

Molecular changes in intraocular fluid: implications for myopia

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Abstract

Myopia is the most common eye disease in the world which is caused by a mismatch between the optical power of the eye and its excessive axial length. Scleral remodeling, oxidative stress, inflammation, pathological states of angiogenesis and fibrosis and metabolism are closely associated with the onset and progression of myopia and the pathological changes that may ultimately result. Intraocular fluid is a collective term for the fluid within the eye, and changes in its composition can reflect the physiological and pathological status within the eye, with aqueous humor and vitreous being the commonly tested specimens. Recent studies have revealed potential changes in a variety of molecules in intraocular fluid during myopia progression. Abnormal expression of these molecules may reflect different stages of myopia and provide new perspectives for disease monitoring and treatment. Therefore, in this review, we systematically review the molecular changes in intraocular fluid associated with myopia, as well as the possible mechanisms, with a view to informing basic myopia research and clinical work.

Keywords: Intraocular fluid, Aqueous humor, Vitreous, Myopia, Molecular changes

Introduction

Myopia represents a prevalent ocular disorder characterized by AL elongation [1], with its incidence escalating globally [2]. As of 2010, the worldwide prevalence was recorded at 28.3%, with projections suggesting an increase to 39.9% by 2030 and 49.8% by 2050 [2]. This persistent rise underscores the imperative for a robust public health intervention. Myopia markedly impairs the educational achievements, quality of life, and employment opportunities of adolescents, imposing substantial burdens on individuals, families, and socioeconomic structures. Significantly, the economic impact attributed to uncorrected myopia was estimated at approximately US\$244 billion globally in 2015, with East Asia experiencing the most significant economic strain [3]. Moreover, myopia, whether low, moderate or high, is associated with a higher risk of several severe ophthalmic complications including cataracts, glaucoma, myopic macular degeneration, retinal detachment and even blindness [4]. Therefore, in-depth understanding of the pathogenesis of myopia and searching for effective preventive and therapeutic methods have become important topics in ophthalmic research.

The intraocular fluid, encompassing AH and vitreous body, are integral in sustaining the ocular microenvironment. They provide essential nutrients to intraocular structures, regulate intraocular pressure, and facilitate the removal of metabolic byproducts [5,6]. Extensive research substantiates that a diverse array of biomolecules within intraocular fluid is

intricately linked to myopia progression through various biological processes such as scleral remodeling, oxidative stress, inflammation, the pathological states of angiogenesis and fibrosis and metabolism (Figure 1). Fluctuations in the molecular composition of intraocular fluid may signify critical phases in the development of myopia (Table 1). This review endeavors to elucidate the correlations between molecular alterations in intraocular fluid and myopia progression, aiming to furnish novel insights and directions for future investigations.

Scleral remodeling

The sclera is instrumental in the initiation and progression of myopia, with alterations in its biomechanical properties, particularly the imbalance between degradation and synthesis of the scleral ECM, forming the structural foundation for AL hyperextension [7]. As a microenvironment interacting directly with the sclera, shifts in the molecular composition of intraocular fluid can reflect the state of scleral remodeling to some extent. Subsequent research has revealed that variations in the expression of MMPs, TIMPs, and TGF- β s within the intraocular fluid are intricately linked to the process of scleral remodeling.

MMPs, a family of zinc-dependent endopeptidases involved in the degradation of a variety of proteins in the ECM, such as collagen and elastin, while TIMPs serve as specific inhibitors of MMPs [8]. Thus, the balance between MMPs and TIMPs is critical for scleral remodeling [7]. Animal model studies on myopia have demonstrated that increased expression of MMP-2 correlates with reorganization of the scleral structure and ECM remodeling [9,10]. Furthermore, enhanced MMP-2 activity and reduced TIMP-2 activity have been observed to contribute to scleral remodeling in guinea pig [11], mouse [12] and chick [13,14] models. Theoretically, TIMPs, as inhibitors of MMPs, should be reduced in myopic progression, but some studies shown that TIMPs expression decreases with increasing MMP-2 [15,16], which may be related to the in vivo homeostasis hypothesis [17,18]. Interestingly, a recent study have shown that low doses of TIMP-2 promote MMP-2 activation, whereas high doses inhibit its activation, reduce collagen degradation, and shorten the AL, suggesting that TIMP-2 supplementation may be helpful in controlling myopia progression [19]. These findings indicate a nuanced regulatory interplay between MMPs and TIMPs in the remodeling of the sclera.

Molecular changes associated with myopia have also been identified in human intraocular fluid as well. In an observational study involving patients with HM or cataract, intraoperatively removed AH was examined and found to contain MMP-2, MMP-3, TIMP-1, TIMP-2, and TIMP-3, but MMP-1 was not detected. Statistical analysis revealed that the levels of MMP-2, TIMP-1, TIMP-2, and TIMP-3 were positively correlated with AL, whereas MMP-3 showed no correlation [20]. Considering the interaction between MMPs and TIMPs, further analysis showed that MMP-2 levels were elevated in AH in patients with HM, while MMP-3 levels remained unchanged. TIMP-1, TIMP-2, and TIMP-3 levels were positively associated with MMP-2 levels, but there was no significant association between MMP-3 and the levels of TIMP-2 or TIMP-3 and the TIMP-1 levels were only slightly correlated with MMP-3 levels [21]. These results align with the hypothesis that an increase in TIMPs is a compensatory response to maintain homeostasis. Additionally, changes in the vitreous body associated with myopia have drawn research interest. Zhuang et al. obtained vitreous samples from highly myopic patients undergoing vitrectomy, finding significantly elevated MMP-2 activity and levels, as well as an increased MMP-2/TIMP-2 ratio compared to controls [22]. In myopic macular degeneration, MMP-2 levels were significantly elevated and positively correlated with AL [23].

