

# Comprehensive bioinformatics analysis of metabolism-related microRNAs in high myopia in young and old adults with age-related cataracts

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**Abstract.** High myopia and age-related cataracts are prevalent ocular disorders that compromise visual acuity. The molecular mechanisms underlying these conditions remain largely unclear. Here, microRNA (miRNA or miR) sequencing was performed on aqueous humor samples obtained from individuals with age-related cataracts and high myopia (AH, n=9), young patients with high myopia (YH, n=9) and a control group of elderly patients with age-related cataracts, matched in terms of sex and age (AN, n=9). miRNA sequencing and differential expression were performed. Intersecting miRNAs were identified, as well as metabolism-related genes from MsigDB were intersected with miRNA target genes. Functional enrichment was performed and disease targets predicted using DisGeNET. A protein-protein interaction network was built with STRING, and hub genes were identified via Cytoscape. GeneMANIA analyzed hub genes, while drug predictions were made using Comparative Toxicogenomics Database. Long non-coding RNAs and transcription factors were predicted via mirNet and ChEA3. Results were validated by RT-qPCR. A total of 18 miRNAs were significantly differential expressed between AH and AN group, of which eight were up- and 10 were downregulated. A total of 23 miRNAs

were significantly differential expressed between the YH and AN group, of which six were up- and 17 were downregulated. hsa-miR-490-3p, hsa-miR-4423-3p and hsa-miR-4485-3p may serve as characteristic miRNAs. A total of 289 target genes were predicted. Functional enrichment analysis yielded 169 terms, with 'herpes simplex virus 1 infection' the most significantly enriched. There were 19 metabolism-associated target genes linked with these miRNAs, suggesting a potential role of metabolic processes in pathogenesis of these conditions. The biosynthetic process of carbohydrate derivatives may serve a key role during the development of high myopia. There were 10 hub genes and Propionyl-CoA Carboxylase Subunit  $\beta$  could potentially serve as a biomarker. Drugs that could modulate their function were predicted; cyclosporine, tretinoin and acetaminophen may exert a broad influence on these hub genes. Hub gene networks based on the miRNAs were constructed to predict 44 associated long non-coding RNAs and 98 transcription factors. The present findings offer novel insights into the molecular mechanisms of age-related cataracts and high myopia and propose potential therapeutic targets.

## Introduction

The global prevalence of myopia has risen in recent decades and is estimated to increase from 30 to 50% (1), with high myopia expected to affect 10-20% of the population by 2050. Myopia often progresses to high myopia, which involves notable elongation of the eyeball and higher refractive error, leading to an increased risk of complications such as retinal detachment. Blindness caused by high myopia affects 3.8% of adults in Singapore and 3.1% of adults in Beijing, China, highlighting the significant public health impact of this condition in these regions (2,3). In early life, myopia prevalence varies by age, typically worsening from adolescence to adulthood and continuing to progress into high myopia later in life (4,5). High myopia is a key risk factor for eye diseases such as open-angle glaucoma, cataracts and myopic macular degeneration, and also leads to irreversible loss of vision such

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as retinal detachment and choroidal neovascularization, both of which are key causes of visual impairment (6-8).

Previous studies have suggested that myopia is associated with metabolic disorders that cause changes in the composition of aqueous humor (9-16). N-3 polyunsaturated fatty acids, which serve as vasodilators with an anti-inflammatory effect, may influence the progression of myopia by suppressing choroidal thinning (9). Hypoxia activates the hypoxia-inducible factor (HIF)-1 $\alpha$  signaling pathway, which promotes scleral fibroblast-myofibroblast transition and remodelling of extracellular matrix (10). Circular RNA zinc finger protein 609 acting as microRNA (miRNA or miR)-615 sponge to regulate retinal neurodegeneration, and thus influencing refractive state, is a promising target for the treatment of retinal neurodegeneration (11). Recent studies have found that abnormal metabolism of thyroid hormones,  $\beta$ / $\gamma$ -crystallin, MMPs, tissue inhibitors of metalloproteases and transforming growth factor- $\beta$  (TGF- $\beta$ ) in the aqueous humor of patients with diabetes mellitus or highly myopic cataracts affects the refractive state by influencing ocular axis length, causing the onset and progression of myopia (12-14). Additionally, as collagen decreases with aging and scleral tensile strength diminishes, this affects ocular accommodation, causing changes in refractive error over time. Altered hyaluronic acid metabolism in vitreous and aqueous humor also contributes to vitreous opacity and myodesopsia of myopia in older adults compared with younger individuals (15,16). Although studies have explored age-related pathways associated with high myopia, such as TGF- $\beta$ 1 and scleral HIF-1 $\alpha$  signalling pathways, to the best of our knowledge, comparative analyses of metabolism-associated genes between young and old adults with high myopia are rare (13,17). Studies have identified miRNAs, a class of small, non-coding RNAs, as key modulators of gene expression with sequence-specific function (18,19). These molecules serve essential roles in regulating key metabolic pathways, including those involved in glucose, lipid and high-density lipoprotein metabolism (20,21) and are ubiquitously expressed in the ocular tissues of humans and other mammals (22). Notably, certain miRNAs, such as miR-3144-3p, miR-320a, miR-9 and miR-22, have been linked to the onset and progression of ocular disease such as glaucoma and age-related macular degeneration (23,24). Previous studies on miRNA sequencing using aqueous humor have used patients with age-associated cataracts as normal controls (25-27). However, the miRNAs uniquely associated with high myopia, particularly those whose expression is independent of cataract-associated factors, remain underexplored.

Numerous studies have investigated pathogenesis and treatment of myopia, such as outdoor activity (28), as well as various treatments for high myopia such as surgery, medication and optical interventions that can help control its progression (29-32). However, to minimize the incidence of high myopia in the future, it is necessary to explore the mechanisms of myopia development at the molecular level. The present study used bioinformatics to compare miRNAs in patients with age-related cataracts with high myopia and cataracts without myopia, as well as compare high myopia in young adults and age-related cataracts without myopia, and to screen for common miRNAs to obtain predicted target genes. Based on metabolism-related genes and target genes, hub genes related to both high myopia and metabolism were identified and the miRNAs that regulate them characterized to offer new insights into the treatment of high myopia.

