Association of *WNT7B* and *RSPO1* with Axial Length in School Children

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Purpose. To evaluate the association between single-nucleotide polymorphisms (SNPs) in the *ZC3H11B*, *RSPO1*, *C3orf26*, *GJD2*, *ZNRF3*, and *WNT7B* genes and myopia endophenotypes in children.

Methods. Seven SNPs identified in previous genome-wide association studies of axial length (AL) were genotyped in 2883 Southern Han Chinese children. Multiple linear regression analyses were conducted to evaluate the genotype association with AL, spherical equivalent (SE), corneal curvature (CC), and central corneal thickness (CCT).

Results. Two SNPs—namely, rs12144790 in *RSPO1* (allele T, $P = 0.0066$, $\beta = 0.062$) and rs10453441 in *WNT7B* (allele A, $P = 8.03 \times 10^{-6}$, $\beta = 0.103$)—were significantly associated with AL. The association of rs4373767 in *ZC3H11B* (allele C, $P = 0.030$, $\beta = -0.053$) could not withstand the correction for multiple testing. *WNT7B* rs10453441 showed a strong association with CC ($P = 1.17 \times 10^{-14}$, $\beta = 0.053$) and with CCT ($P = 0.0026$, $\beta = 2.65$). None of the tested SNPs was significantly associated with SE. The C allele of SNP rs12321 in *ZNRF3* was associated with CC ($P = 0.0060$, $\beta = -0.018$).

Conclusions. This study revealed that the *RSPO1* SNP rs12144790 was associated with AL, whereas *WNT7B* rs10453441 was associated with AL, CC, and CCT in children. A novel association between *ZNRF3* rs12321 and CC was discovered. Our data suggest that the *RSPO1* and *WNT7B* genes might exert their effects on multiple aspects of eye growth during childhood. Potential differences in the genetic profiles of AL between children and adults should be explored in larger cohorts.

Keywords: axial length, children, *WNT7B*, *RSPO1*, genetic association

Refractive errors are common ocular conditions. Uncorrected refractive errors, with significantly increasing prevalence, affect more than 100 million people worldwide and cause economic loss of more than $USD200 billion annually due to lost productivity.[1,2] As the most common type of refractive error, myopia is a leading cause of visual impairment, especially in East Asian populations.[3] In addition, individuals with high myopia, defined as a spherical equivalent (SE) of –6 diopters or above, have a higher risk of blinding complications, such as myopic macular degeneration, choroidal neovascularization, retinal detachment, and glaucoma.[4] Elongation of the axial length (AL) is the major component in myopia development and progression, making AL an essential endophenotype of myopia.[5]

Axial length is a multifactorial trait. Age is a main factor for axial elongation. Postnatal development of the eyeball in human is rapid in the first 24 to 36 months of life, during which the AL normally elongates from about 16 to 17 mm to 22.5 to 23 mm.[6,7] The growth of AL slows down subsequently and gradually reaches its full size, with a further increase of ~1 mm until about 13 years of age, or young adulthood.[8,9] AL elongation is also associated with sex, education, occupation, and height.[10] Among these factors, the cumulative effect of longer time spent in education was shown to pose a causal risk for myopic refractive error.[11] Also, genetic factors may affect the traits to different degrees due to variations in gene expression at different stages of life.[12] If there are accumulative environmental

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effects on AL with age, the inter-person variations in AL and refractive errors in younger people (e.g., children), who have less accumulated exposure to external risk factors, are more likely to be genetically driven. Genetic loci for refractive error with various associations between children and adults have been reported.[13] Age-dependent genetic associations have also been reported in systemic traits, such as height[14] and body mass index.[15] AL genetic loci have been discovered in subjects of different ages.[16–18] The first two genome-wide association studies (GWASs) on AL included child subjects (>10 years of age), but the loci were identified from combined cohorts in which a large proportion of subjects were adults.[16,17] Only one locus discovered by the GWASs, *ZC3H11B*, has been shown in a child-only cohort.[16]

