Phenotypic consequences of the GJD2 risk genotype in myopia development

Haarman, A.E.G.; Enthoven, C.A.; Tedja, M.S.; Polling, J.R.; Tideman, J.W.L.; Keunen, J.E.E.; ... ; Klaver, C.C.W.

Phenotypic Consequences of the *GJD2* Risk Genotype in Myopia Development

Annechien E. G. Haarman,[1,2] Clair A. Enthoven,[1–3] Milly S. Tedja,[1,2] Jan R. Polling,[1,12] J. Willem L. Tideman,[1] Jan E. E. Keunen,[4] Camiel J. F. Boon,[5,6] Janine F. Felix,[2,3,7] H. Raat,[8] Annette J. M. Geerards,[9] Gregorius P. M. Luyten,[5] Gwyneth A. van Rijn,[5] Virginie J. M. Verhoeven,[1,10] and Caroline C. W. Klaver[1,2,4,11]

[1]Erasmus Medical Center, Department of Ophthalmology, Rotterdam, The Netherlands
[2]Erasmus Medical Center, Department of Epidemiology, Rotterdam, The Netherlands
[3]Erasmus Medical Center, the Generation R Study Group, Rotterdam, The Netherlands
[4]University Medical Center St Radboud, Department of Ophthalmology, Nijmegen, The Netherlands
[5]Leiden University Medical Center, Department of Ophthalmology, The Netherlands
[6]Amsterdam University Medical Center, Department of Ophthalmology, University of Amsterdam, The Netherlands
[7]Erasmus Medical Center, Department of Pediatrics, Rotterdam, The Netherlands
[8]Erasmus University Medical Centre, Department of Public Health, Rotterdam, The Netherlands
[9]The Rotterdam Eye Hospital, Rotterdam, The Netherlands
[10]Erasmus Medical Center, Department of Clinical Genetics, Rotterdam, The Netherlands
[11]Institute of Molecular and Clinical Ophthalmology, Basel, Switzerland
[12]Department of Optometry and Orthoptics, Hogeschool Utrecht, University of Applied Science, Utrecht, The Netherlands

Correspondence: Caroline C.W. Klaver, Erasmus Medical Center, room Na-2808, PO Box 2040, 3000 CA, Rotterdam, The Netherlands; c.c.w.klaver@erasmusmc.nl.

Received: October 26, 2020
Accepted: January 26, 2021
Published: August 18, 2021

Citation: Haarman AEG, Enthoven CA, Tedja MS, et al. Phenotypic consequences of the *GJD2* risk genotype in myopia development. *Invest Ophthalmol Vis Sci.* 2021;62(10):16. https://doi.org/10.1167/iovs.62.10.16

Purpose. To study the relatively high effect of the refractive error gene *GJD2* in human myopia, and to assess its relationship with refractive error, ocular biometry and lifestyle in various age groups.

Methods. The population-based Rotterdam Study (RS), high myopia case-control study MYopia STudy, and the birth-cohort study Generation R were included in this study. Spherical equivalent (SER), axial length (AL), axial length/corneal radius (AL/CR), vitreous depth (VD), and anterior chamber depth (ACD) were measured using standard ophthalmologic procedures. Biometric measurements were compared between *GJD2* (rs524952) genotype groups; education and environmental risk score (ERS) were calculated to estimate gene-environment interaction effects, using the Synergy index (SI).

Results. RS adults carrying two risk alleles had a lower SER and longer AL, ACD and VD (AA versus TT, 0.23D vs. 0.70D; 23.79 mm vs. 23.52 mm; 2.72 mm vs. 2.65 mm; 16.12 mm vs. 15.87 mm; all $P < 0.001$). Children carrying two risk alleles had larger AL/CR at ages 6 and 9 years (2.88 vs. 2.87 and 3.00 vs. 2.96; all $P < 0.001$). Education and ERS both negatively influenced myopia and the biometric outcomes, but gene-environment interactions did not reach statistical significance (SI 1.25 [95% confidence interval {CI}, 0.85–1.85] and 1.17 [95% CI, 0.55–2.50] in adults and children).

Conclusions. The elongation of the eye caused by the *GJD2* risk genotype follows a dose-response pattern already visible at the age of 6 years. These early effects are an example of how a common myopia gene may drive myopia.

Keywords: myopia, *GJD2*, biometry, refractive error, gene-environment

Myopia (near-sightedness) is an ocular condition caused by a complex interplay between genetic and environmental risk factors.[1] Over the past decade, genome-wide association studies have revealed hundreds of common genetic variants associated with refractive error and myopia using large population based studies from the Consortium for Refractive Error and Myopia and the UK Biobank.[2–11] Although the majority of these common variants are located intergenically and annotated on the basis of physical distance, they are expressed in a large range of ocular cell types and are involved in biological key processes such as light signaling, pigmentation, circadian rhythm, and extracellular matrix remodeling.[4,9,11] One of the first established common myopia risk variants is near *GJD2*, encoding the gap junction protein connexin 36.[2,12] Single nucleotide polymorphisms (SNPs) annotated to this gene have one of the highest effect sizes of common myopia genes in virtually all genome-wide association studies studies.[2,5–10] The most commonly identified top SNP adjacent to *GJD2* is rs524952, located 39 kb away from its 3′ end on chromosome 15. This variant has a high allele frequency (minor allele frequency 47.5%–49.1%) and a relative strong effect on spherical

equivalent (SER) (Beta −0.06 to −0.29).[4,9,11] Although this variant is not located exonically in *GJD2*, the associated SNP is implicated to have a regulatory effect on *GJD2*.[10,12] Gap junctions like *GJD2* are responsible for transmission of small molecules, ions, and second messengers between adjacent cells and enable metabolic coupling of cells and chemical communication.[13–16] *GJD2* in particular has been implicated in cell communication, cell-cell signaling, visual perception, and transmembrane transport.[17] Functional investigations in animal and cellular studies are currently underway to gain sight into the molecular role of *GJD2* in causing myopia, with the aim to find targets for intervention. Bearing that in mind, detailed information on the effect of *GJD2* on all ocular components of refractive error is needed.

Although the association between *GJD2* and SER and axial length (AL) has been well established, little is known about its effect on anterior chamber depth (ACD) and lens thickness (LT), nor is it clear what the timing of the gene effect is. We therefore studied the influence of this major myopia gene on the entire ocular biometry in large studies of adults, as well as children. We also assessed whether this gene was susceptible for any interaction with environmental factors such as education or lifestyle.

Methods

Study Populations

This study included all study participants with available *GJD2* genotype and SER from adults of the Rotterdam Study (RS-I, RS-II, and RS-III) and MYopia STudy (MYST), and from children of the Generation R study. The Rotterdam Study is a long-running, prospective population-based study conducted in city district Ommoord in Rotterdam, The Netherlands. MYST is a cross-sectional clinic-based case-control study that included highly myopic adult patients (SER ≤ −6D) and emmetropic controls. Generation R is a population-based prospective cohort study of children who were born between April 2002 and January 2006 in Rotterdam, The Netherlands. Detailed description and methodological background of these studies are available elsewhere.[18–21] All measurements 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.

