ACS MEASUREMENT SCIENCE Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

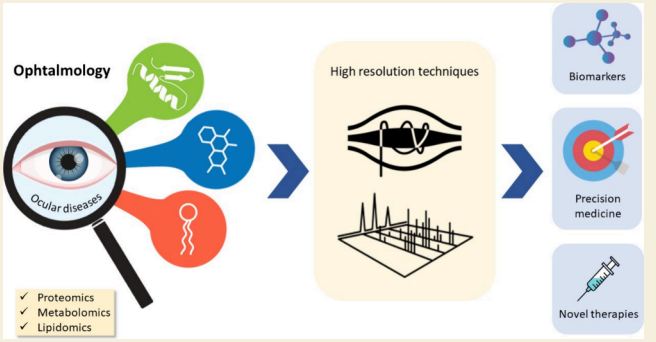
pubs.acs.org/measureau
Review

A Look into Ocular Diseases: The Pivotal Role of Omics Sciences in Ophthalmology Research

Maurine Fucito, Matteo Spedicato, Simona Felletti, Angeli Christy Yu, Massimo Busin, Luisa Pasti, Flavio A. Franchina, Alberto Cavazzini, Chiara De Luca,* and Martina Catani*

Cite This: ACS Meas. Sci. Au 2024, 4, 247−259
Read Online
ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Precision medicine is a new medical approach which considers both population characteristics and individual variability to provide customized healthcare. The transition from traditional reactive medicine to personalized medicine is based on a biomarker-driven process and a deep knowledge of biological mechanisms according to which the development of diseases occurs. In this context, the advancements in high-throughput omics technologies represent a unique opportunity to discover novel biomarkers and to provide an unbiased picture of the biological system. One of the medical fields in which omics science has started to be recently applied is that of ophthalmology. Ocular diseases are very common, and some of them could be highly disabling, thus leading to vision loss and blindness. The pathogenic mechanism of most ocular diseases may be dependent on various genetic and environmental factors, whose effect has not been yet completely understood. In this context, large-scale omics approaches are fundamental to have a comprehensive evaluation of the whole system and represent an essential tool for the development of novel therapies. This Review summarizes the recent advancements in omics science applied to ophthalmology in the last ten years, in particular by focusing on proteomics, metabolomics and lipidomics applications from an analytical perspective. The role of high-efficiency separation techniques coupled to (high-resolution) mass spectrometry ((HR)MS) is also discussed, as well as the impact of sampling, sample preparation and data analysis as integrating parts of the analytical workflow.



KEYWORDS: *omics science, metabolomics, proteomics, lipidomics, ocular disease, ophthalmology, liquid chromatography, mass spectrometry*

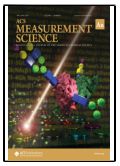
1. INTRODUCTION

The ocular system represents an ideal environment to research novel therapies. Indeed, they are one of the few areas of the human body characterized by a so-called immune privilege together with the brain, testes, placenta, and fetus.[1] Immune privilege refers to the ability of the eye to self-regulate the inflammatory immune response to preserve ocular function. For this reason, the eye represents a potential site for the implantation of stem cells which can in turn restore ocular function with lower risk of rejection than in other parts of the body.[2,3] In addition, this compartmentalization allows investigation of specific biomarkers for a functional evaluation of eye-related diseases.[4,5] Indeed, given the close relationship between the brain and the eye and the fact that many neurodegenerative diseases (such as Alzheimer's and Parkinson's) may initially manifest with ocular symptoms, there is an increasing interest toward the evaluation of novel ocular biomarkers for neurodegenerative disorders to allow early diagnosis.[6,7]

Tears have a fundamental role for the eye. They are produced in very small volume (in a range of a few μL), and they have a very complex structure and composition with an amphiphilic lipid layer between the inner aqueous layer and the external lipidic film, as illustrated in Figure 1. Tears contain thousands of proteins, lipids, electrolytes and small metabolites, whose equilibrium is fundamental to ensure comfort and quality vision.[8] Alterations of the tear composition is commonly associated with specific ocular diseases such as blepharitis and dry eye syndrome.[9−11]

However, eye-related diseases are characterized by complex pathogenic mechanisms depending on many genetic and

Received: November 14, 2023
Revised: February 6, 2024
Accepted: February 7, 2024
Published: February 22, 2024



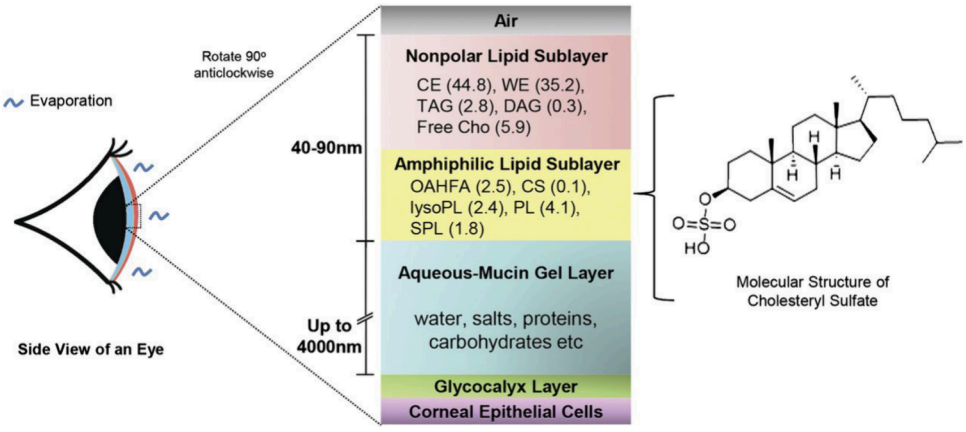


Figure 1. Human tear film model. Reproduced with permission under a Creative Commons CC-BY 4.0 from ref 12. Copyright 2014 Elsevier.

environmental factors, whose combined effect has not been fully understood yet.[13] Indeed, common genetic approaches, used to characterize these pathologies, give only partial information, and they are very often characterized by low throughput. This is the reason why large-scale omics approaches are increasingly used in ophthalmology to have a comprehensive evaluation of the whole system.[14] Omics can be defined as the set of disciplines whose aim is the high-throughput measurement and characterization of biological molecules in a system. In the last years, this branch of science has undergone a rapid advancement thanks to continuous improvements in sequencing technology, high-resolution mass spectrometry (HRMS) and bioinformatic approaches. However, due to the huge amount of data generated by omics studies and the intrinsic variability of diseases, the elaboration of meaningful information by means of these approaches is very challenging, and it requires the combination of different skills and knowledge, including analytical chemistry, biochemistry, medicine, statistical analysis as well as nutritional, environmental or agricultural sciences.[15] The emerging application of omics techniques in the ophthalmology field is focusing not only on diagnostics, to better understand eye diseases at the molecular level, but also on the treatments, to identify new therapeutic targets and to monitor potential or existing treatments. Also, omics techniques have been recently employed at ophthalmological level to map metabolites contained in human tears, revealing that the metabolic profile contained in those samples represents a personal "fingerprint", which can be exploited as a tool for personal identification. This is a breakthrough in the field of forensic analysis since it could help in investigations to ascertain the identity of subjects involved in crimes.[16]

