Metabolomics and biomarkers in ocular matrix: beyond ocular diseases

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Abstract

- According to the recent report, there are 870 million people suffer from ocular diseases worldwide. The present approaches for diagnosis are morphological examination, imaging examination and immunological examination, regrettably, they lack of sensitivity and difficult to make a definite diagnosis in the early stage. Systemic biology as an effective method has been used in clinical diagnosis and treatment for diseases, especially metabolomics which is more attractive with high sensitivity and accuracy. Although previous researches had been confirmed that endogenous metabolites in the ocular matrix play a crucial role in the progress of diseases related diseases, the standard protocols and systematic summary about the biomarker researches based on ocular matrix has not been established. This review article highlights the pretreatment for ocular matrix and the new biomarkers expressed by the eye diseases, expected to promote the application of biomarkers in the diagnosis and treatment of eye diseases.

- KEYWORDS: metabolomics; biomarkers; ocular matrix; ocular diseases; diagnosis

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INTRODUCTION

Eye is a complex sensory organ that can be divided into anterior part composed of cornea, conjunctiva, aqueous humor, iris, ciliary body and lens, the posterior part composed of sclera, choroid, retina and vitreous humor (VH). It is able to receive light and convert it into electrical impulses which are transmitted to the brain through the optic nerve for visual perception⁹. Various injuries in the above ocular matrices will result in different ocular diseases companied with impaired vision function, moreover, systemic diseases such as diabetes⁷, Alzheimer’s disease⁸ and inflammatory bowel disease⁹ may also cause damages in ocular matrix. Morphological, imaging, and immunological examination are the major diagnosis approaches for ocular diseases. However, when there is a significant change in the structure and function of the eye, the diseases have progressed to an irreversible stage. What’s more, affected individuals experience different clinical appearance and progression of the diseases⁵. Given the difficulty in diagnosis and treatment for ocular diseases, there is an urgent need to develop an effective tool.

With the rapid development of analytical technology and bioinformatics and the concept of precision medicine rooted deeply in the people’s mind, systems biology which consist of genomics, proteomics, transcriptomics and metabolomics offers a powerful tool to simulate metabolic reactions in the biological system. Metabolomics is a rapidly evolving field of biochemical research following genomics, transcriptomics and metabolomics was defined as global analysis of the small-molecule metabolites present within the internal environment in an identified and quantified manner⁶. Endogenous metabolites as products or substrates in the process of in vivo metabolism, are jointly influenced by gene, environment and daily diet habits and involved in the organism homeostasis that can act as biomarkers to indicate the normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention⁶. In other words, biomarker is conducive to more deeply understand the in vivo abnormal mechanism and can be used to evaluate the course of disease⁶-¹⁰. At present, biomarker has been applied in prevention, diagnosis and treatment of cancer and diabetes¹¹-¹². Over the last years, increasing number of metabolomics
Metabolomics biomarkers in ocular diseases

analysis in ocular matrix have performed, and gained a pivotal role in comprehending and explaining ocular diseases and systemic diseases. Nevertheless, it still remains problems in research procedure standardization and the clinical conversion of the achievement. Herein, this article will provide an overview of the pretreatment of ocular matrix and the biomarkers related to ocular diseases and systemic diseases, with a view to further study and application of ocular matrix.

**Sample Collection and Pretreatment** A workflow of metabolomics analysis in ocular matrix is shown in Figure 1. Sample pretreatment is a chief step of any bioanalytical workflow and a good sample preparation is considered as a starting point of successful metabolome analysis[13]. Sample preparation includes all procedures and operations applied to the sample prior to analysis. The existing pretreatment methods for ocular matrix fit to different analysis techniques are summarized in Table 1.

Sampling involved collected equipment and materials, which depends on samples status. Ocular matrix can be divided into two status, which includes solid and liquid. Collection of solid samples is usually done by operation while collection of liquid samples should be selected based on their location. Schirmer band and capillary are the more procedures for collection of tears, and anterior chamber puncture is utilized to collect aqueous humor (AH), while VH can be taken with 27G needle and surgery.

Owing to the presence of enzymes, metabolites have rapid turnover, and storage environment influence the composition of origin biological samples hugely. For ocular matrix, several studies had proved that cryo-preservation as the most frequently used method can stabilize metabolite composition well. Back in 1998, Sitaramma et al[14] reported that the collected tears stored at -80℃ for 1mo with the smallest change in metabolites. In 2013, Kryczka et al[15] also stated that after storing the obtained cornea at -80℃ for 8d, metabolites without significantly change.

