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Vitamin D and Its Pathway Genes in Myopia: Systematic Review and Meta-analysis

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Abstract

Objective: To conduct a systematic review and meta-analysis of the association of blood vitamin D (25-hydroxyvitamin D, 25(OH)D) concentration and vitamin D pathway genes with myopia.

Methods: We searched the MEDLINE and EMBASE databases for studies published up to 29 January 2018. Cross-sectional or cohort studies which evaluated the blood 25(OH)D concentration, blood 25(OH)D3 concentration or vitamin D pathway genes, in relation to risk of myopia or refractive errors were included. Standard mean difference (SMD) of blood 25(OH)D concentrations between the myopia and non-myopia groups was calculated. The associations of blood 25(OH)D concentrations and polymorphisms in vitamin D pathway genes with myopia using summary odd ratios (ORs) were evaluated.

Results: We summarized seven studies involving 25008 individuals in the meta-analysis. The myopia group had lower 25(OH)D concentration was lower in the myopia group than the non-myopia group (SMD=-0.27 nmol/L, p=0.001). In the full analysis, the risk of myopia was inversely associated with blood 25(OH)D concentration after adjusting for sunlight exposure or time spent outdoors (AOR=0.92 per 10nmol/L, P<0.0001). However, the association was not statistically significant for the <18 years subgroup (AOR=0.91 per 10nmol/L, P=0.13); and was significant only for 25(OH)D3 (likely to be mainly sunlight derived), but not total 25(OH)D (AOR=0.93 per 10 nmol/L, P=0.00007; AOR=0.91 per 10 nmol/L, P=0.15). We analyzed four single nucleotide polymorphisms in the VDR gene from two studies; there was no significant

association with myopia.

Conclusions: Lower 25(OH)D is associated with increased risk of myopia; the lack of a genetic association suggests that 25(OH)D level may be acting as a proxy for time outdoors.

Introduction

Myopia is a major public health issue worldwide, with its prevalence increasing rapidly in recent decades.[1-3] Although myopic refractive error can be corrected by spectacles, contact lens or refractive surgery, the axial elongation in myopic eyes is irreversible. Moreover, high myopia, i.e., refractive error greater than -6 Diopters, is associated with an increased risk of blinding complications, including retinal detachment, glaucoma and choroidal neovascularization.[4 5] The etiology of myopia is complex, involving both genetic and environmental factors.[6-9] Family linkage analysis, genome-wide association studies (GWAS) and next-generation sequencing studies have identified more than 200 genes and loci for myopia.[10-24] With respect to environmental factors, evidence from observational studies suggests that time spent outdoors protects against myopia development.[9 25 26] A school-based, randomized controlled trial found that an additional 40-minute class of outdoor activities reduced the 3-year cumulative incidence rate of myopia from 39.5% to 30.4%.[25]

While the protective mechanisms of spending time outdoors on myopia remains unclear, it may potentially be explained by 1) the vitamin D hypothesis in that increased ultraviolet (UV) light leads to increased vitamin D production, which directly protects against myopia;[27-31] or 2) the light dopamine hypothesis which suggests an increased intensity of light protects against myopia, via increased dopamine release.[32] This vitamin D hypothesis has gained support from some,[29], [27] but not all,[28] studies. In epidemiological studies, it is difficult to separately

measure exposure to high intensity visible light outdoors, vs. exposure to UV radiation that induces vitamin D synthesis. Questionnaires on time outdoors do not discriminate between exposure to visible light and UV radiation, and 25(OH)D concentration in blood provides a measure of vitamin D status but is also a marker of recent sun exposure/time outdoors. According to the light-dopamine hypothesis, increased time spent outdoors will increase bright light exposure to confer the protective effect against myopia. However, at the same time, children may have received greater exposure of the skin to UVB radiation, to induce a higher 25(OH)D concentration.[33 34]

Distinguishing between causation and association is important for planning appropriate preventive strategies in addressing myopia. Some studies have had concurrent measures of time spent outdoors, blood 25(OH)D concentration and myopia to test statistically independent effects of time spent outdoors and vitamin D. In a large longitudinal cohort study (n=3677), 25(OH)D level was correlated with self-reported time spent outdoors, but there was no independent association with incident myopia.[28] However, in two other studies, lower 25(OH)D levels were associated with increased risk of myopia [31] or longer axial length (AL),[30] and this association persisted after adjustment for some measure of sun exposure. These inconsistent results could be due to the different ways that sun exposure was measured, i.e. self-report [28] [30], an objective measure of the exposure, and further, the detail in the self-report, e.g. hours per day,[30] vs. high/low.[28] In addition, the age of the study participants at which sun exposure, 25(OH)D and

myopia were measured may affect the relationship.

Further insights into a causal role for vitamin D in the development of myopia may be provided from examination of the association between polymorphisms in vitamin D pathway genes and myopia. So far, seven genes in the vitamin D pathway have been studied in relation to risk of myopia: *CYP27B1, CYP2R1, GC, VDR, CYP24A1, RXRA* and *DHCR7*. However, the results have been inconsistent across studies.[35-38]

In light of the inconsistencies in both the association between 25(OH)D concentration and myopia, and vitamin D pathway genes and myopia, we performed a systematic review and meta-analysis of observational studies to assess the evidence supporting a link between myopia and vitamin D metabolism.

Methods

Search Strategy

We searched the MEDLINE and Embase databases using the Ovid platform for relevant reports from their start date to January 29, 2018. We used Boolean logic with the following keywords as free words and controlled vocabularies. Key words for blood 25(OH)D and myopia were [‘‘myopia’’ OR ‘‘refraction’’ OR ‘‘refractive errors’’] AND [‘‘vitamin D’’ OR ‘‘25(OH)D’’] (Supplementary Table 1). Key words for vitamin D pathway genes and myopia were [‘‘myopia’’ OR ‘‘refraction’’ OR ‘‘refractive errors’’] AND [“CYP27B1” OR “CYP2R1” OR “GC” OR ‘‘VDR’’ OR ‘‘CYP24A1’’ OR ‘‘DHCR7’’ OR “vitamin D”] AND [“polymorphism” or ‘‘nucleotide’’ or ‘‘variant’’ or ‘‘genome’’ or ‘‘exon’’ or ‘‘intron’’ or “gene” or ‘‘genetic’’ or ‘‘genotype’’] (Supplementary Table 2).

