 0.061)**	**0.020 (0.013 – 0.027)**	-0.002 (-0.004 – 0.000)
Femur length (n=5,120)	**0.025 (0.006 – 0.044)**	**0.008 (0.002 – 0.015)**	0.000 (-0.002 – 0.002)
Abdominal circumference (n=5,113)	**0.041 (0.022 – 0.061)**	**0.017 (0.010 – 0.024)**	-0.001 (-0.003 – 0.001)
Estimated Weight (n=5,093)	**0.042 (0.023 – 0.062)**	**0.017 (0.010 – 0.024)**	-0.001 (-0.003 – 0.002)
Late pregnancy			
Head circumference (n=5,214)	**0.086 (0.066 – 0.105)**	**0.040 (0.033 – 0.047)**	**-0.004 (-0.006 – -0.002)**
Femur length (n=5,251)	**0.063 (0.044 – 0.082)**	**0.025 (0.018 – 0.032)**	-0.001 (-0.003 – 0.001)
Abdominal circumference (n=5,014)	**0.083 (0.064 – 0.101)**	**0.034 (0.027 – 0.041)**	-0.002 (-0.004 – 0.000)
Estimated Weight (n=5,006)	**0.090 (0.072 – 0.109)**	**0.037 (0.030 – 0.043)**	-0.002 (-0.004 – 0.000)
Birth parameters			
Head circumference (n=2,952)	**0.071 (0.050 – 0.093)**	**0.033 (0.025 – 0.040)**	**-0.003 (-0.005 – -0.000)**
Birthweight (kg) (n=5,923)	**0.227 (0.195 – 0.259)**	**0.093 (0.081 – 0.104)**	**-0.005 (-0.009 – -0.002)**
Weight for gestational age (n=5,884)	**0.132 (0.115 – 0.150)**	**0.050 (0.044 – 0.057)**	-0.002 (-0.004 – 0.000)
Gestational age (weeks) (n=5,895)	**0.019 (0.009 – 0.029)**	**0.010 (0.007 – 0.014)**	-0.001 (-0.003 – -0.000)
Postnatal			
Weight			
3 months (n=3,528)	**0.151 (0.129 – 0.172)**	**0.060 (0.052 – 0.068)**	**-0.003 (-0.005 – -0.000)**
6 months (n=4,407)	**0.149 (0.128 – 0.170)**	**0.063 (0.056 – 0.071)**	**-0.004 (-0.007 – -0.002)**
12 months (n=4,084)	**0.148 (0.126 – 0.170)**	**0.065 (0.057 – 0.073)**	**-0.005 (-0.007 – -0.003)**
24 months (n=3,828)	**0.152 (0.130 – 0.173)**	**0.061 (0.053 – 0.069)**	**-0.003 (-0.006 – -0.001)**
36 months (n=3,633)	**0.133 (0.111 – 0.155)**	**0.056 (0.048 – 0.064)**	**-0.004 (-0.006 – -0.001)**
48 months (n=3,197)	**0.131 (0.108 – 0.154)**	**0.056 (0.048 – 0.065)**	**-0.004 (-0.006 – -0.001)**
72 months (n=5,923)	**0.131 (0.115 – 0.148)**	**0.045 (0.039 – 0.051)**	0.000 (-0.002 – -0.002)
Head circumference			
6 month (n=4,323)	**0.147 (0.125 – 0.169)**	**0.069 (0.061 – 0.077)**	**-0.007 (-0.009 – -0.004)**
12 months (n=3,977)	**0.146 (0.123 – 0.169)**	**0.067 (0.059 – 0.076)**	**-0.006 (-0.009 – -0.004)**
72 months (n=5,778)	**0.141 (0.122 – 0.160)**	**0.059 (0.052 – 0.066)**	**-0.004 (-0.006 – -0.002)**

Values are regression coefficients per SDS (except if otherwise displayed, kg or weeks) and 95% confidence intervals for the beta for increase axial length (AL; mm), corneal radius (CR; mm) or AL/CR ratio from linear regression models. "n =" represents number of total group. Models were adjusted for gender, age of anthropometry measurement, ethnicity and age of eye measurements. $P < 0.05$ are shown in bold.

Table 3 Fetal and infant growth patterns and correlation with ocular biometry at 6 years of age (N = 3,849)

	Beta's (95% CI)		
	Axial length (mm)	**Corneal radius (mm)**	**AL/CR ratio**
Fetal restricted			
Infant restricted	**-0.17 (-0.32 – -0.01)**	**-0.11 (-0.17 – -0.06)**	**0.02 (0.00 – 0.04)**
Infant normal	**-0.19 (-0.27 – -0.11)**	**-0.07 (-0.10 – -0.04)**	0.00 (-0.01 – 0.01)
Infant accelerated	**-0.13 (-0.21 – -0.06)**	**-0.07 (-0.10 – -0.04)**	**0.01 (0.00 – 0.02)**
Fetal normal			
Infant restricted	-0.09 (-0.18 – 0.00)	**-0.04 (-0.08 - -0.01)**	0.01 (-0.01 – 0.01)
Infant normal	**Ref**	**Ref**	**Ref**
Infant accelerated	**0.10 (0.03 – 0.18)**	**0.03 (0.00 – 0.06)**	0.00 (-0.01 – 0.01)
Fetal accelerated			
Infant restricted	**0.12 (0.04 – 0.20)**	0.03 (0.00 – 0.06)	0.00 (-0.01 – 0.01)
Infant normal	**0.19 (0.12 – 0.27)**	**0.07 (0.05 – 0.10)**	-0.00 (-0.01 – 0.01)
Infant accelerated	**0.28 (0.17 – 0.39)**	**0.11 (0.07 – 0.15)**	-0.00 (-0.01 – 0.01)

Values are regression coefficients and 95% confidence intervals for the beta for increase axial length (mm), corneal radius (mm) or AL/CR ratio from linear regression models. "n =" represents number of total group. $P <$ 0.05 are shown in bold. Models were adjusted for gender, age at visit, ethnicity and SDS estimated fetal weight at second trimester. Restricted growth, normal growth and accelerated growth were defined as respectively <-0.67, >-0.67 and <0.67 and >0.67 SDS difference in SDS weight between second trimester and birth and birth and 6 months post-natal.

Figure 1 Non-linearity in the association between axial length (left), corneal radius (middle) and AL/CR ratio (right) and birth weight for gestational age adjusted for age, gender and ethnicity

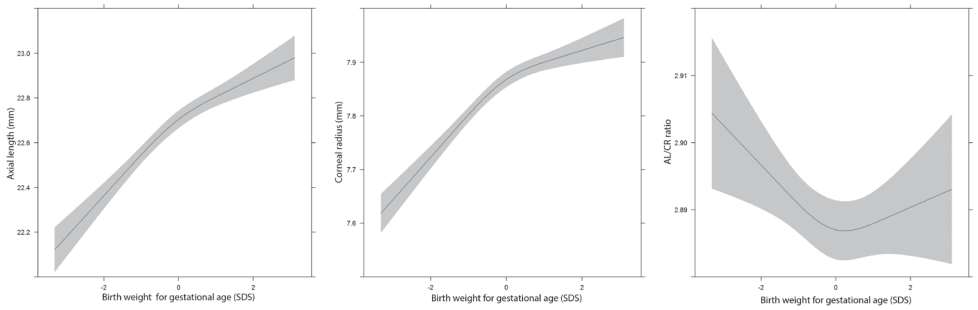
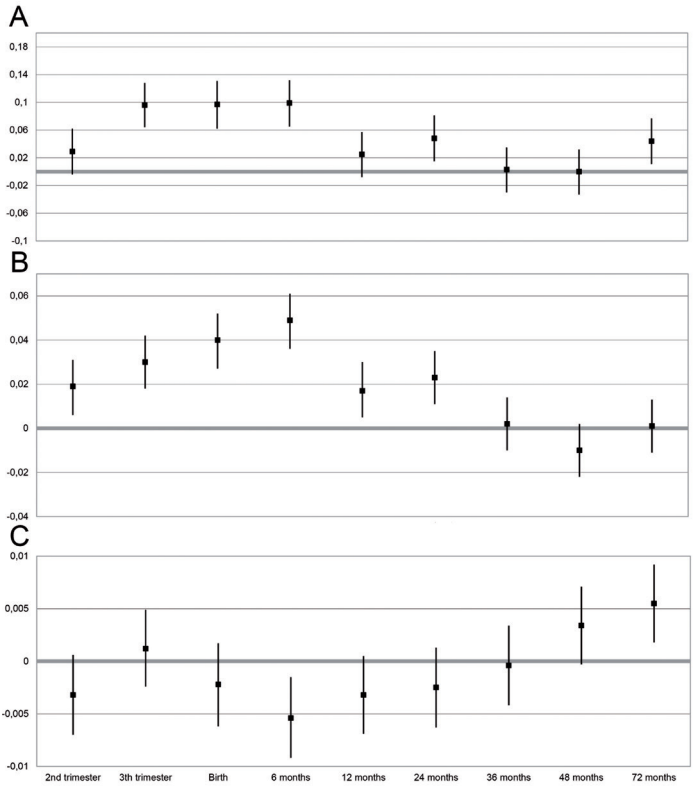


Figure 2 The association between fetal and infant weight (SDS score) per time period with (A) axial length (mm), (B) corneal radius of curvature (mm) and (C) AL/CR ratio (mm/mm) (N = 1595)



Body growth measurements and emmetropisation

Gestational age, birth weight and weight for gestational age were all positively associated with AL and CR at 6 years. Associations for weight increased with age until 3 months postnatally for AL, up to 12 months for CR. We found evidence for non-linear associations between birth weight for gestational age and AL or CR (Figure 1; supplemental table 2). HC and weight measurements from two to six years showed evidence for non-linearity for AL and CR, but not for AL/CR ratio with a significant third degree polynomial suggesting an S-shaped function (supplementary table 2).

The most important period for the association between body growth and ocular biometry was growth up to 24 months in the conditional analysis. Up to this time, higher weight was associated with longer AL and larger CR. At the 72 months' time point, significant additive associations were found only for AL and AL/CR, but not for CR (Figure 2a-c). The low birthweight children had a lower axial length (-0.22 mm; P <0.001) as well as a smaller corneal radius (-0.13 mm; P <0.001) which resulted in a higher AL/CR ratio.

Table 4 Genetic risk score of height and birth weight and the correlation with ocular biometry at 6 years of age

	Genetic risk scores					
	Height (SDS) (n = 3,880)			**Birthweight (SDS) (n = 3,880)**		
Outcomes variables	β (se)	P	R^2	β (se)	P	R^2
Birthweight (kg)	0.059 (0.012)	3.7*10^-7	0.006	-0.047 (0.008)	8.7*10^-9	0.012
SDS Niklasson (SDS)	0.119 (0.022)	4.3*10^-8	0.007	-0.110 (0.015)	7.2*10^-13	0.013
Height (cm)	2.134 (0.107)	1.4*10^-84	0.064	-0.214 (0.079)	0.007	0.001
Axial length (mm)	0.046 (0.016)	0.004	0.001	-0.023 (0.011)	0.04	0.001
Corneal radius (mm)	0.026 (0.006)	4*10^-6	0.005	-0.013 (0.004)	0.002	0.002
AL/CR ratio	-0.004 (0.002)	0.03	0.001	0.002 (0.001)	0.20	0.000

Values are regression coefficients (se) for the beta for association between genetic risk scores and birthweight, SDS Niklasson, height, axial length (mm), corneal radius (mm) or AL/CR ratio from linear regression models. "n =" represents number of total group. $P < 0.05$ are shown in bold. Models were adjusted for gender, age at visit and ethnicity (4 principle components).

Genetics

To identify the genetic overlap in ocular biometry and growth, we created a weighted genetic risk score of 695 known SNPs associated with height and 60 SNPs associated with birth weight.[123,124] The many SNPs for height explained this trait better (6.4%) than the relatively low number of SNPs for birthweight explained birthweight (1.3%). Both genetic risk scores were significantly associated with AL as well as CR (table 4). The genetic risk score for height explained 0.2% of the variance of AL and 0.5% of CR, and was significantly associated with AL/CR ratio (P 0.03). The genetic risk score for birthweight explained 0.23% and 0.1% for CR and AL, respectively, and was not significantly associated with AL/CR.

Proportionally to the variance explained for its own trait, the genetic risk score for birthweight explained a higher variance of CR (15.4%) than the genetic risk score for body height (7.8%). To test for causality, Mendelian randomization was performed with the two-stage least square method. Using the genetic risk scores as instrumental variables, we found significant support for a causal association between the determinants birthweight and height, and ocular biometric outcomes. The presence of more risk alleles for a taller height or higher birthweight was associated with higher AL and CR (all $P < 0.03$; table 4).

DISCUSSION

This study explored whether ocular biometry is related to body growth patterns prenatally until six years of age and whether this is genetically determined. Body growth pat-

terns occurring from mid pregnancy up to 24 months after birth were highly associated with ocular biometry at 6 years of age. Restricted prenatal and postnatal growth resulted in a smaller AL and CR, and accelerated growth resulted in a longer AL and larger CR. Genetic variants associated with taller body height and higher birthweight also predisposed to longer AL and larger CR, providing evidence for genetic overlap between these traits. These results can explain variance in ocular biometry measurements in children with the same refractive error.

Strengths and weaknesses

Strengths of this study were the large sample size and the unique dataset of pre- and postnatal growth measurements. In addition, we had measurements of ocular biometry at a young age, and genetic data to perform Mendelian Randomization. Still, some limitations have to be taken into account. First, lens parameters were not available, which hampered the study of all refractive components. Second, we cannot distinguish whether height or weight at birth is the dominant factor driving the association with ocular biometry as both are highly correlated. Height is difficult to measure accurately before and at birth, but the Mendelian randomization suggests that height is the most important factor. Third, although cycloplegic refraction was performed in a substantial number of children, the prevalence of myopia (2.4%) was too low to reliably study associations with this outcome. We therefore focussed on eye size measurements rather than refractive error categories.

Larger neonates have a higher AL and CR

The results of anthropometric birth parameters were comparable with cross sectional studies in Sydney,[116] Singapore,[113] and in the United Kingdom.[117] This study adds prenatal measurements and found that the associations between weight for gestational age and ocular biometry were non-linear; in particular children with a below average weight have smaller AL. The effect estimates of the association between body weight, height and head circumference measurement and ocular biometry was most significant with the measurements at 3 months postnatally. The conditional analysis validated this notion, and revealed that growth in the first two years of life was most important period for a longer AL and larger CR. This was similar to results found in the ALSPAC study, which also reported an association with weight up to 10-80 months of age. ALSPAC also found a higher effect of the genetic risk score on CR than on AL.[117] The genetic risk score was not significant for AL in ALSPAC, as they probably incorporated less genetic markers and had a lower sample size.

Weight change between four and six years of age was associated with AL and AL/CR, but not with CR. As CR stabilizes around 18 months, this is not surprising.[125] Although we cannot rule out that the association between weight increase and AL may be due to lens growth leading to a myopic shift, it is more likely that shared behavioural risk factors to weight gain and myopia such as less time outdoors explain the relationship.[126]

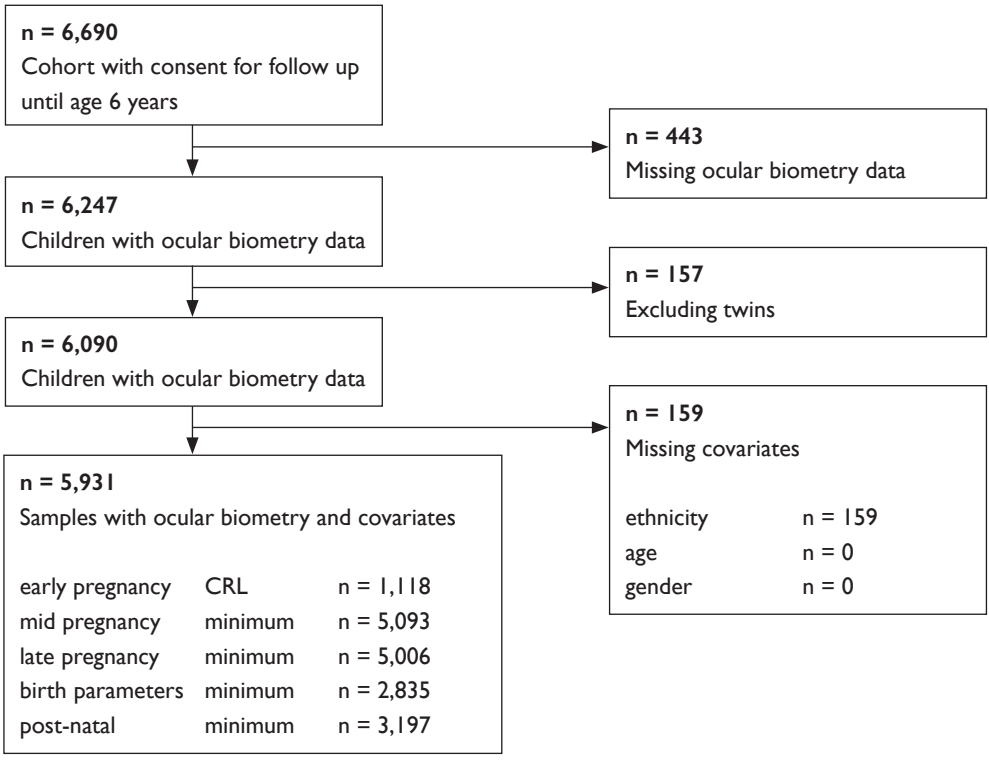
It has been demonstrated that the corneal radius of curvature stops increasing around18 months,[125 127] whereas axial length can increase up to teenage years and adolescence.[111,128] Our observation that the highest association with CR was with weight at one year of age is in line with this finding. Emmetropisation is hypothesized to be an active process of ocular scaling resulting from environmental influences,[22,23,60,129] release of retinal neurotransmitters[42,58,62] and feedback mechanisms.[130,131] The results of this study feed into this hypothesis, because we found a high correlation between body growth, corneal curvature, and AL without influence on AL/CR ratio. The small effect between birth weight and AL/CR ratio may be explained by lens parameters, as the lens is thinner with an increased birthweight.[113] The lack of association in older ages suggests that body growth may determine refractive components up to two years of age, subsequently overtaken by visual input which brings the focal point on the retina by changing lens refraction and axial length.

CONCLUSION

The effect of body growth on ocular biometry was particularly prominent for body measurements up to two years of age. Body growth and ocular biometry at a young age may have a shared genetic background. However, with the fading effect of body growth on ocular biometry as the child grows older, image projection on the retina may become a more dominant trigger for changes in ocular biometry.

SUPPLEMENTAL MATERIAL

Supplemental Figure 1 Flowchart of participants



Supplemental table 1 Pregnancy related determinants and association with **axial length and corneal curvature**

	Values Average (SD) / %(N)	**Axial length** B (SE)	**Corneal curvature** B (SE)
Age mother (years) (n=5,647)	30.6 (5.1)	-0.001 (-0.005 – 0.002)	0.000 (-0.002 – 0.001)
Low maternal education (n=5,260)	53.4 (2,810)	0.014 (-0.053 – 0.025)	-0.007 (-0.007 – 0.022)
Pre-pregnancy BMI (n=3,803)	24.1 (5.0)	0.002 (-0.003 – 0.006)	0.000 (-0.002 – 0.001)
Parity (n=5,176)			
First	56.7 (3,105)	Ref	Ref
Second	30.5 (1,671)	0.019 (-0.022 – 0.059)	0.007 (-0.008 – 0.022)
Third or more	12.8 (400)	0.029 (-0.028 – 0.085)	0.002 (-0.019 – 0.023)
Smoking during pregnancy (n=4,992)	16.4 (821)	-0.032 (-0.083 – 0.019)	-0.002 (-0.021 – 0.016)
Alcohol during pregnancy (n=4,579)	40.1 (1,839)	0.002 (-0.039 – 0.044)	0.007 (-0.008 – 0.022)
Season of birth (n=5,647)			
Winter	22.7 (1,284)	Ref	Ref
Spring	23.3 (1,316)	0.014 (-0.038 – 0.066)	-0.006 (-0.025 – 0.013)
Summer	26.7 (1,509)	-0.018 (-0.069 – 0.032)	-0.013 (-0.031 – 0.005)
Autumn	27.2 (1,538)	-0.015 (-0.065 – 0.035)	-0.014 (-0.032 – 0.004)

Values are betas and 95% confidence intervals for ocular biometry from linear regression models. *$p < 0.05$ and **$p < 0.01$. Models were adjusted for gender, age at visit, ethnicity, and birth weight (SDS).

Supplemental table 2 Non-linear associations of fetal and infant growth characteristics with ocular biometry (AL, CR and AL/CR ratio)

Birth parameters	AL (mm)	CR (mm)	AL/CR ratio
Birth weight (kg) (n=5,923)	0.057 (-0.153 – 0.267)	0.106 (0.030 – 0.183)	-0.032 (-0.056 – -0.009)
Birthweight^2	0.026 (-0.006 – 0.057)	-0.002 (-0.013 – 0.009)	0.004 (0.001 – 0.008)
Birth weight for gestational age (n=5,884)	0.131 (0.114 – 0.148)	0.050 (0.043 – 0.056)	-0.002 (-0.004 – 0.000)
Birth weight for gestational age^2	-0.017 (-0.028 – -0.006)	-0.010 (-0.013 – -0.006)	0.001 (0.000 – 0.003)
Postnatal			
HC 6 month (n=4,323)	0.177 (0.144 – 0.196)	0.079 (0.070 – 0.089)	-0.007 (-0.010 – -0.005)
HC 6 month^2	-0.017 (-0.030 – -0.004)	-0.007 (-0.012 – -0.002)	0.000 (-0.001 – 0.002)
HC 6 month^3	-0.007 (-0.011 – -0.004)	-0.003 (-0.004 – -0.002)	0.000 (0.000 – 0.001)
Weight 24 months (n=3,828)	0.153 (0.132 – 0.175)	0.062 (0.054 – 0.070)	-0.003 (-0.006 – -0.001)
Weight 24 months^2	-0.022 (-0.036 – -0.007)	-0.009 (-0.014 – -0.004)	0.000 (-0.001 – 0.002)
Weight 36 months (n=3,633)	0.167 (0.133 – 0.200)	0.069 (0.057 – 0.081)	-0.004 (-0.008 – -0.000)
Weight 36 months^2	-0.020 (-0.035 – -0.005)	-0.009 (-0.014 – -0.003)	0.000 (-0.001 – 0.002)
Weight 36 months^3	-0.009 (-0.017 – -0.001)	-0.003 (-0.006 – -0.001)	0.000 (-0.001 – 0.001)
Weight 48 months (n=3,197)	0.177 (0.145 – 0.210)	0.079 (0.067 – 0.091)	-0.006 (-0.0010 - -0.002)
Weight 48 months^2	-0.013 (-0.027 – 0.000)	-0.006 (-0.011 – -0.001)	0.001 (-0.001 – 0.002)
Weight 48 months^3	-0.011 (-0.017 – -0.006)	-0.006 (-0.008 – -0.003)	0.001 (0.000 – 0.001)
HC 72 months (n=5,778)	0.180 (0.154 – 0.207)	0.071 (0.061 – 0.081)	-0.003 (-0.006 – -0.000)
HC 72 months^2	-0.015 (-0.029 – -0.001)	-0.005 (-0.010 – -0.000)	0.000 (-0.001 – 0.002)
HC 72 months^3	-0.008 (-0.014 – -0.002)	-0.002 (-0.005 – 0.000)	0.000 (-0.001 – 0.000)
Weight 72 months (n=5,923)	0.144 (0.127 – 0.162)	0.050 (0.044 – 0.057)	0.000 (-0.002 – 0.002)
Weight 72 months^2	-0.029 (-0.039 – -0.018)	-0.012 (-0.016 – -0.008)	0.001 (-0.000 – 0.002)

Values are regression coefficients per SDS (except if otherwise displayed, kg or weeks) and 95% confidence intervals for the beta for increase in axial length (AL; mm), corneal radius (CR; mm) or AL/CR ratio from linear regression models. "n =" represents number of total group. Models were adjusted for gender, age of anthropometry measurement, ethnicity and age of eye measurements. $P < 0.05$ are shown in bold.

CHAPTER 5

AXIAL LENGTH GROWTH AND THE RISK OF DEVELOPING MYOPIA IN EUROPEAN CHILDREN

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ABSTRACT

Purpose: To generate percentile curves of axial length (AL) for European children, which can be used to estimate the risk of myopia in adulthood.
Methods: A total of 12,386 participants from the population-based studies Generation R (Dutch children measured at both 6 and 9 years of age; N=6934), the Avon Longitudinal Study of Parents and Children (British children 15 years of age; N=2495), and the Rotterdam Study III (Dutch adults 57 years of age; N=2957) contributed to this study. AL and corneal curvature data were available for all participants; objective cycloplegic refractive error was available only for the Dutch participants. We calculated a percentile score for each Dutch child at 6 and 9 years of age.
Results: Mean (SD) AL was 22.36 (0.75) mm at 6 years, 23.10 (0.84) mm at 9 years, 23.41 (0.86) mm at 15 years, and 23.67 (1.26) at adulthood. AL differences after the age of 15 occurred only in the upper 50%, with the highest difference within the 95th percentile and above. A total of 354 children showed accelerated axial growth and increased by more than 10 percentiles from age 6 to 9 years; 162 of these children (45.8%) were myopic at 9 years of age, compared to 4.8% (85/1781) for the children whose AL did not increase by more than 10 percentiles.
Conclusions: This study provides normative values for AL that can be used to monitor eye growth in European children. These results can help clinicians detect excessive eye growth at an early age, thereby facilitating decision-making with respect to interventions for preventing and/or controlling myopia.

INTRODUCTION

Refractive errors such as myopia, hyperopia, and astigmatism are the most common ocular disorders worldwide. The prevalence of these conditions varies with both age and geographic location.[59,132-134] Myopia is most prevalent in Eastern Asia[49] and in the Western world,[50,72] whereas hyperopia is more prevalent in developing countries.[132]

Refractive error is the result of a mismatch between the various optical components of the eye, the most important of which are the cornea, the crystalline lens, and the eye's axial length (AL). In the first few years of age, the cornea's refractive power is reduced; the lens also loses refractive power during childhood.[6,135] In contrast, AL increases during childhood and in the teenage years, leading to myopia if this growth in AL exceeds the eye's focal point.[128] High myopia, which is defined as spherical equivalent (SE) of -6D or worse, generally corresponds to AL ≥26 mm, which drastically increases the risk of severe complications later in life, including myopic maculopathy, retinal detachment, and glaucoma.[18,115,136] High myopia in adulthood usually has a myopia onset before the age of 10, which progresses during teenage years and early twenties;[53,129,137,138] therefore, the ability to identify young at-risk children would provide clinicians the opportunity to apply preventative measures in order to minimise further increases in AL [139]. These measures can include changes in lifestyle (e.g., increasing outdoor exposure 60), pharmacological agents such as atropine,[65,66] and optical applications such as multifocal contact lenses.[140]

Normative values as a function of age are available for a variety of measurements, such as height and weight, and these values are generally visualised using percentile curves. These curves are a powerful tool used by clinicians for sensitively detecting aberrant growth at an early age. Percentile curves for most body measurements, such as height and weight in childhood, have been generated using cross-sectional data from extremely large cohorts;[120,141] however, no such normative data currently exist for ocular biometry components or refractive error.

The aim of this study was to generate a growth chart for AL based on large epidemiological cohorts of European children and adults. We assessed the risk of developing myopia and/or high myopia per percentile, and we examined how growth curves from Western Europe relate axial length measurements in other geographic regions.

METHODS

Study population

The study included three population-based studies: the Generation R study, the Avon Longitudinal Study of Parents and Children (ALSPAC), and the Rotterdam Study III (RS-III).

The Generation R study

The Generation R study is a population-based prospective cohort study of pregnant women and their subsequent children, conducted in Rotterdam, the Netherlands. The complete methodology for this study has been described elsewhere.[45,46] In brief, a total of 9,778 pregnant women were included in the study, and their children were born from April 2002 through January 2006. At 6 and 9 years of age, the children were invited for an examination by trained nurses at a research centre. From the initial cohort, 6,690 (68.4%) children participated in the physical examination at 6 years of age, and 5,862 (60.0%) participated at 9 years of age. Follow-up data regarding AL were available for 4,787 children at both ages.

The Avon Longitudinal Study of Parents and Children

ALSPAC is a prospective population-based birth cohort study based in the former Avon health authority area in Southwest England. This study was designed to investigate the determinants for development, health, and disease in childhood and adulthood. Subject recruitment for this study has been described previously.[142] In brief, pregnant women with an expected date of delivery from 1 April, 1991 through 31 December, 1992 were eligible to participate, and 14,541 eligible women were recruited. These pregnancies resulted in 14,062 live births, and 13,988 of the infants were still alive at 1 year of age. Eye examinations were performed in these children from 7 years of age onwards, and ocular biometry measurements were included at age 15.

The Rotterdam Study III

RS-III is a prospective, population-based cohort study of subjects ≥45 years of age living in Ommoord, a suburb of Rotterdam, the Netherlands. In this study, researchers examined cardiovascular, endocrine, neurological, respiratory, and ophthalmic outcomes. Baseline examinations – including best-corrected visual acuity and refractive error measurements – were performed from 2006 through 2008. AL was measured in a random subset of the RS-III cohort at baseline and in a different random subset during follow-up examinations in 2011-2012.[86]

Ethical approval

Written informed consent was obtained from all participants or parents in all three cohorts. The study protocols for the Generation R study and RS-III were approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam, the Netherlands. Ethics approval for the ALSPAC study was obtained from the Law and Ethics Committee and the respective local research ethics committees (http://www.bris.ac.uk/alspac/

researchers/data-access/data-dictionary). All research was conducted in accordance with the Declaration of Helsinki.

Data collection

In the Generation R and ALSPAC studies, ocular biometry was measured using a Zeiss IOLMaster 500 (Carl Zeiss, Jena, Germany or Welwyn Garden City, UK). In RS-III, AL was measured using an A-scan ultrasound device (Pacscan 300AP, Sonomed Escalon, MEyeTech GmbH, Hardegsen Germany) or LenStar device (Laméris Ootech, Haag-Streit, UK). Corneal curvature was measured using a Topcon RM-A2000 auto-refractor (Topcon Optical Company, Tokyo, Japan). For measuring AL, five measurements were obtained per eye and were then averaged to obtain a mean AL value. For the corneal radius three measurements of K1 and K2 were obtained per eye and averaged to obtain a mean corneal radius of curvature (CR). AL/CR ratio was calculated by dividing AL (in mm) by CR (in mm).

To calculate axial elongation and the change in corneal radius in mm/year, and the change in AL/CR ratio in mm/mm/year, the measurement at 6 years of age was subtracted from the measurement at 9 years of age, and divided by the number of years between the two measurements. Refractive error was available in Generation R at 9 years and in the Rotterdam Study III. In the Generation R cohort, automated cycloplegic refraction was measured in a random subsample at 9 years of age using a Retinomax-3 device (Bon, Lübeck, Germany). At least thirty minutes prior to measuring refractive error, 2 drops (3 with dark irises) of cyclopentolate (1%) were administered, and a pupil diameter ≥6 mm was required before SE was determined. SE was calculated as the average sphere + 1/2 cylinder for both eyes. In the RS-III cohort, refraction was measured objectively using a Topcon RM-A2000 (Topcon Optical company, Tokyo, Japan), and then subjectively adjusted with +0.25D or -0.25D steps, spherically as well as cylindrically to achieve the best possible visual acuity. Myopia was defined as SE of ≤-0.5D, emmetropia was defined as SE between -0.5D and +2.0D, and hyperopia was defined SE ≥+2.0D. At the age of 6 years in Generation R, cycloplegic refractive error was only obtained when visual acuity was worse than 0.2 LogMAR, detecting myopia ≤-0.5 but not hyperopia; we therefore did not use refractive error data at age 6 for analyses. In contrast, cycloplegic refractive error was collected in all 9-year-olds, and non-cycloplegic refraction was collected in all adults.

Statistical methods

Average values of AL, CR, and AL/CR were calculated. Differences between genders were analysed using the Student's t-test or the chi-square test. The association between biometry variables and SE were determined using linear regression models. For the growth curves of AL and AL/CR, we used the 2^{nd}, 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, 95^{th}, and 98^{th} percentile values for the children in the Generation R and ALSPAC studies, with the measurements in the RS-III cohort as the final refractive state in adults. AL was plot-

ted against age, and an interpolation line was created between the matching percentiles of each age. Individual percentiles for AL at 6 and 9 years of age were calculated relative to the entire cohort, and the absolute difference between 6 and 9 years was calculated. To test for concordance of our results with other studies conducted in other geographic regions, we extracted data from 15 other population-based and school-based studies that were conducted in North America,[128] Europe,[11,12,143] Asia,[6,9,14,144-146] Australia and Vanuatu[10,15,147] for which gender-stratified data were available. The association between SE and either AL or AL/CR ratio was determined using linear regression models and ordinary least squares linear regression models, with restricted cubic splines with three knots (the 10th, 50th, and 90th percentiles) in the 9-year-old children in the Generation R cohort. All models were adjusted for both age and gender. Ordinary least squares linear regression models were generated using the program R; all other statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY).

RESULTS

Ocular biometry and refractive error

Analyses were performed at the cohort level. In the Generation R cohort, complete ocular biometry data were available for 6084 and 5295 children at 6 and 9 years of age, respectively. In the ALSPAC cohort, complete ocular biometry data were available for 2495 children 15 years of age. In the RS-III cohort, data were available for 2957 adults with a mean age of approximately 57 years. The general demographic characteristics of all participants in all four age categories are shown in Table 1. In the children 6 and 9 years of age, mean (SD) AL was 22.36 (0.75) and 23.10 (0.84) mm, respectively. AL was 23.41 (0.86) mm in the 15-year-olds and 23.67 (1.26) mm in the adults. Among all four cohorts, the minimum and maximum AL values were 17.54 and 30.12 mm, respectively. Mean (SD) CR was 7.77 (0.26) and 7.78 (0.26) mm in the 6-year-old and 9-year-old children, respectively, 7.82 (0.27) mm in the 15-year-olds, and 7.74 (0.26) mm in the adults. Among all four cohorts, the minimum and maximum CR values were 6.91 and 9.61 mm, respectively. The mean (SD) AL/CR ratio was 2.88 (0.08) in the 6-year-olds and 3.05 (0.15) in the adults; among all four cohorts, the minimum and maximum AL/CR values were 2.38 and 4.07, respectively. On average, the females in each age group had significantly shorter AL, steeper CR, and lower AL/CR ratios compared to the males in their respective age groups ($p<0.001$). The gender-stratified mean and SD values for general and ocular characteristics are shown in Table 1. Height had the strongest correlation with AL in the 6-year-old group ($\beta=0.028$; $p<0.001$), and this correlation decreased slightly – but remained significant – in the 9-year-old group ($\beta=0.024$; $p<0.001$). No significant difference in height was found between the refractive error groups in boys (ANOVA $p=0.40$) as well as girls (ANOVA $p=0.24$).

Refractive error had a relatively narrow distribution in both the 9-year-olds and the adults (Supplemental Figure S1), with mean SE values of +0.74D (SD: 1.30; range: -9.8D

Table 1 General and ocular characteristics of the four study cohorts

	All	Male	Female	P-value[2]
Generation R at 6 years of age (N=6084)				
Age in years	6.17 (0.52)	6.18 (0.55)	6.16 (0.50)	0.03
Gender, N (%)	6084 (100)	3033 (49.9)	3051 (50.1)	NA
European ethnicity, N (%)	3983 (65.5)	1965 (64.8)	2018 (66.1)	0.27
Height in cm	119 (6)	120 (6)	119 (6)	<0.001
European ethnicity, N (%)	4089 (67.2)	2023 (66.7)	2066 (67.7)	0.41
Axial length in mm	22.36 (0.75)	22.63 (0.73)	22.09 (0.7)	<0.001
Corneal radius in mm	7.77 (0.26)	7.84 (0.26)	7.70 (0.24)	<0.001
AL/CR ratio	2.88 (0.08)	2.89 (0.08)	2.87 (0.08)	<0.001
Generation R at 9 years of age (N=5296)				
Age in years	9.79 (0.33)	9.80 (0.36)	9.77 (0.31)	0.02
Gender, N (%)	5296 (100)	2617 (49.4)	2679 (50.6)	NA
European ethnicity, N (%)	3770 (71.2)	1842 (70.4)	1928 (72.0)	0.21
Height in cm	142 (6)	142 (6)	141 (7)	0.05
Axial length in mm	23.10 (0.84)	23.36 (0.82)	22.84(0.78)	<0.001
Corneal radius in mm	7.78 (0.26)	7.85 (0.26)	7.72 (0.24)	<0.001
AL/CR ratio	2.97 (0.09)	2.98 (0.10)	2.96 (0.09)	<0.001
SE in dioptres1	0.74 (1.30)	0.73 (1.28)	0.75 (1.31)	0.66
ALSPAC cohort (N=2495)				
Age in years	15.47 (0.32)	15.45 (0.29)	15.49 (0.34)	0.001
Gender, N (%)	2495 (100)	1167 (46.7)	1328 (53.3)	NA
European ethnicity, N (%)	2447 (98.1)	1145 (98.1)	1302 (98.0)	0.79
Height in cm	169 (8)	175 (7)	165 (6)	<0.001
Axial length in mm	23.41 (0.86)	23.68 (0.88)	23.18 (0.84)	<0.001
Corneal radius in mm	7.82 (0.27)	7.88 (0.27)	7.77 (0.25)	<0.001
AL/CR ratio	2.99 (0.1)	3.01 (0.1)	2.98 (0.10)	<0.001
RS-III cohort (N=2957)				
Age in years	56.8 (6.4)	56.8 (6.3)	56.8 (6.3)	0.83
Gender, N (%)	2957 (100)	1290 (43.6)	1667 (56.4)	NA
European ethnicity, N (%)	2745 (92.8)	1215 (94.2)	1530 (91.8)	0.01
Height in cm	170.5 (10)	178 (6)	164 (7)	<0.001
Axial length in mm	23.67 (1.26)	23.99 (1.26)	23.42 (1.20)	<0.001
Corneal radius in mm	7.74 (0.26)	7.81 (0.25)	7.69 (0.25)	<0.001
AL/CR ratio	3.05 (0.15)	3.07 (0.16)	3.04 (0.15)	<0.001
SE in dioptres	-0.31 (2.5)	-0.39 (2.5)	-0.26 (2.5)	0.16

Notes: Except where indicated otherwise, all data are presented as the mean (SD). AL, axial length; CR, corneal radius of curvature; SE, spherical equivalent. 1N=2408 (1204 males and 1204 females).

2*P*-values were calculated using the Student's t-test or the chi-square test.

Figure 1 Association between spherical equivalent (in dioptres) and axial length (in mm; left) and AL/CR ratio (right) at 9 years of age. The mean and 95% CI were adjusted for age, gender, and height

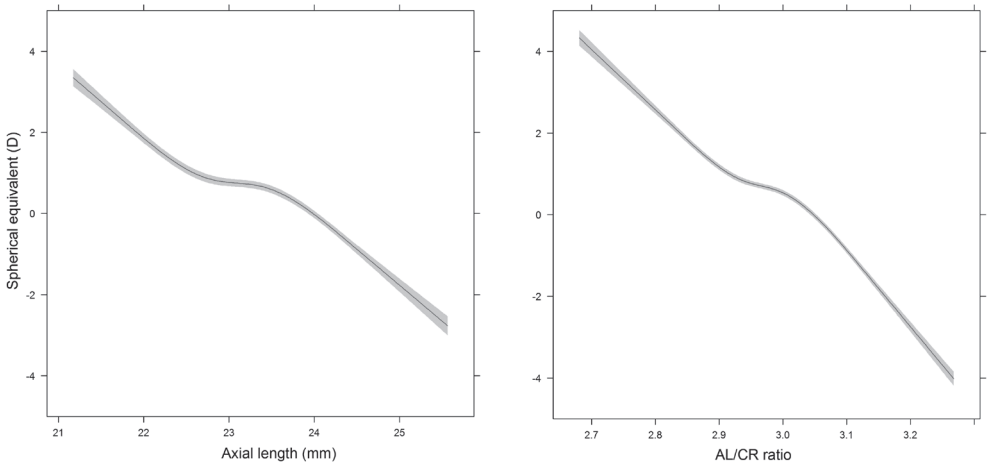
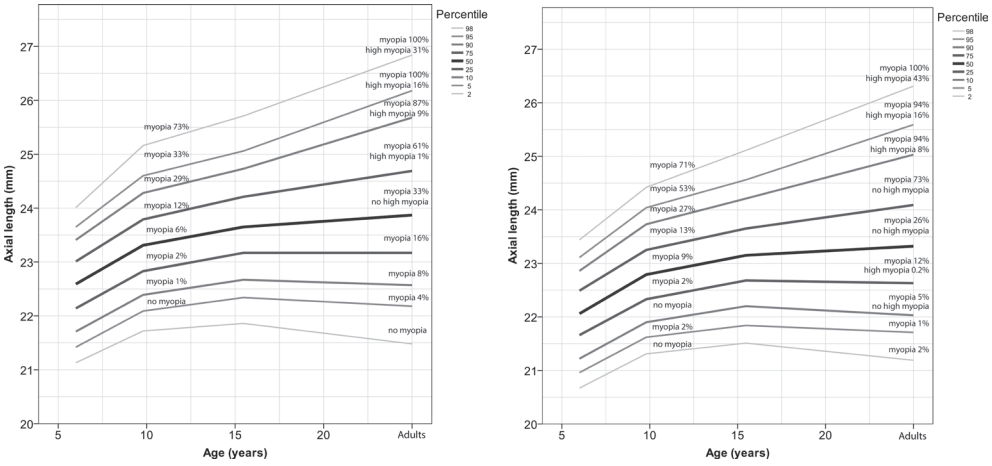


Figure 2 Growth chart depicting axial length (in mm) versus age for European study subjects, males (left) and females (right), with the risk of myopia in adulthood. The myopia percentage represents the proportion of myopia in halfway above and below the percentage line



to +8.3D) and -0.31D (SD: 2.53; range: -13.8D to +9.1D), respectively. At 9 years of age, there was no significant difference in SE between boys and girls (mean SE was +0.73D and +0.75D, respectively; p=0.66); we also found no significant difference between the adult males and females (-0.39D vs. -0.26D, respectively; p=0.16). Among the 9-year-old children, 11.4% (N=274) and 8.4% (N=203) had myopia and hyperopia, respectively; among the adults, 37.0% (N=1093) and 11.9% (N=352) had myopia and hyperopia, respectively.

Table 2 summarises the differences in ocular biometry and the association between SE and the various refractive error groups in the Generation R and RS-III cohorts. Our analysis revealed that SE was inversely correlated with both AL and the AL/CR ratio in both the Generation R (Figure 1) and RS-III cohorts. Interestingly, the relationship between SE and AL/CR ratio was non-linear (quadratic term p<0.001). The correlation between SE and both AL and AL/CR ratio was weakest in the emmetropic participants and strongest in the myopic participants (Table 2).

In addition, SE was significantly correlated with CR. On average, the myopic children had a steeper CR (7.73 mm) compared to both the emmetropic (7.79 mm; p=<0.001) and hyperopic (7.80 mm; p=<0.001) children. Similar results were obtained in the adult cohort (Table 2).

