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DNA methylation biomarkers and myopia: a multi-omics study integrating GWAS, mQTL and eQTL data

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Abstract

Background This study aimed to identify DNA methylation biomarkers associated with myopia using summary-data-based Mendelian randomization (SMR).

Methods A systematic search of the PubMed, Web of Science, Cochrane Library, and Embase databases was conducted up to March 27, 2024. SMR analyses were performed to integrate genome-wide association study (GWAS) with methylation quantitative trait loci (mQTL) and expression quantitative trait loci (eQTL) studies. The heterogeneity in the dependent instrument (HEIDI) test was utilized to distinguish pleiotropic associations from linkage disequilibrium.

Results The systematic review identified 26 DNA methylation biomarkers in five studies, with no overlap observed among those identified by different studies. After integrating GWAS with multi-omics data of mQTL and eQTL, six genes were significantly associated with myopia: *PRMT6* (cg00944433 and cg15468180), *SH3YL1* (cg03299269, cg11361895, and cg13354988), *ZKSCAN4* (cg01192291), *GATS* (cg17830204), *NPAT* (cg04826772), and *UBE2I* (cg03545757 and cg08025960).

Conclusions We identified six methylation biomarkers associated with the risk of myopia that may be helpful to elucidate the etiology mechanisms of myopia. Further experimental validation studies are required to corroborate these findings.

Keywords DNA methylation, Myopia, Biomarker, Multi-omics, Mendelian randomization

Background

Myopia is a prevalent refractive error that leads to blurred distance vision as the focal point of parallel light rays falls in front of the retina [1]. Myopia has emerged as a significant public health concern globally, with a prevalence rate ranging from 10 to 30% among the adult population [2–4] and reaching as high as 80% to 90% among young adults in East Asia [5]. Myopia not only impairs distant vision but also compromises quality of life. A higher degree of myopia is associated with an increased risk of sight-threatening conditions, such as myopic macular degeneration, cataracts, glaucoma, and retinal

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detachment, which have profound implications for individuals' lifelong development and exacerbate societal and healthcare burdens [6]. Therefore, further exploration of the molecular mechanisms underlying myopia is imperative to improve prevention, control, and treatment strategies.

Most myopic individuals are affected by multifactorial inheritance, which is influenced by genetic and environmental factors, as well as interactions between genes and the environment. In recent years, genome-wide association studies (GWASs) have identified numerous genetic loci and candidate regions associated with myopia [7, 8], advancing our understanding of its genetics. Additionally, environmental factors, such as educational pressure, urbanization, more near work, and less outdoor activity, are recognized as risk factors associated with myopia [9, 10]. However, the precise mechanisms by which genetic and environmental factors interact to influence the onset and progression of myopia remain to be fully elucidated.

Epigenetics, based on changes in gene expression levels not caused by alterations in gene sequences, regulates transcription and mediates interactions between genes and the environment [11]. DNA methylation, one of the most extensively studied epigenetic mechanisms, involves the covalent binding of a methyl group to the cytosine of a cytosine-guanine dinucleotide (CpG) [12]. A recent study conducted in Poland found that *ADAM20*, *ZFAND6*, *ETS1*, *ABHD13*, *SBSPON*, *SORBS2*, *LMOD3*, *ATXN1*, and *FARP2* exhibited decreased methylation in young children with high myopia [13]. A case-control study conducted among Chinese, Malay, and Indian populations suggested that variations in the neonatal cord epigenome might influence the risk of developing early-onset myopia in children [14]. However, the study of DNA methylation and myopia is limited by limited sample sizes, insufficient verification of results, and evaluation of long-term biomarkers.

Summary-data-based Mendelian randomization (SMR) integrates GWAS data with molecular trait data, such as cis-DNA methylation quantitative trait loci (cis-mQTL) and cis-expression QTL (cis-eQTL) studies, which have been successfully applied to prioritize DNA methylation or gene expression sites that are pleiotropically or potentially causally associated with complex traits [15, 16]. While tissue of origin is the primary driver of methylation variation, there is a significant correlation between the methylation profiles of ocular tissues and those of peripheral blood [17]. Previous studies have utilized peripheral blood-derived mQTL and eQTL data to explore the methylation and expression profiles in myopia and other ocular diseases [18]. However, existing QTL data for ocular tissue lack methylation information, and the sample sizes for eQTL data are insufficient [18, 19]. Human blood serves as the preferred sample in the majority of population studies examining the association between DNA methylation and myopia [13, 20]. Consequently, this study focused on QTL data derived from peripheral blood samples.

Therefore, we systematically summarized potential methylation biomarkers and employed the SMR method to explore the relationships among methylation, gene expression in human blood, and the myopia phenotype at the gene level. The results of this study may enhance the understanding of the pathogenesis of myopia and provide a robust scientific foundation to support future prevention and treatment strategies.

Methods

A comprehensive overview of the current study is presented in Fig. 1. Following a rigorous systematic literature search, we synthesized the association between DNA methylation and myopia. Subsequently, we employed SMR analysis to explore the pleiotropic relationships among DNA methylation, gene expression, and myopia, aiming to identify mQTL and eQTL sites associated with the myopia phenotype. Furthermore, we investigated the multi-omics perspective to examine whether susceptibility genes influence the expression of related genes by modulating the methylation level of specific regions, ultimately impacting individuals' myopia status.