Eligibility Criteria

The inclusion criteria for studies evaluating the association between blood 25(OH)D and myopia were: (1) cross-sectional, case-control, or cohort studies; (2) diagnosis of myopia based on auto-refraction by ophthalmologists or optometrists; (3) blood 25(OH)D concentration or blood 25(OH)D_3 concentration was evaluated as a risk factor for myopia and (4) unadjusted odds ratio (OR) or adjusted odds ratio (AOR) and 95% confidence interval (95% CI) were provided, or the mean and standard deviation (SD) of 25(OH)D concentration in the myopia and non-myopia groups were reported or could be estimated, or the ß-coefficient and 95% CI for the linear

association between blood 25(OH)D concentration and refraction was given.

We included the genetic association studies that met the following criteria: (1) the original study evaluated the genetic association of vitamin D pathway genes with myopia; (2) the study subjects were unrelated individuals recruited from explicitly defined populations; and (3) allele or genotype counts or frequencies in both the myopia and non-myopia groups were provided or could be calculated, or the ORs and 95% CIs or standard errors (SEs) were available. Animal studies, case reports, reviews, abstracts, and editorials were excluded.

Data extraction

All retrieved records were reviewed by two independent reviewers (T.S.M. and L.T.). Uncertainties were resolved via discussion with another two reviewers (Y.C.S.J. and R.S.S.). Data extracted from each study for the analysis of the association between 25(OH)D concentration and myopia included: (1) study information including first author, year of publication, country of study, age range of participants, ethnicity, definition of myopia, and sample sizes; (2) mean and SD of 25(OH)D in the myopia and non-myopia groups; (3) reported ORs and AORs and 95% CIs (or SEs), and adjusted co-variables; and/or (4) reported unadjusted and adjusted ß-coefficients and 95% CIs (or SEs). With respect to the vitamin D pathway gene and myopia analysis, data extracted included: (1) study information as above; (2) reported ORs and 95% CIs (or SEs) of SNPs for myopia or (3) allelic and genotypic counts for the myopia and non-myopia groups.

We requested raw data from authors of all eligible studies and successfully obtained data

from Yazar *et al.* and Guggenheim *et al.*[28 30],[31] The cross-sectional data of Guggenheim's study[28] were obtained from the ALSPAC Data Buddy Team (http://www.bristol.ac.uk/alspac/, accessed on November 2015). All cross-sectional data of participants at 7 years old and 11 years old were collected, including total 25(OH)D concentration, $25(OH)D_3$ concentration, refraction, time spent on near work, time spent outdoors, and parental educational level.

Assessment of Risk of Bias

We used the Newcastle Ottawa Scale (NOS) and the modified Estabrooks' Quality Assessment and Validity Tool to evaluate the quality of the case-control and cohort studies. Studies were assessed by two independent reviewers (T.S.M. & L.T.). Discrepancies were resolved through discussion with a third reviewer (Y.C.S.J.). Studies were assessed on three dimensions: 1) the selection of the study groups; 2) the comparability of the groups; and 3) the ascertainment of either the exposures or outcomes of interest for case-control or cohort studies, respectively. The NOS provides an overall score for methodological quality of up to nine stars. In the assessment of comparability, one star was awarded if the article accounted for time spent outdoors or exposure to sunlight. Another star would be given if it accounted for age. We included only studies with five or more stars. The modified Estabrooks' tool for cross-sectional studies contains 14 items in two groups.[39] Group I includes the probabilistic sample used, sample size appropriate for power, response rate exceeding 50%, validity, appropriate tests used, and CI reported. Group II includes representative sample, sample drawn from multiple sites, cluster/stratified design, multiple

adjusted, detective variable [primary outcome] directly measured/administrative, reliability, P values reported, and missing data managed appropriately. A study was considered to be of high risk of bias when one item in Group I was marked as ‘‘No’’ or two items marked as ‘‘N/A’’, or any two items from Group II were marked as ‘‘No’’ or three items marked as ‘‘N/A’’.[39] Articles with high risk of bias were excluded from the analysis.

Statistical Analysis

We first analyzed the cross-sectional data acquired from ALSPAC Data Buddy Team. We used the student t test to compare the difference of mean blood 25(OH)D concentration between the myopia and non-myopia groups and logistic regression to assess the association between 25(OH)D concentration and myopia, adjusting for time spent outdoors and time spent on near work. Simple and multiple linear regressions were adopted to test the relationship between blood 25(OH)D concentration and refraction. Results for the 7-year-old and 11-year-old groups were separately synthesized with data from the other studies.

In the meta-analysis, we first evaluated the association between blood 25(OH)D and myopia. The results included standard mean difference (SMD) in 25(OH)D concentration between the myopia and non-myopia groups, ORs and 95% CIs of 25(OH)D concentration for myopia, and ß coefficient and 95% CIs between 25(OH)D concentration and refraction. Anzures-Cabrera *et al.* reported that SMD could be transformed into an OR using the formula: $\mathrm{InOR} = \frac{-\pi}{\sqrt{3}} * \mathrm{SMD} \approx$

−SMD ≈ form.[40] Therefore, SMD was converted into unadjusted ORs, if ORs were not presented in the article. The AORs that were adjusted for the time spent outdoors and/or exposure to sunlight were combined and meta-synthesized. We performed subgroup analysis by ethnicity, vitamin D metabolite measured (total 25(OH)D; $25(OH)D_3$), and across different age groups (<18 years; ≥18 years). For the evaluation of the association between vitamin D pathway SNPs and risk of myopia, the association of each SNP with myopia in the pooled samples, along with the pooled odds ratios (ORs) and 95% CIs, were evaluated using a Mantel-Haenszel method in both fixed- and random-effects models.

We used the Cochran Q statistic to test for heterogeneity across studies and the I^2 statistic to quantify the proportion of total variation attributable to between-study heterogeneity. The P value of the Q statistics lower than 0.1 and I^2 above 50% indicated high heterogeneity. If significant heterogeneity was detected, results from the random-effects model were adopted, otherwise the fixed-effect model was used. Sensitivity analysis was performed by sequentially omitting each study one at a time and recalculating the results. The modified Egger's regression test was used to assess the potential publication bias. The Review Manager software (RevMan, version 5.2; the Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen; 2012) was used for the meta-analysis. The Stata software (version 12; Stata Corp LP, College Station, TX) was used to conduct the Egger's test and generate outcomes from Guggenheim *et al.*'s dataset. A p value of less than 0.05 was considered statistically significant. In the meta-analysis of genetic studies, a P

value of less than 0.05 was considered nominally significant. The Bonferroni method was used to correct the P values for multiple testing. Thus, a P value of <0.0125 (P = 0.05/4, where 4 was the number of comparisons that were made (4 SNPs) was considered as statistically significant.