This manuscript will review the latest advancements of omics approaches in ophthalmology in the last ten years, especially related to the discovery of new biomarkers for diseases. Although other recent reviews exist on the topic,[17−26] they do not provide a comprehensive view on specific (ultra) high performance liquid chromatography ((U)HPLC) coupled with (HR)MS applications on all the three branches of omics science (proteomics, lipidomics and metabolomics), which is the specific focus of this Review.

2. OMICS SCIENCES

All omics disciplines share the common aim of investigating and analyzing large amounts of data related to the structure and function of a given biological system in a particular condition. Multiomics approaches are increasingly adopted to understand the relationship between molecular biomarkers and disease phenotypes.

Omics sciences are interconnected, and they can be represented by means of the so-called "omics" cascade (see Figure 2) which describes the hierarchical flow of information

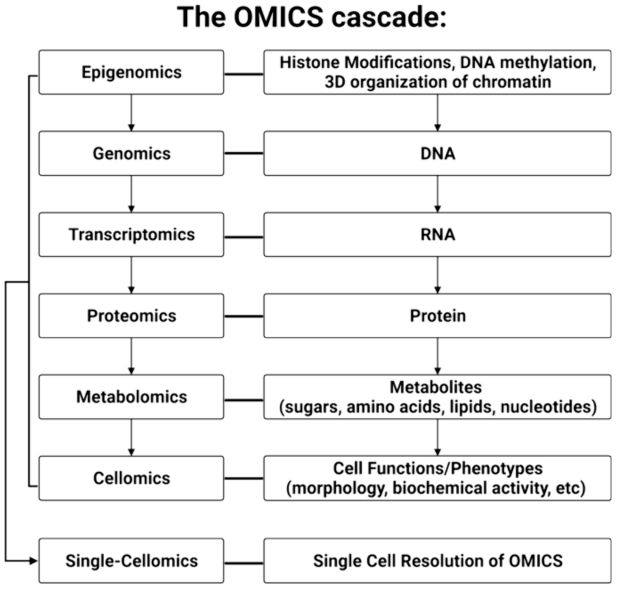


Figure 2. Overview of the omics cascade. All omics fields are intercorrelated with each other and can provide complementary information on a given biological system. Reproduced with permission under a Creative Commons CC-BY 4.0 from ref 27. Copyright 2023 Springer.

in a biological system.[27] This Review will be particularly focused on the latest two omics sciences at the bottom of the cascade, i.e., proteomics and metabolomics. The role of the first one is the study of the proteome, i.e., the entire set of proteins encoded by mRNA, while metabolomics is aimed at measuring all the small intermediate molecules (e.g., amino acids, lipids, sugars, organic acids, etc.) produced by metabolic reactions. Lipidomics is a branch of metabolomics.

2.1. Analytical Workflow

Omics sciences require the employment of highly informative and sensitive approaches. This is why nuclear magnetic

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

resonance (NMR) or MS are mostly employed, with the latter often combined with a separation technique. As mentioned before, this Review will only focus on MS-based techniques; therefore, NMR will not be considered. Despite the very high resolution provided by state-of-the-art chromatographic methods, the separation of all the metabolites present in a sample by means of chromatography coupled to MS is very challenging due to their chemical diversity as well as their different concentrations. Volatile biomolecules, such as those required for breath analysis,[28] are well separated by means of gas chromatography (GC) coupled to MS; however, the largest part of metabolites (as well as proteins/peptides) are nonvolatile, and therefore, they could be analyzed through GC only prior derivatization. This is the reason why (U)HPLC-MS is considered to be the most versatile separation technique to identify proteins and metabolites (including lipids), especially in tandem mode (MS/MS).

In omics sciences, two different approaches can be used: (i) *untargeted* analysis, whose attempt is a comprehensive evaluation of all the measurable species in a sample, including unknowns and, (ii) *targeted* analysis, which are directly focused on the separation (and quantification) of specific categories of chemically characterized and biochemically annotated analytes (whose m/z ratio is known).

Reversed-phase liquid chromatography (RPLC) with gradient elution is by far the most widely used method for omics applications since it is MS compatible and it allows the separation of the largest number of metabolites/proteins except polar or ionic compounds, which are better separated under hydrophilic interaction liquid chromatography (HILIC) conditions.[29,30] This elution mode provides an orthogonal separation with respect to RPLC; indeed, they are often combined in multidimensional LC approaches.[31,32] Anyway, in some specific cases other chromatographic modes can also be used, as it will be highlighted along the manuscript.

Given the intrinsic complexity of biological samples, especially for untargeted approaches, the use of high-efficiency columns is particularly advisable. As a result, columns packed with superficially porous particles (SPPs) or sub-2 μm fully porous particles (FPPs) are commonly employed in omics applications since they allow increasing the sensitivity of the method as well as decreasing matrix effects.[33,34]

Tremendous advancements in omics science have been boosted by continuous development of MS instrumentation, especially with the introduction of HRMS analyzers such as Orbitrap or Q Time of Flight (Q-TOF)[35] which have enabled the identification of isobaric compounds at trace levels.[36]

Compared to other experiments, omics research generates a much larger data output, so that bioinformatic approaches are fundamental to retrieve the correct information from omics data. Indeed, experimental raw data collected from LC-MS experiments include a number of signals and information which are often redundant and superfluous with respect to the analytes or biomarkers under study.[23]

The raw data must be first simplified and reduced to necessary and significant data, through the generation of a raw data matrix where the different rows represent different samples and the columns represent different variables, such as metabolites of interest (metabolite feature). After preprocessing, "clean" data can be possibly normalized and scaled through statistical tools, e.g., providing alignment of retention time and accurate mass (deconvolution).[37]

After this, advanced statistical methods are applied to interpret data and identify correlation between variables and experimental response, especially univariate and multivariate statistical methods.[26] Many software now allows to find features which can discriminate between two or more different sample sets (experimental groups), for instance healthy and diseased subjects, or subjects undergoing different treatments.

