Comprehensive analysis of transcriptome-wide m\(^6\)A methylome in the anterior capsule of the lens of high myopia patients

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**ABSTRACT**

To assess the m\(^6\)A methylome in the anterior capsule of the lens of high myopia patients. MeRIP-seq and RNA-seq were performed to identify differences in the m\(^6\)A methylomes and gene expression between anterior capsule of the lens of simple nuclear cataract patients (N) and nuclear cataract patients with high myopia (G). Expression of m\(^6\)A-related enzymes was confirmed by quantitative real-time-PCR. ALKBH5 was downregulated in G. The observed m\(^6\)A peak was identical to the conserved RRACH motif and was markedly correlated with two distinct coordinates. Differentially methylated genes were enriched in some pathways regulating the formation of extracellular matrix. These findings suggest that upregulation of m\(^6\)A methylation may change fundus anatomy by regulating the composition of the extracellular matrix through encoding protein.

**Introduction**

With the change in lifestyle, great modifications have taken place in human eye-use habits, and the incidence of high myopia is increasing. At present, 163 million of people, who account for 2.7% of the world total population, are suffering from high myopia [1–3]. Worriedly, this number is rising sharply [2–4]. High myopia, defined as myopia exceeding 6.00 dioptries or axis length ≥26 mm, is a disorder that affects almost the entire human eye, from the anterior pole to the posterior pole, including high ametropia, cataract, open-angle glaucoma, and retinopathy [5]. Excessive axial elongation of the eye in high myopia can cause mechanical stretching of the outer coats of the eyeball, resulting in various pathologic changes such as staphyloma, choroidal atrophic lesions, lacquer cracks, and choroidal neovascularization.

N\(^6\)-methyladenosine (m\(^6\)A), as the most prevalent internal form of modification in polyadenylated mRNAs and long noncoding RNAs in higher eukaryotes, was first identified in the 1970s [6]. Studies in bioscience and medicine have revealed that RNA m\(^6\)A plays important biological roles in the regulation of cellular metabolic processes. In 2014, Batista et al. have found that RNA m\(^6\)A controls cell transition fate in mammalian embryonic stem cells [7]. The next year, Zhao et al. have discovered that RNA m\(^6\)A regulates pluripotency in murine stem cells [8]. In addition, Shen et al. have studied the role of m\(^6\)A in shoot stem cell fate in Arabidopsis in 2016 [9]. Furthermore, m\(^6\)A methylation plays important roles in human disease such as control of HIV-1 replication and interaction with the host immune system during HIV-1 infection of T cells [10], promoting translation of oncoproteins in human lung cancer [11] and induction of breast cancer stem cell phenotype [12]. Thus, more medical fields will reveal the role of m\(^6\)A methylation. In mammals, m\(^6\)A methylation modification involves three enzymes: methylase (METTL3, METTL14, and WTAP), demethylase (FTO and ALKBH5), and methylation recognition enzyme (YTHDF1, YTHDF2, and YTHDF3). Enzyme abnormality causes a series of diseases, including tumour, neurological diseases, and circulatory disorders [13–15]. However, the role of m\(^6\)A methylation in high myopia is still unclear.
To investigate the functions of m^6A, facilitate future studies of mammalian m^6A, and explore the pathogenic mechanism of the eye caused by high myopia, we detected the m^6A methylomes of anterior lens capsule in high myopia patients. This enabled us to acquire a set of transcriptome-wide m^6A profiles in patients with high myopia and investigate tissue and breed generality and selectivity of methylated genes and their functional implications.

Materials and methods

Acquisition of biological samples

Twelve anterior lens capsule samples were enrolled and divided into two groups. One group was characterized by simple nuclear cataract patients (N, 6 samples), while the other group was characterized by nuclear cataract patients with high myopia (G, 6 samples). Each patient underwent conventional cataract phacoemulsification combined with intraocular lens implantation. During continuous circular capsulorhexis, the anterior capsule was collected into a cryopreservation tube and stored in −80°C. Patients with nuclear cataract and an axis length ≥29 mm were included in this study. Whereas patients with other eye diseases and other systemic diseases such as hypertension, diabetes, coronary heart disease, and autoimmune disease were excluded from the investigation. Patient information is shown in Table 1. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Preparation and sequencing of RNA library