Systematic review

Search strategy

The systematic review was reported following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [21] and registered with PROSPERO (CRD42024532355). Original studies were searched in PubMed, Web of Science, the Cochrane Library, and Embase up to March 27, 2024. Complete search strategies are provided in Supplementary Table 1. In brief, the main search terms included "DNA Methylation" OR "Methylation" and "Myopia" OR "Nearsightedness" OR "Shortsightedness" OR "Refractive Error" OR "Ametropia". Related studies were screened independently by two trained investigators (DLC and HMC), and any discrepancies were resolved through discussion until a consensus was reached.

Inclusion and exclusion criteria

We included studies that met the following criteria: (a) clear diagnostic criteria for myopia and DNA methylation analysis methods; (b) investigation of the relationship between DNA methylation and myopia risk; (c) cross-sectional, case-control, randomized controlled trials, and cohort studies; (d) in vitro and animal studies; and (e) reporting of specific genes. Studies were excluded

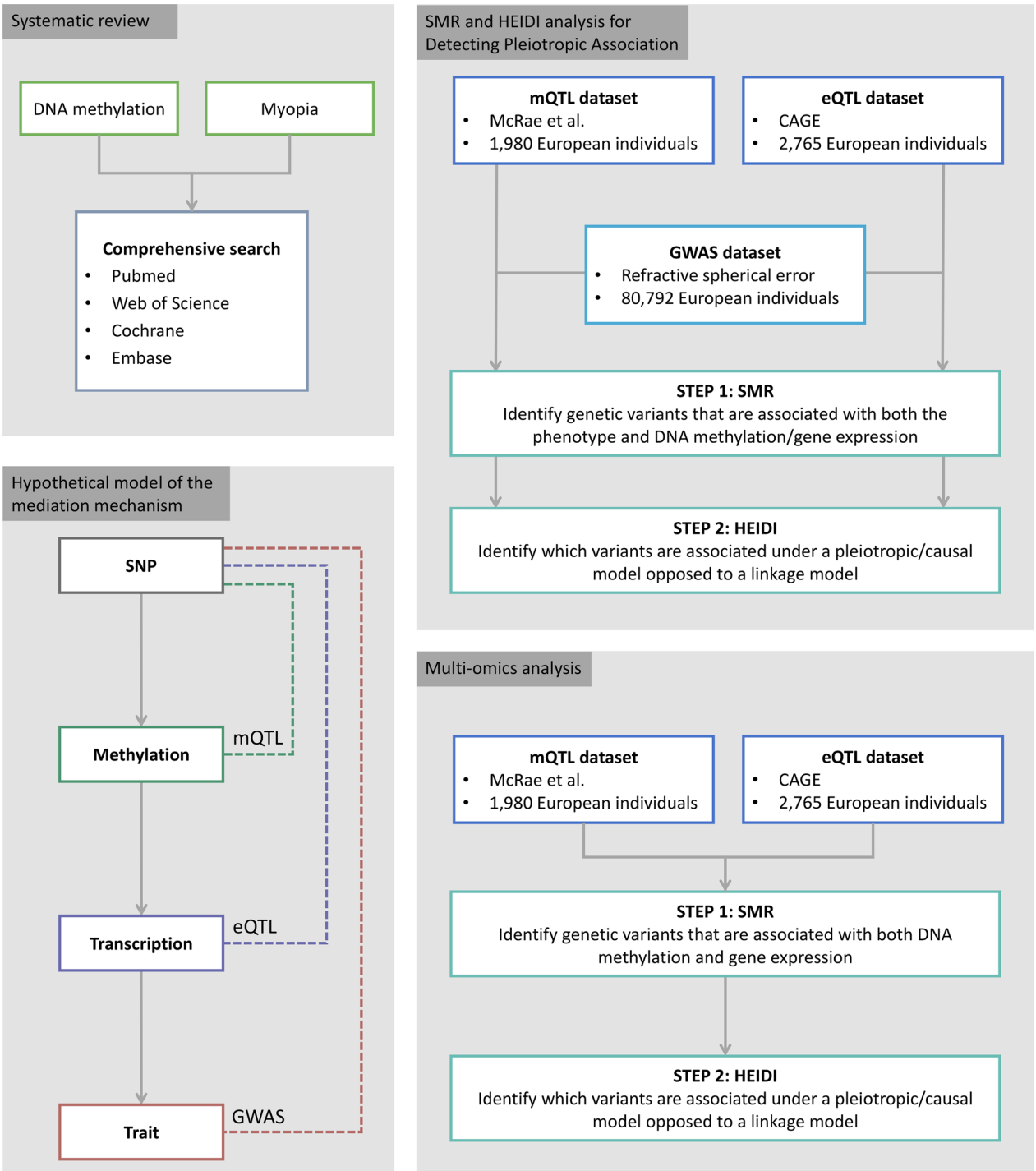

Fig. 1 Overview of summary-data-based Mendelian randomization analyses of eQTL, mQTL, and GWAS datasets. *Note* SNP: single nucleotide polymorphism; GWAS: genome-wide association study; mQTL: methylation quantitative trait locus; eQTL: expression quantitative trait locus; SMR: summary-data-based Mendelian randomization; HEIDI: heterogeneity in dependent instruments

if they (a) were conference abstracts, case reports, editorial comments, letters or reviews; (b) lacked information on DNA methylation and myopia; (c) did not specify target tissues for methylation analysis; or (d) were not written in English. In cases where multiple studies reported the same population, the one with the largest sample size was included.