There are three major TGF- β isoforms in mammals, which are crucial in regulating cell growth, differentiation, immune responses, and ECM formation [24]. TGF- β s have a strong association with myopia. For instance, TGF- β 2 levels are significantly elevated in both the retina and sclera of chicks with FDM [25]. In tree shrews with experimentally induced myopia, a decline in various TGF- β isoforms correlates with AL elongation [26]. In human studies, Zhu et al. analyzed AH sample from post-cataract surgery using real-time polymerase chain reaction and immunofluorescence staining, and found that TGF- β 2 expression in the AH of patients with HM exceeded that of controls [27]. Consistent results were also found in a recent study, which demonstrated that TGF- β 2 levels in AH increased by 3.43% for each 1 mm increase in AL [28]. Moreover, a prospective study revealed significantly higher TGF- β 2 levels in the AH of patients with highly myopic cataracts compared to those with non-highly myopic cataracts. While TGF- β 1 levels were not significantly altered, they

were found to correlate with the patient's age and AL [29]. Notably, this study found that TGF- β 2 levels in the second eye of patients with HM were higher than those in the first eye, which may be related to the fact that cataract surgery promoted TGF- β 2 expression in the other eye. Although the majority of research underscores a significant role for TGF- β , particularly TGF- β 2, in myopia development, some studies report no significant differences. Zhuang et al. used enzyme-linked immunosorbent assay to measure TGF- β 2 concentrations in the vitreous of HM patients and found them comparable to controls [22]. This disparity might be attributed to differences in the environments of the AH and vitreous or variations in control group selection. Given the importance of TGF- β in the development of myopia, a Meta-analysis explored the relationship between TGF- β gene polymorphisms and myopia, and found that the rs4803455 and rs1800469 loci of TGF- β 1 were associated with myopia, suggesting that further research and treatment of myopia based on these findings may be important. Yet rs1982073 and rs2241716 for TGF- β 1 and rs7550232 for TGF- β 2 were not significantly associated with myopia [30], indicating that further studies are still needed to explain these inconsistent results.

In summary, altered biomechanical properties of the sclera, particularly an imbalance between the degradation and synthesis of the ECM, form the basis of myopic AL hyperextension. Changes in MMPs, TIMPs, and TGF- β s in intraocular fluid are closely associated with scleral remodeling, and these molecules may serve as potential indicators of myopia progression. Given the complexity of myopia mechanisms and the inconsistency of findings, more in-depth studies are still needed in the future.

Oxidative Stress

Oxidative stress is a state of imbalance between the production of ROS in the intra- and extracellular environment and the antioxidant defense system [31]. An excessive buildup of ROS in the intraocular fluid has been linked to the onset of various ophthalmic conditions, including cataracts, glaucoma, and retinal disorders [31]. Studies have pointed to a significant association between oxidative stress and the development of myopia. At the retinal level, proteomic analysis of samples from rabbit models of myopia revealed that oxidative stress may be a key mechanism in the progression of myopia [32]. Another investigation in a chicken model induced by FDM revealed that the expression of neuronal NO synthase was increased, and that antioxidant intervention could mitigate myopia progression [33]. At the scleral level, intense ROS release during oxidative stress may induce apoptosis in scleral cells, thereby fostering the growth of myopic sclera [34]. In patients with HM, the substantial reduction of antioxidant enzymes and other protective agents can lead to cellular damage, apoptosis, and potentially impair visual functions [35].

In recent studies, the relationship between oxidative stress levels in the intraocular fluid and myopia development has garnered attention. T-AOC, an indicator of all antioxidant substances, and total nitrite/nitrate levels, an important indicator for assessing the state of oxidative stress, were measured in the eyes of middle-aged and elderly HM patients with AH. It was found that T-AOC values were significantly lower and total nitrite/nitrate levels were significantly higher in patients with HM, reflecting higher levels of oxidative stress in the intraocular environment. Further analysis revealed that T-AOC was negatively correlated with refraction and AL, indicating that the lower the T-AOC value, the greater the degree of myopia. Whereas, total nitrite/nitrate levels were positively correlated with refraction and AL, indicating that higher levels of oxidative stress were associated with more myopia [36].

Similar results were obtained in our study, where T-AOC levels were significantly lower in patients with AL \geq 28 mm compared to patients with AL \leq 26 mm in the testing of AH samples collected from 40 young patients (70 eyes) undergoing implantable collamer lens surgery. Additional correlation analysis confirmed a negative association between T-AOC and AL [37]. The above results were consistent in both young and elderly patients with HM, emphasizing the potential role of oxidative stress in the development of myopia. Regarding the vitreous, examination of samples from animal models of myopia combined with bioinformatics analysis suggests that oxidative stress and lipid metabolism are pathways involved in compensatory eye elongation [38].

Overall, oxidative stress appears to play a significant role in myopia development within the intraocular fluid environment, particularly in HM patients. The reduced antioxidant capabilities and increased oxidative markers highlight a pathogenic process that potentially accelerates myopia by altering the biological functions of the retina and sclera.

Inflammation

Inflammation is the body's natural response to harmful stimuli and typically benefits the organism when controlled [39]. It serves as a key factor in the development of myopia, as evidenced by studies indicating that the expression of ocular inflammatory cytokines escalates with the progression of myopia in various animal models including tree shrews [40], guinea pigs [41], hamsters [42,43], and chicks [44]. These studies suggest that an increased state of ocular inflammation accelerates the development of myopia. Additionally, patients with inflammatory diseases have been reported to exhibit a higher prevalence of myopia [43,45]. In comparison, various markers of inflammation such as elevated circulating neutrophils, reduced monocytes, eosinophils, and lymphocytes [46], along with increased neutrophil-to-lymphocyte ratios and platelet-to-lymphocyte ratios, demonstrate an imbalance in patients with high and pathological myopia. These findings suggest that individuals with myopia may experience a systemic inflammatory state [47,48].