Ophthalmological and Genetic Data

Adult Population. All participants from the Rotterdam Study and MYST underwent extensive ophthalmological examinations, including SER and corneal radius (CR) measured with the Topcon RM-A2000 Auto-Refractor (Topcon Optical Company, Tokyo, Japan). Biometric measurements including AL, corneal thickness (CT), ACD, and LT were measured with Lenstar LS900 (Laméris Ootech, Gelderland, The Netherlands). Three measurements of biometry per eye were averaged to a mean value. Participants with an AL greater than 30 mm were additionally measured with the A-scan function of the PacScan 300 AP (Sonomed Escalon, New Hyde Park, NY, USA) to guarantee accurate measurements on the higher end of the axial length spectrum. In the Rotterdam Study, biometry measurements were introduced at a later stage than refractive error and were therefore available in a subgroup of participants. Participants with bilateral pseudophakia, aphakia, or refractive surgical procedures at baseline without knowledge of refractive error before surgery were excluded for analysis regarding SER. Measurements of the two eyes were averaged; when these were missing in one eye, the measurement of the other eye was used. SER was calculated as the sum of the full spherical value and half of the cylindrical value. Mild and moderate myopia was defined as SER ≤ −0.5D and high myopia as SER ≤ −6D, according to the recent International Myopia Institute guidelines.[22] Data on LT of pseudophakic patients were excluded. Mean CR (in radius mm) was calculated as the sum of the mean values of keratometry readings of vertical and horizontal corneal meridian (K1 and K2) per eye divided by two. Vitreous depth (VD) was calculated by subtracting CT, ACD, and LT from total AL.

Children Population. For the Generation R Study, we included the research visits of the children aged six and nine years. At both ages, automated cycloplegic refractive error was measured using a Topcon KR8900 instrument (Topcon). Two drops (three in case of dark irises) of cyclopentolate (1%) with a five-minute interval were dispensed, and refractive error measurements were performed at least 30 minutes thereafter when the pupil diameter was ≥6 mm. In children at age six, automated cycloplegic refractive error was measured in a subset of children with a visual acuity of >0.1 logarithm of the minimum angle of resolution (LogMAR) in at least one eye, or in children with an ophthalmologic history. Those with visual acuity of ≤0.1 LogMAR, no glasses, and no ophthalmologic history were classified as nonmyopic.[23,24] In children at age nine, automated cycloplegic refractive error was measured in all children. Ocular biometry was measured by a Zeiss IOL-master 500 (Carl Zeiss Meditec, Jena, Germany) and included AL, ACD, K1, and K2. Myopia was defined as SER ≤ −0.5D in at least one eye. Five measurements of AL per eye were averaged to mean AL. Mean CR was calculated (CR) on the basis of three measurements of CR (K1 and K2) per eye. The AL/CR was used as a proxy for refractive error, because SER was not available for all children. Mean AL/CR ratio was calculated by dividing AL (mm) by CR (mm) for both eyes, divided by two. Furthermore, AL elongation (mm/year) was calculated by dividing the difference in AL between measurements at age six and age nine by the time in years. Mean AL elongation of two eyes was used in the analyses. Finally, because LT was not available in children, we calculated lens power in children aged nine years and in the adults using the previously validated modified Stenström and Bennett-Rabbetts methods, which uses spherical refraction, CR, ACD, and a customized value of the c-constant (2.550 and 2.560, respectively) for estimation.[25,26]

Genetic Data

The *GJD2* genotype was assessed according to the genotype of SNP rs524952 (TT, TA, AA) as described before.[9,27,28] The A allele is the risk allele (A) associated with a more negative refractive error; T is the reference allele. This SNP was chosen for the current analyses, because it was most frequently associated with refractive error and in complete linkage disequilibrium ($R^2 = 1$ and $D' = 1$) with the second most identified SNP annotated to *GJD2* (rs634990). Furthermore, analysis of other SNPs in high LD ($R^2 > 0.4$) that were previously associated with refractive error showed the same results (data not shown). Quality control procedures were performed. This SNP did not deviate from Hardy–Weinberg equilibrium ($P < 10^{-6}$) and its call rate was >0.05.

Investigative Ophthalmology & Visual Science

Environmental Factors

In the adult population, we focused on education as an environmental risk factor. Level of education was determined with a questionnaire using the United Nations Educational, Scientific and Cultural Organization classification for educational attainment.[29] We distinguished four levels of education: primary education, lower education (lower/intermediate general education or lower vocational education), intermediate education (intermediate vocational education or higher general education), and higher education (higher vocational education or university).

In the children population, we focused on the combined exposures of outdoor time and near work. These exposures were measured using a questionnaire filled in by the parents. Outdoor exposure was calculated as the sum of time playing outside and walking or cycling to and from school, and was averaged per day. Number of books read per week (<1 or ≥1 per week) and reading distance (in <30 cm or ≥30 cm) was asked and dichotomized. For desktop computer use, the question "How much time does your child use the computer in the morning/afternoon/evening" was asked for weekdays and weekend days separately. Total hours computer use per week was computed as the sum of five times weekdays and two times weekend days. To assess the combined effect of environmental factors, we calculated an environmental risk score (ERS) by performing a multivariate regression including outdoor exposure, books per week, and reading distance as described previously.[30]

Statistical Analysis

Differences in SER and ocular biometry between genotypes in adults and children were compared using ANOVA or post-hoc independent *t*-test for normally distributed data (CT, LT, CR, and lens power), and Kruskal Wallis test or Mann Whitney test for skewed data (SER, AL, AL/CR, ACD, and VD). Because age and gender influence biometry in children, we performed age- and gender-adjusted regression analysis in this group as well. To assess whether the different biometric components differed proportionally to total AL between genotypes, biometric components were divided by AL. To investigate whether refractive error or ocular biometry changed linearly with increasing number of risk alleles, a linear trend test was performed. In the MYST case control study, we performed logistic regression analysis, adjusted for age, sex, and education to estimate the effect of *GJD2* genotype. To assess the impact of the *GJD2* genotype as a risk factor for myopia in the adult population (RS and MYST), we calculated odds ratios (OR) using logistic regression analysis, adjusted for age and sex.

In the RS adults, the effect of education on the association between *GJD2* genotype and SER was determined using a stratified analysis and linear regression analysis. In children, we investigated the effect of the ERS in every *GJD2* stratum using ANOVA and linear regression analysis, adjusting for age, sex and ethnicity. In addition we calculated the Synergy Index (SI) and relative excess risk due to interaction (RERI), adjusted for age and sex, as proposed by Rothman, where a SI >1 or RERI >0 indicates a positive interaction.[31,32]

For all analyses, $P < 0.05$ was considered statistically significant. The IBM SPSS Statistics version 25 (IBM Corp. Armonk, NY, USA) was used for the statistical analyses. We used the epiR package of the R statistical software version 1.1.456 for calculation of the SI and RERI.