As an endophenotype of refractive errors, AL may have some shared associated genes with refractive errors. Previous GWASs on AL have identified associated variants in seven genes/loci: *ZC3H11B*, *RSPO1*, *C3orf26*, *GJD2*, *LAMA2*, *ZNRF3*, and *WNT7B*.[16–18] The single-nucleotide polymorphisms (SNPs) that showed the most significant associations with AL in these loci were rs4373767 (chr1:219586340, Human Genome Assembly GRCh38.p13), rs4074961 (chr1:37627051), rs9811920 (chr3:100125449), rs11073058 (chr15:34697425), rs12193446 (chr6:129498893), rs12321 (chr22:29057205), and rs10453441 (chr22:45967859), respectively.[16–18] Among them, *ZC3H11B*, *RSPO1*, *GJD2*, and *LAMA2* have also been associated with refractive errors.[19–21] Moreover, AL has higher heritability estimates (0.67–0.94) than SE (0.58–0.88) in different ethnicities.[22–24] Apart from refractive errors, AL also shares some genetic factors with other ocular traits. For example, the *WNT7B* SNP rs10453441 was associated with corneal curvature (CC)[18] and central corneal thickness (CCT)[25,26] in adults. However, whether these genes are also associated with AL, refractive errors, CC, and/or CCT in children remains largely unknown. In this study, we evaluated the effects of reported AL-associated loci in a cohort of children.

Methods

Study Subjects and Phenotype Measurements

A total of 2883 Chinese children were involved in this study. They were recruited from the Hong Kong Children Eye Study (HKCES) between January 2016 and July 2017. The HKCES is a population-based cohort study to longitudinally investigate the development of ocular quantitative traits and occurrence of childhood eye diseases based on comprehensive ophthalmic examinations and questionnaires on detailed information of daily activities and diet. Participants in the HKCES were randomly recruited from primary schools in all districts of Hong Kong. The details of the HKCES were previously described.[27,28]

All study subjects were given complete ophthalmic examinations, physical examinations, and a standardized interview, followed by the collection of buccal swabs for DNA extraction. Axial length for both eyes was measured with a Zeiss IOL Master optical biometer (Carl Zeiss Meditec, Jena, Germany). Spherical equivalent (diopters, equal to sphere + cylinder/2) and corneal curvature (millimeters, average of corneal radius) were measured after cycloplegia using an ARK-510A auto-refractor (Nidek, San Jose, CA, USA). CCT was measured using a Corvis ST non-contact tonometer (Oculus, Wetzlar, Germany).

This study was approved by the ethics committee of The Chinese University of Hong Kong and performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parents, and verbal consent was obtained from each child.

Selection of SNPs and Genetic Analysis

We selected seven candidate SNPs in seven loci reported in previous GWASs of AL—namely, rs4373767 in *ZC3H11B*, rs4074961 in *RSPO1*, rs9811920 in *C3orf26*, rs11073058 near *GJD2*, rs12193446 in *LAMA2*, rs12321 in *ZNRF3*, and rs10453441 in *WNT7B*. Each of these SNPs had the strongest association with AL in the respective genes.[16–18] Because the assay for *RSPO1* rs4074961 was not commercially available, an alternative SNP, rs12144790, was selected as a replacement based on linkage disequilibrium ($R^2 = 0.8803$ in Southern Han Chinese; phase 3 of the 1000 Genomes Project, http://www.internationalgenome.org/home). Genomic DNA was extracted from the buccal swab sample of each subject using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The seven SNPs was genotyped in all of the 2883 child subjects using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on a Light Cycler 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's instructions.

Statistical Analysis

The Hardy–Weinberg equilibrium (HWE) test and genotype–phenotype association analyses were performed in PLINK 1.9 (https://www.cog-genomics.org/plink).[29] A SNP with $P < 0.05$ in the HWE test was removed from further statistical analysis. The allelic associations of each SNP with the required ocular quantitative traits were evaluated by a multiple linear regression model adjusted for other covariates. The mean value of the parameters in both eyes was applied as the dependent variable, and the allele dosage coding (i.e., 0, 1 or 2, indicating the number of minor alleles) was taken as the independent variable in the regression model. The covariates in the regression analysis for AL association included age, gender, and height,[30] whereas the regressions of SE, CC, and CCT were adjusted for age and gender only. The Bonferroni correction was adopted for multiple testing. Conditional analysis of the SNPs was conducted by additional covariate linear regression. $P < 0.0083$ (= 0.05/6, where 6 is the number of SNPs tested) was considered to be statistically significant study wide. Correlation analysis of AL and CC on SE was conducted using multiple linear regression. All of the linear regression analyses were performed using PLINK 1.9 and SPSS Statistics 23 (IBM, Armonk, NY, USA).