Intraocular fluid is one of the main sites of inflammatory response in myopia development. Research shows that myopic eyes exhibit elevated levels of inflammatory cytokines in the vitreous or AH compared to non-myopic eyes. For instance, significant increases in cytokines such as MCP-1, IL-5, IL-6, IFN- γ , IP-10, eotaxin, macrophage inflammatory protein-1 α , IL-4, and granulocyte colony-stimulating factor have been documented in the vitreous of patients with HM [49]. Similarly, increased levels of IL-6, IL-8, IL-10, IP-10, and MCP-1 have been observed in HM and its complications, further indicating a pro-inflammatory state in highly myopic eyes [50]. In the AH of patients with highly myopic cataracts, the expression of IL-1 α was decreased, whereas the expression of MCP-1 was elevated, suggesting that the anterior chamber of the eye may be in a pro-inflammatory state [51]. Moreover, the positive correlation of MCP-1 with AL reinforces its association with myopia progression [28]. IL-6 and IL-8, as typical inflammatory factors, are similarly significantly altered in the atrium in addition to the vitreous. It has been found that IL-6 and IL-8 are significantly higher in highly myopic eyes than in non-highly myopic eyes and have a significant positive correlation with the AL [52,53]. Notably, the tight junctions of the cornea are diminished in eyes suffering from allergic conjunctivitis, leading to increased levels of intraocular inflammatory cytokine expression, which may be related to cytokine penetration, with high levels of MCP-1, IL-6, IL-8, and TNF- α being recognized as a potential contributor to the promotion of retinal inflammation and myopic progression [54], indicating a more profound effect of inflammation on myopic progression. The development of mCNV is closely associated with the upregulation of inflammatory cytokines. It has been shown that the inflammatory cytokines IL-6, IL-8, IL-10, IP-10 and MCP-1 were significantly increased in AH in the eyes of highly myopic patients with and without mCNV compared to controls [50]. However, the evidence for an association between myopia and inflammation is not consistent. One study found no significant correlation between AL and concentrations of inflammatory cytokines such as IL-1 β , IL-6, IL-8, IL-10, IL-12p, and TNF- α in AH of cataract patients with AL ranging from 22.6 to 31.5 mm [55]. Similarly, our research detected no significant changes in TNF- α [37]. Additionally, other investigations reported that IL-6 and IL-8 levels were not significantly elevated compared to controls [23,51], and that log-transformed concentrations of MCP-1, sICAM-1, and svCAM-1 showed no correlation with refractive error or AL [56]. Such discrepancies could stem from variations in sample size, testing methodologies, control group selection, and inclusion criteria. In particular, most of the samples were from cataract patients, which can have an effect on the molecular changes of intraocular fluid, and this may be one of the reasons for the inconsistent results of the study. Therefore, younger patients should be included as controls in future studies. Our recent study took this aspect into account by including non-cataract patients aged 18-45 years and found that IL-1 β and IL-6 concentrations were significantly higher in the AH of patients with AL \geq 28 mm compared with those with AL \leq 26 mm, with IL-6 positively correlating with AL [37], presenting a consistent performance with previous studies [52,53].

Given the role of inflammation in various ocular diseases and the complex mechanisms underlying myopia, further research is essential to clarify the relationship between inflammation and myopia and to uncover the underlying mechanisms.

Angiogenesis

VEGF is a critical bioactive protein integral to angiogenesis [57]. Studies have shown that aberrant VEGF expression

171 is strongly associated with a variety of serious ocular diseases [57]. The involvement of VEGF in myopia development is
172 increasingly acknowledged. A study reported that intravitreal injection of VEGF165 effectively slowed myopia progression
173 in guinea pig models of FDM-induced myopia [58]. Significant changes in VEGF have also been found in the human eye.
174 Most studies noted that in patients with HM and mCNV, VEGF levels in the AH were significantly decreased and showed a
175 negative correlation with AL [23,36,52,53,55,56,59–62]. Notably, there were variations in the experimental procedures of
176 the researchers. The study by Sawada et al. excluded patients with uveitis, myopic atrophy, and diabetes mellitus to
177 minimize confounding by VEGF expression, but did not establish a normal control group and was a cross-sectional study,
178 which limited the inference of causality [61]. Shin et al. enhanced the robustness of their findings through subgroup analysis
179 but included patients with conditions that could compromise the generalizability of the results [62]. Yuan et al. reported a
180 decrease in AH VEGF levels corresponding to increases in AL, but did not account for the impact of intraocular volume on
181 these levels [53]. Conversely, Wong et al. controlled for AL in their analysis, yet faced limitations due to the small scale and
182 cross-sectional nature of their study [23]. In vitreous samples, Zhu et al. and Hu et al. found that VEGF concentrations
183 decreased as AL increased [55,59]. However, one study showed significantly higher levels of VEGF in the vitreous of
184 patients with HM, and the authors hypothesized that this may be related to a low-grade inflammatory state in the eye rather
185 than differences in the type of retinopathy [49].

186 In mCNV patients, the role of VEGF has also received attention from researchers. It was reported that VEGF levels in
187 AH of mCNV patients were lower compared to controls, but there was no correlation between VEGF and AL. It is
188 hypothesized that VEGF may be mainly localized in the retina and choroid, resulting in low levels in AH. Another theory
189 proposes that due to the extended AL and consequently larger intraocular volume in HM patients, VEGF might be diluted in
190 the anterior chamber and vitreous cavity [63]. Notably, low VEGF levels in the AH of patients with mCNV have also been
191 found to be accompanied by other cytokine imbalances[64]. Moreover, VEGF levels were notably higher in the vitreous of
192 highly myopic patients with mCNV compared to controls, although no disparity was noted in AH levels. This may reflect
193 altered hemodynamics in the dilated retinal pigment epithelium cells and choroid in the posterior pole [50]. In response to
194 the inconsistency of test results in AH as well as vitreous samples, the study introduced a novel approach by considering
195 VEGF mass—calculating the total amount of VEGF (concentration multiplied by volume) rather than concentration alone,
196 accounting for changes in AL and vitreous volume to more accurately assess VEGF’s bioactivity within the eye [50].

197 PEDF is a potent inhibitor of angiogenesis [65]. Typically, in normal ocular tissues, the regulation of angiogenesis is
198 governed by the equilibrium between VEGF and PEDF [66]. Despite expectations of elevated PEDF levels in highly
199 myopic eyes to counterbalance reduced VEGF, it has observed diminished PEDF levels in the AH of patients with HM [67].
200 Conversely, while one investigation found no significant disparity in PEDF levels between highly myopic individuals and
201 control subjects, the VEGF/PEDF ratio was notably reduced in the highly myopic group [62]. This suggests potential
202 damage to the fundus and may partially clarify the predominant influence on VEGF secretion relative to PEDF in scenarios
203 of fundus alterations triggered by HM, thus accounting for the unanticipated lower PEDF levels [23,62].