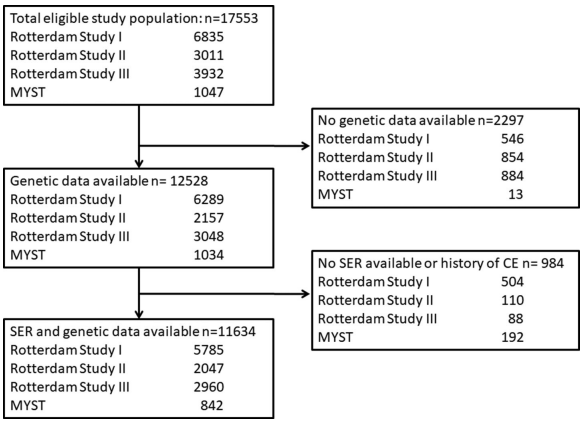

Figure 1. Selection process of study participants for this study for spherical equivalent (SER) analysis. CE, cataract extraction.

Results

The selection process of adult participants included in this analysis is shown in Figure 1. The final adult sample consisted of 11,634 participants (RS1 n = 5785, RSII n = 2047, RSIII n = 2930, and MYST n = 462 cases and n = 380 controls) (Fig. 1). In the adult RS study population, 42.5% were male, and mean (SD) age was 64.8 (9.4) (Table 1). Frequency of carrying zero, one, or two risk alleles was 27.3%, 49.5%, and 23.3%, respectively. Mean (SD) SER was 0.46 (2.60) D, and the prevalence of myopia and high myopia was 25.1% and 2.2%, respectively. In the MYST population, 39.4% and 45.8% of cases and controls were male; mean (SD) age was 45.9 (12.6) and 49.2 (12.5) years, respectively. The mean (SD) SER was −10.4 (3.38) D in cases and −0.50 (1.77) D in controls. The children population consisted of 4132 children examined at baseline at six years (50.1% male, mean [SD] age 6.18 [0.51]), and of 3133 children examined at follow-up performed at nine years (49.5% male, mean [SD] age 9.81 [0.38]) (Table 2). Frequency of carrying zero, one, or two risk alleles was 26.2%, 50.7%, and 23.1%, respectively. The prevalence of myopia was 2.2% and 11.6%, respectively (Table 2).

Effect on Refractive Error and Biometry

RS adults carrying two risk alleles had a lower SER, longer AL, longer ACD and longer VD (AA vs TT 0.23D vs 0.70D; 23.79 mm vs 23.52 mm; 2.72 mm vs 2.65 mm; 16.12 mm vs 15.87 mm; $P = 4.33 \times 10^{-7}$, $P = 1.10 \times 10^{-5}$, $P = 4.85 \times 10^{-4}$ and $P = 5.30 \times 10^{-5}$, respectively) (Table 3). In heterozygous carriers we observed a similar, but less strong effect in comparison to those carrying two risk alleles (AT versus TT, 0.43D vs. 0.70D; 23.64 mm vs. 23.52 mm; 2.69 mm vs. 2.65 mm; 15.97 mm vs. 15.87 mm; $P = 4.91 \times 10^{-6}$, $P = 1.61 \times 10^{-3}$, $P = 0.022$, and $P = 0.005$, respectively). AL was linearly longer with increasing number of risk alleles ($P = 6.67 \times 10^{-9}$). In MYST, carrying one GJD2 risk allele increased the risk of being a high myopia case (OR 1.486 [95% CI, 1.083–2.039]). This risk was even higher when carrying two risk alleles (OR 2.183 [95% CI, 1.516–3.143]) (Supplemental Table S1).

In adults (RS and MYST taken together), the OR for common myopia was 1.245 and 1.426 for heterozygous and homozygous risk carriers, respectively ($P = 2.71 \times 10^{-5}$ and