Longitudinal changes in AL were also measured in the Generation R cohort between the 6-year-old and 9-year-old children. On average, AL increased by 0.21 mm/year (SD: 0.08 mm/year), and the AL/CR ratio increased by 0.025 units/year (SD: 0.011 units/year). The myopic children had more rapid eye growth rate (0.34 mm/year) than both the emmetropic (0.19 mm/year; p<0.001) and hyperopic (0.15 mm/year; p<0.001) children. At 9 years of age, the increases in AL and AL/CR ratio were significantly associated with a shift in refractive error towards increased myopia; this result was present in all refractive error categories. We found no significant change in CR from 6 to 9 years of age (Table 2).

AL growth curves

Figure 2 shows the growth chart for AL versus age in percentiles. From 6 to 9 years of age, all of the percentiles examined increased in AL; however, none of the percentiles below the median increased further after the age of 15. In particular, the lowest percentiles of AL increased relatively little after the age of 6, and the 5th percentile values changed by less than 1 mm with age. The AL of all of the median and above-median percentiles increased until adulthood. The median percentile in the male participants increased by 1.28 mm (22.59 mm vs. 23.87 mm at 6 years of age and adulthood, respectively; Figure 2 and (Supplementary Table S1a), and the 95th percentile increased by 2.5 mm (23.65 mm vs. 26.18 mm at 6 years of age and adulthood, respectively). Similar results were observed for AL in the female participants (Figure 2 and Supplementary Table S1b) and for the AL/CR ratio in both genders (Supplementary Figure S2). The above-median percentiles of AL were associated with a >50% risk of developing myopia in adulthood age; moreover, the highest 10th percentile was associated with a 97% risk of myopia and a 23% risk of high myopia. CR was relatively consistent across all age groups (Supplementary Figure S3).

Table 2 Ocular biometry and correlation with spherical equivalent (SE) in children and adults

	Children at 9 years of age (N=2408)		Adults ≥45 years of age (N=2957)	
	Mean (SD; 90% range)	β (95% CI) of association with SE	Mean (SD, 90% range)	β (95% CI) of association with SE
Axial length (mm)				
All	23.10 (0.81; 21.79 – 24.42)	-1.06 (-1.12 – -1.01)	23.67 (1.26; 21.82 – 25.90)	-1.61 (-1.66 – -1.56)
Hyperopia	22.08 (0.69; 21.20 – 23.28)	-0.82 (-1.02 – -0.62)	22.30 (0.90; 20.70 – 23.72)	-1.04 (-1.16 – -0.91)
Emmetropia	23.08 (0.67; 22.02 – 24.23)	-0.25 (-0.28 – -0.21)	23.30 (0.85; 21.95 – 24.71)	-0.23 (-0.23 – -0.19)
Myopia	23.98 (0.83; 22.75 – 25.37)	-0.98 (-1.15 – -0.82)	24.62 (1.19; 22.86 – 26.58)	-1.24 (-1.34 – -1.16)
P-value	<0.001		<0.001	
Corneal radius of curvature (mm)				
All	7.78 (0.25; 7.38 – 8.22)	0.70 (0.49 – 0.91)	7.74 (0.26; 7.33 – 8.18)	1.10 (0.74 – 1.46)
Hyperopia	7.80 (0.26; 7.38 – 8.26)	1.11 (0.52 – 1.69)	7.79 (0.25; 7.39 – 8.23)	0.13 (-0.47 – 0.74)
Emmetropia	7.79 (0.25; 7.39 – 8.22)	0.19 (0.01 – 0.29)	7.75 (0.26; 7.33 – 8.20)	0.12 (-0.13 – 0.24)
Myopia	7.73 (0.25; 7.38 – 8.26)	0.63 (-0.05 – 1.31)	7.72 (0.26; 7.30 – 8.15)	0.44 (-0.05 – 0.93)
P-value	<0.001		0.008	
AL/CR ratio				
All	2.97 (0.09; 2.84 – 3.13)	-11.56 (-11.89 – -11.23)	3.05 (1.51; 2.83 – 3.32)	-14.43 (-14.73 – -14.13)
Hyperopia	2.83 (0.08; 2.40 – 3.01)	-9.77 (-10.91 – -8.62)	2.86 (0.11; 2.69 – 3.02)	-9.94 (-10.96 – -8.92)
Emmetropia	2.96 (0.06; 2.87 – 3.06)	-4.43 (-4.76 – -4.11)	3.01 (0.08; 2.87 – 3.14)	-3.35 (-3.73 – -2.97)
Myopia	3.10 (0.09; 2.97 – 3.25)	-11.07 (-12.24 – -9.90)	3.19 (0.14; 3.00 – 3.42)	-12.43 (-13.03 – -11.84)
P-value	<0.001		<0.001	
Axial length growth (mm/year)				
All	0.21 (0.08; 0.11 – 0.37)	-10.54 (-11.05 – -10.04)	NA	NA
Hyperopia	0.15 (0.06; 0.06 – 0.26)	-5.01 (-7.31 – -2.71)	NA	NA
Emmetropia	0.19 (0.05; 0.12 – 0.29)	-3.64 (-4.07 – -3.21)	NA	NA
Myopia	0.34 (0.11; 0.17 – 0.53)	-5.86 (-7.30 – -4.44)	NA	NA
P-value	<0.001		NA	

Corneal radius of curvature growth (mm/year)				
All	0.004 (0.01; NA0.010 – 0.015)	1.46 (-3.60 – 6.52)	NA	NA
Hyperopia	0.003 (0.01; -0.010 – 0.015)	4.80 (-7.79 – 17.40)	NA	NA
Emmetropia	0.004 (0.01; -0.009 – 0.015)	-0.42 (-2.69 – 1.85)	NA	NA
Myopia	0.003 (0.01.; -0.013 – 0.015)	-3.34 (-21.07 – 14.39)	NA	NA
P-value	0.37		NA	
AL/CR change (units/year)				
All	0.025 (0.011; 0.012 – 0.046)	-72.73 (-76.55 – -68.92)	NA	NA
Hyperopia	0.018 (0.010; 0.005 – 0.034)	-31.97 (-47.33 – -16.60)	NA	NA
Emmetropia	0.023 (0.008; 0.013 – 0.037)	-22.82 (-25.84 – -19.80)	NA	NA
Myopia	0.043 (0.014; 0.021 – 0.068)	-41.31 (-51.99 – -30.63)	NA	NA
P-value	<0.001		NA	

Notes: Except where indicated otherwise, all data are presented as the mean (SD). AL, axial length; CR, NA, not applicable (no follow-up data were available); SE, spherical equivalent. Sample size in the refractive error categories at 9-year-old: hyperopia, N=203; emmetropia, N=1926; myopia, N=279. Sample size in the refractive error categories in the adults: hyperopia, N=352; emmetropia, N=1512; myopia N=1093. In the regression models, SE was used as the dependent variable, and the ocular biometry measurements were used as the independent variable. The models were adjusted for age, gender, ethnicity, and height. *P*-values reflect the differences in ocular biometry measurements between the refractive groups and were calculated using an ANOVA.

The median absolute difference in AL was 5.6 percentiles (IQR: 2.4–11.2), indicating that a given child's percentile at age 6 is a reliable predictor of that child's percentile at age 9. Moreover, we found a significant correlation in percentile position between 6 and 9 years of age (Spearman correlation coefficient: 0.92; $p<0.001$). Higher change in percentile position was highly correlated to myopia prevalence (figure 3). Of the 354 children who had an increase in percentile score of ≥10, 45.8% (N=162) were myopic at 9 years of age; in contrast, only 4.8% (85/1781) of the children who had an increase in percentile score <10 were myopic at 9 years of age.

Support for our growth curves based on previous publications

Finally, we used gender-stratified AL measurements obtained from published population-based and school-based studies in order to confirm our growth curves. As shown in Figure 4, the median AL growth rates in studies of European children were similar to our own median values. The mean AL value in Asian populations was larger after 7 years of age. In addition, the mean AL values in the children measured in both Vanuatu study and in an older study of Norwegian children were smaller than our median value.[143,147]

DISCUSSION

The aim of this study was to provide normative growth values for ocular biometry and the associated risk of developing myopia in European children. Our analysis revealed that median AL increased with age until 15 years of age, after which AL continued to increase into adulthood in the top 50th percentile. CR was relatively similar across age groups, with only a slightly smaller corneal radius in the adult cohort. At 9 years of age, the children in the European cohorts were generally emmetropic, with an average SE of +0.74 D, and 11.4% of these children were already myopic. The correlation between SE and AL/CR ratio and was not linear as a whole; rather, it was weaker around the emmetropic values. This was likely due to compensation by other optical features such as the crystalline lens and anterior chamber depth.[7]

Strength and limitations

Our study has several strengths. First, we included more than 12,000 measurements of ocular biometry in European children and adults in four discrete age categories. Second, the studies from which we collected our data used autorefraction to measure refractive error. Third, the age ranges of the children were extremely narrow, allowing for highly robust analysis. Finally, the data were stratified by gender.

Despite these strengths, several possible weaknesses warrant discussion. First, the ALSPAC study involving 15-year-old children was conducted in the UK, whereas the

Figure 3 The change in percentile score of axial length between 6 and 9 years of age (x-axis) and the percentage of myopia at 9 years of age (y-axis)

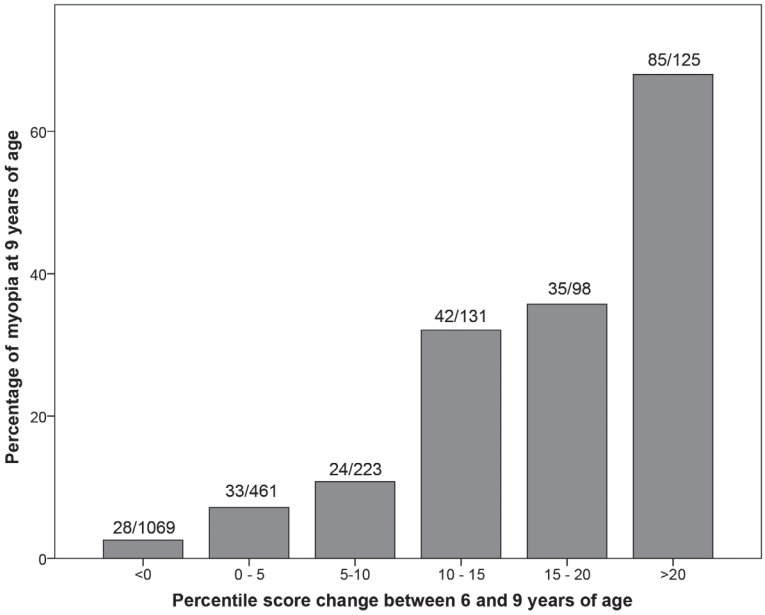
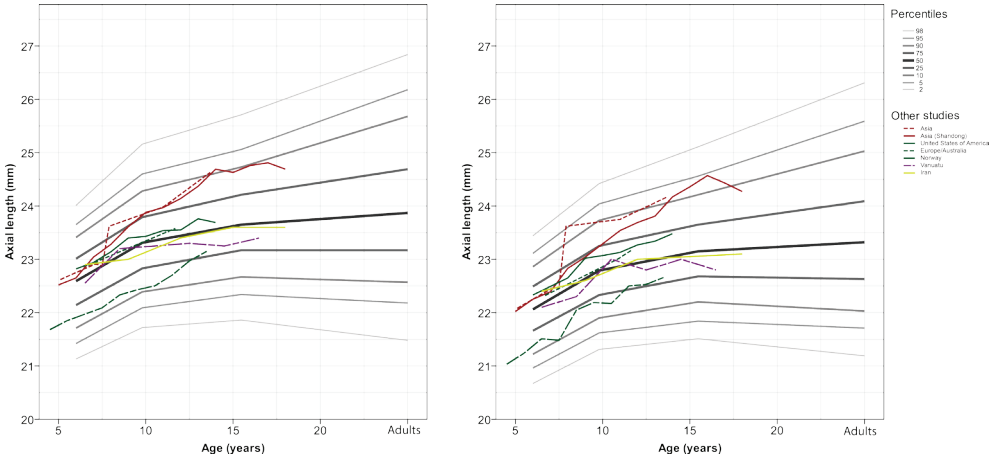


Figure 4 Axial length is plotted against age for male (left) and female (right) children from various geographic locations. For comparison, the data from the present study are copied from Figure 2 and are shown here in grey. Gender-stratified data were collected from Australia, Europe, the United States, Greenland, Iran, Vanuatu, and Norway. The European and Australian children were clustered as being predominantly of European descent



Generation R and RS-III studies were conducted in the Netherlands; therefore, geographic and/or other factors may have affected our analysis. Second, we lacked a study population of young adults, and actual measurements of refractive error for ages 20-25 years would have corrected for small alternations of axial length changes from early to late adulthood, whereas most of the axial elongation will occur between 15-25 years of age.[148] Third, the birth years differed among the three cohorts, and younger cohorts may have a higher risk of myopia in adulthood compared to older cohorts.[50,72] Such a cohort effect may have led to an underestimation of the upward trend of the growth curve at age 15 and older. Fourth, differences in the instruments used (e.g., IOLMaster vs. keratometry/A-scan ultrasonography) for the various cohorts may have generated a systematic error in biometry measurements. Although AL measurements do not differ between instruments, CR values can differ by up to 0.03 mm between Topcon Keratometry and IOLMaster.[100,149-153] Lastly, the published studies predominantly reported mean AL values, rather than median AL values. However, this likely had only had a slight effect on the trajectories, as the difference mean and median AL values was relatively low (0.03–0.12 mm) in all of our study cohorts.

European versus Asian children

Our findings are similar to other cohort data in several respects. First, we observed a gender difference in AL, CR, and AL/CR ratio, which is consistent with previous observations.[9-11,154] In addition, we found that AL increased more rapidly in the myopic children than in the children with hyperopia, a finding consistent with the NICER (Northern Ireland Childhood Errors of Refraction) study.[155] We also compared the AL growth rates in our study with data obtained from other geographic regions and found several interesting ethnic and cohort effects. For example, children in East Asia generally have higher AL after the age of 6 years compared to both European and Iranian children, reflecting higher risk for developing myopia.[10-12,144] Compared to the 6-year-old children in our Dutch study, 3-year-old Asian children have shorter AL and lower AL/CR ratios, but similar CR values.[13] At 5 years of age, children in Singapore had similar AL values as the 6-year-old children in our study;[14] however, at 8 years of age, the children in Singapore had longer AL values and higher AL/CR ratios than our 9-year-old children. In contrast, compared with our results, Northern European children in a study conducted in 1971 had lower AL values at all ages,[143] which can be caused by a lower myopia prevalence as well as a lower body height, or a combination of these.

The prevalence of myopia among European children has only been examined in relatively few studies.[133,134,156] The multi-ethnic CHASE (Child Heart and Health Study in England) study in the UK reported a prevalence of 11.9% (≤-0.50D) at approximately 11 years of age,[11] and the NICER study in Northern Ireland reported a prevalence of 17.7% (≤-0.50D) at approximately 13 years of age.[157] The multi-ethnic CLEERE (Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error) study conducted in the US found a prevalence of 11.6% (≤-0.75D in both meridians) in 10-year-olds,[128] and the. Australian Sydney Myopia Study found a prevalence of 11.9% (≤-0.50D) in 13-year-

olds.[15] These values are similar to the prevalence of 11.4% that we found in our Dutch cohort of 9-year-olds. We and others have found that height is associated with axial length, and this needs to be taken into account when interpreting the growth curves.

Interestingly, our analysis revealed a large difference in eye growth between children at risk for developing myopia and children with low risk; specifically, the rate of eye growth was twice as high in the children who developed myopia compared to the children who remained hyperopic. Follow-up studies are needed to determine whether children born after 2010 have a steeper growth curve than suggested by our growth chart. In addition, the growth curves can be improved further by focussing on children who differ in ages from those in our study, thereby providing complementary data.

CONCLUSIONS

Our normative data regarding AL may serve as a key instrument for monitoring eye growth in children with progressive myopia in European and other populations. Paediatric ophthalmologists, optometrists, and orthoptists can use these charts to determine whether a child's axial length is above average for his/her age, and this information can be used to estimate the risk of developing high myopia. In addition, children with a rate of AL growth higher than expected based on their percentile line can be identified relatively early, allowing these children to benefit from the increasing number of therapeutic options for preventing myopia.

SUPPLEMENTAL MATERIAL

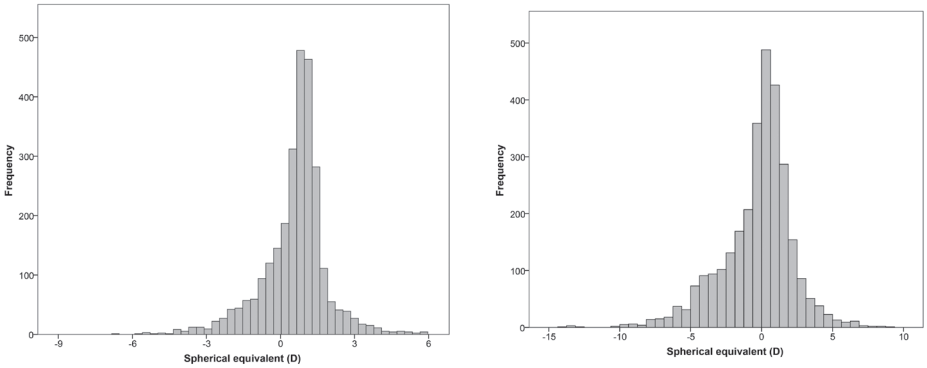
Supplementary Table S1a Percentiles of axial length, corneal radius and AL/CR ratio in 6 and 9 year old European boys

Percentile	AL	CR	AL/CR ratio
6 years visit (N = 1965)			
2	21.13	7.33	2.71
5	21.42	7.42	2.75
10	21.71	7.52	2.79
25	22.14	7.68	2.84
50	22.59	7.84	2.89
75	23.01	8.00	2.92
90	23.41	8.16	2.96
95	23.65	8.27	2.99
98	24.01	8.39	3.03
9 years visit (N = 1842)			
2	21.72	7.34	2.77
5	22.09	7.43	2.84
10	22.39	7.53	2.87
25	22.83	7.69	2.92
50	23.31	7.84	2.97
75	23.79	8.02	3.02
90	24.28	8.17	3.07
95	24.60	8.27	3.12
98	25.16	8.41	3.20
15 years (ALSPAC; N = 1145)			
2	21.86	7.36	2.80
5	22.34	7.48	2.85
10	22.67	7.57	2.90
25	23.17	7.70	2.95
50	23.65	7.86	3.00
75	24.21	8.05	3.06
90	24.73	8.25	3.12
95	25.06	8.31	3.16
98	25.71	8.46	3.26
45+ years visit (RS III; N = 1215)			
2	21.48	7.29	2.76
5	22.18	7.40	2.83
10	22.57	7.50	2.90
25	23.17	7.64	2.97
50	23.87	7.81	3.05
75	24.69	7.97	3.16
90	25.68	8.14	3.28
95	26.18	8.26	3.35
98	26.84	8.35	3.44

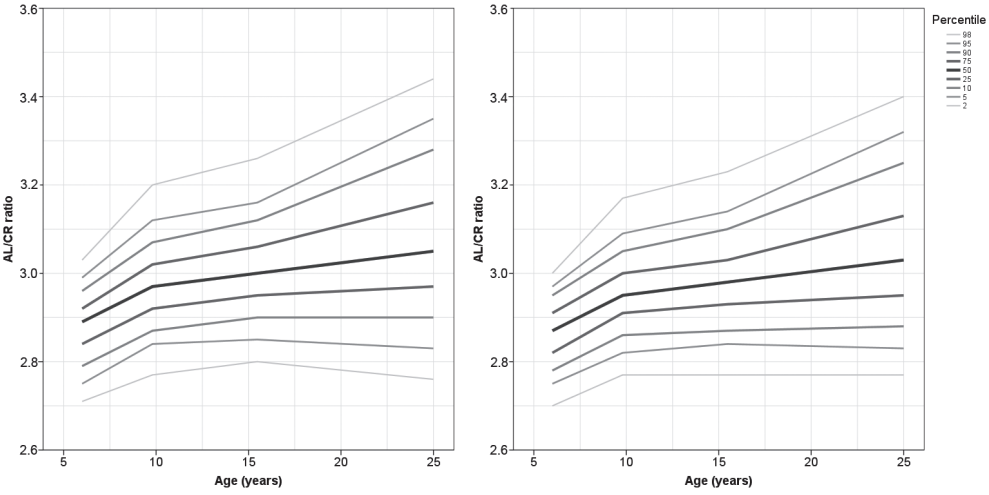
Supplementary Table S1b Percentiles of axial length, corneal radius and AL/CR ratio in 6 and 9 year old European girls

Percentile	AL	CR	AL/CR ratio
6 years visit (N = 2018)			
2	20.67	7.22	2.70
5	20.96	7.32	2.75
10	21.22	7.41	2.78
25	21.66	7.54	2.82
50	22.06	7.70	2.87
75	22.49	7.85	2.91
90	22.86	8.00	2.95
95	23.11	8.11	2.97
98	23.44	8.21	3.00
9 years visit (N = 1928)			
2	21.31	7.24	2.77
5	21.62	7.34	2.82
10	21.90	7.42	2.86
25	22.33	7.56	2.91
50	22.79	7.72	2.95
75	23.25	7.88	3.00
90	23.73	8.02	3.05
95	24.04	8.13	3.09
98	24.42	8.23	3.17
15 years visit (ALSPAC; N = 1302)			
2	21.51	7.27	2.77
5	21.84	7.37	2.84
10	22.20	7.46	2.87
25	22.68	7.61	2.93
50	23.15	7.76	2.98
75	23.65	7.93	3.03
90	24.21	8.10	3.10
95	24.56	8.21	3.14
98	25.11	8.31	3.23
RS III 45+ years visit (N = 1530)			
2	21.19	7.18	2.77
5	21.71	7.29	2.83
10	22.03	7.37	2.88
25	22.63	7.53	2.95
50	23.32	7.68	3.03
75	24.09	7.85	3.13
90	25.03	8.02	3.25
95	25.59	8.11	3.32
98	26.31	8.22	3.40

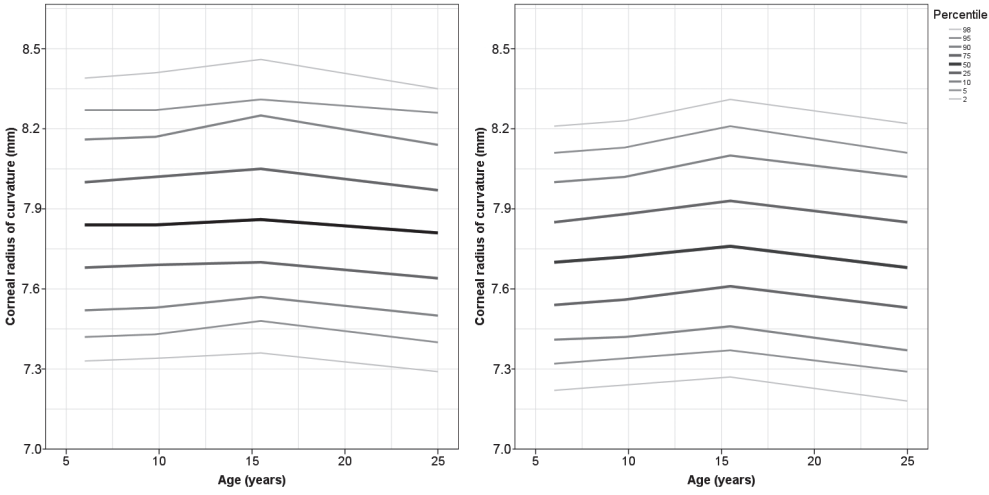
Supplementary Figure S1 Distribution of refractive error at age 9 years (left) and in adults (right)



Supplementary Figure S2 AL/CR as a function of age in boys (left) and girls (right)



Supplementary Figure S3 CR as a function of age boys (left) and girls (right)



CHAPTER 6

EYE SIZE AND SHAPE IN 10-YEAR OLD CHILDREN IN RELATION TO REFRACTIVE ERROR: A MAGNETIC RESONANCE IMAGING STUDY

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ABSTRACT

Purpose: To determine the eye shape and volume measured with MRI, and its association with refractive error ocular biometry in school children.

Methods: A total of 3,757 children from the Dutch population-based birth-cohort study Generation R underwent ocular biometry (Zeiss IOL-master 500) with axial length (AL) and T2-weighted MRI scanning at 10 years of age (height, width and volume). Cycloplegic refractive error was determined by automated refraction. Eyes were segmented from MRI using an automated algorithm combining atlas registration with voxel classification. Associations between ocular biometry, anthropometry, MRI measurements, and refractive error were tested using Pearson correlation. Shape was calculated as $height^2/AL^2$ -1 and $width^2/AL^2$-1, where shape >0.005 was considered oblate; shape with <0.005 as prolate; and else as spherical. Differences between myopic, emmetropic and hyperopic eyes were tested using ANOVA.

Results: Mean volume of the posterior segment was 6.35(±0.68) cm^3. Myopic eyes (SE ≤-0.5D) had a 0.47 cm^3 (P <0.001) larger posterior segment volume than emmetropic eyes, and 0.97 cm^3 (P <0.001) larger volume than hyperopic (SE≥ +2.0D) eyes. Mean horizontal shape was 0.056 (SD 0.066); hyperopic eyes and myopic eyes were on average still oblate, but 45% of the myopic eyes were prolate, whereas 88% of the hyperopic eyes were oblate. The correlation between refractive error and MRI-derived posterior segment length (r -0.51; *P* <0.001) was stronger than the association between refractive error and MRI-derived height (r -0.30; *P* <0.001) or width of the eye (r -0.10, *P* <0.001).

Conclusion: In this study, eye shape at 10 years was predominantly oblate, even in eyes with myopia. The association between MRI-based posterior segment length was higher than with the height and width of the eye. Whether eye shape is an independent predictor for development of myopia should be investigated in longitudinal studies.

INTRODUCTION

Refractive errors affect a large part of the world population, and the prevalence of myopia, or nearsightedness, increases worldwide.[50,72,98] Myopia develops during childhood and teenage years up to adolescence, predominantly by elongation of the vitreous chamber.[158] A proportion of the myopes will develop high myopia (≤-6 D), in which the axial length (AL) can grow beyond 30 mm.[114] This can lead to the development of staphylomas and result in morphological changes of the optical nerve, retina and sclera with increased risk of visual impairment and blindness.[18,53,69]

A study among young pilots was the first to show that those with more peripheral hyperopic defocus had more severe myopia progression.[159] Animal studies confirmed this observation,[160] and this created interest in eye shape, peripheral refraction and development of myopia. Eye shape can be measured with magnetic resonance imaging (MRI), as height, width and volume of the eye cannot be obtained with regular ocular biometry techniques. MRI studies in adults showed that eyes with high myopia have a prolate shape and the eye is more curved in the posterior pole than in the periphery, in contrast to the more oblate shaped emmetropic eyes, where the eye is more curved in the periphery than in the posterior pole.[69,161-165] A prolate shape has been hypothesized to be a risk factor for axial eye growth as the degree and retinal surface area of hyperopic defocus is larger in the periphery, which may attribute to growth stimuli for foveal myopia.[159,166,167] Studies investigating eye shape on MRI are scarce, focused mainly on myopia, and were performed on a relatively small set of either very young children or adults.[9,52,128,143,144,161,168] Large pediatric studies evaluating MRI-based biometry and shape for the entire spectrum of refractive errors are lacking.

This study is the largest study to date to describe eye shape determined on MRI images and investigate the association between shape parameters and refractive error in school children.

MATERIAL AND METHODS

General design

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands. A total of 9,778 pregnant women were included in the study. All children were born between April 2002 and January 2006.[45,46] The children were invited at age 6 and 10 years with their mothers for examination on the research center by trained nurses. Of the 9,778 included pregnant women, 5872 participated with their children for physical examination at the research centre at 10 years of age, respectively. A total of 3637 children underwent a T2 eye scan. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20). Written informed consent was obtained from all participants.

Magnetic resonance imaging (MRI)

All participants underwent brain MRI at a 3 Tesla scanner (Discovery 750, General Electric, Milwaukee, WI) with an 8-channel receive-only head coil .[169] The protocol included a 3D stabilized/variable flip angle 3D Fast spin echo T2-weighted fat suppressed scan (TR = 1440 $_{ms}$; TE = 129.59 $_{ms}$; FOV = 256 x 256 mm; matrix size = 256 x 256; 176 sagittal slices with 1 mm, voxel size 1x1x1 mm^3) with a scan time of 56 seconds and an echo train length of 256. Both eyes were included in the FOV of this sequence. The scans were acquired with the children in supine position using an overhead 45° inclined mirror with a focus point to avoid movement artifacts in the eyes. Scans were included based on visual quality inspection with an overlying automated segmentation raster.

Segmentation method

The eyes were segmented into six regions using a combination of atlas segmentation and pixel-wise classification.[170] First, 30 images were manually segmented by an experienced observer (JT). These segmentations were used as atlases and to train a random forest classifier. The 30 atlases were registered to each subject image to produce a map with class probabilities for posterior segment (PS), anterior chamber, and lenses for left and right eyes (Figure 1). Then, a random forest classifier was applied to produce a second map with class probabilities. To prevent the classifier from overruling the segmentations inside the eyes, which might locally look like background, a bias was added to the classifier's probabilities. Lastly, the two maps were multiplied and for each voxel the class with the max probability was used as the final segmentation. Table 1 shows the mean Dice

Figure 1 Segmentation method of the eye with on the left side a high myopic eye and the left side an emmetropic eye

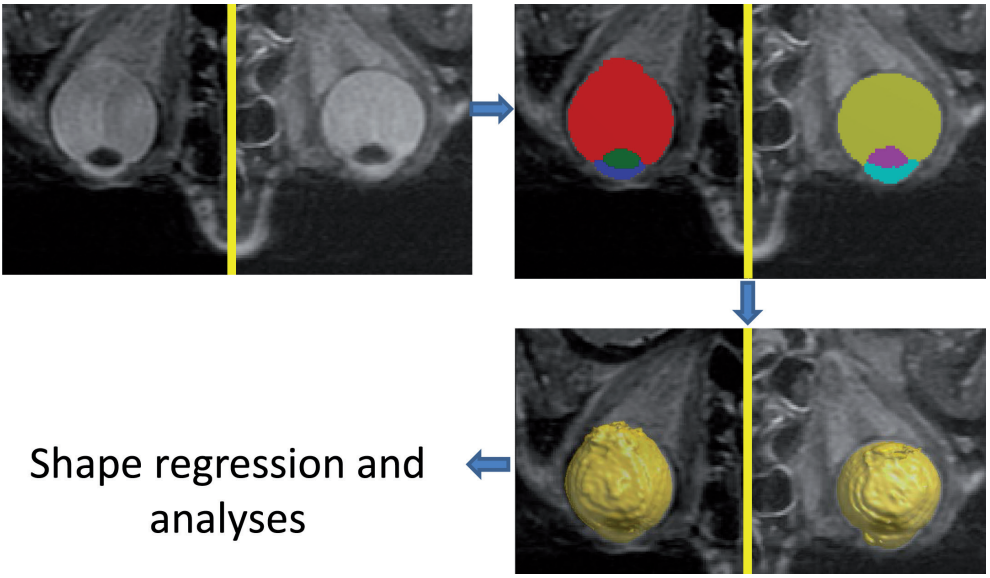


Table 1 Results of the 3 fold cross-validation of the segmentation method. For each class we report the mean and the standard deviation of Dice similarity coefficients computed from all test images in all folds. Dice similarity of 1 is perfect overlap

Tissue	Dice Similarity Coefficients
Right PS	0.97±0.02
Left PS	0.97±0.01
Right Anterior Chamber	0.83±0.1
Left Anterior Chamber	0.83±0.08
Right Lens	0.82±0.9
Left Lens	0.83±0.83

Similarity Coefficient for 3-fold cross-validation of this method.
To train the classifier, segmentation labels were used as the ground truth and the following 48 features were used to characterize each pixel: the first- and second order derivatives, the gradient magnitude, the Laplacian, the Eigenvalues of the Hessian matrix, and the determinant of the Hessian matrix; all these features were computes on multiple scale levels of 1mm, 1.6mm, and 4mm respectively.

Anatomical Directions

To determine eye height and eye width, we defined an anatomical coordinate system using the segmentations in the subject image space. The Anterior-Posterior (AP) axis was defined as the direction between the centroid of the vitreous chamber and the centroid of the lens. The Superior-Inferior (SI) axis was defined as the direction orthogonal to the plane spanned by AP and the direction between the centroid of left PS and the right PS. The Left-Right (LR) axis was defined as the direction orthogonal to the plane spanned by AP and SI. The height of the eye was measured along the SI axis at the center of mass of PS and width of the eye was measured along LR axis at the center of mass of PS.

Shape and volume on MRI

Height, width, posterior segment length and volume were measured on MRI. Shape was calculated as $\text{height}^2/\text{axial length}^2$ -1 and $\text{width}^2/\text{axial length}^2$-1, where shape >0.005 was considered oblate; shape <0.005 as prolate and a shape of 0 ± 0.005 as spherical. Eye volume (cm^3) per region was computed from the number of segmented voxels.

Refractive error and ocular biometry

Ocular biometry was measured by Zeiss IOL-master 500 (Carl Zeiss MEDITEC IOL-master, Jena, Germany) and included AL, corneal radius of curvature (CR), and anterior chamber

depth (ACD). For AL five measurements per eye were averaged to a mean AL. Three measurement of the keratometry (K1 and K2) were taken of the right and left eye, and were averaged to a mean corneal radius of curvature (CR). AL/CR ratio was calculated by dividing AL (mm) by CR (mm). Axial elongation was calculated in mm/year ((measurement 10 years visit – measurement 6 years visit) / (age at 10 years visit – age at 6 years visit)). Ophthalmological examination included automated cycloplegic autorefraction (Retinomax-3, Bon, Lübeck, Germany). Two drops (three in case of dark irises), with 5 minutes time interval, of cyclopentolate (1%) were administered at least 30 minutes before refractive error measurement in all children. Spherical equivalent (SE) was calculated as the average sphere + 1/2 cylinder of both eyes. Pupil diameter was ≥6 mm at the time of the measurement. Children with inadequate cycloplegia (pupil diameter <6.0 mm) were excluded for the analysis with refractive error or spherical equivalent.

Covariates

Body height was measured without shoes. Gestational age and birth weight were obtained using medical records and hospital registries. As a proxy for ethnicity, countries of birth of the parents were obtained and determined by questionnaire using the method developed by Statistic Netherlands and grouped into European and non-European.

Statistical analysis

Differences in variables between boys and girls or the three groups (hyperopia ≥+2.0D, emmetropia <+2.0 - >-0.5 D and myopia ≤-0.5D) were tested using chi square and ANOVA test. Correlation between the MRI variables (height, width, PS depth, PS volume, lens volume, anterior chamber volume, prolateness) and ocular measurements (SE, AL/CR, AL, CR, anterior chamber depth and axial length growth) or gestational age and anthropometry (birthweight, body height) were tested with Pearson correlation. The association between spherical equivalent and horizontal shape was determined using linear ordinary least squares linear regression models, with restricted cubic splines with three knots (the 10^{th}, 50^{th}, and 90^{th} percentiles). The associations between axial length, height or width of the eye with SE were tested using linear regression models adjusted for age and gender. Statistical tests were performed using SPSS (version 21.0.0.0).

RESULTS

A total of 2963/3637 (81.5%) children were included in the analyses. Excluded were N = 523 (14.4%) children with low quality of the MRI scan (motion artifacts N = 441, braces N = 56 and incorrect positioning of the participant N = 26). Ocular biometry had not been measured in N = 151 (4.2%) of the children. Children with good quality

and low quality scans did not show significant differences in AL (P = 0.28), AL/CR ratio (P = 0.56), or SE (P = 0.32). Children were on average 10.1 (0.6) years of age and 51.5% (1525) were girls. The characteristics of the cohort, ocular biometry and volume measurements are summarized in table 2. Of the 1703 children with refractive error, axial length and MRI data, 209 (12.2%) were myopic and 128 (7.5%) hyperopic.

Table 2 General and ocular characteristics from 10-year-old boys and girls from the Generation R study

	All N=2963	Boys N=1438	Girls N= 1525	P**
General measurements				
Age child (years)	10.1 (0.59)	10.2 (0.61)	10.1 (0.56)	0.002
European ethnicity (%)*	70.0 (2074)	69.0 (990)	71.0 (1084)	0.13
Body height (cm)	141.7 (6.5)	141.8 (6.3)	141.6 (6.7)	0.48
Birthweight (grams)	3434 (564)	3521(555)	3352 (561)	<0.001
Gestational age (weeks)	39.8 (1.8)	39.9 (1.8)	39.7 (1.9)	0.006
IOL master and auto refractor				
Axial length (mm)	23.11 (0.83)	22.86 (0.77)	23.39 (0.80)	<0.001
Axial length growth (mm/year)	0.21 (0.09)	0.21 (0.09)	0.21 (0.08)	0.69
Corneal radius (mm)	7.78 (0.26)	7.85 (0.25)	7.72 (0.24)	<0.001
AL/CR ratio	2.97 (0.09)	2.98 (0.10)	2.96 (0.09)	<0.001
Spherical equivalent (D)$	0.74 (1.30)	0.68 (1.28)	0.72 (1.31)	0.40
MRI measurements				
Posterior segment length (mm)	17.02 (0.80)	17.25 (0.80)	16.80 (0.74)	<0.001
Posterior segment height (mm)	23.57 (0.95)	23.72 (0.95)	23.43 (0.93)	<0.001
Posterior segment width (mm)	23.73 (0.95)	23.97 (0.93)	23.51 (0.91)	<0.001
Posterior segment volume (cm^3)	6.35 (0.68)	6.18 (0.63)	6.53 (0.68)	<0.001
Lens volume (cm^3)	0.084 (0.013)	0.085 (0.0013)	0.084 (0.0013)	0.03
Anterior chamber volume (cm^3)	0.24 (0.035)	0.24 (0.036)	0.23 (0.032)	<0.001
IOL master and MRI				
Vertical prolateness	0.042 (0.068)	0.031 (0.067)	0.052 (0.066)	<0.001
Horizontal prolateness	0.056 (0.066)	0.053 (0.067)	0.059 (0.065)	0.005
Oblate eye shape (%)*^	78.6 (2328)	77.6 (1116)	79.5 (1212)	0.01
Spherical eye shape (%)*^	4.5 (133)	3.7 (53)	5.2 (80)	–
Prolate eye shape (%)*^	16.9 (502)	18.7 (269)	15.3 (233)	–

All data are presented as the mean (SD). AL, axial length; CR, corneal radius of curvature; SE, spherical equivalent, except where indicated otherwise. ^Shape was the horizontal eye shape.

* data are presented as % (N).

**P-values were calculated using the Student's t-test or the chi-square test.

$ children with cycloplegic refractive error and MRI data, N = 1732 (837 boys and 895 girls).

Biometry on IOL-master and MRI

The axial length measured with the IOL-master was highly correlated (r 0.87; P <0.001) with the PS length measured on MRI, and likewise, PS length on MRI had almost similar correlation with spherical equivalent (r = 0.52 vs 0.61). Mean AL was 23.11 (0.83) mm.

On MRI, eyes had a larger width than height (mean 23.73 (SD 0.94) vs 23.57 (0.95) mm); and mean PS length was 17.02 (0.80) mm. The mean total volume of the eyes was 6.67 cm^3 with a PS volume of 6.35 cm^3. Boys had longer, higher, and wider PS than girls with a higher volume of PS, lens, and anterior chamber (lens volume P = 0.03, all others P <0.001; table 2). The correlation between the axial length and the height and the width of the eye was 0.63 (P <0.001) and 0.66 (P <0.001), respectively. The correlation between height and width was 0.67 (P <0.001). AL and anterior chamber depth performed with IOL master showed the highest correlation with PS length, followed by height, and the least with width of the eye (Table 3, figure 2). Hyperopic eyes had volume 6.37 cm^3, emmetropic eyes 6.61 cm^3, and myopic eyes had the largest volume, i.e., 7.24 cm^3 (P <0.001). The difference between the myopic and the hyperopic eyes was lowest for width (0.7 mm), whereas the difference in height was 1.2 mm, and in PS length 1.5 mm (Table 4). The correlation between SE and AL was r -0.61, with height r -0.31 and with width of the eye r -0.22 (figure 2).

Figure 2 Spherical equivalent as a function of width and height of the posterior segment, and axial length of the eye

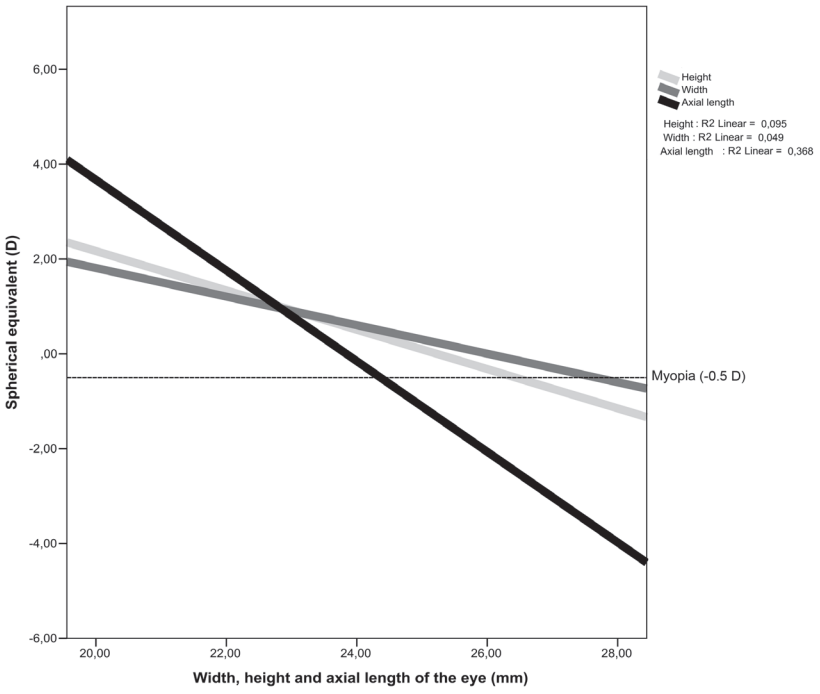


Table 3 Correlation between ocular measures obtained with IOL master and ocular biometric parameters measured on MRI in children aged 10 years

	Pearson correlation Coefficient of MRI measurements of the eye							
	Height	**Width**	**PS depth**	**PS volume**	**Lens volume**	**AC volume**	**Vertical shape**	**Horizontal shape**
Ocular measurements								
SE (D)	-0.303**	-0.222**	-0.507**	-0.383**	-0.115**	-0.226**	0.266**	0.396**
AL/CR	0.204**	0.100**	0.413**	0.265**	0.086**	0.224**	-0.326**	-0.477**
AL (mm)	0.627**	0.656**	0.868**	0.807**	0.286**	0.475**	-0.307**	-0.297**
CR (mm)	0.499**	0.629**	0.571**	0.639**	0.232**	0.328**	-0.024	-0.133**
ACD (mm)	0.150**	0.071**	0.203**	0.164**	0.133**	0.335**	-0.255**	-0.367**
AL growth (mm/year)	0.232**	0.185**	0.442**	0.321**	0.100**	0.205**	-0.275**	-0.347**
Other measurements								
Body height (cm)	0.253**	0.248**	0.180**	0.261**	0.055**	0.186**	0.115**	0.107**
Birthweight (kg)	0.151**	0.183**	0.141**	0.190**	0.042*	0.137**	0.013	0.052**
Gestational age (weeks)	0.047*	0.042*	0.021	0.051**	0.011	0.032	0.019	0.012

Correlations were tested using Pearson correlation. *P < 0.05 **P <0.01. PS = posterior segment, AC = anterior chamber, AL = axial length, SE = spherical equivalent, CR = corneal radius of curvature, ACD = anterior chamber depth.