RNA high-throughput sequencing was performed by Cloud-Seq Biotech (Shanghai, China). Briefly, total RNA was used for removing the rRNAs using the NEBNext rRNA Depletion kit (New England Biolabs, Inc., MA, USA) following the manufacturer’s instructions. RNA libraries were constructed by using NEBNext Ultra™ II Directional RNA Library Prep kit (New England Biolabs) according to the manufacturer’s instructions. Libraries were controlled for quality and quantified using the Bioanalyzer 2100 system (Agilent Technologies, Inc., USA). Library sequencing was performed on an illumina HiSeq instrument with 150 bp paired-end reads.

Preparation and sequencing of MeRIP library

m^6A RNA-seq service was provided by Cloud-seq Biotech Inc. Briefly, m^6A RNA immunoprecipitation was performed using the GenSeq™ m^6A RNA IP kit (GenSeq Inc., China) by following the manufacturer’s instructions. The quality and quantity of total RNA were assessed by using NanoDrop ND-2000 (Thermo Fisher Scientific, USA). RNA purity was qualified when OD_{260}/OD_{280} values ranged from 1.8 to 2.1. RNA integrity and gDNA contamination were measured using denatured agarose gel electrophoresis. Agilent 2100 Bioanalyzer was used to detect library quality. RNA quantification and quality assurance were assessed using Qubit3. Both the input sample without immunoprecipitation and the m^6A IP

Table 1. Patient characteristics of G and N.

| Samples | Gender | Ages | Axial | Eye |
|---------|--------|------|-------|-----|
|         | N      | G    | N    | G  | N   | G  |
| 1       | F      | M    | 73   | 60 | 23.69 | 29.20 | OS | OS |
| 2       | F      | M    | 59   | 78 | 23.67 | 29.69 | OD | OS |
| 3       | F      | M    | 73   | 59 | 23.17 | 29.95 | OD | OS |
| 4       | F      | M    | 58   | 59 | 25.42 | 29.71 | OS | OS |
| 5       | F      | M    | 68   | 78 | 22.92 | 29.56 | OS | OS |
| 6       | M      | F    | 61   | 70 | 24.68 | 29.32 | OS | OD |
| P       |        |      | 0.680 | 0.000 |       |       |     |     |
| Mean    | 65.33 ± 6.99 | 67.33 ± 9.24 | 23.93 ± 0.95 | 29.57 ± 0.27 |

N: simple nuclear cataract; G: nuclear cataract complicated with high myopia; F: female; M: male; OD: right eye; OS: left eye.
samples were used for RNA-seq library generation using NEBNext Ultra II Directional RNA Library Prep kit (New England Biolabs). The library quality was evaluated using the BioAnalyzer 2100 system (Agilent Technologies, Inc.). Library sequencing was performed on an illumina HiSeq instrument with 150 bp paired-end reads.

Data analysis

Data analysis of mRNA sequencing results

Paired-end reads were obtained from the Illumina HiSeq 4000 sequencer and were quality-controlled using Q30. After 3′ adaptor-trimming and low-quality reads removing using the cutadapt software (v1.9.3) [16], the high-quality clean reads were aligned to the reference genome (UCSC hg19) using the Hisat2 software (v2.0.4) [17]. Guided by the Ensembl gtf gene annotation file, the cuffdiff software [18] (part of cufflinks) was used to get the gene-level FPKM as the expression profiles of mRNA, fold change and p-value were calculated based on FPKM, and differentially expressed mRNA were identified. Gene ontology (GO) and pathway enrichment analysis were performed based on the differentially expressed mRNAs. GO and pathway enrichment analysis were performed using the Database for Annotation, Visualization, and Integrated Discovery [19,20]. The ontology covers three parts: cellular component (CC), molecular function (MF), and biological process (BP). The p-value denotes the significance of GO term enrichment of the genes. Pathway enrichment analysis is a functional analysis that maps genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The Fisher p-value denotes the significance of the pathway correlated to the conditions.