An ideal sample pretreatment method should be as far as possible to keep the original metabolite composition and determined by the character of the target metabolite and the platform selected. Separating the analytes from protein is the vital procedure for sample pretreatment, generally methanol, ethanol, chloroform, water and their mixtures with different proportion were used in ocular matrix, take retina for example[16-17]. Subsequently, liquid-liquid extraction (LLE) and solid phase extraction (SPE)[18] can be employed to extract the targeted metabolites. Up to now, SPE has not been reported in the studies of endogenous metabolites from ocular matrix but applied in exogenous metabolites[16,20].

In detection platform, nuclear magnetic resonance (NMR), liquid chromatograph-mass spectrometer (LC-MS) and gas chromatograph-mass spectrometer (GC-MS) are the prevalent techniques. In the early stage, NMR is the major technology for the researches of ocular matrix. There are some key advantages of NMR such as relative ease sample preparation, high reproducibility and inherently nondestructive. Furthermore, NMR is particularly suited to characterize highly polar compounds such as sugars, organic acids, alcohols, polyols and unique classes of metabolites such as protein-bound metabolites and ions[21]. For ocular matrix, before analysis, the free-protein supernatant was often lyophilized and then reconstituted with D$_2$O containing TSP[22] or DSS[23]. In recent years, a number of emerging NMR technologies are being used to strengthen its utility in metabolomic applications such as solid-state NMR (ssNMR) and magic-angle sample spinning (MAS-NMR) which can offer broader possibilities for detecting intact tissues, organs, and other solid or semisolid samples[21].

Since the robust, reproducible, selectivity and recently increasing number of well-established metabolite libraries as well as new types of GC column, GC-MS is an efficient and well used analytical platform suited for metabolomics. Non-volatile metabolites containing carboxylic acids (-COOH), alcohols (-OH), amines (-NH$_2$), and thiols (-SH) that can be derivatized, low molecular weight compounds (ca. 50-600 Da), and volatile metabolites are amenable to separating and identifying with GC-MS[24]. Yet present metabolomics studies in ocular matrix, GC-MS just applied in AH and retina.

Before injecting to detect, endogenous metabolites often should be derivatized to render them volatile. Derivatized will be processed followed with lyophilizing the free-protein supernatant, and the mainstay method is methy-lsilylation used MSTFA and BSA[25-27]. Not only LC-MS as the complementary to NMR and GC-MS, but also has particular features to allow it become a powerful metabolomic tool that could be adapted to nearly all kind of compounds and provide rich structure information. Ultra-high performance liquid chromatography (UHPLC) can annotation metabolites within the short spans and possible to analyze a completely different set of metabolites by simply changing the chromatographic column and mobile phase[28]. For LC-MS, following with protein precipitation, either direct injection analysis using protein-removed supernatant, or resolve after lyophilizing in the mobile phase or water[29].

**Biomarkers in Ocular Diseases** Current metabolomics studies involved almost all ocular matrices such as tear, AH, VH, cornea, lens and retina. Biomarkers of diseases found in metabolomics based on ocular matrix are listed in Table 2.

**Tear** Tear is extracellular biofluid covers the anterior surface of the eyeball, which provide lubrication, protection and
Figure 1 The workflow in metabolomics analysis based on ocular matrix.
## Table 1 Pretreatment for ocular matrix