Table 1. Demographic Features of the Study Participants

Demographic	Total ($N = 2883$)
Age (y), mean ± SD	7.55 ± 0.94
Male (%)	51.2
Average AL (mm), mean ± SD	23.13 ± 0.94
Height (cm), mean ± SD	124.53 ± 7.72
Average SE (diopter), mean ± SD	0.14 ± 1.55
Average CC (mm), mean ± SD	7.79 ± 0.26
Average CCT (μm), mean ± SD	550.22 ± 30.70

Table 2. Genotyping Results of SNPs in 2883 Children

SNP	Chromosome	Position*	Gene/Locus	A1/A2†	Genotype Count‡	Call Rate	HWE *P*	Minor Allele Frequency
rs12144790	1	37623442	*RSPO1*	T/C	422/1367/1086	0.997	0.84	0.385
rs4373767	1	219586340	*ZC3H11B*	C/T	252/1181/1444	0.998	0.65	0.293
rs9811920	3	100125449	*C3orf26*	A/G	382/1304/1186	0.996	0.44	0.360
rs11073058	15	34697425	*GJD2*	T/G	505/1404/964	0.997	0.91	0.420
rs12321	22	29057205	*ZNRF3*	C/G	520/1408/946	0.997	0.94	0.426
rs10453441	22	45967859	*WNT7B*	A/G	353/1266/1257	0.998	0.21	0.343

*Genomic position is based on Genome Reference Consortium Human Build 38 (GRCh38).
†Minor allele/major allele, where minor allele is the effect allele for the effect size β.
‡The numbers represent the counts of samples with genotypes A1A1, A1A2, and A2A2.

Table 3. Allelic Association of SNPs with Axial Length

SNP	Chromosome	Position*	Gene/Locus	A1/A2†	β (SE)‡	*P*§	Reported β‖	Ref.‖
rs12144790	1	37623442	*RSPO1*	T/C	0.062 (0.023)	**0.0066**	0.07	17
rs4373767	1	219586340	*ZC3H11B*	C/T	–0.053 (0.024)	0.030	–0.16	16
rs9811920	3	100125449	*C3orf26*	A/G	–0.031 (0.023)	0.17	0.08	17
rs11073058	15	34697425	*GJD2*	T/G	0.044 (0.023)	0.051	0.07	17
rs12321	22	29057205	*ZNRF3*	C/G	–0.027 (0.022)	0.23	–0.05	17
rs10453441	22	45967859	*WNT7B*	A/G	0.103 (0.023)	**8.03×10^{-6}**	0.12	18

*Genomic position is based on GRCh38.
†Minor allele/major allele, where minor allele is the effect allele for the effect size β.
‡β is the coefficient of linear regression indicating the effect sizes on axial length (in mm).
§*P* values are derived from the linear regressions adjusted for age, gender, and height. The *P* values in bold are less than the significance level cutoff of 0.0083 after multiple testing correction.
‖Comparison of effect size with the previous genetic reports. Reported β is the reported coefficient and the Ref. column shows reference numbers for the previous publications.

Results

Features of Study Subjects and Genotyping of SNPs

A total of 2883 Chinese children from 5 to 10 years of age were included in this study (Table 1). The mean age of the subjects was 7.55 ± 0.94 years, and 51.2% of the subjects were boys. The mean AL (i.e., averaged AL of both eyes) was 23.13 ± 0.94 mm, and mean height was 124.53 ± 7.72 cm. Also, the mean SE, CC, and CCT were 0.14 ± 1.55 diopter, 7.79 ± 0.26 mm, and 550.22 ± 30.70 µm, respectively (Table 1). All seven candidate SNPs were genotyped in the 2883 children. SNP rs12193446 in *LAMA2* was nonpolymorphic in the samples and was removed from further data analysis. The call rates of the other six SNPs were >99.6%. None of the SNPs showed deviation from the HWE ($P > 0.05$) (Table 2).

SNP Associations with AL

Table 3 shows the SNP association results for AL. Two SNPs (i.e., rs10453441 and rs12144790) were significantly associated with AL, withstanding the Bonferroni correction ($P < 0.0083$). The minor allele A of the SNP rs10453441 in *WNT7B* showed a robust association ($P = 8.03 \times 10^{-6}$), with an effect size of 0.103 ± 0.023 mm. Such an effect size is comparable to a previous report in adults ($\beta = 0.12$).[18] Another SNP, rs12144790, in *RSPO1* was also associated with AL ($P = 0.0066$); its minor allele T was correlated with an increase of 0.062 ± 0.023 mm in AL, an effect size that is similar to that in adults ($\beta = 0.07$).[17]

In the conditional analysis of rs10453441 and rs12144790 regarding AL association, the effect sizes were 0.102 for rs10453441 ($P = 9.56 \times 10^{-6}$) and 0.060 for rs12144790 ($P = 0.0085$) (Supplementary Table S1). These were comparable to the univariate SNP association (Table 3), suggesting an independent effect of the two SNPs in AL variation.