Table 1. Baseline Characteristics of the Adult Study Population

	Cohort						
	RS1	**RS2**	**RS3**	**RS Total**	**MYST – Cases**	**MYST–Controls**	**Total Adults**
Total population N	6835	3011	3932	13778	1047		17553
Genotype and SER known	5785	2047	2960	10729	462	380	11634
Risk allele (% A)	48.3	47.5	47.8	48.0	55.1	44.8	48.2
TT	1560 (27.0)	570 (27.8)	812 (27.4)	2942 (27.3)	116 (21.2)	126 (31.7)	3160 (27.2)
AT (one risk allele)	2865 (49.5)	1009 (49.3)	1465 (49.5)	5339 (49.5)	258 (47.3)	187 (47.0)	5736 (49.3)
AA (two risk alleles)	1360 (23.5)	468 (22.9)	683 (23.1)	2511 (23.3)	172 (31.5)	85 (21.4)	2738 (23.5)
Age (y), N	5785	2047	2960	10729	462	380	11634
Mean (SD)	68.8 (8.8)	64.4 (7.5)	57.1 (6.5)	64.8 (9.4)	45.9 (12.6)	49.2 (12.5)	63.6 (10.7)
Range	55.1–99.3	55.2–95.5	45.7–90.1	45.7–99.3	16.73–79.62	16.73–79.62	16.73–99.27
Sex (% men)	2350 (40.6)	940 (45.9)	1303 (44.0)	4591 (42.5)	182 (39.4)	174 (45.8)	4947 (42.5)
Education, N	5703	2027	2950	10680	448	364	11492
Primary education (%)	1339 (41.2)	150 (7.4)	303 (10.3)	1792 (16.8)	8 (1.8)	4 (1.1)	1804 (15.7)
Lower education (%)	2351 (41.2)	942 (46.5)	1061 (36.0)	4354 (40.8)	32 (7.1)	51 (14.0)	4437 (38.6)
Intermediate education (%)	1545 (27.1)	594 (29.3)	804 (27.3)	2943 (27.6)	103 (23.0)	105 (28.8)	3154 (27.4)
Higher education (%)	468 (22.3)	341 (16.8)	782 (26.5)	1591 (14.9)	305 (68.1)	204 (56.0)	2100 (18.3)
SER (D), mean (SD)	0.83 (2.59)	0.48 (2.51)	−0.29 (2.59)	0.46 (2.60)	−10.4 (3.38)	−0.50 (1.77)	−0.004 (2.26)
Range	−19.13 to 15.13	−15.25 to 10.50	−16.13 to 9.13	−19.13 to 15.13	−23.88 to 6.00	−5.94 to 4.00	−23.88 to 15.13
Myopia (%)*	1111 (19.2)	505 (24.7)	1091 (36.9)	2707 (25.1)	462 (100)	126 (33.2)	3295 (28.3)
High myopia (%)†	111 (1.9)	36 (1.8)	87 (2.9)	234 (2.2)	462 (100)	0 (0)	696 (6.0)
Biometry, N	915	1238	2635	4788	544	398	5730
AL (mm), N	915	1238	2635	4788	544	398	5730
Mean (SD)	23.49 (1.24)	23.53 (1.19)	23.74 (1.30)	23.64 (1.27)	27.56 (1.72)	23.89 (1.33)	24.03 (1.74)
Range	15.49–31.29	19.87–31.35	18.96–31.30	15.49–31.35	24.22–37.58	21.15–33.24	15.49–37.58
LT (mm), N	682	1075	2492	4249	459	378	5086
Mean (SD)	4.81 (0.44)	4.62 (0.38)	4.49 (0.39)	4.58 (0.41)	4.10 (0.40)	4.18 (0.37)	4.50 (0.44)
Range	3.79–6.66	2.61–6.04	3.19–6.29	2.61–6.66	1.92–5.16	2.56–5.18	1.92–6.66
CT (μm), N	567	1216	2391	4174	543	396	5113
Mean (SD)	547.15 (34.61)	547.66 (34.17)	550.28 (33.92)	549.09 (34.11)	542.33 (37.35)	545.02 (35.87)	548.0 (34.7)
Range	450.00–678.50	443.00–669.00	404.00–691.00	404.00–691.00	454.50–718.00	387.50–645.50	387.5–718.0
CR (radius mm), N	1493	1277	2396	5166	546	398	6115
Mean (SD)	7.72 (0.26)	7.73 (0.25)	7.75 (0.27)	7.74 (0.26)	7.73 (0.28)	7.79 (0.32)	7.75 (0.30)
Range	6.94–8.61	6.93–8.52	7.03–9.08	6.93–9.08	6.76–9.08	6.66–10.20	6.66–10.20
AL/CR, N	915	1238	2394	4778	544	398	5486
Mean (SD)	3.03 (0.16)	3.05 (0.15)	3.07 (0.16)	3.05 (0.16)	3.57 (0.20	3.07 (0.17)	3.11 (0.22)
Range	1.98–4.14	2.44–3.92	2.54–4.03	1.98–4.14	3.00–4.81	2.75–4.69	1.98–4.81
ACD (mm), N	733	1103	2524	4360	515	389	5264
Mean (SD)	2.75 (0.61)	2.66 (0.47)	2.68 (0.37)	2.68 (0.44)	3.08 (0.43)	2.82 (0.38)	2.73 (0.45)
Range	1.64–5.25	1.60–5.43	1.50–5.17	1.50–5.43	1.81–5.07	1.99–4.83	1.50–5.43
VD (mm), N	420	1054	2265	3739	494	384	4616
Mean (SD)	15.76 (1.00)	15.74 (1.06)	16.13 (1.18)	16.00 (1.14)	19.79 (1.63)	16.32 (1.17)	16.41 (1.68)
Range	12.56–18.80	13.03–21.15	12.32–23.53	12.32–23.53	16.71–26.56	13.78–23.89	12.32–26.56
Lens power mS (D), N	718	1086	2490	4294	457	374	5125
Mean (SD)	19.62 (2.11)	19.26 (1.73)	19.79 (1.91)	19.63 (1.91)	20.16 (1.85)	20.52 (2.10)	19.76 (1.95)
Range	13.73–26.95	13.44–27.08	12.70–26.61	12.70–27.08	12.78–26.42	12.94–26.15	12.70–27.08
Lens power BR (D), N	718	1086	2490	4294	457	374	5125
Mean (SD)	19.47 (2.09)	19.11 (1.71)	19.64 (1.89)	19.48 (1.89)	20.25 (1.87)	20.68 (2.12)	19.61 (1.93)
Range	13.63–26.71	13.44–26.84	12.60–26.37	12.60–26.84	12.83–26.60	13.04–26.35	12.60–26.84

mS, modified Stenström; BR, Bennett-Rabbetts.
* Myopia was defined as SER ≤ −0.5D.
† High myopia was defined as SER ≤ −6D.

Table 2. Baseline Characteristics of the Children Study Population

	Examination at 6 Years		Examination at 9 Years	
Genotype and myopia status known	N	4132	N	3390
Risk allele (% A)	4132	48.5	3390	48.3
TT	1082 (26.2)		899 (26.5)	
AT (one risk allele)	2094 (50.7)		1702 (50.2)	
AA (two risk alleles)	956 (23.1)		789 (23.3)	
Ethnicity (% European)	4132	3559 (86.1)	3390	2979 (87.9)
Gender (% male)	4132	2072 (50.1)	3390	1679 (49.5)
Age (y)				
Mean (SD)	4132	6.2 (0.5)	3390	9.8 (0.3)
Range		4.8–9.0		8.5–12.0
Myopia (n (%yes))	4132	92 (2.2)	3390	392 (11.6)
SER (D)				
Mean (SD)	—	—	1453	0.72 (1.32)
Range				−9.81 to 8.31
AL (mm)				
Mean (SD)	3877	22.37 (0.75)	3284	23.11 (0.84)
Range		17.54–28.88		17.68–27.72
CR (radius mm)				
Mean (SD)	3660	7.78 (0.26)	3278	7.79 (0.26)
Range		6.94-9.07		6.93–8.79
AL/CR				
Mean (SD)	3824	2.87 (0.08)	3276	2.97 (0.10)
Range		2.38–3.90		2.40–3.53
ACD (mm)				
Mean (SD)	3539	3.31 (0.32)	3218	3.57 (0.26)
Range		1.46–6.31		2.01–5.14
AL elongation (mm)				
Mean (SD)	—	—	2894	0.41 (0.17)
Range				0–1.65
Lens power (D) mS				
Mean (SD)	—	—	1339	23.33 (1.51)
Range				18.60–29.40
Lens power (D) BR				
Mean (SD)	—	—	1278	23.35 (1.52)
Range				18.63–29.38

mS, modified Stenström; BR, Bennett-Rabbetts.