204 Angiogenesis is a prominent feature of PM, and anti-VEGF therapy has been widely used to inhibit this pathological
205 process. Although measuring VEGF concentrations in intraocular fluid can shed light on angiogenic activity, it does not
206 fully encapsulate the complex dynamics of angiogenesis. Emerging research suggests that evaluating the total VEGF
207 content might offer a more holistic view of angiogenic processes. Moreover, maintaining the balance between VEGF and
208 PEDF is crucial for the regulation of angiogenesis, making the VEGF/PEDF ratio a potentially superior metric for assessing
209 the state of angiogenesis compared to the levels of individual factors.

210 Changes in growth factors

211 Experimental animal studies have demonstrated that both topical and intraocular administration of multiple growth
212 factors can influence externally induced AL elongation [68–70]. These growth factors undergo specific changes in the
213 intraocular fluid during the development of myopia. EGF is a biologically active polypeptide whose primary role is to
214 promote cell growth, proliferation, and differentiation [71]. One study identified a negative correlation between the total
215 intraocular EGF and AL, indicating that EGF may be linked to pathological changes in the fundus in cases of HM [72]. This

was calculated by multiplying the EGF concentration by the intraocular volume in a method consistent with Zhang et al. [50]. IGFs, crucial for tissue growth, cellular proliferation, and repair, are believed to play a significant role in myopia development [69,73]. Insulin microarray analysis of AH and vitreous samples from patients with PM showed that insulin, IGF-2, IGF-2R, and IGF-BPs were significantly increased in patients with PM [74]. GDF-15, part of the TGF- β superfamily, participates in the TGF- β signaling pathway, influencing inflammatory responses and promoting fibrosis [75,76]. HGF, extensively studied in various ocular diseases, including corneal and retinal disorders, has been shown to upregulate MMP-2 expression in scleral fibroblasts of myopic guinea pigs, potentially contributing to myopia development through the degradation of scleral ECM [70]. PDGF was first isolated from platelet extracts in the early 1970s and classified as a mitogen for fibroblasts and mesenchymal-derived cells [77]. Notably, levels of GDF-15, HGF, and PDGF-AA were found to be significantly higher in the AH of patients with HM compared to controls. Given the pro-fibrotic effects of these growth factors, researchers have hypothesized their involvement in the fibrotic processes associated with HM [36,60].

Other potential molecules

With the advancement of omics technologies, researchers have obtained significant insights by analyzing AH or vitreous samples from myopic or highly myopic patients (Figure. 2). Transcriptomics and proteomics, two vital branches of omics research, have offered fresh perspectives in understanding the molecular underpinnings of myopia (Table 2). Transcriptomics have revealed an abundance of miRNAs in AH [78], often encapsulated within exosomes [79]. The altered miRNA expression profiles are closely linked to myopia progression. Chen et al. first analyzed the miRNA expression profiles of exosomes in myopic patients' AH and found that the total RNA content in the myopic group was 2.78 times higher than that in the control group. By Open Array, they identified five myopia-specific miRNAs (has-miR-582-3p, has-miR-17-5p, has-miR-885-3p, has-miR-19b-3p, and has-miR-450b-5p), which may be six common myopia-related genes as potential targets [80]. NGS was used to detect a total of 249 mature miRNAs and 17 novel miRNAs that were differentially expressed in myopia, which may be involved in the regulation of the TNF, MAPK, PI3K- AKT, and HIF-1 signaling pathways, and were verified by quantitative polymerase chain reaction [81]. In another study, also using the NGS combined with immunofluorescence and western blot validation, it was concluded that hsa-miR-142-3p may be involved in the pathologic progression of HM by reducing collagen I in scleral fibroblasts by targeting TGF- β 1 [82]. The researchers also noted whether there were corresponding changes in the vitreous of the patients. Detection of miRNAs in the vitreous using Low Density Arrays analysis revealed that let-7c was significantly upregulated in the vitreous of highly myopic eyes, while miR-200a was significantly downregulated. Further analysis showed that the up-regulated miRNA target genes involved MAPK and multiple inflammatory signaling pathways; the down-regulated miRNA target genes involved the PI3K/AKT pathway [83].

Proteomics serves as the one of the final steps downstream of the omics cascade, focusing on global protein measurement. An iTRAQ-based quantitative proteomics study identified 210 proteins differentially expressed in HM patients, with 123 proteins up-regulated and 87 proteins down-regulated in patients with HM [84]. However, results from other ITRAQ-based studies varied, possibly due to differences in sample size and inclusion criteria. One found that 146 proteins were differentially expressed among proteins associated with the HM group compared with the control group, of which 97 proteins were upregulated and 49 proteins were downregulated in patients with HM [85]. In another study, 58 differentially expressed proteins were identified, of which 32 were up-regulated and 26 were down-regulated. Bioinformatic analysis and confirmation by enzyme-linked immunosorbent assay suggested that PLG may be a candidate biomarker for HM. Its overexpression may lead to scleral thinning by affecting the composition of collagen fibers and ECM of the sclera [86]. APOA1 has emerged as a significant protein in several studies, associated with myopia arrest in animal models [87] and identified in both chick embryo vitreous [38] and AH exosomes of HM patients [88]. APOA1's role is supported by its implication in oxidative stress and lipid metabolism pathways [38]. A proteomics study using old experimental methods indicated that DBP, albumin, and transthyretin were significantly increased in highly myopic eyes. The researchers suggested that DBP may be involved in the APOA1 arrest pathway and thus play a specific role in the development of myopia [89]. The results of a study on PM also ultimately point to APOA1 [90]. Additionally, proteomics has extended to

261 studying mCNV, with elevated levels of glial fibrillary acidic protein and complement-related proteins identified, primarily
262 involving the JAK-STAT pathway [91].