Data analysis of MeRIP sequencing results

Briefly, paired-end reads were obtained from the Illumina HiSeq 4000 sequencer and were quality-controlled using Q30. Then, 3′ adaptor-trimming and low quality reads removing using the cutadapt software (v1.9.3) [16] were performed. Clean reads of all libraries were aligned to the reference genome (HG19) using the Hisat2 software (v2.0.4) [17]. Reads were matched to the results on the genome (bam file), and the IGV software [21] was used for visualization to observe the abundance of reads at specific locations in the genome of each sample. methylated sites on RNAs (peaks) were identified using the MACS software [22]. Differentially methylated sites were identified using diffReps [23]. These peaks identified by both software overlapping with exons of mRNA were identified and selected using home-made scripts. GO and pathway enrichment analysis were performed for the differentially methylated protein-coding genes. MEME software was used for motif analysis [24].

RNA extraction and quantitative real-time PCR

Levels of the mRNA m6A-related genes METTL3, METTL14, FTO, ALKBH5, YTHDF1, and YTHDF2 were analysed in G and N groups. TRizol reagent (Invitrogen, USA) was used to isolate total RNA, which was then used to synthesize complementary DNA by using the SuperScript™ III Reverse Transcriptase (Invitrogen). Real-time PCR was performed by using qPCR SYBR Green Master Mix (CloudSeq, Shanghai) and QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, USA). All procedures were performed according to the manufacturer’s protocols. The sequences of primers used are presented in Table 2.

Statistical analyses

The significance of differences between G and N groups was tested by using unpaired two-tailed

| Gene   | Primer | Gene sequence               |
|--------|--------|----------------------------|
| ALKBH5 | Forward | CACGGTGATCTGGAGATGCG      |
|        | Reverse | TCCTCCAACCTTTGCGGCA       |
| METTL3 | Forward | CAAAGGCAAGCCACAGCAAGA     |
|        | Reverse | CTGAGAAGCGATCCGGAAGC      |
| METTL14| Forward | CAGGCGCTGTGTTGGAAGT       |
|        | Reverse | CAAAGGCGCTGTGACGACT       |
| FTO    | Forward | GAGTGACACCGAGCCCTCT       |
|        | Reverse | CTGGAATACTGGGGACAGGG       |
| YTHDF1 | Forward | TCACATGGAGGTGGGATT        |
|        | Reverse | GTGAGCGGAGGATGG           |
| YTHDF2 | Forward | ACCACGAGGCTCCTCAG         |
|        | Reverse | CATTACCTACCCACCAAGC       |
| GAPDH  | Forward | GGCCCTCAAGGAGTAAGAC      |
|        | Reverse | AGGGGAGATTCAGTGCGGT       |
Student’s t-test. All statistical analyses were conducted using GraphPad Prism v7.00 software. The differences were considered significant if p-value was <0.05.

Results

Overview of m6A methylation map in anterior capsular of G lens

We calculated the methylation sites in the two groups and found that the number of methylation sites in the G group was higher than that in the N group (Figure 1a).

More than 80% and 70% of the m6A peaks were consistently detected in three biological replicates of N and G groups, respectively. These recurrent peaks should be regarded as highly enriched m6A peaks for further analysis. The results showed that 13,617 m6A recurrent peaks among 8,196 expressed genes in N and 8,569 m6A recurrent peaks among 5896 expressed genes in G were detected (Figure 1a, b). We performed genome-wide profiling of m6A-modified m6A sites in the G and N groups. The methylation difference between N and G is shown in Figure 1b. The distribution of differentially methylated m6A sites with significance in chromosomes of high myopia is shown in Figure 1c.

Figure 1. (a) The number of methylation sites and genes of G and N. (b) The methylation difference between N and G. (c) Distribution of differentially methylated m6A sites with significance in chromosomes of high myopia.
mRNA in G and N. Compared to N, 2,083 significantly hypermethylated m\(^6\)A peaks and 907 significantly hypomethylated m\(^6\)A peaks were identified in G (fold change ≥2 and \(p \leq 0.0001\)). The top 20 altered m\(^6\)A peaks are listed in Table 3. The hypermethylated m\(^6\)A peaks were identified in all chromosomes (especially chr1), while no hypomethylated m\(^6\)A peak was found in chrY (Figure 1c).