Global metabolic composition of biological human samples can be significantly influenced, for instance, by diet, stress, age, gender, as well as disease states.[38] A widely employed statistical approach to treat large numbers of experimental information related to the whole metabolomic profile is the so-called Principal Component Analysis (PCA), which provides a simplification of large data sets into new systems of coordinates, which represent the components, derived from the experimental data. The data variance is therefore accounted for by some of the principal components. This method is particularly suitable during data analysis since it helps classify data and make conjectures concerning the differences in metabolic profile of study and control groups.

2.2. Proteomics

Proteomics focuses on the identification and quantification of the proteins from a biological sample.[39] Their abundance is controlled by the rates of translation and degradation, while their function and stability are regulated by the covalent addition of functional groups or subunits called post-translational modifications (PTMs), such as glycosylation, methylation, acetylation and phosphorylation. The identification and understanding of PTMs can highlight changes involved in the onset or the development of complex and heterogeneous diseases, including ophthalmic disease. Proteomics analysis is usually performed with the use of dedicated nano-LC columns and instruments to enhance the sensitivity of the technique. This is due to multiple reasons, such as low concentrations of the analytes, wide dynamic range and high sample complexity. A reduction in column internal diameter allows an increase in the concentration of the sample injected in the system, thereby decreasing chromatographic dilution.[40]

Two different methods can be employed in proteomics, named top-down and bottom-up. The latter is the most frequently used since it is based on the digestion of proteins into peptides, which are then analyzed by means of MS/MS. The possibility of analyzing peptides instead of proteins is greatly advantageous since peptides are more easily separated by RPLC and their fragmentation can be readily predicted.[41] In top-down proteomics, intact proteins are injected into the MS. Although it has high potential to achieve information that are not accessible through bottom-up approaches (such as the identification of proteoforms from single genes) and continuous improvements in dedicated instrumentation and software, top down proteomics is still affected by several challenges regarding sensitivity and dynamic range of measurements, data analysis and interpretation.[42]

2.3. Metabolomics

Metabolites are small molecules (<1.5 kDa) that are involved in the chemical reactions and metabolic processes occurring within living organisms. These molecules are the intermediates, products or byproducts of various biochemical pathways taking place in cells, tissues, and organs,[23] and often metabolites produced by a bioreaction represent the reagents for successive reactions. Metabolites are diverse in nature and can include compounds such as sugars, amino acids, lipids, nucleotides,

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

organic acids, hormones, vitamins, secondary metabolites, and many others.[43]

Metabolomics, as a scientific discipline, aims to comprehensively analyze and quantify these metabolites present in a biological system. By profiling the concentrations and changes in metabolite levels, metabolomics provides insights into the biochemical activity, metabolic state, and physiological condition of cells, tissues, or organisms.[44]

An issue associated with complex samples of biological origin is that they contain several compound classes with different chemico-physical features, ranging from polar neutrals (e.g., sugars), to acid, basic or amphoteric compounds, to nonpolar species. For this reason, it is impossible to employ a single technique or method able to separate all these metabolites.[45] RPLC is the main chromatographic mode employed for the separation and analysis of nonpolar metabolites, like flavonoids and phenolic compounds. The separation takes place based on the different hydrophobicity of analytes by using columns coated with a C18 or C8 chains.[45] Polar species, such as sugars, amino acids and nucleotides, are poorly retained on C18 resins; therefore, in some cases, the use of columns with HILIC stationary phase is also reported.[46,47]

2.4. Lipidomics

Among other classes of biomarkers that can provide information concerning the health of the ocular system, lipids must be mentioned. These molecules play fundamental roles in human life, being the constituents of cellular membranes, providing energy storage and acting as precursors for other endogenous molecules.[48]

General structure of lipids includes a nonpolar portion, which is hydrophobic and insoluble in water, and a polar portion, more hydrophilic. According to the Lipid MAPS Consortium, they have been classified into eight classes: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, polyketides (Figure 3).[48,49]

The branch of -omics sciences which studies lipids involved in cellular reactions and metabolic mechanisms is called "lipidomics". Lipidomic investigations can be exploited not only to characterize lipidic profiles contained in a biological sample from both a qualitative and a quantitative point of view but also to monitor their variations over time.

Concerning lipids in tears, they are mainly produced by the meibomian glands (present along the rims of the eyelids), but also by the cornea and the conjunctiva, and are contained especially in the most superficial layer of the liquid film covering the eyes.[50] This layer contains both hydrophilic and hydrophobic lipids, and it has a protective role, since it represents a barrier against foreign bodies and avoids the evaporation of the intermediate aqueous layer.[51] Regarding the separation of the lipid component, it must be highlighted that no standardized procedures exist. A basic approach to separate lipids by classes, e.g., fatty acids, sterols, glycerolipids and phospholipids, is solid phase extraction (SPE).[52,53]

On the other hand, the most efficient and sensitive technique is (U)HPLC both in normal and reversed phase. The first mode separates lipids based on their polarity, so for instance it is useful for glycerophospholipids having different headgroup polarity,[54] but it is hard to couple with MS detection. On the other hand, fatty acids can be separated through RPLC depending on their chain length and degree of unsaturation.[55]

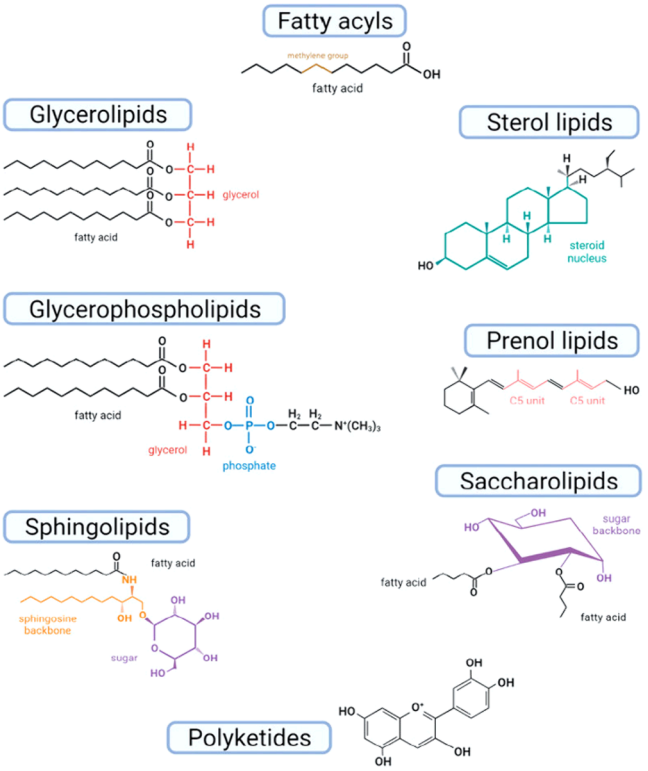


Figure 3. Representation of lipids classes according to the Lipid MAPS Consortium. Reproduced with permission from ref 49. Copyright 2022 Elsevier.