With regard to the other candidate SNPs, the *ZC3H11B* rs4373767 (allele C; $\beta = -0.053$, $P = 0.030$) showed a nominally significant association with AL, but it could not withstand the Bonferroni correction. The reported regression coefficient of allele C was –0.21 in Singapore Chinese adults,[16] which is around four times greater than that in children, as revealed in our current study (Table 3). None of the other SNPs (*C3orf26* rs9811920, *GJD2* rs11073058, or *ZNRF3* rs12321) reached nominal significance in AL association ($P < 0.05$). The direction of effect of the minor allele A in rs9811920 in children ($\beta = -0.031$) was opposite that in adults ($\beta = 0.08$), whereas the other two SNPs ($\beta_{rs11073058} = 0.044$ and $\beta_{rs12321} = -0.027$) showed approximately half the effect sizes of those in adults ($\beta_{rs11073058} = 0.07$ and $\beta_{rs12321} = -0.05$).[17]

SNP Associations with Other Ocular Traits

The A allele of *WNT7B* rs10453441 was strongly correlated with a larger CC ($\beta = 0.053$, $P = 1.17 \times 10^{-14}$) and thicker CCT ($\beta = 2.65$, $P = 0.0026$), but not with SE ($\beta = 0.088$, $P = 0.032$) after Bonferroni correction (Table 4). SNP rs12321 in *ZNRF3* was associated with CC ($P = 0.0060$), and the effect size of allele C was –0.018 ± 0.0068 mm (Table 4). No significant associations with CC, SE, and CCT were detected in other SNPs. Notably, the association of *ZC3H11B* rs4373767 with refractive error, which was significant in the adult study

Table 4. Allelic Association of SNPs with Corneal Curvature, Spherical Equivalent, and Central Corneal Thickness

SNP	Gene/Locus	A1/A2*	Corneal Curvature (mm)		Spherical Equivalent (diopter)		Central Corneal Thickness (μm)	
			β (SE)†	P‡	β (SE)†	P‡	β (SE)†	P‡
rs12144790	*RSPO1*	T/C	0.011 (0.0068)	0.096	–0.035 (0.041)	0.39	–0.90 (0.87)	0.30
rs4373767	*ZC3H11B*	C/T	–0.0041 (0.0068)	0.57	0.075 (0.043)	0.083	–1.17 (0.92)	0.21
rs9811920	*C3orf26*	A/G	–0.00079 (0.0068)	0.91	0.059 (0.041)	0.15	0.32 (0.87)	0.71
rs11073058	*GJD2*	T/G	0.00053 (0.0068)	0.94	–0.070 (0.040)	0.080	0.95 (0.85)	0.27
rs12321	*ZNRF3*	C/G	–0.018 (0.0068)	**0.0060**	–0.041 (0.040)	0.31	–0.39 (0.86)	0.65
rs10453441	*WNT7B*	A/G	0.053 (1.0068)	**1.17×10^{-14}**	0.088 (0.041)	0.032	2.65 (0.88)	**0.0026**

SE, standard error.
*Minor allele/major allele, where minor allele is the effect allele for the effect size β.
†β is the coefficient of linear regression indicating the effect sizes on corneal curvature, spherical equivalent, and central corneal thickness.
‡P values are derived from linear regressions adjusted for age and gender. The P values in bold are less than the significance level cutoff of 0.0083 after multiple testing correction.

in Hong Kong,[20] was not significant in children (SE, β = 0.083 and P = 0.088) (Table 4).

Discussion

In this study, SNPs in seven AL-associated loci were investigated for their associations with AL, SE, CC, and CCT in Chinese children. A significant association was identified between AL and SNPs rs10453441 in *WNT7B* and rs12144790 in *RSPO1*. The effects of the minor alleles of both SNPs were in the same direction as those reported in adults, and the effect sizes were also comparable.[17,18] Each additional copy of the minor allele in rs10453441 and rs12144790 was correlated with increases of 0.103 mm and 0.062 mm, respectively, in AL among children, compared to 0.12 mm and 0.07 mm in adults.[17,18] Therefore, our data suggest that the *WNT7B* and *RSPO1* genes might affect AL development during childhood.