Data extraction and quality assessment

Two independent investigators (DLC and HMC) were responsible for the data extraction of eligible studies. In the case of disagreements, the two investigators strived to achieve consensus through thorough discussion. If consensus could not be reached, a third reviewer was consulted to ensure agreement on the results. We referenced Newcastle–Ottawa Scale (NOS) to evaluate the quality of case-control studies [22]. The NOS comprises three components: selection, comparability, and outcome, with scores of 7–9 indicating high-quality studies, scores of 4–6 indicating medium-quality studies, and scores below 4 indicating low-quality studies. The Stroke Therapy Academic Industry Roundtable (STAIR) was employed to assess the quality of animal experiments [23]. The STAIR developed "Recommendations for Ensuring Good Scientific Inquiry", which includes sample size calculation, inclusion and exclusion criteria, randomization, allocation concealment, reporting of animals excluded from analysis, blinded assessment of outcome, and reporting potential conflicts of interest and study funding.

Summary-data-based Mendelian randomization

Data sources for integrative analysis

Detailed information on GWAS, mQTL, and eQTL is provided in Supplementary Table 2. Summary-level data for refractive spherical equivalent (RSE) were sourced from the largest publicly available GWAS, which included 351,091 individuals of European ancestry from the UK Biobank, the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, and the Consortium for Refractive Error and Myopia (CREAM) [24, 25]. The detailed description of the RSE phenotype in these study cohorts can be found elsewhere [24]. Although other refractive errors, such as hyperopia and astigmatism, may influence the RSE value, myopia diagnosis is predominantly based on the RSE value (threshold of -3.0D). As the RSE value becomes more negative, it indicates an increase in refractive power, which correspondingly elevates the risk of myopia. Compared with simple dichotomous variables (such as the presence or absence of myopia), the RSE offers a more precise measure of myopia severity. Although the "hyperopia reserve" or "emmetropization" process is a critical factor in myopia development, it was not considered in this study because all participants were adults. Thus, we used the RSE phenotype as a representative of myopia, which has also been used in previous studies [18].

The associations between single nucleotide polymorphisms (SNPs) and CpG sites in human blood were extracted from mQTL data reported by McRae et al. [26] involving 1980 individuals of European ancestry. The mQTL study utilized the Illumina Human Methylation 450K array to generate genome-wide CpG methylation data. Summary-level statistics for eQTL were obtained from the Consortium for the Architecture of Gene Expression (CAGE) study, which included 2765 individuals, predominantly of European descent, with gene expression measured at the transcriptional level in peripheral blood [27].

SMR analysis to identify relationships between DNA methylation and myopia

SMR analysis was utilized to estimate the association between DNA methylation and RSE (https://cnsgenomics.com/software/smr/). SMR analysis is a Mendelian randomization (MR) approach in which the genetic variant, DNA methylation and trait are defined as the top-associated cis-mQTL, exposure, and the outcome, respectively (Fig. 1). This analysis follows a two-step least-squares approach, incorporating the effect size of the top-associated cis-mQTL (within a 2-Mb window) and its corresponding effect in the GWAS. Only probes with a P value threshold of 5.0×10^{-8} were included to select the top-associated mQTLs for SMR analysis, as one of the assumptions for MR analysis is that the instrumental variable has a strong effect on the exposure. The heterogeneity in the dependent instrument (HEIDI) test was conducted to investigate the presence of heterogeneity in the SMR association statistics, with an mQTL P value threshold of 1.57×10^{-3} set to select mQTLs for the HEIDI test, equivalent to a chi-square value ($df=1$) of 10. The linkage disequilibrium (LD) r-squared threshold used to prune SNPs (mQTLs) in the HEIDI test aimed to remove SNPs in low LD, not in LD, or in very strong LD with the top-associated mQTL ($LD<0.05$ and $LD>0.9$). LDs were estimated from individual-level SNP genotype data from the 1000 Genomes Project [28]. Significant associations of methylation probes with RSE were identified through an SMR test, with associations considered significant at a Bonferroni-corrected significance level of 5.36×10^{-7} (0.05/93359) to account for multiple comparisons. SMR is unable to distinguish between causality and pleiotropy. Thus, any identified pleiotropic association should be interpreted as an inference regarding causality.

SMR analysis to identify relationships between gene expression and myopia

Similar to the SMR analysis examining DNA methylation and RSE, we conducted SMR analysis investigating the association between gene expression and RSE (Fig. 1). The instrumental variable was defined as the top-associated cis-eQTL, with gene expression levels as the exposure and the trait as the outcome. The same methodology outlined above for the SMR analysis between mQTL and RSE was followed. To control for the genome-wide type I error rate, *P* values were Bonferroni-corrected and set at 5.89×10^{-6} (0.05/8494) to adjust for multiple testing.