## Materials and methods

**Subjects.** Aqueous humor samples from 54 patients, aged 17-79 years, with a male-to-female ratio of 1:2, were collected from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) between June 2022 and September 2023. Patients aged  $\leq 30$  years were assigned to the younger group, and those aged  $\geq 60$  to the older group. Samples from younger patients were obtained during implantable collamer lens surgery, while those from older patients were collected during cataract surgery. To ensure adequate volume for sequencing accuracy while minimizing individual variability, aqueous humor samples were carefully collected during the surgery, with each sample size  $\leq 150$   $\mu$ l to maintain normal intraocular pressure. Samples were combined from samples of three patients matched according to age, sex, presence or absence of cataracts and refractive status, resulting in a total of 18 mixed samples. These samples were then divided into three groups ( $n=6$ /group): Young with high myopia (YH), age-related cataracts with high myopia (AH) and age-related cataracts without high myopia (AN). A total of three mixed samples from each group was taken for sequencing and the remaining mixed samples used for subsequent validation. High myopia inclusion criteria were spherical equivalent (SE)  $\leq -6.0$  diopters or an axial length (AL)  $>26$  mm in either eye (33). The diagnosis of age-associated cataracts followed internationally recognized criteria from the American Academy of Ophthalmology's Preferred Practice Pattern guidelines (34,35). Inclusion criteria were patients with a need for vision correction or age-related cataracts who exhibited clinical signs of nuclear, cortical or posterior subcapsular lens opacities. Exclusion criteria were patients who had undergone vitreous cavity injections, anterior chamber paracentesis or any treatments that could potentially alter the aqueous humor environment prior to surgery. Additionally, patients with history of hypertension, abnormal blood glucose levels, autoimmune disease or immunodeficiency were excluded.

**miRNA sequencing and differential analysis.** Total RNA was extracted using Total RNA Purification Kit (cat. no. TRK1001; LC Sciences, Houston, USA). Bioanalyzer 2100 and RNA 6000 Nano LabChip kit (Agilent Technologies, Inc.) were utilized to analyse the quantity and purity of total RNA. A total of  $\sim 1$   $\mu$ g total RNA was used to construct a small RNA library, using TruSeq Small RNA Library Prep kits (cat. no. RS-200-0012; Illumina, Inc.) according to the manufacturer's instructions. HiSeq SBS Kit v4 (250 cycles; cat. no. FC-401-4003; Illumina, San Diego, USA) was used for sequencing. The final library loading concentration was 3-4 nM, calculated based on the actual quality of each sample. Single-end sequencing (50 bp) was performed on an Illumina HiSeq2500 at Lc-Bio Technologies according to the manufacturer's protocol. For the miRNA-seq data, Reads with reference sequences of human miRNA (miRBase mature human.fa). Differentially expressed miRNAs (DEmiRNA1), DEmiRNA2, and DEmiRNA3 between AH and AN, YH and AN, YH and AH groups were sifted out by DESeq2 package (version 1.32.0) setting  $|\log_2FC| > 1$  and  $P < 0.05$ , respectively. In order to explore the miRNAs that played a role in both AH and AN, YH and AN, we intersected DEmiRNA1 and DEmiRNA2 to yield intersected miRNAs, and the remained miRNAs were treated as non-intersected miRNAs. The intersected

Table I. Basic information of enrolled patients.

Group	Sample	Age, years	Sex	SE, diopter	AL, mm
YH	YH1	23.33±5.51	F	-10.92±1.81	29.57±2.32
	YH2	20.33±2.89	F	-10.83±1.23	28.67±1.55
	YH3	24.33±6.03	M	-12.17±2.01	30.37±1.82
AH	AH1	72.67±3.06	F	-9.00±2.00	28.77±1.61
	AH2	65.67±4.93	F	-13.08±3.47	30.03±2.70
	AH3	65.00±4.00	M	-13.83±5.01	30.07±1.89
AN	AN1	70.67±2.45	F	-1.42±2.45	24.23±0.72
	AN2	72.33±1.00	F	+1.00±1.00	23.87±0.71
	AN3	68.33±0.58	M	+0.33±0.58	23.50±1.04

SE, spherical equivalent; AL, axial length; YH, young with high myopia; AH, age-related cataracts and high myopia; AN, age-related cataracts without high myopia; M, male; F, female. Age: YH vs. AN ( $P<0.001$ ), AH vs. AN ( $P=0.850$ ), AH vs. YH ( $P<0.001$ ); SE: YH vs. AN ( $P<0.001$ ), AH vs. AN ( $P<0.001$ ), AH vs. YH ( $P>0.05$ ); AL: YH vs. AN ( $P<0.001$ ), AH vs. AN ( $P<0.001$ ), AH vs. YH ( $P>0.05$ )

DEmiRNAs were considered high myopia characteristic miRNAs and their target genes were predicted via mirtarbase data of mirNet database (<https://www.mirnet.ca>).

**Screening of metabolism-related hub genes for high myopia.** Metabolism-related genes were downloaded from MsigDB database (<https://www.gsea-msigdb.org/gsea/msigdb>) (36), and the genes intersecting with miRNA target genes were considered metabolism-related high myopia genes. Functional enrichment analysis of target genes using Metascape (<http://metascape.org/gp/index.html#/main/step1>) and prediction of disease targets using DisGeNET database (<https://disgenet.com>) was performed. The metabolism-associated high myopia genes were enriched into the Reactome passages, and the network was visualized using Cytoscape software (version 3.10.1, <https://cytoscape.org>). Protein interaction (PPI) network construction was performed for using the STRING (<https://string-db.org>) website with a confidence level of 0.1 to screen the genes. Next, the cytohubba function of Cytoscape (version 3.10.1) was used for identifying hub genes and the top ten metabolism-related high myopia hub genes were obtained by sorting degree function.