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Pretreatment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tear</td>
<td>LC-MS</td>
<td>Vortex mixing Schirmer’s strips in 9:1 MeOH/H₂O; centrifuge and collect supernatant; dry; reconstitute</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tear sample were centrifuged (14000 g, 10min, 4°C) in ice-cold 80% MeOH; supernatants were incubated on dry ice; evaporated; reconstitute</td>
<td>[38]</td>
</tr>
<tr>
<td>AH</td>
<td>LC-MS</td>
<td>Vortex-mixing for 1min equal volumes on the AH sample and freeze cold (-20°C) methanol/ethanol (1:1) mixture; stored on ice for 10min; centrifuge; collect supernatant and filter for analysis</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One volume AH mix with four volumes 100% ethanol; centrifuge; collect supernatant; lyophilization; reconstitute with aqueous solution</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One volume AH mix with five volumes ultrapure water; centrifuge; collect supernatant; analysis</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>GC-MS</td>
<td>One volume AH mix with seven volumes 75% MeOH; vortex; centrifuge; collect supernatant and derivatize for analysis</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td>One volume AH mix with four volumes 100% ethanol; centrifuge; collect supernatant; lyophilization; reconstitute with DSS.</td>
<td>[23]</td>
</tr>
<tr>
<td>VH</td>
<td>LC-MS</td>
<td>One volume VH mix with four volumes acetone; store at -20°C overnight; centrifuge; collect supernatant; precipitation extract used 80% methanol and merge supernatant; freeze-drying; reconstitute with acetonitrile:methanol:isopropanol (4:4:1); sonic; centrifuge; collect supernatant and analysis</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One volume VH mix with four volumes 100% ethanol; centrifuge; collect supernatant; add 1/2 chloroform and same volume water; centrifuge; lyophilization; reconstitute with water</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td>One volume VH mix with four volumes 100% ethanol; centrifuge; collect supernatant; add 1/2 chloroform and same volume water; centrifuge; lyophilization; reconstitute with D₂O containing DSS and phosphate buffer</td>
<td>[23]</td>
</tr>
<tr>
<td>Cornea</td>
<td>LC-MS</td>
<td>Samples were rinsed in 1×PBS and lysed with ice-cold 80% methanol; incubated on dry ice for 15min and homogenized; centrifuged; collect supernatant; analysis</td>
<td>[80]</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td>The proteins in cornea samples were precipitated by EtOH; the lipids were removed from the protein-free cornea extracts using the chloroform/EtOH/water mixture; centrifuged; lyophilization; reconstitute with D₂O containing DSS and phosphate buffer</td>
<td>[67]</td>
</tr>
<tr>
<td>Lens</td>
<td>LC-MS</td>
<td>Lens homogenate with pre-cooled EtOH; centrifuged; collect supernatant; pellet extracted again; merge supernatant; dry; reconstitute with water</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lens homogenate with pre-cooled EtOH; centrifuged; collect supernatant; pellet extracted again; merge supernatant; to remove lipids from the extract, H₂O and CHCl₃ was added to the combined supernatant, shaken, then H₂O was added; centrifuged; collect supernatant; lyophilized; re-dissolved in aqueous solution</td>
<td>[71-72]</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td>Lens homogenate with pre-cooled EtOH; centrifuged; collect supernatant; pellet extracted again; merge supernatant; dry; reconstitute</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lens homogenate with pre-cooled EtOH; centrifuged; collect supernatant; pellet extracted again; merge supernatant; to remove lipids from the extract, H₂O and CHCl₃ was added to the combined supernatant, shaken, then H₂O was added; centrifuged; collect supernatant; lyophilized; re-dissolved in D₂O containing DSS and phosphate buffer</td>
<td>[71-72]</td>
</tr>
<tr>
<td>Retina</td>
<td>LC-MS</td>
<td>Retina homogenate with 80% MeOH; incubate on ice; centrifuge; collect supernatant; lyophilized; reconstitute with mobile phase (A:B=4:6)</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add 800 µL of chloroform:methanol (50:50, pre-cooled to -20°C) to retina samples; homogenate; 400 µL of water was added to the mixture; centrifuge; collect bottom lipophilic layer; lyophilized; reconstituted in 200 µL 50:50</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retina homogenization with 40 µL water; centrifuged; 5 µL of the supernatant were transferred for protein quantitation and 140 µL of methanol were added; homogenize; centrifuge; collect supernatant and spin-dried for 24h; reconstitute with water</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add 140 µL extraction buffer [methanol:chloroform:H₂O (700:200:50)] to the retina sample; homogenize; centrifuge; collect supernatant; spin-dry; suspended in 100 µL of mobile phase (40% of A and 60% of B) with vortex for 10s</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>GC-MS</td>
<td>Retina homogenate with 80% MeOH; incubate on ice; centrifuge; collect supernatant; lyophilized; derivatize</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add 800 µL of chloroform:methanol (50:50, pre-cooled to -20°C) to retina samples; homogenate; 400 µL of water was added to the mixture; centrifuge; collect top hydrophilic layer; lyophilized; derivatize</td>
<td>[27]</td>
</tr>
</tbody>
</table>

LC-MS: Liquid chromatograph-mass spectrometer; GC-MS: Gas chromatograph-mass spectrometer; NMR: Nuclear magnetic resonance; AH: Aqueous humor; VH: Vitreous humor.
nutrition for the eye and is also the carrier to remove local waste, metabolic drugs and inflammatory mediators generated in eye diseases. It has been proven that tear contains thousands of molecules, including amino acids, amino ketones, amino alcohols, aromatic acids, carbohydrates, acylcarnitine, nucleotides[30].