Table 4 Ocular biometry measured on MRI in relation to refractive error in 10 year old children

	Refractive error category			
	Hyperopia	**Emmetropia**	**Myopia**	***P*-value**
MRI measurements				
Height	22.9 (0.8)	23.5 (0.9)	24.1 (0.9)	<0.001
Width	23.3 (0.9)	23.7 (0.9)	24.0 (1.0)	<0.001
PS depth	16.2 (0.6)	16.9 (0.7)	17.7 (0.9)	<0.001
PS volume	5.80 (0.5)	6.30 (0.6)	6.77 (0.8)	<0.001
Lens volume	0.081 (0.01)	0.084 (0.01)	0.088 (0.01)	<0.001
AC volume	0.22 (0.03)	0.24 (0.03)	0.25 (0.04)	<0.001
Vertical shape	0.077 (0.06)	0.041 (0.06)	0.008 (0.06)	<0.001
Horizontal shape	0.113 (0.08)	0.058 (0.06)	0.005 (0.06)	<0.001
Oblate shape	87.5	71.5	53.1	<0.001
Sphere shape	0.8	4.8	1.9	<0.001
Prolate shape	11.7	23.6	45.0	<0.001
IOL-master measurements				
Axial length	22.04 (0.6)	23.07 (0.7)	23.98 (0.83)	<0.001
Corneal radius (mm)	7.76 (0.25)	7.79 (0.25)	7.72 (0.25)	0.002
AL/CR ratio	2.84 (0.07)	2.96 (0.06)	3.11 (0.09)	<0.001

Average (SD) of the ocular biometry measurements per refractive error category. PS = posterior segment, AC = anterior chamber. *P*-values were calculated using ANOVA or chi square test.

Eye shape

Mean eye horizontal shape was 0.056 (0.068) and vertical 0.042 (0.066). The proportion of horizontal oblates was 78.6%, of spheres 4.5% (shape = 0 +/-0.005), and of prolates 16.9% (figure 3). Myopic refractive errors were more often observed in eyes with a spherical and prolate shape, indicating that myopic refractive error increases as eyes becomes less oblate (figure 4). Of myopes, 99 (47.4%) had a prolate shape; of hyperopes 5 (3.9%) had a prolate shape. Conversely, prolate shape was present in 31.6% of myopic eyes; sphere and oblate shape in 16.7% and 7.4%, respectively. Faster axial length growth was associated with more horizontal and vertical prolateness.

Birth parameters

Body height, birthweight, and gestational age had a higher correlation with the height and width of the eye than with the PS length. Age, gender, body height, body weight and birthweight together explained 16% of the variance in PS volume, 15% of the variance in width, 13% in the variance of the height of the eye and 13% of the PS depth.

Figure 3 The distribution of the horizontal shape with a spherical shape between the dotted lines

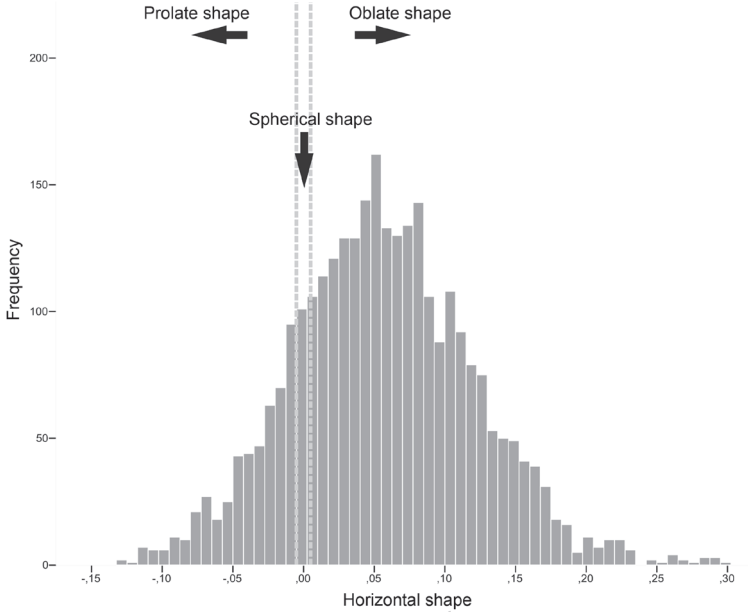
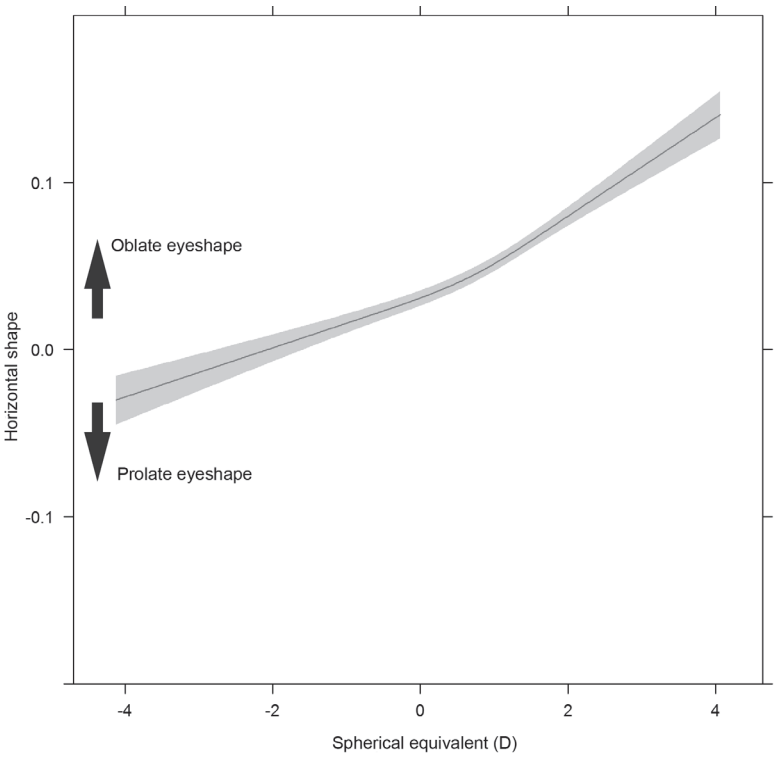


Figure 4 The association between spherical equivalent and horizontal eye shape



DISCUSSION

This study is the first to provide normative data on ocular shape in a large group of school children presenting with a wide spectrum of refractive errors. We found that at this age, most children have an oblate eye shape. Hyperopic eyes and emmetropic eyes were predominantly oblate shaped; this was also true for most myopia except for the higher refractive errors and longer axial lengths. Posterior segment depth had the highest association with refractive error, but also height and width increased with more myopia. Apart from myopic refractive error, eye volume was associated with age, male gender, birth weight, and genetic risk score for myopia. Conversely, width of the eye was more related to anthropometry measurements such as body height and birthweight.

MRI measurements

The study of ocular biometry on MRI images has several advantages, such as the possibility to study dimensions of the eye in all directions as well as volumes. A few previous studies reported eye measurements on MRI in babies and adults. Newborns were reported to have an average eye volume of 2428 mm^3, while our 10 year old children measured 6670 mm^3,[163] this suggests an increase by factor 2.75 in the first decade. Ethnic differences may play a role, as children of the Singapore STARS study already had eye volume 6690 mm^3 at the age of 6.5 years. This is similar to our children aged 10 years, which corresponded to the same mean spherical equivalent.[165] Our mean posterior segment length was slightly shorter than that found in a study of adults, but this population consisted of predominantly females who are known to have shorter axial lengths.[164] With higher myopic refractions, axial length increased with a factor three compared to width of the eye, and with a factor one and a half compared to the height of the eye. This has been reported before.[161]

Various anthropometric measurements (i.e. body height and body weight) have been associated with axial length of the eye in young children and adults.[113,116,117] Our study shows that width and height of the eye appear to be more correlated with birthweight and body height than axial length, and these measures are less prone to change when myopic refractive error increases. The relatively larger axial length growth with increasing myopia may result in a more curved posterior pole and prolate shape in myopic eyes. This may increase peripheral hyperopic defocus, which can trigger further axial elongation.[159,167] Peripheral hyperopic defocus is currently already a target for treatment by the use of multifocal soft contact lenses and orthokeratology (ortho-K).[171-173]

Strength and limitations

The strengths of this study are the population-based setting, the three dimensional biometric eye measurements based on MRI images, the large group of participants of homogeneous age encompassing the whole spectrum of cycloplegic refractive error and early life growth data. Considering that the highest incidence of myopia in Western-European

children is between 10 and 15 years of age, follow up measurements of eye shape as the children grow older will provide further insights in the consequences of eye shape.[53] Among the limitations are the cross sectional design of this analysis, the relatively low number of children with refractive errors, and the limited quality of MRI scans in 15% of our population. MRI imaging is a relatively difficult examination for children at this age and sensitive for braces wear, and drop out seemed unbiased as axial length, corneal radius, and refractive error was similar in children with good quality and low quality scans.

CONCLUSION

In conclusion, our study showed that eyes of 10-year old children in Europe are largely oblate shaped, even in those with myopia. Nevertheless, the eye volume, height, width, and shape were strongly related to refractive error. Posterior segment length showed the stronger association with refractive error, whereas width showed the highest correlation with body height and birthweight. Longitudinal studies are required to reveal whether eye size and shape determine the rate of eye growth and development of refractive error.

PART IV

MYOPIA RISK FACTORS

CHAPTER 7

ENVIRONMENTAL FACTORS EXPLAIN SOCIO-ECONOMIC PREVALENCE DIFFERENCES IN MYOPIA IN SIX-YEAR-OLD CHILDREN

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ABSTRACT

Objective: High myopia (≤-6 D) usually has its onset before 10 years of age and can lead to blinding complications later in life. We examined whether differences in myopia prevalences in socio-economic risk groups could be explained by differences in lifestyle factors.

Methods: A total of 5711 six-year-old children participating in the prospective population-based birth-cohort study Generation R underwent a stepwise ophthalmic examination, which included visual acuity and objective cycloplegic refraction to identify children with myopia (≤-0.5D). Daily activities, ethnicity, factors representing family socio-economic status and housing were ascertained by questionnaire. Risk assessments of myopia and mediation analyses were performed using logistic regression; attenuation of risks was calculated by bootstrapping.

Results: Prevalence of myopia was 2.4% (n=137). Myopic children spent more time indoors and less outdoors than non-myopic children ($P<0.01$), had lower vitamin D ($P=0.01$), had a higher BMI, and participated less in sports ($P=0.03$). Children of non-European descent (OR 2.60; 95%CI 1.84–3.68), low maternal education (OR 2.27; 95%CI 1.57–3.28) and low family income (OR 2.62; 95%CI 1.8 –3.74) were more often myopic. Lifestyle factors explained the majority of the increased risk for ethnicity (82%; 95%CI 55–120), maternal education (69%; 95%CI 45–109), and family socio-economic status (71%; 95% CI 46-104).

Conclusion: This study found environmental factors to be strong risk factors for myopia already at the age of 6 years. The myopia prevalence differences in socio-economic groups were greatly determined by differences in distribution of these environmental risk factors, highlighting the importance of lifestyle adjustments in young children developing myopia.

INTRODUCTION

Myopia (nearsightedness) is an eye disorder with increasing prevalence,[72] burden,[18] and corresponding economic costs[78] during the past two decades. Most challenging for public health are the visual consequences, in particular of pathological myopia[72] due to myopic macular degeneration, glaucoma, and retinal detachment.[18] These changes often lead to irreversible visual impairment, emphasizing the need to unravel the underlying causes.

The classical risk profile for myopia includes education,[93,174] other socioeconomic factors,[175] and ethnicity.[11,16,50] Higher education coincides with an almost three times increased risk,[93] In East-Asia prevalences reach 85% among school leavers,[16] whereas in Europe prevalence rates are now approaching 50% in 25 years olds.[50] The biological basis for these risk factors is unclear, as is the consistency across age groups and countries. This lack of insight in the causal relationship hinders the development of effective clinical and public health campaigns. Recent research focus has shifted to the study of behavioral factors; of these, time spent outdoors,[22,59] reading, and indoor activities have been launched as the most prominent determinants.[22] Whether these behavioral factors are consistent, and whether they mediate in the association between classical risk factors and myopia has not been settled.

This study addresses the consistency of currently known environmental and socioeconomic risk factors in a cohort of young multi-ethnic children. We conducted mediation analysis to decipher the relevant components underlying the associations, and estimated to what extent these mediators explain the differential occurrence of myopia.

POPULATION AND METHODS

Study population

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands. The complete methodology has been described elsewhere.[45] Briefly, a total of 9,778 pregnant women were included in the study, and children were born between April 2002 and January 2006. The children were invited at age 6 years for examination by trained nurses at the research center. Of the initial cohort, 6,690 (68.4%) children participated in the physical examination. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20), and written informed consent was obtained from all participants.

Assessment of myopia

A two step approach was performed to identify children with myopia. First step included an ophthalmological examination consisting of visual acuity according to LogMAR using

LEA-charts at 3 meter distance by means of the ETDRS method. Step 2 was carried out in children with a LogMAR visual acuity of >0.1 in at least one eye or in children with an ophthalmologic history (visit to eye care practitioner), and included performance of automated subjective cycloplegic refraction (Topcon auto refractor KR8900 (Topcon, Japan)) in both eyes. Two drops (three in case of dark irises), with 5 minutes time interval, of cyclopentolate (1%) were administered ≥30 minutes before refractive error measurement. Pupil diameter was ≥6 mm at the time of the measurement. Spherical equivalent (SE) was calculated as sphere + ½ cylinder, and myopia was defined as SE ≤-0.5D in at least one eye. Children with LogMAR visual acuity ≤0.1 in both eyes, no glasses or ophthalmic history were classified as non-myopic.[176]

Ethnicity, education and income

As a proxy for ethnicity, country of birth of the parents was obtained and determined by questionnaire using the method developed by Statistic Netherlands.[121] Country of parental origin was grouped into Morocco, Turkey, Dutch Antilles and Surinam, and 'other' for risk estimation; and for final analysis grouped into European and non-European (Supplementary Table 1). Educational level of the mother and household income at age 6 years of the children were used to estimate social economic status. The highest educational level accomplished by mother and net household income were obtained by questionnaire and categorized into high (university or bachelor degree) or low (>3 years general secondary school, or lower) and <2400 euros/month was categorized as low income (lowest tertile).

Potential mediators

Type of house was categorized into a rental house or private property. Marital status was stratified into a single parent or living with a partner. Time spent playing outdoors, biking and walking to school, time spent watching television/playing (handheld)computer games. Total time spent on activities was calculated in average hours/day. All outdoor activities (hr/day) were combined, and all indoor activities (hr/day) were subtracted to make a daily activity score of outdoor time relatively to indoor time per day, to avoid overfitting of the model.[177] Sport participation was obtained using questionnaire ("Does your child participate in a sport?"), The measurements of 25(OH)D (vitamin D, nmol/L) were conducted on blood samples collected at the research center at 6 years of age, using the gold standard liquid chromatography/tandem mass spectrometry (LC-MS/MS) method at the Endocrine Laboratory of the Vrije Universiteit Medical Center (VUMC), Amsterdam, The Netherlands between July 2013 and January 2014. Height and weight were measured at the research center.

Statistical analysis

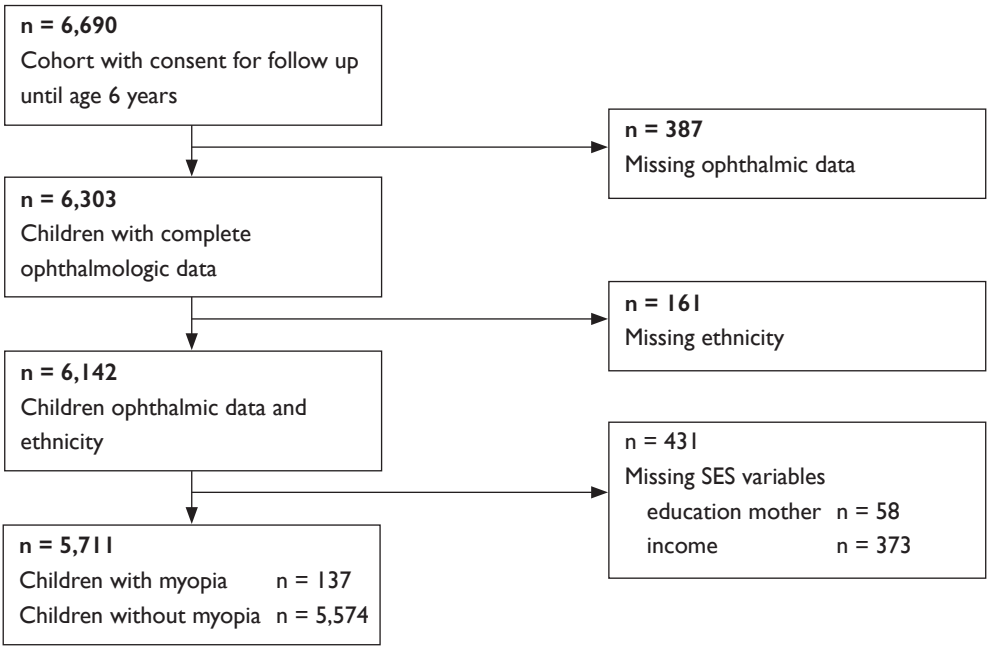
Differences in the European and Non-European groups were calculated using chi-square and student t-test or Mann Whitney U Test. Four models were performed for testing associations between ethnicity, low income and low educational level versus myopia with logistic regression analysis. Model 1 included only adjustment for age and sex. Model 2 added inclusion for low household income vs myopia and low maternal educational level vs myopia social economic factors such as marital status of the parents and rental house, and for ethnicity vs myopia marital status of the parents, rental house, and also family income and educational level of the mother. Model 3 included Model 2 with additional inclusion of activity factors such as outdoor time relatively to indoor time per day and participation in sports. Model 4 included Model 3 with additional inclusion of 25(OH) D and BMI. Selected mediators were ordered and added to the model based on an hierarchical approach in which theoretically more distal mediators to the trait were first added to the model (supplemental figure 1).[178] Multiple imputation procedures were used to replace missing covariates for the most likely values to avoid potential bias that may result from missing data,[179] using Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach.[180] Data on playing outdoors (24.1%), data about housing (21.4%), and 25(OH)D (36.3%) were missing, all other covariates had missing values <20%. Mediation analyses was performed using the Baron and Kenny method,[181] which requires mediators to fulfill the following criteria: a) only factors associated with myopia independent of ethnicity or income/education were included in the model (table 1); and b) mediators were unequally distributed over the ethnic groups (supplemental table 2), or between income/education groups (supplemental table 3). Differences in distributions were tested using logistic regression models. To calculate the attenuation of the effect estimate after adjusting for the mediator(s), the following formula was used: (100 x (B model 1 – B model 1 with explanatory factor) / (B model 1)). The bootstrap method was used to calculate a 95% CI around the percentage of attenuation with 1000 re-samplings per imputed dataset using the statistical program R. All other analyses were performed in SPSS (version 21.0.0.1).

RESULTS

A total of 5,711 children were available for the analysis for ethnicity, maternal educational level, and family income (figure 1). Of the total group, 31% (N = 1764) were of a non-European descent. Cycloplegia was slower in children of non-European descent due to dark irises, however, this did not lead to a differential distribution of SE (Mann-Whitney U *P* 0.96). As shown in table 1, children with myopia were more likely to live with unmarried parents, to live in a rental home, spend less time outdoors and more time indoors, have lower 25(OH)D levels, less participation in sports, and a higher BMI.

Data on serum levels (such as 25(OH)D had the highest proportion (36%)) of missing values as result of refusal of blood withdrawal. Children with at least one predictor vari-

Figure 1 Flowchart of participants eligible for analysis



able missing (n = 3845) were less likely than children with complete data (n = 1866) to participate in sports (P = 0.04), to live in private property house (P <0.001), to have lower vitamin D (P < 0.001) and have lower BMI (P <0.001). Other mediators did not differ between the two groups. As this may cause selection bias we imputed missing values and used data from all 5711 children for analysis.

Of the total group 2.4% (n=137) children were myopic. Children of Dutch-Antilles, Surinamese (OR 3.29; 95%CI 2.13 – 5.10) and Moroccan descent (OR 2.35; 95%CI 1.34 -4.13) were more likely to be myopic compared to their European peers. The total group of non-European children were more often myopic (table 2). When adjusting only for age, ethnicity, and gender, low educational level of the mother and low family income were associated with a higher frequency of myopia (table 3).

The association between family income or maternal education and risk for myopia was independent of ethnicity. In a sensitivity analysis performed for Europeans and non-Europeans, effect estimates showed similar effect in both groups: low education of the mother in Europeans (OR 1.79; 95% CI 1.07 – 2.99) and in non-European (OR 1.66; 95% CI 0.93 - 2.96) and low family income in Europeans (OR 1.81; 95% CI 1.05 – 3.12) and in non-European (OR 1.97; 95% CI 1.12 – 3.45).

All variables which remained significantly associated with myopia after adjustment for ethnicity, maternal educational level, or family income entered the mediation analysis (table 1). As shown in table 2, 56% (95% CI 31 – 86) of the increased risk for Non-European children was explained by differences in socio-economic factors, and 82% (95% CI 55 – 120) could be explained by all mediators. For maternal education, 34%

Table 1 Distribution of mediators for myopia, independent of ethnicity, maternal educational level, or family income

	Myopia N = 137	No myopia N = 5,574	*P*-value adjusted for ethnicity	*P*-value adjusted for education	*P*-value adjusted for income
Child characteristics					
Age (years)	6.37 (0.7)	6.16 (0.5)	**<0.001**	**<0.001**	**<0.001**
Sex, female (%)	46.7 (64)	50.0 (2,785)	0.50	0.45	0.49
BMI (kg/m2)	16.7 (2.2)	16.2 (1.8)	**0.03**	**0.01**	**0.02**
Social economic factors					
European (%)	45 (61)	69.7 (3,886)	–	**<0.001**	**<0.001**
Maternal education, low (%)	67.9 (93)	45.6 (2,542)	**0.001**	–	**0.007**
Income, low (%)	59.9 (82)	33.8 (1,883)	**<0.001**	**<0.001**	–
Habituation					
Rental home (%)	64.8 (90)	38.9 (2,297)	**0.001**	**<0.001**	**0.003**
Married/Registered partnership (%)	55.3 (76)	67.4 (3,744)	**0.03**	**0.03**	0.22
Activities daily life					
Outdoor – indoor (hr/day)	-1.1 (2.1)	-0.08 (1.7)	**<0.001**	**<0.001**	**<0.001**
Vitamin D (nmol/L)	53 (27)	67 (30)	**0.006**	**<0.001**	**0.003**
Participation in sports (%)	30.4 (42)	44.4 (2,476)	**0.03**	**0.03**	**0.04**

Values are means (SD), or percentages (absolute numbers).

P values are calculated with logistic regression models adjusted for ethnicity/educational level of mother/income on the imputed datasets.

Data missing on BMI (<1%), rental house (21%), marital status (9,4%), playing outdoors (24%), biking to school (12%), walking to school (12%), watching television (16%), computer use (17%), vitamin D (36%), and participation in sports (9,8%). Outdoor time was calculated as sum of playing outdoors + biking to school + walking to school. Nearwork was calculated as time spent on computer + time spent watching television.

(95% CI 17 – 59) of the increased risk was explained by housing and marital status of the parents; an additional 29% was explained by daily activities and playing sports (table 3). The proportion of increased risk of myopia for low maternal education explained by all mediators was 69% (95% CI 45 – 109). For low family income, similar trends were observed, and the proportion of increased risk explained by all mediators was 71% (95% CI 46 – 104) (table 3).

As shown in table 2 and 3, the differences in socio-economic factors disappeared and became non-significant after adjusting for the more proximal lifestyle factors (ethnicity OR 1.29, 95% CI 0.83 – 1.99; maternal education OR 1.40, 95% CI 0.92 – 2.12; and low family income OR 1.47, 95% CI 0.96 – 2.25). The OR was slightly higher if in Model 4 only 25(OH)D was added than with only BMI (low maternal education OR 1.45 95% CI 0.96 – 2.18 with BMI only vs. 1.42 95% CI 0.94 – 2.15 with 25(OH)D only; and low family income OR 1.55 95% CI 1.02 – 2.36 with BMI only vs 1.49 95% CI 0.97 – 2.28

Table 2 Association between ethnicity and risk of myopia with adjustment for mediators using various models

	Basic model	Basic + SES model	Basic + SES + Activities model	Basic + SES + Activities + 25(OH)D, BMI model
Determinant	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
European	Ref	Ref	Ref	Ref
Non-European	**2.60**	**1.71**	1.42	1.29
	(1.84 – 3.68)	**(1.15 – 2.54)**	(0.94 – 2.15)	(0.83 – 1.99)
Attenuation	–	-56%	-74%	-82%
		(-86 – -31)	(-109 – -48)	(-120 – -55)

Values are OR (95% CI) and represent risk of myopia.

P values are corrected for age and gender; *P* values <0.05 are shown in bold.

Attenuations represents the attenuations of effect estimates for Non-European ethnicity, relative to model 1 after adjustment for the mediators in model 2 -4 (100 x (B model 1 – B model with mediators) / (B model 1)).

Basic model is adjusted for age and gender.

Basic + SES model is adjusted for basic model and education mother, family income, marital status and rental home.

Basic + SES + Activities model is adjusted for Basic + SES model and outdoor activities – nearwork activities and participation in sports.

Basic + SES + Activities + 25(OH)D, BMI model is adjusted for Basic + SES + Activities model and serum 25(OH)D levels and BMI of the child.

with 25(OH)D only). The mediating effect of 25(OH)D was higher for ethnicity, but additional adjustment for 25(OH)D or BMI did not alter significance of the association between ethnicity and myopia (with BMI only OR 1.40 $P = 0.12$; with 25(OH)D only OR 1.31 $P = 0.23$).

DISCUSSION

This study, which was performed in a multi-ethnic cohort in a densely populated area of the Netherlands, found environmental risk factors to be major determinants of myopia already occurring at the age of 6 years. We also found a higher frequency of myopia in children from families with low income, low maternal education, and non-European ethnicity. Adjustment of these socio-economic risk profiles for environmental factors caused the association to disappear, indicating a mediating effect of lifestyle.

Surprisingly, young children from families with a non-European ethnic background and/or a low socioeconomic status appear to be more often myopic in Rotterdam. To demystify the underlying causal structure of the profiles, we conceptualized the potential mediating pathways and ranked them in a conceptual framework (supplemental figure 1). We considered living circumstances such as housing as more distal mediators,

Table 3 Association between education and family income versus risk of myopia, and attenuation of the risk by mediators using various models

	Basic model	**Basic + SES model**	**Basic + SES + Activities model**	**Basic + SES + Activities + 25(OH)D, BMI model**
Determinant	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
High education	Ref	Ref	Ref	Ref
Low education	2.27	1.84	1.48	1.40
	(1.57 – 3.28)	(1.25 – 2.71)	(0.98 – 2.21)	(0.92 – 2.12)
Attenuation	–	-34%	-63%	-69%
		(-59 – -17)	(-98 – -41)	(-109 – -45)
High income	Ref	Ref	Ref	Ref
Low income	2.62	1.99	1.58	1.47
	(1.84 – 3.74)	(1.34 – 2.95)	(1.04 – 2.39)	(0.96 – 2.25)
Attenuation	–	-39%	-64%	-71%
		(-66 – -17)	(-96 – -41)	(-104 – -46)

Values are OR (95% CI) and represent risk of myopia.

P values are corrected for age and gender; *P* values <0.05 are shown in bold.

Attenuations represents the attenuations of effect estimates for ethnicity, low maternal education and low income relative to model 1 after adjustment for the mediators in model 2 -4 (100 x (B model 1 – B model with mediators) / (B model 1)).

Basic model is adjusted for age and gender.

Basic + SES model is adjusted for basic model and marital status and rental home.

Basic + SES + Activities model is adjusted for Basic + SES model and outdoor activities – nearwork activities and participation in sports.

Basic + SES + Activities + 25(OH)D, BMI model is adjusted for Basic + SES + Activities model and serum 25(OH)D levels and BMI of the child.

and daily activities such as near work and outdoor exposure as more proximal mediators. Children from families with a non-European ethnic background and/or a low socioeconomic status appeared to spend more time performing indoor activities, and to have less compensation by outdoor exposure, participated less in sports, had more often lower vitamin D levels, higher BMI and were living more often in rental houses than children from more advantaged families. We enriched the model step by step with these factors in the mediation analysis, and the decomposition of the increased risk was most profound when all factors were taken into account. Consequently, this suggests that the risk profiles based on education, and income,[97,174,175,182-184] do not cause myopia, but represent certain living conditions and habits that are more directly involved in the pathogenesis of myopia. Our findings add to the growing bulk of evidence that daily activities of children are an important cause of myopia,[22] and specifically shows that these activities also underlie associations with ethnic background and socioeconomic status.

The myopia profiles found in this study may be specific for young children growing up in a big city in Europe. As daily habits change with age, the profiles may be modified as the children grow older. They may also remain in the same direction, because awareness of lifestyle risks may become greater in the highly educated parents. This was also the case for the association between socioeconomic status and smoking after the discovery of its detrimental health effects.[185,186]

Strength and limitations

Strengths of this study were the prospective design which decreased the chance of selection bias, the mix of ethnicities, and the wide range of variables available for analysis. Limitations were the relatively low number of myopes due to the young age of the children and the limited number of covariates that could added to the mediation analysis. Another limitation was the lack of data on parental myopia, a well-known myopia risk factor, and incomplete data in some of the variables which were not randomly distributed. To avoid selection bias, we applied the Fully Conditional Specification method to replace missing variables, a widely accepted method for imputation.[179]

The myopia prevalence in the 6-year-olds of our study was somewhat higher than in Australian children of comparable age (1.5%),[22] but much lower than in 7 year old Chinese children (6.7%).[9] We found an increased risk of myopia in non-European, more specific in Dutch-Antillean or Surinamese and Moroccan ethnicity. Our group of children with an Asian descent was small, which hampered direct comparison to other ethnic comparison studies. Multi-ethnic studies with large number of Asians usually estimate the highest prevalence of myopia in the Asians, even at very young ages.[187]

CONCLUSION

In summary, this study in a large cohort of young children living in Western Europe, demonstrated an important role for lifestyle in the development of myopia at a young age. Risks for socio-economic groups should be deconstructed and deciphered into living circumstances and daily activities. For clinicians and researchers in the field of myopia, it is important to bear in mind that socio-economic risk groups may differ between populations, but that mediators proximal to the trait are likely to remain the same. The more proximal risk factors can be modified at an individual level by with an increasing the level of outdoors activity in children, or as a population intervention with more time dedicated to outdoor exposure at schools.

SUPPLEMENTAL MATERIAL

Supplemental Table 1 Subdivision of ethnicities within subgroups per geographic region (N=5,711)

	Number	% of subgroup	% of total
European	3,855		67.5
Dutch	3,366	87.3	58.9
European	440	11.4	7.7
American Western	38	1.0	0.7
Oceania	11	0.3	0.2
Non-European			
Turkish	430	100	7.5
Moroccan	293	100	5.1
Surinamese and Dutch Antilles	571		10.0
Dutch Antilles	171	29.9	3.0
Surinamese - Creole	161	28.2	2.8
Surinamese - Hindustani	167	29.2	2.9
Surinamese - Unspecified	72	12.6	1.3
Other	562		9.8
Cape Verdean	162	28.8	2.8
Other African	122	21.7	2.1
Indonesian	29	5.2	0.5
Asia – Western	5	0.9	0.1
Asia – non-Western	152	27.0	2.7
American – non-Western	92	16.4	1.6

Values are absolute numbers or percentages.

Supplemental Table 2 Demographic characteristics of study participants in Generation R with respect to ethnicity (N=5,711)

	All N=5,711	**European N = 3,947**	**Non-European N =1,764**	***P*-value**
Child characteristics				
Age (years)	6.16 (0.5)	6.11 (0.4)	6.28 (0.6)	<0.001
Sex, female (%)	49.9 (2,849)	50.3 (1,984)	49.0 (865)	0.41
BMI (kg/m2)	16.2 (1.8)	16.0 (1.5)	16.7 (2.3)	<0.001
Myopia (%)	2.4 (137)	1.5 (61)	4.3 (76)	<0.001
Social economic factors				
Maternal education, low (%)	46.1 (2,635)	35.2 (1,389)	70.6 (1,246)	<0.001
Income, low (%)	34.4 (1,965)	20.4 (807)	65.6 (1,158)	<0.001
Habituation				
Rental home (%)	41.8 (2,386)	29.0 (1,145)	70.4 (1,241)	<0.001
Married/Registered partnership (%)	66.9 (3,820)	68.9 (2,721)	62.3 (1,099)	<0.001
Activities daily life				
Playing outdoors (hr/day)	1.57 (1.1)	1.67 (1.1)	1.32 (1.1)	<0.001
Biking to school (hr/day)	0.049 (0.08)	0.058 (0.09)	0.029 (0.07)	<0.001
Walking to school (hr/day)	0.086 (0.11)	0.068 (0.10)	0.125 (0.14)	<0.001
Watching television (hr/day)	1.45 (1.07)	1.21 (0.8)	1.98 (1.4)	<0.001
Computer use (hr/day)	0.35 (0.46)	0.29 (0.4)	0.49 (0.6)	<0.001
Outdoor – nearwork (hr/day)	-0.10 (1.7)	0.30 (1.49)	-0.99 (1.94)	<0.001
Vitamin D (nmol/L)	67 (30)	74 (30)	50 (25)	<0.001
Participation in sports (%)	44.1 (2,518)	49.4 (1,951)	32.1 (567)	<0.001

Values are means (SD), or percentages (absolute numbers).

P values are calculated using chi squared test for categorical variables and student-t test or Mann Withney U test for continues variables. Data missing on BMI (<1%), rental house (21%), marital status (9,4%), playing outdoors (24%), biking to school (12%), walking to school (12%), watching television (16%), computer use (17%), vitamin D (36%), and participation in sports (9,8%).

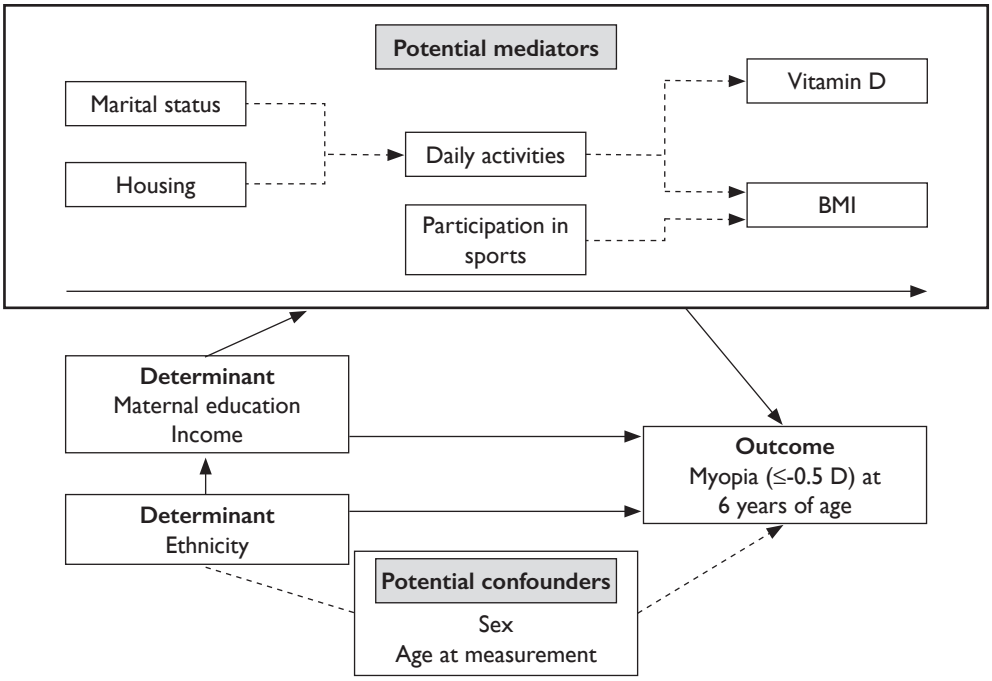
Supplemental Table 3 Demographic characteristics of study participants in Generation R with respect to maternal education and family income (N=5,711)

	Low education N = 2,635	High education N = 3,076	*P*-value	Low income N = 1,965	No low income N = 3,746	*P*-value
Child characteristics						
Age (years)	6.25 (0.6)	6.08 (0.4)	<0.001	6.29 (0.6)	6.09 (0.4)	<0.001
Sex, female (%)	50.0 (1,318)	49.8 (1,532)	0.46	49.2 (967)	50.2 (1,882)	0.24
BMI (kg/m2)	16.5 (2.1)	15.9 (1.5)	<0.001	16.5 (2.1)	15.9 (1.6)	<0.001
Social economic factors						
Maternal education, low (%)	–	–	–	76.4 (1,502)	30.2 (1,113)	<0.001
Income, low (%)	57.0 (1,502)	15.1 (463)	–	–	–	–
Habituation						
Rental home (%)	57.8 (1,523)	28.0 (863)	<0.001	70.9 (1,394)	26.5 (992)	<0.001
Married/Registered partnership (%)	62.5 (1,646)	70.7 (2,173)	<0.001	51.3 (1,007)	75.1 (2,812)	<0.001
Activities daily life						
Playing outdoors (hr/day)	1.58 (1.3)	1.56 (1.0)	0.54	1.45 (1.2)	1.63 (1.1)	<0.001
Biking to school (hr/day)	0.04 (0.08)	0.06 (0.09)	<0.001	0.04 (0.08)	0.06 (0.08)	<0.001
Walking to school (hr/day)	0.11 (0.13)	0.06 (0.09)	<0.001	0.12 (0.13)	0.07 (0.10)	<0.001
Watching television (hr/day)	1.81 (1.3)	1.14 (0.7)	<0.001	1.89 (1.3)	1.22 (0.82)	<0.001
Computer use (hr/day)	0.46 (0.5)	0.27 (0.4)	<0.001	0.45 (0.5)	0.30 (0.4)	<0.001
Outdoor – nearwork (hr/day)	-0.54 (2.0)	0.34 (1.4)	<0.001	-0.74 (2.0)	0.24 (1.5)	<0.001
Vitamin D (nmol/L)	50 (30)	72 (30)	<0.001	55 (28)	73 (30)	<0.001
Participation in sports (%)	34.7 (915)	52.1 (1,603)	<0.001	31.5 (620)	50.7 (1,898)	<0.001

Values are means (SD), or percentages (absolute numbers).

P values are calculated using chi squared test for categorical variables and student-t test or Mann Withney U test for continues variables. Data missing on BMI (<1%), rental house (21%), marital status (9,4%), playing outdoors (24%), biking to school (12%), walking to school (12%), watching television (16%), computer use (17%), vitamin D (36%), and participation in sports (9,8%).

Supplemental Figure 1 Conceptual framework of mediators in the association between ethnicity, maternal education, and family income versus risk of myopia



CHAPTER 8

LOW SERUM VITAMIN D IS ASSOCIATED WITH AXIAL LENGTH AND RISK OF MYOPIA IN YOUNG CHILDREN

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ABSTRACT

Objective: To investigate the relationship between serum 25(OH) D levels and axial length (AL) and myopia in 6-year-old children.
Methods: A total of 2,666 children aged 6 years participating in the birth-cohort study Generation R underwent a stepwise eye examination. First, presenting visual acuity (VA) and AL were performed. Second, automated cycloplegic refraction was measured if Logmar VA >0.1. Serum 25-hydroxyvitamin D (25(OH) D) was determined from blood using liquid chromatography/ tandem mass spectrometry. Vitamin D related SNPs were determined with a SNP array; outdoor exposure was assessed by questionnaire. The relationships between 25(OH)D and AL or myopia were investigated using linear and logistic regression analysis.
Results: Average 25(OH)D concentration was 68.8 nmol/L (SD ± 27.5; range 4 - 211); average AL 22.35 mm (SD ± 0.7; range 19.2 – 25.3); and prevalence of myopia 2.3% (n=62). After adjustment for covariates, 25(OH)D concentration (per 25 nmol/L) was inversely associated with AL (β -0.043; *P* <0.01), and after additional adjusting for time spent outdoors (β -0.038; *P* <0.01). Associations were not different between European and non-European children (β -0.037 and β -0.039 respectively). Risk of myopia (per 25 nmol/L increase) was OR 0.65 (95% CI 0.46 – 0.92). None of the 25(OH)D related SNPs showed an association with AL or myopia.
Conclusion: Lower 25(OH)D concentration in serum was associated with longer AL and a higher risk of myopia in these young children. This effect appeared independent of outdoor exposure and may suggest a more direct role for 25(OH)D in myopia pathogenesis.

INTRODUCTION

In the last decades, the prevalence of myopia has increased dramatically in Asia as well as in the Western world.[72,99,188] Prevalence estimates are now around 2% in 6 year old children with European ethnicity, and 12% in children of Asian descent.[189,190] These figures rise to 50% in young European adults[50] and up to 96% in students from South Korea.[98] Although myopic refractive error can be corrected optically by glasses, contact lenses, or refractive surgery, the longer axial length (>26 mm) increases the life-time risk of severe visual impairment and blindness due to retinal complications.[18] The basis of myopia is a developmental mismatch between the optical components of the eye,[10,112] of which excessive elongation of axial length (AL) in early youth is the most important.[191]

The need to unravel the etiology of myopia and develop preventive measures is urgent from a public health perspective. Associations with genetic risk variants[38,174] and environmental factors such as time spent outdoors[22,23,192] and education[174,189] have been well established.[193,194] Recent studies reported an association with serum 25-hydroxy vitamin D (25(OH)D) concentration and myopia in adolescents.[195,196] Whether this reflects the association between outdoor exposure and myopia, or whether vitamin D itself plays a role in the pathophysiology is unclear. Studies investigating the potential relation with vitamin D receptor (VDR) polymorphisms found no consistent relationships.[197,198]

Serum 25(OH)D is derived from multiple sources. Cholecalciferol (vitamin D3) is formed in the skin after sunlight exposure, and also absorbed by the gut after dietary intake of e.g. fatty fish. Ergocalciferol (vitamin D2) results from intake of foods containing yeasts and fungi.[199,200] Both precursors are hydroxylated in the liver into 25(OH)D. Its active metabolite 1,25(OH)2D is formed after transformation in the kidney[201] and is distributed to other sites of the body thereafter. In non-supplemented individuals, sunlight exposure is thought to be the main determinant of 25(OH)D.[200,202-204] The main function of 1,25(OH)2D is regulation of calcium and phosphate metabolism in bone tissue and plasma, but it also has metabolic functions in insulin metabolism.[205,206] In neuronal disease such as cognitive decline and Parkinson disease,[207,208] it can be involved in immune responses[209] and in DNA transcription and methylation.[210,211] Whether 1,25(OH)2D has a direct effect on eye growth is currently unclear.

The aim of this study was to investigate the association between 25(OH)D levels, AL, and the risk of myopia in children at age 6 years in a large population-based study. Additionally, influence of time spent outdoors on these relationships, and vitamin D related genotypes was studied.

METHODS

Study population

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands.

The complete methodology has been described elsewhere.[45,46]A total of 4,154 children underwent an ophthalmologic examination by trained nurses at the research center at age 6 years and underwent blood withdrawal for serum measurements. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20), and written informed consent was obtained from all participants. Research was conducted according to the declaration of Helsinki.