**Network construction of hub genes.** The GeneMANIA database (<https://genemania.org/>) was used for the analysis of hub genes and prediction of their function. The comparative toxicogenomics database (<https://ctdbase.org>) was utilized to predict which drugs hub genes would be affected by. The mirNet website was used to predict long non-coding (lnc) RNAs of miRNAs related to the hub genes. Regulatory relationships of characteristic miRNA-hub genes and predicted transcription factors (TFs) of hub genes via ChEA3 website (<https://maayanlab.cloud/chea3>). All network diagrams were visualized with Cytoscape software (version 3.10.1).

**Reverse-transcription quantitative (RT-q)PCR.** Total RNA was isolated from intraocular aqueous humor using a miEASY microRNA Serum/Plasma kit (cat. no. RN4601; Aidlab Biotechnologies, Ltd.). miRNA was reverse-transcribed into cDNA using the miRNA 1st Strand cDNA Synthesis kit (Stem-loop) [cat. no. AG11743; Hunan Aikerui Bioengineering

Co., Ltd.) according to the manufacturer's protocol. qPCR was performed using the 2X Universal SYBR Green Fast qPCR Mix (cat. no. RK21203; ABclonal Biotech Co., Ltd.) according to the manufacturer's instructions. The thermocycling conditions comprised initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 30 sec and annealing/extension at 60°C for 35 sec. The relative expression levels were ascertained by the  $2^{-\Delta\Delta C_q}$  method (37), with the expression levels normalized to U6. The primer sequences were as follows: miR-490 forward, 5'-CAACCTGGAGGA CTCCATGC-3' and reverse, 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGACTGGATACGACCAGCAT-3'; miR-4423 forward, 5'-CGCGATAGGCACCAAAAAG-3' and reverse, 5'-GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGACTGGGATACGACTTGTG-3' and U6 forward, 5'-GGAACGATACAGAGAAGATTAGC-3' and reverse 5'-TGGAACGCTTCACGAATTTGCG-3'.

**Statistical analysis.** Statistical analysis and diagram generation were conducted using SPSS (version 26.0, IBM Corp.), TBtools (version 2.047, <https://github.com/CJ-Chen/TBtools-Manual>) and GraphPad Prism (version 10.1.2, Dotmatics). Data are presented as the mean  $\pm$  SD, with three independent experimental repeats. Shapiro-Wilk test was employed to assess normality. Comparisons of  $>2$  groups were performed by one-way ANOVA and Bonferroni post hoc tests. When comparing two groups, data following a normal distribution were analysed using a t-test, while the Mann-Whitney U test was employed for non-normally distributed data.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Patient characteristics.** There were significant differences in age, refraction or ocular axis length between each group (Table I). AN and AH groups were significantly older than the YH group. For SE, both the YH and AH groups showed significant differences compared with the AN group. For AL, both the YH and AH groups had significantly longer AL compared with the AN group.