Dry eye disease (DED)[31] is a multifactorial disorder of the ocular surface with the main feature of imbalance of tear film homeostasis and the instability of extra lacrimal lipids. A research applied HPLC-MS to correlate DED to tear steroid levels[32], the results showed that the content of cortisol (CORT), 4-Androstene-3,17-dione, 17-α-hydroxyprogesterone is decreased. Ocular inflammation is the factor responsible for DED. Cell-based studies have found that cortisol can exert its biological role through several different molecular mechanisms, thereby reducing the production of eicosanoid-like substances and inhibiting various white blood cell-related inflammations[33-34]. In addition, due to cortisol (CORT) is one of the products derived from 17-α-hydroxyprogesterone (17-OHP), the decrease in 17-OHP levels and the CORT levels is interconnected. ADIONE is a precursor of synthetic androgen, and previous literatures have reported that reduced androgen levels may cause structural dysfunction of the glands and meibomian glands[35-37]. The research made it possible to study steroid profiling directly in tear for diagnose of DED.

Keratoconus (KC) is a non-inflammatory disease companied with progressive, asymmetric corneal ectasia. The mechanism of KC is complicated and still remain mystery so that the discovery of the differences in the metabolic composition of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diseases</th>
<th>Up</th>
<th>Down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tear</td>
<td>Dry eye disease</td>
<td>Isocitrate,aconitate, malate,acetyl-phosphate, ornithine, aspartate, lactate</td>
<td>Cortisol, 4-Androstene-3,17-dione, 17-α-hydroxyprogesterone</td>
</tr>
<tr>
<td></td>
<td>KC</td>
<td>Glutamic,glutamine, H-L-proline,lysine, valine,very-low-density lipoproteins</td>
<td>GSH</td>
</tr>
<tr>
<td></td>
<td>AH</td>
<td>Alanine,arginine,citrulline,sphingosine, amino-decanoic,cis-phylth,thyme,oxalic acid, glutamine</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Myopia</td>
<td>-</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td>Cataract</td>
<td>-</td>
<td>Methyl-tetrahydrofolate,taurine,nicotinamide,xanthine,uric acid</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>-</td>
<td>Uric acid</td>
<td>-</td>
</tr>
<tr>
<td>DR</td>
<td>Arginine, ornithine, proline, citrulline</td>
<td>Lactic acid, succinic acid, 2-hydroxybutyric acid, ascorbic acid, formic acid</td>
<td></td>
</tr>
<tr>
<td>VH</td>
<td>DR</td>
<td>Tyrosine, ascorbic acid</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RRDCD</td>
<td>Lactate, glucose</td>
<td>Galactitol, ascorbic acid</td>
</tr>
<tr>
<td></td>
<td>RRDCD</td>
<td>Succinic acid, lactic acid,phenylpyruvate,L-carnitine</td>
<td>Sphingosine, sphingosine, dihydro-sphingosine, arachidonic acid</td>
</tr>
<tr>
<td>Cornea</td>
<td>DM</td>
<td>Glucosamine, piperonic acid, spermidine, betaine, sphingosine, 2-hydroxyphosphine, Indole-3-carboxylic acid, aminoacidic acid</td>
<td>Pyruvate, glyceraldehyde-3-phosphate</td>
</tr>
<tr>
<td></td>
<td>KC</td>
<td>Acetate, citrate</td>
<td>GSH</td>
</tr>
<tr>
<td>Lens</td>
<td>Age-related-cataract</td>
<td>Asparagine, histidine,glutamine,threonine, dimethylamine, isoleucine</td>
<td>-</td>
</tr>
<tr>
<td>Retina</td>
<td>Myopia</td>
<td>Mannose, glucose</td>
<td>Tyrosine, threonine, valine, isoleucine, aminobutyric acid</td>
</tr>
<tr>
<td></td>
<td>Glaucoma</td>
<td>Hypo-taurine, urea, choline phosphate, sorbitol, fructose, N-acylethanolamines</td>
<td>-</td>
</tr>
<tr>
<td>Hypoxic ischemic encephalopathy</td>
<td>-</td>
<td>CDP-choline</td>
<td>-</td>
</tr>
</tbody>
</table>