Assessment of AL and myopia

The examination included a stepwise ophthalmological examination. Step 1 consisted of monocular visual acuity with LogMAR based LEA-charts at 3 meter distance by means of the ETDRS method, and ocular biometry including AL (mm) was measured by Zeiss IOL-master 500 (Carl Zeiss MEDITEC IOL-master, Jena, Germany) per eye; five measurements were averaged to a mean AL.[212] Step 2 was carried out in children with a LogMAR visual acuity of >0.1 in at least one eye and in children wearing prescription glasses, and included performance of automated cycloplegic refraction (Topcon auto refractor KR8900 (Topcon, Japan)) and a complete ophthalmologic work up by an ophthalmologist. Two drops (three in case of dark irises) of cyclopentolate (1%) were administered at least 30 minutes before refractive error measurement. Pupil diameter was ≥6 mm at time of the measurement. Spherical equivalent (SE) was calculated as the sum of the full spherical value and half of the cylindrical value in accordance with standard practice, and myopia was defined as SE ≤-0.5D in at least one eye. Children with LogMAR visual acuity ≤0.1, no glasses or ophthalmic history were classified as non-myopic.[176,213]

Assessment of 25(OH)D

At a median age of 6.0 y (95% range 5.6–7.9), nonfasting blood samples were drawn by antecubital venipuncture and stored at -80°C until analysis. Serum samples were collected in all children on the examination day at the research center. The measurements of 25(OH)D (nmol/L) in the samples (110μmL serum per sample) were DEQAS certified and were conducted at the Endocrine Laboratory of the VU University Medical Center, Amsterdam, The Netherlands between July 2013 and January 2014.[214] Serum 25(OH) D was measured with the use of isotope dilution online solid phase extraction liquid chromatography-tandem mass spectrometry, the 'gold standard' (LC-MS/MS)[215] using a deuterated internal standard (IS: 25(OH)D3- d6) (Synthetica AS, Oslo, Norway). This method is highly sensitive and has been widely used in 25(OH)D studies.[216,217] The limit of quantitation was 4.0 nmol/L; intra-assay CV was <6%, and interassay CV was <8% for concentrations between 25 and 180 nmol/L.

Questionnaire

Each mother completed a questionnaire regarding the daily life activities of their child. Time spent playing outdoors and time spent watching television was obtained using questions such as "how much time does your child spend outdoors/watching television in the morning/afternoon/evening". Questions were asked for weekdays and weekend days separately, and answers were multiple choice (never, 0 – ½ hour, ½ – 1 hour, 1 – 2 hours, 2 – 3 hours, 3 – 4 hours). Total time spent in a week was summed and divided by seven to make an average hours/day.

Genotyping of SNPs in Vitamin D pathway

Samples were genotyped using Illumina Infinium II HumanHap610 Quad Arrays following standard manufacturer's protocols. Intensity files were analyzed using the Beadstudio Genotyping Module software v.3.2.32, and genotype calling based on default cluster files. Any sample displaying call rates below 97.5%, excess of autosomal heterozygosity (F<mean-4SD) and mismatch between called and phenotypic gender were excluded. Genotypes were imputed for all polymorphic SNPs from phased haplotypes in autosomal chromosomes using the 1000 Genomes GIANTv3 panel. SNPs located in genes involved in the Vitamin D metabolic pathway were studied for association with AL and presence of myopia; i.e., genes determining serum 25(OH)D levels (GC, DHCR7, CYP2R1), a gene involved in activation of serum 25(OH)D (CYP27B1), the vitamin D receptor gene (VDR), and the gene involved in deactivation of 1,25-(OH)2D in mitochondria (CYP24A1). A total of 33 SNPs[197,218,219] were tested, and analyses were adjusted for multiple testing using Bonferroni adjusted *P*-value 0.05/33, $P=0.0015$.

Measurement of covariates

Height and weight of children were measured by trained nurses, and BMI (weight/height^2) was calculated. Age was determined at the time of the visit. Income was obtained using the questionnaire and was clustered in low income (lowest tertile) and higher income. If income at the time of the visit was not available, income at birth was used. Ethnicity was obtained in the questionnaire, according to standardized criteria employed by 'Statistics Netherlands', the official national statistics agency,[121] concerning the country of birth of parents and child: (1) if both parents were born in the Netherlands, the ethnicity is Dutch; (2) if one of the parents was born in another country than the Netherlands, that country was considered country of birth; (3) if both parents were born in the same country other than the Netherlands, that country was represented; (4) if the parents were born in different countries outside the Netherlands, then the country of the mother was represented; and (5) if that child and both parents were born in different countries outside the Netherlands, the country of birth of the child was represented. Ethnicity was grouped into European and non-European. To adjust for sea-

sonality, four seasons were formed on basis of the month in which the children participated in the study (Winter: December – February, Spring: March – May, Summer: June – August, Autumn: September – November).

Statistical analysis

Separate analyses were performed for AL and myopia. Differences in covariates between myopia and children without myopia were tested using logistic regression analysis adjusting for potentially confounding effects of age and gender. The relation between 25(OH) D and AL was investigated using multivariable linear regression analysis; the relation with myopia (SE ≤-0.5D) was analyzed using multivariable logistic regression analysis, Covariates were only added to the model if they were significantly related with the outcome as well as with 25(OH)D. Three models were tested: model 1 only adjusted for age and gender; model 2 for age, gender, BMI, ethnicity, television watching, family income, and season visiting the research center; model 3 additionally adjusted for time spent playing outdoors. Effect estimates were determined per 25 nmol/L 25(OH)D. Beta's are presented with SE; Odds Ratios (ORs) with 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version 21.0 for Windows software (SPSS Inc).

RESULTS

Demographics

A flow diagram presenting the selection of children for the current analysis is shown in Supplement Figure 1. A total of 2,666 children were available for analysis of serum Vitamin D and myopia; 2,636 children were available for analysis of serum 25(OH)D and AL. Demographic characteristics are presented in Table 1. Children with myopia were on average somewhat older. Adjusted for age and height, girls had smaller AL than boys but not a lower frequency of myopia. Myopic children had a higher BMI, watched more television, and spent less time outdoors. Myopia occurred more frequently in children of non-European ethnicity.

Serum 25(OH)D

The average serum 25(OH)D in the total study population was lower than the optimal level of 75 nmol/l.[199] Only 37.2% (1,023) of the children reached this optimal level; these were mostly (41.1%) children who had been examined in summer time (Table 2). Figure 1 shows an inverse relation between serum 25(OH)D and AL for the entire population ($P < 0.001$). Most myopic children had high AL and low serum 25(OH)D levels; only 18% (11/62) of myopic children reached serum levels which corresponded to the optimal level.

Table 1 Demographic characteristics of study participants in Generation R (N=2,666)

	All N = 2,666	No myopia N = 2,604	Myopia N = 62	*P*-value
Characteristics				
Age (years)	6.12 (0.44)	6.12 (0.44)	6.28 (0.65)	0.001
Sex, female (%)	49.1 (1,308)	49.1 (1,278)	48.4 (30)	0.99
BMI (kg/m2)	16.09 (1.71)	16.07 (1.69)	16.86 (2.14)	0.005
Low family income (%)	28.0 (747)	27.5 (715)	51.6 (32)	<0.001
Axial length (mm)	22.35 (0.7)	22.33 (0.7)	23.14 (0.86)	<0.001
Ethnicity (%)				
European	75.5 (2,013)	76.3 (1,986)	56.5 (35)	<0.001
Non-European	24.5 (653)	23.7 (618)	43.5 (27)	
Activities daily life				
Time spent outdoors (hr/day)	1.59 (1.14)	1.60 (1.14)	1.16 (0.96)	0.003
Watching television (hr/day)	1.34 (0.99)	1.33 (0.97)	1.83 (1.48)	0.001

Values are means (SD), or percentages (absolute numbers).

P values are corrected for age, gender, height in logistic regression.

Table 2 Average serum 25(OH)D (nmol/L) per season in myopic and non-myopic children

Serum 25(OH)D concentration (nmol/L)	N	All	No myopia	Myopia
Child				
All seasons	2,666	68.8 (27.5)	69.2 (27.4)	50.2 (24.1)
Spring	751	60.8 (21.7)	61.3 (21.6)	42.5 (17.5)
Summer	693	84.2 (28.4)	84.4 (28.4)	69.2(22.6)
Autumn	686	72.9 (26.8)	73.1 (26.8)	63.3 (24.7)
Winter	536	54.7 (23.0)	55.3 (22.9)	36.8 (19.7)

Values are means (SD).

P values are corrected for age, gender, height. *P* values <0.05 are shown in bold.

Figure 1 Distribution of axial length as a function of serum level of 25(OH)D

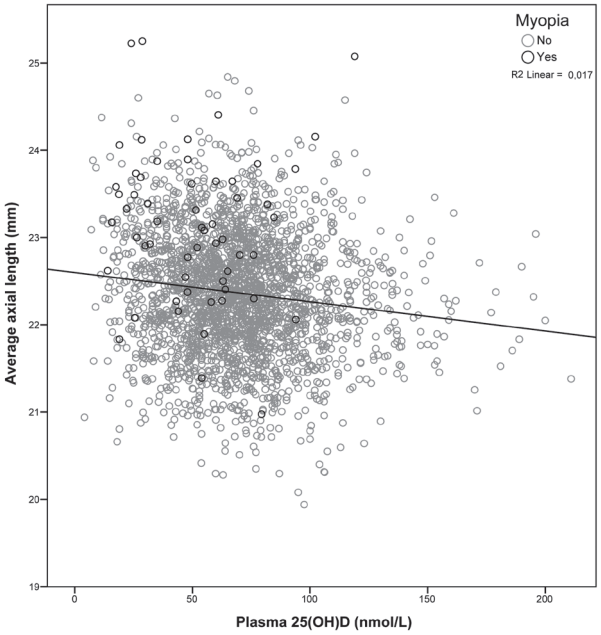


Table 3 shows associations between serum 25(OH)D and AL and myopia. Lower serum levels were associated with higher AL and higher risks of myopia. The estimates remained statistically significant after adjustment for covariates. The effect between serum 25(OH)D and AL remained (beta -0.033 (SE 0.012; *P* 0.02)) after exclusion of myopic children. The association was similar in children of European and non-European descent, but the association with AL in the relatively small non-European group failed to reach statistical significance.

Search for possible explanations

We hypothesized that our findings could be explained by outdoor exposure. Figure 2 shows the positive relation between time spent outdoors and serum 25(OH)D (Pearson, $P = <0.001$). Independent of serum 25(OH)D, time spent outdoors (hr/day) was a risk factor for AL (beta -0.034 (SE 0.012; *P* 0.003). It was not a significant risk factor for myopia (OR 0.81 (95% CI 0.61 – 1.07), possibly due to the small number of myopes. The association between serum 25(OH)D and AL and myopia remained significant after adjustment for time spent outdoors (model 3). We explored possible interactions as well, but there was no significant interaction effect between 25(OH)D, ethnicity or income. Additionally, the association was tested separately in the small subgroup with missing data on time spent outdoors. The effect was similar to the effect in the group with data.

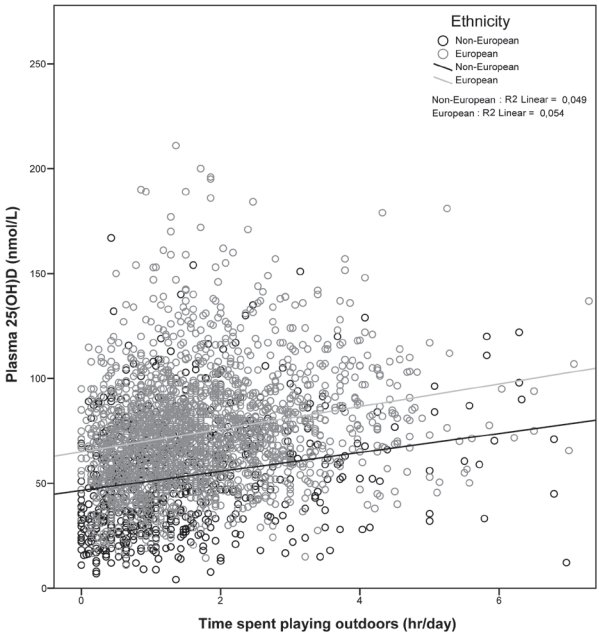
To investigate a possible genetic association between Vitamin D and eye growth, we studied genes incorporated in the Vitamin D pathway. We considered single nucleo-

Table 3 Multivariate regression analysis of the association between 25(OH)D and axial length and myopia in children at age 6 years

	Model 1: Age and sex adjusted model		Model 2: Multivariate model excluding outdoor exposure		Model 3: Multivariate model including outdoor exposure	
	Association	***P***	**Association**	***P***	**Association**	***P***
Axial length (mm), beta (SE) of association with 25(OH)D, per 25 nmol/L						
	N=2,636		N=2,636		N=2,636	
All participants	-0.054 (0.012)	<0.001	-0.043 (0.014)	0.002	-0.038 (0.014)	0.007
European ethnicity	-0.051 (0.014)	<0.001	-0.043 (0.016)	0.006	-0.037 (0.016)	0.02
Non-European ethnicity	-0.034 (0.027)	0.20	-0.043 (0.030)	0.16	-0.039 (0.031)	0.20
Myopia, OR (95% CI) of association with 25(OH)D, per 25 nmol/L						
	N=2,666		N=2,666		N=2,666	
All participants	0.47 (0.35 – 0.62)	<0.001	0.63 (0.45 – 0.89)	0.008	0.65 (0.46 – 0.92)	0.01
European ethnicity	0.61 (0.39 – 0.95)	0.02	0.69 (0.42 – 1.11)	0.13	0.71 (0.44 – 1.16)	0.17
Non-European ethnicity	0.56 (0.37 – 0.85)	0.006	0.59 (0.37 – 0.95)	0.03	0.61 (0.38 – 0.98)	0.04

The multivariate model for axial length includes adjustment for model 1 and BMI, season of blood withdrawal, ethnicity, television watching, family income. The multivariate model for myopia includes adjustment for model 1 and BMI, ethnicity, television watching, education mother. Outdoor exposure indicates time spent outdoors.

Figure 2 Distribution of serum level of 25(OH)D as a function of time spent outdoors



tide polymorphisms (SNPs) in genes that determine serum 25(OH)D levels, in genes involved in activation of serum 25(OH)D, in the vitamin D receptor gene (VDR), and in the gene involved in deactivation of 1,25-$(OH)_2D_3$ in mitochondria (CYP24A1) (supplemental table 1). One SNP (rs2245153) in the CYP24A1 gene showed a significant association with AL (beta 0.039; *P* 0.04) and myopia (OR 1.55; 95% CI 1.04 – 2.31), 2 SNPs in CYP24A1 (rs4809959 beta 0.032; *P* 0.04 and rs3787557 beta 0.046; *P* 0.04) and one in the VDR (rs11568820 beta -0.042; *P* 0.03) only showed a significant association with axial length. *P*-values were all insignificant after adjustment for multiple testing.

DISCUSSION

In this study children with lower serum levels of 25(OH)D had longer AL (Beta -0.038 per 25 nmol/l), and those with higher 25(OH)D had a lower risk of myopia (OR 0.65; 95% CI 0.46 – 0.92 per 25nmol/L). The association remained significant after adjusting for outdoor exposure, indicating that these two closely related determinants may have some overlapping as well as separate effects on the development of myopia. Genetic variants in the vitamin D pathway genes appeared not to be related: although SNPs in the VDR and CYP24A1 genes showed some association with AL and myopia, this did not remain after adjustment for multiple testing.

Strength and limitations

Our study had strengths and weaknesses. Assets were the particularly large study sample, the inclusion of the combination of measurements of AL and myopia, and the correction for many potential confounders. The young age of our study population was a benefit as well as a potential drawback. It allowed for measurements of the determinant very close to the onset of myopia, leaving less room for confounding bias. On the other hand, it hampered the study of large effects as most children did not develop excessive eye growth yet. There were other drawbacks. We performed cycloplegia only in children with a diminished visual acuity. Reports show that our cut off value of LogMAR VA of >0.1 had a 97.8% sensitivity to diagnose myopia.[176,213] We therefore think that our approach did not substantially affect the number of myopes in our study, nor biased the observed associations. Finally, as the correlation between serum 25(OH)D level and time playing outdoors was relatively low in our study, our questionnaire may not have fully assessed all time spent outdoors. Not all participants filled in the questionnaire completely and data on time spent outdoors was partially missing. However, association in the sample of children without data on time spent outdoors was similar to the association in those with complete data.

Vitamin D and axial length

A novel finding of our study was that the increase in AL in children with low 25(OH) D was already present in the physiological range of refractive error, before the onset of myopia. This implies that Vitamin D has a continuous effect on AL, and not only determines the development of myopia. We confirmed that the risk of myopia decreased with increasing 25(OH)D levels (OR 0.65) with each 25 nmol/L. The association between 25(OH)D and axial length was also significant in the European children; but failed to reach significance in the Non-European group due to low statistical power. Correction for time spent outdoors demonstrated some attenuation of the association, but did not explain it entirely. Whether this is due to residual confounding of time spent outdoors or whether Vitamin D is truly causally related with AL and myopia remains an open question. The evidence for a role of time spent outdoors in myopia is available from cross sectional studies, intervention and randomized clinical trials as well as from animal studies.[22,62,192,220] Vitamin D production is triggered by UV-exposure, not by light exposure per se. Animal studies have shown that artificial light, free of UV, can inhibit development of myopia development.[220] This may suggests that outdoor exposure and Vitamin D are independent risk factors for axial elongation and myopia. However, true causality cannot be concluded from a cross sectional study; longitudinal and functional studies are needed to provide more profound evidence.

A few previous studies have investigated the role of serum 25(OH)D in myopia. A South-Korean and an Australian study found a positive association in adolescents and young adults.[195,221] The ALSPAC study found an association with development of refractive error only for $25(OH)D_2$, not for $25(OH)D_3$ in 15 years old children. A potential drawback of this study was the measurement of refraction without any cycloplegia.[222] Mutti et al. found an association between SNPs in the VDR gene and myopia in a smaller study.[197] We could not validate this association, as none of the Vitamin D related SNPs were significant after adjusting for multiple testing.

Potential mechanisms

Various hypotheses underscribe a function of 25(OH)D in eye growth. One theory focusses on Vitamin D in relation to dopamine. The current view is that light exposure initiates the release of dopamine in retinal amacrine cells.[62,223,224] The released dopamine appears to influence the function of gap junctions and the size of receptive fields,[225] an important determinant of eye growth. Vitamin D is known to influence dopamine metabolism in neurological disorders, such as Morbus Parkinson and restless legs syndrome.[226] In particular in Parkinson, Vitamin D protects against cell death in the substantia nigra of the dopamine secreting neuron.[208,227] Increased dopamine metabolism[228] was found in the rat brain under influence of vitamin D. In the developing rat brain, Vitamin D was found to upregulate glial derived neurotrophic factor (GDNF) which increases dopamine neurons.[229] Taken together, Vitamin D appears to strengthen the function of dopamine or dopamine secreting cells in neuronal tissues. Whether this

also accounts for dopamine secreted by amacrine cells in the retina remains an intriguing question.

Another mechanism may be the regulation of DNA transcription in genes containing vitamin D response elements (VDRE, supplemental figure 2). In this case, the active intracellular $1,25(OH)_2D$ binds to VDR binding protein, enters the nucleus, and forms a complex with retinoid X receptor in order to bind to VDRE and initiate transcription. VDREs are located in many genes.[230] It has been shown that retinal cells can metabolize $1,25(OH)_2D$; and this active form of vitamin D may interfere with transcription of genes that promote the myopia signaling cascade.[231]

CONCLUSION

In conclusion, we found that serum levels of 25(OH)D were inversely related to AL, and that low levels increased the risk of myopia. Our data suggest that this relationship may be independent from time spent outdoors. The potential role for 25(OH)D in myopia pathogenesis should be further explored by intervention research and functional studies.

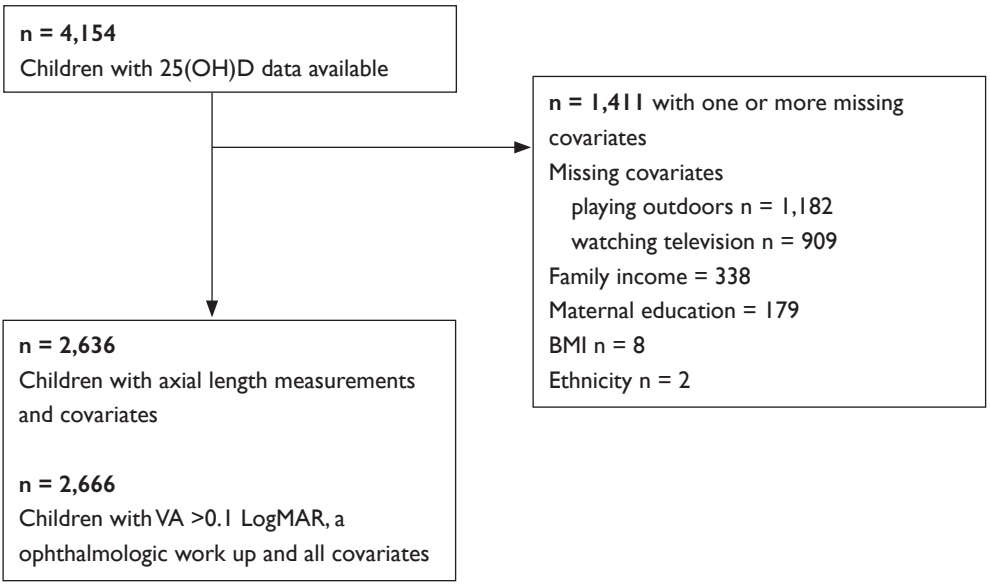
SUPPLEMENTAL MATERIAL

Supplemental Table 1 Association between 25(OH)D related SNPs and axial length and myopia in children at age 6 years

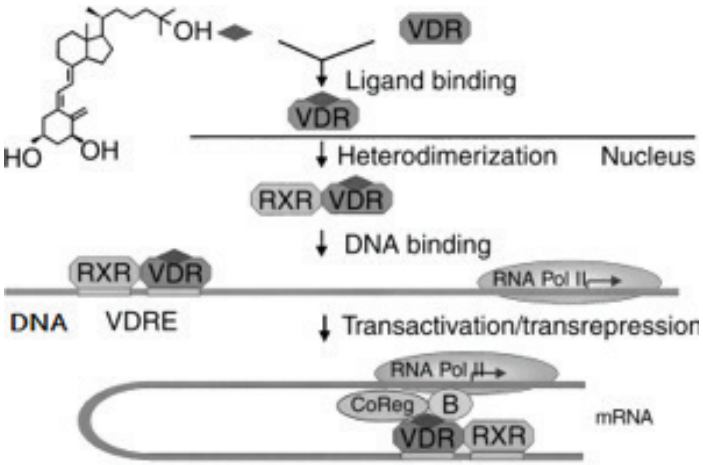
					Axial length (N=3,938)		Myopia (N=3,928)
Gene	rs number	A1	A2	Freq A1	Beta (SE)	*P*	OR myopia (95% CI)
Determinants of serum 25(OH)D level							
GC	rs2282679	T	G	0.76	0.018 (0.019)	0.35	1.01 (0.69 – 1.48)
DHCR7	rs7944926	A	G	0.41	0.001 (0.017)	0.95	0.87 (0.62 – 1.21)
CYP2R1	rs10741657	G	A	0.64	0.010 (0.016)	0.53	0.82 (0.60 – 1.11)
Activation of 25(OH)D							
CYP27B1	rs8176345	C	T	0.97	-0.020 (0.045)	0.65	0.82 (0.34 – 1.96)
CYP27B1	rs4646536	A	G	0.69	0.008 (0.017)	0.65	1.25 (0.89 – 1.77)
Intracellular vitamin D receptor							
VDR	rs7975232 (ApaI)	C	A	0.45	-0.012 (0.016)	0.47	1.10 (0.82 – 1.49)
VDR	rs1544410 (BsmI)	C	T	0.61	-0.026 (0.016)	0.11	1.07 (0.79 – 1.47)
VDR	rs731236 (TaqI)	A	G	0.62	-0.032 (0.016)	0.05	1.11 (0.80 – 1.54)
VDR	rs11568820 (CDX2)	C	T	0.71	**-0.042 (0.019)**	**0.03**	0.71 (0.50 – 1.00)
VDR	rs2228570 (FOK1)	G	A	0.65	0.052 (0.035)	0.14	1.60 (0.78 – 3.28)
VDR	rs2239182	T	C	0.48	-0.024 (0.016)	0.14	1.19 (0.88 – 1.61)
VDR	rs3819545	A	G	0.62	0.027 (0.016)	0.10	0.78 (0.58 – 1.05)
VDR	rs2853559	G	A	0.63	0.001 (0.017)	0.97	0.94 (0.68 – 1.31)
Mitochondrial inactivation of 1,25-(OH)2D3							
CYP24A1	rs2248359	C	T	0.56	0.018 (0.016)	0.25	1.22 (0.90 – 1.65)
CYP24A1	rs6022999	A	G	0.70	0.008 (0.019)	0.65	1.10 (0.77 – 1.58)
CYP24A1	rs2585428	C	T	0.54	0.020 (0.016)	0.19	1.27 (0.95 – 1.72)
CYP24A1	rs2245153	T	C	0.79	**0.039 (0.019)**	**0.04**	**1.55 (1.04 – 2.31)**
CYP24A1	rs2296241	G	A	0.47	0.026 (0.016)	0.10	1.30 (0.97 – 1.75)
CYP24A1	rs4809960	T	C	0.77	0.019 (0.018)	0.29	1.27 (0.87 – 1.87)
CYP24A1	rs4809959	A	G	0.49	**0.032 (0.016)**	**0.04**	1.25 (0.93 – 1.68)
CYP24A1	rs2181874	G	A	0.72	-0.030 (0.018)	0.10	0.98 (0.71 – 1.37)
CYP24A1	rs3787557	T	C	0.87	**0.046 (0.023)**	**0.04**	1.12 (0.70 – 1.81)
CYP24A1	rs3787555	C	A	0.74	0.032 (0.018)	0.08	1.27 (0.88 – 1.82)
CYP24A1	rs3787554	G	A	0.90	0.031 (0.024)	0.25	1.35 (0.76 – 2.39)
CYP24A1	rs4809958	T	G	0.84	0.019 (0.021)	0.38	1.07 (0.70 – 1.62)
CYP24A1	rs2762939	G	C	0.69	-0.012 (0.019)	0.52	0.97 (0.68 – 1.39)
CYP24A1	rs6068816	C	T	0.89	-0.004 (0.026)	0.87	1.00 (0.62 – 1.62)
CYP24A1	rs6127118	G	A	0.79	0.010 (0.022)	0.65	1.08 (0.70 – 1.68)
CYP24A1	rs2209314	T	C	0.76	-0.010 (0.021)	0.63	1.07 (0.70 – 1.64)
CYP24A1	rs1570669	A	G	0.63	0.009 (0.017)	0.59	1.35 (0.97 – 1.86)
CYP24A1	rs927650	C	T	0.65	0.004 (0.016)	0.79	0.74 (0.55 – 1.01)
CYP24A1	rs2762934	G	A	0.80	-0.018 (0.020)	0.36	1.04 (0.71 – 1.52)
CYP24A1	rs6097807	A	G	0.72	0.013 (0.018)	0.49	1.38 (0.98 – 1.96)
CYP24A1	rs6068810	G	T	0.95	0.025 (0.035)	0.47	0.90 (0.48 – 1.71)

25(OH)D level. Values are in increase in AL (mm) from linear regression models and odds ratios for myopia (95% confidence interval) from logistic regression models. Models are adjusted for age, gender and 10 principal components. A1 is allele 1 and A2 is allele 2. *P* values <0.05 are shown in bold.

Supplemental figure 1 Flowchart participants in analysis of 25(OH)D and axial length at age 6 years



Supplemental figure 2 Vitamin D receptor and DNA transactivation/transrepression



VDR: Vitamin D receptor, RXR: retinoïd X receptor. 1,25(OH)2D signaling is through the VDR in the nucleus. It forms a heterodimer with the RXR receptor. On the DNA strand this complex binds to the VDRE at the promotor of many genes. This results in transactivation of transrepression of genes. (adapted from http://www.nature.com/ki/journal/v63/n85s/full/4493809a.html).

CHAPTER 9

ENVIRONMENTAL RISK FACTORS CAN REDUCE AXIAL LENGTH ELONGATION AND MYOPIA INCIDENCE IN 6 TO 9 YEAR OLD CHILDREN

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ABSTRACT

Objective: Identify risk factors for axial length (AL) elongation and incident school myopia.
Design: Population-based prospective birth-cohort study.
Subjects: 4,734 children examined at age 6 and 9 years from the Generation R study in Rotterdam, The Netherlands
Methods: AL and corneal radius (CR) were measured with an IOL-master 500 and daily life activities and demographic characteristics were obtained by questionnaire. 3,362 (71%) children were eligible for cycloplegic refractive error measurements. Linear regressions models on AL elongation were used to create a risk score based on the regression coefficients from environmental and ocular factors. The predictive value of the prediction score for myopia (≤-0.5D) was estimated using receiver operation characteristics. To test if regression coefficients differed for baseline AL/CR ratio, interaction terms were calculated with baseline AL/CR ratio and environmental factors.
Main outcomes: AL elongation and incident myopia.
Results: From age 6 to age 9, average AL elongation was 0.21(±0.009) mm/year and 223/2136 (10.4 %) developed myopia, leading to a myopia prevalence at 9 years of 12.0%. Seven parameters were independently associated ($P < 0.05$) with faster AL elongation: parental myopia, ≥1 books read per week, time spent reading, no participation in sports, non-European ethnicity, less time spent outdoors and baseline AL/CR ratio. The discriminative accuracy for incident myopia based on these risk factors was 0.78. AL/CR ratio at baseline showed statistically significant interaction with books read per week ($P < 0.01$) and parental myopia ($P < 0.01$). Almost all predictors showed the highest association with AL elongation in the highest quartile of AL/CR ratio; incidental myopia in this group was 24% (124/513).
Conclusion: Determination of a risk score can help identify schoolchildren at high risk of myopia. Our results suggest that behavioral changes can offer protection particularly in these children.

INTRODUCTION

Myopia (nearsightedness) is a common refractive error that is reaching epidemic proportions worldwide.[50,72,91,98,232,233] Concomitantly with the myopia boom, high myopia (spherical equivalent (SE) ≤-6D) has also burgeoned,[50,72,98,234] which is worrisome as the underlying excessive axial elongation increases the risk of maculopathy, glaucoma, and other myopia-related complications leading to blindness later in life.[18,114] Current prevalence estimates of high myopia are already reaching 7-10% among 14-16 year olds in East-Asia, and these children often had their onset of myopia at school age or before.[106,234] Identifying risk factors for eye growth at a young age may help characterize children at risk for whom lifestyle advice and interventions could be beneficial.[48,60,65]

Many studies have identified risk factors that are associated with an increased risk of myopia in children.[22,59,129,187,235] Several follow up studies have prospectively investigated risk factors to assess their contribution to the onset of myopia, and found the best predictive value for baseline SE and ocular biometry.[236,237] Up to now lifestyle factors, such as time spent outdoors, did not appear to have additional predictive value, potentially as differences in SE are the result of previous behavioral patterns. In addition, the number of environmental risk factors studied was limited.[238] Nonetheless, this is remarkable, as it is becoming more and more clear that an important cause of the myopia rise in the world is the changing lifestyle in school children.[22,59,129,187,235] This is also unfortunate, because in contrast to baseline ocular parameters, lifestyle factors can be modified.

In this study, we investigated the effect of a large set of variables measured in children at the age of six years on axial length eye growth, refractive error, and onset of myopia at age 9 years. We calculated the predictive value of ocular and non-ocular factors, and evaluated the risk of incident myopia for various risk profiles.

METHODS

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands. The complete methodology has been described elsewhere.[45,46] Briefly, a total of 9,778 pregnant women were included in the study, and children were born between April 2002 and January 2006. The children were invited at age 6 and 9 years for examination at the research center. Of the initial cohort, 6,690 (68.4%) children participated in the physical examination at 6 years of age and 5862 (60.0%) participated at 9 years. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20), and written informed consent was obtained from all participants. Research was conducted according to the declaration of Helsinki.

Ocular biometry at 6 and 9 years was measured by Zeiss IOL-master 500 (Carl Zeiss MEDITEC IOL-master, Jena, Germany). For axial length (AL) five measurements per eye were averaged to a mean AL. Three measurement of the cornea curvature (K1 and K2)

were taken of right and left eye, and were averaged to a mean corneal radius of curvature (CR). AL/CR ratio, a measurement highly related to SE, was calculated by dividing AL (mm) by CR (mm).[239] Axial elongation was calculated in mm/year ((AL 9 years – AL 6 years) / (age at 9 years – age at 6 years)). At 9 years, 1.5 year after the start of this follow up round the IRB approved the installation of cycloplegic eye drops, and automated cycloplegic refractive error was introduced (Topcon auto refractor KR8900 (Topcon, Japan)). Two drops (three in case of dark irises) of cyclopentolate (1%) with 5 minutes interval were administered at least 30 minutes before refractive error measurement. Pupil diameter was ≥6 mm at the time of the measurement. Spherical equivalent (SE) was calculated as the sum of the full spherical value and half of the cylindrical value in accordance with standard practice. Myopia was defined as SE ≤-0.5D.

At age 6 years, the method of automated cycloplegic refractive error was performed in children with a LogMAR visual acuity of >0.1 with LogMAR based LEA-charts at 3 meter distance by means of the ETDRS method[212] in at least one eye or in children with an ophthalmologic history to identify children with myopia. Children with LogMAR visual acuity ≤0.1, no glasses or ophthalmic history were classified as non-myopic at 6 years.[176,213] Myopia incidence was the proportion of all new cases of myopia in children without myopia at the first visit who underwent cycloplegic refractive error at 9 years of age and had axial length measurements at both ages.

Predictor variables

Each mother completed a questionnaire at 6 years regarding the daily life activities of their child. Time spent playing outdoors was obtained using questions such as "how much time does your child spend outdoors" separately for the morning, afternoon and evening for both weekdays and weekend days. Answers were multiple choice (never, 0 – ½ hour, ½ – 1 hour, 1 – 2 hours, 2 – 3 hours, 3 – 4 hours). Total time spent in a week was summed and divided by seven to make an average hours/day. Computer and television use was processed likewise as time spent outdoors. Maternal education was defined according to statistics Netherlands and categorized in primary and secondary or higher education. Income was obtained using the questionnaire and was clustered in low income (lowest tertile, <2400 euros/month) and higher income. As proxy for ethnicity country of birth of the parents was obtained and grouped into European and non-European. At 6 years reading habits were not assessed, at 9 years questions about books read per week (<1 or 1≥ per week), time spent reading (> 5 hours/week), interval duration of reading (≥30 minutes), reading distance (<30 cm or ≥30 cm) and parental myopia were asked. Child height and weight were measured at 6 years of age without shoes and heavy clothing, BMI (kg/m^2) of children was calculated. 25(OH)D (25-hydroxy vitamin D) was measured using the 'golden standard' liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. Birth parameters and gestational age were obtained using medical records and hospital registries. SDS for weight for gestational age were calculated according to Northern European growth Standards.[120]

Statistical methods

Univariate associations between candidate predictors and myopia were tested using logistic regression. Univariate and multivariate associations between candidate predictors and axial elongation were tested using linear regression models. A relatively high proportion of the environmental determinants had missing values. Parental myopia (39%), reading habits (32%), and 25(OH)D (36%) had the highest rate of missing values. Time spent outdoors was missing for 24% of the cohort. Other predictors had <20% missing values. To avoid any bias due to missing candidate predictors, Fully Conditional Specification, an iterative of the Markov Chain Monte Carlo approach was used for imputation. Multivariable linear regression was performed with backward selection to select combinations of predictor variables. All variables with a *P*-value <0.05 in univariate analysis were tested in a multivariate analysis. All variables with a *P*-value <0.05 in the multivariate analysis were added to the prediction model. AL/CR ratio and time spent outdoors were categorized in the prediction model. We tested interaction effect with AL/CR ratio at baseline by adding multiplicative interaction terms with the environmental risk factors. AL/CR ratio was divided into four quartiles to compare regression coefficients between groups with increasing myopic SE. To identify the predictive value of the risk factors, independent of the ocular measurements at baseline, we used the quartile specific beta's of the significant associated factors in the complete sample to calculate a prediction score in the two highest quartiles.

A prediction score was created by multiplying regression coefficients by 100. Calibration of the model was evaluated with the Hosmer-Lemeshow $\chi 2$ statistic and the final model's ability to discriminate between myopic and non-myopic children was assessed by using the area under the curve in the receiver operating characteristic curve. Analyses were performed in SPSS (version 21.0).

RESULTS

General characteristic

A total of 4734 children, 50.7% girls, had ocular biometry measurements at both 6.0 (±0.5) and 9.8 (±0.3) years of age (Figure 1). Despite a difference in eye size, the increase in axial length was not different between boys and girls (P = 0.95), and averaged 0.21 mm/year (SD 0.09). Children with myopia at the last visit had significantly greater axial elongation compared to non-myopic participants (0.34 vs 0.19 mm/year; *P* <0.001).

Cycloplegic measurements of refractive error were introduced 1.5 year after the start of the examinations at age 9. After that time point, 2464/3362 (73%) children had reliable measurements of SE at age 9 (Figure 1); they did not differ significantly in AL/CR ratio from children who refused cycloplegia (2.970 vs 2.966; *P* = 0.32). SE at age 9 was +0.73 D (SD 1.29) on average. Myopic children at 9 years were more often from low socio-economic families, non-European descent, had more often myopic parents, spent less time outdoors, read more books, and had higher AL and AL/CR ratio at 6 years (Table 1).

Table 1 Demographic characteristics of the study population and the risk of myopia at 9 years of age

	All children with refractive error data N = 2464	Myopia at 9 years N = 287	OR (95%CI) of school myopia	*P*-value
Characteristics				
Age (years)	6.00 (0.32)	6.02 (0.37)	1.45 (0.99 – 2.11)	0.05
Sex, female (%)	50 (1236)	53 (152)	1.14 (0.89 – 1.45)	0.31
BMI (kg/m2)	16.2 (2.0)	16.3 (2.1)	1.05 (0.98 – 1.12)	0.18
Low family income (%)	31 (762)	45 (129)	2.00 (1.47 – 2.72)	<0.001
Low education mother (%)	45 (1106)	56 (159)	1.62 (1.23 – 2.13)	0.001
Vitamin D (nmol/L)	68 (29)	60 (28)	0.99 (0.98 – 0.99)	<0.001
Myopic parents (0 – 2)				
No myopic parent (%)	41 (1017)	31 (88)	ref	–
1 myopic parent (%)	38 (938)	40 (115)	1.51 (0.98 – 2.32)	0.06
2 myopic parents (%)	21 (509)	29 (84)	2.18 (1.38 - 3.44)	0.002
Gestational age (weeks)	39.8 (2)	39.6 (2)	0.94 (0.89 – 1.00)	0.06
Birthweight (kg)	3.4 (0.6)	3.4 (0.6)	0.83 (0.67 – 1.03)	0.09
Weight for gestational age (SD)	-0.06 (1.0)	-0.09 (1.0)	0.95 (0.84 – 1.08)	0.46
Activities daily life				
Time spent outdoors (hr/day)	1.6 (1.1)	1.3 (0.95)	0.78 (0.64 – 0.95)	0.02
Watching television (hr/day)	1.4 (1.0)	1.6 (1.1)	1.16 (1.02 – 1.31)	0.02
Computer use (hr/day)	0.3 (0.4)	0.4 (0.5)	1.29 (0.95 – 1.74)	0.10
No participating in sports (%)	57 (1394)	64 (183)	1.40 (1.05 – 1.88)	0.03
Books read (≥1/week)	43 (1066)	50 (144)	1.38 (1.00 – 1.90)	0.05
Time spent reading (>5hr/week)	37 (923)	40 (115)	1.13 (0.80 – 1.59)	0.48
Continuous nearwork (>30 min)	16 (382)	21 (61)	1.54 (1.03 – 2.31)	0.04
Reading distance (<30 cm)	47 (1175)	56 (160)	1.44 (1.12 – 1.68)	0.01
Ocular biometry				
Axial length (mm)	22.3 (0.72)	22.7 (0.78)	2.18 (1.81 – 2.64)	<0.001
AL/CR ratio (per 0.01 increase)	2.87 (0.07)	2.94 (0.08)	1.21 (1.18 – 1.24)	<0.001
Ethnicity (%)				
Non-European	30 (749)	53 (151)	2.95 (2.30 – 3.80)	<0.001

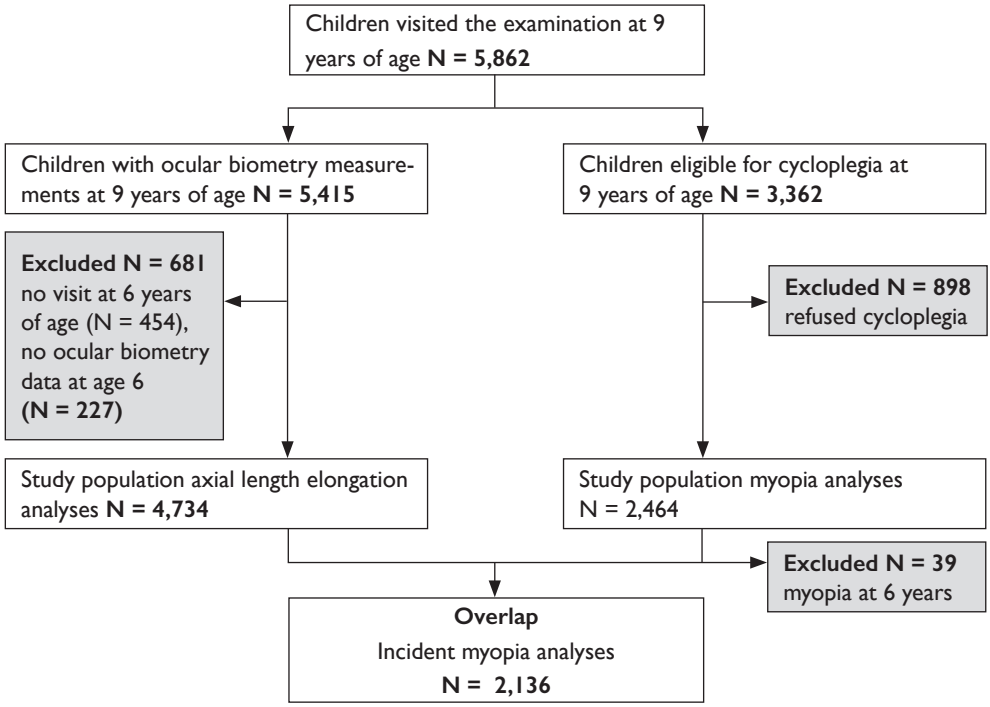
All numbers and odds ratios refer to the imputed dataset in children with cycloplegic refractive error available (n =2464).

OR, Odds ratio. All variables were measured at 6 years of age except books read, time spent reading, continues nearwork and reading distance.

Values are means (SD), or percentages (absolute numbers).

P-values are unadjusted *p*-values of logistic regression models.

Figure 1 Flowchart showing distribution of study population



Children with at least one predictor variable missing (n = 3192) were less likely to be from European descent (67% vs 81%), have a mother with high education (56% vs 66%) and to come from high income families (69% vs 78%). To prevent results based on selection bias, we imputed data to the total cohort of 4734 children. No large differences in regression coefficients were found between the results for axial length elongation in the imputed and non-imputed dataset (Supplemental Table 1).