Multidimensional liquid chromatography (2D-LC) also offers the opportunity to combine two separation mechanisms with different selectivity (orthogonal) into a single run, enhancing the separation power especially for complex samples, especially by working in heart-cutting mode. This strategy is particularly suitable when it is desirable to conduct the chromatographic separation with solvents not compatible with MS (e.g., salts or organic modifiers not compatible with ESI). In that case, they can be used in the first dimension.[56,57]

3. OMICS APPLICATIONS IN OPHTHALMOLOGY

This section will be specifically focused on the latest achievements in ophthalmology research by using omics approaches in the past decade.

Since the analytical workflow for omics applications starts from sampling procedures followed by different steps of sample preparation, this fundamental part of the experimental procedures is also reviewed before focusing on proteomics, metabolomics and lipidomics applications.

3.1. Sampling

Sampling has a crucial role in omics approaches since errors or differences in this step could affect the entire analytical workflow and omics data that are obtained. In the case of ophthalmic research, different types of samples can be collected from the eye for omics experiments, namely, vitreous and aqueous humor, tears, cornea, lens, and retina.[23]

An issue associated with the sampling step in omics experiments associated with ophthalmology is that only very small volumes and quantities of material could be available. Indeed, for instance, volumes often smaller than 10 μL of tears

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

are usually produced, and their composition could change with varying stimuli.[18]

Two main sampling procedures exist to collect tears samples, i.e. (i) by using an absorbent material or (ii) by capillarity.[18,58] Schirmer's strips are also very diffused as adsorbent material, since they are simply put in contact with the closed eye without the need of anesthesia. They are made of Whatman filter paper and are mainly used as a tool to detect ocular dryness, based on the degree of wetness of the strip after 5 min of contact with the eye.[59] However, after the collection, the strip can be possibly cut into smaller pieces to undergo solvent extraction and centrifugation. Regarding the proteins, Koduri et al. discussed their elution capacity from Schirmer's strips using different buffers and showed the highest protein yield after incubation of the Schirmer's strip in 100 mM ammonium bicarbonate with 0.25% Nonidet P-40 at 4 °C for an hour.[60] Regarding metabolites, a crucial aspect to consider during the sample collection step with Schirmer's strip is the potential reduction in metabolite concentration due to increased tear flow, a result of stimulation.[61] However, it has been shown by Small et al. that this method is accurate, fast, easy and, especially, less invasive when compared with microcapillary tubes; moreover, it does not require any kind of aggressive extraction.[62]

Since the direct contact of the adsorbent with the eye could cause the dilution of the collected sample and its contamination with other tissues, other sampling methods based on capillarity, i.e. collection of unstimulated tears, have been developed, in order to allow also for a more precise volume quantification of the sample. Obviously, the volumes collected are tiny, in the order of microliters (from 1 to 10 μL).[63] In this case, glass microcapillary tubes are used, even though lately also polytetrafluoroethylene materials are being investigated.[64,65] It has been found out that similar tear lipidome profile is obtained with Schirmer strips and microcapillaries, but some contamination deriving from blood and skin can possibly occur with the strips, causing the detection of higher concentrations of some lipids.[50] Other collection strategies involve the use of cellulose, cotton or paper materials.

Also, there is no standardized way to perform the tears collection which makes difficult not only the data comparison between published manuscripts but also the translation of experimental results into clinical use and trials.

3.2. Sample Preparation

Sample preparation highly varies depending on the analytes to be extracted. In the case of proteins, they are usually extracted from the sample by using buffers, such as phosphate buffered saline in the case of tears collected by Schirmer's strips.[66] A lysis step then follows, either physical or chemical in nature, through sonication and the use of detergent agents.[67] Finally, proteins are digested with a protease. The most established protease is the porcine trypsin due to its high specificity and the relative abundance of cleavage sites in protein sequences after a lysine or an arginine, on the C-terminal side, performed at 37 °C.[68] In the case of the use of Schirmer's strips for tear fluid analysis, a single unit filter-aided method was developed by Aaas et al. which allowed the identification of 1526 proteins.[69] A study also compared four different workflows for the proteomic analysis of tear fluid collected via Schirmer's strips by employing two distinct protein digestion methods (postextraction protein digestion and in-strip protein digestion) as well as two fragmentation techniques (collision induced dissociation, CID and higher-energy collisional dissociation, HCD). It was found that the combination of HCD fragmentation with in-strip digestion was the most effective, allowing the identification of over 3000 proteins in human tear samples from 11 healthy subjects.[70]

To obtain a comprehensive snapshot of the metabolome associated with a specific biological condition, it is important to use a sample preparation method that can extract a wide range of metabolites without introducing bias toward specific chemical families or physical locations. However, no single extraction solution can capture all metabolites present in a given biospecimen.[76] In some cases, such as with plasma samples, centrifugation or molecular weight cutoff filters are employed.[71,72] These filters are designed to retain molecules above a specific molecular weight, and their usage in sample preparation is straightforward: it is sufficient to simply load the sample into the filter, centrifuge, and analyze the eluent containing species with molecular mass below the cutoff weight.[72]

In the case of the lipids, an extraction step is usually required to suppress the matrix effect and prepurify the sample. This step is based on the high solubility of hydrophobic lipids in organic solvents, which can be traditionally exploited in liquid–liquid extraction using solvent such as chloroform, methanol and water, also mixed together.[73] Folch extraction, for instance, is a well-established extraction method which exploits partitioning of lipids in chloroform and methanol, which are used in 2:1 ratio.[74] This choice allows to separate lipids from more polar metabolites. Steps of centrifugation and freeze-drying can be performed subsequently.

The sampling and preparation steps play a key role for the outcome of the analysis, due to the high variability of the sample composition related to the potential partial evaporation of the small amount of sample collected.[16] In any case, for all the samples cited, storage prior to LC-MS/MS analyses can be done at −20 °C or at −80 °C.

3.3. Omics in Ophthalmology

In the next paragraphs, the latest advancements in omics research applied to ophthalmology diseases is presented. The main pathologies that will be taken into account are briefly discussed in the following.