Results

Association between blood 25(OH)D concentration and myopia

A total of 175 publications were retrieved from the EMBASE and MEDLINE databases; 25 of these were eligible for detailed screening and evaluation. Among them seven articles[27-31 41 42] met our inclusion criteria for meta-analysis (Figure 1) based on our search strategy (Supplementary Table 1). Data on a total of 25,008 participants (n=8244 myopes and n=16,764 non-myopes) were included in the meta-analysis. Table 1 summarizes the characteristics of the included studies. The quality assessments suggested that all the included studies were of good quality (Supplementary Table 3 & 4). Results obtained from ALSPAC Data Buddy Team were summarized in Supplementary Table 5. Six studies [27-31 42] reported blood 25(OH)D concentration in myopes and non-myopes; four studies reported 25(OH)D concentration in relation to refraction[27 28 31 41] .

Difference of blood 25(OH)D concentration between subjects with and without myopia

The mean blood 25(OH)D concentration was significantly lower in the myopia group compared to the non-myopia group regardless of whether the results from ALSPAC at 7 years or 11 years old were used in the meta-analysis (Table 2).

Table 1. Summary of Included Studies Evaluating the Serum 25(OH)D Level and Myopia / Vitamin D Related Genes and Myopia

First Author	Years	Study-design	Location of Study	Myopia	Non-myopia	Age (year)	Measure of Vitamin D	Assay for vitamin D measurement	Definition of Myopia	Cycloplegic refraction	Adjusted factors in the analysis	Ref
Mutti*	2011	Case control	USA	14	8	13-25	$25(OH)D_3$, serum	HPLC	Refraction in each meridian ≤-0.75D	yes	age and dietary intakes	29
Choi*	2014	Cross-sectional	Korea	1633	405	15-16	$25(OH)D_3$, serum	Radioimmunoassay	SE≤-0.5D	no	age, sex, area of residence, parental income, total energy intake, milk consumption, daily calcium intake, and smoking	27
Guggenheim*	2014	Cross-sectional (raw data)	UK	93 / 139	963 / 869	7 / 11	25(OH)D, $25(OH)D_3$, serum	HPLC	SE≤-0.5D	no	age, gender, time spent outdoors, near works and parental educational level	28
Yazar*	2014	Cross-sectional	Australia	221	725	20 ± 0.4	$25(OH)D_3$, serum	LC-MS/MS	SE≤-0.5D	yes	age, sex, ethnicity, parental myopia, education status, and ocular sun-exposure biomarker	31
Williams*	2016	Cross-sectional	UK	371	2797	72	$25(OH)D_3$, serum	HPLC	SE≤-0.75D	no	age, sex, study center and season	38
Kwon*	2016	Cross-sectional	Korea	5864	9262	20	25(OH)D, serum	Radioimmunoassay	SE≤-0.5D	no	age, sex, household income, BMI, life habitat factors, IOP, education level, and sun exposure	41
Tideman † *	2016	Cross-sectional	Netherland	62	2604	6.12 ± 0.44	25(OH)D, serum	LC-MS/MS	SE≤-0.5D	yes	age, sex, BMI, season of blood withdrawal, ethnicity, and time spent outdoors, education status of parents	30
Mutti †	2010	Case control	USA	289	81	18-50	N.A.	N.A.	Refraction in each meridian ≤-0.75D	yes	N.A.	43

*paper studied serum 25(OH)D and myopia; † Paper studied vitamin D related genes and myopia; HPLC: high performance liquid chromatography system; LC-MS/MS: liquid chromatography–tandem mass spectrometry

Table 2. Meta-analysis of the Association between 25(OH)D Level and Myopia

Age group of ALSPAC Included	Type of Analysis	No of Studies	Sample size	all effect SMD, OR or Coefficient (95%CI)	unit	z score	P Value	Heterogeneity I^2,%	Heterogeneity Q (P)	Egger's	Reference
7 year	**SMD of 25(OH)D level between Myopia and Non-myopia**										
	SMD	6	8445	-0.27 (-0.43 to -0.11)	nmol/L	3.28	0.001	74%	0.002	0.267	27-31,38
	OR of 25(OH)D with Myopia										
	Unadjusted OR	6	8445	0.85 (0.77 to 0.93)	10 nmol/L	3.33	0.0009	67%	0.0009	0.276	27-31,38
	Adjusted OR	4	7836	0.92 (0.88 to 0.96)	10 nmol/L	3.96	< 0.0001	0%	0.41	0.445	28, 30, 31, 38
	Coefficient of 25(OH)D with Refraction										
	Unadjusted Coefficient	4	17128	9.24E-03 (-3.20E-03 to 0.022)	nmol/L	1.46	0.146	98%	1.99E-06	8.14E-08	27, 28, 31, 41
	Adjusted Coefficient	3	17040	3.40E-03 (-1.00E-03 to 7.81E-03)	nmol/L	1.51	0.130	83%	1.25E-03	0.086	28, 31, 41
11 year	**SMD of 25(OH)D level between Myopia and Non-myopia**										
	SMD	6	8397	-0.25 (-0.42 to -0.08)	nmol/L	2.96	0.003	78%	0.0005	0.297	27-31,38
	OR of 25(OH)D with Myopia										
	Unadjusted OR	6	8397	0.85 (0.76 to 0.96)	10 nmol/L	2.60	0.009	75%	0.001	0.495	27-31,38
	Adjusted OR	4	7788	0.92 (0.88 to 0.96)	10 nmol/L	3.43	0.0006	45%	0.14	0.803	28, 30, 31, 38
	Coefficient of 25(OH)D with Refraction										
	Unadjusted Coefficient	4	17128	9.36E-03 (-2.77E-03 to 0.021)	nmol/L	1.51	0.131	97%	9.92E-0.5	N.A.	27, 28, 31, 41
	Adjusted Coefficient	3	17040	4.57E-03 (2.59E-03 to 6.55E-03)	nmol/L	4.53	6.01E-06	0.46%	0.37	N.A.	28, 31, 41

SMD: Standard Mean Difference of Vitamin D Level between Myopia and Non-myopia; Adjusted results have been adjusted for sun exposure or time spent outdoors.