Risk factors for axial eye growth

In the univariate analysis, greater axial elongation was associated with a younger age of the participant, low family income, non-European descent, lower 25(OH)D levels, one or two myopic parents, less time spent outdoors and sport participation, more computer use and time spent reading, and increased AL or AL/CR ratio at age 6. In the multivariate analyses, the predictors one or two myopic parents, less time spent outdoors, no participation in sports, more books read per week, more time spent reading, an increased AL/CR ratio at baseline, and ethnicity remained significantly associated with increased axial elongation (Table 2). AL at baseline was not taken into account in the multivariate model, as this measure was highly correlated with AL/CR ratio. The regressions coefficients of the predictors were not significantly different in a model with and without AL/CR ratio in the model (Supplemental Table 2).

Table 2 Univariate and multivariate regression analysis of the coefficients (standard deviation) between axial elongation between 6 and 9 years of age and potential predictors

	Model 1 Association axial elongation (mm/year)	***P*-value model 1**	**Model 2 Association axial elongation (mm/year)**	***P*-value model 2**
Characteristics at 6 years				
Age (years)	**-0.010 (0.003)**	**<0,001**	**-0.021 (0.003)**	**<0.001**
Sex, female	0.000 (0.002)	0,95	0.002 (0.002)	0.35
BMI (kg/m2)	0.001 (0.001)	0.88	–	–
Low family income	**0.008 (0.003)**	**0.006**	0.001 (0.004)	0.82
Low education mother	0.001 (0.003)	0.71	–	–
Vitamin D (/20 nmol/L)	**-0.002 (0.001)**	**0.006**	0.000 (0.000)	0.77
Myopic parents (0 – 2)				
No myopic parent	Ref		Ref	–
1 myopic parent	**0.014 (0.003)**	**0.001**	**0.11 (0.003)**	**0.002**
2 myopic parents	**0.026 (0.005)**	**<0.001**	**0.19 (0.004)**	**<0.001**
Gestational age (weeks)	-0.001 (0.001)	0.17	–	–
Birthweight (g)	0.000 (0.000)	0.52	–	–
Size for gestation (SDS)	0.000 (0.001)	0.98	–	–
Environmental risk factors				
Time spent outdoors (hr/day)	**-0.005 (0.001)**	**0.004**	**-0.003 (0.001)**	**0.007**
Watching television (hr/day)	0.002 (0.001)	0.10	–	–
Computer use (hr/day)	**0.007 (0.003)**	**0.03**	0.002 (0.003)	0.46
No sports participation	**0.010 (0.003)**	**<0.001**	**0.008 (0.002)**	**0.001**
Books read per week (1>)	**0.021 (0.003)**	**<0.001**	**0.013 (0.003)**	**<0.001**
Time reading at 9 years (>5 hours)	**0.017 (0.003)**	**<0.001**	**0.012 (0.003)**	**0.001**
Continuous reading at 9 years (≥30 min)	0.008 (0.005)	0.12	–	–
Reading distance at 9 years (<30 cm)	0.007 (0.003)	0.08	–	–
Ocular biometry at 6 years				
Axial length (mm)	**0.024 (0.002)**	**<0.001**	–	–
AL/CR ratio (mm/mm)	**0.332 (0.016)**	**<0.001**	**0.32 (0.016)**	**<0.001**
Ethnicity (%)				
Non-European	**0.015 (0.003)**	**<0.001**	**0.010 (0.03)**	**0.001**

Model 1 is adjusted only for age and gender; model 2 is also adjusted for significant variables from model 1.

Prediction of myopia

A total of 2175 children had complete ocular biometry data at 9 years and cycloplegic refractive error. At baseline, 39 (1.8%) were diagnosed with myopia at age 6, and were discarded in further risk analyses. Myopia incidence was 223/2136 (10.4%) between the two visits. A total of 2136 children were included in the prediction analyses.

A prediction score for each child was calculated by the sum of the beta's of the multivariate association multiplied by 100 (Table 3). The score ranged from 0 – 19.4, with a mean of 5.5 (SD 3.0). Total prediction score was associated with axial elongation (β 0.010; SD 0.0004; $P < 0.001$). Each point increase of the score had an OR 1.43 (95% CI 1.35 – 1.52) of incident myopia. The area under the curve (AUC) of the uncategorized prediction score was 0.78; without ocular biometry this was 0.65 (Figure 2). The model was well calibrated according to the Hosmer-Lemeshow *P*-value of 0.67. Supplemental table 3 reports sensitivity, specificity, positive predictive values and negative predictive values at the different cutoff values. Of the 571 children with a predictor score of ≤3.5, only 8 (1.4%) developed myopia between 6 and 9 years of age. In contrast, 54% (27/50) of the children with a score of ≥11 developed myopia in this age period (Figure 3). The prediction score (AUC 0.79) in study participants with complete data (83 cases and 959 controls) was comparable to the data set including imputed data (AUC 0.78). The prediction of axial eye growth between 6 – 9 years old for incident myopia was highest with an AUC of 0.85.

Figure 2 ROC curve for prediction of incident myopia between 6 and 9 years of age. Receiver operation characteristic: cutoff values of prediction score are reported as dots in the curve. The area under the receiver operating characteristic curve (C-index) for the categorized score is 0.78 for the prediction score, 0.75 for the AL/CR ratio only, and 0.63 for the risk score without ocular biometry

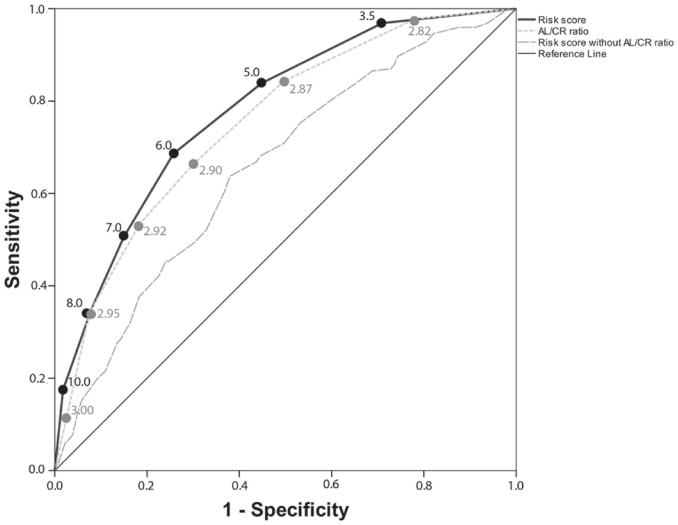


Table 3 Multivariate prediction model for axial elongation

Predictor variables	Complete model (β, (95%CI))	Assigned points for prediction score
Characteristics at 6 years		
Myopic parents (0 – 2)		
No myopic parent	Ref	0
1 myopic parent	0.012 (0.005 – 0.019)	1.2
2 myopic parents	0.019 (0.010 – 0.028)	1.9
Environmental factors		
Time spent outdoors (<2hr/day)	0.005 (0.000 – 0.011)	0.5
No sports participation	0.008 (0.003 – 0.013)	0.8
Books read per week	0.012 (0.006 – 0.018)	1.2
Time spent reading at 9 years (>5 hours)	0.012 (0.006 – 0.018)	1.2
Ocular biometry at 6 years		
AL/CR ratio		
<=2.80	Ref	0
2.80 – 2.85	0.008 (0.000 – 0.016)	0.8
2.85 – 2.90	0.019 (0.011 – 0.027)	1.9
2.90 – 2.95	0.034 (0.026 – 0.042)	3.4
2.95 – 3.00	0.055 (0.046 – 0.065)	5.6
>3.00	0.128 (0.114 – 0.142)	12.8
Ethnicity (%)		
Non-European	0.010 (0.004 – 0.016)	1.0
Total		19.4
Hosmer-Lemeshow (*P*-value)		0.67
Area under the Curve		0.78

Model is adjusted for potential confounding effects of age and gender.

Points calculated based on regression coefficients (regression coefficient multiplied by a factor 100). Individual prediction score can be calculated by using the following equation: Individual score = 1.2 × one myopic parent (1 myopic parent = 1, no or two myopic parents = 0) + 1.9 * two myopic parents (two myopic parents = 1, no or one myopic parent = 0) + 0.5 * time spent outdoors (<2 hours a day = 1, ≥2 hours a day = 0) + 0.8 * sport participation (no = 1, yes = 0) + 1.2 * books read per week (1 = ≥ 1/week, 0 = <1/week) + 1.2 * time spent reading (1 = ≥5 hours/week, 0 = <5 hours/week) + 0 to 12.8 * AL/CR ratio category (1 = category, 0 = other category) + 1.0 * ethnicity (1 = non-European, 0 = European).

Figure 3 The proportions of children with incident myopia and children who remained non-myopic based on the risk score for axial length elongation

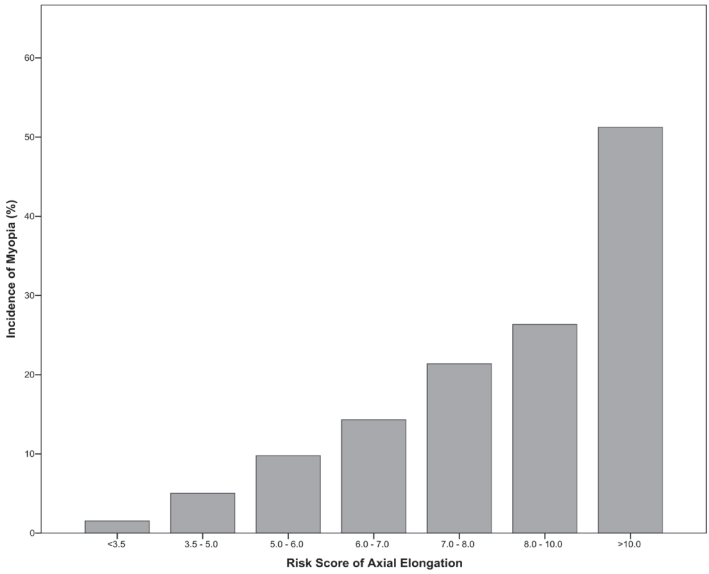
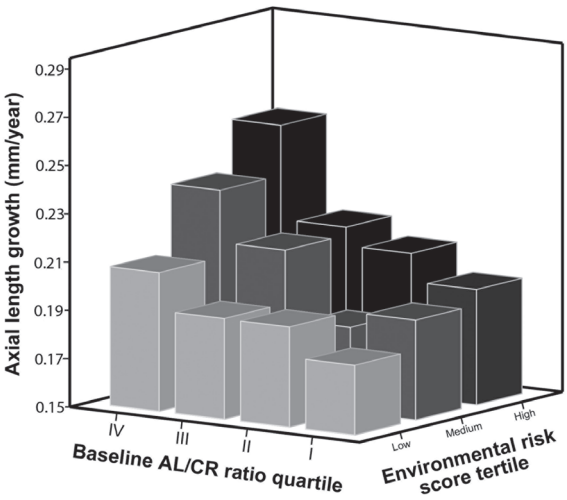


Figure 4 Axial length elongation in relation to baseline AL/CR ratio and environmental risk factors



Baseline AL/CR ratio were divided into quartiles. Environmental prediction score were based on beta's of time spent outdoors, sports participation, books read per week and time spent reading in table 3 and divided into tertiles.

Table 4 Multivariate prediction models for axial elongation per quartile of AL/CR ratio at baseline

Quartile of AL/CR ratio at baseline	**I**	**II**	**III**	**IV**
Predictor variables	β	β	β	β
Characteristics at 6 years				
Myopic parents (0 – 2)				
No myopic parent	Ref	Ref	Ref	Ref
1 myopic parent	**0.019 (0.002 – 0.036)**	**0.013 (0.002 – 0.024)**	0.010 (-0.005 – 0.025)	0.005 (-0.006 – 0.015)
2 myopic parents	**0.037 (0.019 – 0.054)**	**0.024 (0.011 – 0.037)**	0.010 (-0.004 – 0.024)	0.007 (-0.015 – 0.028)
Activities daily life				
Time spent outdoors (<2hr/day)	0.010 (-0.010 – 0.030)	0.004 (-0.006 – 0.015)	-0.003 (-0.012 – 0.006)	0.008 (-0.001 – 0.018)
No sports participation	0.009 (-0.003 – 0.022)	**0.012 (0.003 – 0.022)**	**0.011 (0.002 – 0.019)**	0.002 (-0.006 – 0.015)
Books read per week (>1/wk)	**0.023 (0.008 – 0.0038)**	**0.011 (0.001 – 0.022)**	0.007 (-0.005 – 0.018)	0.006 (-0.006 – 0.018)
Time spent reading (>5 hr/wk)	0.013 (-0.003 – 0.029)	0.010 (-0.001 – 0.021)	**0.016 (0.003 – 0.023)**	0.009 (-0.001 – 0.018)
Reading distance (<30 cm)	**0.026 (0.008 – 0.044)**	0.002 (-0.008 – 0.012)	-0.003 (-0.005 – 0.012)	-0.004 (-0.020 – 0.011)
Ethnicity (%)				
Non-European	**0.025 (0.011 – 0.038)**	0.004 (-0.006 – 0.015)	0.006 (-0.004 – 0.015)	0.009 (-0.001 – 0.018)
Incidental myopia % (N)	24 (124 /513)	11 (60/546)	6 (31/540)	1 (8/537)
Hosmer-Lemeshow (*P*-value)	0.48	0.39	–	–
Area under the Curve*	0.66	0.64	–	–

*Calculated based on the prediction score with quartile specific regression coefficients. The AL/CR ratio in group I was ≥2.916, in group II 2.876 - 2.916, group III 2.833 - 2.876 and group IV ≤2.833.

Effects in children with high values of AL/CR ratio at baseline

To test if predictors were independent of AL/CR ratio at baseline, we tested multiplicative interaction terms. AL/CR ratio at baseline showed statistical significant interaction with parental myopia (P <0.01), books read per week (P <0.01), reading distance (P 0.04), ethnicity (P <0.01) and the environmental risk score (<0.001; Figure 4). The multivariate analyses were repeated in a stratified analysis of the four quartiles of baseline AL/CR ratio (Table 4). All predictors except for sports participation showed the highest association with AL elongation in the highest quartile of AL/CR ratio; incidental myopia in this group was 24% (124/513).

DISCUSSION

In this study, we identified ocular as well as environmental factors risk factors for axial eye growth. By combining these risk factors, we calculated a prediction score for myopia onset between 6 and 9 years of age, and found a predictive value of 0.78. Axial length elongation had the highest predictive value for onset of myopia with an AUC of 0.85. Environmental factors were significantly associated with both increase in AL and incident myopia, and had the greatest effect in children with the highest quartile of AL/CR ratio at baseline, suggesting that this group of children may benefit the most from behavioral and lifestyle interventions.

Previous studies

The values for eye growth are lower in the current European study than those estimated in Singapore with children of comparable age. Average eye growth in Singapore was 0.30 mm/year, and likewise, myopia had a higher incidence.[240]

Algorithms to predict the development of myopia have been reported previously, predominantly in young children.[238,241] These previous studies reported that those with low values of refractive error but still emmetropic had the highest risk of incident myopia. Our predictive value of axial length growth was comparable to their predictive value of baseline refractive error, as well as the predictive value of a model including only non-ocular data resulting in AUC 0.63 compared to the model of the CLEERE study including only non-ocular factors (UAC 0.58 – 0.68). However, the other studies did not find an additional effect for environmental factors. In this study the highest effect of environmental factors was found for those children with the highest risk of myopia. Based on these factors the AUC was almost 0.70 in this group, suggesting that these children have the most benefit of lifestyle changes.

Strengths and limitations

Strengths of this study are the large sample size, longitudinal nature of the data, the homogeneous group of children and the wide variety of predictors in this study. Using axial length growth allowed us to study a continuous phenotype and the entire spectrum of the trait and detect more subtle changes than merely the dichotomous myopia, but some limitations have to be discussed. There is a potential selection bias in response to cycloplegia. Children with dark irises were more often non-responders to cyclopentolate (1%) and more often non-European children. This response was probably not related to the current SE within this group, and therefore only affected power to detect an association with non-European ethnicities. Another limitation is that baseline ocular biometry and refractive error as a predictor of incident myopia have the disadvantage that these factors are not only a result of genetic variation or susceptibility for eye growth, but also reflect previous risk behavior. This may result in an underestimation of the profit that can be gained by behavioral change. Ideally the same participants were used for the axial elongation analysis as well as for the myopia analysis, but due to later implementation of cycloplegia this was not possible. The reading habit measurements were not measured at baseline, but are important behavioral factors and for this reason we added them to the model.

Interpretation of the results

This prediction score has the highest validity in urban children with AL and refractive error in the normal range before the age of ten years. Eye growth is highest in the first years of life. At birth the average AL is 17.3 mm, which increases to 22.3 mm at 6 years, 23.1 mm in 9 years old and 23.5 mm in the current adult population, which might become higher as result of the cohort effect.[52,242] Nonetheless, the decrease in eye growth rate with increasing age will lower the validity of the prediction score in children below six or above ten years of age.

Hence, the prediction score is most suitable for primary or secondary prevention by detecting children at risk for developing high myopia. Two studies described the effect of time spent outdoors on myopia development. More time spent outdoors during class recess has a positive effect in a two school comparison as well as in a randomized controlled trial.[60,192] Furthermore, an experimental glass classroom has been developed to investigate the effect of more light during school hours, but results needs to be awaited.[243] According to our results, interventions are especially beneficial for the high-risk group. Based on the expected eye growth, additional other options for secondary prevention are available for inhibiting progression of myopia.[244] Orthokeratology decreases eye growth by 30 – 50% and atropine 1% can even decrease progression by 75%.[245,246]

CONCLUSION

The risk score developed by this study helps identify schoolchildren at high risk of myopia. Future applications in schoolchildren may initiate behavioral changes and other interventions that delay myopia onset and reduce the risk of high myopia.

SUPPLEMENTAL MATERIAL

Supplementary Table 1 Effect and p-values in the imputed vs the non-imputed datasets

	Imputed dataset	*P*-value model 1	Non imputed dataset	*P*-value
Characteristics at 6 years				
Age (years)	**-0.010 (-0.003)**	**<0.001**	**-0.010 (0.003)**	**<0.001**
Sex, female	0.000 (0.002)	0.95	0.000 (0.002)	0.95
BMI (kg/m2)	0.001 (0.001)	0.88	0.001 (0.001)	0.39
Low family income	**0.008 (0.003)**	**0.006**	**0.010 (0.003)**	**0.001**
Low education mother	0.001 (0.003)	0.71	0.000 (0.003)	0.88
Vitamin D (mmol/L)	**-0.0001 (0.00005)**	**0.006**	**-0.0001 (0.00006)**	**0.02**
Myopic parents (0 – 2)				
No myopic parent	Ref		Ref	–
1 myopic parent	**0.014 (0.003)**	**0.001**	**0.17 (0.003)**	**<0.001**
2 myopic parents	**0.026 (0.005)**	**<0.001**	**0.37 (0.005)**	**<0.001**
Gestational age (weeks)	-0.001 (0.001)	0.17	-0.001 (0.001)	0.14
Birthweight (grams)	0.000 (0.000)	0.52	0.000 (0.000)	0.65
Size for gestation (SDS)	0.000 (0.001)	0.98	0.0000 (0.001)	0.99
Environmental risk factors				
Time spent outdoors (hr/day)	**-0.005 (0.001)**	**0.004**	**-0.005 (0.001)**	**<0.001**
Watching television (hr/day)	0.002 (0.001)	0.10	0.003 (0.001)	0.06
Computer use (hr/day)	**0.007 (0.003)**	**0.03**	0.006 (0.003)	0.05
No sports participation	**0.010 (0.003)**	**<0.001**	**0.009 (0.003)**	**<0.001**
Books read per week (1>)	**0.021 (0.003)**	**<0.001**	**0.023 (0.003)**	**<0.001**
Time reading at 9 years (>5 hours)	**0.017 (0.003)**	**<0.001**	**0.019 (0.003)**	**<0.001**
Continuous reading at 9 years (≥30 min)	0.008 (0.005)	0.12	0.008 (0.004)	0.09
Reading distance at 9 years (<30 cm)	0.007 (0.003)	0.08	**0.010 (0.003)**	**0.002**
Ocular biometry at 6 years				
Axial length (mm)	**0.024 (0.002)**	**<0.001**	**0.024 (0.002)**	**<0.001**
AL/CR ratio (mm/mm)	**0.332 (0.016)**	**<0.001**	**0.332 (0.016)**	**<0.001**
Ethnicity (%)				
Non-European	**0.015 (0.003)**	**<0.001**	**0.015 (0.03)**	**<0.001**

Supplementary Table 2 Effect and p-values in the axial length elongation model with and without AL/CR ratio at baseline

	Model without AL/CR ratio at baseline	*P*-value	Model with AL/CR ratio at baseline	*P*-value
Characteristics at 6 years				
Age (years)	**-0.012 (0.003)**	**<0.001**	**-0.021 (0.003)**	**<0.001**
Sex, female	-0.002 (0.003)	0.35	0.002 (0.002)	0.35
Low family income	0.004 (0.004)	0.41	0.001 (0.004)	0.82
Vitamin D (mmol/L)	-0.000 (0.000)	0.42	0.000 (0.000)	0.77
Myopic parents (0 – 2)				
No myopic parent	Ref		Ref	–
1 myopic parent	**0.014 (0.003)**	**<0.001**	**0.11 (0.003)**	**0.002**
2 myopic parents	**0.025 (0.006)**	**0.002**	**0.19 (0.004)**	**<0.001**
Environmental risk factors				
Time spent outdoors (hr/day)	**-0.003 (0.001)**	**0.007**	**-0.003 (0.001)**	**0.007**
Computer use (hr/day)	0.005 (0.003)	0.12	0.002 (0.003)	0.46
No sports participation	**0.008 (0.003)**	**0.004**	**0.008 (0.002)**	**0.001**
Books read per week (1>)	**0.014 (0.003)**	**<0.001**	**0.013 (0.003)**	**<0.001**
Time reading at 9 years (>5 hours)	**0.011 (0.004)**	**0.005**	**0.012 (0.003)**	**0.001**
Ocular biometry at 6 years				
AL/CR ratio (mm/mm)	**–**	**–**	**0.32 (0.016)**	**<0.001**
Ethnicity (%)				
Non-European	**0.012 (0.003)**	**<0.001**	**0.010 (0.03)**	**0.001**

Supplementary Table 3 Accuracy of prediction of myopia for various cut off values of the prediction score

Cut off value	% of positive test results (N)*	Sensitivity	Specificity	Positive predictive value	Negative predictive value
>3.5	73.3 (1565)	96	29	14	99
>5.0	48.4 (1035)	84	56	18	97
>6.0	30.2 (646)	67	74	23	95
>7.0	18.4 (394)	51	85	29	94
>8.0	10.6 (226)	34	92	35	92
>10.0	3.7 (73)	17	98	51	91
>11.0	2.3 (50)	12	99	54	91

*Number (percentage) of the dataset with a score of more than the cut off value. Total N was 2136 children without myopia at 6 years of age.

PART V

GENETIC RISK OF MYOPIA IN CHILDREN

CHAPTER 10

WHEN DO MYOPIA GENES HAVE THEIR EFFECT? COMPARISON OF GENETIC RISKS BETWEEN CHILDREN AND ADULTS

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ABSTRACT

Purpose: Previous studies have identified many genetic loci for refractive error and myopia. We aimed to investigate the effect of these loci on ocular biometry as a function of age in children, adolescents and adults.
Methods: The study population consisted of three age-groups identified from the international CREAM consortium: 5,490 individuals aged <10 years; 5,000 aged 10-25 years; and 16,274 aged >25 years. All participants had undergone standard ophthalmic examination including measurements of axial length (AL) and corneal radius (CR). We examined the lead SNP at all 39 currently known genetic loci for refractive error identified from genome-wide association studies (GWAS), as well as a combined genetic risk score (GRS). The beta coefficient for association between SNP genotype or GRS versus AL/CR was compared across the 3 age groups, adjusting for age, sex, and principal components. Analyses were Bonferroni-corrected.
Results: In the age-group <10 years, 3 loci (*GJD2, CHRNG, ZIC2*) were associated with AL/CR. In the age-group 10-25 years, 4 loci (*BMP2, KCNQ5, A2BP1, CACNA1D*) were associated; and in adults 20 loci were associated. Association with GRS increased with age; $\beta = 0.0016$ per risk allele ($P = 2E\text{-}08$) in <10 years, 0.0033 ($P = 5E\text{-}15$) in 10-25 year-olds, and 0.0048 ($P = 1E\text{-}72$) in adults. Genes with strongest effects (*LAMA2, GJD2*) had an early effect that increased with age.
Conclusion: Our results provide insights on the age span during which myopia genes exert their effect. These insights form the basis for understanding the mechanisms underlying high and pathological myopia.

INTRODUCTION

The prevalence of myopia (nearsightedness) has increased dramatically in developed countries in recent decades.[72,188] Myopia is a complex, multifactorial disease with increasing public health burden due to a strong rise worldwide. In particular high myopia is associated with blinding complications such as myopic macular degeneration, glaucoma and retinal detachment.[18,115,247] High myopia mostly has its onset in early childhood before age 10 years.[53]

The eye's dimensions alter markedly during the peak development phase between birth and the late teenage years, ultimately exerting very strong effects on final refractive error (RE) in later adult life. A complex process called emmetropisation aims to coordinate ocular development, bringing light into clear focus on the retina. Early life myopia is characteristically associated with excessive axial length (AL) increase. This results in a mismatch of the optical effects of the various refractive components of the eye, resulting in a focal point in front of the retina. Such a mismatch can be described by the ratio of AL to corneal radius (CR), AL/CR ratio, which has a high correlation with RE[112,239] and is independent of cycloplegia which may vary between studies.

Various studies have examined the heritability of myopia showing increased risk for first-degree relatives of affected individuals[24,27] and twins.[25,26] Numerous genetic loci that cause familial high myopia (*MYP1-18*) have been discovered using linkage analysis.[29] More recently, genome wide association studies (*GWAS*) in large cohorts have been performed to identify further determinants for REs in the general population. The first single nucleotide polymorphisms (SNPs) identified were near *GJD2*[39] and *RASGRF1*.[40] Later many more loci were found in studies of large populations (CREAM; 23andMe).[42-44]

All previously published refractive error GWAS studies were performed in cohorts enrolling participants aged 25 years and older. We aimed to study the effect size of the 39 GWAS-identified genetic regions associated with refractive error to date, as a function of age.

METHODS

Study specific analysis

We included 18 cohorts from 8 different countries in Europe, Asia and Oceania, with a total of 5,490 children <10 years, 5,000 individuals of 10-25 years, and 16,274 adults, all with phenotypic and genome-wide genotypic data available. Age cut off points were based on prior knowledge regarding eye growth. The eye has the highest growth rate before the age of 10 years, and generally does not grow in axial length after age 25 years.[128] Details on subject recruitment procedures can be found in the supplemental materials (online). Each study participant was genotyped with either an Affymetrix or Illumina SNP array (supplemental table I). All studies were conducted according to the Declaration of Helsinki. The studies were approved by the local review boards. Written, informed consent for the collection and analysis of measurements of all study participants was obtained.

SNPs

A total of 39 SNPs were included in this analysis. The SNPs were selected based on their known association with RE and myopia in the GWAS carried out by CREAM[42] and 23andMe[43](supplementary table 2). An unweighted genetic risk score (GRS) was calculated for each participant by summing the dosage of risk alleles (scale 0-2) for all 39 SNPs. The risk score was normally distributed.

Ocular biometry

The ocular biometry measurements included AL and CR, and the AL/CR ratio was calculated. Multiple measurements of AL and CR were taken of the right eye and left eye, were averaged to calculate a mean AL and CR for each eye. The average AL of both eyes was divided by the average CR of both eyes to calculate the AL/CR ratio. Details of the phenotypic assessment protocols/instruments used in each study can be found in the supplemental material (online).

Meta-analysis

All studies performed linear regression models with each SNP or the GRS as determinants, and the AL/CR ratio as outcome. Analyses were adjusted for the potentially confounding effects of age and gender, and additionally – to account for ancestry differences within the sample – for principal components where applicable. A meta-analysis was performed to estimate the beta effects using an inversed variance weighted fixed effect model with METAL.[248] Meta-analyses were performed in each age stratum separately, and in combined strata of all participants <25 years. Several children measured in TEST (Twins Eye Study Tasmania) and GTES (Guangzhou Twin Eye Study) had follow up measurements at an older age; therefore, only data from the oldest age were used in the combined analysis. In the Asian studies the following SNPs were excluded due to low minor allele frequency (MAF) <0.05 in the Chinese population: rs17428076, rs1656404, rs14165, rs13091182, rs12205363, rs11145465, rs10882165, and rs17183295.

Pathway analysis

Loci with significant effects (P <0.05) were further explored to identify differences in effect of early-onset genes (significant loci identified in groups <10 years, 10-25 years or the combined analysis) and late-onset genes (adult subjects). Data were analysed through the use of QIAGEN's Ingenuity®.

Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) and the online software tool Database for Annotation, Visualization and Integrated Discovery (DAVID).[249,250]

RESULTS

Our study sample of children <10 years comprised 5,490 participants derived from 5 studies; one of European ancestry (TEST), three of Asian ancestry (SCORM, STARS, and Guangzhou Twins), and one of mixed European, African, and Asian ancestry (Generation R). Our sample of individuals aged 10-25 years included 5,000 participants derived from 6 studies; 4 of European ancestry (TEST, ALSPAC, BATS and RAINE), and 2 of Asian (STARS, Guangzhou Twins) ancestry. Our sample of adults >25 years compromised 16,274 participants derived from 10 studies; 9 of European ancestry (Croatia Split, -Kurcula and – Vis study, Gothenburg Health Study, EPIC-Norfolk and the Rotterdam Study I-III), and one Asian study (Nagahama). General characteristics per study are shown in Table I.

Genetic risk score

The genetic risk score was associated with a higher AL/CR ratio even in children aged <10 years (table 2), and this association increased in magnitude with older age. Specifically, AL/CR increased with each age category from 0.0019 (SD 0.0003) per risk allele in children <10 years, to 0.0033 (SD 0.0004) in participants aged 10-25 years, to 0.0051 (SD 0.0003) in adults (Figure 1). Only the adult group showed evidence for heterogeneity (heterogeneity *P*-value 0.0005) between studies, therefore, meta-analyses for this age category were also performed using the random effect model (0.0048; SD 0.0007; supplementary table 3 and 4). The variance explained by the genetic risk score increased from 0.7% in the children aged 6 from the Generation R study, to 3.7% for the adult participants in the RS I-III (Figure 2).

Genetic loci

In children <10 years, 9/39 loci were significant at $P < 0.05$, and 3/39 were significant after correction for multiple-testing for 39 SNPs ($P < 0.00128$). The 3 loci significant after Bonferroni correction were in the vicinity of the genes *GJD2, ZIC2* and *CHRNG*. The 2 nominally-significant loci with the greatest effect size (beta) were close to the CHRNG and PRSS56 genes. The other 5 loci were near *KCNQ5, SHISA6, KCNMA1, BMP2* and *BICC1*. Interestingly, the SNP at the *BMP2* locus had a reversed effect from that observed in adult samples, i.e., the risk allele was associated with a lower AL/CR ratio. In individuals aged 10 – 25 years, 10/39 loci showed nominally significant association with AL/CR ratio, of which 5 survived Bonferroni correction (*BMP2, TOX, KCNQ5, A2BP1* and *CACNA1D*). Five of the 10 SNPs above were already nominal significantly associated with AL/CR ratio in children <10 years (*GJD2, BICC1, ZIC2, BMP2* and *PRSS56*); of the remaining nominally-significant loci, the variant with the greatest effect in 10-25 year-olds was the SNP at the *LAMA2* locus. One variant differed significantly in effect between children <10 years and those aged 10-25 years. This was the SNP at the *BMP2* locus which, as mentioned above, showed an opposite effect to that expected in chil-

Table 1 Participating studies and characteristics stratified per age group

Study	N	AL/CR (SD; range)	Age (SD)	Gender, % Female
Age <10 years				
STARS	207	2.99 (0.150; 2.76 – 3.46)	5.45 (2.11)	47.3
Generation R	3,874	2.87 (0.083; 2.38 – 3.90)	6.18 (0.51)	50.3
SCORM	898	3.02 (0.112; 2.63 – 3.45)	7.48 (0.87)	47.7
TEST	166	2.94 (0.101; 2.65 – 3.25)	7.53 (1.21)	52.4
GTES	345	2.97 (0.100; 2.62 – 3.45)	8.73 (0.79)	50.1
Total	**5,490**			
Age 10-25 years				
STARS	96	3.23 (0.127; 2.95 – 3.60)	12.23 (1.7)	58.3
GTES	699	3.13 (0.147; 2.58 – 3.82)	14.83 (1.2)	52.9
TEST	182	2.99 (0.108; 2.68 – 3.51)	15.16 (4.0)	60.4
ALSPAC	1,996	2.99 (0.099; 2.57 – 3.52)	15.46 (0.3)	53.6
BATS	983	3.03 (0.106; 2.67 – 3.82)	19.07 (3.2)	53.8
RAINE	1,044	3.05 (0.104; 2.63 – 3.54)	20.04 (0.4)	48.9
Total	**5,000**			
Age >25 years				
Nagahama	2,762	3.13 (0.153; 2.62 – 3.86)	52.05 (13.8)	49.0
Croatia-Split	730	3.02 (0.128; 2.38 – 3.90)	52.16 (13.0)	61.2
Croatia Korcula	832	2.99 (0.203; 2.26 – 5.73)	56.62 (13.3)	64.7
Croatia-Vis	573	2.99 (0.121; 2.50 – 3.83)	55.93 (13.8)	60.4
GHS 2	936	3.07 (0.160; 2.50 – 4.01)	59.26 (10.6)	50.0
GHS 1	1,919	3.06 (0.151; 2.30 – 3.88)	60.17 (10.7)	47.1
EPIC-Norfolk	6,051	3.05 (0.146; 2.42 – 3.95)	68.90 (8.0)	54.3
RS I-III	2,471	3.05 (0.143; 2.43 – 3.86)	70.02 (8.8)	53.6
Total	**16,274**			

*GTES= Guangzhou Twin Eye Study, RS I-III = Rotterdam Study I-III, GHS=Gutenberg Health Study.

dren aged <10 years (Figure 3). One of the loci (*TOX*) showed evidence for heterogeneity (supplementary table 3) in effect between study cohorts in the age category 10-25 years (Heterogeneity $P = 0.001$). With random effect model the effect of this SNP decreased to β 0.0062 (SE 0.0073; *P* 0.40) (supplementary table 4). In the combined analysis of all studies <25 years, *BICC1* and *PRSS56* reached Bonferroni adjusted significance; one additional locus (*PDE11A*) showed a nominally significant effect for AL/CR ratio. In adults, 31/39 loci showed a significant effect, of which 19/39 were Bonferroni significant. All loci, except for *ZBTB38* (β -0.0004; SE 0.0019), showed an association in the expected direction (i.e. risk allele associated with a higher AL/CR ratio). As in 10-25 years, one locus significant in adults showed evidence for heterogeneity (LOC100506035); with random effect model this locus lost statistical significance (supplementary table 3 and 4). Figure 3 displays all estimated effect sizes per age group.

Figure 1 Association between genetic risk score and myopia in the three age groups

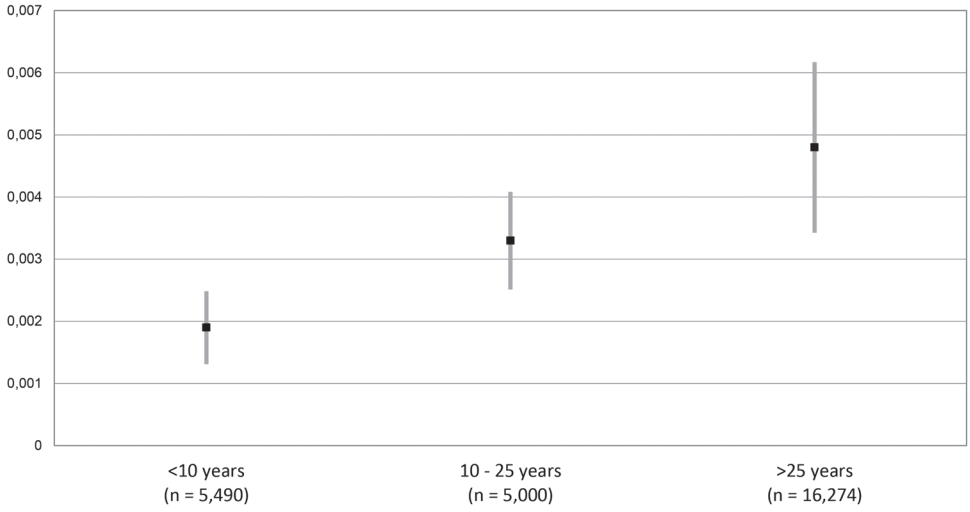
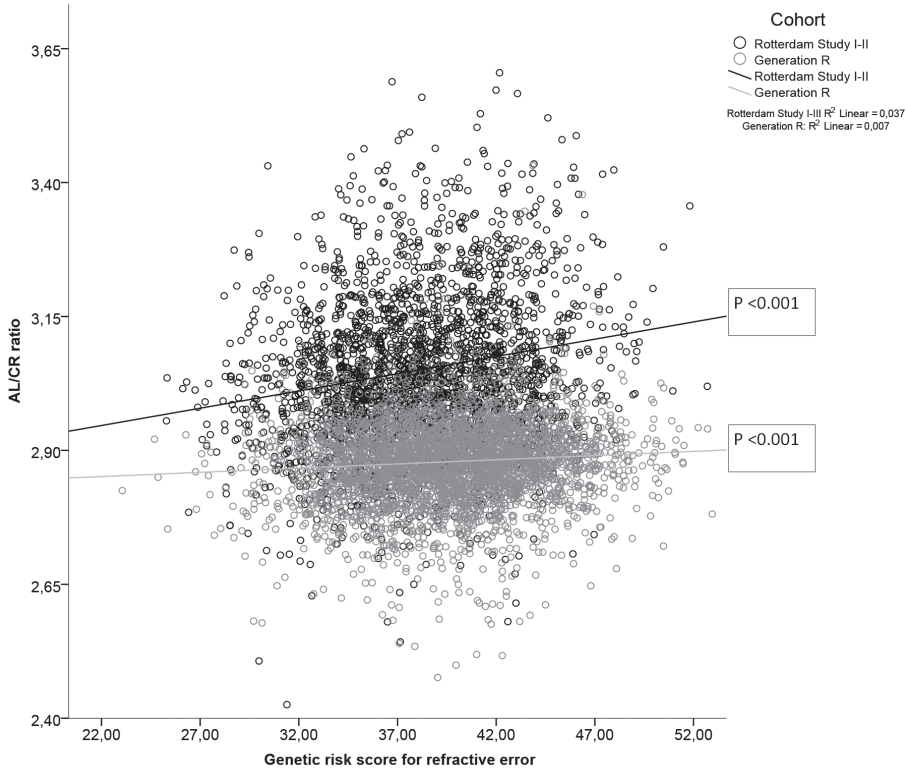


Figure 2 Association between non-weighted genetic risk score and AL/CR ratio in children and adults.



Pathway analysis

Pathway analyses were performed to gain insight into the mechanisms for early versus late-onset eye growth and myopia development. We hypothesized that loci with at least a moderate (nominally significant $P<0.05$) effect in children and adolescents most likely had an early onset. Hence, a locus was defined as early onset when nominally significant ($P<0.05$) in the group<10 years of age or the group 10-25 years and no evidence for heterogeneity (in Figure 4 all loci above the green line). Loci nominally significant in the adult population without a significant effect in the group<10 years of age or the group 10-25 years were grouped as late onset genes (in Figure 4 all loci below the green line). We utilized two types of pathway analysis software.

Figure 3 Increased effect on AL/CR ratio with age for BMP2 gene

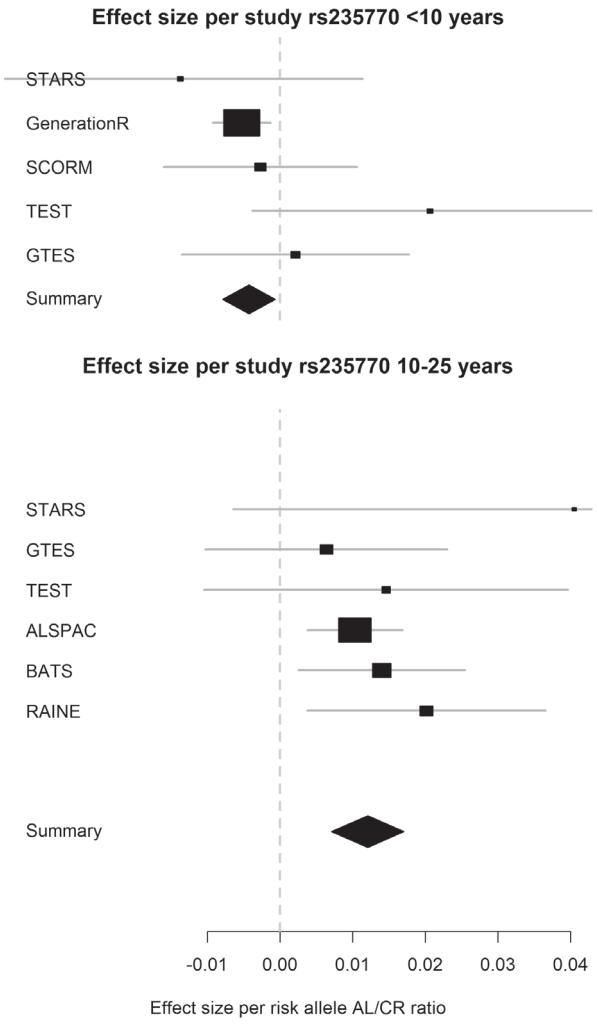
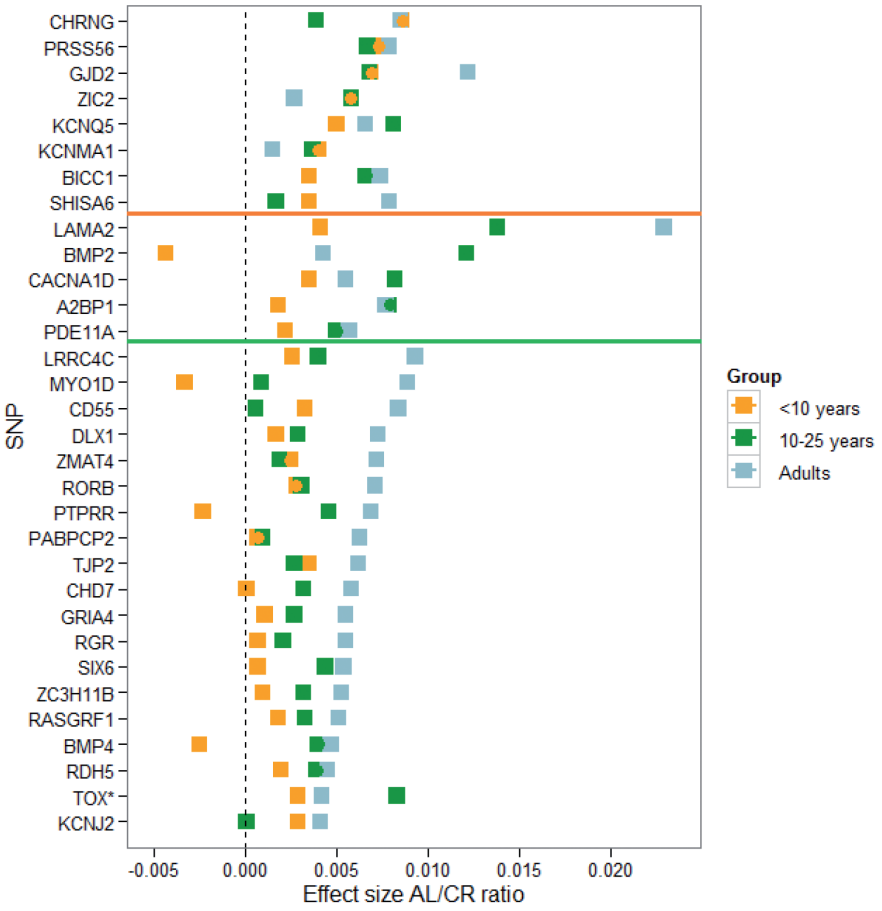


Figure 4 Distribution of effects on AL/CR ratio per myopia-related gene in three age groups



Ingenuity Pathway Analysis (IPA)

IPA is a web-based software to analyse and integrate the identified SNPs based on biological functions. Analyses were performed in two separate analyses, one analysis with genes with an early onset and one analysis with late onset genes. We used the program's diseases and disorder table to identify associated diseases. Genes with an early onset in the age groups <25 years were enriched in pathways of auditory disease, organismal injury and abnormalities, and gastrointestinal disease (at FDR <5%). The genes that were significantly associated in adults predisposed to connective tissue disorders, developmental disorder (e.g. microphthalmia; with the genes *BMP4* and *SIX6*), and also gastrointestinal disease (supplementary table 5).