KC: Keratoconus; DR: Diabetic retinopathy; RRD: Rhegmatogenous retinal detachment; RRDCD: Choroidal detached rhegmatogenous retinal detachment; DM: Diabetes mellitus; PDR: Proliferative diabetic retinopathy; GSH: Reduces glutathione; GSSG: Oxidative glutathione; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate. -: Not be detected.
Metabolomics biomarkers in ocular diseases

KC is of great significance. Karamichos et al. used LC-MS to identify biomarkers between KC patients and normal people, as a result, a total of 296 endogenous polar metabolites were detected of which more than 40 were significantly changes in KC patients. The metabolites involved in glycolysis and gluconeogenesis such as 1,3-glycerophosphate and 3-phosphoglycerate increased significantly in the tear of KC and induced the up-regulated in isocitrate, aconitate, malate, and acetyl-phosphate involved in citric acid cycle (TCA). What's more, ornithine and aspartate were accumulated which indicated that urea cycle was affected. In terms of oxidative stress, the ratio of reduces glutathione (GSH) to oxidative glutathione (GSSG) was decreased while the ratio of lactate to pyruvate was increased. Previous researches reported that the ratio of lactate to pyruvate is positively correlated with oxidative stress while the ratio of reduces GSH to GSSG is adverse. Notably, the metabolites that associated with inflammation was not found, which is in line with the nature of the disease. Overall, the results suggested that KC may alter the metabolites related to urea cycle, TCA cycle and oxidative stress.

**Aqueous humor** AH is a transparent liquid with a complex mixture of electrolytes, organics, growth factors, cytokines and proteins. The circulating AH nourishes the cornea and lens and removes the metabolic waste from the avascular tissues. The major types of metabolite contains lipids, amino acids, carnitines, alkaloids, nucleotides, carbohydrates, involved in variety of metabolic pathways and related to some kinds of ophthalmopathy.

Glaucoma is a chronic irreversible disease and the leading cause of blindness in human characterized by increased intraocular pressure (IOP), degeneration of retinal ganglion cells (RGC) and optic nerve fibers (ONF). It is manifested by progressive changes in retinal sensitivity and visual field performance. Mayordomo-Febrer et al. analyzed the rat AH in glaucoma model established by injection of sodium hyaluronate solution used NMR spectra. The results indicated the accumulation of alanine, glutamic, glutamine, H-L-proline, lysine, valine and very-low-density lipoproteins (VLDLs) while a significant decreased in glucose on the side of sodium hyaluronate injection. Degeneration of RGC is one of the major mechanisms for the progression of glaucoma. Excessively accumulation of glutamic will lead to the over-expression of N-methyl-D-aspartate (NMDA) receptor and consequently decrease the expression of retinal anti-apoptotic factor Bel-2 which will reduce pro-apoptotic factor Bax and enzyme caspase-3 related to RGC apoptosis. Abnormal IOP represents the primary risk factor for developing glaucoma and ATP is involved in regulating IOP. The reduction of glucose may elevate IOP by decreasing the generation of ATP and in turn the elevated IOP will lead to less ATP into the capillaries. The changes in VLDLs is also linked with the pathogenesis of glaucoma. According to the previous researches, VLDLs can prompt the expression of fibronectin, laminin and collagen type IV which contributes to reduce cell adhesion to the basement membrane of the trabecular meshwork. The identified metabolites in this study could enhance our knowledge of glaucoma biomarkers and new biotherapy.