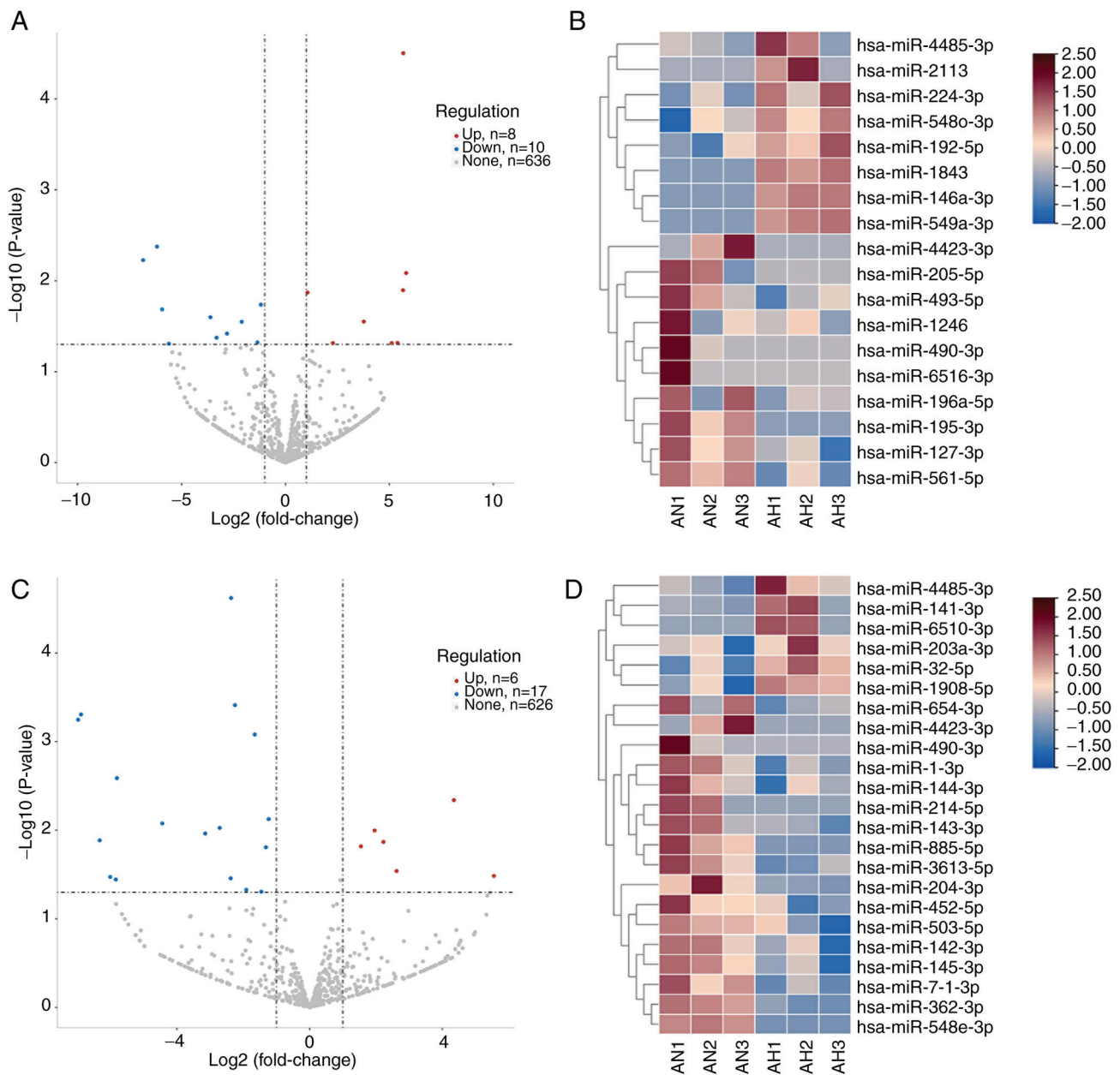


Figure 1. Differential miR expression. (A) Volcano plot and (B) heatmap of differentially expressed miRs in AH and AN. (C) Volcano plot and (D) heatmap of differentially expressed miRs in YH and AN. AH, age-related cataracts and high myopia. AN, age-related cataracts without high myopia. YH, young and high myopia; miR, microRNA.

**Sequencing data and differential expression analysis.** For sequencing data of transcriptome miRNAs, reads were compared with the reference sequence of human miRNA (miRBase\_mature\_human.fa). Count values for 2,656 miRNAs were obtained from 9 samples. A total of 18 miRNAs were significantly DE between AH and AN group, of which eight were up- and 10 were downregulated (Fig. 1A and B). A total of 23 miRNAs were significantly DE between the YH and AN group, of which six were up- and 17 were downregulated (Fig. 1C and D). Differences between YH and AH group are shown in Fig. S1.

**Analysis of high myopic characterized miRNAs and their target genes.** Intersecting DE miRNAs in AH and YH were hsa-miR-490-3p, hsa-miR-4423-3p and hsa-miR-4485-3p, which were defined as the high myopic miRNAs (Fig. 2A).

Enrichment of non-intersecting miRNAs is shown in Fig. S2. The target genes of the three characterized miRNAs were predicted from the mirtarbase data of mirNet database and 289 target genes were obtained. Functional enrichment analysis of the 289 genes was performed using Metascape and a total of 169 terms was enriched (Fig. 2B and C).

**Metabolism-related target gene screening and analysis.** A total of 1,717 metabolism-related genes were obtained, included 19 that intersected with the aforementioned 289 target genes (Fig. 3A). Functional enrichment analysis showed 22 terms were enriched (Fig. 3B-D). DisGeNET database for disease target prediction revealed 24 terms were enriched, of which the top 20 diseases related to target genes were highlighted (Fig. 3E).



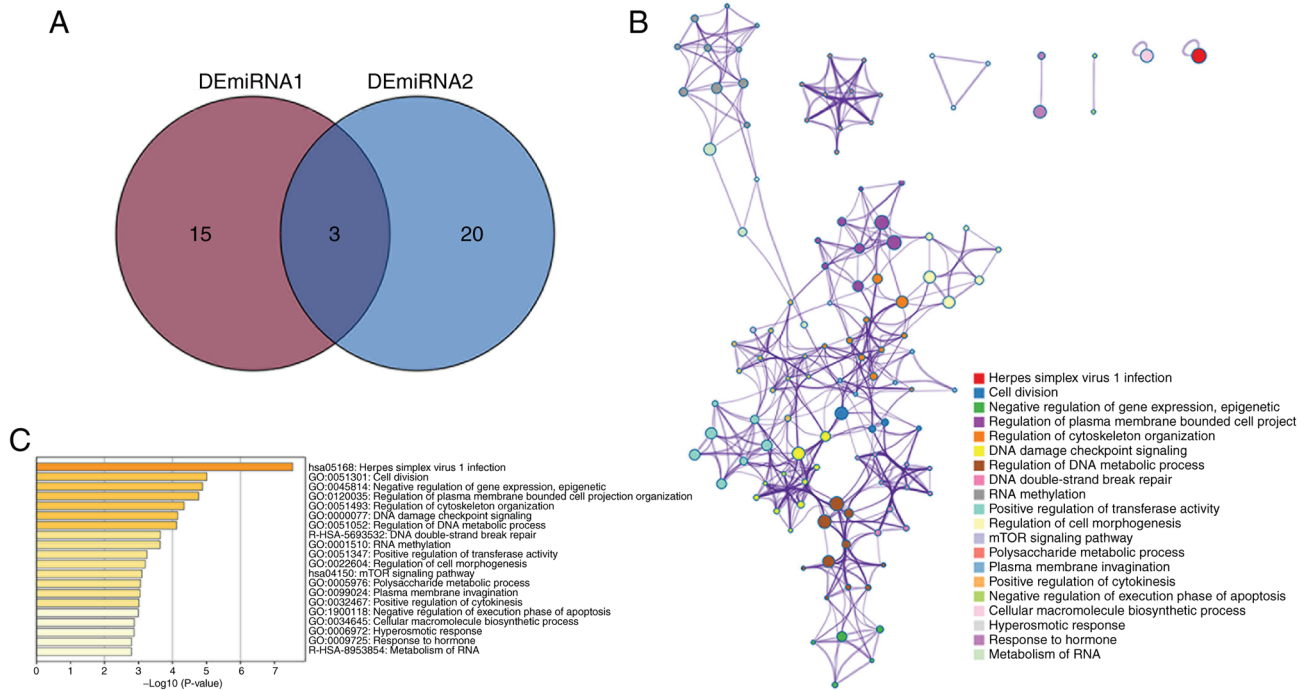


Figure 2. Analysis of high myopic miRNAs and their target genes. (A) Venn diagram of intersecting of DE miRNAs. (B) Functional enrichment of 289 myopic target genes of the three miRNAs. (C) Top 20 enriched functional pathways of 289 target genes.