263 Metabolomics, as another step in the final stage o in the cascade of omics, is increasingly elucidating the association
264 between metabolite alterations in intraocular fluid and the progression of myopia (Table 3). Both AH and vitreous have been
265 central to myopia-related metabolomic studies. Through untargeted approaches using CE-MS and LC-MS, over 40
266 metabolites have been identified. Higher levels of aminooctanoic acid, arginine, citrulline and sphinganine were observed in
267 patients with HM compared to those with low myopia. These metabolites play pivotal roles in NO synthesis and cellular
268 signaling, suggesting their involvement in myopia development by influencing intraocular pressure regulation and ocular
269 growth. Additionally, low myopia patients exhibited higher levels of aminoundecanoic acid, dihydro-retinoic acid and
270 cysteinylglycine disulfide. These metabolites may be linked to retinal health and antioxidant defense related to retinoid
271 signal transduction, indicating a potential protective role at different myopia stages [92]. Using GC-TOF-MS in an
272 untargeted manner, 29 metabolites showed significant changes between HM patients and controls, with 27 increasing and 2
273 decreasing. Further metabolic network analysis highlighted the prominence of amino acid metabolism pathways. Specific
274 amino acid metabolites, such as glutamine, N-alpha-acetyl-L-ornithine, nicotinoylglycine, and o-hydroxyhippuric acid, were
275 markedly upregulated. Levels of oxalic acid, linoleic acid methyl ester, and thymidine also significantly increased, with the
276 latter two being associated with enhanced ROS generation and oxidative stress in HM patients. The rise in oxalic acid might
277 relate to calcium binding and subsequent impacts on calcium concentration, contributing to myopia progression. Thymidine
278 levels are thought to correlate strongly with certain features of AL elongation [93]. Shao et al. utilized LC-MS to analyze
279 AH metabolites in patients with AL less than 24 mm (Group A), between 24-26 mm (Group B), and greater than 26 mm
280 (Group C). Comparative and enrichment analyses across these groups revealed that differential metabolites were involved in
281 Taurine and hypotaurine metabolism, vitamin B6 metabolism, pantothenate, and coenzyme A biosynthesis. Further trend
282 analysis and determination of differential metabolites based on FC values, coupled with receiver operating characteristic
283 curve analysis and correlation analysis, identified 5-methoxytryptophol and cerulenin as potential biomarkers significantly
284 associated with abnormal axial myopia [94]. A study on PM, using an untargeted UHPLC-MS/MS approach, analyzed AH
285 and vitreous humor samples from PM and non-myopic patients. The analysis revealed significant differences in 104 AH
286 metabolites and 114 VH metabolites between the groups. Enrichment analysis identified four metabolomic pathways
287 associated with PM: bile secretion, insulin secretion, thyroid hormone synthesis, and the cGMP-PKG signaling pathway.
288 Additionally, ten amino acids were significantly associated with AL [74]. Another study employing similar techniques
289 investigated the metabolomic characteristics of vitreous in PM patients, identifying significant changes in 19 metabolites
290 within the tryptophan metabolism pathway, notably elevated levels of L-kynurenine, indicative of increased oxidative stress
291 and inflammatory response. Furthermore, significantly elevated uric acid levels were correlated with PM complications,
292 suggesting its potential as a PM biomarker. The study also observed changes in amino acid (gamma-glutamyltyrosine and 4-
293 guanidinobutyric acid) and lipid metabolites (L-carnitine, acetyl-L-carnitine and propionylcarnitine), associated with
294 heightened oxidative stress in PM [95].

295 It is worth noting that multi-omics research has been gradually utilized in the exploration of disease mechanisms. As a
296 multifactorial disease, the combined application of multi-omics can provide a more comprehensive and in-depth
297 understanding of the changes in the development of myopia, especially in the screening of certain meaningful biomarkers.
298 In conclusion, the application of omics technologies to AH and vitreous samples has uncovered crucial molecular alterations
299 and potential biomarkers. These findings are instrumental in deepening our understanding of myopia's mechanisms, aiding
300 in its diagnosis, and fostering the development of targeted treatment strategies.

301 Conclusion

302 The etiology of myopia is shaped by an intricate interplay of genetic predispositions and environmental exposures,
303 thereby complicating the elucidation of its underlying mechanisms. Despite considerable advancements in the field, a
304 definitive account of the factors driving myopia progression remains elusive. Current findings on myopia are mostly based
305 on animal and cellular experiments due to the difficulty of obtaining specimens from the human eye. With the

306 popularization of cataract surgery and advances in vitrectomy surgery, we are able to obtain intraocular fluid, including AH
307 and vitreous, in an ethical manner. However, the widespread adoption of cataract extraction and the refinements in
308 vitrectomy techniques have facilitated the ethical collection of intraocular fluid, including AH and vitreous body, thus
309 enriching our understanding of myopia at the molecular level. According to existing studies, there are corresponding
310 changes in the molecules in the intraocular fluid during the progression of myopia as well as in the later stages of the
311 pathology, which point to scleral remodeling, oxidative stress, inflammation, pathological states of angiogenesis and fibrosis
312 and metabolism. Yet researchers face certain challenges in interpreting these changes. On the one hand, this may be due to
313 the fact that the source of AH and vitreous molecules is not limited to the eye, but can partly come from plasma [96,97]. On
314 the other hand, limitations of the studies themselves, such as insufficient sample sizes, varying inclusion criteria, differences
315 in statistical methods, and failure to adequately take into account the dilutional effect of AL elongation, may lead to
316 inconsistent results. Moreover, the majority of human ocular specimens are derived from elderly patients undergoing
317 cataract surgery, which may not offer comprehensive insights comparable to those obtained from younger individuals.
318 Fortunately, researchers are gradually overcoming these challenges with improvements in study design and advances in
319 assay technology. Developments in microscopic specimens and high-throughput assay technologies have led to more
320 precise analysis of molecular changes in intraocular fluid. Researchers are increasingly recognizing the importance of robust
321 study designs and have begun to consider the effect of AL on results, expand sample sizes, and include low myopia and
322 younger patients as controls to improve the scientific validity of their studies. In summary, the study of molecular changes
323 in intraocular fluid has been instrumental in shedding light on the mechanisms of myopia. Despite existing challenges,
324 ongoing technological advancements and methodological improvements provide optimism for a more thorough and direct
325 comprehension of myopia at the human level in the foreseeable future. Such advancements are poised to enhance the
326 strategies for prevention and management of myopia.