**Myopia** is a public health problem, moreover the severe myopia (high degree myopia) has more likely to develop into eye disorders overtime. Barbas-Bernardos et al. had investigated on the AH in myopia by LC-MS suggesting that higher abundant metabolites, which include amino-caprylic, arginine, citrulline and sphenamine, occurred in highly myopic person. Arginine and citrulline are contacted by the citrulline cycle that jointly regulated by the concentrations of arginine and citrulline. The high concentration of these amino acid will compete for the enzyme center to inhibit NO production and impaired blood flow, ultimately chronic ischemic injury of optic nerve. Surprisingly, the study found that amino-decanoic in AH showed a significant difference between high myopia and low myopia, but did not appear in the metabolic profile of normal human aqueous humor. It suggested that amino-decanoic acid plays a vital role in the progression of myopia. Another study in AH based on GC-MS in 2017 identified four metabolites discriminated normal and myopia groups, cis-phtyl, thymine, oxalic acid and glutamine respectively. As reported, thymidine is related to the phenotype of the eye, and oxalic acid concentration is associated with the content of calcium ion. Additionally, altered in glutamine indicated that high myopia may cause changes in active oxygen concentration. These works provided potential biomarkers for the diagnosis of myopia and a new insight into the underlying mechanisms of the high myopia formation.

Cataract mainly caused by crosslinking, aggregation and deposition of proteins in crystalline bodies, is a leading cause to blindness. Epidemiological studies revealed that people with diabetes have five times the risk of cataracts than normal and the incidence increase with age. A metabolomics study of AH in diabetic and non-diabetic cataract patients based on LC-MS was conducted and several antioxidants (methyl-tetrahydrofolic acid, taurine, niacinamide, xanthine, and uric acid) were found decreased in AH of diabetics. It has been found in animal models that taurine can prevent diabetic cataract caused by tetraoxopyrimidine. Nicotinamide has been reported as an effective inhibitor of protein glycosylation and subsequent advanced glycation end products. Xanthine and uric acid are regulated by xanthine oxidoreductase, catalyzing the oxidation of hypoxanthine to...
xanthine, xanthine to uric acid, and the reduction of NAD\(^+\) or molecular oxygen. The differences in antioxidants observed indicated that increased oxidative stress may be contributed to earlier cataract onset in diabetic patients.

Retinoblastoma (Rb) is a primary intraocular cancer in children with high rate of recurrence, tissue metastasis and fatality rate, which is difficult to diagnose\(^{[60]}\). As early as 1998\(^{[61]}\), it was reported that the uric acid in the aqueous humor of patients with retinoblastoma was increased. During cell replacement, nucleic acids and nucleotides are degraded to form xanthine and uric acid. Increased uric acid levels in body fluids are associated with many malignancies and also with the rapid destruction of malignant tissues after chemotherapy or radiotherapy. Uric acid in AH has a potential to be a biomarker for retinoblastoma.

\**Vitreous humor**\n
VH is a transparent liquid located at the back of the eyeball which the major composition of metabolites are amino acids, sugars, alkaloids, sphingosine and others, able to separates the lens from the retina\(^{[65]}\). Rhegmatogenous retinal detachment (RRD)\(^{[63]}\) is a serious eye disease. It had been reported the occurrence of the up-regulated of tyrosine, urea and ascorbic acid in the VH of people with RRD. With development of disease, RRD can complicated with choroidal detachment which named choroidal detached rhegmatogenous retinal detachment (RRDCD)\(^{[64]}\). LC-Q-TOF/MS technology was used in research by Wu\(^{[65]}\) on VH. After multiple data analysis, 24 differential metabolites were identified. According to the comparison, in RRDCD patients, the expression levels of succinic acid, lactic acid, and phenylpyruvate that are directly involved in energy metabolism were significantly increased, which indicated that the progression of RRDCD has a greater demand for energy than RRD. In contrast, the concentration of sphingosine, sphingosine, and dihydro-sphingosine were reduced. The most common product in sphingolipid metabolism is ceramide, which play an important role in cell apoptosis and proliferation. Decreasing the concentration of these three compounds may lead to cell proliferation. What’s more, aromatic acid was decreased while L-carnitine was increased in the RRDCD group suggesting a severe inflammatory response in RRDCD patients.

\**Cornea**\n
Cornea is the outermost structure of the eye without vascular tissue so that the main nutrients are supplied by the aqueous humor. Lipids, amino acids, fatty acids, purines are the major metabolites of cornea\(^{[25]}\).

KC is a non-inflammatory disease and had been confirmed that the occurrence of KC is closely related to the process of oxidative stress\(^{[46]}\). Snytnikova et al\(^{[67]}\) applied 1H-NMR and LC-MS to perform a quantitative study which aim at comparing the metabolomic compositions of cornea taken from KC patients and normal. The results showed that the metabolomics of the cornea in KC patients was characterized by an increase in acetate and citrate concentrations and a decrease in the ratio of GSH to GSSG which are indicated the enhanced oxidative stress in KC and it is an important angle for the intervention of KC.