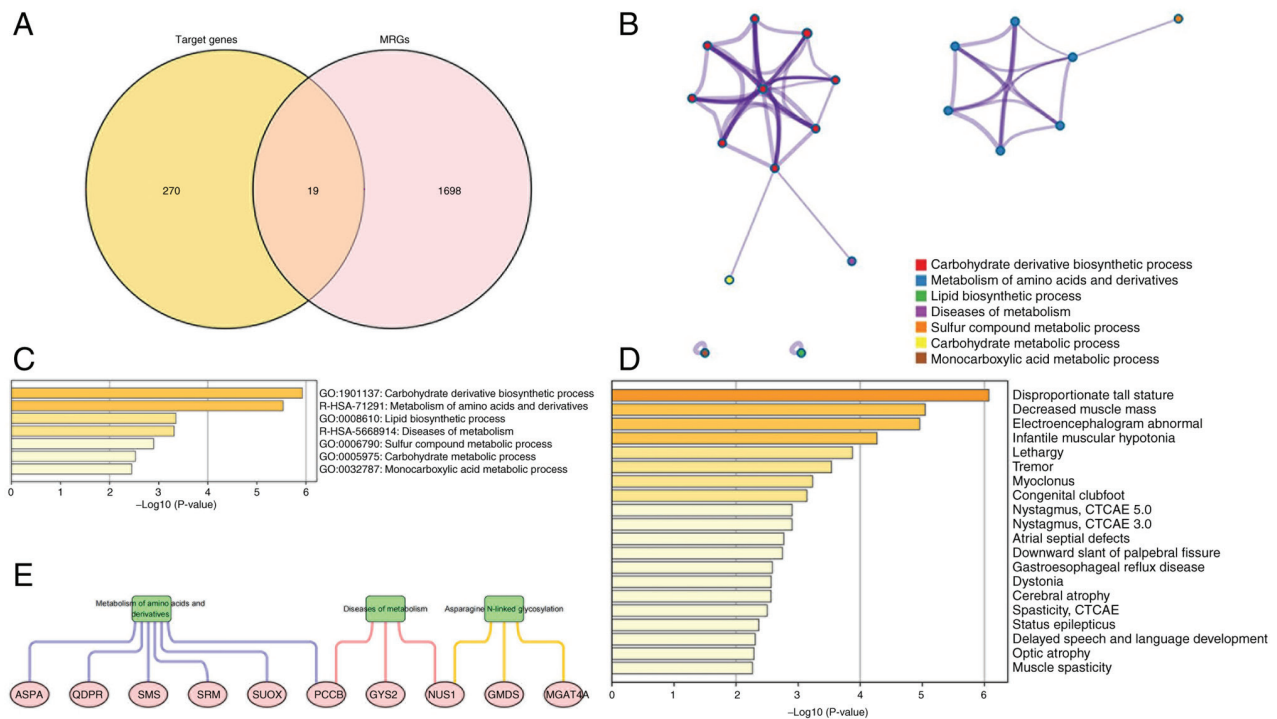


Figure 3. Metabolism-associated target genes and functional enrichment. (A) Venn diagram of the intersection of MRGs and 289 high myopic target genes. (B) Functional enrichment of 19 metabolism-associated target genes. (C) Top seven enriched functional pathways of metabolism-associated target genes. (D) Reactome pathways enriched in 19 metabolic target genes. Green rectangles indicate Reactome pathways enriched, red dots indicate genes involved in the Reactome and different colours of the lines indicate different Reactomes. (E) Top 20 disease terms associated with metabolism-related target genes. MRG, metabolism-related gene.

**Screening and analysis of hub target genes.** STRING was utilized to explore the interactions between 19 metabolism-related target genes. During PPI network construction, three discrete proteins were revealed without

any edges or sub-networks, so only 16 proteins were displayed in the interaction network (Fig. 4A). The top 10 hub target genes, propionyl-CoA Carboxylase Subunit  $\beta$  (PCCB), Glucosaminyl (N-Acetyl) Transferase 3 (GCNT3),