SMR analysis to identify relationships between DNA methylation and gene expression of selected probes

The steps of the SMR analysis above did not directly reveal the association between the identified DNA methylation and the gene expression levels of specific genes. As described by Xu et al. [29], the relationship between methylation and gene expression can be investigated using SMR analysis between mQTLs and eQTLs. To gain a comprehensive understanding of the genes potentially influenced by the DNA methylation sites identified in the SMR analysis above, we conducted a multi-omics analysis of the identified methylation and transcript probes to examine the relationship between DNA methylation levels and gene expression levels (Fig. 1). In testing for a DNAm-transcript association, DNA methylation, gene expression, and the top-associated cis-mQTL were considered as exposure, outcome, and instrumental variables, respectively. *P* values from the SMR analysis between DNA methylation and gene expression were Bonferroni-corrected and set at 1.76×10^{-5} (0.05/2838, all genes included in both mQTL and eQTL were tested).

Results

Systematic review

Screening and characteristics of the included studies

In total, 273 records were retrieved, 159 from PubMed, 47 from Web of Science, 1 from the Cochrane Library, and 66 from Embase (Supplementary Fig. 1). After removing duplicates ($n=45$), the remaining articles were screened for eligibility based on title and abstract ($n=228$). Subsequently, 30 articles were excluded based on the following criteria: conference abstracts ($n=11$), case reports ($n=11$), reviews ($n=7$), editorials ($n=1$), and non-English studies ($n=5$). The remaining articles ($n=198$) were then evaluated for eligibility based on their full text. Of these, an additional 11 were excluded due to inconsistency with the research topic ($n=4$) and overlap in sources of evidence ($n=2$). Ultimately, 5 studies were included in the systematic review [13, 14, 20, 30, 31].

Among the five studies, one focused exclusively on human subjects [13], two investigated animal subjects [30, 31], and two studied both [14, 20]. In terms of population studies, the studies encompassed Chinese individuals aged 18 years and above, Caucasians aged 3 to 12 years, and infants in Singapore. Two studies had sample sizes exceeding 400 participants and focused on high myopia and early-onset myopia [14, 20]. In terms of biological sample collection, peripheral blood and infant cord blood were primarily collected in population studies. As for animal experiments, mice, White-Leghorn chickens, and guinea pigs were utilized predominantly, with sample sizes ranging from 10 to 70. These animal experiments, which involve the collection of retinal tissue, eye tissue, blood, and scleral tissue samples, have focused primarily on the development of deprivation myopia. The methylation of *LINE-1*, *PQLC1* and *KRT12* has been validated to be associated with myopia.

Quality assessment of the included studies

The quality assessment of the included studies is shown in Table 1, and detailed information is provided in Supplementary Tables 3 and 4. Only one population study met the criteria for a high-quality study. The other two population studies did not explicitly mention the representativeness and non-response rate during the reporting process, which may have affected the integrity and reliability of the study results. Moreover, in animal experimental studies on DNA methylation and myopia, poor study quality is a common problem. Specifically, these studies did not report significant results in terms of sample size calculation, inclusion and exclusion criteria, randomization, allocation concealment, reports of animals excluded from analysis, or blind assessment of outcomes, which limited our understanding and evaluation of these animal experimental studies.

Methylation sites and their associated loci identified for myopia

After a comprehensive summary of the included studies, we identified 26 DNA methylation biomarkers. Among them, 21 markers (*PCDHA10*, *ADAM20*, *PAG1*, *ZFAND6*, *ETS1*, *ABHD13*, *LIG4*, *SBSPON*, *SORBS2*, *SLC25A3P1*, *TANC1*, *LMOD3*, *ATXN1*, *FARP2*, *OR6B3*, *LINE-1*, *8p23*, *12q23.2*, *FGR*, *PQLC1* and *KRT12*) have been effectively verified by population studies, and 6 markers (*EGR1*, *FOS*, *NAB2*, *IGF-1*, *MMP-2* and *LINE-1*) have been confirmed in animal experiments. Most of these markers are located in the promoter region, among which EGR1, FOS, and NAB2 are located in the regulatory region and promoter region.

Table 1 Characteristics of the five studies included in this systematic review

First author (year)	Study characteristics						Evaluation of DNA methylation				Quality scores	Verification
	Country	Ancestry	Sample size (case/control)	Research type	Tissue type	Types of myopia	Detection method	Biomarker	Location	Percentage of Methylation		
Thomson et al. [30]	Australia	White-Leghorn chickens	10 (5/5)	Animal experiments	Retinal tissue	Myopia	Illumina MiSeq platform	*EGR1*, *FOS*, and *NAB2*	Regulatory and promoter	*EGR1* and *FOS*: < 20% methylation; NAB2: > 20% methylation	–	No

Table 1 (continued)