Table 3. Biometric Measurements in the Three Different Genotype Groups in the Adult Rotterdam Study Population

GJD2 Genotype (rs524952)	Homozygous Risk Allele (AA) (N = 2511)	Heterozygous (AT) (N = 5339)	Homozygous Reference Allele (TT) (N = 2942)	*P* Value
SER (D)	0.23 (2.04)	0.43 (2.59)	0.70 (2.57)	4.33×10^{-7}*
% Myopia	696 (27.7)	1363 (25.5)	648 (22.0)	5.0×10^{-6}†
% High Myopia	61 (2.4)	125 (2.3)	48 (1.6)	0.062†
AL (mm)	23.79 (1.33)	23.64 (1.25)	23.52 (1.22)	1.10×10^{-5}*
CR (radius mm)	7.74 (0.27)	7.74 (0.26)	7.73 (0.26)	0.595‡
AL/CR	3.07 (0.16)	3.05 (0.16)	3.04 (0.15)	2.10×10^{-5}*
CT (µm)	548.64 (3.08)	548.71 (33.86)	550.17 (35.39)	0.452‡
ACD (mm)	2.72 (0.46)	2.69 (0.45)	2.65 (0.42)	4.85×10^{-4}*
LT (mm)	4.56 (0.41)	4.58 (0.41)	4.59 (0.41)	0.299‡
VD (mm)	16.12 (1.23)	15.97 (1.10)	15.87 (1.22)	5.30×10^{-5}*
Lens power mS (D)	19.60 (1.98)	19.62 (1.91)	19.66 (1.87)	0.790‡
Lens power BR (D)	19.45 (1.96)	19.47 (1.89)	19.50 (1.85)	0.802‡

Values are shown as mean (SD). The number (N) is the total number with a certain genotype with available SER.
mS, modified Stenström; BR, Bennett-Rabbetts.
* Calculated using Kruskal Wallis test.
† Calculated using χ^2 test.
‡ Calculated using ANOVA test.

$P = 3.99 \times 10^{-9}$); for high myopia the OR was 1.300 and 1.654, respectively ($P = 0.015$ and $P = 2.50 \times 10^{-5}$, respectively).

We were interested in the effect of *GJD2* on the different biometric components in relation to the enlarged AL. Therefore we assessed proportional changes, that is, the ratio

Table 4. Effect of GJD2 Genotype (rs524952) on Refractive Error and Biometric Measurements in the Children Population

Eye Measurement	Homozygous Risk Allele (AA)	Heterozygous (AT)	Homozygous Reference Allele (TT)	Beta *GJD2* (rs524952) Genotype*	*P* Value
Age 6	N = 956	N = 2094	N = 1082		
Myopia (% yes)	26 (2.7)	48 (2.3)	18 (1.7)	0.324 (0.28), 0.529 (0.31)†	0.235, 0.247
SER (D)	—	—	—	—	—
AL (mm)	22.39 (0.75)	22.38 (0.76)	22.34 (0.73)	0.041 (0.016)	0.009
CR (radius mm)	7.77 (0.26)	7.78 (0.26)	7.78 (0.26)	−0.001 (0.006)	0.897
AL/CR	2.88 (0.078)	2.88 (0.081)	2.87 (0.079)	0.006 (0.002)	3.11×10^{-4}
ACD (mm)	3.32 (0.33)	3.31 (0.31)	3.31 (0.34)	0.008 (0.008)	0.305
Age 9	N = 789	N = 1702	N = 899		
Myopia (%yes)	106 (13.4)	190 (11.2)	96 (10.7)	0.051 (0.13), 0.265 (0.150)†	0.698, 0.078
SER (D)	0.64 (1.4)	0.73 (1.3)	0.77 (1.3)	−0.064 (0.050)	0.194
AL (mm)	23.16 (0.85)	23.12 (0.84)	23.08 (0.83)	0.054 (0.019)	0.005
CR (radius mm)	7.78 (0.26)	7.79 (0.26)	7.80 (0.26)	−0.006 (0.006)	0.346
AL/CR	2.98 (0.096)	2.97 (0.094)	2.96 (0.097)	0.009 (0.002)	3.92×10^{-5}
ACD (mm)	3.59 (0.26)	3.57 (0.25)	3.56 (0.27)	0.021 (0.006)	0.001
AL elongation (mm)	0.42 (0.18)	0.41 (0.18)	0.41 (0.17)	0.005 (0.004)	0.288
Lens power mS (D)	23.25 (1.42)	23.38 (1.53)	23.32 (1.56)	−0.069 (0.054)	0.207
Lens power BR (D)	23.29 (1.41)	23.39 (1.53)	22.34 (1.59)	−0.059 (0.057)	0.301
ERS	−0.0055 (0.95)	0.035 (1.00)	−0.0087 (1.07)	−0.007 (0.031)	0.813

Values are shown as mean (SD). N is the number of individuals with available myopia diagnosis per genotype.
mS, modified Stenström; BR, Bennett-Rabbetts.
* Calculated using linear regression analysis, adjusted for age and sex.
† Calculated using logistic regression analysis, adjusted for age and sex: AT vs. TT and AA vs. T.

between a biometric component and total AL, in the adult RS population and identified a disproportionally decreased LT and CT (AA versus TT, 0.191 vs. 0.195; 0.0231 vs. 0.0234; $P = 1.93 \times 10^{-4}$ and $P = 2.19 \times 10^{-4}$, respectively) and a longer VD (AA versus TT, 0.676 vs. 0.674, $P = 0.017$); we observed no disproportional difference in ACD (AA versus TT, 0.114 vs. 0.112, $P = 0.100$ (Supplemental Table S2).

In children homozygous for *GJD2* risk alleles, AL was significantly longer, after correction for age and gender, at both 6 and 9 years (AA versus TT, 22.39 mm vs. 22.34 mm, $P = 0.009$, and 23.16 mm vs. 23.08 mm, $P = 0.005$ for ages six and nine, respectively) (Table 4). ACD was larger in children carrying any number of *GJD2* risk alleles, but this effect was only significant in children aged nine (AA versus TT, 3.32 mm vs. 3.31 mm, $P = 0.305$, and 3.59 mm vs. 2.56 mm, $P = 0.001$ for ages six and nine, respectively). In addition, children carrying two risk alleles had larger AL/CR at ages six and nine years (AA versus TT, 2.88 vs. 2.87, and 2.98 vs. 2.96, $P = 3.11 \times 10^{-4}$ and $P = 3.92 \times 10^{-5}$, respectively). *GJD2* genotype was not significantly associated with CR ($P = 0.897$ and $P = 0.346$ for ages six and nine), AL elongation between ages six and nine ($P = 0.288$) or lens power (modified Stenström $P = 0.207$; Bennett-Rabbetts $P = 0.301$).