WNT7B rs10453441 was also associated with corneal curvature and central corneal thickness in children. The effect size of the A allele for CC was 0.053 mm in our cohort of children, similar to 0.051 mm in adults.[18] In contrast, its effect size on CCT was smaller in children (β = 2.65) than that in adults (β = 4.51 in the discovery cohort and β = 2.95 in the replication cohort of the GWAS).[26] Because that GWAS was conducted among Latino adults with a mean age of 54.2 years, it is not clear whether or not there were age and ethnicity effects. Nevertheless, the correlation of *WNT7B* rs10453441 with AL, CC, and CCT suggests that *WNT7B* should play a role in the development of multiple ocular traits during childhood.

Because axial length is a major determinant of spherical refractive error, it is likely that the genes for AL may also play a role in SE. Interestingly, however, we found in this study that *WNT7B* rs10453441 was not associated with SE in children. A lack of association between this SNP and SE was also reported in adults.[18] Both AL and CC are correlated with SE,[31] but AL has a negative correlation and CC has a positive correlation.[32] Similarly, in our cohort of children, AL was negatively correlated with SE (β = –1.24, $P < 1 \times 10^{-200}$), but CC showed a positive correlation (β = 0.41, $P = 3.72 \times 10^{-4}$) (Supplementary Table S2). Because rs10453441-A was positively correlated with both AL (β = 0.103) and CC (β = 0.053), and SE is a compositional parameter mainly resulting from spherical power (determined by AL) and cylindrical power (determined by CC), the effect of this SNP on SE might have been neutralized such that it was not significantly correlated with SE.

In addition, we discovered a novel association between the SNP rs12321 in *ZNRF3* and CC. Neither this SNP nor the gene has been reported in any genetic association study of CC. It might be specifically associated with CC in children or in the Southern Chinese population. A recent GWAS study reported *RSPO1* as being a robust locus associated with CC; the P value was 6.51×10^{-100} in the meta-analysis of a total of 132,260 European and Asian subjects.[33] The β in this GWAS was 0.022, whereas it was 0.011 in our cohort of children (Table 4). Although the association in our study was not significant (P = 0.096), the coefficient values indicated a smaller effect size in children as compared to adults, resulting in insufficient power in our study. Therefore, further studies in larger cohorts of children are warranted.

Results of the present study suggest that *WNT7B* may play a role in the pathway of ocular development and growth, regulating the eyeball volume and related biometric parameters. SNP rs10453441 is an intronic variant for which the function has not been determined. *WNT7B*, a member of the *WNT* gene family, plays a role in the regulation of lung development by participating in the Wnt/β-catenin signaling pathway.[34] This signaling pathway is also involved in eye organogenesis. *WNT7B* is expressed in embryonic neural retina, postnatal retina, and cornea in mice.[18] It could be related to the development and maintenance of central nervous system vasculature, including the blood–brain barrier and blood–retina barrier.[35,36] Moreover, there is indirect evidence regarding how *WNT7B* affects eye growth. Missense (rs1475762618) and stop-gained (rs1569119395) variants have been associated with anophthalmia–microphthalmia syndrome in the Ensembl Genome database (www.ensembl.org). WNT7B potentially interacts with other key members in the WNT signaling pathway, including FZD4, LRP5, and Norrin, in congenital eye disorders such as persistent hyperplastic primary vitreous, neural tube defects, and Norrie disease.[35,37,38] Further studies are warranted.

In this work, we also identified a significant correlation between AL and the SNP rs12144790 in *RSPO1*, which is expressed in human retina (see the Human Protein Atlas database, http://www.proteinatlas.org).[39] *RSPO1* encodes a secreted protein, R-spondin-1, which also plays a role in the Wnt/β-catenin signaling pathway and in sex determination and skin differentiation.[40] Although both Wnt7b

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and R-spondin-1 mediate Wnt/β-catenin signaling,[41] the functional link between them is unclear. Their colocalization was observed in luminal cells of the mammary gland,[41] cholangiocarcinoma,[42] and lung adenocarcinoma.[43] R-spondin-1 also regulates corneal endothelial cell proliferation and maintains corneal endothelium homeostasis with Wnt3a.[44] R-spondin-1 has been shown to be responsive to mammalian target of rapamycin (mTOR) signaling.[45] Additionally, mTOR complex 1, a functional protein complex for mTOR signaling, could be activated by WNT7B.[44,46] Unlike *WNT7B* rs10453441, *RSPO1* rs12144790 was associated only with AL and not CC or CCT. Thus, the *RSPO1* gene is more likely to affect AL growth specifically, whereas *WNT7B* is involved in the development of AL and other ocular traits. In addition, a novel association was detected between SNP rs12321 and corneal curvature. This SNP is located at the 3′ untranslated region of the *ZNRF3* gene. The protein encoded by this gene is an important negative feedback regulator of the WNT pathway. R-spondin proteins, including R-spondin-1, are natural antagonists of ZNRF3. The variants in these genes potentially affect ocular traits and eye development via WNT signaling.