First author (year)	Study characteristics						Evaluation of DNA methylation				Quality scores	Verification
	Country	Ancestry	Sample size (case/control)	Research type	Tissue type	Types of myopia	Detection method	Biomarker	Location	Percentage of Methylation		
Swierkowska et al. [13]	Poland	Caucasians	36 (18/18)	Cross-sectional study	Peripheral blood	High myopia: with a minimum RE of -6.0 D in at least one eye and a minimum axial length of 26 mm	Infinium MethylationEPIC BeadChip	*PCDHA10*, *ADAM20*, *PAG1*, *ZFAND6*, *ETS1*, *ABHD13*, *LIG4*, *SBSPON*, *SORBS2*, *SLC25A3P1*, *TANC1*, *LMOD3*, *ATXN1*, *FARP2*, *and OR6B3*	Promoter	*PCDHA10*: 29.63 ± 27.2; 56.70 ± 28.9; *ADAM20*: 63.66 ± 26.0; 89.87 ± 2.4; *PAG1*: 62.06 ± 22.5; 86.48 ± 2.6; *ZFAND6*: 59.63 ± 23.4; 83.28 ± 4.6; *ETS1*: 65.25 ± 23.6; 88.02 ± 2.7; *ABHD13*: 62.27 ± 25.1; 84.72 ± 3.4; *LIG4*: 62.27 ± 25.1; 84.72 ± 3.4; *SBSPON*: 67.98 ± 26.0; 90.18 ± 1.8; *SORBS2*: 64.35 ± 26.1; 86.16 ± 3.5; *SLC25A3P1*: 49.46 ± 23.6; 70.84 ± 3.8; *TANC1*: 53.42 ± 20.2; 74.74 ± 4.9; *LMOD3*: 66.22 ± 21.5; 87.39 ± 4.9; *ATXN1*: 43.33 ± 18.3; 64.38 ± 4.7; *FARP2*: 45.02 ± 21.7; 65.44 ± 11.1; *OR6B3*: 55.98 ± 18.8; 76.12 ± 3.7	Medium quality	No

Table 1 (continued)

First author (year)	Study characteristics						Evaluation of DNA methylation				Quality scores	Verification
	Country	Ancestry	Sample size (case/control)	Research type	Tissue type	Types of myopia	Detection method	Biomarker	Location	Percentage of Methylation		
Ding et al. [31]	China	Male pigmented guinea pigs	70 (Zero-week group28/treatment28/control14)	Animal experiments	Posterior scleral tissue	Form-deprivation myopia	Bisulfite conversion followed by PCR	*IGF-1*, *MMP-2*	Promoter	The methylation level at four of these CpG sites was found to be 20% lower in the form-deprivation eyes when compared with the control eyes after four weeks of treatment. In addition, the mRNA expression of *IGF-1* was 37% higher	–	No
Hsi et al. [20]	China	Chinese descent; Mice	440 (220/220); 17 (6/11)	Cross-sectional study and animal experiments	Leukocytes, ocular tissue; blood, retinal and scleral tissues	High myopia: have myopia in both eyes and the worse eye had a spherical refraction ≤ -6.0 diopter D; Form-deprivation myopia	Bisulfite conversion followed by PCR	*LINE-1*	NA	81.44 ± 0.21; 80.53 ± 0.21	Medium quality	Yes (LINE-1)
Seow et al. [14]	Singapore	Chinese, Malay and Indian origin; C57BL/6 J wild type mice	519 (29/490)	Case-control study and animal experiments	Infant umbilical cords; Retina and sclera	Early-onset myopia; Lens-induced myopia	Illumina Infinium HumanMethylation450K chip microarray	*8p23*,*12q23.2*,*FGR*, *PQLC1* and *KRT12*	NA	NA	High quality	Yes (PQLC1 and KRT12)

NA, not available

The detailed information is provided in Supplementary Table 4

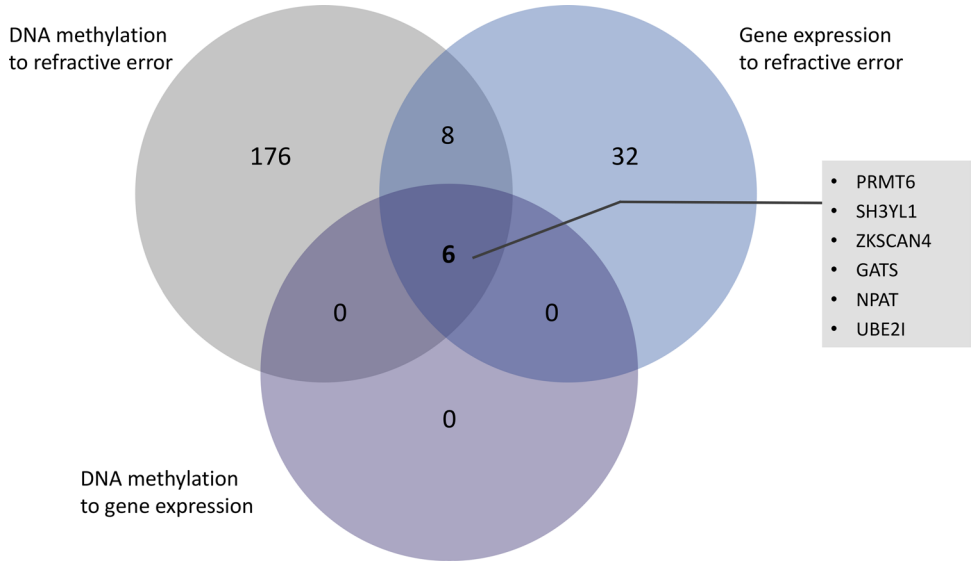

Fig. 2 Results of summary data-based Mendelian randomization. Shown are -log10 (*P* values) from the summary-data-based Mendelian randomization (SMR) tests for AF against the physical positions of DNA methylation (left) or gene expression probes (right). The red lines represent the significance thresholds of the SMR tests