To understand the preferential location of m\(^6\)A in mRNA, we investigated the metagene profiles of m\(^6\)A peaks in the mRNA

**Table 3.** The top 20 differently methylated m\(^6\)A peaks.

| Gene   | Gene ID | Fold change | Regulation | Chromosome | Start     | End       | Peak_length | P-value     |
|--------|---------|-------------|------------|------------|-----------|-----------|-------------|-------------|
| C11orf96 | 4279    | 44.76811594 | Up         | chr11      | 43,964,201| 43,965,420| 1219        | 7.14069E-14 |
| CSF1    | 1365    | 11.87987988 | Up         | chr1       | 110,464,468| 110,464,616| 403         | 2.24665E-13 |
| TMEM176B | 24,762  | 35.12587413 | Up         | chr3       | 447,177   | 447,580   | 403         | 3.40939E-13 |
| PTP4A3  | 26,335  | 9.891826923 | Up         | chr8       | 142,441,561| 142,441,620| 59          | 2.11684E-13 |
| CHL1    | 16,617  | 36.8        | Up         | chr1       | 471,772   | 471,772   | 403         | 2.24665E-13 |
| COL6A3  | 14,856  | 9.425531915 | Up         | chr2       | 238,258,789| 238,258,852| 63          | 2.57265E-13 |
| COL6A3  | 14,857  | 10.49201278 | Up         | chr2       | 238,259,772| 238,259,835| 63          | 3.0255E-13  |
| CHL1    | 1997    | 5.684013051 | Up         | chr1       | 203,152,768| 203,152,919| 151         | 3.40939E-13 |
| PXDN    | 12,802  | 16.53529412 | Up         | chr2       | 1,635,658 | 1,636,780 | 1122        | 3.90611E-13 |
| IGF8P4  | 10,837  | 11.05427975 | Up         | chr17      | 38,599,741| 38,600,336| 595         | 4.01326E-13 |
| RIMS1   | 22,499  | 306.1       | Down       | chr6       | 72,892,441| 72,892,852| 411         | 1.40E-13    |
| PTCHD4  | 22,367  | 22.209302   | Down       | chr6       | 48,078,784| 48,078,800| 16          | 1.757E-13   |
| C20orf57| 16282   | 9.1723077   | Down       | chr22      | 30,116,343| 30,116,522| 179         | 2.625E-13   |
| OCLN    | 20,687  | 334.8       | Down       | chr5       | 68,849,396| 68,849,820| 424         | 3.016E-13   |
| PTCHD4  | 22,355  | 12.119048   | Down       | chr6       | 47,847,061| 47,847,420| 359         | 3.384E-13   |
| ANKRD24 | 12,139  | 6.303719    | Down       | chr19      | 4,224,424 | 4,224,811 | 387         | 4.95E-13    |
| ALS2CL  | 17,103  | 5.9847037   | Down       | chr3       | 46,711,821| 46,712,140| 319         | 6.597E-13   |
| HEY2    | 22,926  | 7.2871901   | Down       | chr6       | 126,070,731| 126,071,005| 274         | 7.967E-13   |

![Figure 2](image-url) (a) Preferential location of m\(^6\)A in mRNA. Each transcript is divided into three parts including 5\(^\prime\) untranslated region, coding DNA sequence and 3\(^\prime\) untranslated region. (b) Pie charts showing m\(^6\)A peaks distribution in different gene context. (c) Data visualization of MYL12A mRNA m\(^6\)A modification in G.
transcriptome. m⁶A peaks were markedly correlated with two distinct coordinates: immediately following near the end of the 5' untranslated regions (5'UTRs) and start of the coding sequence (CDS) and near the end of the CDS and the beginning of the 3' untranslated region (3'UTRs) (Figure 2a).

The stop codon of peaks was more pronounced than the start codon of peaks (31.63% vs 27.10%). To assess the enrichment methodically, we assigned each m⁶A peak to one of the five non-overlapping transcript segments: 5'UTRs, start codon, CDS, stop codon, and 3'UTR. As a result, 87% of m⁶A peaks were within genic regions, and more than 60% of genic peaks were localized near the stop codon and CDS, while 12% were found in the 5'UTRs and 3'UTRs (Figure 2b). The topological patterns distributing within genes were highly similar in both samples, suggesting that recognition of motif for m⁶A methylation was conserved among anterior capsule of human lens. MYL12A, a considerably hypermethylated peak, is shown in Figure 2c.