Table 2 Effect size of myopia related genes in age groups <10 years, 10-25 years, 25> years

				<10 years		10 - 25 years		Combined		>25 years	
Variant	Chr	Gene	RA	Beta (SE)	P	Beta (SE)	P	Beta (SE)	P	Beta (SE)	P
Allele Score	–	–	–	0.0019 (0.0003)	10^-11	0.0033 (0.0004)	10^-15	0.0024 (0.0002)	10^-24	0.0051(0.0003)	10^-72
rs1652333	1	*CD55*	G	0.0033 (0.0017)	0.05	0.0006 (0.0024)	0.80	0.0026 (0.0014)	0.07	0.0084(0.0017)	10^-6
rs4373767	1	*ZC3H11B*	T	0.0010 (0.0017)	0.55	0.0032 (0.0023)	0.16	0.0019 (0.0014)	0.16	0.0053(0.0017)	0.002
rs17412774	2	*PABPCP2*	A	0.0007 (0.0017)	0.69	0.0010 (0.0023)	0.67	0.0008 (0.0014)	0.57	0.0063(0.0017)	10^-4
rs17428076	2	*DLX1*	C	0.0017 (0.0021)	0.43	0.0029 (0.0027)	0.28	0.0024 (0.0017)	0.16	0.0073(0.0021)	10^-4
rs1898585	2	*PDE1 1A*	T	0.0022 (0.0019)	0.26	0.0050 (0.0029)	0.09	0.0034 (0.0017)	0.04	0.0057(0.0021)	0.007
rs1656404	2	*PRSS56*	A	0.0073 (0.0024)	0.002	0.0067 (0.0033)	0.04	0.0069 (0.0019)	10^-4	0.0079(0.0024)	0.001
rs1881492	2	*CHRNG*	T	0.0086 (0.0024)	10^-4	0.0039 (0.0031)	0.21	0.0064 (0.0020)	0.001	0.0085(0.0022)	10^-5
rs14165	3	*CACNA1D*	G	0.0035 (0.0020)	0.08	0.0082 (0.0026)	0.001	0.0055 (0.0016)	0.001	0.0055(0.0020)	0.005
rs13091182	3	*ZBTB38*	G	0.0008 (0.0020)	0.69	-0.0001 (0.0024)	0.98	0.0007 (0.0015)	0.66	-0.0004(0.0019)	0.83
rs9307551	4	*LOC100506035*	A	0.0007 (0.0019)	0.70	0.0037 (0.0026)	0.16	0.0020 (0.0016)	0.20	0.0051(0.0020)	0.008
rs5022942	4	*BMP3*	A	0.0014 (0.0018)	0.44	-0.0016 (0.0026)	0.54	0.0007 (0.0015)	0.63	0.0006(0.0020)	0.78
rs7744813	6	*KCNQ5*	A	0.0050 (0.0017)	0.004	0.0081 (0.0023)	10^-4	0.0060 (0.0014)	10^-5	0.0066(0.0018)	10^-4
rs12205363	6	*LAMA2*	T	0.0041 (0.0041)	0.31	0.0138 (0.0046)	0.003	0.0094 (0.0031)	0.003	0.0229(0.0036)	10^-10
rs7829127	8	*ZMAT4*	A	0.0025 (0.0020)	0.22	0.0019 (0.0028)	0.49	0.0025 (0.0017)	0.13	0.0072(0.0021)	0.001
rs7837791	8	*TOX*	G	0.0029 (0.0016)	0.06	0.0083 (0.0022)	10^-4	0.0050 (0.0013)	10^-4	0.0042(0.0017)	0.012
rs4237036	8	*CHD7*	T	0.0001 (0.0018)	0.96	0.0032 (0.0024)	0.18	0.0013 (0.0014)	0.37	0.0058(0.0018)	0.001
rs11145465	9	*TJP2*	A	0.0035 (0.0022)	0.11	0.0027 (0.0028)	0.33	0.0029 (0.0017)	0.09	0.0062(0.0021)	0.004
rs7042950	9	*RORB*	G	0.0028 (0.0019)	0.14	0.0031 (0.0026)	0.24	0.0027 (0.0016)	0.08	0.0071(0.0020)	10^-4
rs7084402	10	*BICC1*	G	0.0035 (0.0016)	0.03	0.0066 (0.0023)	0.004	0.0050 (0.0013)	10^-4	0.0074(0.0017)	10^-6
rs6480859	10	*KCNMA1*	T	0.0040 (0.0018)	0.02	0.0037 (0.0023)	0.10	0.0040 (0.0014)	0.004	0.0015(0.0017)	0.38
rs745480	10	*RGR*	G	0.0007 (0.0016)	0.67	0.0021 (0.0022)	0.34	0.0011 (0.0013)	0.40	0.0055(0.0017)	0.001
rs10882165	10	*CYP26A1*	T	0.0012 (0.0018)	0.49	0.0002 (0.0024)	0.93	0.0007 (0.0014)	0.61	0.0011(0.0018)	0.54
rs1381566	11	*LRRC4C*	G	0.0026 (0.0020)	0.21	0.0040 (0.0034)	0.23	0.0028 (0.0018)	0.12	0.0093(0.0022)	10^-5
rs2155413	11	*DLG2*	A	0.0022 (0.0017)	0.18	0.0027 (0.0022)	0.23	0.0023 (0.0013)	0.09	0.0021(0.0017)	0.21
rs11601239	11	*GRIA4*	C	0.0011 (0.0016)	0.50	0.0027 (0.0022)	0.22	0.0014 (0.0013)	0.30	0.0055(0.0017)	0.001
rs3138144	12	*RDH5*	G	0.0020 (0.0021)	0.35	0.0039 (0.0027)	0.16	0.0028 (0.0017)	0.10	0.0045(0.0019)	0.02

rs12229663	12	*PTPRR*	A	-0.0023 (0.0019)	0.21	0.0046 (0.0026)	0.08	0.0000 (0.0016)	1.00	0.0069(0.0019)	10^-4
rs8000973	13	*ZIC2*	C	0.0058 (0.0017)	10^-4	0.0058 (0.0023)	0.01	0.0059 (0.0014)	10^-5	0.0027(0.0017)	0.10
rs2184971	13	*PCCA*	A	0.0008 (0.0016)	0.61	0.0006 (0.0023)	0.80	0.0009 (0.0014)	0.48	0.0021(0.0017)	0.21
rs66913363	14	*BMP4*	G	-0.0025 (0.0017)	0.15	0.0040 (0.0024)	0.10	0.0006 (0.0014)	0.68	0.0047(0.0017)	0.006
rs1254319	14	*SIX6*	A	0.0007 (0.0017)	0.68	0.0044 (0.0024)	0.07	0.0017 (0.0014)	0.22	0.0054(0.0018)	0.002
rs524952	15	*GJD2*	A	0.0069 (0.0016)	10^-5	0.0068 (0.0023)	0.003	0.0067 (0.0013)	10^-7	0.0122(0.0016)	10^-14
rs4778879	15	*RASGRF1*	G	0.0018 (0.0017)	0.29	0.0033 (0.0023)	0.15	0.0019 (0.0014)	0.17	0.0051(0.0017)	0.002
rs17648524	16	*A2BP1*	C	0.0018 (0.0018)	0.33	0.0079 (0.0024)	0.001	0.0039 (0.0015)	0.01	0.0077(0.0019)	10^-5
rs2969180	17	*SHISA6*	A	0.0035 (0.0016)	0.03	0.0017 (0.0023)	0.46	0.0027 (0.0014)	0.05	0.0079(0.0017)	10^-6
rs17183295	17	*MYO1D*	T	-0.0033 (0.0023)	0.16	0.0009 (0.0030)	0.76	-0.0018 (0.0018)	0.33	0.0089(0.0023)	10^-4
rs4793501	17	*KCNJ2*	T	0.0029 (0.0016)	0.08	0.0001 (0.0022)	0.95	0.0019 (0.0013)	0.16	0.0041(0.0017)	0.015
rs12971120	18	*CNDP2*	A	0.0002 (0.0019)	0.93	0.0048 (0.0026)	0.07	0.0017 (0.0015)	0.27	0.0024(0.0019)	0.22
rs235770	20	*BMP2*	T	-0.0043 (0.0018)	0.02	0.0121 (0.0025)	10^-6	0.0008 (0.0015)	0.60	0.0043(0.0017)	0.013

Values are betas (SE) and *P*-values, from linear regression models adjusted for sex, age and principal components if applicable meta-analysed with inversed variance meta-analysis in METAL. Bold: $P < 0.05$.

Database for Annotation, Visualization and Integrated Discovery (DAVID)

The software program DAVID is an online knowledge database to identify overlapping functions of genes. We performed the analyses separately for early and late onset genes. Using the categories defined above, early-onset genes were significantly more than expected annotated to ion channels and ion transport. The genes annotated to these categories were *CACNA1D, CHRNG, GJD2, KCNMA1* and *KCNQ5*. Late onset genes appeared to be significantly more related to neuron differentiation and visual perception. The genes involved in these categories were *RORB, SIX6, RASGRF1, CHD7, RGR, RDH5* and *GRIA4* (supplementary table 6).

DISCUSSION

This study identifies the age span during which the known GWAS-identified loci for refractive error have their greatest effect. The current meta-analysis suggests that specific loci had their greatest effect in young children (*CHRNG, ZIC2, KCNMA1*), while others reached the greatest effect during early teenage years (*BMP2, CACNA1D, A2BP1*). However, most appeared to have a gradual effect during the entire age span of myopia development (*LAMA2, LRRC4C, DLX1, RDH5, GRIA4, RGR, SIX6*).

Strengths and limitations

Strengths of this study were the large sample size, the comparison across 3 distinct age categories, and the precision in measurements of ocular biometry. A drawback was the lack of complete cycloplegic refraction in children in several studies, which jeopardized valid measurements of RE in this age category. Thus, we used AL/CR ratio as an indicator of RE to avoid heterogeneity in the outcome. This ratio has a high correlation with RE[112,239] and was available from all studies in the consortium. Another limitation was the lack of power to detect statistically significant differences between the age groups for most genes. A pooled analysis would have increased statistical power, but raw data from individual participants were not available. Ideally, a study using longitudinal data of the same children over different age periods would have the best study design for the current analysis.

Little has been reported on the development and progression of myopia as a function of age; however, a number of studies investigated the relationship between development of ocular biometry related to age. Until the age of 25 years, corneal curvature, the crystalline lens, and axial length all evolve with age, and thereby influence refractive error. The cornea increases in radius until preschool age leading to flattening of the corneal curvature and decrease in refractive power;[125] the crystalline lens grows until 10 years of age, also reducing refractive power.[135,251] This decrease in refractive power is compensated by axial elongation which increases from 17 mm in newborns[52] to 23.3 mm in 12-13 year olds.[90] The average AL in emmetropic adults is 23.5 mm.[242,252] The highest growth rate

of AL occurs in the first years of life and relates to emmetropisation; the growth rate after early teens is more gradual but mainly relates to myopisation.[252] The exact age at which eye growth stops is not known; generally this occurs before age 20 years, but increase in AL has been described up to the age of 25 years in university students.[53,253]

Identified genes and functions

One of the key detected GWAS-identified loci for refractive error is on chromosome 15 near the *GJD2* gene, which encodes a gap junction protein known as CX36. This protein not only processes cone-to-cone and cone-to-rod signals[254] but also directs signaling between other retinal cells.[255,256] This cell-to-cell communication appears to be under regulation of light exposure and dopamine,[225] two factors that have an established role in eye growth and myopia development. Our data suggest that *GJD2* has an early-onset, indicating that altered retinal cell signaling, perhaps via reduced light exposure and low dopamine levels, may be a first step in myopia development. As expected, some early-onset genes also had a reported role in eye development. Knockout of *LAMA2*, a gene encoding the large extracellular glycoprotein laminin-α2; causes growth retardation including smaller eyes with compressed cellular layers.[257] Mutations in the serine protease gene *PRSS56* cause a severe decrease of AL leading to microphthalmia.[258] Another developmental gene is *ZIC2*, an enhancer-binding factor required for embryonic stem cell specification.[259] This gene may be important for development of retinal architecture, as it is known to be involved in differentiation and proliferation of retinal progenitor cells,[260] and development of retinal ganglion cell trajectories.[261] Strikingly, several other genes involved in eye development, such as *SIX6, CDH7*, and *DLX1*, did not show an early onset but were more significant after the age of 10 years. Other early-onset genes were ion channels such as *KCNQ5*, a potassium channel present in cone and rod photoreceptors,[262] and *CACNA1D*, a calcium channel present in photoreceptors.[263] *CHRNG* has as yet an unknown role in myopia development. It encodes the γ subunit of the embryonal acetylcholine receptor, which is widely expressed in the retina,[264,265] and is associated with multiple pterygium syndrome.[266]

Several remarkable patterns of effect were notable. For instance, the lead SNPs at the *BMP2, MYO1D, PTPRR*, and *BMP4* loci showed an opposite effect in children <10 years than in those who were older. This is not uncommon in biology, as such a trajectory has also been described for the *FTO* locus in relation to body mass index in children.[267] Interestingly, gene expression studies of *BMP2* in chickens showed that mRNA of this gene in the retinal pigment epithelium is up- or down-regulated depending on the location of the image plane.[268] When the image was focused behind the retina, mRNA was downregulated and the vitreous chamber enlarged. This underscores a bidirectional role for *BMP2* in modulation of eye growth.

Most genes had a late onset. *BMP4* has a similar function to *BMP2* as it is also responds to optical defocus with bidirectional regulation of eye growth.[269] *SIX6* is a DNA-binding homeobox and has a SIX domain, which binds downstream effector molecules. It is known to influence eye size in zebrafish with knocked down *SIX6* expression

[270]. Other genes play a less obvious role in myopiagenesis. MYO1D is involved in membrane trafficking in the recycling pathway and expressed in oligodendrites.[271] *RORB*, a gene encoding a nuclear receptor-directing photoreceptor differentiation, is known to activate and generate S-opsin.[272,273] *DLX1* belongs to the DLX family of homeobox transcription factors, and produces GABAergic interneurons during embryonic development.

CONCLUSION

In conclusion, our study suggests that only a small proportion of the currently known GWAS-identified loci for RE exert their full effect at a young age. Furthermore, some of the pathways previously-identified by GWAS meta-analyses[42] can now be separated into early- and late-onset pathways. For example, genes coding for ion channels typically had an early onset, while genes related to connective tissue and visual feedback mechanisms appeared to become more important at a later age. As the currently known genes play only a minor role in early-onset myopia, we question whether this type of myopia is caused by common variants in other genes, or whether rare variants with large effects determine early-onset. Future research may shed more light on genes for early-onset myopia, and unravelling these genes will open up strategies for prevention of high myopia.

Detailed acknowledgments and online resources can be found in the published article online: https://onlinelibrary.wiley.com/doi/full/10.1002/gepi.21999

SUPPLEMENTAL MATERIAL

Supplementary Table S1 Genotyping and imputation details

Study	Genotyping platform	Imputation	Reference population (1000G)
ALSPAC	Illumina HumanHap550	MACH/minimac	GIANT phase1 release v3
BATS/TEST	Illumina HumanHap610/660-Quad	MACH	1000G Phase 1 release on Aug 4, 2010
RAINE	Illumina 660W-Quad	MACH/minimac	1000G Phase 1 release on Nov 23, 2010
TEST	Illumina HumanHap610/660-Quad	MACH	1000G Phase 1 release on Aug 4, 2010
Generation R	Illumina Infinium II HumanHap610 Quad Arrays	MACH	1000 Genomes GIANTv3 panel
GTES	Affymetrix Gene Titan	IMPUTE2 v2.3.0	1000G Phase 1 release on Nov 23,2010
SCORM	Illumina HumanHap550/550-Duo	MACH/minimac	1000G Phase 1 release March 2012
STARS	Illumina HumanHap610-Quad	MACH/minimac	1000G Phase 1 release March 2012
GHS 1/2	Affymetrix Genome-Wide Human SNP Array 6.0	MACH/minimac	1000G Phase 1 release on Nov 23, 2010
Rotterdam Study	RS I: Illumina Infinium II HumanHap550 chip v3.0 array. RS II: HumanHap550 Duo Arrays + Human610-Quad Arrays Illumina, RS-III: Human 610 Quad Arrays Illumina	MACH	NCBI build 36, HapMap release #22
Croatia	Korcula: Illumina CNV370v1 and CNV370-Quadv3 Vis: Illumina HumanHap 300v1 Split: Illumina CNV370-Quadv3 and Illumina OmniExpress Exome-8v1_A	IMPUTEv2 (phasing using shapeit v2)	1000G Phase 1 integrated v3 release March 2012 (Vis and Korcula) release June 2014 (Split)
Nagahama	Human 610 Quad Arrays Illumina / Human Omni 2.5 Arrays Illumina	MACH	NCBI build 36, HapMap release #28
EPIC-Norfolk	Affymetrix UK Biobank Axiom Array	IMPUTE version 2.3.2.	1000G Phase 3 (October 2014)

Abbreviations: 1000G, One thousand genomes project.

Supplementary Table S2 All SNPs previously associated with myopia and refractive error

SNP	Chr	Pos	Gene	Citation
rs1652333	1	207470460	*CD55*	Verhoeven et al. 2013
rs4373767	1	219759682	*ZC3H11B*	Cheng et al. 2013
rs17412774	2	146773948	*PABPCP2*	Kiefer et al. 2013
rs17428076	2	172851936	*DLX1*	Kiefer et al. 2013
rs1898585	2	178660450	*PDE11A*	Kiefer et al. 2013
rs1656404	2	233379941	*PRSS56*	Verhoeven et al. 2013
rs1881492	2	233406998	*CHRNG*	Verhoeven et al. 2013
rs14165	3	53847408	*CACNA1D*	Verhoeven et al. 2013
rs13091182	3	141133960	*ZBTB38*	Kiefer et al. 2013
rs9307551	4	80530671	*LOC100506035*	Verhoeven et al. 2013
rs5022942	4	81959966	*BMP3*	Kiefer et al. 2013
rs7744813	6	73643289	*KCNQ5*	Verhoeven et al. 2013
rs12205363	6	129834628	*LAMA2*	Verhoeven et al. 2013
rs7829127	8	40726394	*ZMAT4*	Verhoeven et al. 2013
rs7837791	8	60179086	*TOX*	Verhoeven et al. 2013
rs4237036	8	61701057	*CHD7*	Verhoeven et al. 2013
rs11145465	9	70989531	*TJP2*	Verhoeven et al. 2013
rs7042950	9	77149837	*RORB*	Verhoeven et al. 2013
rs7084402	10	60265404	*BICC1*	Verhoeven et al. 2013
rs6480859	10	79081948	*KCNMA1*	Kiefer et al. 2013
rs745480	10	85986554	*RGR*	Kiefer et al. 2013
rs10882165	10	94924324	*CYP26A1*	Verhoeven et al. 2013
rs1381566	11	40149607	*LRRC4C*	Kiefer et al. 2013
rs2155413	11	84634790	*DLG2*	Kiefer et al. 2013
rs11601239	11	105556598	*GRIA4*	Verhoeven et al. 2013
rs3138144	12	56114768	*RDH5*	Verhoeven et al. 2013
rs12229663	12	71249996	*PTPRR*	Verhoeven et al. 2013
rs8000973	13	100691367	*ZIC2*	Verhoeven et al. 2013
rs2184971	13	100818092	*PCCA*	Verhoeven et al. 2013
rs66913363	14	54413001	*BMP4*	Kiefer et al. 2013
rs1254319	14	60903757	*SIX6*	Verhoeven et al. 2013
rs524952	15	35005885	*GJD2*	Verhoeven et al. 2013
rs4778879	15	79372875	*RASGRF1*	Verhoeven et al. 2013
rs17648524	16	7459683	*A2BP1*	Verhoeven et al. 2013
rs2969180	17	11407901	*SHISA6*	Verhoeven et al. 2013
rs17183295	17	31078272	*MYO1D*	Verhoeven et al. 2013
rs4793501	17	68718734	*KCNJ2*	Verhoeven et al. 2013
rs12971120	18	72174023	*CNDP2*	Verhoeven et al. 2013
rs235770	20	6761765	*BMP2*	Verhoeven et al. 2013

Supplementary Table S3 Heterogeneity per P-value per SNP for each age group

Variant	Chr	Gene	RA	<10 years Hetero-geneity P	10 – 25 years Hetero-geneity P	Combined Hetero-geneity P	>25 years Hetero-geneity P
Allele Score	–	–	–	0.07	0.08	**0.0002**	**0.0005**
rs1652333	1	*CD55*	G	0.40	0.25	0.23	0.18
rs4373767	1	*ZC3H11B*	T	0.18	0.69	0.29	0.38
rs17412774	2	*PABPCP2*	A	0.50	0.39	0.46	0.25
rs17428076	2	*DLX1*	C	0.26	0.02	0.05	0.70
rs1898585	2	*PDE11A*	T	0.40	0.86	0.76	0.77
rs1656404	2	*PRSS56*	A	0.45	0.15	0.25	0.53
rs1881492	2	*CHRNG*	T	0.69	0.34	0.45	0.95
rs14165	3	*CACNA1D*	G	0.48	0.70	0.51	0.26
rs13091182	3	*ZBTB38*	G	0.13	0.89	0.94	0.16
rs9307551	4	*LOC100506035*	A	0.94	0.78	0.92	0.02
rs5022942	4	*BMP3*	A	0.82	0.91	0.94	0.98
rs7744813	6	*KCNQ5*	A	0.31	0.66	0.53	0.65
rs12205363	6	*LAMA2*	T	0.12	0.07	0.06	0.54
rs7829127	8	*ZMAT4*	A	0.24	0.75	0.54	0.92
rs7837791	8	*TOX*	G	0.82	**0.001**	**0.002**	0.12
rs4237036	8	*CHD7*	T	0.35	0.94	0.84	0.89
rs11145465	9	*TJP2*	A	0.17	0.24	0.38	0.13
rs7042950	9	*RORB*	G	0.83	0.41	0.70	0.12
rs7084402	10	*BICC1*	G	0.58	0.38	0.52	0.83
rs6480859	10	*KCNMA1*	T	0.27	0.63	0.62	0.81
rs745480	10	*RGR*	G	0.38	0.88	0.68	0.10
rs10882165	10	*CYP26A1*	T	0.51	0.31	0.45	0.03
rs1381566	11	*LRRC4C*	G	0.40	0.60	0.49	0.78
rs2155413	11	*DLG2*	A	0.21	0.52	0.31	0.29
rs11601239	11	*GRIA4*	C	0.58	0.96	0.96	0.05
rs3138144	12	*RDH5*	G	0.67	0.72	0.83	0.43
rs12229663	12	*PTPRR*	A	0.41	0.18	0.06	0.97
rs8000973	13	*ZIC2*	C	0.44	0.61	0.65	0.01
rs2184971	13	*PCCA*	A	0.75	0.19	0.37	0.55
rs66913363	14	*BMP4*	G	0.62	0.22	0.10	0.57
rs1254319	14	*SIX6*	A	0.76	0.24	0.31	0.78
rs524952	15	*GJD2*	A	0.73	0.36	0.52	0.49
rs4778879	15	*RASGRF1*	G	0.15	0.99	0.79	0.30
rs17648524	16	*A2BP1*	C	0.14	0.52	0.07	0.72
rs2969180	17	*SHISA6*	A	0.59	0.24	0.30	0.23
rs17183295	17	*MYO1D*	T	0.47	0.99	0.83	0.37
rs4793501	17	*KCNJ2*	T	0.42	0.03	0.03	0.10
rs12971120	18	*CNDP2*	A	0.21	0.34	0.22	0.36
rs235770	20	*BMP2*	T	0.24	0.67	**4*E-5**	0.48

Supplementary Table S4 Random effect analysis of SNPs with P < 0.05 and heterogeneity P <0.05

				10 – 25 years		>25 years	
Variant	Chr	Gene	RA	Effect (SE)	P	Effect (SE)	P
GRS	–	–	–	–	–	0.0048 (0.0007)	<0.001
rs9307551	4	*LOC100506035*	A	–	–	0.0066 (0.0034)	0.06
rs7837791	8	*TOX*	G	0.0062 (0.0073)	0.40	–	–

GRS = Genetic risk score

Supplementary Table 5 IPA Analysis of diseases and disorders associated with early and late onset genes for myopia with p-values and molecules

Diseases and Disorders of early onset genes

Name	*p-value range*	*Molecules*
Auditory Disease	1.80E-02 – 1.13E-05	2
Organismal Injury and Abnormalities	4.62E-02 – 1.13E-05	11
Gastrointestinal Disease	4.71E-02 – 5.75E-05	8
Hematological Disease	1.22E-02 – 1.18E-04	3
Metabolic disease	4.71E-02 – 1.18E-04	3

Diseases and Disorders of late onset genes

Name	*p-value range*	*Molecules*
Connective tissue disorders	4.60E-02 – 1.14E-04	4
Developmental disorders	4.60E-02 – 1.14E-04	7
Gastrointestinal Disease	4.66E-02 – 1.14E-04	16
Skeletal and Muscular disorders	4.60E-02 – 1.14E-04	4
Cancer	4.66E-02 – 8.24E-04	16

Supplementary Table 6 DAVID pathway analysis of functional annotation with early and late onset genes for myopia with p-values and molecules

Functional annotation of early onset genes

GO Term	*p-value*	*Molecules*
Channel activity	1.8E-4	5
Passive transmembrane transporter activity	1.8E-4	5
Ion channel complex	3.2E-4	4
Ionic channel	6.7E-4	4
Cation channel activity	1.0E-3	4

Functional annotation of late onset genes

GO Term	*p-value*	*Molecules*
Neurological system process	5.0E-4	7
Visual perception	1.0E-3	4
Sensory perception of light stimulus	1.0E-3	4
Cognition	1.1E-3	6
Vision	5.8E-3	3

CHAPTER 11

CHILDHOOD GENE-ENVIRONMENT INTERACTIONS AND AGE-DEPENDENT EFFECTS OF GENETIC VARIANTS ASSOCIATED WITH REFRACTIVE ERROR AND MYOPIA: THE CREAM CONSORTIUM

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ABSTRACT

Myopia, currently at epidemic levels in East Asia, is a leading cause of untreatable visual impairment. Genome-wide association studies (GWAS) in adults have identified 39 loci associated with refractive error and myopia. Here, the ageofonset of association between genetic variants at these 39 loci and refractive error was investigated in 5200 children assessed longitudinally across ages 7-15 years, along with gene-environment interactions involving the major environmental risk-factors, nearwork and time outdoors. Specific variants could be categorized as showing evidence of: (a) early-onset effects remaining stable through childhood, (b) early-onset effects that progressed further with increasing age, or (c) onset later in childhood (N=10, 5 and 11 variants, respectively). A genetic risk score (GRS) for all 39 variants explained 0.6% (P=6.6E-08) and 2.3% (P=6.9E-21) of the variance in refractive error at ages 7 and 15, respectively, supporting increased effects from these genetic variants at older ages. Replication in multi-ancestry samples (combined N=5599) yielded evidence of childhood onset for 6 of 12 variants present in both Asians and Europeans. There was no indication that variant or GRS effects altered depending on time outdoors, however 5 variants showed nominal evidence of interactions with nearwork (top variant, rs7829127 in *ZMAT4*; P=6.3E-04).

INTRODUCTION

The refractive errors myopia and hyperopia are common visual disorders that typically require correction with spectacles, contact lenses, or refractive eye surgery. Myopia – particularly with increasing severity – is a leading cause of irreversible visual impairment and blindness due primarily to stretching and thinning of the ocular tissues within the posterior segment of the eye. These changes are associated with an increased risk of retinal detachment, chorioretinal atrophy, choroidal neovascularisation, myopic maculopathy, glaucoma and cataract.[16,274] Myopia is rare in infancy, usually developing during school age or in early adulthood.[275] For current generations of young adults, approximately 30-40% of individuals in Western countries[276,277] and 80% of those in urban areas of East Asia have myopia.[278,279]

Genome-wide association studies (GWAS) in primarily population-based samples[280-286] and next-generation sequencing (NGS) studies of carefully selected high myopia pedigrees harbouring extremely rare, high penetrance disease-causing mutations[287-292] have improved our understanding of the genetics of refractive error and myopia. To date at least 39 distinct loci harbouring common genetic variants showing genome-wide significant association with refractive error have been identified through GWAS. For the genetic variants that contribute most to the burden of myopia in the general population (i.e. the GWAS-identified variants) it is not yet known whether the variants act during very early life, childhood, or in adulthood. This is an important question given that knowledge of the time and mode of action of the causal variants at the associated loci is necessary for detecting children at-risk of myopia (who would benefit most from treatment intervention), and would aid the design of new therapies capable of halting myopia progression.

For environmental risk factors to which most children are exposed, inter-individual differences in genetic susceptibility may account for some of the phenotypic variance.[293] Exposure to nearwork, i.e. reading and other tasks requiring prolonged near vision, has long been proposed as an environmental risk factor for myopia to which children are ubiquitously exposed during their schooling. The total duration of reading, the period of continuous reading, the reading distance between the text and the eyes, and variation in nearwork exposure outside of the school day have each been shown to be associated with refractive error or myopia progression.[294,295] The other most strongly implicated environmental risk factor for myopia is insufficient time spent outdoors,[296-298] and it has been suggested that time spent outdoors and time spent performing nearwork activities together underlie the robust association between myopia and educational achievement.[16,299] Gene-environment (GxE) interactions – which in this project we define as marker-phenotype associations whose effects differ statistically depending on whether individuals have been exposed to a high vs. low level of an environmental risk factor – may contribute extensively to variation in disease susceptibility.[300] Given the recent identification of gene-environment interactions involving nearwork or level of education,[301-303] a key question in myopia research currently is whether GxE interactions con-

tribute to the rising prevalence of myopia and to the higher incidence rate observed in young Asian populations as compared to their European counterparts.

We carried out analyses of pediatric/adolescent cohorts collaborating in the Consortium for Refractive Error And Myopia (CREAM) to investigate whether the top index variants at the 39 loci previously identified in GWAS meta-analyses of adults have early-onset effects manifest during childhood. We also tested for evidence of GxE interactions involving either nearwork or time spent outdoors. A single large cohort with longitudinal measurements of refractive error over much of childhood was used for the primary analyses. Meta-analyses of cross-sectional samples were then used to test for replication.

METHODS

Participants and phenotypes

All participants were aged <25 years-old and none had been included in the earlier CREAM meta-analysis of refractive error,[281] which only included individuals >25 years of age. Details of the participant recruitment and phenotypic assessment are presented in the Supplementary Information (online). The study was conducted in accordance with the Declaration of Helsinki, and all participants provided informed consent. The experimental protocols for the study were approved by the respective ethical review boards at host institutions, as follows. ALSPAC, the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees; BATS, the Human Research Ethics Committee at the QIMR Berghofer Medical Research Institute; GZT, the Ethics Review Board of the Zhongshan Ophthalmic Center of Sun Yat-Sen University; RAINE, the Human Research Ethics Committee at the University of Western Australia; SCORM and STARS, the Institutional Review Boards of the Singapore Eye Research Institute, Singapore General Hospital, National University of Singapore, and the National Healthcare Group, Singapore; TEDS, the Institute of Psychiatry ethics committee; TEST, the Royal Victorian Eye and Ear Hospital, the University of Tasmania, and the Australian Twin Registry; WESDR, the Health Sciences Institution Review Board of the University of Wisconsin, Madison.

Participants underwent cycloplegic autorefraction (RAINE, TEST, BATS, GZT, SCORM, STARS) or non-cycloplegic autorefraction (ALSPAC) or subjective refraction (TEDS, WESDR) and the spherical equivalent refractive error averaged between the two eyes was calculated. Parental questionnaires that included items on time spent engaged in nearwork outside of school, and time spent in outdoor activities were used to classify children as spending a high or low amount of time performing nearwork or outdoors each day. Classification was done within each cohort separately, using a median split ("low" group, exposure below median level; "high" group, exposure above median level).

Genetic analysis

DNA samples obtained from blood or saliva were genotyped using either an Illumina or Affymetrix high-density single nucleotide polymorphism (SNP) array, and genotypes at untyped markers were imputed using the 1000-Genomes Project reference panel (see Supplementary Information online for details). Stringent quality control procedures (e.g. imputation quality r^2 or info score >0.5) were applied to each cohort separately (Supplementary Information online). 39 SNPs that showed genome-wide significant association with refractive error in the general adult population in two previous GWAS analyses[280,281] were selected for evaluation (Table S1).

Cross-sectional models and meta-analyses

For each of the 8 cross-sectional cohorts separately, single SNP tests of association with refractive error were conducted using the following linear regression model:

$$y_i = \mu + a_i\beta_{Age} + s_i\beta_{Sex} + g_i\beta_{SNP} + \varepsilon_i \quad (1)$$

Where yi is the spherical equivalent refractive error of the i^{th} participant, of age ai and sex si and with gi their risk allele dosage on the scale 0-2 for the test SNP, and εi the residual. Regression coefficients are indicated as βAge, βSex, and βSNP for the model parameters age, sex and SNP genotype, respectively. Additional G x E interaction models were tested for samples with information available on environmental exposures, nearwork or time outdoors (both exposures coded: 0=low, 1=high). For the ith participant, using ni to denote nearwork and ti for time outdoors:

$$y_i = \mu + a_i\beta_{Age} + s_i\beta_{Sex} + g_i\beta_{SNP} + n_i\beta_{NW} + g_in_i\beta_{SNP\cdot NW} + \varepsilon_i \quad (2)$$
$$y_i = \mu + a_i\beta_{Age} + s_i\beta_{Sex} + g_i\beta_{SNP} + t_i\beta_{TO} + g_it_i\beta_{SNP\cdot TO} + \varepsilon_i \quad (3)$$

Results from the individual cohorts were meta-analyzed in 5599 individuals comprising 5 cohorts of European ancestry (BATS, RAINE, TEDS, TEST, WESDR; N=3,143; Table 1) and 3 cohorts of Asian ancestry (GZT, SCORM, STARS; N=2,456; Table 1) using a weighted inverse-variance, fixed effects model.[304] A random effects model was used if Cochran's Q-test for heterogeneity yielded a *P*-value below 0.05.

Longitudinal study (ALSPAC)

Refractive error was included in the clinical assessments for ages 7, 10, 11, 12 and 15 years in ALSPAC children.[305] Linear mixed models for refractive trajectory were fit as described[305] using the nlme package in R[306] for individuals (N=5,200; Table 1) who underwent at least 3 refractive assessments and whose genotype data passed quality control filters (Supplementary Information online). Briefly, SNP dosage, age and higher-order

age terms (age^2 and age^3) were modelled as fixed effects while for each child, the difference from the average refractive error at baseline and the linear rate of change in refractive error were modelled as individual-level random effects, using an autoregressive correlation structure. To examine GxE interactions, initially, 3-way interaction models were tested that included the interaction between SNP, change-from-baseline in age, and environmental exposures (nearwork or time outdoors). If the *p*-value for the 3-way interaction was >0.05 then models including only 2-way interactions were tested.

Quanto[307] was used to gauge the power to detect main and interaction effects in the ALSPAC cohort. These calculations assumed a minor allele frequency (MAF) of 0.25, a sample size of 4461 (corresponding to 5,200 minus 739 participants with missing information about time spent performing nearwork), a binary exposure affecting 39% of the cohort (equivalent to that for high vs. low nearwork exposure in ALSPAC) and a refractive error distribution with a mean of zero and a standard deviation of 1.50 D. The estimated power would be conservative given that a linear mixed model analysis will have greater power than a linear model analysis.

Genetic risk score for all 39 SNPs

A genetic risk score was computed by summing the dosage of risk alleles for all 39 SNPs. In individuals of Asian ancestry only 31 of the 39 SNPs were polymorphic (MAF> 0.05) and therefore contributed to the genetic risk score calculation. The frequency distribution of genetic risk score in each sample was normally distributed with a mean of 36 (95% C.I. 29 to 42) alleles in Europeans and 40 (95% C.I. 37 to 42) alleles in Asians. To calculate the variance in refractive error explained by the genetic risk score at a specific age for participants in the ALSPAC cohort, refractive error at age 7.5 years (or at age 15 years) was regressed on genetic risk score using a linear model. Inclusion of the covariates age and sex did not improve the fit of the model, and hence these covariates were omitted. The variance explained by the genetic risk score was therefore taken as the adjusted R^2 value for a model that included the genetic risk score as the only predictor variable.

Pathway analysis

The genes (Table 2) implicated in having early-onset effects (N=10 genes) or later-onset effects (N=11 genes) in the ALSPAC discovery sample were evaluated using PANTHER Version 10.0 (release date May 15, 2015)[308] and DAVID Version 6.7 (release date 27 Jan, 2010)[309] to identify potential functional pathways.

RESULTS

Early-onset and later-onset effects in childhood

Nine cohorts of children/adolescents were studied (Table 1). The largest of these, ALSPAC (N=5,200), which had longitudinal data for refractive error, was used for discovery analyses, and 8 cross-sectional cohorts were used for validation. The discovery cohort had ~80% power to detect an association for a SNP with an effect size of 0.1 D and MAF of 0.25.
Of the 39 SNPs examined, 16 showed evidence of onset in childhood (Table 2 and Table S2). Early-onset associations already manifest at 7.5 years of age were present for 10 SNPs (*P*=4.8E-02 to *P*=5.3E-03). Later-onset associations that emerged between the ages of 7.5 and 15 were noted for 11 SNPs (*P*=4.9E-02 to 8.8E-04 for SNP x Age interaction). Five SNPs showed a main effect at baseline as well as later progressive effects. Examples of SNPs showing evidence of early-onset and later-onset effects are presented in Figure 1 for early-onset *CHRNG* SNP rs1881492, later-onset *A2BP1* (also known as *RBFOX1*) rs17648524, and PRSS56 rs1656404 with both effects. For all associated SNPs the "direction of effect" was the same as in the original GWAS.[280,281]

The genetic risk score was very strongly associated with refractive error both at 7.5 years of age (β=0.018 D, 95% CI -0.012 to -0.024, *P*=2.2E-9) and with increasing age (β=0.003 D/yr, 95% CI -0.002 to 0.004, *P*=5.8E-14). By the age of 15 years, the model suggested that the 39 SNPs together would produce a more than 1.0 D difference in refractive error between participants carrying the lowest and highest number of risk alleles observed (Figure 2). At age 7.5 years the genetic risk score explained 0.6% of the variation in refractive error (N=4,566; *P*=6.6E-08); at age 15 years the corresponding figure was 2.3% (N=3,666; *P*=6.9E-21).

For validation we tested the genetic risk score and 12 of the 16 above SNPs (4 were nearly monomorphic in Asians) in the 8 multi-ethnic cross-sectional study cohorts (combined N=5,599; Table 1). The average age of the participants varied from 6.6 years-old in the STARS cohort to 20.0 years-old in RAINE. The genetic risk score and 4 SNPs – rs7744813 (KCNQ5), rs7837791 (*TOX*), rs8000973 (*ZIC2*) and rs17648524 (*A2BP1*) – were associated with refractive error (*P*<0.05; Table 3). All 4 SNPs had the expected direction of effect and none exhibited evidence of between-cohort heterogeneity. Interestingly, 3 of the 4 SNPs had evidence of both early-onset and later progressive effects in the discovery cohort. Meta-analysis summary plots for the genetic risk score and the individual SNPs tested for replication are presented in Figure S1 (online). There was suggestive evidence that SNPs had larger effect sizes in Asian than in European ancestry participants (Figure S2).

Tests in the Discovery Cohort for SNP x SNP interactions for all 741 possible pairs of the 39 SNPs revealed no evidence for interactions exceeding that expected by chance (not shown).

Table 1 Demographics of study samples. Values in brackets are standard deviations

Longitudinal cohort (N=5,200)

Study	Ethnicity	N	Female (%)	Age-at-baseline	Years follow-up
ALSPAC*	European	5200	51.0	7.5 (0.3)	7.0 (1.5)

Cross-sectional cohorts (N=5,599)

Study	Ethnicity	N	Female (%)	Age (years)	Refraction (D)
TEDS	European	698	56.0	16.2 (1.8)	-0.38 (1.70)
WESDR	European	289	50.5	17.7 (4.6)	-1.09 (1.79)
TEST	European	410	57.2	11.8 (5.0)	0.36 (1.24)
RAINE	European	754	50.9	20.0 (0.4)	-0.06 (1.53)
BATS	European	992	53.6	19.1 (3.2)	-0.33 (1.42)
GZT	Asian	1055	51.8	15.6 (2.8)	-1.97 (2.49)
SCORM	Asian	994	48.4	7.5 (0.9)	-0.55 (1.73)
STARS	Asian	407	49.4	6.6 (3.9)	-2.00 (2.09)

*Refraction details at each age for the longitudinal cohort are provided in the supplementary material (Table S8).