Risk of myopia and blood 25(OH)D concentration

Six studies provided data for calculation of unadjusted OR of myopia in relation to the 25(OH)D concentration.[27-31 42] Higher 25(OH)D concentration was associated with a lower risk of myopia (Table 2). Four [28 30 31 42] studies provided AORs for the association of 25(OH)D concentration with myopia, adjusted for time spent outdoors and/or a measure of sun exposure. Higher 25(OH)D concentration remained associated with a lower risk of myopia (Table 2).

Association between blood 25(OH)D concentration and refraction

Four articles[27 28 31 41] reported the ß-coefficient for the association of 25(OH)D concentration with refraction. When including the 7-year-old cross-sectional data from the study of Guggenheim *et al.*,[21] the association between blood 25(OH)D concentration and refraction was not statistically significant in either the unadjusted or adjusted analyses (Table 2). However, when the results of the 11-year-old group were included instead, blood 25(OH)D concentration was significantly positively associated with refraction in the adjusted (but not unadjusted) analysis (Table 2).

Association of vitamin D pathway genes with myopia

A total of 76 articles were retrieved from EMBASE and MEDLINE, involving six vitamin D pathway genes (Figure 2). After screening for eligibility, two papers reporting results for SNPs within the *VDR* and *GC* genes were included in the meta-analysis.[30 43] Four SNPs (i.e., rs3819545, rs7975232, rs2853559 and rs2239182) in *VDR* were reported (Supplementary Table 6).

The combined OR for the C allele of SNP rs3819545 showed a nominal association with myopia (OR: 1.30, 95% CI: 1.04 to 1.64, I^2 = 0%, P = 0.02; Figure 3A), but could not withstand the Bonferroni correction. (P< 0.0125) None of the other SNPs in the VDR or any of the SNPs in the GC gene showed a significant association with myopia (Figure 3B, 3C, 3D).

Subgroup analysis

Studies with cycloplegic refraction

We performed subgroup analysis including only studies with cycloplegic refraction; only three studies [37 44 45] provided data and were eligible for inclusion. The association between blood 25(OH)D concentration and myopia remained significant (SMD: -0.47, 95% CI: -0.81 to -0.13, I^2=73%, P = 0.006; OR: 0.81 per 10nmol/L, 95% CI: 0.68 to 0.95, I^2 = 71%, P = 0.01; AOR: 0.90 per 10nmol/L, 95% CI: 0.84 to 0.95, I^2 = 71%, P = 0.0004) and of a similar magnitude.

Ethnicity: Caucasian vs non-Caucasian

The study subjects were divided into Caucasian and non-Caucasian for ethnicity analysis. Blood 25(OH)D concentration was inversely associated with myopia in both non-Caucasians[27 29] (OR: 0.77 per 10nmol/L, 95% CI: 0.67 to 0.88, I^2 = 0%, P = 0.0001) and Caucasians[28 30 31] (OR: 0.91 per 10nmol/L, 95% CI: 0.87 to 0.95, I^2=47%, P < 0.0001) (Table 3). The ORs of both groups remained significant after adjustment for time outdoors (Caucasian: OR: 0.93 per 10nmol/L, 95% CI: 0.89 to 0.98, I^2 = 0%, P = 0.004; Table 3; non-Caucasian: OR: 0.71 per 10nmol/L, 95% CI: 0.51 to 0.99, I^2 = 66%, P = 0.05; Table 3).

Age: younger than 18 years vs older

The association between 25(OH)D and myopia was borderline non-significant in the younger age group (<18 years) including 337 myopes and 3972 non-myopes (Figure 4A & 4B), but was significant in the older age group (≥18 years) including 592 myopes and 3522 non-myopes (Figure 4C & 4D), despite very similar effect estimates.

Type of vitamin D: Total 25(OH)D vs 25(OH)D_3

Among the seven included articles, three reported total 25(OH)D concentration[27 28 41] and four 25(OH)D_3.[28 30 31 42] The association with myopia was statistically significant for 25(OH)D_3, but not total 25(OH)D (Table 4), possibly due to the smaller sample size in the latter; the effect estimates were of similar magnitude.

Risk of bias assessment and sensitivity analysis

We performed sensitivity analysis by omitting each study at a time subsequently to confirm the results. The heterogeneity was reduced when data from the ALSPAC Study[28] were excluded. None of the other results was significantly altered in the sensitivity analysis. Egger's tests were not statistically significant in any of the analyses (Tables 2 and 3).

272 **Table 3. Subgroup Analysis of Different Ethnicities**

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Type of Analysis	No of Studies	Myopia	Non-myopia	Overall effect				Heterogeneity		Egger's	Ref
				OR or coefficient (95%CI)	unit	z score	P Value	I^2,%	Q (P)		
Caucasian											
Unadjusted OR	4	661	6374	0.91 (0.87 to 0.95)	10 nmol/L	4.24	<0.0001	47%	0.13	0.028	29,30,31,38
Adjusted OR	4	661	6374	0.93 (0.89 to 0.98)	10 nmol/L	2.89	0.004	0%	0.73	0.251	29,30,31,38
Unadjusted Coefficient	2	263	1591	2.37E-03 (-4.27E-03 to 9.02E-03)	nmol/L	0.70	0.484	90%	1.83E-03	3.40E-07	28,31
Non-Caucasian											
Unadjusted OR	3	268	1120	0.77 (0.67 to 0.88)	10 nmol/L	3.85	0.0001	0%	0.74	0.338	27,30,31
Adjusted OR	2	86	715	0.71 (0.51 to 0.99)	10 nmol/L	1.99	0.05	66%	0.08	N.A.	30,31
Unadjusted Coefficient	2	86	715	1.96E-02 (-9.07E-03 to 4.83E-2)	nmol/L	1.34	0.180	88%	3.47E-03	3.40E-07	31,38