Interaction With Environmental Factors

The effect of education in adults and ERS in children on the association between *GJD2* and myopia is shown in Figures 2 and 3. Adults with more *GJD2* risk alleles had a lower SER in every education stratum, except for primary education ($\text{Beta}_{\text{primary education}} = -0.131$ ($P = 0.110$); $\text{Beta}_{\text{lower education}} = -0.251$; $\text{Beta}_{\text{intermediate education}} = -0.249$; $\text{Beta}_{\text{higher education}} = -0.22$ ($P = 2.79 \times 10^{-6}$, $P = 2.11 \times 10^{-4}$ and $P = 0.014$ for other education level groups, respectively) (Fig. 2). However, when adjusting for age and sex, the interaction effect was not significant (Beta = −0.025, $P = 0.503$). The SI and RERI examining the biological interaction between education and *GJD2* genotype did not reach statistical significance (SI 1.25 [0.85–1.85] and RERI 0.185 [−0.087 to 0.459]). The combined effect of outdoor exposure and near work calculated as ERS in children followed the same trend that was observed with education in adults: in children with an increased ERS, we observed a more myopic AL/CR in children carrying one or two risk alleles (AL/CR below versus above median ERS: 2.95 vs. 2.96, $P = 0.281$; 2.96 vs. 2.98, $P = 1.33 \times 10^{-4}$; 2.97 vs. 2.99, $P = 0.028$ for none, one, and two *GJD2* risk alleles, respectively (Fig. 3). The SI and RERI for interaction in children was not significant (1.17 [95% CI 0.55–2.50] and 0.25 [95% CI −0.85 to 1.36], respectively).

Discussion

Our combined analysis of large studies on Dutch adult and children revealed that the *GJD2* risk genotype is associated with myopia mainly by an enlarged VD and ACD and not by changes in LT or CT. The effect of the *GJD2* genotype on total AL was already visible in children aged six and nine years old, suggesting that *GJD2* has an effect at early age. The effect of the risk alleles was consistent with a dose response relationship, implying that alleles have an additive effect. The large risks of myopia and high myopia among carriers of a single-risk SNP suggests that this gene can be a showcase of how common myopia genes affect ocular phenotype at an early age.

We identified the most prominent effect of *GJD2* on total AL: the entire length of the eye was longer in risk carriers compared to non-risk carriers in both adults and children. This is a typical characteristic found in myopia, the ocular globe is mainly enlarged in length and not height or width.[33–35] The ACD enlargement was only significant in adults and children risk carriers at the age of nine, implying that VD precedes ACD in myopia development. Several studies also investigated biometric components in *GJD2*.[3,28,36,37] Cheng et al.[3] found a longer AL in adult carriers, and Li

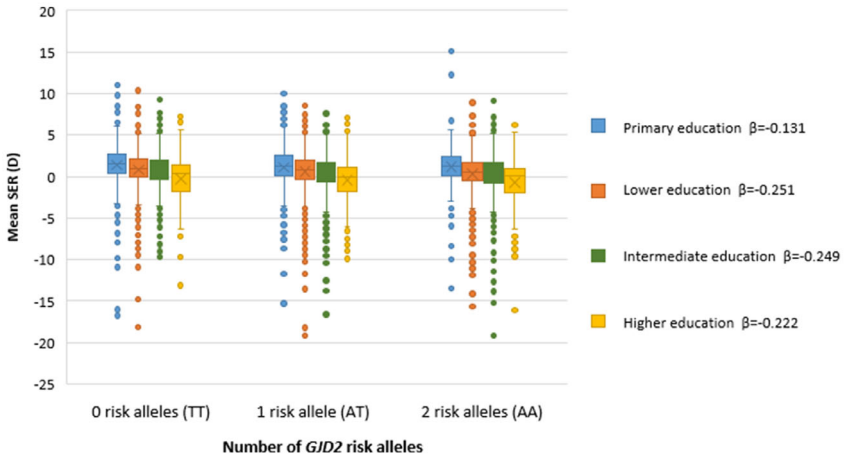

Figure 2. Effect of the *GJD2* genotype (rs524952, 0–2 risk alleles) on mean SER (in diopters [D]) in RS adult population, stratified by educational level. X-axis represents number of *GJD2* risk alleles (rs524952, 0–2) and genotype. Colors represent the four educational levels. Beta coefficients are the effect of *GJD2* genotype, adjusted for age and sex, within every educational level. Beta = −0.131 ($P = 0.110$); Beta = −0.251 ($P = 2.79 \times 10^{-6}$); Beta = −0.249 ($P = 2.11 \times 10^{-4}$); Beta = −0.222 ($P = 0.014$) for primary, lower, intermediate, and higher education, respectively.

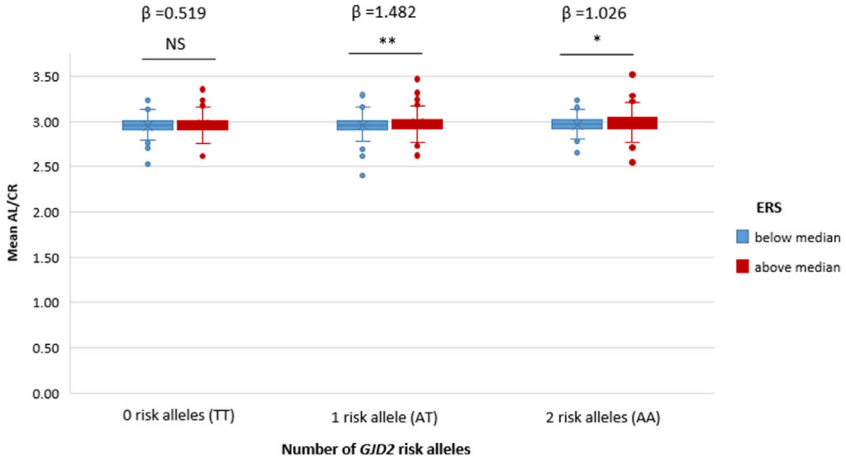

Figure 3. Effect of the ERS on AL/CR in children, stratified by *GJD2* genotype (rs524952, 0–2 risk alleles). Betas indicate regression coefficients demonstrating the effect of ERS on AL/CR within every genotype, derived from linear regression analysis adjusted for age, sex, and ethnicity. Beta_0 risk alleles = 0.519 ($P = 0.203$); Beta_1 risk allele = 1.482 ($P = 9.67 \times 10^{-7}$); Beta_2 risk alleles = 1.026 ($P = 0.014$). NS, not significant. *$P < 0.05$; **$P < 0.001$.

and coworkers[37] identified an effect of *GJD2* genotype on both SER and AL in children. Tideman et al.[28] analyzed children cohorts from the TEST, SCORM, STARS, and Guangzhou Twins studies, in addition to Generation R, and found a significant association between *GJD2* and AL/CR. Only Chen at al.[36] did not find evidence for a relationship, not with AL nor with other biometric markers. This Chinese cohort study examined *GJD2* SNP rs634990, a variant in full LD with our SNP, and we therefore had anticipated similar results. However, the sample size included only 814 participants; hence, it is likely that lack of power explains the lack of significant findings.[36]

In contrast to the elongating effect on AL, ACD and VD, *GJD2* genotype did not influence LT or CT. This is in contrast to our expectations, because in particular LT is known to have adaptation potential to refractive changes and can become thinner with myopia progression.[38–40] Therefore we investigated whether LT and CT were disproportionally altered in thickness or were in line with the AL enlargement. We found a somewhat thinner-than-expected cornea and lens in persons carrying *GJD2* risk alleles, but this did not result in functional consequences with respect to refractive power. In addition, the absence of *GJD2* expression in human lens or corneal tissue makes a genuine effect on these structures unlikely.