\**Lens**\n
Lens is a transparent tissue that can transmit and focus incident light onto the retina to provide clear vision\(^{[68]}\). There is no vascular system to scatter light and with a lack of nucleus and organelles in the fibrous cells. Based on the special structure, the main energy and nutrition of the lens comes from the VH. Lens plays an important role in maintaining the balance of the intraocular environment\(^{[60]}\). The metabolites in lens mainly include amino acids, nucleotides and sphingolipids\(^{[70]}\).

The main lesion of lens is cataract, which is divided into age-related cataract and diabetic cataract. Increasing number of studies showed that the oxidative stress caused by hyperglycemia in diabetic will increase the risk of cataract\(^{[71]}\). Yanshole et al\(^{[72]}\) performed a series of metabolomic studies on the lens. In 2014, they performed quantitative metabolomics study on rat lens for the first time that combined LC-MS and NMR. More than 40 low molecular weight compounds were found and quantified in the lens. Among them, the most abundant metabolites in the three-month-old of rat lens are correlated with oxidative stress include taurine, hypotaurine, lactic acid, choline phosphate, and GSH. The study also performed that with age, alanine and arginine decreased by 300% while asparagine, glutamic acid, isoleucine, proline, threonine, glycine, carnitine, and glycerol phosphate decreased by 100%. What’s more, the experiment reported that the statistically significant difference in the OXYS lens is higher concentration of tryptophan, tyrosine, carnitine, glycerocephosphat, GSH and GSSG and lower concentration of choline, point out the imbalance of the kynurenine pathway and the compensatory response of the OXYS rat lens to oxidative stress. Afterwards, two other researches seem to be more helpful in understanding the effect of age on the cataracts. The results displayed that the most pronounced difference is observed for compounds playing a key role in the lens cell protection and metabolic activity such as AMP, ADP, inositol, creatine, carnitine and UV filters\(^{[73-74]}\). Results from above studies consistently elucidated the influence of age on the metabolic in lens and provided new intervention idea for age-related cataract.

\**Retina**\n
Retina as a tissue that can receive light stimulation, transform light into pulses of neurons and reach the brain through visual pathways which plays a vital role in the process of forming vision. Metabolites in the retina mainly include amino acids, glucose, purine peptides and lipids, which were involved in multiple metabolic pathways\(^{[75]}\).
Yang *et al*[^76] applied the form deprivation myopia animal model and GC-TOF-MS platform to observe how retinal metabolomic changes during the myopia development. The results showed that mannose and glucose levels in the retina are elevated, which suggested that aerobic glycolysis is reduced during the development of myopia. Additionally, seven intermediates that involved in lipid metabolism were decreased indicated fatty acid biosynthesis inhibition. The study also found in amino acid pathway, tyrosine, threonine, valine, isoleucine and aminobutyric acid showed a decrease trend during the progression of myopia. Such changes provided new idea to identify possible drug targets to suppress myopia development.

Optic nerve cell damage and axon degenerative changes leading to vision degradation are the main mechanisms of glaucoma[^77^-^78]. A study[^79] analyzed the changes of metabolites in retina after optic nerve injury 24h and 14d, which represents two stages after injury and finally identified 9, 19, and 32 regulated metabolites respectively when comparison of 24h versus control, 14d versus control samples, and 24h versus 14d. The metabolites which change significantly in 24h are involved in (L)-proline metabolism and phosphatidylcholine pathway. After 14d, it found that the content of hypo-taurine, urea, choline-phosphate, sorbitol and fructose were significant increased. In addition, at 24h and 14d, an inverse regulation of N-acylethanolamines (NAEs) was observed. NAEs are endogenous lipids that are synthesized and accumulated in response to tissue injury and considered as neuroprotectants[^80]. These metabolites showed a clear difference between the early and late stages of degeneration and may have potential to act as prognostic factors or therapeutic target molecules during retinal or neuronal degeneration.

**Ocular Biomarkers Indicating Systemic Diseases** Systemic diseases such as diabetes mellitus (DM), multiple sclerosis (MuS), hypoxic brain damage and Alzheimer’s disease have been proven that will induce ocular manifestations. Therefore, exploring biomarkers based on the ocular matrix may also be important for understanding systemic diseases.