- Dry eye (DE) disorder or syndrome or disease is a very common condition associated with insufficient lubrication and moisture on the surface of the eye, instability of the tear film, hyperosmolarity and inflammation, which can result in damage to the ocular surface.[75] Lately, it has been hypothesized that DE can worsen because of oxidative stress caused by oxidizing agents, such as reactive oxygen species (ROS).[76]
- A joint cause of the DE disorder is meibomian gland dysfunction (MGD), which involves an obstruction of the meibomian gland orifice, which causes a decrease in the meibum volume secreted by these glands and/or a modification in its composition.[77]
- Myopia, or nearsightedness, occurs when distant objects appear blurry because the images focus in front of the retina.[78] Individuals with high myopia are at a greater risk of developing serious eye diseases such as glaucoma and cataracts.
- Glaucoma is a group of optic neuropathies which can cause retinal degeneration and damage to the optic

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

nerve, leading potentially to blindness, especially in people older than 50. Also, it is often associated with increased pressure within the eye, known as intraocular pressure (IOP).[79,80] A particular kind of glaucoma is primary open angle glaucoma (POAG).

- Diabetic retinopathy (DR) is a progressive eye condition that affects individuals with diabetes. It occurs when long-term high blood sugar levels damage the small blood vessels in the retina. There are two main types of diabetic retinopathy: nonproliferative diabetic retinopathy (NPDR) that occurs in early stage, which is characterized by small changes in the blood vessels; and proliferative diabetic retinopathy (PDR) that occurs in advanced stages and involves the growth of new, abnormal blood vessels in the retina.[81]
- Keratoconus (KC) is a progressive eye disorder that affects the cornea, the clear front surface of the eye. In patients with keratoconus, the cornea gradually thins and bulges into a cone-like shape, leading to distorted vision.[82]
- Autoimmune retinopathy (AIR) is a rare and therefore still very much unknown autoimmune disease that affects the ocular system, particularly due to autoantibodies acting against the retina and therefore causing inflammation. It can be nonparaneoplastic (not related to a cancer) or paraneoplastic (related to a form of cancer).[83]

3.3.1. Proteomics Applications in Ophthalmology. Various proteomics methods have been used to investigate changes in the ocular proteome induced by treatments or pathological conditions, underlying alterations in the quantity of different proteins.[84] For the sake of simplicity, this section has been divided into different subsections according to the area of the research.

Measurement of Proteins Assessing Healthy Ocular Functions. One of the main scopes for which proteomics studies have been employed in ophthalmology research is to assess ocular function by evaluating protein content in human vitreous healthy tissue. Aretz et al. identified 1111 unique proteins associated with various functions including enzymatic activities, complement cascades, and visual perception, including 262 proteins found as a constitutive pattern across three samples.[85] Eight years later, the number of proteins identified increased notably to 6511 proteins, using an Orbitrap Fusion Tribrid mass spectrometer. It included 302 crucial for energy metabolism, and various other structural and nutrient transport proteins.[86]

The tear proteome was also evaluated under normal healthy ocular conditions. Likewise in this case, it can be observed that over the years, with continuous improvements in MS analyzers, the number of identified proteins has tremendously increased, going from 747 distinct tear proteins in 2015[93] to over 3000 in 2022.[75] This latter study revealed that the most prevalent proteins in tears are the immunoglobulins, particularly immunoglobulin A which defends the eye against pathogens; keratins which are essential for ocular protection, complement families whose role has not been determined; yet and other ocular surface filaments which have a role in immune response and barrier protection. Lactotransferrin (LTF), lipocalin-1 (LCN1), albumin (ALB), and prolactin-inducible protein (PIP) were identified as the top four proteins in the tear film, with LTF role in mucosal defense and inflammation reduction, PIP's function in enhancing aquaporin placement for ocular lubrication, and LCN1's protective role against desiccation.[70] Lacrimal proline-rich protein 4 and zymogen granule protein 16 homologue B both play a role in the protection and maintenance of the dynamic balance of the ocular surface, and their upregulation was highlighted in human reflex tears.[87] A study by de Souza et al. identified 18 antioxidant enzymes which protect the eye from detrimental effects of oxygen exposure.[88] The most recent investigation identified for the first time active ghrelin and gastric inhibitory polypeptide in tears from healthy individuals. Additionally, the consistent reproducibility of leptin levels suggested its potential as a candidate biomarker to evaluate the impact of metabolic disorders on the ocular surface.[89]

Measurement of Proteins Involved in Ocular Diseases. The largest part of omics applications in ophthalmology research is devoted to the evaluation of protein biomarkers for ocular diseases. Indeed, it is well-known that eye pathologies could induce alteration of a variety of proteins, whose identification can serve to identify putative biomarkers. For instance, a recent study has allowed the identification of the neuronal cell adhesion molecule as a potential biomarker to help in the diagnosis of AIR.[90] On the other hand, Balaiya et al. characterized the PDR vitreous and aqueous proteome in humans to help elucidate the pathogenesis of PDR.[67] They found distinct proteins linked to the coagulation, complement, and kallikrein-kinin systems solely in vitreous from PDR patients and not in the control group. Notably, within the coagulation category, fibrinogen and prothrombin were most probably highlighting the significance of angiogenesis in the progression of PDR. In the aqueous humor, they identified proteins in PDR patients associated with transport, coagulation, and inflammatory responses.[67]

Proteomics studies have been applied to also better understand GL, DE and MGD diseases. By exploring the proteome alterations in aqueous humor of primary open angle GL patients it has been found that several proteins including apolipoprotein D, complement C3, pigment epithelium-derived factor, dickkopf-related protein 3, and wingless-related integration inhibitory factor 1 exhibited significant upregulation compared to the control group.[91] With respect to DE and MGD, Soria et al. analyzed the tear film proteome of healthy patients and people affected by these pathologies.[84] Differences in concentrations of antileukoproteinase, phospholipase A2, and lactoperoxidase were detected in patients affected by MGD and DE.[84,92]

Various proteins from tears and corneal tissues have been implicated in the development of KC. These proteins are associated with pathways linked to the cytoskeleton, cell matrix, TGFβ signaling, and extracellular matrix remodeling.[21] The current research regarding serves as a point of reference for future investigations, establishing a myopia proteome database for diverse biological specimens.[22]

Three proteins are translational biomarkers used in ophthalmology in clinical diagnosis of various ocular diseases namely matrix metalloproteinase 9 (MMP-9), IgE and lactoferrin.[93] MMP-9 is a well-established nonspecific inflammatory marker, consistently validated across various ocular surface diseases, indicating its effectiveness in predicting inflammatory changes. It has been integrated into the InflammaDry diagnostic system, designed for general practitioners, demonstrating high sensitivity and specificity in identifying DE conditions.[94] IgE is a crucial protein in allergic

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

diseases, playing a direct role in triggering allergic reactions on the ocular surface. Several studies have supported its effectiveness as a marker for allergy, particularly in the context of allergic conjunctivitis, which has been associated with elevated levels of tear IgE in patients compared to controls.[93] Lactoferrin, recognized as a significant iron-binding protein with immune-modulating and antimicrobial properties, has been consistently linked to aqueous-deficient DE. Various studies have validated its utility as a biomarker, particularly when combined with Schirmer's test, exhibiting high specificity (95%) and good sensitivity (72%) in diagnosing Sjögren's syndrome. Additionally, both IgE and lactoferrin are used in the TearScan MicroAssay for diagnostic tests, enabling improved differentiation between DE and allergies.