Current evidence for the link between the gene *GJD2* and refractive error are based on risk SNPs with a probably regulatory function and a location in the proximity of *GJD2*. Mechanisms through which *GJD2* alters eye growth are yet unclear. *GJD2* plays a pivotal role in retinal signal transduction and is expressed in gap junctions between cones, rods, and bipolar cells.[41–43] Furthermore, animal studies demonstrated expression in AII amacrine cells and ganglion cells.[44,45] Changes in expression may cause disruption of the normal channel permeability and affect signal transduction and size of receptive fields. A blurry image and

axial length elongation may be the result.[46] The investigated SNP is located in a regulatory region, which may influence gene expression.[12] Although the GTEX database shows both decreased and increased expression in neuronal tissue for this SNP, it has a clear expression quantitative trait locus (eQTL) effect in pituitary, pancreas, skeletal muscle, and heart-atrial appendage tissue with the A risk allele associated with decreased expression of *GJD2*. Functional studies showing the direction of this SNP effect in ocular tissue are currently lacking.

Our study has strengths and limitations. Among the strengths are the large study population including all RS cohorts, MYST, and the Generation R study aiming to maximize statistical power, enriching the number of persons with high myopia and increasing generalizability of findings. Extensive biometric data was available to evaluate the gene effect on various eye components in both adults and children. All studies of adults were performed at the same research center using identical study protocols, thus increasing homogeneity across studies and validating a combined analysis of outcomes. Another strength is the inclusion of both adults and children. The adults revealed gene effects on the final refractive state; the children allowed the study of changes during emmetropization and early myopization. A limitation was the lack of measurements on LT and CT in children. Fortunately, we were able to estimate lens power for these missing data using independent methods. We and others lack data on biometry in children from the first years after birth and cannot identify whether *GJD2* effects are congenital or mostly result from early environmental triggers.

In conclusion, the *GJD2* risk genotype leads to myopia mainly by an elongated VD and ACD in a dose-response fashion already at an early age. The early onset and significant risk of high myopia make this gene interesting for further examination of myopia mechanisms. Deciphering how this gap junction protein drives eye elongation may reveal promising drug targets and open the way to risk management in myopia.

Acknowledgments

The authors thank all ophthalmologists who have referred high myopic cases to the Myopia Study, including J.G.M. van Beek, MD (Department of ophthalmology, Albert Schweitzer Hospital Dordrecht, The Netherlands), I. Bleyen, MD, B.T. van Dooren, MD, PhD, E. Kilic, MD, PhD, S.E. Loudon, MD, PhD, J.R. Polling, BoH, H. J. Simonsz, MD, PhD, and R.C. Wolfs, MD, PhD (Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands); G.L. Porro, MD, PhD, and J.J. Willemse Assink, MD, PhD (Department of Ophthalmology, Amphia Hospital, Breda, The Netherlands); C.B. Hoyng, MD, PhD (Department of Ophthalmology, Radboud University Medical Center, The Netherlands); M.J. Jager, MD, PhD (Department of Ophthalmology, Leiden University Medical Center, The Netherlands); R. van Leeuwen, MD, PhD (Department of Ophthalmology, University Medical Center Utrecht, The Netherlands); A.M.J. Roefs, MD (Oogkliniek Drechtsteden (Papendrecht), The Netherlands), N.W.R. Slingerland, MD and K.L. de Roon Hertoge, MD (Oogartsenpraktijk Delfland, Delft, The Netherlands); and F.D. Verbraak, MD, PhD (Department of Ophthalmology, Amsterdam Medical Center, Amsterdam, The Netherlands).

Supported by the following foundations: Oogfonds, ODAS, Uitzicht 2017-28 (LSBS, MaculaFonds, Oogfonds), Netherlands Organization for Scientific Research (NWO); Grant 91617076 (VJMV) and Grant 91815655 (CCWK), and European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme Grant 648268 (CCWK).

Disclosure: **A.E.G. Haarman,** None; **C.A. Enthoven,** None; **M.S. Tedja,** None; **J.R. Polling,** None; **J.W.L. Tideman,** None; **J.E.E. Keunen,** None; **C.J.F. Boon,** None; **J.F. Felix,** None; **H. Raat,** None; **A.J.M. Geerards,** None; **G.P.M. Luyten,** None; **G.A. van Rijn,** None; **V.J.M. Verhoeven,** None; **C.C.W. Klaver,** None

References

1. Stambolian D. Genetic susceptibility and mechanisms for refractive error. *Clin Genet*. 2013;84:102–108.
2. Verhoeven VJ, Hysi PG, Saw SM, et al. Large scale international replication and meta-analysis study confirms association of the 15q14 locus with myopia. The CREAM consortium. *Hum Genet*. 2012;131:1467–1480.
3. Cheng CY, Schache M, Ikram MK, et al. Nine loci for ocular axial length identified through genome-wide association studies, including shared loci with refractive error. *Am J Hum Genet*. 2013;93:264–277.
4. Hysi PG, Choquet H, Khawaja AP, et al. Meta-analysis of 542,934 subjects of European ancestry identifies new genes and mechanisms predisposing to refractive error and myopia. *Nat Genet*. 2020;52:401–407.
5. Schache M, Richardson AJ, Mitchell P, et al. Genetic association of refractive error and axial length with 15q14 but not 15q25 in the Blue Mountains Eye Study cohort. *Ophthalmology*. 2013;120:292–297.
6. Simpson CL, Wojciechowski R, Yee SS, Soni P, Bailey-Wilson JE, Stambolian D. Regional replication of association with refractive error on 15q14 and 15q25 in the Age-Related Eye Disease Study cohort. *Mol Vis*. 2013;19:2173–2186.
7. Kiefer AK, Tung JY, Do CB, et al. Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia. *PLoS Genet*. 2013;9:e1003299.
8. Yoshikawa M, Yamashiro K, Miyake M, et al. Comprehensive replication of the relationship between myopia-related genes and refractive errors in a large Japanese cohort. *Invest Ophthalmol Vis Sci*. 2014;55:7343–7354.
9. Tedja MS, Wojciechowski R, Hysi PG, et al. Genome-wide association meta-analysis highlights light-induced signaling as a driver for refractive error. *Nat Genet*. 2018;50:834–848.
10. Verhoeven VJ, Hysi PG, Wojciechowski R, et al. Genome-wide meta-analyses of multiancestry cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nat Genet*. 2013;45:314–318.
11. Tedja MS, Haarman AEG, Meester-Smoor MA, et al. IMI–Myopia Genetics Report. *Invest Ophthalmol Vis Sci*. 2019;60:M89–M105.
12. Solouki AM, Verhoeven VJ, van Duijn CM, et al. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nat Genet*. 2010;42:897–901.
13. Saez JC, Connor JA, Spray DC, Bennett MV. Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. *Proc Natl Acad Sci U S A*. 1989;86:2708–2712.
14. Carter TD, Chen XY, Carlile G, Kalapothakis E, Ogden D, Evans WH. Porcine aortic endothelial gap junctions: identification and permeation by caged InsP3. *J Cell Sci*. 1996;109(Pt 7):1765–1773.
15. Goldberg GS, Lampe PD, Sheedy D, Stewart CC, Nicholson BJ, Naus CC. Direct isolation and analysis of endogenous transjunctional ADP from Cx43 transfected C6 glioma cells. *Exp Cell Res*. 1998;239:82–92.