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335 Competing interests

336 The authors have no competing interests to declare.

337 Abbreviations

338 AH: aqueous humor

339 AKT: serine-threonine kinase

340 AL: axial length

341 APOA1: apolipoprotein A1

342 cGMP: cyclic guanosine monophosphate

343 DBP: vitamin D-binding protein

344 ECM: extracellular matrix
345 EGF: epidermal growth factor
346 FDM: form-deprivation myopia
347 GDF: growth differentiation factor
348 G-CSF: granulocyte colony stimulating factor
349 HM: high myopia
350 HGF: hepatocyte growth factor
351 HIF-1: hypoxia-inducible factor-1
352 IGF: insulin-like growth factor
353 IGFBP: IGF-binding protein
354 IL: interleukin
355 IL-1ra: IL-1 receptor antagonist
356 IFN- γ : interferon- γ
357 IP-10: interferon- γ -induced protein 10
358 JAK: janus tyrosine kinase
359 MAPK: mitogen-activated protein kinase
360 mCNV: myopic choroidal neovascularization
361 MCP-1: monocyte chemoattractant protein-1
362 MIP-1 α : macrophage inflammatory protein-1 α
363 MMP: matrix metalloproteinase
364 NGS: next-generation sequencing
365 NO: nitric oxide
366 PDGF: platelet-derived growth factor
367 PEDF: pigment epithelium-derived factor
368 PI3K: phosphatidyl-inositol 3-kinase
369 PM: pathologic myopia
370 PKG: protein kinase G
371 ROS: reactive oxygen species
372 sICAM-1: soluble intercellular adhesion molecule-1
373 svCAM-1: soluble vascular cell adhesion molecule-1
374 STAT: signal transducer and activator of transcription
375 T-AOC: total antioxidant capacity
376 TGF- β : transforming growth factor- β
377 TIMP: tissue inhibitors of metalloproteinase
378 TNF: tumor necrosis factor
379 VEGF: vascular endothelial growth factor

380 References

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580 humour. *Eye.* 2002;16(4):454–60.

581 Table 1. Myopia-related molecular changes in human intraocular fluid

Molecules	Sample source	Molecular changes	Ref.1	AL association	Ref.2
MMP-2	AH, Vitreous	Increased	[20-23]	Positive correlation	[20,23]
TIMP-1	AH	Increased	[20,21]	Positive correlation	[20]
TIMP-2	AH, Vitreous	Increased	[20-22]	Positive correlation	[20]
TIMP-3	AH	Increased	[20,21]	Positive correlation	[20]
TGF-β1	AH	No Significant Change	[29]	Positive correlation	[29]
TGF-β2	Vitreous	No Significant Change	[22]	—	
	AH	Increased	[27,29]	Positive correlation	[28]
T-AOC	AH	Decreased	[36,37]	Negative correlation	[36,37]
Total nitrate	AH	Increased	[36]	Positive correlation	[36]
MCP-1	AH, Vitreous	Increased	[28,49-51]	Positive correlation	[28]
		—		No correlation	[56]
sICAM-1	AH	Increased	[28]	Positive correlation	[28]
	AH	—		No correlation	[56]
svCAM-1	AH	—		No correlation	[56]
IL-1β	AH	Increased	[37]	No correlation	[37,55]
IL-1ra	AH	Decreased	[51]	—	
IL-4	Vitreous humor	Increased	[49]	—	
IL-5	Vitreous humor	Increased	[49]	—	
IL-6	AH, Vitreous	Increased	[37,49,50,52,53]	Positive correlation	[37,52,53]
	AH	No Significant Change	[23,51]	No correlation	[55]

IL-8	AH	No Significant Change	[23,51]	No correlation	[55]
	AH, Vitreous	Increased	[50,52]	Positive correlation	[52]
IL-10	AH, Vitreous	Increased	[50]	No correlation	[55]
IL-12p	AH	—		No correlation	[55]
TNF- α	AH	No Significant Change	[37]	No correlation	[37,55]
IP-10	Vitreous	Increased	[49,50]	—	
IFN- γ	Vitreous	Increased	[49]	—	
Eotaxin	Vitreous	Increased	[49]	—	
MIP-1 α	Vitreous	Increased	[49]	—	
G-CSF	Vitreous	Increased	[49]	—	
VEGF	AH, Vitreous	Decreased	[23,36,52,53,62-64]	Negative correlation	[23,36,52,53,55,56,59,61-63]
	Vitreous	Increased	[49,50]	Positive correlation	[50]
PEDF	AH	No Significant Change	[23,62]	No correlation	[23,62]
	AH	Decreased	[67]	—	
VEGF/PEDF	AH	Decreased	[62]	—	
EGF	AH	Increased	[72]	Positive correlation	[72]
IGF-2 IGF-2R IGFBP-1 IGFBP-2 IGFBP-3 IGFBP-4 IGFBP-6	Vitreous	Increased	[74]	—	
GDF-15	AH	Increased	[60]	Positive correlation	[60]
HGF	AH	Increased	[36,60]	Positive correlation	[60]
PDGF-AA	AH	Increased	[60]	Positive correlation	[60]

Table 2. Transcriptomics and proteomics applied to the detection of myopia-related intraocular fluid molecules

Sample	Detection	Changes	Relevant mechanisms involved	Specific	Ref.
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	source	methods			miRNA or protein	
miRNAs	AH	Open Array	The amount of AH RNA was up 2.78-fold in the highly myopic group	—	Has-miR-582-3p, has-miR-17-5p, has-miR-885-3p, has-miR-19b-3p and has-miR-450b-5p	[80]
miRNAs	AH	NGS	249 miRNAs and 17 novel miRNAs differentially expressed in myopia	TNF, MAPK, PI3K-Akt and HIF-1 signaling pathways	Has-let-7i-5p, has-miR-127-3p and has-miR-98-5p	[81]
miRNA	AH	NGS	miR-142-3p was upregulated and positively correlated with ocular AL	Targeting TGF- β 1 to reduce collagen I in scleral fibroblasts	miR-142-3p	[82]
miRNAs	Vitreous	Low Density Array	Let-7c miRNA was upregulated and miR-200a was downregulated	Up-regulated: 23 pathways including MAPK pathways; down-regulated: 32 pathways including PI3K/AKT	Let-7c, miR-200a	[83]
Proteins	AH	iTRAQ	123 were up-regulated and 87 were down-regulated proteins	—	—	[84]
Proteins	AH	iTRAQ	97 were up-regulated and 49 were down-regulated	—	—	[85]
Proteins	AH	iTRAQ	32 up-regulated and 26 down-regulated proteins	PLG affects the composition of collagen fibers and ECM leading to scleral thinning	PLG	[86]
Proteins	AH	Conventional Method	Albumin; transthyretin, DBP were significantly elevated.	DBP may be involved in the APOA1 arrest pathway	Albumin, transthyretin, DBP	[89]
Proteins	AH	Label-free	63 up-regulated and 38 down-regulated	APOA1 as a stop signal for myopia progression may be a key protein and therapeutic target for PM	APOA1	[90]
Proteins	AH	Label-free	Glial fibrillary acidic proteins and complement-related proteins were elevated	JAK-STAT signaling pathway	Glial fibrillary acidic proteins and complement-related proteins	[91]