Notably, more than 3,500 of m⁶A-methylated coding genes contained only one m⁶A peak, while a relatively small number of genes contain two or more peaks (Figure 3a), which was consistent with the trend of the proportions previously reported in the mouse brain and pig liver [25].

An unbiased search for motifs enriched in regions surrounding m⁶A peaks was performed to determine whether the identified m⁶A peak was identical to the conserved RRACH motif (where R represented purine, A was m⁶A, and H was a non- guanine base) [26,27]. Clustering of significantly enriched sequences perfectly confirmed the previously established m⁶A RRACH consensus sequence in both groups (Figure 3b). The identification of a strong consensus reinforces the authenticity of the discovered m⁶A peaks and supports the existence of a predominant methylation machinery.

**m⁶A-containing genes are involved in important biological pathways**

To further determine general biological functional pathways that the significance of m⁶A methylation in high myopia development, the genes containing significantly altered m⁶A peaks (differentially methylated genes, G) were analysed by performing GO and KEGG pathway analysis. We systematically screened these different and common peaks and the related genes and identified the GO terms with the help of the GO consortium database (Figure 4).

GO analysis showed that the hypermethylated m⁶A peak-related genes encoding m⁶A-containing mRNAs were mainly involved in a variety of biological processes, including anatomical structure morphogenesis (ontology: biological process; Figure 4a), proteinaceous extracellular matrix (ontology: cellular component; Figure 4b), extracellular matrix structural constituent (ontology: molecular function; Figure 4c). The hypomethylated genes were significantly associated with regulation of ion transport (ontology: biological process; Figure 4d), plasma membrane part (ontology: cellular component; Figure 4e), and ion channel activity (ontology: molecular function; figure 4f). GO biological process classification indicated that most of the m⁶A methylation was enriched in the cellular process (Figure 4a). These genes were involved in the formation of extracellular matrix (Figure 4c), suggesting that extracellular matrix was associated with the formation of pathological damage to high myopia.

Outstandingly, KEGG pathway analysis showed the result of unique G and unique N (Figure 5c), in addition to information on hypermethylated genes (Figure 5a) and hypomethylated genes (Figure 5b).

**Conjoint analysis of RNA-seq and MeRIP-seq**

Transcriptome profiles of altered genes in the anterior capsule of patients with high myopia were determined using RNA-seq and shown in Figure 6a. Compared to N, 20,385 genes were differentially expressed in G (fold change ≥1.5 and p < 0.05), including 7,834 upregulated genes and 1,2524 downregulated genes. The top 20 altered genes are listed in Table 3. The top 10 GO and KEGG pathways are shown in
Supplementary Figures 1 and 2. Based on conjoint analysis of differentially expressed genes and differentially methylated genes, the higher the transcription expression, the higher the methylation ratio (Figure 6b, c).

**ALKBH5 and FTO were downregulated in G**

Based on quantitative real-time-PCR, the expressions of six major enzymes involved in m^6^A methylation, including METTL3, METTL14, FTO, ALKBH5,
YTHDF1, and YTHDF2, were verified between G and N. As shown in Figure 7, in G, the levels of demethylases ALKBH5 and FTO, demethylases acting to remove m⁶A modification, significantly decreased compared to those in N (ALKBH5: G/N = 0.245, P < 0.05; FTO: G/N = 0.234, P < 0.05), whereas METTL3 and METTL14, the key methyltransferases responsible for m⁶A modifications, significantly decreased (G/N = 0.346, P < 0.05) and increased (G/N = 2.565, P < 0.05), respectively. The levels of two methylation recognition enzymes (YTHDF1 and YTHDF2) significantly decreased in G compared to N (YTHDF1: G/N = 0.284, P < 0.05; YTHDF2: G/N = 0.226, P < 0.05).
Figure 6. (a) Scatter plots showing the differentially expressed genes (fold changes ≥2 and \( p < 0.05 \)). (b) m6A and gene expression of transcript. (c) Data visualization of methylation and gene expression.