Summary-data-based Mendelian randomization

Relationships of DNA methylation and gene expression with the refractive spherical equivalent

SMR analysis, combined with the HEDI test, was utilized to identify pleiotropic associations of DNA methylation and gene expression on RSE. The probes for DNA methylation and gene expression that surpassed the Bonferroni-corrected significance threshold are depicted in Fig. 2. The results of the SMR analysis indicating the identified pleiotropic associations between DNA methylation and gene expression regarding RSE are provided in Supplementary Tables 5 and 6. A total of 190 methylation biomarkers and 46 transcript biomarkers were identified. Notably, 14 duplicate genes were identified: *PRMT6*, *CD34*, *SH3YL1*, *CTNNB1*, *ZKSCAN4*, *GATS*, *KIAA1967*, *NPAT*, *UBE2I*, *TNFSF13*, *ORMDL3*, *MGC57346*, *C17orf69* and *CNDP2* (Fig. 3). The direction of effect estimates was not always consistent for different CpG sites located in the same gene. For example, a one standard deviation (SD) increase in genetically predicted *PRMT6* methylation at cg00944433 was associated with a higher RSE ($b=-0.10$, $P=8.04\times10^{-8}$), whereas a one SD increase in genetically predicted *PRMT6* methylation at cg15468180 was associated with a lower RSE ($b=0.19$, $P=4.12\times10^{-7}$) (Supplementary Table 5). The direction of effect estimates for gene expression probes on the same gene were consistent. For instance, the expression of *PRMT6* at ILMN_1813834 ($b=-0.17$, $P=2.04\times10^{-7}$) and ILMN_2234229 ($b=-0.13$, $P=1.41\times10^{-7}$) was associated with a higher RSE (Supplementary Table 6).

Relationships between DNA methylation and gene expression

To gain further insights into the genes potentially affected by the DNA methylation sites identified, we conducted an additional SMR analysis to establish links between DNA methylation levels and gene expression levels. Our analysis revealed six genes: *PRMT6* (methylation probes: cg00944433 and cg15468180; expression probes: ILMN_1813834 and ILMN_2234229), *SH3YL1* (methylation probes: cg03299269, cg11361895, and cg13354988; expression probe: ILMN_1712231), *ZKSCAN4* (methylation probe: cg01192291; expression probe: ILMN_1804571), *GATS* (methylation probe: cg17830204; expression probe: ILMN_1699631), *NPAT* (methylation probe: cg04826772; expression probe: ILMN_2120965), and *UBE2I* (methylation probes: cg03545757 and cg08025960; expression probe: ILMN_1810474) (Fig. 3). The results of the SMR analysis of the identified pleiotropic associations between DNA methylation and gene expression are presented in Supplementary Table 7. The omics plots of the six identified genes are provided in Supplementary Figs. 2–7.

Discussion

The present study investigated the associations between DNA methylation biomarkers and myopia through a combined approach of systematic review and SMR analysis. Five studies examining the link between DNA methylation and myopia were thoroughly reviewed, indicating a promising role of epigenetics in myopia. Six genes were