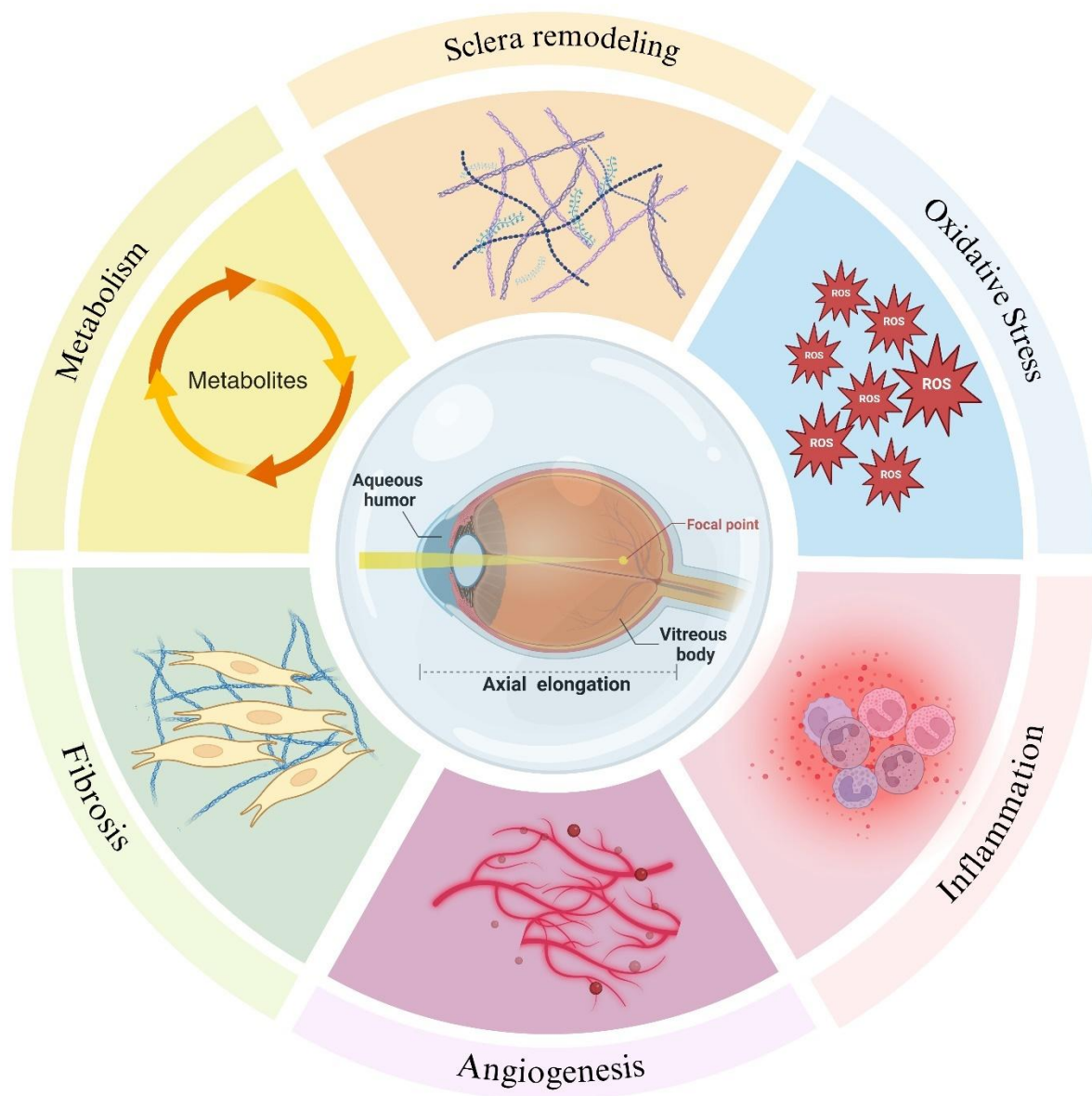
583 Table 3 Metabolomics of intraocular fluid components

Detection methods	Sample source	Altered biomarkers	Potential mechanisms	AL association	Ref.
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CE-MS and LC-MS	AH	Aminooctanoic acid, L-arginine, citrulline, aminoundecanoic acid, L-cysteinylglycine disulfide trihydroxyphenyl-gamma-valerolactone, dihydropteroic acid, dodecanedioic acid, aminocyclohexanecarboxylic acid, butyryl-L-carnitine, pantothenic acid, didehydroretinoic acid, sphinganine, histidinyl-phenylalanine, dimethylnonanoyl carnitine, PC(O-32:2)//PC(P-32:1), PC (42:6), C24 sulfatide, PC(P-42:2)//PC(O-42:3), laccerc(d40:0), trihexosylceramide (d36:2), neuacagalcer(d42:2)	Oxidative stress, NO synthesis and retinoic acid signaling	—	[92]
GC-TOF-MS	AH	N-alpha-acetyl-L-ornithine, nicotinoylglycine, oxalacetic acid, o-hydroxyhippuric acid, oxalic acid, ribose, cis-gondoic acid, linoleic acid methyl ester, thymidine, phosphate, indole-3-acetamide, 2-aminophenol, 2-ketoadipate, 3-phenyllactic acid, cis-phytol, conduritol b epoxide, salicin	Amino acid metabolic pathways, oxidative stress and calcium ion binding	Thymidine	[93]
UHPLC-MS/MS	AH	SQH, inosine, uridine, hypoxanthine, pseudouridine, 3-nitro-L-tyrosine, phosphopyruvic acid, L-threo-3-phenylserine, DL-0-tyrosine, APK	Bile secretion, insulin secretion, thyroid hormone synthesis and cGMP-PKG signaling pathway	Serine, methionine, proline, creatine, lysine, arginine, tyrosine, threonine, glutamine, asparagine	[74]
	Vitreous	D-(-)-glutamine, hypoxanthine, prostaglandin G2, decanoylcarnitine, D-glutamic acid, L-threonic acid, L-(+)-citrulline, citramalate, L-pyroglutamic acid, KPH	Bile secretion, thyroid hormone synthesis, insulin secretion and cGMP-PKG signaling pathway		
LC-MS	AH	(s)-actinidine, 1,2,3,4,6,7,8-heptachlorodibenzofuran, 2,2'-iminodipropan-1-ol, 2(n)-methyl-norsalsolinol, 3-methoxytyramine, 5-methoxytryptophol, acetaminophen, cerulenin, decanoylcarnitine, hippuric acid, indoleacetaldehyde, I-octanoylcarnitine, levofloxacin, imsp01080031, n-(2,4-dimethylphenyl)formamide, n-acetylarlyamine, n-acetylorithine, n,n-dimethyl-safingol, nadolol, netilmicin, orsellinic acid, oxybuprocaine, p-cresol sulfate, pantothenic acid, paracetamol sulfate, pyridoxal, pyrocatechol sulfate, sodium tetradecyl sulfate, spisulosine, theophylline, tryptophol	Taurine and hypotaurine metabolism, vitamin B6 metabolism, pantothenate, and coenzyme A biosynthesis	5-methoxytryptophol and cerulenin	[94]