**Diabetes mellitus** Sustained hyperglycemia in DM will lead to microvascular complications and eye is the major organ affected by DM. There are many reports on metabolic behaviors of DM complication.

In diabetic patients, hyperglycemia may change the corneal epithelium basement membrane before more serious lesions[^81]. Glucose metabolism disorder in diabetic patients is observed in corneal metabolites. In the glucose metabolism of diabetic patients, the highest concentrations of metabolites were glucosamine, piperonic acid, spermidine and betaine, but the content of glycolysis-related metabolites such as pyruvate and glyceraldehyde-3-phosphate were reduced. Lipidomics found that the levels of sphingosine and 2-hydroxy sphingosine were significantly increased, which is consistent with the increase in ceramide and sphingomyelin reported in previous research[^82]. Additionally, Indole-3-carboxylic acid as a derivative of tryptophan increased significantly in the cornea of patients with type 2 diabetes. Reporter speculated the phenomenon is linked with oxidative stress induced by high glucose status. Due to tryptophan is a vital metabolite that is regulated by the kynurenic pathway and is used to produce NAD, which is the basic substrate for NADH in the glycolysis and citric acid cycle. Another metabolite with significant change is aminoadipic acid, which has been detected in the plasma and skin of diabetic patients[^83]. Since aminoadipic acid is derived from lysine metabolism that unlike the easily metabolized glucose derivative, it is considered as a biomarker in cornea.

Diabetic retinopathy (DR) is the most common microvascular complication of DM. This metabolic disorder is a chronic inflammatory state that damages both the photoreceptors and the blood vessels of the retina[^84]. One study conducted with $^1$H-NMR compared the AH between people with DR, DM and age-related cataract[^85]. The results illustrated the lower level of lactic acid, succinic acid, and 2-hydroxybutyric acid in patients with DR than those in patients with DM alone, while higher level of asparagine, histidine, glutamine, threonine and dimethylamine. Compared with age-related cataract patients, it showed that lactic acid, succinic acid, ascorbic acid, and formic acid were reduced in DR, and asparagine and isoleucine were increased. Lactic acid and succinic acid are the intermediate products of the tricarboxylic acid cycle[^86]. Hyperglycemia induces mitochondrial dysfunction will cause cell division and decreased cellular which explains the decrease in lactic acid and succinic acid content in DR patients. Additionally, hyperglycemia also induces oxidative stress pathways and promotes the consumption of NADPH that contribute to the pathogenesis of DR. This process increases the levels of NADH and NADH/NAD, reduces the TCA cycle of patients with DR, thereby reducing catabolism and increasing asparagine, glutamine, histidine and threonine[^87^-^88].

In this study, ascorbic acid was reported significantly different between DR and senile cataract patients, while DR and DM with no differentiate. Another study used VH demonstrated that the main metabolic fingerprints of vitreous fluid are the higher abundance of lactate and glucose and the significant deficiency of galactitol and ascorbic acid[^89]. To our knowledge, ascorbic acid can inhibit angiogenesis[^90], the modulation of ascorbic acid may be considered as a therapeutic option in DR.

During the process of DR, proliferative retinopathy is severe blinding stages. It has been proven that arginine metabolism shows severe disturbances in the VH of both human and mice during the early DR phase[^91^-^92]. Based on the LC-MS
Ocular diseases may be caused by multi-factor and still remain huge problems for diagnose and treatment worldwide. With the continuous development of precision medicine and technology, metabolomics has been widely used in the study of ocular matrix. With literature reviewed, we discovered that metabolites associated with oxidative stress, energy and inflammation may represent a new hope for diagnosis in ocular diseases and systemic diseases. However, there are some challenges remain to be addressed in such studies. Firstly, compared with the common samples such as plasm, urea, feces and other tissues, trace metabolites in ocular matrix is the inherent limitation for pretreatment, detection and analysis. Therefore, continuous researches should be done to develop a standard research protocol. Secondly, in terms of metabolites level, the association and mutual metastasis of ocular diseases and other systemic diseases will be an arduous but deserved challenge. Finally, the majority of current data comes from animal experiment and the cohort of individuals, in order to transform the biomarkers from laboratory to clinic, it is necessary to plan future studies in the condition of large sample, multicenter, blindness and randomization.

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