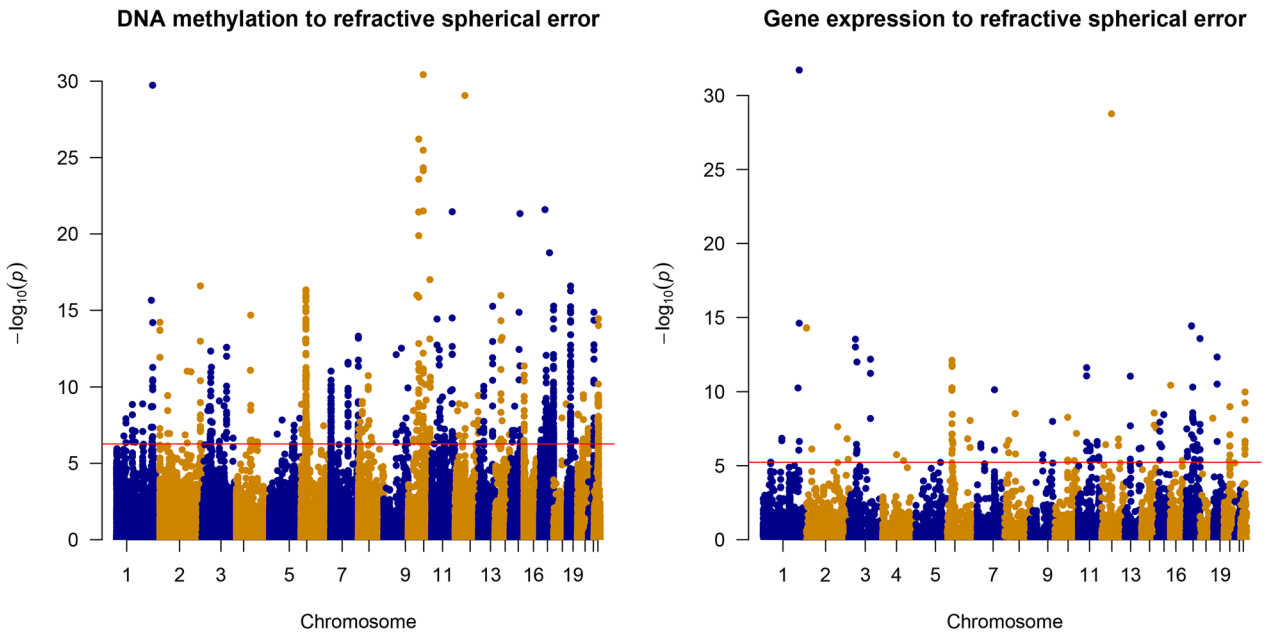

Fig. 3 Identified genes associated with refractive error of three-step summary data-based Mendelian randomization

found to be associated with myopia development, which was supported by multi-omics evidence.

In our systematic review, no overlap was found between the biomarkers identified by different studies. Therefore, current research on the relationship between DNA methylation and myopia is insufficient, and no consistent conclusions have been reached. When comparing the various studies, we noticed significant methodological differences, particularly in the application of DNA methylation detection technology, the selection of research subjects, and the criteria for grouping. These variations may impact the study outcomes, emphasizing the need for stricter experimental control in subsequent studies to enhance reliability and accuracy.

PRMT6 (protein arginine methyltransferase 6) can catalyze the formation of both omega-N monomethylarginine and asymmetrical dimethylarginine, with a preference for the latter [32]. *GATS* (CASTOR3P, CASTOR Family Member 3, pseudogene) is predicted to be involved in cellular responses to L-arginine and in the negative regulation of *TORC1* signaling. Previous research has shown that the expression level of arginine in individuals with myopia or high myopia is significantly higher compared to the control groups [33, 34]. We previously conducted a systematic review of the metabolomics of myopia and performed enrichment analysis of differentially expressed metabolites, identifying enrichment of the arginine biosynthesis pathway in aqueous humor metabolomics studies of individuals with high myopia [35]. We propose that elevated methylation of *PRMT6* at cg15468180 site and *GATS* at cg17830204 site may regulate arginine metabolism through reduced expression of the relevant gene, potentially influencing visual signal transduction or retinal homeostasis and thereby reducing the risk of myopia. A study combining four independent datasets suggested that *PRMT6* is a promising candidate for geographic atrophy, indicating a potential role for *PRMT6* in treating eye-related diseases [36]. However, the direct relationships among *PRMT6*, *GATS* and myopia, as well as the specific mechanism of their role in the development of myopia, remain to be fully elucidated. Future research should accordingly pay attention to this area to acquire more comprehensive insights and understanding.

We found that increased methylation of *SH3YL1* at the cg13354988 site was associated with a reduced risk of myopia via downregulation of the expression of this gene. *SH3YL1* (SH3 and SYLF domain-containing 1) facilitates phosphatase and phosphatidylinositol binding activities and is anticipated to act upstream or within the phosphatidylinositol biosynthetic process. Myopic axial elongation is related to non-pathological changes such as photoreceptor and retinal pigment epithelium (RPE) cell density decreases and retinal layer thinning [37]. Phosphatidylinositol and its phosphorylated derivatives, precursors for the second messengers inositol (1,4,5) trisphosphate and diacylglycerol, are crucial for signal transduction and the regulation of retinal cell membrane dynamics [38]. Phosphoinositides, phosphorylated derivatives of phosphatidylinositol, regulate membrane

trafficking in response to cellular and environmental changes, thereby playing a crucial role in disk morphogenesis in rods, which is essential for retinal and RPE membrane function [38]. Future studies should explore the mechanism by which methylation at the cg13354988 site in the SH3YL1 gene modulates the functions of phosphatidylinositols and their derivatives in RPE signal transduction and cellular morphological alterations.

UBE2I (ubiquitin-conjugating enzyme E2 I) contributes to protein ubiquitination, suggesting the potential role of ubiquitination in the onset and progression of myopia. An analysis of datasets GSE112155 and GSE15163 from the GEO database identified myopia-associated differential genes that involved in the regulation of the ubiquitin-mediated proteolysis pathway [39]. Previous studies have highlighted the critical role of ubiquitination in ocular diseases, including diabetic retinopathy and age-related macular degeneration [40, 41]. We speculated that increased methylation at cg03545757 and cg08025960 sites reduces gene expression of *UBE2I*, thereby modulating the ubiquitination process and potentially contributing to the development of myopia. This study revealed a protective effect of high methylation against myopia at cg01192291 site in *ZKSCAN4*. *ZKSCAN4* (zinc finger with KRAB and SCAN domains 4) is a zinc finger protein involved in genomic stability, stem cell generation, and telomere elongation [42, 43]. A previous study revealed that *ZKSCAN3* plays a crucial role in the coordinated regulation of lysosomal function and autophagy in RPE cells [44]. It has been established that the interplay between autophagy and the ubiquitin–proteasome system influences age-related macular degeneration [45]. Based on our findings, the methylation of *UBE2I* and *ZKSCAN4* may also impact myopia through similar mechanisms. However, the specific roles of *UBE2I* and *ZKSCAN4* in myopia remain unclear and require further investigation.