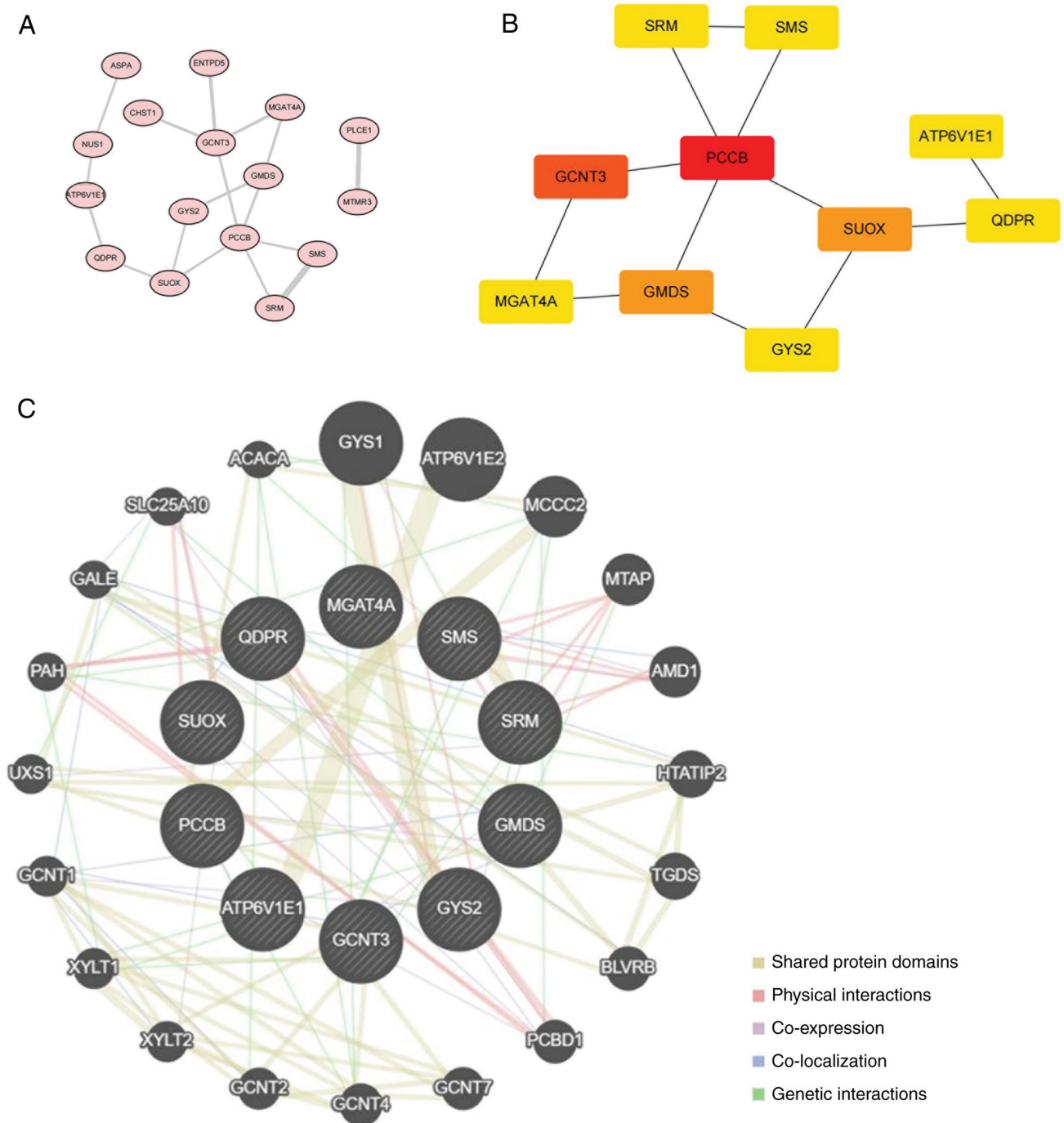


Figure 4. Screening and analysis of hub genes. (A) Protein-protein interaction network of metabolism-related target genes. The lines in the figure represent the interactions and thickness of the lines represents the degree of their binding; the thicker the lines are, the tighter the binding. (B) A total of 10 hub genes was obtained through degree sorting. The lines represent interactions, and the colour represents degree value (red, large; yellow, small). (C) Other genes associated with hub genes.

GDP-Mannose 4,6-Dehydratase (GMD5), sulfite Oxidase (SUOX), Spermidine Synthase (SRM), Spermine Synthase (SMS), ATPase H+ Transporting V1 Subunit E1 (ATP6V1E1), Quinoid dihydropteridine Reductase (QDPR), Glycogen Synthase 2 (GYS2), Alpha-1,3-Mannosyl-Glycoprotein 4-Beta-N-Acetylglucosaminyltransferase A (MGAT4A), were obtained (Fig. 4B). The GeneMANIA database was used to obtain 20 genes that may share protein structural domains, have physical interactions, co-expression, co-localization and gene interactions with these hub target genes (Fig. 4C).

*Drug prediction of hub target genes.* After obtaining hub target genes, CTD database was used to predict drugs that would affect their function. Drugs applicable to the human species that would cause an increase or decrease in the expression of the corresponding gene were selected (Table II). A total of 17 drugs was identified. Based on the number of genes affected, cyclosporine, tretinoin, acetaminophen, tetrachlorodibenzodioxin, and benzopyrene had the most extensive impact.

*Characterized miRNA-hub target gene-network construction and validation.* A network was constructed

Table II. Drug predictions of the hub genes.

Drug/ID	Gene	Regulation
Cyclosporine/D016572	SUOX	Down
	QDPR	Down
	GCNT3	Up
	SRM	Up
Tretinoin/D014212	SRM	Down
	SUOX	Up
	MGAT4A	Up
Acetaminophen/D000082	PCCB	Down
	GCNT3	Down
	GMDS	Down
Estradiol/D004958	GCNT3	Up
	SRM	Up
Perfluoro-n-nonanoic acid/C101816	SUOX	Down
Dihydrotestosterone/D013196	SMS	Up
Bisphenol A/C006780	SMS	Up
Cobaltous chloride/C018021	SRM	Down
Valproic acid/D014635	GMDS	Up
	QDPR	Up
	GMDS	Down
Cisplatin/D002945	GMDS	Down
Tetrachlorodibenzodioxin/D013749	GMDS	Down
	GYS2	Down
	GCNT3	Up
	GMDS	Down
Benzo(a)pyrene/D001564	GYS2	Down
	GCNT3	Up
	SRM	Up
Nickel/D009532	MGAT4A	Up
	GMDS	Down
Aflatoxin B1/D016604	GMDS	Down
Trichostatin A/C012589	MGAT4A	Down
Zoledronic acid/D000077211	GCNT3	Up
Azathioprine/D001379	GCNT3	Up

around the hub target gene, based on the three characterized miRNAs screened; two miRNAs corresponding to the hub target gene were extracted, and then the mirNet website was used to predict lncRNAs of the miRNAs. A total of six genes, one miRNA and 44 lncRNAs were screened (Fig. 5A). TFs of 10 hub target genes were analysed by ChEA3 and a total of 98 TFs were predicted to be associated (Fig 5B; Table SI). Expression levels of the primary characterized miRNAs in the network, hsa-miR-490-3p and hsa-miR-4423-3p, in aqueous humor were assessed. Consistently, miR-490 and miR-4423 expression levels were decreased in both AH and YH groups in comparison with AN group (Fig. 6A and B).

## Discussion

The present study used miRNA sequencing in patients with AH, AN and YH and found three shared miRNAs, hsa-miR-490-3p, hsa-miR-4423-3p and hsa-miR-4485-3p, which were defined as high myopic miRNAs. Furthermore,

19 target genes were associated with metabolism and high myopia were explored for interactions via STRING website and a PPI network was constructed. Potential drugs affecting 10 hub genes function were predicted and a characterized miRNA/hub gene/TFs network was visualized. DE genes were associated with high myopia; intersecting DE miRNAs were identified to exclude the influence of age, which, to the best of our knowledge, has not been performed previously. Adults without cataracts and high myopia are not indicated for surgery and juvenile cataracts are mostly due to lens opacity caused by other genetic disorder (38), therefore these patients were not included.