Table 4. Subgroup Analysis of Different Measurements of Vitamin D

Type of Analysis	No of Studies	Myopia	Non-Myopia	Overall effect				Heterogeneity		Reference
				OR (95%CI)	unit	z score	P Value	I^2,%	Q (P)	
25(OH)D										
Unadjusted OR	4	672	3959	0.82 (0.67 to 1.00)	10 nmol/L	1.82	0.06	81%	0.001	27-30
Adjusted OR	3	490	3554	0.91 (0.80 to 1.03)	10 nmol/L	1.46	0.15	61%	0.11	28-30
$25(OH)D_3$										
Unadjusted OR	3	685	4485	0.91 (0.84 to 0.98)	10 nmol/L	2.54	0.01	51%	0.13	28,31,38
Adjusted OR	3	685	4485	0.93 (0.89 to 0.97)	10 nmol/L	3.37	0.0007	0%	0.55	28,31,38

Discussion

Our meta-analysis was to study the association between blood 25(OH)D concentration and myopia. From seven studies we synthesized the association of myopia with blood 25(OH)D concentration and from another two observational studies we tested the association of myopia with polymorphisms in genes of the vitamin D pathway. We demonstrated a significantly lower mean 25(OH)D concentration in the myopic group when compared with the non-myopic group; significantly reduced odds of myopia with higher 25(OH)D concentration in logistic regression analysis, including after adjustment for time outdoors or sun exposure; and a significant positive association between 25(OH)D concentration and refraction in linear regression. There was no significant association between VDR polymorphisms and myopia.

There are several strengths in our meta-analysis. We included only studies of high quality and low risk of bias according to published guidelines. Sensitivity analysis was conducted to further confirm our findings and no significant publication bias was found. Where possible, we obtained original data from eligible research groups, to maximize the quality of the data analysis, including the data of Guggenheim et al from ALSPAC.[28] Nevertheless, data from some other groups remained unavailable for the analysis. On the other hand, our study is not without limitations. First, a range of different assays were used to measure 25(OH)D concentration in the included studies. However, for these analyses assessing risk in relation to incremental change in 25(OH)D, rather than trying to define a specific 25(OH)D level associated with increased risk,

lack of standardization is less problematic. Second, heterogeneity among studies affected our meta-analysis. Some studies measured total 25(OH)D concentration whereas others measured $25(OH)D_3$. To account for this, we used SMD in the analysis rather than MD. Subgroup analysis for total 25(OH)D concentration and $25(OH)D_3$ concentration was also conducted. Another source of heterogeneity was variations in the multiple regression analysis. Some studies adjusted for sunlight exposure, others for time spent outdoor, or an objective measure of sun exposure.

The definition of myopia was not consistent between the studies (Table 1). We used a random-effects model to account for heterogeneity when necessary, but standardized definitions would improve future meta-analyses. In addition, non-cycloplegic refraction was used in some studies.[27 28 41 42] We therefore conducted subgroup analysis to include only those studies with cycloplegic refraction and the results were consistent.

The small number of eligible studies available in the literature; in particular, with only two eligible genetic association studies, also limited our meta-analysis. Notably, the majority of the included studies for the association between blood 25(OH)D concentration and myopia were cross-sectional studies, therefore their causative relationship could not be determined.

The association between myopia risk and 25(OH)D concentration was reduced but remained significant after adjustment for outdoor exposure or sunlight exposure. The association after adjustment could be due to residual confounding factors or a direct effect of vitamin D on myopia. Precise (and accurate) measurement of confounders is essential in evaluating the true

independence of an association after the adjustment. With imprecise measurements an association may be reduced but not abolished after adjustment, even though there is in fact no independent effect. Notably, self-report methods used for measuring past outdoor/sunlight exposure are likely to be imprecise, and collapsing the data to two categories (high vs. low) within the analysis further increases the risk of residual confounding. Yazar and colleagues sought to overcome self-report bias by using conjunctival UV auto-fluorescence (CUVAF) photography as a marker of cumulative exposure to UV radiation.[46] However, the time course of development of damage detected by CUVAF has not yet been well-defined. CUVAF was more strongly associated with reduced risk of myopia than was self-reported sun exposure, possibly because it reflects sun exposure over a longer time course (more relevant to the development of myopia) than self-reported sun exposure or 25(OH)D levels.[47] Wearable UV sensors are now commonly used as an objective measure of exposure to UV radiation, but are generally only used for a relatively short (recent) time period.[47 48] Of note, during time outdoors, we are exposed to both UV radiation and visible light; wearable UV sensors, and probably also CUVAF, measure only the former but not the latter. Therefore, even these objective measures of exposure cannot differentiate the roles of UV radiation from those of visible light.

The association with myopia was statistically significant only for $25(OH)D_3$ concentration and not total 25(OH)D. This support a hypothesis that 25(OH)D concentration is simply a proxy for time outdoors, although not all $25(OH)D_3$ is derived from sun exposure of the skin and most of

the total 25(OH)D is likely to be 25(OH)D_3. In addition, the effect estimates were of similar magnitude for 25(OH)D_3 and total 25(OH)D, and the borderline non-significance in the total 25(OH)D analysis might be explained by the smaller sample size.

We found a significant association between vitamin D and myopia for individuals aged older than 18 years, by which myopia generally would have developed, but a borderline non-significant association for those aged less than 18 years. Again, this may have been due to the lower sample size in the <18 years group, compared to the ≥18 years group. Of note, the findings in the older age group are dominated by the paper by Yazar and colleagues where the average was 20 years.

We found no significant association between polymorphisms in the *VDR* gene and myopia. In addition, other vitamin D pathway genes involving in activation and deactivation of serum 25(OH)D and determination of serum 25(OH)D level (including *GC, DHCR7, CYP2R1, CYP27B1, CYP24A1,* and *RXRA*) have also been investigated their association with myopia (Supplementary Table 7),[35-38] but none of them was associated with myopia. This was in line with a recent Mendelian randomization study of 37,382 and 8,376 adult participants of European and Asian ancestry respectively, in the Consortium for Refractive Error And Myopia (CREAM).[35] SNPs in *DHCR7*, *CYP2R1*, *GC* and *CYP24A1* genes with known effects on 25(OH)D concentration were used as instrumental variables. The estimate for the effect of 25(OH)D on refractive error was only -0.02 (95% CI -0.09 to 0.04) D per 10nmol/l increase in 25(OH)D concentration in Caucasians and 0.01 (95% CI -0.17 to 0.19) D per 10nmol/l increase in Asians. With these tight confidence

intervals on the estimates, the authors concluded that the true contribution of vitamin D levels to the degree of myopia is very small and indistinguishable from zero. They attributed the previous findings from observational studies linking 25(OH)D levels to myopia to the effects of confounding by time spent outdoors.