The other four candidate SNPs in *ZC3H11B*, *GJD2*, *C3orf26*, and *ZNRF3* did not show significant correlation with AL in children, suggesting that these genes might not affect AL variation in the early stages of life, or they might have a time-accumulative effect on the axial eye growth. In our previous study, the T allele of rs4373767 in *ZC3H11B* was significantly associated with increased AL ($\beta = 0.23$, $P = 0.003$) in Chinese adults with myopia.[20] The effect size is similar to that in a GWAS ($\beta = 0.16$).[16] Although the association of rs4373767-T was mild in our cohort of children and could not withstand the Bonferroni correction ($\beta = 0.053$, $P = 0.030$) (Table 3), its effect is in the same direction as that in adults. Notably, the effect of the T allele in children was about a quarter of that in adults; thus, the lack of statistical significance could be due to reduced statistical power resulted from a smaller effect size. However, our data also suggest that the *ZC3H11B* gene might exert a small effect on AL growth before the age of 10, and a larger effect may occur later in life. This is different from the case for *WNT7B* and *RSPO1* SNPs, which might have reached their full effect sizes before the age of 10. In addition, *ZC3H11B* is a susceptibility gene for high and extreme myopia in adults from the same population.[20] As such, *ZC3H11B* may influence AL elongation continuously later in life and confer increased risk to myopia progression. Further studies are warranted to elucidate the time-specific and/or cumulative effect of this gene in axial length growth and myopia progression. A previous AL GWAS reported findings for rs4373767 (allele C: $\beta = -0.16$, $P = 0.0018$) in a cohort of children from the Singapore Cohort Study of the Risk Factors for Myopia. In this cohort ($n = 929$), the age range was from 10 to 12 years, and the average SE was –2.02 diopters, which differs from our cohort (0.14 diopter) (Table 1). Therefore, findings regarding the genetic associations of AL might be affected by different distributions of refractive error across the cohorts.[16]

There are several limitations in our study. Because the effect size of some SNPs was smaller in children, a larger sample size is required to increase the statistical power. Apart from the possible influences of time-specific effects, false negatives in the detection of associations might also be caused by a relatively low sample size. Therefore, no definite conclusions can be drawn regarding the differential associations between children and adults. The epistatic effects among SNPs can be estimated by the introduction of an interaction term in the linear regression; however, the sample size in the current study did not offer the necessary additional degrees of freedom. This study selected only one SNP from each locus. Although the selected SNPs were the ones showing the strongest associations with AL in previous adult studies, the inclusion of more SNPs from each locus may reveal a more complete association profile of the gene with AL and other ocular traits in children. Moreover, there is no direct evidence linking the selected SNPs and the biological functions of the genes. Thus, further fine-mapping studies (e.g., haplotype-tagging SNP analysis) are necessary to provide better coverage of each locus and to potentially identify functional SNPs.

This study may provide some insights into future studies of AL elongation. Although the subjects were young children, their traits might have already been affected by environmental factors such as outdoor activity and reading.[47] Genetic models involving environmental factors may provide more precise association patterns and predictions for such traits. In future studies, not only gene–gene interaction but also gene–environment interaction analyses should be performed to gain a better understanding of the mechanisms of AL elongation. Our results indicate age effects of several gene SNPs on AL. Further studies using genome-wide approaches in both children and adults are warranted to better understand the genetic architecture of ocular trait development and identify the differential associations between children and adults.

In conclusion, this study revealed an association between SNPs in *WNT7B* and *RSPO1* and axial length in children, suggesting that these two genes are important in early axial eye growth. *WNT7B* rs10453441 also has a robust association with CC and CCT. To the best of our knowledge, the association between rs12321 in *ZNRF3* and CC has been revealed for the first time in this study. Similar effect sizes of the *WNT7B* and *RSPO1* SNPs in AL were observed in children and adults; however, the other SNPs showed smaller effects in children. These results suggest potential time-specific or time-accumulative effects of these genes on AL growth, which must be validated by longitudinal studies in larger cohorts of children.

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