Among the characterized miRNAs, hsa-miR-490-3p and hsa-miR-4423-3p were downregulated in both AH and YH groups. In previous studies, hypoxia was an important pathological mechanism of high myopia, especially the scleral hypoxia and changes in reactive oxygen species-associated metabolites in aqueous humor (39,40). In agreement with the present results, hsa-miR-490-3p is significantly downregulated in patients with squamous lung carcinoma, a disease associated with systemic hypoxia (41). miR-4423 is a regulator of airway epithelial differentiation and its diminished function contributes to development of lung cancer (42), suggesting this miRNA may affect the myopic process by regulating hypoxic mechanisms. However, hsa-miR-4485-3p was upregulated in both AH and YH groups. It has been shown that the source of miR-4485-3p is one of the transcripts of antisense nc mitochondrial RNA (ASncmtRNA), ASncmtRNA-2, which is derived from the mitochondrial 16S gene (43). Increase in miR-4485 induces downregulation of cell cycle proteins cyclin B1 and D1 (44). These two proteins are key regulators of the cell cycle, with cyclin D1 serving a key role in G1 to S phase transition and cyclin B1 affecting G2 to M phase transition; their downregulation may lead to decreased cell proliferation, suggesting that high myopia may be associated with changes in intraocular cell cycle regulation. In addition to the intersecting miRNAs, there were non-intersecting miRNAs related to high myopia. The mechanism of high myopia is complex and requires further study.

A total of 289 target genes were predicted by three characterized miRNAs and functional enrichment yielded 169 terms. Among them, 'human herpes simplex virus 1 infection' (HSV-1) was the most enriched KEGG pathway. HSV-1 is a common human virus and it can infect the eye, especially the cornea, resulting in herpes simplex keratitis and uveitis. HSV-1 can infect trabecular meshwork cells in rats, causing viral anterior uveitis, which causes elevated intraocular pressure, tissue damage in the anterior chamber angle and inflammatory cell infiltration (45). It is suggested that high myopia may also contribute to the pathological state by affecting aqueous humor circulation pathway and inducing inflammatory processes (41). In 19 metabolism-related target genes, 'carbohydrate derivative biosynthetic process' was the GO pathway with most pronounced enrichment, which is consistent with earlier studies (46,47). A total of 12 altered metabolic pathways are identified in patients with myopia combined with choroidal neovascularization, five of which are related to carbohydrate derivative metabolism, suggesting an important role for their involvement in disease development (48). There are also articles suggesting the carbohydrate derivative biosynthetic process is associated with a variety of diseases, including Alzheimer's disease, diabetic retinopathy and glaucoma, largely



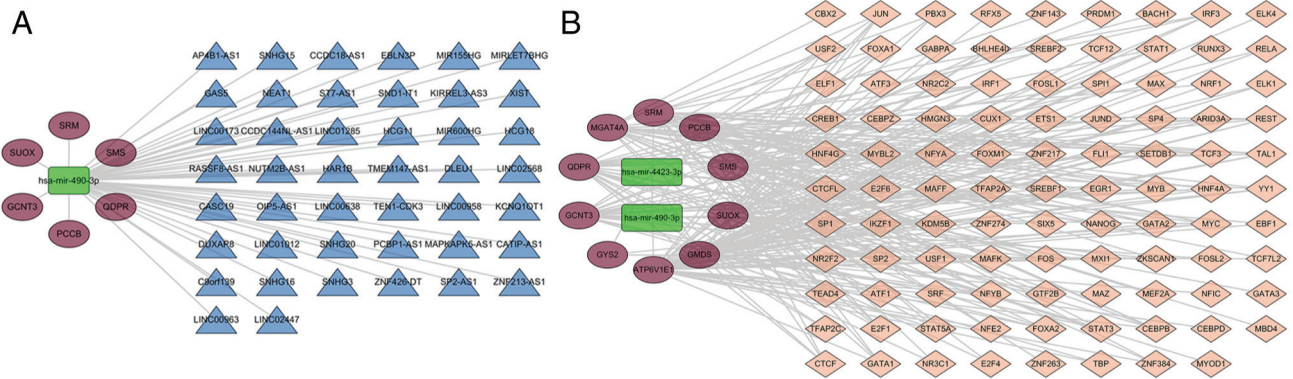


Figure 5. miR/hub gene/lncRNA and TF networks. miR (rectangle)/hub gene (circle)/(A) lncRNA (triangle) and (B) TF (diamond) network. lnc, long non-coding; TF, transcription factor; miR, microRNA.

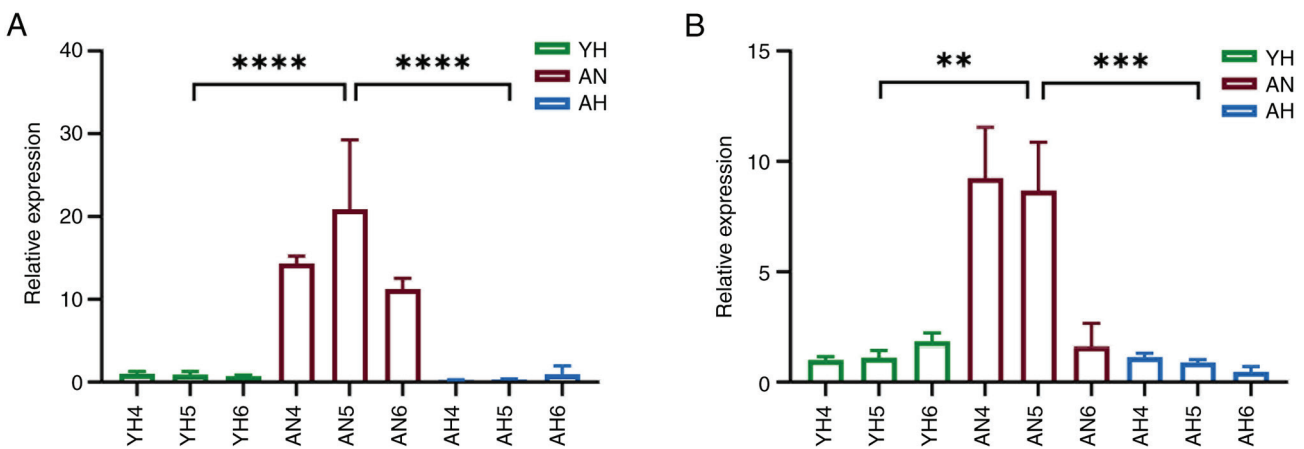


Figure 6. Reverse transcription-quantitative PCR validation of characterized miRs. Expression of (A) *hsa-mi-490-3p* and (B) *hsa-mi-4423-3p*. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . YH, young and high myopia; AN, age-related cataracts without high myopia; AH, age-related cataracts and high myopia; miR, microRNA.