On the other hand, results of animal studies provide some support for the light-dopamine hypothesis, which suggests that an increase in light intensity induces dopamine release to alter retinal gene expression and signalling for axial elongation.[49 50] Elevated light levels have been shown to prevent the development of form-deprivation myopia and the axial elongation in chicks (40,000 lux),[51-53] rhesus monkeys (28,000 lux), [54] and tree shrews (15,000 lux).[55] In chicks, a greater protection effect was found with higher light intensities.[56] Notably, this protective effects was abolished by administering a dopamine D2 receptor antagonist,[53] which suggested its mechanism is via the dopaminergic system. Importantly, these animal studies involved a bright light system that was free of UV radiation.[51-56] These studies suggest that it is exposure to bright light during time outdoors that is important, rather than exposure to UV radiation. This evidence from animal studies further suggests that it is time outdoors, rather than vitamin D that is important for the development of myopia, and that 25(OH)D concentration is serving as a proxy for children's outdoor time, in these observational studies.

In summary, the blood 25(OH)D concentration is inversely associated with risk of myopia. Although this association remained after adjusting for various measures of time spent outdoors,

these measurements were imprecise. It is not clear what either 25(OH)D level or time outdoors are really measuring, that is relevant to myopia. Polymorphisms in the *VDR* gene were not associated with myopia. Animal studies support the anti-myopia effect of bright light but not UV radiation. The association of lower 25(OH)D concentrations with myopia probably reflects that 25(OH)D concentrations are a proxy for children's time spent outdoors.

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Author contribution Statements

S.M.T. conceived the study design, and did the data collection, data analysis, and data interpretation. She wrote the main manuscript text and prepared the tables and figures.

T.L. did the data collection and data interpretation.

S.S.R. did the data collection and data analysis

S.Y. provided some raw data and critically revised the manuscript

L.J.C. critically revised the manuscript

D.A.M. critically revised the manuscript

R.M.L. critically revised the manuscript

C.P.P. critically revised the manuscript

J.C.S.Y. conceived the study design, supervised the data collection and data analysis and critically revised the manuscript.

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Figure legends

Figure 1: Flowchart of including studies on the association between blood 25(OH)D concentration and myopia

Figure 2: Flowchart of including studies on the association of vitamin D pathway genes with myopia

Figure 3: Meta-analysis of the association of vitamin D pathway genes with myopia. The bars with squares in the middle represent 95% confidence intervals (95% CIs) and odds ratios (ORs). The central vertical solid line indicates the ORs for the null hypothesis. Diamond indicates summary OR with its corresponding 95% CI. **(3A)**. rs3819545, **(3B)**. rs7975232, **(3C).** rs2853559, **(3D)** rs2239182.

Figure 4: Subgroup analysis of the association between blood 25(OH)D concentration and myopia in different age group. The bars with squares in the middle represent 95% confidence intervals (95% CIs) and odds ratios (ORs). The central vertical solid line indicates the ORs for the null hypothesis. Diamond indicates summary OR with its corresponding 95% CI. **(4A)**. less than 18 years (unadjusted ORs); **(4B)**. less than 18 years (adjusted ORs); **(4C)**. more than 18 years (unadjusted ORs); **(4D)**. more than 18 years (adjusted ORs)

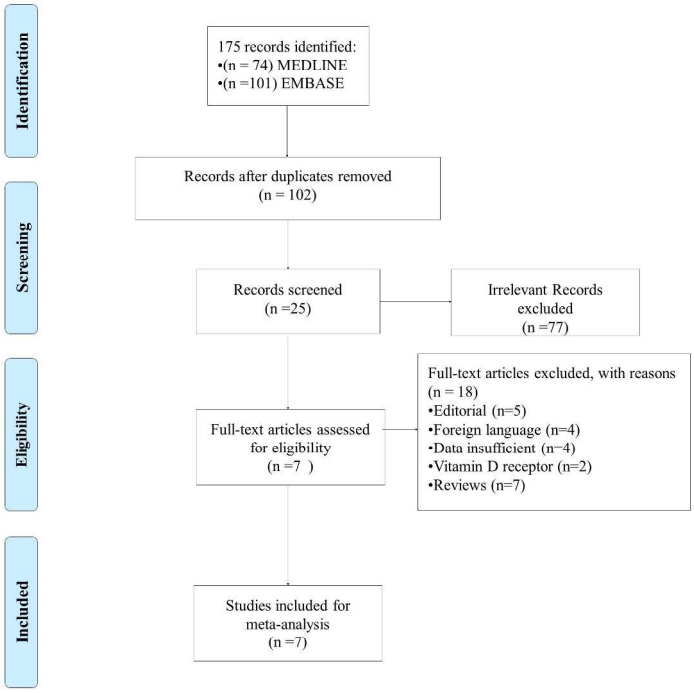

Figure 1: Flowchart of including studies on the association between blood 25(OH)D concentration and myopia

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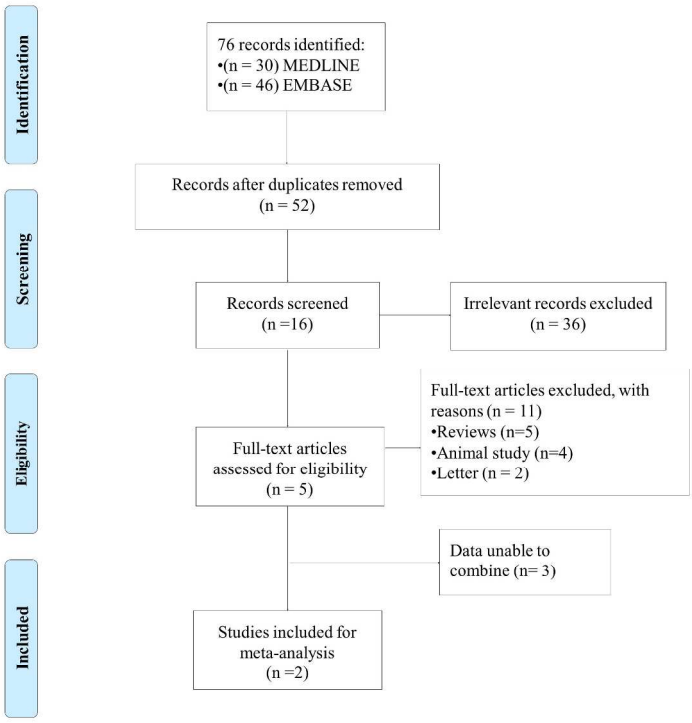