As already stated before, omics science does not only allow finding novel potential biomarkers, but also possibly understanding the impact of treatments on ocular diseases to evaluate their efficacy. These kinds of studies have been mainly carried out by investigating the proteome in human tears and vitreous.

The alterations in the human tear proteome were monitored after the application of cyclosporine A and diquafosol tetrasodium for DE disease.[95] The study revealed 54 differentially expressed proteins in the topical cyclosporine A treatment group and 106 in the diquafosol tetrasodium treatment group. Gene ontology analysis pointed toward the augmentation of both innate and adaptive immune responses as well as cellular detoxification in response to both treatments. Another tear proteome study for DE disease analyzed the therapeutic efficacy of topical fluorometholone compared to a poly(vinyl alcohol) control treatment.[96] They quantified 758 proteins, finding 9 proteins with varying expression between fluorometholone and poly(vinyl alcohol) treatments after 3 weeks and 7 proteins after desiccating stress. Notably, complement C3 and calmodulin like 5 were identified as key proteins differentiating the severity of DE Disease, potentially serving as biomarkers to identify patients benefiting most from fluorometholone treatment. Rossi et al. emphasized an increase of the activity of extracellular vesicles exosomes in POAG tears compared to control biofluids.[66] This is mainly due to the tear film being an acellular biofluid with protein and lipid components primarily released by glandular and epithelial cells through exosomes.[70] The study highlighted the involvement of promyelocytic leukemia protein in the etiology of POAG, which could be a potential new target for following POAG treatment.[66]

Human vitreous proteome was studied for ranibizumab efficacy in PDR patients. A total of 339 proteins showing differential expression were identified in reaction to the treatment and associated with immune response, platelet degranulation, and complement activation.[97]

3.3.2. Metabolomics Applications in Ophthalmology.

Metabolomics is another essential tool to evaluate various ocular conditions since the eye is thought to have its own metabolome. Indeed, the composition of aqueous and vitreous humor can be considered as a representation of the local metabolism with negligible influence from the systemic environment. However, at present, there are still some open questions on the extent of variations of metabolites in the ocular environment, requiring further investigation.

This section has also been divided into different subsections according to the area of the research.

Measurement of Metabolites Assessing Healthy Ocular Functions. Metabolites in ocular tissues play various essential functions in maintaining the health and proper functioning of the eye. For this reason, it is crucial to assess normal conditions for healthy tissues, in order to have a valuable baseline for research on eye diseases. Metabolomic changes can be influenced by many factors such as inflammation or oxidative stress, diet and nutrition, aging, disease or genetic factors.[98]

In a recent work, 60 metabolites in tear fluid have been identified, including acylcarnitines, amino acids, biogenic amines, and glycerophospholipids, which are well-known to be involved in processes like structural maintenance and membrane stability and signaling.[99] Moreover, ocular tissue metabolism serves an important role in maintaining corneal homeostasis. A study conducted by Zhang et al. demonstrated the significance of various metabolites, including glucose and glutamine, which are involved in metabolic pathways such as aerobic glycolysis and glutaminolysis through the tricarboxylic acid cycle. These metabolites play essential roles in supporting the normal functioning of the cornea.[100]

Measurement of Metabolites Involved in Ocular Diseases. Detection of differences in metabolite concentrations and distribution may be fundamental to understand the development of various ocular diseases. Literature in the past ten years contains several reports on the application of metabolomics experiments on different ocular diseases.

One of the first pathologies to be studied has been DE.[101] Due to the diverse range of symptoms associated with DE, the currently used diagnostic and prognostic methods are nonspecific; therefore, metabolomics studies are a fundamental tool to understand the complex biochemical changes happening in the tear film. By analyzing tear samples, researchers can detect alterations in metabolite profiles linked to inflammation, oxidative stress, tear film instability, hyperosmolarity, and ocular surface inflammation. These findings provide insights into the underlying mechanisms of DE and could aid in the development of targeted therapies.[58,75] For instance, Chen et al. have analyzed the metabolic profile of tears in DE patients and a healthy control group, identifying 156 metabolites, with 32 of them showing significant changes in individuals with DE.[102] These specific metabolites belonged to various classes such as fatty acids, nucleosides, nucleotides, carboxylic acids and their derivatives, which provided potential biomarkers for DE. The study also revealed the involvement of metabolic processes such as glycolysis/gluconeogenesis, amino acid metabolism, and the complement and coagulation cascades in the development of DE.

Although high myopia is a very common condition, the key metabolic alterations in patients affected by this disease have not been fully understood.[103] By examining the metabolic profile of aqueous humor samples, it has been recently found that some of the prominent metabolites found in high myopia include aminooctanoic acid, arginine, and citrulline. On the other hand, aminoundecanoic acid and cysteinylglycine disulfide were found to be less abundant.[104] However, aqueous humor is not the only type of sample that can be employed to detect metabolites associated with myopia, in fact serum samples have also been investigated. In a study conducted by Kearney et al., myopic individuals were found to have significantly higher concentrations of melatonin and dopamine in their serum compared to nonmyopic individuals. Furthermore, nine metabolites closely associated with myopia have been recently discovered, suggesting disruption in

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

diacylglycerol metabolism, amino acid processing, and vitamin metabolism. According to this study, γ-glutamyltyrosine and 12-oxo-20-trihydroxy-leukotriene B4 have emerged as potential biomarkers for high myopia.[103]

Metabolomics has also improved the understanding the alterations induced by glaucoma, which, if left untreated, could lead to vision loss and, in severe cases, blindness. By evaluating the metabolic profile in aqueous humor of 28 primary open-angle glaucoma (POAG) patients and 25 controls, 22 metabolites were found to be expressed differently.[105] Cyclic adenosine monoposphate (cAMP), 2-methylbenzoic acid, and 3′-sialyllactose in the aqueous humor were suggested as potential biomarkers for POAG. Moreover, the analysis of aqueous humor and plasma samples of 26 patients with POAG and 26 control individuals has revealed lower levels of spermine and taurine in POAG patients compared to controls. These metabolites, known for their neuroprotective properties, indicated neuronal damage and oxidative stress in POAG.[106] In another work focused on the investigation of plasma samples from 34 POAG patients and 30 controls, notable changes in nicotinamide, N-acetyl-L-leucine, and arginine concentrations were observed between the two groups of patients.[107] The LC-MS/MS analysis of serum samples from 211 glaucoma patients and 295 controls, has allowed to reveal that asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) concentrations were significantly higher in serum samples of patients with glaucoma at an advanced level,[108] thus supporting a possible relationship between elevated ADMA and SDMA levels and glaucoma severity.[109,110]