Figure 7. The expressions of six major enzymes involved with m6A methylation including METTL3, METTL14, FTO, ALKBH5, YTHDF1, and YTHDF2 between G and N.
**Expression of candidate genes correlated with pathological damage in high myopia**

To further investigate the mechanisms of pathological damage in high myopia, we performed statistical analysis of differentially expressed m^6^A methylation-modified genes, including C11orf96 (uncharacterized protein), CSF1 (macrophage colony-stimulating factor 1), PTP4A3 (protein tyrosine phosphatase type IVA 3), COL6A3 (collagen alpha-3(VI) chain), CHI3L1 (chitinase-3-like protein 1), PXDN (peroxidase homolog). However, COL6A3, CHI3L1, and PXDN were most likely to be our target genes.

**Discussion**

High myopia is a blinding disease with a high incidence, which seriously affects the quality of life of patients. In this study, we obtained the first high myopia transcriptome-wide m^6^A modification profile using MeRIP-Seq and discovered that mRNA m^6^A sites were mainly enriched around stop codons, CDS, and 3^′^UTRs, consistent with the distribution characteristics of mammal transcriptomes, although the modifications also occurred in 5^′^UTRs [7,25,28]. The m^6^A distribution in our study was the typical m^6^A topological pattern in most of the mature mRNA. The extensively higher m^6^A signals at the stop codon or 3^′^UTRs contributed to RNA stability, signalling for transport and translocation, or acted as regulatory elements for protein translation through the recruitment of specific factors onto the m^6^A sites for RNA transport or protein synthesis [29,30]. High-throughput m^6^A RNA sequencing databases showed that the distribution of m^6^A modifications on mRNA was sequence-specific and tended to occur in the conserved motif RRACH [25,28,31–35]. Accordingly, in our study, we successfully identified the consensus motif sequence in patients with high myopia transcriptome.

m^6^A mRNA modifications can be dynamically regulated by a multicomponent methyltransferase complex with opposite modifying activities, including m^6^A writers, m^6^A erasers, and m^6^A readers. Increasing evidence suggests that m^6^A modification plays a significant role in the proliferation, migration, and invasion of tumour cells. METTL3 was reported to be an oncogene in human lung cancer cells by promoting the translation of certain mRNAs such as epidermal growth factor receptor and Hippo pathway effector TAZ [11]. A study verified that METTL14 interacted with the microprocessor protein DGCR8 and positively modulated the primary microRNA 126 process in an m^6^A-dependent manner [36]. In ophthalmology, Jia et al. have found that YTHDF1 suppresses ocular melanoma by modulating HINT2 mRNA translation [37]. Luo et al. have confirmed that silencing METTL3 significantly suppressed uveal melanoma cell proliferation and colony formation through cell cycle G1 arrest, as well as migration and invasion [38]. Recently, it was reported that METTL3 silencing promoted the proliferation and alleviated the apoptosis of high glucose-induced human lens epithelial cells [39]. These studies have proved that RNA m^6^A methylation also plays an important role in the development of ophthalmic diseases. In our study, METTL14 was upregulated and METTL3, FTO, and ALKBH5 were downregulated in the anterior capsule of high myopia patient lens as determined using qPCR. This inconsistency of changes disrupts the dynamic equilibrium of writers and erasers of m^6^A modification. Our data suggest that m^6^A methylation is strongly associated with the pathogenic mechanism of high myopia.