Table 2 Age-of-onset of SNP associations with refractive error in the discovery cohort (ALSPAC)

					SNP main effect at baseline (D)			SNP x Age interaction (D/yr)		
Marker	Chr	Gene	RA	RAF	Beta	SE	*P*	Beta	SE	*P*
GR Score	–	–	–	–	**-0.018**	**0.003**	**2.2E-09**	-0.003	0.000	5.8E-14
rs1652333	1	*CD55*	G	0.32	-0.002	0.019	9.3E-01	-0.005	0.003	4.0E-02
rs1656404	2	*PRSS56*	A	0.21	**-0.066**	**0.024**	**5.7E-03**	-0.008	0.003	1.3E-02
rs1881492	2	*CHRNG*	T	0.23	**-0.058**	**0.024**	**1.7E-02**	-0.005	0.003	1.5E-01
rs14165	3	*CACNA1D*	G	0.70	**-0.040**	**0.020**	**4.2E-02**	-0.001	0.003	7.7E-01
rs7744813	6	*KCNQ5*	A	0.59	**-0.048**	**0.019**	**9.9E-03**	-0.005	0.003	3.5E-02
rs12205363	6	*LAMA2*	T	0.92	**-0.097**	**0.035**	**5.7E-03**	-0.008	0.005	1.2E-01
rs7837791	8	*TOX*	G	0.53	**-0.045**	**0.018**	**1.1E-02**	-0.005	0.002	2.7E-02
rs4237036	8	*CHD7*	T	0.66	0.020	0.019	2.9E-01	-0.007	0.003	5.6E-03
rs7042950	9	*RORB*	G	0.22	0.018	0.022	4.1E-01	-0.009	0.003	2.5E-03
rs6480859	10	*KCNMA1*	T	0.37	-0.029	0.018	1.1E-01	-0.008	0.002	1.3E-03
rs10882165	10	*CYP26A1*	T	0.40	**-0.035**	**0.018**	**4.8E-02**	0.001	0.003	7.6E-01
rs8000973	13	*ZIC2*	C	0.52	**-0.042**	**0.018**	**1.8E-02**	-0.008	0.002	1.5E-03
rs66913363	14	*BMP4*	G	0.51	**-0.051**	**0.018**	**5.3E-03**	0.001	0.003	7.2E-01
rs524952	15	*GJD2*	A	0.46	-0.018	0.018	3.3E-01	-0.008	0.003	8.8E-04
rs17648524	16	*A2BP1*	C	0.33	-0.001	0.019	9.4E-01	-0.007	0.003	5.6E-03
rs2969180	17	*SHISA6*	A	0.35	**-0.039**	**0.019**	**3.9E-02**	-0.005	0.003	4.9E-02

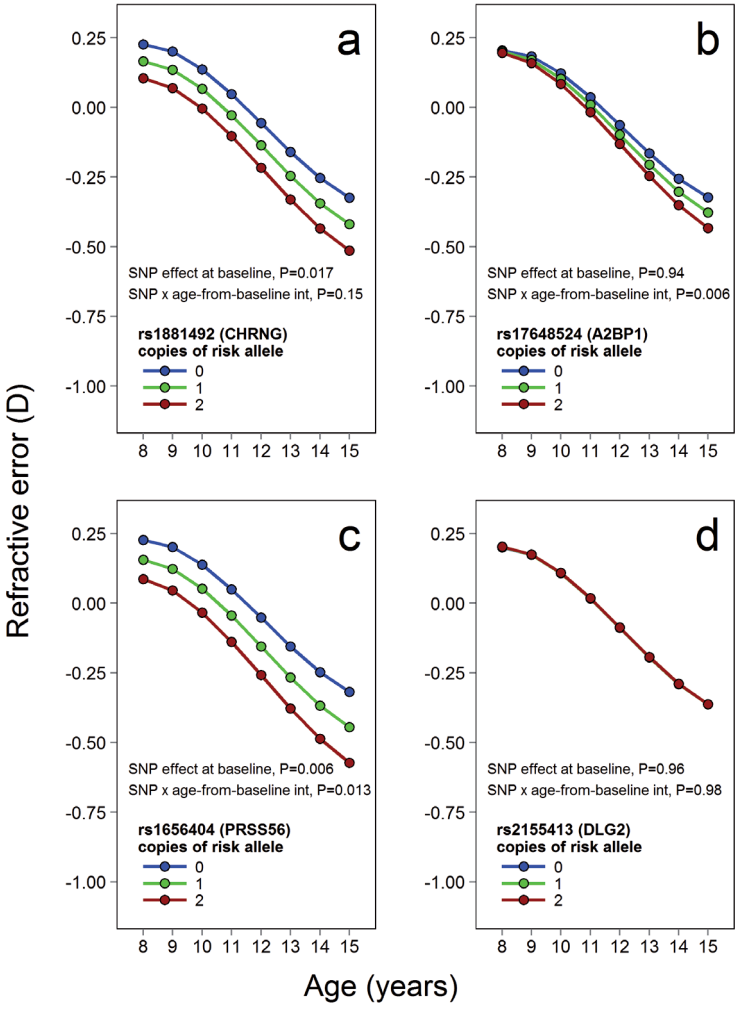
Associations were tested at baseline (age of 7.5 years-old) and over the next 7 years (SNP x Age interaction). Results for all 39 SNPs are shown in Table S2. Abbreviations: Chr=Chromosome. GR=Genetic risk. RA=Risk allele. RAF=Risk allele frequency.

Table 3 Replication meta-analysis results for SNP main effects

				Europeans (N=3,143)				Asians (N=2,456)				Europeans + Asians (N=5,599)				
Marker	**Chr**	**Gene**	**RA**	*RAF*	**Beta**	**SE**	*P*	*RAF**	**Beta**	**SE**	*P*	I^2	*Het_P*	**Beta**	**SE**	*P*
GR Score	–	–	–	–	**-0.026**	**0.007**	**3.8E-04**	–	**-0.048**	**0.011**	**1.4E-05**	0.57	***0.023***	**-0.034**	**0.006**	**1.4E-08**
rs1652333	1	*CD55*	G	*0.32*	0.042	0.042	0.315	*0.52*	-0.101	0.056	0.073	0.27	*0.210*	-0.004	0.034	0.899
rs1881492	2	*CHRNG*	T	*0.23*	-0.001	0.054	0.986	*0.12*	0.197	0.102	0.054	0.00	*0.926*	0.033	0.048	0.483
rs7744813	6	*KCNQ5*	A	*0.59*	**-0.110**	**0.042**	**0.008**	*0.81*	0.001	0.071	0.993	0.41	*0.107*	**-0.083**	**0.036**	**0.021**
rs7837791	8	*TOX*	G	*0.53*	0.011	0.040	0.772	*0.53*	**-0.185**	**0.055**	**0.001**	0.49	*0.059*	**-0.063**	**0.032**	**0.049**
rs4237036	8	*CHD7*	T	*0.66*	-0.077	0.041	0.062	*0.74*	0.102	0.069	0.140	0.40	*0.112*	-0.033	0.035	0.358
rs7042950	9	*RORB*	G	*0.22*	0.041	0.047	0.391	*0.74*	-0.004	0.070	0.956	0.00	*0.903*	0.020	0.039	0.618
rs6480859	10	*KCNMA1*	T	*0.37*	-0.022	0.041	0.579	*0.16*	**-0.229**	**0.074**	**0.002**	0.52	*0.042*	-0.063	0.036	0.075
rs8000973	13	*ZIC2*	C	*0.52*	-0.067	0.040	0.093	*0.21*	-0.092	0.070	0.190	0.00	*0.470*	**-0.081**	**0.035**	**0.019**
rs66913363	14	*BMP4*	G	*0.51*	-0.021	0.044	0.628	*0.73*	0.061	0.066	0.354	0.00	*0.790*	0.002	0.037	0.953
rs524952	15	*GJD2*	A	*0.46*	-0.008	0.041	0.839	*0.48*	**-0.171**	**0.057**	**0.003**	0.53	***0.036***	-0.064	0.033	0.058
rs17648524	16	*A2BP1*	C	*0.33*	**-0.143**	**0.042**	**7.2E-04**	*0.06*	-0.140	0.106	0.186	0.49	*0.057*	**-0.146**	**0.039**	**2.0E-04**
rs2969180	17	*SHISA6*	A	*0.35*	0.028	0.042	0.499	*0.51*	-0.036	0.056	0.521	0.00	*0.553*	0.003	0.033	0.926

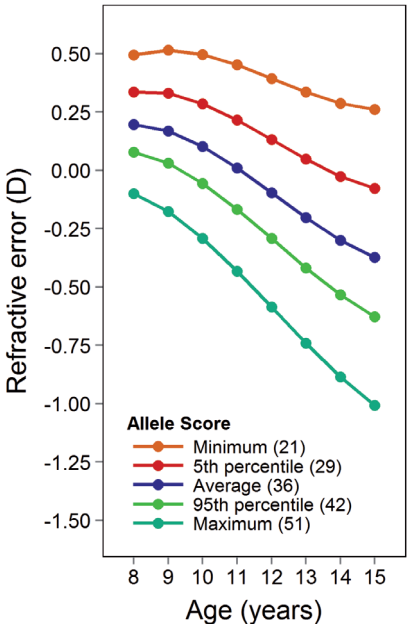
SNPs associated with refractive error in the ALSPAC age-of-onset analyses were tested for association with refractive error in 8 independent cohorts of children (5 European ancestry, 3 Asian ancestry). Abbreviations: Chr=Chromosome. GR Score= Genetic risk score. RA=Risk allele. RAF=Risk allele frequency. *SNPs with minor allele frequencies <0.05 were not examined due to low statistical power.

Figure 1 SNPs associated with early-onset and later-onset effects on refractive development during childhood



Analyses were carried out using data from longitudinal eye examinations in 5,200 ALSPAC participants. Each panel shows how refractive error trajectory varied with SNP genotype, for 4 different SNPs: rs1881492, rs17648524, rs1656404 and rs2155413. The lines in each panel show the refractive error trajectories predicted by the best-fit linear mixed model (LMM) for participants carrying the number of risk alleles indicated (0, 1 or 2). The SNPs in panels a & c showed an association with refractive error at baseline, i.e. evidence of early onset in childhood. The SNPs in panels b & c showed an age-dependent interaction with refractive error over later childhood. The SNP in panel D did not show evidence of effects during childhood.

Figure 2 Association between a genetic risk score for 39 SNPs and refractive error trajectories in ALSPAC participants



The genetic risk score was calculated as the sum of the number of risk alleles (0 2) carried by an individual at each of the 39 myopia-susceptibility SNPs. The coloured lines show the trajectories for children carrying the number of risk alleles indicated, as predicted by the best-fit linear mixed model.

Interactions with time engaged in nearwork

Two types of interactions between SNP genotype and nearwork exposure were evaluated in the ALSPAC discovery cohort: An interaction already present at the baseline age of 7.5 years-old (a 2way SNP x nearwork interaction) and an interaction that developed progressively during later childhood (a 3way, SNP x nearwork x age-from-baseline interaction). For a SNP with a risk allele frequency of 0.25, and ignoring the repeated measures nature of the data, the analysis of ALSPAC participants had >90% power to detect an interaction effect of 0.25 D at α=0.05 (and >50% power at α=1.28E-3, corresponding to a Bonferroni correction for testing all 39 SNPs).

Nominal support for 3-way SNP x nearwork x age-from-baseline interactions was observed for 4 markers (Figure 3A-D): rs17428076 upstream of *DLX1* (P=0.049), rs7829127 within *ZMAT4* (P=6.3E-04), rs7084402 upstream of *BICC1* (P=0.043) and rs17648524 within *A2BP1* (P=2.3E-03). In models that considered just 2-way interactions at baseline, only rs1254319 upstream of SIX6 showed nominal evidence of an interaction (P=0.042; Figure 3E). Of these 5 interactions, only that involving rs7829127 (ZMAT4) survived correction for multiple testing (corrected P=0.025). Consistent with

the limited evidence for individual SNP x nearwork interactions, no evidence of interaction between the genetic risk score and ALSPAC children's level of nearwork was observed (2-way interaction, $P=0.20$; 3-way interaction, $P=0.086$).

Four of the cross-sectional study cohorts, 1 of European ancestry (TEDS) and 3 of Asian ancestry (GZT, SCORM and STARS), had information available regarding the time participants spent engaged in nearwork (Table S6), allowing tests for replication. In the meta-analysis of all 4 replication studies (Table S3) none of the SNPs that showed nominal evidence of an interaction with nearwork in the ALSPAC discovery cohort showed evidence of replication (all $P>0.16$). Likewise, the genetic risk score did not show evidence of an interaction with nearwork in the cross-sectional cohorts ($P=0.49$).

Interactions with time spent outdoors

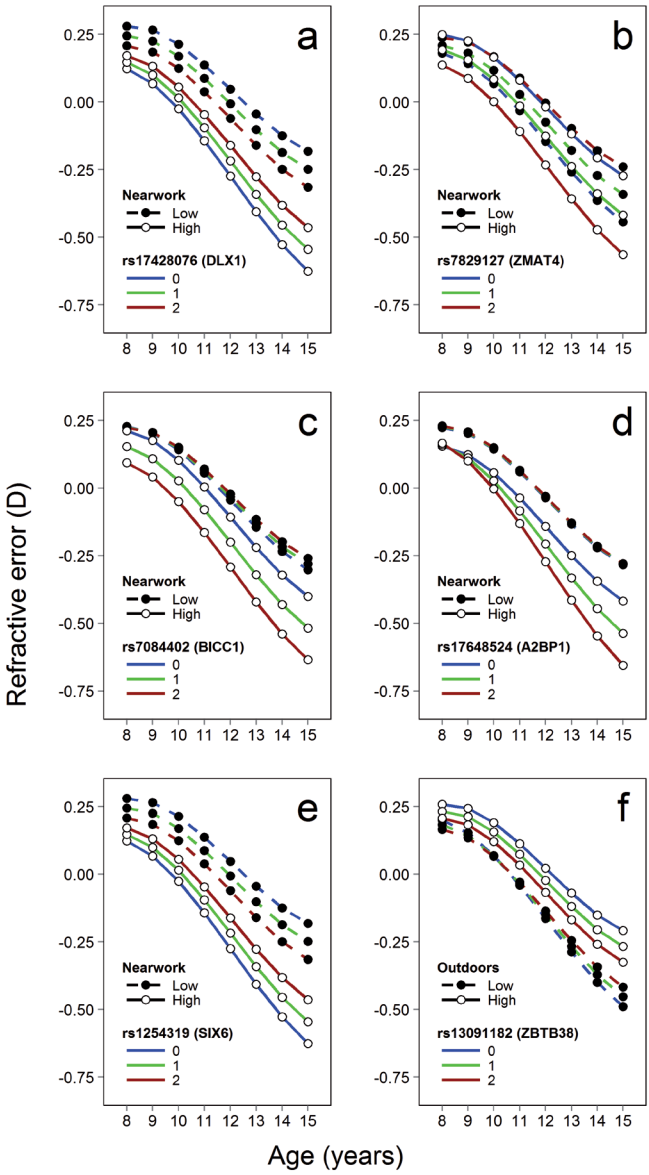
In the discovery cohort, only rs13091182 within *ZBTB38* showed nominal evidence of a 3-way interaction involving time outdoors (uncorrected $P=0.028$; corrected $P>0.05$; Figure 3F). Surprisingly, the risk allele of rs13091182 was associated with *slower* progression towards myopia (or less hyperopia) in general and with faster progression towards myopia in children who spent *more* time outdoors, suggesting a potentially false-positive result. There was no evidence for 2-way SNP x time outdoors interactions (uncorrected $P>0.20$ for all 39 SNPs). Similarly, for the genetic risk score, there was no indication of an interaction with time spent outdoors (2-way interaction, $P=0.16$; 3-way interaction, $P=0.49$).

Five of the cross-sectional samples had information available on the time participants spent outdoors (TEDS, RAINE, GZT, SCORM and STARS). The single SNP, rs13091182, showing evidence of an interaction with time outdoors in the discovery cohort showed no evidence of replication (indeed, none of the 31 SNPs with MAF >0.05 in both ancestry groups showed evidence of an interaction with time outdoors; all $P>0.17$; Table S4). Similarly, the genetic risk score did not show evidence of an interaction with time spent outdoors in the replication cohorts.

Pathway analysis

Pathway analysis identified a single functional pathway for the set of 10 genes (Table 2) implicated in having early-onset effects, namely "*hedgehog signalling*" (Panther $P=0.043$; key genes *ZIC2* and *BMP4*). The set of 11 genes implicated in having later-onset effects did not show enrichment for specific pathways.

Figure 3 Refractive error trajectories in ALSPAC participants for SNPs showing evidence of an interaction with nearwork or time outdoors



Levels of nearwork activity and time spent outdoors were assessed at 8-9 years of age and classified as high or low (above or below the median level). Panels a-d show how refractive error trajectories varied depending on nearwork level and the number of risk alleles (0 – 2) carried for 4 different markers that showed SNP x nearwork x age-from-baseline (3-way) interactions. Panel e: Refractive trajectories for the only marker to show a SNP x nearwork (2-way) interaction at baseline age. Panel f: Refractive trajectories for the only marker to show a SNP x time outdoors x age-from-baseline (3-way) interaction. The coloured lines show the trajectories predicted by the best-fit linear mixed model for children carrying the number of copies of the risk allele indicated in the legend.

DISCUSSION

Early-onset and later-onset SNP effects

Sixteen SNPs showed evidence of effects in childhood in ALSPAC participants (Table 2); 10 SNPs had early-onset effects manifest by age 7.5 years, 11 SNPs had later-onset effects, and 5 SNP had early-onset effects that progressed further during later childhood. For the 12 of these 16 SNPs available in the cross-sectional cohorts, 4 showed evidence of replication (Table 3). There was suggestive evidence that SNP effect sizes were approximately 2 times larger in Asian as compared to European ancestry children/adolescents (Figure S2). A genetic risk score that captured the effects of all 39 GWAS-identified variants confirmed the involvement of genetic influences acting at an early age (7.5 years) and then increasing further in magnitude across later childhood.

We sought to discover whether the early-onset and later-onset variants clustered according to functional pathway (for example, if GWAS SNPs A and B are causal variants that affect the expression levels of genes X and Y, respectively, and X acts downstream of Y to regulate refractive development, then one might expect the onset age for SNPs A and B to coincide). However, as summarised in Table 4, SNPs associated with early-onset or later-onset effects did not clearly cluster according to the known function(s) of the genes implicated in mediating the SNPs' effects. Pathway analysis confirmed this impression, with only a single functional pathway being identified. Potential reasons for this lack of functional clustering are, first, that many genes in the genome have diverse functions, which are sometimes poorly understood. For instance, during development of the human visual system, an ion channel may play a vital role during early embryonic development of the retina, be a necessary component of the visual cycle, and yet also contribute to neuronal plasticity. Second, precisely which gene or genes mediate the effect of a specific GWAS-identified SNP is not known with certainty for any of the refractive error GWAS SNPs identified to date: While the nearest gene to a GWAS SNP is usually considered the most likely to be involved, this does not always hold true.[310]

The 39 SNPs examined were identified in adult GWAS meta-analyses with sample sizes of approximately 45,000 individuals, and all had small effects (typically 0.1 D per copy of the risk allele). The ALSPAC longitudinal cohort (N=5,200) had ~80% power to detect an association for a SNP with an effect size of 0.1 D and MAF of 0.25 (but note that the true power would likely have been lower because: refractive development would not be complete by 15 years of age, our models tested primarily for yearly effects rather than cumulative effects, and the "winner's curse" phenomenon,[311] i.e. the over-estimation of effect sizes in the original GWAS investigations). Therefore, a likely reason why some of the 39 SNPs we studied failed to show childhood-onset associations in the longitudinal cohort is limited statistical power. Thus, we cannot conclude that the SNPs that did not show observable childhood-onset associations have an age-of-onset beyond 15 years-old even though they might well do: much larger studies will be required to definitively address this issue. Similarly, the limited concordance between the longitudinal and cross-sectional studies was also likely due to limited statistical power, although 8 of the 12 SNPs tested for replication showed the expected direction of effect (Table 3).

Table 4 Summary of findings. SNPs with evidence ($P<0.05$) of early-onset, later onset, or GxE interaction effects on refractive error in one or more analysis are highlighted

SNP	Gene	Role	Longitudinal Early-onset	Longitudinal Later-onset	Cross-sectional	Interaction
GR score	–	–	Y	Y	Y	
rs7837791	*TOX*	ED	Y	Y	Y	
rs4237036	*CHD7*	ED		Y		
rs7084402	*BICC1*	ED				NW
rs8000973	*ZIC2*	ED	Y	Y	Y	
rs66913363	*BMP4*	ED	Y			
rs1254319	*SIX6*	ED				NW
rs1656404	*PRSS56*	ED, EM	Y	Y		
rs17428076	*DLX1*	ED,NP				NW
rs12205363	*LAMA2*	EM	Y			
rs1652333	*CD55*	IT		Y		
rs1881492	*CHRNG*	IT	Y			
rs14165	*CACNA1D*	IT	Y			
rs6480859	*KCNMA1*	IT		Y		
rs7744813	*KCNQ5*	IT,VC	Y	Y	Y	
rs17648524	*A2BP1*	NP		Y	Y	NW
rs13091182	*ZBTB38*	U				TO
rs9307551	*LOC100506035*	U				NW
rs7829127	*ZMAT4*	U				NW
rs2969180	*SHISA6*	U	Y	Y		
rs7042950	*RORB*	VC		Y		
rs10882165	*CYP26A1*	VC	Y			
rs524952	*GJD2*	VC		Y		

Abbreviations: Y=Yes, NW=Nearwork, TO=Time outdoors, VC = Visual cycle, NP = Neuronal plasticity, IT = Ion transport, EM = Extracellular matrix, ED = Eye development, U = Unknown.

Interactions with environmental exposures

In general there was scant evidence for GxE interactions, especially for SNP x time spent outdoors effects. Given the expected power of >90% to detect interaction effects with a magnitude 0.25 D or more, this argues against SNP x nearwork or SNP x time outdoors interactions of this size being present for the majority of variants studied, rather than lack of statistical power precluding their discovery.

In the ALSPAC longitudinal analysis the gene-environment interaction between ZMAT4 SNP rs7829127 genotype and nearwork survived correction for multiple-testing ($P_{corr}=0.025$). Although this interaction was not replicated in the cross-sectional meta-analyses, variants at this locus have previously been reported to show an interaction with

the duration of education in a meta-analysis of 5 studies from Singapore (SNP x education interaction=-0.42 D, 95% C.I. -0.15 to -0.69, $P=0.002$)[301]. We did not explore interactions between SNPs and years of education, since in several cohorts the participants were still students. The functional role of ZMAT4 is not known.

Why might GxE interactions involving these 39 SNPs be so scarce? First, differences in environmental risk exposures were not considered in the original GWAS investigations carried out by CREAM[281] and 23andMe.[280] Thus, SNPs with strong interaction effects but no main effects may not have been detected using those GWAS designs. Second, the age range and ethnic diversity of the original GWAS discovery samples were highly varied. Given the substantial increase in the prevalence of myopia in the past few decades, which strongly implicates a major role for environmental risk factors, it seems almost certain that the individuals studied in the CREAM and 23andMe GWAS meta-analyses would have grown up in environments with a wide range of risk exposure profiles depending on the participants' years of birth: young (recently born) individuals would have been exposed to a much more myopiagenic environment than older (more distantly born) adults. Therefore, a variant that increases the risk of myopia only in children who perform excessive nearwork may have shown an (apparent) main effect association with refractive error in a GWAS carried out in a young adult cohort, in which participants were ubiquitously exposed to high nearwork during childhood. However, this same variant may not have shown an association with refractive error in a GWAS on an older cohort, due to the lower nearwork exposure during childhood of the older individuals. Thus, support for the association of such a variant in the CREAM and 23andMe GWAS samples may have been diluted rather than strengthened during the meta-analysis of younger and older cohorts.

Separate from tests for gene-environment interactions, time spent outdoors itself was not associated with myopia in 3 of the 5 cross-sectional studies (GZT, STARS, and TEDS) and the association was of borderline significance in another (TEDS). This lack of an association with time outdoors implies that detecting a SNP x time outdoors interaction would also have been challenging, even after meta-analysis of data from all 5 cohorts.

Interestingly, a large-effect GxE interaction predisoposing children to myopia was identified recently, involving a rare variant at the *APLP2* gene locus and time spent reading.[303] APLP2 was implicated in myopia development through studies in an animal model,[312] which – given the statistical challenge of identifying GxE interaction effects in human populations – suggests that combining findings from animal models and human studies could be a fruitful future approach.

We reasoned that correction for multiple testing *was not* appropriate when examining the age-of-onset of the 39 SNPs investigated, because of compelling existing evidence that by adulthood these SNPs truly are associated with refractive error. That is, our analyses sought to discover whether or not each SNP had an effect during childhood, not whether a group of candidate SNPs were associated with refractive error *per se*. By contrast, in view of very limited evidence for interactions with environmental exposures for most of the SNPs examined, correction for multiple testing was considered appropriate when evaluating SNP x nearwork and SNP x time outdoors interactions: In these analyses, a large number of independent hypothesis tests were carried out, with little or no prior knowledge that an interaction must be present at some age.

Limitations of the present work

The present work had a number of other limitations. The cross-sectional samples were not matched for age, which prevented us from testing for "early" and "later" onset effects in the replication stage. The level of exposure to nearwork and time outdoors also varied across samples, which meant that imprecisely-matched interaction effects were meta-analysed, potentially reducing statistical power. We chose to categorise time spent performing nearwork and time spent outdoors relative to the median activity level in each study sample because the measurement scales used in the various studies were not standardised (precluding the use of an absolute measure). If in reality these environmental risk factors exert their influence non-linearly – for instance if spending more than a certain threshold number of hours per day outdoors is needed to protect against myopia development – then our approach may have poorly captured the effects of the environmental exposures. For the combined meta-analysis of European and Asian cross-sectional studies, we assumed that each lead SNP tagged the underlying causal variant(s) equally well in European and Asian ancestry individuals, which is an oversimplification. Finally, we chose to examine only a simple, binary GxE model, whereas more complex scenarios may exist.[313-315]

CONCLUSIONS

Specific myopia-predisposing SNPs were found to differ in the age at which they had their effects, and whether or not these effects got progressively stronger during later childhood. Thus, SNPs implicating the genes *CHRNG, CACNA1D, LAMA2, CYP26A1* and *BMP4* were associated with early onset changes in refractive error that did not progress further, while SNPs close to *PRSS56*, *KCNQ5, TOX, ZIC2* and *SHISA6* showed early-onset effects that became greater still at older ages. Effects that only appeared in later childhood – after the age of 7.5 years – implicated the genes *CD55, CHD7, RORB, KCNMA1, A2BP1* and *GJD2*. Gene-environment interactions involving nearwork or time outdoors were rare or absent for the vast majority of the GWAS-identified SNPs, and indeed a genetic risk score that demonstrated very convincing association with early-onset (P=2.2E-9) and later progressive (P=5.8E-14) changes in refractive error appeared to act independently of the time children spent in these activities. However, one robust interaction between rs7829127 in ZMAT4 and time spent performing nearwork (nominal P=6.3E-04, corrected P=0.025) was observed, replicating a previously-identified interaction involving rs7829127 and years of education.[301]

Detailed acknowledgments and online resources can be found in the published article online: https://www.nature.com/articles/srep25853

SUPPLEMENTAL MATERIAL

Supplementary Table S1 SNPs examined

SNP	Chr	Pos	Gene	Citation
rs1652333	1	207470460	*CD55*	Verhoeven et al. 2013
rs4373767	1	219759682	*ZC3H11B*	Cheng et al. 2013
rs17412774	2	146773948	*PABPCP2*	Kiefer et al. 2013
rs17428076	2	172851936	*DLX1*	Kiefer et al. 2013
rs1898585	2	178660450	*PDE11A*	Kiefer et al. 2013
rs1656404	2	233379941	*PRSS56*	Verhoeven et al. 2013
rs1881492	2	233406998	*CHRNG*	Verhoeven et al. 2013
rs14165	3	53847408	*CACNA1D*	Verhoeven et al. 2013
rs13091182	3	141133960	*ZBTB38*	Kiefer et al. 2013
rs9307551	4	80530671	*LOC100506035*	Verhoeven et al. 2013
rs5022942	4	81959966	*BMP3*	Kiefer et al. 2013
rs7744813	6	73643289	*KCNQ5*	Verhoeven et al. 2013
rs12205363	6	129834628	*LAMA2*	Verhoeven et al. 2013
rs7829127	8	40726394	*ZMAT4*	Verhoeven et al. 2013
rs7837791	8	60179086	*TOX*	Verhoeven et al. 2013
rs4237036	8	61701057	*CHD7*	Verhoeven et al. 2013
rs11145465	9	70989531	*TJP2*	Verhoeven et al. 2013
rs7042950	9	77149837	*RORB*	Verhoeven et al. 2013
rs7084402	10	60265404	*BICC1*	Verhoeven et al. 2013
rs6480859	10	79081948	*KCNMA1*	Kiefer et al. 2013
rs745480	10	85986554	*RGR*	Kiefer et al. 2013
rs10882165	10	94924324	*CYP26A1*	Verhoeven et al. 2013
rs1381566	11	40149607	*LRRC4C*	Kiefer et al. 2013
rs2155413	11	84634790	*DLG2*	Kiefer et al. 2013
rs11601239	11	105556598	*GRIA4*	Verhoeven et al. 2013
rs3138144	12	56114768	*RDH5*	Verhoeven et al. 2013
rs12229663	12	71249996	*PTPRR*	Verhoeven et al. 2013
rs8000973	13	100691367	*ZIC2*	Verhoeven et al. 2013
rs2184971	13	100818092	*PCCA*	Verhoeven et al. 2013
rs66913363	14	54413001	*BMP4*	Kiefer et al. 2013
rs1254319	14	60903757	*SIX6*	Verhoeven et al. 2013
rs524952	15	35005885	*GJD2*	Verhoeven et al. 2013
rs4778879	15	79372875	*RASGRF1*	Verhoeven et al. 2013
rs17648524	16	7459683	*A2BP1*	Verhoeven et al. 2013
rs2969180	17	11407901	*SHISA6*	Verhoeven et al. 2013
rs17183295	17	31078272	*MYO1D*	Verhoeven et al. 2013
rs4793501	17	68718734	*KCNJ2*	Verhoeven et al. 2013
rs12971120	18	72174023	*CNDP2*	Verhoeven et al. 2013
rs235770	20	6761765	*BMP2*	Verhoeven et al. 2013

Supplementary Table S2 Age-of-onset of SNP associations in discovery cohort (ALSPAC)

					SNP main effect at baseline (D)			SNP x Age interaction (D/yr)		
Marker	**Chr**	**Gene**	**RA**	***RAF***	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**
GR Score	–	–	–	–	-0.018	0.003	2.2E-09	-0.003	0.000	5.8E-14
rs1652333	1	*CD55*	G	0.32	-0.002	0.019	9.3E-01	-0.005	0.003	4.0E-02
rs4373767	1	*ZC3H11B*	T	0.38	-0.005	0.018	8.0E-01	-0.001	0.003	7.9E-01
rs17412774	2	*PABPCP2*	A	0.57	-0.026	0.018	1.5E-01	-0.004	0.003	1.7E-01
rs17428076	2	*DLX1*	C	0.74	-0.026	0.021	2.1E-01	0.000	0.003	8.7E-01
rs1898585	2	*PDE11A*	T	0.17	0.005	0.025	8.3E-01	-0.006	0.003	1.1E-01
rs1656404	2	*PRSS56*	A	0.21	-0.066	0.024	5.7E-03	-0.008	0.003	1.3E-02
rs1881492	2	*CHRNG*	T	0.23	-0.058	0.024	1.7E-02	-0.005	0.003	1.5E-01
rs14165	3	*CACNA1D*	G	0.70	-0.040	0.020	4.2E-02	-0.001	0.003	7.7E-01
rs13091182	3	*ZBTB38*	G	0.67	-0.032	0.019	8.4E-02	0.001	0.003	6.4E-01
rs9307551	4	*LOC100506035*	A	0.20	-0.026	0.022	2.4E-01	-0.005	0.003	1.3E-01
rs5022942	4	*BMP3*	A	0.22	-0.003	0.021	8.7E-01	-0.004	0.003	1.8E-01
rs7744813	6	*KCNQ5*	A	0.59	-0.048	0.019	9.9E-03	-0.005	0.003	3.5E-02
rs12205363	6	*LAMA2*	T	0.92	-0.097	0.035	5.7E-03	-0.008	0.005	1.2E-01
rs7829127	8	*ZMAT4*	A	0.75	-0.006	0.022	7.7E-01	0.002	0.003	4.2E-01
rs7837791	8	*TOX*	G	0.53	-0.045	0.018	1.1E-02	-0.005	0.002	2.7E-02
rs4237036	8	*CHD7*	T	0.66	0.020	0.019	2.9E-01	-0.007	0.003	5.6E-03
rs11145465	9	*TJP2*	A	0.21	-0.036	0.021	9.6E-02	-0.004	0.003	2.4E-01
rs7042950	9	*RORB*	G	0.22	0.018	0.022	4.1E-01	-0.009	0.003	2.5E-03
rs7084402	10	*BICC1*	G	0.49	-0.019	0.018	3.0E-01	-0.001	0.003	7.7E-01
rs6480859	10	*KCNMA1*	T	0.37	-0.029	0.018	1.1E-01	-0.008	0.002	1.3E-03
rs745480	10	*RGR*	G	0.48	-0.021	0.018	2.3E-01	-0.003	0.002	2.6E-01
rs10882165	10	*CYP26A1*	T	0.40	-0.035	0.018	4.8E-02	0.001	0.003	7.6E-01
rs1381566	11	*LRRC4C*	G	0.18	-0.023	0.026	3.8E-01	-0.002	0.004	5.6E-01
rs2155413	11	*DLG2*	A	0.45	0.001	0.018	9.6E-01	0.000	0.002	9.8E-01
rs11601239	11	*GRIA4*	C	0.49	0.004	0.018	8.0E-01	-0.001	0.002	6.9E-01
rs3138144	12	*RDH5*	G	0.54	-0.027	0.021	1.9E-01	-0.002	0.003	5.2E-01
rs12229663	12	*PTPRR*	A	0.76	-0.033	0.022	1.3E-01	0.000	0.003	8.8E-01
rs8000973	13	*ZIC2*	C	0.52	-0.042	0.018	1.8E-02	-0.008	0.002	1.5E-03
rs2184971	13	*PCCA*	A	0.60	0.002	0.018	8.9E-01	0.000	0.002	9.1E-01
rs66913363	14	*BMP4*	G	0.51	-0.051	0.018	5.3E-03	0.001	0.003	7.2E-01
rs1254319	14	*SIX6*	A	0.29	-0.011	0.020	5.8E-01	-0.002	0.003	3.8E-01
rs524952	15	*GJD2*	A	0.46	-0.018	0.018	3.3E-01	-0.008	0.003	8.8E-04
rs4778879	15	*RASGRF1*	G	0.42	-0.017	0.018	3.7E-01	-0.004	0.003	9.4E-02
rs17648524	16	*A2BP1*	C	0.33	-0.001	0.019	9.4E-01	-0.007	0.003	5.6E-03
rs2969180	17	*SHISA6*	A	0.35	-0.039	0.019	3.9E-02	-0.005	0.003	4.9E-02
rs17183295	17	*MYO1D*	T	0.19	0.006	0.023	7.8E-01	-0.004	0.003	1.5E-01
rs4793501	17	*KCNJ2*	T	0.53	0.000	0.018	9.8E-01	-0.002	0.003	4.2E-01
rs12971120	18	*CNDP2*	A	0.82	0.017	0.021	4.1E-01	-0.003	0.003	3.2E-01
rs235770	20	*BMP2*	T	0.37	-0.010	0.019	5.8E-01	-0.005	0.003	5.3E-02

Abbreviations: Chr=Chromosome. RA=Risk allele. RAF=Risk allele frequency.

Supplementary Table S3 Meta-analysis of SNP x near work interaction effects in cross-sectional cohorts

SNP	Chr	Gene	RA	Beta	SE	P	I^2	PQ-test
Allele score	–	–	A	-0.014	0.021	0.489	0	0.584
rs1652333	1	*CD55*	G	-0.049	0.108	0.649	0	0.460
rs4373767	1	*ZC3H11B*	T	-0.217	0.116	0.061	0	0.979
rs17412774	2	*PABPCP2*	A	0.157	0.114	0.169	0	0.877
rs1898585	2	*PDE11A*	T	-0.189	0.117	0.108	0	0.769
rs1881492	2	*CHRNG*	T	0.253	0.185	0.170	0	0.609
rs9307551	4	*LOC100506035*	A	-0.237	0.113	0.035	9	0.348
rs5022942	4	*BMP3*	A	-0.088	0.117	0.450	0	0.621
rs7744813	6	*KCNQ5*	A	0.251	0.134	0.061	0	0.856
rs7829127	8	*ZMAT4*	A	-0.104	0.166	0.529	55	0.084
rs7837791	8	*TOX*	G	-0.031	0.106	0.771	9	0.351
rs4237036	8	*CHD7*	T	-0.133	0.129	0.304	43	0.152
rs7042950	9	*RORB*	G	0.009	0.133	0.946	0	0.927
rs7084402	10	*BICC1*	G	-0.002	0.108	0.985	0	0.915
rs6480859	10	*KCNMA1*	T	-0.242	0.135	0.073	0	0.832
rs745480	10	*RGR*	G	0.020	0.109	0.854	0	0.712
rs1381566	11	*LRRC4C*	G	-0.060	0.129	0.644	0	0.502
rs2155413	11	*DLG2*	A	0.215	0.138	0.120	28	0.379
rs11601239	11	*GRIA4*	C	-0.008	0.111	0.943	0	0.765
rs3138144	12	*RDH5*	G	-0.083	0.170	0.625	0	0.409
rs12229663	12	*PTPRR*	A	0.042	0.111	0.704	0	0.832
rs8000973	13	*ZIC2*	C	-0.039	0.128	0.759	0	0.581
rs2184971	13	*PCCA*	A	0.091	0.127	0.473	0	0.896
rs66913363	14	*BMP4*	G	0.205	0.125	0.099	0	0.403
rs1254319	14	*SIX6*	A	-0.078	0.120	0.513	0	0.698
rs524952	15	*GJD2*	A	-0.033	0.110	0.761	15	0.317
rs4778879	15	*RASGRF1*	G	0.033	0.110	0.766	0	0.631
rs17648524	16	*A2BP1*	C	0.178	0.176	0.312	22	0.279
rs2969180	17	*SHISA6*	A	0.010	0.108	0.927	0	0.435
rs4793501	17	*KCNJ2*	T	0.047	0.110	0.671	56	0.078
rs12971120	18	*CNDP2*	A	-0.049	0.120	0.682	0	0.581
rs235770	20	*BMP2*	T	-0.031	0.131	0.814	0	0.847

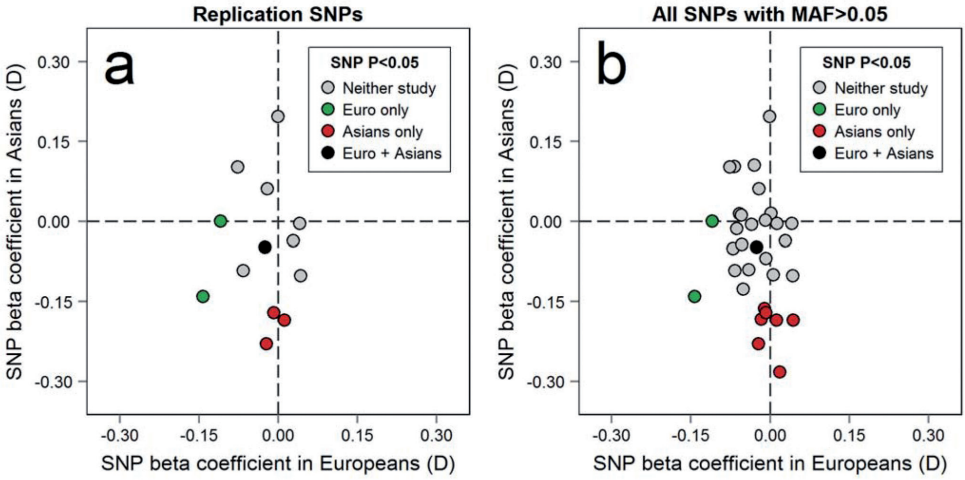
Beta shows the difference in refractive error (D) associated with each copy of the risk allele in individuals exposed to high versus low levels of nearwork. Meta-analysis was conducted for 4 cohorts (TEDS, GZT, SCORM and STARS) combined N=3,154. Abbreviations: Chr=Chromosome. RA=Risk allele. I2=Heterogeneity statistic. PQ-test=*P*-value for Cochran's Q-test.

Supplementary Table S4 Meta-analysis of SNP x time outdoors interaction effects in cross-sectional cohorts

SNP	Chr	Gene	RA	Beta	SE	P	I^2	PQ-test
Allele score	–	–	A	-0.003	0.019	0.892	29	0.231
rs1652333	1	*CD55*	G	0.108	0.104	0.301	2	0.394
rs4373767	1	*ZC3H11B*	T	0.132	0.104	0.202	0	0.974
rs17412774	2	*PABPCP2*	A	0.064	0.107	0.549	0	0.841
rs1898585	2	*PDE11A*	C	-0.038	0.120	0.754	0	0.706
rs1881492	2	*CHRNG*	G	0.011	0.156	0.946	48	0.101
rs9307551	4	*LOC100506035*	C	0.088	0.110	0.421	0	0.675
rs5022942	4	*BMP3*	G	0.028	0.114	0.804	0	0.550
rs7744813	6	*KCNQ5*	A	-0.097	0.116	0.404	8	0.361
rs7829127	8	*ZMAT4*	A	0.015	0.137	0.915	0	0.951
rs7837791	8	*TOX*	T	-0.032	0.099	0.746	0	0.528
rs4237036	8	*CHD7*	T	-0.081	0.114	0.477	0	0.927
rs7042950	9	*RORB*	A	0.101	0.122	0.411	0	0.708
rs7084402	10	*BICC1*	G	0.009	0.103	0.928	0	0.864
rs6480859	10	*KCNMA1*	C	-0.157	0.113	0.165	0	0.663
rs745480	10	*RGR*	C	-0.070	0.100	0.486	0	0.492
rs1381566	11	*LRRC4C*	T	-0.121	0.141	0.388	23	0.269
rs2155413	11	*DLG2*	A	-0.006	0.113	0.961	33	0.198
rs11601239	11	*GRIA4*	C	0.028	0.102	0.782	0	0.674
rs3138144	12	*RDH5*	G	-0.137	0.149	0.358	14	0.326
rs12229663	12	*PTPRR*	G	-0.045	0.109	0.681	0	0.587
rs8000973	13	*ZIC2*	T	-0.140	0.111	0.205	0	0.698
rs2184971	13	*PCCA*	G	-0.054	0.109	0.623	7	0.366
rs66913363	14	*BMP4*	G	0.016	0.122	0.896	0	0.703
rs1254319	14	*SIX6*	A	0.023	0.110	0.834	23	0.269
rs524952	15	*GJD2*	T	-0.055	0.106	0.606	0	0.829
rs4778879	15	*RASGRF1*	A	0.068	0.104	0.513	52	0.082
rs17648524	16	*A2BP1*	G	0.044	0.129	0.733	0	0.816
rs2969180	17	*SHISA6*	A	0.037	0.103	0.720	0	0.910
rs4793501	17	*KCNJ2*	C	-0.139	0.102	0.174	0	0.672
rs12971120	18	*CNDP2*	A	-0.027	0.116	0.813	6	0.372
rs235770	20	*BMP2*	C	-0.062	0.134	0.642	0	0.648

Beta shows the difference in refractive error (D) associated with each copy of the risk allele in individuals exposed to high versus low levels of time outdoors. Meta-analysis was conducted for 5 cohorts (TEDS, RAINE, GZT, SCORM and STARS) combined N=3,908. Abbreviations: Chr=Chromosome. RA=Risk allele. I2=Heterogeneity statistic. PQ-test=*P*-value for Cochran's Q-test.

Supplementary Figure S2 SNP effects in European and Asian meta-analyses samples



PART VI

GENERAL DISCUSSION, SUMMARY AND APPENDICES

CHAPTER 12

GENERAL DISCUSSION

Refractive errors, particularly myopia, are the most common ocular disorders. Adults with high myopia usually have a myopia onset before the age of 10 years,[53] and these individuals encounter increased risk of visual impairment and blindness during adulthood.[18,48] This makes it an essential part of every ophthalmic practice as high myopia is related to glaucoma, retinal detachment and myopic macular degeneration.[18]

Growth of the eyes' axial length during childhood and teenage years is the most important determinant of high myopia.[6,10,112] Consequences and prevalence of myopia can be explored in adult studies, even so to study the causes of myopia prospectively, research in children is compelled with as ultimate goal developing strategies to stop myopia progression and reduce risk of visual impairment in adulthood.