Figure 2: Flowchart of including studies on the association of vitamin D pathway genes with myopia

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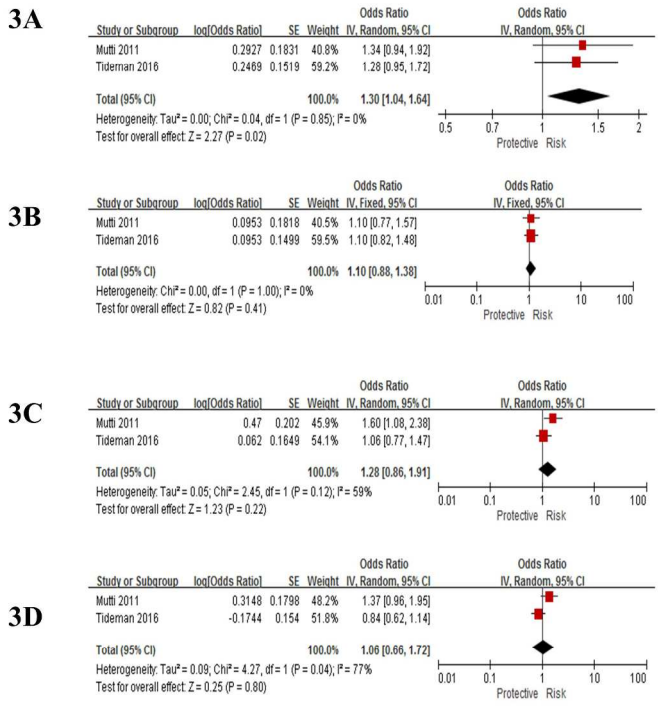

Figure 3: Meta-analysis of the association of vitamin D pathway genes with myopia. The bars with squares in the middle represent 95% confidence intervals (95% CIs) and odds ratios (ORs). The central vertical solid line indicates the ORs for the null hypothesis. Diamond indicates summary OR with its corresponding 95% CI. (3A). rs3819545, (3B). rs7975232, (3C). rs2853559, (3D) rs2239182.

260x201mm (300 x 300 DPI)

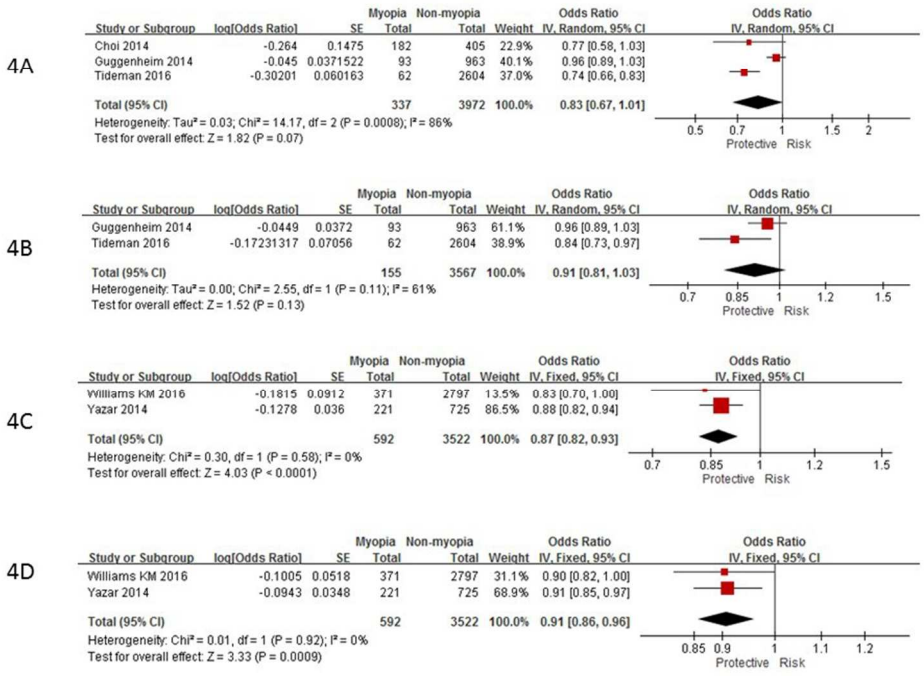

Figure 4: Subgroup analysis of the association between blood 25(OH)D concentration and myopia in different age group. The bars with squares in the middle represent 95% confidence intervals (95% CIs) and odds ratios (ORs). The central vertical solid line indicates the ORs for the null hypothesis. Diamond indicates summary OR with its corresponding 95% CI. (4A). less than 18 years (unadjusted ORs); (4B). less than 18 years(adjusted ORs); (4C). more than 18 years (unadjusted ORs); (4D). more than 18 years (adjusted ORs)

81x60mm (300 x 300 DPI)

Supplementary table1. Search strategy for vitamin D and myopia

1. exp vitamin D/ or vitamin D.mp. or exp vitamin D deficiency/
2. vitamin D3.mp. or exp colecalciferol/ or exp calcitriol/ or 25-OH D.mp. or exp calcifediol/
3. 24-Hydroxylase.mp.
4. 1,25-Dihydroxyvitamin D3 24-Hydroxylase.mp.
5. 1 or 2 or 3 or 4
6. exp high myopia/ or myopia*.mp. or exp myopia/
7. refractive error.mp. or exp refraction error/
8. nearsighted*.mp.
9. exp refraction index/ or refraction.mp.
10. 6 or 7 or 8 or 9
11. 5 and 10