RNA m^6^A methylation plays a key role in the regulation of post-transcriptional gene expression [25,31,40,41]. The importance of m^6^A in post-transcriptional regulation of gene expression is further reinforced by the discovery and characterization of mammalian reader proteins that recognize m^6^A modifications of mRNA and subsequently affect the stability of the target transcripts. YTHDF1 and YTHDF2, members of YTH domain-containing proteins, closely resembled each other and were predominantly cytoplasmic, but their mechanisms could be different. YTHDF2 mediated the decay of target mRNAs, whereas YTHDF1 interaction with eIF3 and other translation initiation factors suggested that it might affect translation rather than affect the half-lives of mRNAs [42,43]. In our study, compared to N, we confirmed that the YTHDF1
and YTHDF2 were downregulated in G. This result indicated that two readers might participate in the progress of high myopia through regulation of post-transcriptional gene expression. The gene FGF10 (Figure 6c), as a differentially methylated region, may have a certain impact on its downstream proteins, including nELavl and RBM10, which are RNA-binding proteins that directly interact with target RNAs and regulate several aspects of RNA metabolism. Studies have reported that nELAV proteins are unique to neurons and associated with Alzheimer’s disease [44]. Therefore, we speculate that hypomethylated FGF10 may enhance the ability to bind nELAV protein, playing an important role in the mechanism of high myopia brain injury. RBM10, another FGF10-binding protein, promotes many transformation and hypoxia-associated processes and events, including angiogenesis [45–47]. The occurrence of choroidal neovascularization in high myopia may be related to hypomethylation, weakening the ability of FGF10 to bind to RBM10.

One of the main functions of m^6^A is to mediate mRNA degradation in mammalian cells [29,48–50], suggesting a possible negative relationship between the m^6^A methylation extent and the transcript level. However, this observation somewhat differed from our present result in the anterior capsule of human lens, which showed that most of the highly expressed transcripts were relatively more modified by m^6^A. These differences may be due to different methodologies, different biological species, or different tissue samples. The results further indicated that different tissues might possess different characteristics in m^6^A methylation sites, suggesting a regulatory role for m^6^A in gene expression.

Differential m^6^A methylation has proved responsible for tissue or organ differentiation and development. Genes encoding m^6^A-containing RNAs in adult mouse brain tissue are linked to neurodevelopmental and neurological disorders [25,31]. Moreover, m^6^A RNA methylation in Drosophila and Zebrafish early embryogenesis shows a conserved mechanism of neuronal mRNA regulation contributing to brain function [43]. Our study uncovers regulation roles of m^6^A modification in the anterior lens capsule of high myopia patients. Differentially methylated genes mostly participate in the regulation of anatomical structure morphogenesis pathways. GO analysis showed that the upregulated m^6^A peak-related genes encoding m^6^A-containing mRNAs were mainly involved in the formation of extracellular matrix, consistent with the results shown by Wang [51]. In differentially expressed m^6^A methylation-modified genes, CHI3L1 (chitinase-3-like protein 1), encoding protein YKL-40, is thought to play a role in tissue remodelling and in the capacity of cells to respond to and cope with changes in their environment [52]. In addition, YKL-40 is an inflammatory marker that plays an important role in pathological processes such as cell proliferation, migration, differentiation, and tissue remodelling of inflammatory reactions [53–55]. Therefore, we suspect that the upregulation of methylase (METTL14) and the downregulation of demethylase (FTO and ALKBH5) catalyse the hypermethylation of gene CHI3L1, which may affect the expression level of its encoded protein YKL-40 and promotes the pathological state of high myopia by regulating the composition of the extracellular matrix. Posterior scleral staphyloma is one of the most basic changes in fundus anatomy in a series of interrelated degenerative changes in high myopia [56]. Thus, high expression of m^6^A methylation of mRNA may be involved in the formation of posterior scleral staphyloma by affecting the components of the extracellular matrix.

The choroid circulation receives approximately 95% of blood from the ophthalmic artery and provides oxygen and nutrition to the outer retinal layers. Therefore, the choroidal circulation may play an important role in the retinal dysfunction and vision loss. GO analysis showed that the upregulated m^6^A peak-related genes encoding m^6^A-containing mRNAs were also associated with circulatory system development, blood vessel development, and vasculature development (Figure 4). This suggests that the increase in methylation level of mRNA may cause damage to the fundus of patients with high myopia by affecting the choroidal circulation.

**Conclusion**

For the first time, we provide the integral human lens transcriptome m^6^A map in high myopia patients. Our map reveals features of m^6^A distribution in the high myopia of
human transcriptome and identifies generality as well as selectivity of methylated genes and their functional implications. Additionally, the abnormal expression of methylation-related enzymes destroys the dynamic homeostasis of methylation. This comprehensive methylome profiling provides a solid basis for the determination of potential functional roles for RNA m⁶A modification in pathological damage to the eye caused by high myopia.

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**Disclosure statement**

The authors declare that they have no conflict of interest.

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