due to accumulation of advanced glycation end products (AGEs) (49,50). Hyperglycaemia, alterations in oxidative environment and cell proliferation status all affect the formation of AGEs, suggesting development of high myopia may be related to ocular tissue being affected by damage similar to that caused by toxic products such as AGEs. The disease targets predicted by these 19 genes were primarily associated with musculoskeletal, neurological, cardiovascular, digestive and developmental disorder; 'decreased muscle mass' was associated with the metabolism-associated high myopia target genes. Muscle status serves a key role in maintaining the normal physiological function of the eye. For example, patients with myasthenia gravis have ocular symptoms such as extraocular muscle weakness and eye pain (51,52). In people with high myopia, abnormalities in accommodation are prevalent (53), which are mainly influenced by structures such as the lens, Zinn's zonule and ciliary muscles; visual function training targeting the muscles and ligaments inside and outside the eye may restore the eye to a healthy state. The identified diseases, such as nystagmus, cerebral atrophy, and optic nerve atrophy, may be eye-related but are not directly linked to high myopia. Future studies could explore their potential associations with high myopia.

Among 10 ranked hub genes, the most widely associated gene was *PCCB*, which, to the best of our knowledge, has not

been described in high myopia to date. The protein encoded by *PCCB* gene is reported to be a subunit of propionyl coenzyme A carboxylase (PCC), which is associated with metabolism of fatty acids, amino acids and other metabolites in mitochondria (54). Decreased expression of the *PCCB* gene leads to impairments in the  $\gamma$ -aminobutyric acid (GABA) signalling pathway. Specifically, mutations in *PCCB* gene may lead to loss of function of PCC, which affects the production of succinic acid semialdehyde, the precursor of GABA, and indirectly affects synthesis of GABA (55). A previous study has shown that the administration of baclofen (GABABR agonist) intravenously to the eyes of chicks significantly decreases myopic excursion and AL growth in eyes with deprivation and lens-induced myopia (56). In myopic guinea pigs, it was found that compared with normal controls, retinal concentrations of dopamine and GABA are decreased, glutamate, 3-methoxytyramine and glycine are increased and myopic refractive error and AL increase (57). This suggests that by interfering with *PCCB*, GABA concentration is regulated to maintain the balance between excitatory and inhibitory neurotransmitters in the eye, which may be effective in slowing development of high myopia.

In drug analysis, cyclosporine involved four hub genes. Cyclosporine is an immunosuppressant that suppresses



activity of the immune system primarily by decreasing the activity and proliferation of T lymphocytes. Cyclosporine binds to cyclophilin, which decreases the transcriptional activation of cytokine genes such as IL-2, TNF- $\alpha$ , IL-3 and IL-4 to reduce the proliferation of T lymphocytes (58). This medication is currently used as a first-line agent for uveitis, especially in patients with Behcet's disease who need to take it orally on a regular basis in conjunction with hormonal medication (59), but it is more commonly used in myopia as an anti-inflammatory topical agent following photorefractive keratectomy or laser *in situ* keratomileusis (60). The screening of cyclosporine, acetaminophen and other medications may suggest that inflammation exists in high myopia, providing a novel direction for treatment; larger studies are needed to validate these findings.

lncRNAs can act as sponges to bind miRNAs, thus preventing miRNAs from binding to target mRNAs, and can also influence the expression of their target genes by controlling miRNA expression (61). TFs control RNA transcription, localization and stability through binding (62). The present data demonstrated lncRNAs and TFs corresponding to the characterized miRNAs that may serve as potential biomarkers of therapeutic response. DE miRNA expression pattern was also observed in the aqueous humor of the patients. The present study results indicated that the selected characterized miRNAs were associated with high myopia, as well as with common viral infections, metabolic processes, muscle alterations and upstream genetic factors related to ophthalmology. Notably, cyclosporine was a key drug linked to the hub genes of interest. Cyclosporine is widely used in the treatment of various ocular diseases (63,64), underscoring the validity of the present analysis and the potential feasibility of future therapeutic applications. Numerous metabolism-related characterized miRNAs and hub genes were involved in the pathological progression of high myopia. However, the present study has limitations. First, due to the difficulty of obtaining individual aqueous humor samples, miRNAs were extracted by mixed sampling. Second, non-metabolism-associated genetic alterations in samples may also cause different expression changes in the screened genes. Third, patients with AH represent a minority of patients with cataracts; to match age and sex between the groups, the present sample size was relatively small.

To the best of our knowledge, the present study is the first to perform miRNA sequencing for the exploration of age-related cataracts and high myopia in patients. hsa-miR-490-3p, hsa-miR-4423-3p, and hsa-miR-4485-3p may serve as characterized miRNAs in high myopia. Prediction of metabolism-associated target genes and functional enrichment analysis indicate the biosynthetic process of carbohydrate derivatives may serve a pivotal role during the development and progression of high myopia. Furthermore, decreased muscle mass might be implicated in the aetiology of high myopia. PCCB could serve as a potential biomarker in management of high myopia. The hub genes affected by cyclosporine provide new avenues for therapeutic strategies in high myopia.

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### Availability of data and materials

The data generated in the present study may be found in the Genome Sequence Archive for Human (<https://ngdc.cnca.ac.cn/gsa-human/>) using the accession number HRA009123.

### Authors' contributions

FH, WW and KH conceived and designed the study. FH, YC, SZ and RH performed experiments. JW acquired data. FH and YC analyzed data. FH wrote the manuscript. WW and KH revised the manuscript. FH, WW and KH confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval no. 2023-1; Chongqing, China) and written informed consent was obtained from patients or their parent/guardian before sample collection.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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