The aim of this thesis was to elucidate the progress of eye growth and myopia development and to gain more insight in early life eye growth and early onset myopia and related risk factors. The results per study with the individual merits and limitations have been discussed individually. This general discussion will intertwine the separate chapters, focus on the most important and common findings, place them in the currently available knowledge, relate them to disease risk and etiology, and will reflect on general methodological issues as well as future directions for research.

MAIN FINDINGS AND CLINICAL RELEVANCE

Consequences of high myopia

The importance of studying the development of myopia is the increased risk of complications at adult age. We studied the late effects of myopia, by showing the effect of high myopia and longer axial length on the visual acuity in adulthood. The alterations in the morphology of the eye and retina have been described extensively.[18] Myopic eye growth triggers retinal, choroidal and scleral thinning.[247] The effect on visual acuity has not been quantified heretofore and is indispensable for policy making when the generations with more than 50% myopia prevalence grow older.[98] We found exponential increased risk of visual impairment with increasing axial length above 26 mm. Age related visual impairment increases around the age of 75 years, whereas the group with high myopia endures an increase in visual impairment at an age as young as 55 years. The group with an axial length above 30 mm had a cumulative risk of >90% to acquire visual loss. The underlying causes have been sorted out in previous research. Axial length gives an increased risk for a manifold of histopathological findings, such as optic nerve crescent with an inci-

dence of more than 90% above 25.5 mm, chorio-retinal atrophy, lacquer cracks, fuchs spot and staphyloma's.[115] In a subset of the Rotterdam study the retinal change with highest visual morbidity was myopic macular degeneration.[48] To reduce the risk of visual impairment, early normal and pathological growth and risk factors for axial length elongation should be elucidated.

Early growth and ocular biometry

The eye develops from as early as the first weeks of fetal life as the optic pits develops from the neural ectoderm. At this point axial length can be measured as the size of the optic vesicle. Until the 5th week of fetal life growth is slow but will accelerate from the 6th week onward with a steady increase up to 7 months in pregnancy.[316] Axial length is around 15-17 mm at birth and increases rapidly in the first months.[52,316] Between 3 and 9 months the increase is around 1,2 mm with a similar increase between 9 month and 3 years of age.[52,110]

There are differences in axial length and in axial length growth between children. Part of this variation is compensated by differences in corneal curvature and lens power, the other part results in differences in refractive error. Important determinants underlying inter individual differences in ocular biometry, such as axial length and corneal radius of curvature, are birthweight and anthropometry at birth,[113,116] as well as anthropometry measurement and growth trajectories at later ages.[117] Children with higher birth weight had longer axial length and anterior chamber depth, and a higher corneal radius of curvature.[113,116,117] These association may be mediated by for examples smoking by the mother or diet during pregnancy,[317,318] or can be causally related. The indispensable age period for the association between body growth and change in ocular biometry is unknown.[319]

These questions were studied in Part II. First, we examined cross sectionally the associations between ocular biometry at six years of age with prenatal ultrasound measurements, birth parameters and postnatal growth. This revealed an increasing effect of growth and growth patterns on ocular biometry from mid-pregnancy until one to two years post-natally. A conditional analysis, which included growth measurements adjusted for previous measurements to have the independent effect per age period, showed an effect of growth up to two years of age on ocular biometry. Later growth measurements did not show an independent effect. Two risk scores of genes involved in adult height and birth weight revealed a causal effect with Mendelian Randomization with axial length as well as with corneal curvature.

Secondly, we provided normative growth data of the eye from the age as a young as 6 years up to adulthood with growth curves. These growth curves can be used to help in clinical decision-making in myopia treatment or myopia prevention. The growth curves also revealed that children with an increase of ten percentiles or more between 6 and 9 years of age were in 46% of the cases already myopic at age 9, whereas in the rest of the group this was only 5%. After the age of 9 years axial length in the 25th percentile and higher increased, and after the age of 15 years the 50th percentile and higher increased.

The change in axial length above the 95th percentile was 2.5x the change of the lowest 5 percentiles after the age of 6 years. Besides, we observed a myopia prevalence of 12% in 9-year-old children.

Third we compared all eye dimensions and eye volume using MRI scans and provided normative values for 9-year-old children. Eye volume, height, width and shape transform across the whole spectrum of emmetropisation and myopisation. The corpus vitreous length has the highest association with refractive error, whereas the width of the eye showed the highest correlation with body height and birthweight. However, longitudinal studies are required to test the effect of the shape of the globe on refractive error change.

Our study was the first examining the effect of prenatal growth on ocular biometry with numerous follow up measurements. Results presented in the literature combined with our results, disentangled that body size is correlated to ocular biometry, but this associations develops during pregnancy and the first years post-natal.[117] During this period all components of the eye evolve, in which refractive power reduction of the cornea and lens is compensated by axial elongation to remain the large majority of eyes in an equilibrium of emmetropia or low hyperopia.[125] The refractive power of the lens declines during childhood and changes the focal point more posteriorly and concomitantly the axial length elongates.[6-8] The myopia incidence is still relatively low at this young age. Eye growth most likely continuous based on local regulated genetic and environmental determinants in the emmetropisation process to match the different ocular components precisely.[42,60,220,320] Eye growth has been reported in several studies with subjects from the same area, and revealed a deceleration after the age of 10 years.[128,143,144,147] The growth after the age of 10 years was distinctly attributable to the change in the highest fifty percentiles of the spectrum and correlated to an increase prevalence of myopia. There was no difference in the lowest fifty percentiles after the age of 15 years; whereas the eye growth and myopia development can continue up to adolescence in the myopic population and the deceleration of the eye growth is marginal or absent in this group.

Not only the equilibrium of the different refractive components is unbalanced, also the shape of the globus is altered in myopic eyes. Eye shape is oblate in emmetropes and more prolate in myopes.[69,165,321] Myopic eyes are characterized by a more apparent increase in the axial length compared to the height and width of the globus.[161] Our study revealed that the volume is related to the refractive error, but also determined by birth parameters. Height and width have a higher correlation with birthweight than the length of the eye.

Risk factors for axial length and myopia

Which factors are underlying or responsible for the disproportionate and unbalanced eye growth beyond the focal plane and the development of myopia? Many environmental and genetic risk factors for myopia have been described in literature. The most consistent risk factor during childhood in different study designs is time spent outdoors.[22,60,183,322] A risk factor with a strong association in adults is educational achievement.[174,323] Time

spent reading is found in some studies to be associated,[63,235] but is difficult to quantify.[324,325] Recent decades, in which it became possible to study the genome, revealed various genes involved in the development of refractive error. Participants of population-based studies were genotyped and single nucleotide polymorphisms (SNP), intra person variations in the DNA sequence, were determined. 39 SNPs were identified to be responsible for 5% of the variation in refractive error.[42,43] All the participants in these studies were older than 25 years, whereas myopia develops before 25 years. Unknown is at which age these SNPs sort their effect.

Environmental factors

We found that children from families with low socio-economic status and mothers with low education more often suffered myopia at 6 years of age. These associations were mediated by environmental factors earlier identified in adults and children studies, such as more time spent indoors and less time outdoors and sport participation. These mediators illuminated more than two thirds of these associations. Secondly, we studied the association between vitamin D or time spent outdoors, and axial length and myopia. We found decreased levels of vitamin D and time spent outdoors was associated with a longer axial length. And lastly, we identified 9 factors associated with axial elongation and based on the factors myopia incidence between 6 and 9 years of age was predicted with 78% accuracy. Corroborating the dominant role of previous refraction or ocular biometry measurement in the prediction of incident myopia,[238] our study revealed that environmental risk factors had mainly effect in the groups already at high risk for myopia: the children with or most close to myopia.

Most noteworthy was the contradictory risk profiles of myopia in young children, but with similar underlying risk factors as found in the literature. The role of vitamin D in myopia development remains questionable as it might have a causal role or if it represents residual confounding for time spent outdoors with studies describing varying results.[195,221,222] The question of a causal role for vitamin D or if it represents residual confounding for time spent outdoors remains an open question. Many studies in older children revealed the higher prevalence of myopia in children with less time spent outdoors,[22,322] we identified a role of those factors on axial length already at an age as young as 6 years. Although environmental factors have additional value in predicting myopia, the ocular biometry or spherical equivalent at baseline have currently higher precision.[237,238] Notwithstanding their predictive value for myopia is only moderate; the effect on axial growth is obvious.

Genetic factors

We studied the effect of all 39 SNPs and the genetic risk score on AL/CR ratio, the best proxy for refractive error without cycloplegia.[112] We found that only a small proportion of those SNPs exerted their full effect at a young age. We found 13 genes with a nominal

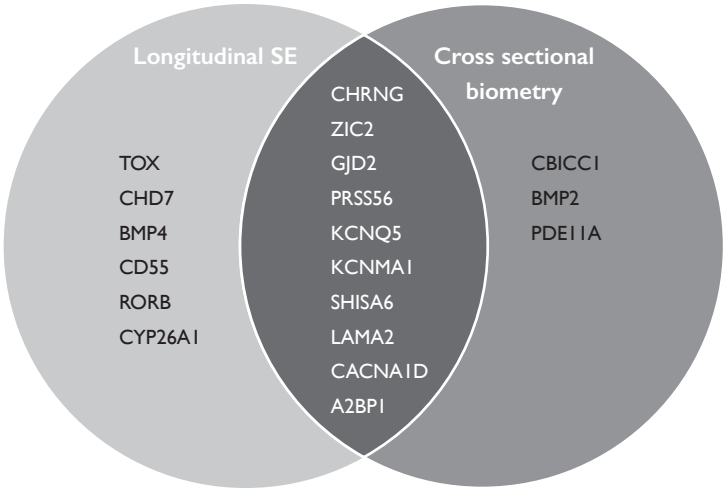
significant effect at an average age of 15 years. The ALSPAC study measured non-cycloplegic refractive error repeatedly between 7 and 15 years of age. We investigated in this study the effect of genes and the change in effect size with increasing age. With this different method 10 genes overlapped with the cross sectional data on ocular biometry (figure 1). Additionally we studied the effect of time spent outdoors and time spent on nearwork and the effect of the genes. One SNP, ZMAT4, showed a Bonferroni adjusted significant interaction effect with nearwork.

Some loci with an early onset, which increased with age i.e. GJD2 and *LAMA2*, were the loci with the largest effect in adulthood. Implicating a continuous effect during childhood and teenage years up to adolescence.[42] GJD2 is an important gap junction and tangled in the retinal-signaling cascade, which potentially may trigger differences in refractive error development from the moment visual input starts. Conversely, some genes, i.e. ZIC2 and CACNA1D, had already a relatively large effect in the children below ten years and teenage group. ZIC2 is known to be involved in the development of neuron trajectories in the retina during embryology,[260,326] and may consequently be involved in refractive error development at a young age. CACNA1D is a calcium channels present in the retina.[263] A larger number of genes could potentially reveal more age specific pathways, nevertheless the interaction between nearwork and the locus near ZMAT4 has been found before in adults, and suggests that this association finds its origin early in life.[327]

Family clustering of myopia suggests a genetic background, but the rapid increase of myopia in the last decades cannot be explained by genetics alone and suggests an essential role for environmental factors. Time spent outdoors and parental myopia were important risk factors in the development of myopia and axial length growth in this thesis. These factors were earlier associated with myopia in other studies,[22,322] but in animal studies causality is further explored.

Two important mechanisms are found to induce myopia in young animals. Form deprived myopia is a method in which a diffuser is placed in front of the eye, and lens induced myopia in which a negative lens in placed in front of the eye to create a hyperopic defocus.[328] Both mechanisms have in common that a degraded the retinal image is formed on the retina. Early studies with chickens located the emmetropisation and myopisation process in the retina with local diffusers that resulted in local scleral growth.[329] Similar studies confirmed this retinal regulation of refractive error development with sectioned optic nerves. These animals still showed myopic progression with both form deprived as well as lens induced myopia.[330-332] Furthermore, the effect of induced myopia is higher in genetic susceptible animals, indicating an interaction between genetic susceptibility and quality of the retinal image.[333] The similarities of results between animal studies and gene environment interactions in humans,[174] make animal models a good method to study the pathways of risk factors in myopia development.

Figure 1 Venn diagram of overlap in results with two different methods of the early onset genes



SE = non cycloplegic spherical equivalent.

Less time spent outdoors was an important risk factor in myopia and axial length elongation in this thesis. Many animal studies also focused on the different aspects of the outdoor environment, such as amount of light and composition of light to test biological pathways for potential intervention. The importance of light is shown in study in chicks fitted with diffusers and were held in different lighting conditions. The chicks raised in the brightest light showed less myopia development and axial length growth.[220] The experiment was repeated but with the injection of spiperone (a dopamine antagonist) which abolished the effect of the light showing the importance of dopamine in the myopia pathway.[334] In a large GWAS different pathways were found as well, including also dopamine genes.[335] The other factor which differs between outdoor environment and indoor environment is the composition of the light. Outdoor light has consists of more short wavelength light (blue) whereas in the indoor environment is more long wavelength light (red), which can be a cue for defocus.[336] The peripheral hyperopic defocus and eye shape was also tested in animals, that suggested an important role for the peripheral retina as well. In monkeys the central macular region was eliminated but, still the peripheral retina induced eye growth based on hyperopic defocus.[160,337]

Result from studies in this thesis combined with other human and animal studies make time spent outdoors and genetic susceptibility important and established risk factors for myopia. Environmental factors found in young children were similar to factors in animal studies with potential mechanisms for a causal pathway. The retina an important structure in myopia development which can find the sign of defocus, not only in the macula but also in the peripheral retina, and actively change the focal point by a retinal scleral signaling cascade through the choroid.[61]

METHODOLOGICAL CONSIDERATIONS

Study design

Most studies described in this thesis were embedded in the Generation R Study and ALSPAC, both population-based prospective birth cohort studies. Cohort studies are observational studies of a pre-defined group of subjects to detect disease occurrence and differences in exposure or genes between diseased and non-diseased participants. This type of study design can be time consuming and costly, but is very effective to study common disease. Potential pitfalls are that this type of studies are vulnerable to different biases, such as selection bias, information bias, confounding, and are sensitive to potential model misspecification. For example, confounders for which is adjusted in the model can modify the outcome of the statistic test. It is therefore important to consider what factors are used in the model and how confounders relate to each other and to the determinant. As well, exposed participants can differ in many ways from non-exposed in your cohort, which you cannot all measure. This all can lead to residual confounding or inflated effects and will subsequently influence the conclusion drawn from the data and can potentially decrease the validity of the results.

Validity

The validity of a study can be separated into internal and external validity. Internal validity of a study describes to what extent the determinant and outcome are causally related or if other variables are responsible for the found correlation. Systematic errors, biases, chance or confounders can threaten internal validity. These systematic errors should be minimalized.

Selection bias

Selection bias may occur when there is a difference in the association between outcome and determinant in subjects who participated and subjects who did not participate or are lost to follow up in the study, but were eligible.[338] The Generation R study started with 9778 mothers which was an estimated participation rate of 61% of all eligible pregnant woman in Rotterdam during the inclusion period.[339] This might bias results, but is less likely because biased results in cohort studies are theorized to be more induced by loss to follow up than from non-response at the start of the study.[338] A total of 6690 (68%) participants completed follow up visits at 6 years and 5882 (60%) at 9 years of age.[46] The non-response was not likely to be random during the follow up visits; the participants were more often from high socio-economic status and were more often of European descent, compared to what would be expected based on baseline data and the structure of the Rotterdam population.[46,339,340] Although it is unlikely that participation was related to ophthalmologic phenotypes, the selective non-participation may be a threat for

prevalence estimates and internal validity. Prevalence of myopia can be underestimate as result of the non-participation of the non-European children who had a higher prevalence of myopia. The potential bias is difficult to quantify for all variables, but sensitivity analysis revealed no different associations of risk factors for myopia between European and non-European participants in chapter 3.1 and 3.2.

Information bias

An information bias is an error caused by misclassified data and can lead to a systematic error, i.e. invalid conclusions are drawn from incorrect data. An information bias can occur when groups are different in providing and answering questions, under or overestimating certain risk behavior in relation to the disease under study. Information bias can be non-differential, independent of the outcome, which will mostly dilute the effect estimate; or it can be differential in which it is related to the outcome and an invalid conclusion is drawn. Within our studies the chance of a differential information bias is small, mostly the determinants were collected before the outcome measurements, and participants were unaware of the outcome under study. This most commonly may create non-differential information bias; in this case the effect size of the association will be underestimated or diluted. Some risk factors under study were collected with questionnaire. Time spent outdoors is difficult to quantify, and previous research has shown that objective and subjective measure differ.[341,342] This will likely have result in random misclassification in our study as well, and may potentially have diluted effect sizes.

Confounding

A potential limitation in epidemiological research is confounding. A confounder is a variable that is associated with determinant and outcome (independent of the determinant), and is not in the causal pathway. Ignoring confounders can lead to spurious associations between determinant and outcome and over or underestimation of the effect. Confounders in this thesis were based on literature and the association with the determinant and outcome. Residual confounding cannot be excluded due to potential inaccuracies in the questionnaires. The most important confounder concerning myopia in children is age. Behavior changes with increasing age as well as the myopia prevalence. We adjusted our multivariable analyses for age or stratified our genetic analyses in age categories. Other factors taken into account were socio-economic status, and time spent outdoors if vitamin D was the determinant under study.

SNPs

Two chapters in this used SNPs identified and replicated in multiple studies. Genome wide association studies are a powerful tool to identify DNA regions associated with phe-

notypic variance. Some drawbacks of the use of those SNPs are that the SNPs are usually not the causal variants but only variants in linkage disequilibrium. SNPs can be located in intergenic regions and the most nearby genes are not necessarily the genes associated with the disease, but can be located at a different part of the chromosome.

External validity

The external validity of a study reflects who well the results obtained from the study cohort can be applied to other populations, i.e. if the result is generalizable. The Generation R population-based birth cohort from Rotterdam. Rotterdam is the second largest city in The Netherlands, with an ethnic variation, but the largest group was Europeans. Risk factors found in our study did not differ notably with studies performed in other continents. Results were in general comparable between children with European background and non-European background. Presumably findings presented in this thesis are generalizable to other pediatric populations.

IMPLICATIONS & FUTURE DIRECTIONS

The prevention and reduction of visual morbidity as result of myopia should be an important objective for the individual patient care and prominent public health goal. Currently irrefutable risk factors for the development of high myopia are deficient and it remains challenging to identify children at risk for myopia and give them the best-personalized treatment. Eye growth is a dynamic process and a very important indicator in the clinic to monitor refractive error progression. Implications of this thesis and future research should have a dual policy, and focus on individual patient as well as on the public health level.

Favorable individual patient outcomes can be best accomplished by development of effective therapies without side effects to prevent excessive axial elongation and to improve treatments for myopia related complications such as myopic maculopathy. To prevent excessive eye growth, the first goal is to define when eye growth is too rapid in relation to other ocular biometry. In this thesis we provided a first step with data for eye growth, growth curves and eye dimensions. Further studies should expand the growth curves in relation to lens development.[7] It is important for clinical practitioners to keep in mind that there is variation in normal emmetropic eye growth across the age spectrum from birth to 25 years.

Various treatment options are currently under study. Atropine is a non-selective muscarinic receptor antagonist (M-antagonist) and currently the most studied pharmacological agent to inhibit myopia progression.[343] Nevertheless, it is primarily applied in Asian countries.[344] Optical treatments of myopia progression, such as multi focal contact lenses and orthokeratology, are increasingly applied in the clinic. The reduction found for these optical treatment modalities are lower than for high dose atropine, but a ran-

domized clinical trial has never compared the two treatment interventions. The potential additive effect of atropine and optical treatment are also unknown. It is unresolved why some children have a low response to atropine or optical interventions and why others stop in myopia progression. These are certainly questions that should be answered in future research to improve myopia treatment and individual patient care.

The visual consequences of myopia and the increased prevalence require, besides effective treatment, public health interventions. Although the paradoxical risk profiles for myopia in the young generation, the underlying risk pattern is equal to the profiles in older generations and research should therefore focus on the effect of lifestyle change. Currently ongoing randomized clinical trials give promising results on the short term.[60] Further studies are required to evaluate the potential rebound effect, which is seen with atropine and quantify the inhibiting effect on the final refractive error of increased time spent outdoors at particular age categories. Other ongoing trials are education within a glass classroom to increase the amount of incoming lux, but effectivity needs to be awaited.[16] The impact of the digitalization should be elucidated: the effect of education is known to be high, but the effect of near work on digital screens is still indecipherable. Potential groups of intervention are the children at risk for developing myopia. In previous papers, as well as in this thesis, children close to myopia have the highest risk of myopia. Similarly, the groups with the most myopic refractive error or longest axial length appear to have most benefit of more time spent outdoors and a change in reading habits. Presumably to gain the highest cost effectiveness and best effect, interventions will have to focus on these groups. These results also suggest behavioral interventions have an effect on eye growth, and will have benefits in myopic children to reduce progression. Randomized trials are necessary to reveal the effect of other risk factors than time spent outdoors identified by cross-sectional studies.

Our findings provide clues for the genetic risk of common SNPs in children and the early onset loci. It is now a challenge to dissect more loci that cause refractive error. The currently known SNPs explain only a limited part of the phenotypic variance in young children. Current research is aiming to explain more of this variance by common SNPs. GWAS will continue to be a powerful tool for identifying genetic risk, but in children it is difficult to find large groups without large heterogeneity in age.

Furthermore, other effects of the loci have to be found such as gene-environment interactions to have potentially a higher explained variance. The aim of gene-environment interaction is to show that the effect of loci or genes is higher in children with a more myopic environment. An advantage is that this can performed with a risk score of all the SNPS or risk scores of pathway specific loci. Other techniques may be deployed as well, such as more detailed imputation and more in depth genotyping (e.g., whole exome sequencing) to explain more of the genetic variance.

Refractive error is a sum of the different refractive structures of the eye and their shape. A more in depth approach to understand the etiology of refractive errors is to focus on the effect of risk factors on the separate ocular components to identify targets for different risk factors and genetic loci. Better measurements of nearwork can be potentially achieved with apps on electronic screen and watches for measuring light intensity.[325] More recently research focus has shifted to the effect of the choroid in the devel-

opment of refractive error. The choroid is a dynamic vascular structure underneath the retina to provide the retina of nutrients. Some studies have found a dynamic effect of nearwork on the choroidal thickness.[345]

FINAL REMARKS AND CONCLUSION

Myopia is a common condition in young children and the visual morbidity later in life is highest in the most extreme cases, which are mostly the children with an onset at a young age. It remains an intriguing puzzle to unravel the causes and pathways leading to eye growth and myopia. We identified various genes and environmental risk factors associated with eye growth. Yet, ultimately it is important to identify children at risk for high myopia at a young age and develop a treatment without significant side effects to inhibit eye growth before the onset, or to keep the spherical equivalent below -6 D to lower the risk of the consequent visual impairment in adulthood.

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CHAPTER 13

SUMMARY

Part I – Background

Chapter 1 and 2 describe the aims of our studies. Myopia is the eye disorder with the most rapid increase in prevalence worldwide. It develops in childhood with a peak incidence between 13-15 years. Especially high myopia, a refractive error of -6 diopters or more, increases the risk of permanent visual impairment during adulthood due to structural abnormalities of the retina and optic nerve. The causes of myopia are complex. Lifestyle factors in childhood, such as time spent outdoors and close work are risk factors. Moreover, genetic studies have revealed more than 100 SNPs associated with myopia. Pharmacological and optical interventions to inhibit myopia progression are becoming increasingly common. The ultimate goal of this thesis was to gain insight into the causes and consequences of childhood myopia. For this purpose we investigated the environmental, genetic on myopia, eye growth and ocular biometry in subjects of the Generation R and ALSPAC study.

The main objectives of this study are:
1. To assess the effect of early onset myopia on visual impairment, by studying the effect of high myopia and myopia, and axial length on visual acuity above the age of 45 years.
2. To assess the development of ocular biometry from young childhood to adulthood and the association with prenatal and postnatal growth.
3. To assess the association of environmental risk factors on ocular biometry and myopia at a young age. The exposures of interest include outdoors exposure, nearwork, computer and tablet use, vitamin D and reading habits.
4. To assess the effect of genetic factors on different ages on the development of ocular biometry and refractive error and to find gene x environment interactions.

Part II – Consequences of myopia

Chapter 3 reported in a large study of multiple cohorts the association between axial length and refractive error and visual impairment. Of all high myopes, 39% developed visual impairment at age 75 years. In particular those at the more extreme ends of the axial length spectrum were at great risk of visual impairment: risks increased from 3.8% in eyes with axial length <26mm, to 25% in eyes with axial length ≥26mm and to >90% in eyes with axial length ≥30mm. Projections of these risks to areas with a high incidence of myopia indicate that visual impairment will be rising considerably as the population ages, and one in ten persons will develop visual impairment in the most endemic regions.

Part III – Eye development

Chapter 4 provides an overview of ocular biometry and its association with prenatal growth, and revealed an association with growth patterns from mid pregnancy up to 24 months postnatal. Restricted prenatal and postnatal growth resulted in a smaller corneal radius and axial length. A higher risk score for height in adults was associated with a longer axial length and larger corneal radius.

Chapter 5 reports baseline data for ocular biometry and refractive error in European children from 6 years up to adulthood with myopia prevalences. These data may be applied to monitor eye growth and myopia progression in children. Axial length increased up to 10 – 15 years of age, and after this age particularly the highest 50 percentiles continued elongating and developed myopia. The average spherical equivalent in 9-year-old European children is +0.73 D, nevertheless 12% was found to be already myopic.

Chapter 6 showed the complete ocular shape of the eye in a large group of young children. Normative values for 9 year old children were provided for width, height and volume of the eye. The corpus vitreous depth had a higher correlation with refractive error, than the height and width of the eye. Eye volume increases with higher age, male gender, more myopic refractive error, and a higher birth weight. Conversely, width of the eye has higher association with other anthropometry measurements such as body height, birthweight and the genetic risk score for height than the axial length or height of the eye.

Part IV –Risk factors for myopia

Chapter 7 reveals a higher frequency of myopia in 6-year-old children from families with low income, low maternal education, and non-European ethnicity. These paradoxical findings to previous literature found its origin by living circumstances such as housing and marital status of the parents, playing sports, and more time spent indoors relative to outdoors. These mediators disentangled more than two thirds of the risk profiles.

Chapter 8 demonstrates a significant association between serum 25(OH)D levels, AL and myopia. In this study children with lower serum levels of 25(OH)D had longer AL and those with higher 25(OH)D had a lower risk of myopia. The association remained significant after adjusting for outdoor exposure, indicating that these two closely related determinants may have some overlapping as well as separate effects on the development of myopia.

Chapter 9 found several risk factors for eye growth below the age of 10 years. With these factors we created a tool for predicting early onset myopia with a discriminative accuracy of 0.78 in the total group.

Part V – Genetic risk of myopia in children

Chapter 10 describes the effect of genetic variants on ocular biometry in children which were identified in adults. In this meta-analysis some loci had their greatest effect in young children (*CHRNG, ZIC2, KCNMA1*), while others reached the greatest effect during early teenage years (*BMP2, CACNA1D, A2BP1*). However, most appeared to have a gradual effect during the entire age span of myopia development (*LAMA2*, *LRRC4C*, *DLX1*, *RDH5, GRIA4, RGR, SIX6*).

Chapter 11 gives the effect of the genetic variants and the effect on refractive error in children. We show that similar genes as found in early onset of increasing effect at a young age. Above this, we identified a variant with an interaction with nearwork (*ZMAT4*).

Part VI – General discussion and summary

Chapter 12 provides the general discussion of this thesis with ideas and directions for future research.

SAMENVATTING

Deel I – Achtergrond

Hoofdstuk 1 en 2 beschrijven de doelstellingen van dit proefschrift. Myopie is een oogaandoening die ontstaat in de jeugd en heeft momenteel een snel stijgende prevalentie wereldwijd. Hoge myopie, een brilsterkte van -6 dioptrieën of sterker, ontstaat meestal op jonge leeftijd en geeft een sterk verhoogde kans op blijvende slechtziendheid door veranderingen in de morfologie van het netvlies en oogzenuw op volwassen leeftijd. De oorzaak van myopie is complex. Leefstijl factoren in de jeugd, zoals weinig buiten zijn en het verrichten van veel dichtbij werk zijn belangrijke risicofactoren. Er zijn inmiddels >100 genetische factoren geïdentificeerd. Farmacologische en optische interventies om de toename van myopie te remmen worden steeds meer toegepast.

De belangrijkste doelstelling van dit proefschrift zijn:
1. Het bestuderen van het effect van op jonge leeftijd ontwikkelde myopie op slechtziendheid, door te kijken naar het effect van hoge myopie op slechtziendheid boven de 45 jaar.
2. Het onderzoeken van de ontwikkeling oculaire biometrie van jonge kinderen tot volwassenen en de associatie met prenatale groei.
3. Het bestuderen van de associatie tussen omgevingsfactoren en oculaire biometrie en myopie op een jonge leeftijd.
4. Het bepalen van het effect van genetische factoren op verschillende leeftijd en het vinden van gen-omgevings interacties.

Deel II –Consequenties van myopie

Hoofdstuk 3 laat de gevolgen zien van hoge myopie en een lange aslengte op de visus in een grote studie van meerdere cohorten. Van alle hoog myopen ontwikkelde 39% slechtziendheid op 75-jarige leeftijd. Met name diegenen met de hoogste aslengte hadden het allergrootste risico op slechtziendheid: het risico nam toe van 3.8% met een aslengte onder de 26 mm tot 25% in ogen met een aslengte van meer dan 26 mm tot 90% in aslengte van meer dan 30 mm. Extrapolatie van deze getallen naar regio's met een hoge prevalentie van myopie laat zien dat slechtziendheid zal toenemen als de huidige twintigers ouder worden en kan stijgen tot 1/10 personen met slechtziendheid in de meest endemische regio's.

Deel III – Oog ontwikkeling

Hoofdstuk 4 geeft een overzicht van de oculaire biometrie en de associatie met prenatale groei en laat zien dat er een associatie is tussen prenatale groei patronen vanaf halverwege de zwangerschap tot 24 maanden postnataal. Lage prenatale – en postnatale groei

resulteerde in een kleinere cornea radius van de kromming en een kortere aslengte. Een hogere risicoscore voor lengte in volwassenen was geassocieerd met een langere aslengte en grotere cornea radius van de kromming.

Hoofdstuk 5 beschrijft de uitgangswaarden voor oculaire biometrie en refractie in Europese kinderen van 6 jaar tot volwassen leeftijd met prevalenties van myopie. Deze groeicurven kunnen worden gebruikt om ooggroei en myopie progressie te monitoren. Aslengte nam toe tot een leeftijd van 10 -15 jaar, en daarna vond met name verandering plaats boven het 50ste percentiel Het gemiddelde sferische equivalent in 9-jarige kinderen was +0.73 D, maar ook had al 12% myopie.

Hoofdstuk 6 belicht meerdere dimensies van de oculaire biometrie. Normaalwaarde voor 10 jarige kinderen worden gegeven voor breedte, hoogte en volume van het oog. Het corpus vitreous heeft een hogere correlatie met refractie, dan met de hoogte en de breedte van het oog. Oog volume neemt toe met toenemende leeftijd, mannelijk geslacht, toename in myopie en een hoger geboortegewicht. De breedte van het oog heeft juist een hogere correlatie met andere antropometrie metingen zoals lichaamslengte, geboortegewicht en een genetische risicoscore dan de aslengte of hoogte van het oog.

Deel IV – Risicofactoren voor myopie

Hoofdstuk 7 laat een hogere prevalentie van myopie zien in 6-jarige kinderen uit families met een lager inkomen, van moeders met een lager onderwijsniveau en niet-Europese achtergrond. Deze paradoxale bevindingen ten opzichte van voorgaande literatuur vinden hun oorsprong in leefstijlfactoren zoals sporten, buitenspelen en computer gebruik. Deze mediatoren ontrafelde meer dan 2/3 van het verhoogde risico.

Hoofdstuk 8 demonstreerde een associatie met serum 25(OH)D spiegels, aslengte en myopie. In deze studie hadden kinderen met lagere vitamine D spiegels een hogere aslengte en waren vaker myoop. De associatie bleef significant na correctie voor buitenspelen, wat indiceert dat deze twee nauw aan elkaar gerelateerde determinanten een overlappend alsook een apart effect kunnen hebben bij de ontwikkeling van myopie.

Hoofdstuk 9 worden risicofactoren beschreven voor ooggroei voor de leeftijd van 10 jaar. Acht factoren, waaronder leefstijl factoren en myopie bij de ouders waren geassocieerd met een toename in ooggroei. Door middel van deze factoren kon myopie voorspeld worden met een discriminerende waarde van 0.78 in de totale groep.

Deel V – Genetisch risico voor myopie in kinderen

Hoofdstuk 10 beschrijft het effect van genetische varianten gevonden in volwassen op oculaire biometrie in kinderen. In deze meta-analyse hadden sommige loci het groot-

ste effect op jonge leeftijd (*CHRNG, ZIC2, KCNMA1*), terwijl andere het grootste effect bereikten in de tienerjaren (*BMP2, CACNA1D, A2BP1*). Echter, de meeste loci hadden een geleidelijk effect gedurende myopie ontwikkeling (zoals *LAMA2*, *LRRC4C*, *DLX1*, *RDH5, GRIA4, RGR, SIX6*).

Hoofdstuk 11 geeft het effect van genetische varianten op het sferische equivalent in kinderen. Deze studie laat vergelijkbare genen zien op jonge leeftijd en een interactie tussen het locus *ZMAT4* en dichtbijwerk in kinderen.

Deel VI – Algemene discussie en samenvatting

Hoofdstuk 12 geeft een algemene discussie over dit proefschrift met ideeën en richtingen voor toekomstig onderzoek.

CHAPTER 14

APPENDICES

PhD portfolio

Name PhD student:	Willem Tideman
Erasmus University Department:	Ophthalmology and Epidemiology
Research School:	NIHES
PhD period:	2013 - 2017
Promotor:	Prof. Dr. C.C.W. Klaver and Prof Dr. J.R. Vingerling

PhD training	**Year**	**Work-load (ECTS)**
Courses		
– NIHES Master of Science in Genetic Epidemiology	2013-2015	70
– Radiation hygiene and protection level 5R, Erasmus MC	2013	0.7
– MRI Safety Course, Erasmus MC	2014	0.3
– Endnote Course, Medical Library, Erasmus MC	2014	0.3
– Systematic literature retrieval, Medical Library, Erasmus MC	2014	0.3
– Scientific integrity Course, Rotterdam, The Netherlands	2015	0.2
– Course Biomedical Research Techniques XIV, MolMed, Erasmus MC	2015	1.5
– Biomedical English Writing and Communication, Erasmus MC	2015	3.0
– Presentation course, MolMed, Erasmus MC	2016	1.0
Seminar, symposia and workshops		
– Generation R Research meetings, Erasmus MC	2013-2016	1.0
– Molecular Epidemiology meetings, Erasmus MC	2014-2016	1.0
– Retinal Genetics, Gent, Belgium	2013	0.5
– Nederlands Oogheelkundig Gezelschap (NOG) Annual meeting Annual meeting, Groningen, The Netherlands	2013 2013	0.2 1.0
– Clinical Translational Conference on Myopia; Berkeley, California, United States	2013	0.2
– Sophia Wetenschapsdag, Rotterdam, The Netherlands		
– 5th Rotterdam Amblyopia meeting 'infantile esotropia'; Rotterdam, The Netherlands	2014	0.2
– Annual meeting refractive and cataract surgery; Rotterdam, The Netherlands	2014	0.2
– Myopia symposium, Rotterdam, The Netherlands	2015	0.2
– CREAM Consortium meeting, Baltimore, Maryland, United States	2015	0.4
– Symposium Behandeling van Progressieve Myopie, Rotterdam, Utrecht en Heerenveen, The Netherlands. Oral presentation	2015-2017	0.6
Presentations		
Invited speaker		
– Ophthalmic physics meeting, Maastricht. Oral presentation	2013	0.2
– The Association for Research in Vision and Ophthalmology (ARVO) Symposium 'Drilling down from animals building up from humans, will we meet in the middle', Denver, Colorado, United States. Oral presentation	2015	0.2
– Optometristen Vereniging Nederland, Nieuwegein, Utrecht, The Netherlands. Oral presentation	2015	0.2
– Panelmember of interactive discussion Myopia control – the Dutch way, NCC, Veldhoven, The Netherlands	2016	0.2

Presentations on international conferences		
– International myopia conference; Asilomar, California, United States. Poster	2013	1.0
– ARVO, Orlando, Florida, United States. Oral presentation	2014	1.0
– ARVO, Denver, Colorado, United States. Oral presentation	2015	1.0
– International Myopia Conference, Wenzhou, China. Oral presentation	2015	1.0
– American Society of Human Genetics, Baltimore, Maryland, United States. Poster	2015	1.0
– Euretina, Rotterdam, The Netherlands. Oral presentation	2016	1.0
– ARVO, Seattle, Washington, United States. Oral presentation	2016	1.0
– International Orthoptic conference; Rotterdam, The Netherlands. Oral presentation	2016	1.0
– ARVO, Baltimore, Maryland, United States. Poster presentation	2017	1.0
– International Myopia Conference, Birmingham, United Kingdom. Oral presentation	2017	1.0
Presentations on national conferences		
– Dutch Ophthalmology PhD Student Meeting, Nijmegen, The Netherlands. Oral presentation	2014	1.0
– NOG Annual Meeting, Maastricht, The Netherlands. Oral presentation	2014	0.4
– NOG Annual Meeting, Groningen, The Netherlands. Oral presentation	2015	1.0
– Dutch Ophthalmology PhD Student Meeting, Nijmegen, The Netherlands. Oral presentation	2016	1.0
– NOG Annual Meeting, Maastricht, The Netherlands. Oral presentation	2016	1.0
– NOG Annual Meeting, Maastricht, The Netherlands. Oral presentation	2017	1.0
Honors and Grants		
– Chair of myopia session, ARVO annual meeting, Denver, United States	2015	0.1
– Erasmus Trust Fonds Travel Grant	2014-2017	
– Dr. Henkes Stichting, Travel Grant	2014-2015	
– ARVO International Travel Grant	2015	
– International Myopia Conference Travel Grant	2015	
Teaching activities		
– Guest teacher at Hogeschool Utrecht 'Public health and myopia'.	2017	1.4
Supervising Master's Theses		
– Zehra Biyik Kilic, Kopenhagen University	2015	1.4
– Nadine Roth, Vrije Universiteit Amsterdam	2015-2016	1.4
Other		
– Reviewer of Brithish Medical Journal, JAMA Ophthalmology, Optometry and Vision science, Eye, Acta Ophthalmology, Ophthalmic and Physiological Optics 2014 – onwards		1.0

ABOUT THE AUTHOR

Jan Willem Lodewijk Tideman was born on August 17th, 1985 in Groningen, the Netherlands. In 2004 he graduated from secondary school at the KDC in Emmen. He went on to study at the Rijksuniversiteit Groningen, where he completed his Bachelor of Science Degree in medicine in 2009. Hereafter, he completed his Master of Science in Groningen, and did his clinical internships in the Deventer Ziekenhuis. Before starting his PhD in the Erasmus Medical Center he worked for 6 months at the Bethesda Ziekenhuis Hoogeveen at the Emergency department. In 2013, he started a PhD project described in this thesis supervised by prof dr. Caroline Klaver. As part of his PhD, Willem obtained a Master of Science Degree in Genetic Epidemiology from the Netherlands Institute for Health Sciences in 2015. In February 2017, Willem started working as a clinical resident at the department of Ophthalmology at the Erasmus Medical Center.

LIST OF PUBLICATIONS

1 Klaver, CC, Polling, JR, **Tideman JWL**, Meester-Smoor, MA Verhoeven VJM, Why do Eyes Become Myopic? CRST Europe (2014).
2 **Tideman JWL**, Polling, JR, van der Schans A, Verhoeven VJM., Klaver CCW, Bijziendheid, een groeiend probleem. Ned Tijdschr Geneesk. 160(0):D803 (2016)
3 **Tideman JWL**, Polling JR, Voortman T, Jaddoe VWV, Uitterlinden AG, Hofman A, Vingerling JR, Franco OH, Klaver CCW, Low serum Vitamin D is associated with axial length and risk of myopia in young children. Eur J Epidemiol, 31, 491-9. (2016)
4 **Tideman JWL**, Fan Q, Polling JR, Guo X, Yazar S, Khawaja A, Höhn R, Lu Y, Jaddoe VWV, Yamashiro K, Yoshikawa M, Gerhold-Ay A, Nickels S, Zeller T, He M, Boutin T, Bencic G, Vitart V, Mackey DA, Foster PJ, MacGregor S, Williams C, Saw SM, Guggenheim JA, Klaver CCW, The CREAM consortium. When do myopia genes have their effect? Comparison of genetic risks between children and adults. Genet Epidemiol, 40(8): 756-766. Doi: 10.1002/gepi.21999 (2016)
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6 Fan Q, Guo X, **Tideman JWL** (shared first), Williams KM, Yazar S, Hosseini SM, Howe LD, Purcain BS, Evans DM, Timpson NJ, McMahon G, Hysi PG, Krapohl E, Wang YX, Jonas JB, Baird PN, Wang JJ, Chang CY, Teo YY, Wont TY, Ding X, Wojciechowski R, Young TL, Pärssinen O, Oexle K, Pfeiffer N, Bailey-Wilson JE, Paterson AD, Klaver CCW, Plomin R, Hammond CJ, Mackey DA, He M, Saw SM, Williams C, Guggenheim JA, CREAM consortium, Childhood gene-environment interactions and age-dependent effects of genetic variants associated with refractive error and myopia: The CREAM Consortium. Sci Rep 13;6:25853 (2016)
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8 Polling JR, Kok RG, **Tideman JWL**, Meskat B, Klaver CCW, Effectiveness study of atropine for progressive myopia in Europeans. Eye 30, 998-1004. Doi: 10.1038/eye.2016.78. (2016)
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11 Cuellar-Partida G, Williams KM, Yazar S, Guggenheim JA, Hewitt AW, Williams C, Wang JJ, Kho PF, Saw SM, Cheng CY, Wong TY, Aung T, Young TL, **Tideman JWL**, Jonas JB; Consortium for Refractive Error and Myopia (CREAM), Mitchell P, Wojciechowski R, Stambolian D, Hysi P, Hammond CJ, Mackey DA, Lucas RM, MacGregor S. Genetically low vitamin D concentrations and myopic refractive error: a Mendelian randomization study. Int J Epidemiol. doi: 10.1093/ije/dyx068, (2017)

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13 **Tideman JWL**, Polling JR, Jaddoe VWV, Vingerling JR, Klaver CCW. Environmental risk factors can reduce axial length elongation and myopia incidence in 6 to 9 year old children. Ophthalmology. (2018)

DANKWOORD

Vele handen maken licht werk. Met enige weemoed maar voldoening, hierbij het laatste stuk van dit proefschrift. Dit proefschrift was nooit tot stand gekomen met alleen mijn eigen inspanningen, maar is het resultaat veel arbeid, ook ruimschoots voordat ik begon met dit promotieonderzoek. Van het opzetten van Generation R, dataverzameling tot analyse en schrijven, het is onmogelijk iedereen met naam en toenaam te noemen, toch wil ik een aantal mensen in het bijzonder bedanken.

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