Notably, lower methylation at the cg04826772 site in the *NPAT* locus contributes to myopia by decreasing the expression of this gene. *NPAT* (nuclear protein, a coactivator of histone transcription) is an essential factor in histone transcription regulation and significantly influences cell cycle regulation and DNA replication [46]. Histone modification is integral to the epigenetic regulatory mechanism, orchestrating gene expression patterns and cellular functions through acetylation, methylation, phosphorylation, and other modifications [47]. Studies carried out on mice and guinea pigs have demonstrated that scleral glycolysis contributes to the development of myopia by inducing fibroblast-to-myofibroblast transdifferentiation through lactate-induced histone lactylation [48]. Therefore, we hypothesize that methylation of *NPAT* promotes gene transcription and reduces the risk of myopia by influencing the process of histone modification. However, the specific mechanism and pathway connecting *NPAT* methylation to myopia remain elusive, necessitating further experimentation and investigation.

To the best of our knowledge, this study represents the initial effort to summarize the association between DNA methylation and myopia while exploring the pleiotropic correlation through SMR analysis at the multi-omics level. However, several limitations warrant acknowledgment. First, the number of studies included in the systematic review was limited, and the sample sizes were small, with a mix of human and animal studies, which may have contributed to high heterogeneity and low comparability. Second, while summary-based methylome- and transcriptome-wide approaches assist in hypothesizing the potential involvement of specific genes in the pathogenesis of the disorder being investigated, they have limitations in establishing definitive causal relationships between phenotypes and identified genes, requiring further validation. Third, our analysis was confined to the cis-region, aimed at minimizing the likelihood of selecting pleiotropic variants, thereby enhancing causal inference potential. However, this approach sacrifices the discovery of genes or DNA methylation sites demonstrating trans-relationships. Fourth, the populations included in the SMR analysis were of European ancestry. Future GWAS or mQTL/eQTL studies conducted in diverse populations, such as Asians, may broaden the generalizability of our findings.

To verify the accuracy of methylation sites, we recommend conducting validations in multiple independent cohorts and integrating discovery and validation cohorts to ensure the robustness and reproducibility of the results. For in-depth validation of methylation mechanisms and functions, we suggest conducting validation work targeting specific methylation sites based on preliminary high-throughput screening results, including experimental validation of methylation/demethylation of target genes. Specifically, luciferase activity assays can be employed, which involve the methylation of plasmids with CpG methyltransferase in vitro to construct target gene-luciferase expression plasmids containing methylated and unmethylated promoters, thereby assessing the impact of methylation status on gene expression activity. Additionally, CRISPR-Cas9 technology can be utilized for targeted editing experiments of DNA methylation, involving artificially inducing the expression of target genes in a cellular environment or precisely adding/erasing methylation at the target gene via the CRISPR-Cas9 system, to directly investigate the specific effects of methylation modifications on gene function.

Conclusion

In conclusion, the SMR analysis identified six biomarkers that exhibit pleiotropic associations with myopia, DNA methylation levels, or gene expression levels. The results may assist in prioritizing candidate genes implicated in the etiology of myopia and guiding subsequent functional studies, which could potentially inform therapeutic strategies. Further experimental validation is imperative to confirm the precise mechanism underlying the relationship between DNA methylation biomarkers and myopia.

Abbreviations

SMR	Summary-data-based Mendelian randomization
GWAS	Genome-wide association study
mQTL	Methylation quantitative trait loci
eQTL	Expression quantitative trait loci
HEIDI	Heterogeneity in the dependent instrument
CpG	Cytosine-guanine dinucleotide
cis-mQTL	Cis-DNA methylation QTL
cis-eQTL	Cis-expression quantitative trait loci
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
NOS	Newcastle–Ottawa Scale
STAIR	Stroke Therapy Academic Industry Roundtable
RSE	Refractive spherical equivalent
SNPs	Single nucleotide polymorphisms
CAGE	Consortium for the Architecture of Gene Expression
MR	Mendelian randomization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-024-01772-1.

Additional file 1

Additional file 2

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Author contributions

CWP contributed to the conceptualization; XXD, DLC, HMC, and CWP performed the methodology; XXD contributed to the software; XXD, DLC, HMC, DLL, and CWP performed the validation; XXD, DLC, and HMC performed the formal analysis; XXD, DLC, HMC, DLL, and CWP assisted in the investigation; CWP contributed to the resources; XXD, DLC, and HMC curated the data; XXD, DLL, and CWP contributed to writing—original draft preparation; XXD, DLC, HMC, DNH, CL, and AG contributed to writing—review and editing; XXD, DLC, HMC, DNH, and CWP was involved in the visualization; CWP contributed to the supervision; DLL and CWP contributed to the project administration; CWP acquired the funding. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials and are available online from each genome-wide association study. Statistical code is available on the request by directly contacting the corresponding author (email: pcwonly@gmail.com).

Declarations

Ethics approval and consent to participate

Given that this study represents a reevaluation of previously collected and published data, no additional ethical approval or informed consent from the subjects was needed.

Competing interests

The authors declare no competing interests.

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