Early diagnosis is fundamental for people affected by diabetes in order to assess their predisposition to develop diabetic retinopathy. Metabolomics data obtained from vitreous fluid samples from 9 patients with DR and 9 nondiabetic controls showed a decrease in downstream metabolites of glycolysis, specifically glyceraldehyde 3-phosphate and 2/3-phosphoglycerate in diabetic patients.[111] Conversely, there was an increase in the product of the pentose phosphate pathway, namely pentose phosphate, which is consistent with changes observed under conditions of oxidative stress.[112] However, there is another metabolomic pathway that is involved in DR, namely that of arginine. In a study conducted on vitreous fluid samples, it has been found out that overactivity of arginase, the main enzyme involved in synthesis of arginine, can contribute to generating superoxide species.[113] This supports that oxidative stress is a crucial contributor to DR. Detection of metabolites linked to oxidative stress could be useful to identify biomarkers for prediction and treatment.[114]

Finally, recent works are focused on the identification of potential biomarkers for KC. It has been found that dysregulated metabolic pathways including urea cycle, glycolysis and gluconeogenesis can be associated with KC.[115] The concentrations and ratio of specific metabolites involved in glycolysis and Krebs cycle such as lactate/malate and lactate/pyruvate were elevated in human keratoconic cells, while arginine and reduced/oxidized glutathione ratio were reduced.[23] These results seem to suggest that KC is strongly linked to oxidative stress and acute inflammation at the ocular level.[116]

3.2.3. Lipidomics Applications in Ophthalmology. Analysis of tear samples through lipidomic approaches can help highlight potential occurrence of critical situations and clarify the interactions between lipids and other metabolites, whose content is influenced by both systemic (e.g., multiple sclerosis) and ocular diseases (e.g., glaucoma; keratoconus; dry eye condition) as well as personal behaviors (e.g., contact lens wear).[18] The investigation of tear metabolome and lipidome can therefore provide an explanation for the pathogenesis of some diseases through the identification of diagnostic biomarkers. Alternatively, it can help monitor treatments and ongoing therapies.

Measurement of Lipids Assessing Healthy Ocular Functions. Most common nonpolar lipids in the tear film, which are primarily provided by the meibomian glands, are wax esters, triglycerides and cholesterol esters, while polar lipids are mainly constituted by phosphatidylcholine and phosphatidylethanolamine (see Figure 4).[117,118]

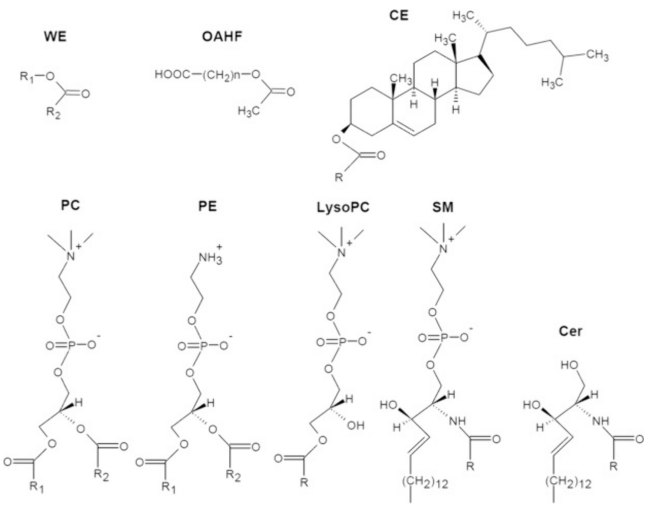


Figure 4. Schematic representation of the most abundant lipids in tear film. Nonpolar: wax esters (WE), cholesterol esters (CE); polar: (O-acyl)-u-hydroxy fatty acids (OAHF), phosphatidylcholines (PC), phosphatidylethanolamines (PE), lysophosphatidylcholines (LysoPC), sphingomyelins (SM), ceramides (Cer). Reproduced with permission from ref 119. Copyright 2016 Elsevier.

Joint research between universities in Beijing and Singapore comprehensively evaluated the lipidomic profile of tear film in 45 patients and 15 volunteers.[76] Absolute amount of lipids was found to be higher with Schirmer's method. HPLC-MS analysis was performed both in normal-phase and in reversed-phase to discriminate between classes of lipids with different polarity. Polar lipids, such as O-acyl-omega-hydroxy-fatty acid (OAHF), phosphatidylserine, phosphatidylglycerol, sphingosine phosphate and many others were separated using a silica column and a mixture of chloroform, methanol, NaOH and water. On the other hand, phospholipids, sphingolipids, glycerol lipids and other less polar species were analyzed by employing a C18 column and a mixture of chloroform, methanol and acetic acid. Authors have hypothesized that lysophospholipidome contained in tears has microbicidal properties; moreover, following the results obtained, they have speculated that cholesteryl sulfate could be the main lipidic species contained in the intermediate amphiphilic sublayer.

When using HPLC coupled to MS, different chromatographic or MS conditions are needed to detect different classes of lipids. The most sensitive technique to obtain a complete characterization of the main lipids involves the use of soft

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

ionization sources in MS to avoid excessive fragmentation of species.[120] Chen and co-workers developed an untargeted lipidomic method optimized to detect lipids in tear and meibum both in positive and in negative ion mode, with the aim of employing it for large-scale clinical translational studies, finding that polar lipids and OAHFA have a role in preserving the stability of the tear film.