Investigative Ophthalmology & Visual Science

16. Kam Y, Kim DY, Koo SK, CO Joe. Transfer of second messengers through gap junction connexin 43 channels reconstituted in liposomes. *Biochim Biophys Acta*. 1998;1372:384–388.
17. Belluardo N, Trovato-Salinaro A, Mudo G, Hurd YL, Condorelli DF. Structure, chromosomal localization, and brain expression of human Cx36 gene. *J Neurosci Res*. 1999;57:740–752.
18. Ikram MA, Brusselle G, Ghanbari M, et al. Objectives, design and main findings until 2020 from the Rotterdam Study. *Eur J Epidemiol*. 2020;35:483–517.
19. Tideman JL, Snabel MC, Tedja MS, et al. Association of axial length with risk of uncorrectable visual impairment for Europeans with myopia. *JAMA Ophthalmol*. 2016;134:1355–1363.
20. Kruithof CJ, Kooijman MN, van Duijn CM, et al. The Generation R Study: Biobank update 2015. *Eur J Epidemiol*. 2014;29:911–927.
21. Kooijman MN, Kruithof CJ, van Duijn CM, et al. The Generation R Study: design and cohort update 2017. *Eur J Epidemiol*. 2016;31:1243–1264.
22. Flitcroft DI, He M, Jonas JB, et al. IMI–defining and classifying myopia: a proposed set of standards for clinical and epidemiologic studies. *Invest Ophthalmol Vis Sci*. 2019;60:M20–M30.
23. Leone JF, Mitchell P, Morgan IG, Kifley A, Rose KA. Use of visual acuity to screen for significant refractive errors in adolescents: is it reliable? *Arch Ophthalmol*. 2010;128:894–899.
24. O'Donoghue L, Rudnicka AR, McClelland JF, Logan NS, Saunders KJ. Visual acuity measures do not reliably detect childhood refractive error—an epidemiological study. *PLoS One*. 2012;7:e34441.
25. Rozema JJ, Atchison DA, Kasthurirangan S, Pope JM, Tassignon MJ. Methods to estimate the size and shape of the unaccommodated crystalline lens in vivo. *Invest Ophthalmol Vis Sci*. 2012;53:2533–2540.
26. Rozema JJ, Atchison DA, Tassignon MJ. Comparing methods to estimate the human lens power. *Invest Ophthalmol Vis Sci*. 2011;52:7937–7942.
27. Medina-Gomez C, Felix JF, Estrada K, et al. Challenges in conducting genome-wide association studies in highly admixed multi-ethnic populations: the Generation R Study. *Eur J Epidemiol*. 2015;30:317–330.
28. Tideman JW, Fan Q, Polling JR, et al. When do myopia genes have their effect? Comparison of genetic risks between children and adults. *Genet Epidemiol*. 2016;40:756–766.
29. United Nations Educational SaCOU. *International Standard Classification of Education (ISCED)*. 1976.
30. Enthoven CA, Tideman JWL, Polling JR, et al. Interaction between lifestyle and genetic susceptibility in myopia: the Generation R study. *Eur J Epidemiol*. 2019;34:777–784.
31. Rothman KJ GS, Lash TJ. *Modern epidemiology*. 3rd edition. Philadelphia, PA: Lippincott Williams and Wilkins; 2008.
32. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. *Epidemiology*. 1992;3:452–456.
33. Meng W, Butterworth J, Malecaze F, Calvas P. Axial length of myopia: a review of current research. *Ophthalmologica*. 2011;225:127–134.
34. Rada JA, Shelton S, Norton TT. The sclera and myopia. *Exp Eye Res*. 2006;82:185–200.
35. Atchison DA, Jones CE, Schmid KL, et al. Eye shape in emmetropia and myopia. *Invest Ophthalmol Vis Sci*. 2004;45:3380–3386.
36. Chen JH, Chen H, Huang S, et al. Endophenotyping reveals differential phenotype-genotype correlations between myopia-associated polymorphisms and eye biometric parameters. *Mol Vis*. 2012;18:765–778.
37. Li FF, Lu SY, Tang SM, et al. Genetic associations of myopia severities and endophenotypes in children [published online ahead of print August 14, 2020]. *Br J Ophthalmol*, https://doi.org 10.1136/bjophthalmol-2020-316728.
38. Mutti DO, Sinnott LT, Lynn Mitchell G, et al. Ocular Component Development during Infancy and Early Childhood. *Optom Vis Sci*. 2018;95:976–985.
39. Iribarren R, Morgan IG, Chan YH, Lin X, Saw SM. Changes in lens power in Singapore Chinese children during refractive development. *Invest Ophthalmol Vis Sci*. 2012;53:5124–5130.
40. Muralidharan G, Martinez-Enriquez E, Birkenfeld J, Velasco-Ocana M, Perez-Merino P, Marcos S. Morphological changes of human crystalline lens in myopia. *Biomed Opt Express*. 2019;10:6084–6095.
41. Kantor O, Benko Z, Enzsoly A, et al. Characterization of connexin36 gap junctions in the human outer retina. *Brain Struct Funct*. 2016;221:2963–2984.
42. Volgyi B, Deans MR, Paul DL, Bloomfield SA. Convergence and segregation of the multiple rod pathways in mammalian retina. *J Neurosci*. 2004;24:11182–11192.
43. Deans MR, Volgyi B, Goodenough DA, Bloomfield SA, Paul DL. Connexin36 is essential for transmission of rod-mediated visual signals in the mammalian retina. *Neuron*. 2002;36:703–712.
44. Feigenspan A, Teubner B, Willecke K, Weiler R. Expression of neuronal connexin36 in AII amacrine cells of the mammalian retina. *J Neurosci*. 2001;21:230–239.
45. Schubert T, Degen J, Willecke K, Hormuzdi SG, Monyer H, Weiler R. Connexin36 mediates gap junctional coupling of alpha-ganglion cells in mouse retina. *J Comp Neurol*. 2005;485:191–201.
46. Kumar NM, Gilula NB. The gap junction communication channel. *Cell*. 1996;84:381–388.

Investigative Ophthalmology & Visual Science