Supplementary table 2. Search strategy for vitamin D pathway genes and myopia

1. exp single nucleotide polymorphism/ or exp DNA polymorphism/ or exp genetic polymorphism/ or polymorphism*.mp.
2. exp nucleotide/
3. gene.mp. or exp gene/
4. exp genetic variation/ or exp genetic risk/ or genetic*.mp.
5. exp allele/ or allele*.mp.
6. genotype*.mp. or exp genotype/
7. exp high myopia/ or myopia*.mp. or exp myopia/
8. refractive error.mp. or exp refraction error/
9. nearsighted*.mp.
10. exp refraction index/ or refraction.mp.
11. vitamin D/ or vitamin d.mp.
12. vitamin D binding protein.mp. or exp vitamin D binding protein/
13. (DBP or GRD3 or VDBG or VDBP or GcMAF).mp. or DBP/gc or Gc-MAF.mp. or HEL-S-51.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]
14. (CYP27B1 or CYP1 or CP2B or PDDR or VDD1 or VDDR or VDDRI or CYP27B or P450c1 or CYP1alpha).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]
15. exp cytochrome P450/ or cytochrome P450 family 27 subfamily B member 1.mp.
16. exp vitamin D receptor/
17. 1,25- dihydroxyvitamin D3 receptor.mp. or exp calcitriol receptor/
18. CYP2R1.mp.
19. 7-dehydrocholesterol reductase.mp.
20. DHCR7.mp. or exp 7 dehydrocholesterol/
21. (CYP24A1 or CP24 or HCAI or CYP24 or HCINF1 or P450-CC24).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]
22. exp vitamin D/ or exp colecalciferol/ or exp vitamin D deficiency/
23. 25OH D.mp. or exp 25 hydroxyvitamin D/
24. 1 or 2 or 3 or 4 or 5 or 6
25. 7 or 8 or 9 or 10
26. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
27. 24 and 25 and 26

Supplementary table 3. Quality Assessment

First Author	Sample							Measurement			Statistical Analysis				
(year of publication)	Probabilistic sample used	Representative	Sample size appropriate for power	Sample drawn > 1 site	Cluster/stratified design	Multiple adjusted	Response rate > 50%	DV directly measured/administrative	DV reliability [c]	DV validity	Appropriate tests used	p values reported	CI reported	Missing data managed appropriately	High Risk or not
Choi (2014)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	N/A	No
Guggenheim (2014)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	N/A	No
Yazar (2014)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	No
Williams (2016)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	N/A	No
Kwon (2016)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	N/A	No
Tideman (2016)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes	Yes	Yes	Yes	N/A	No

Supplementary table 4. Quality Assessment for Included Case-control Study (NOS)

Author (Year of Publication)	Newcastle - Ottawa Quality Assessment Scale for Case-control Study*									
	Selection				Comparability		Exposure			
	1	2	3	4	1(a)	1(b)	1(a)	1(b)	2	3
Mutti (2011)	*	-	-	*		*	*	*	*	n.g.
Mutti (2010)	*	*	-	*	*	*	*	*	*	n.g.
Tideman (2016)	*	*	*	*	*	*	*	*	*	n.g.

n.g.: not given

Supplementary table 5. Summarized Results from the ALSPAC Data Buddy Team

	Age	Sample size	Myopia	Non-myopia	OR (95%CI)		Coefficient	
		Myopia/Non-myopia	Mean ± SD (nmol/L)	Mean ± SD (nmol/L)	unadjusted (10 nmol/L)	adjusted (10 nmol/L)	unadjusted (Diopter per nmol/L)	adjusted (Diopter per nmol/L)
25(OH)D	7	93 / 963	75.62 ± 29.14	79.40 ± 30.83	0.96 (0.89 to 1.03)	0.96 (0.88 to 1.04)	-5.98E-04 (-2.38E-03 to 1.18E-03)	-2.5E-04 (-2.53E-03 to 2.03E-03)
25(OH)D3	7	93 / 963	70.89 ± 28.41	74.93 ± 31.01	0.96 (0.89 to 1.03)	0.96 (0.88 to 1.05)	-7.96E-04 (-2.57E-03 to 9.76E-03)	-6.35E-04 (-2.91E-03 to 1.64E-03)
25(OH)D	11	139 / 869	58.85 ± 18.64	59.07 ± 19.54	1.006 (0.91 to 1.11)	1.022 (0.91 to 1.15)	-5.12E-04 (-3.51E-03 to 2.49E-03)	2.84E-03 (-5.74E-04 to 6.26E-03)
25(OH)D3	11	139 / 869	53.19 ± 18.44	53.95 ± 19.32	1.022 (0.90 to 1.15)	1.026 (0.90 to 1.16)	-1.13E-04 (-3.14E-03 to 2.92E-03)	2.81E-03 (-5.3E-04 to 6.15E-03)

Adjusted for age, gender, time spent outdoors, near works and parental educational level

Supplementary table 6. Characteristics of studies on the association between vitamin D pathway genes and myopia

First Author	Year	SNP ID	Gene Name	Ethnicity	Sample size	MAF	Minor allele
Tideman	2016	rs7975232	*VDR*	Mixed	3928	0.45	C
		rs2239182			3928	0.48	T
		rs3819545			3928	0.38	G
		rs2853559			3928	0.37	A
Mutti	2011	rs7975232	*VDR*	Caucasian	370	0.5	A
		rs2239182			370	0.49	G
		rs3819545			370	0.41	C
		rs2853559			370	0.36	T

Supplementary table 7. Summary of reported paper on vitamin D pathway genes and myopia

Genes	Author	Year	Sample size	Study design	Ethnicity	Results
VDR						
	Mutti	2011	370	Case-control study	Mixed	rs2853559 (OR:1.99), rs2239182 (OR:2.17), and rs3819545 (OR: 2.34) associated with myopia
	Tideman	2016	4154	Case-control study	European	no association
	Williams	2017	4166	Case-control study	European	no association
GC						
	Tideman	2016	4154	Case-control study	European	no association
	Williams	2017	4166	Case-control study	European	no association
	Cuellar	2017	45758	Meta-analysis of GWAS data		no association
	Mutti	2011	370	Case-control study	Mixed	no association
DHCR7						
	Tideman	2016	4154	Case-control study	European	no association
	Williams	2017	4166	Case-control study	European	no association
	Cuellar	2017	45758	Meta-analysis of GWAS data		no association
CYP2R1						
	Tideman	2016	4154	Case-control study	European	no association
	Williams	2017	4166	Case-control study	European	no association
	Cuellar	2017	45758	Meta-analysis of GWAS data		no association
CYP27A1						
	Tideman	2016	4154	Case-control study	European	no association
	Williams	2017	4166	Case-control study	European	no association
	Cuellar	2017	45758	Meta-analysis of GWAS data		no association
CYP27B1						
	Tideman	2016	4154	Case-control study	European	no association
RXRA						
	Williams	2017	4166	Case-control study	European	no association
	Cuellar	2017	45758	Meta-analysis of GWAS data		no association