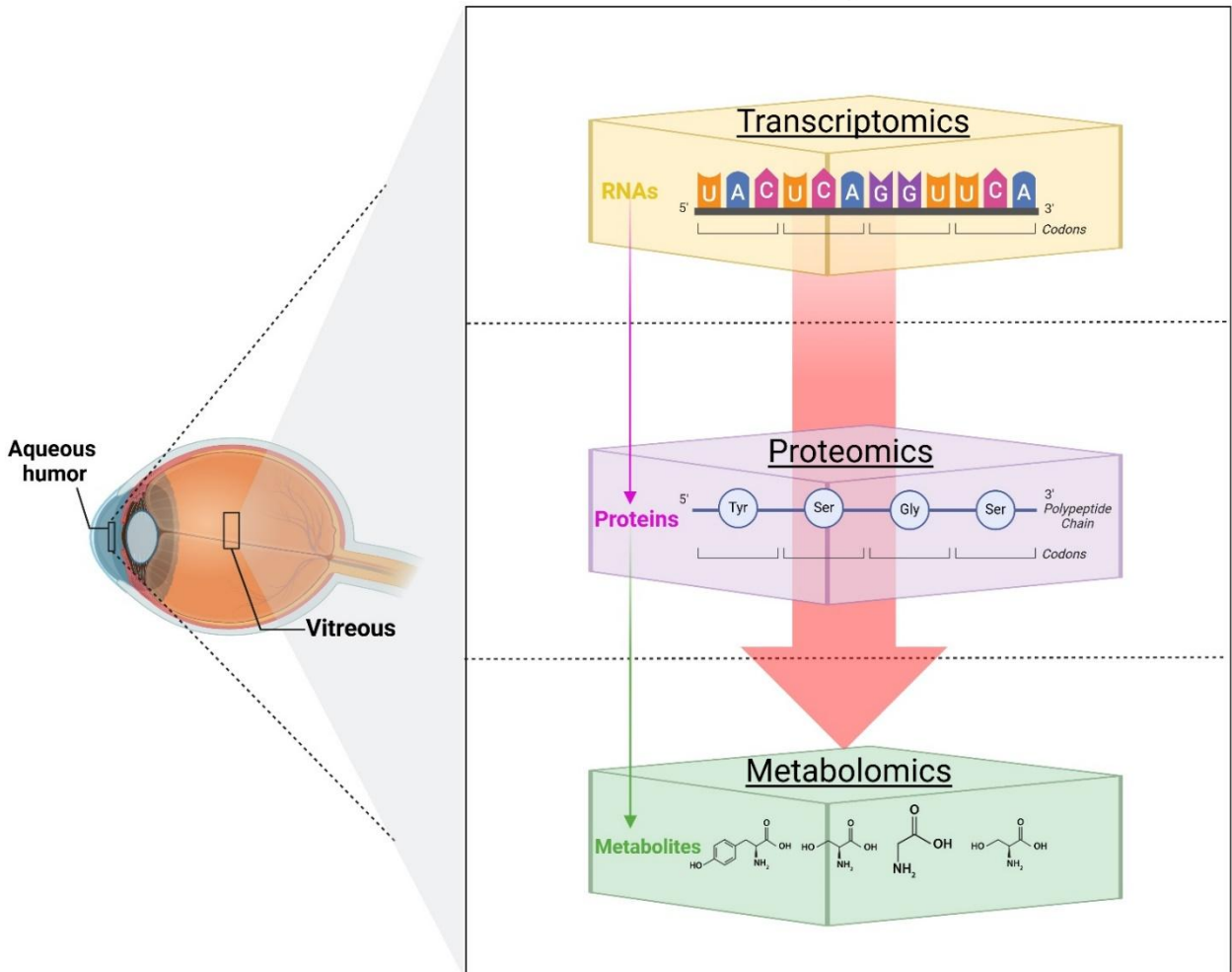
UHPLC-MS/MS	Vitreous	L-carnitine, propionylcarnitine, theophylline, glycerol tripropanoate, theobromine, gamma glutamyltyrosine, L-kynurenine, pivaloylcarnitine, 1-(beta-D-R-ribofuranosyl)-1,4-dihydrionicotinamide, isobutyryl-L-carnitine, N-acetylmethionine, L-2,3-dihydrodipicolinate, N-acetylhistidine, monoethylglycineethylidide, 5-hydroxyindoleacetic acid, aspartame, L-acetylcarnitine, dehydroneotene, 4-guanidinobutanoic acid, uric acid, 16-methylheptadecanoic acid, indoxyl sulfate, estradiol	Tryptophan metabolic pathway, uric acid metabolic pathway, oxidative stress and inflammation	[95]
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584 **Figure Legends**



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586 Figure 1. Molecular changes in intraocular fluid during the development of myopia reflect scleral remodeling, oxidative stress,
587 inflammation, pathological states of angiogenesis and fibrosis and metabolism. Created with Biorender.com

Three Omics in Myopia Intraocular Fluid



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Figure 2. Overview of transcriptomics, proteomics, and metabolomics in analyzing molecular changes in intraocular fluid related to myopia. Created with Biorender.com