Different types of tears present a different lipidic profile. Rohit et al. have demonstrated that basal tears have a much higher lipid concentration than flush or reflex tears and therefore are more recommended for tear lipid analysis. The mole % of each lipid class is also different, the phospholipids content being higher in reflex and flush tears, while nonpolar lipids (cholesterol, wax esters, triacylglycerols) are the major components in basal tears.[121] Also, no differences have been observed between the fluids produced by both eyes of the same individual with no ocular diseases, allowing for the two samples to be pooled together.[99,122] Similarly, intraindividual variation in the content of lipids and metabolites in basal tears of healthy persons is low over 3 days, as opposed to the significant variation demonstrated in interindividual analysis, suggesting the possibility to discriminate between individuals based on tears composition.[123,124]

Measurement of Lipids Involved in Ocular Diseases. Lipidomics can provide information concerning the degree of inflammation in DE. For instance, prostaglandin E2 and D2 are involved in many inflammatory processes, the concentration of the former increasing and that of the latter decreasing immediately during the acute phases of inflammation. As a consequence, an increase in the ratio PGE2/PGD2 correlates to eye irritation, as observed in a study by Keun Lee where nano-LC was coupled with tandem MS.[125]

Steroids levels in tears have been shown to be related to DE by Pieragostino and co-workers who, in 2017, published research focused on the development of an analytical method HPLC-MS/MS to detect and identify seven steroids, including cortisol, testosterone and progesterone, in basal tears. By analyzing and comparing tears of 13 healthy women and 14 DE women, they found that the levels of three of the steroids tested were significantly lower in patient affected by DE and could therefore be a possible diagnostic tool for this disorder.[126]

Another class of potential biomarkers for DE could be represented by low molecular mass wax esters containing saturated fatty acyl groups. Indeed, their concentration decreases notably as the disease arises, with respect to waxes with higher molecular weight and unsaturation, underlining the role of structures (molecular mass and degree of unsaturation) of these lipids. Therefore, this disease seems to be related also to a combination of modifications in some lipidic components in addition to actual lipid deficiency.[127]

DE disease is directly related to meibomian gland dysfunction which leads to a decrease in the meibum volume secreted by these glands. Since tear fluid contains mainly lipids, which prevent evaporation, the direct consequence of decreased lipids is faster rate of tear evaporation, thereby causing dry eye.[128] The severity of these symptoms worsens with age, but it is not influenced by sex.[129,130] It was recently found that in MGD patients' meibum, nonpolar lipids content is significantly lower with respect to healthy subjects, especially for cholesterol esters, whereas the content of more polar lipids, such as cholesterol, OAHFA, free fatty acids, as well as triglycerides, is significantly higher.[131] It is suspected that abnormal lipid levels in serum (dyslipidemia), especially high levels of triglycerides and low-density lipoproteins, or low levels of high-density lipoproteins are associated with risks of cardiovascular disease. Nowadays, it seems that there could be an association between[130] dyslipidemia and MGD, meaning that analysis of tear film could help detect dyslipidemia and therefore identify potential risk factors for cardiovascular events such as stroke.[130]

Lipidomics was also applied to verify if there are differences between the lipid profile of healthy people and people with open-angle glaucoma (POAG), Cabrerizo et al. analyzed aqueous humor of 20 subjects.[132] Lipidomic analysis revealed that for 37 out of 110 lipids considered there was a significant difference between the two groups, including cholesterol esters and sphingomyelins, whose concentration was much lower for healthy subjects. This could be ascribed to the metabolic response to oxidative stress in individuals suffering from POAG. Moreover, Chauhan et al. found that the content of total lipids, total phospholipids, total ceramide, and total sphingolipids were similar between healthy controls and glaucoma patients, while glucosylsphingosine increased notably in patients with POAG.[79] In the latter case, lipids were extracted using Bligh−Dyer method[133] starting from optic nerve matrices provided by human donors after their death. HRMS was performed by employing a Q-exactive mass spectrometer.

Also, some signaling lipids contained in aqueous humor have been linked to glaucoma including oxylipins such as derivatives of linoleic and arachidonic acid. Particularly, their concentration increases from the diagnosis of glaucoma.[134,135]

4. CONCLUSION AND PERSPECTIVES

The application of omics science to ophthalmology research is quite recent, but it has already provided a better understanding of the development of ocular diseases. Despite the tremendous advancements of high-resolution MS analyzers and high-efficiency chromatographic methods, there are still many challenges to be overcome before this kind of investigation could be effectively integrated into clinical research and routine analysis. First of all, obtaining a suitable amount of ocular tissue or fluid is not straightforward. Second, the large data sets obtained from omics investigations require the highly qualified personnel as well as more advanced software and technologies that can be able to process the information and to provide meaningful results.

In any case, omics sciences are increasingly becoming an essential tool for ophthalmologists not only to discover potential novel biomarkers, but also to monitor and understand the efficacy and operating principles of medical treatments toward ocular diseases. It can be envisaged that multiomics analysis will be increasingly applied in the future, which, coupled to continuous improvement in data management and analysis and microsampling techniques, can potentially increase the understanding of eye diseases as well as propose novel personalized therapies and early diagnostic approaches.

AUTHOR INFORMATION

Corresponding Authors

Chiara De Luca − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121*

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

Ferrara, Italy; orcid.org/0000-0002-8001-3287; Email: chiara.deluca@unife.it

Martina Catani − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy;* orcid.org/0000-0003-4217-8766; Email: martina.catani@unife.it

Authors

Maurine Fucito − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy;* orcid.org/0000-0002-9913-3705

Matteo Spedicato − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy*

Simona Felletti − *Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara 44121, Italy;* orcid.org/0000-0002-4192-2074

Angeli Christy Yu − *Department of Translational Medicine and for Romagna, University of Ferrara, 44124 Ferrara, Italy*

Massimo Busin − *Department of Translational Medicine and for Romagna, University of Ferrara, 44124 Ferrara, Italy*

Luisa Pasti − *Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara 44121, Italy*

Flavio A. Franchina − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy*

Alberto Cavazzini − *Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, 44121 Ferrara, Italy; Council for Agricultural Research and Economics, Rome 00184, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmeasuresciau.3c00067

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the National Recovery and Resilience Plan (NRRP), Mission 04 Component 2 Investment 1.5 - NextGenerationEU, Call for tender n. 3277 dated 30/12/2021; Award Number: 0001052 dated 23/06/2022. C.D.L. benefitted from funding FSE REACT-EU, within the program PON "Research and Innovation" 2014−2020 (PON R&I), DM1062/2021.

■ REFERENCES

(1) Streilein, J. W. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. *J. Leukoc Biol.* **2003**, *74* (2), 179−185.

(2) Ma, M.; Li, B.; Zhang, M.; et al. Therapeutic effects of mesenchymal stem cell-derived exosomes on retinal detachment. *Exp. Eye Res.* **2020**, *191*, No. 107899.

(3) Boulton, M.; Albon, J. Stem cells in the eye. *Int. J. Biochem. Cell Biol.* **2004**, *36* (4), 643−657.

(4) Ahmad, M. T.; Zhang, P.; Dufresne, C.; Ferrucci, L.; Semba, R. D. The Human Eye Proteome Project: Updates on an Emerging Proteome. *Proteomics.* **2018**, *18* (5−6), No. 1700394.

(5) Hains, P. G.; Truscott, R. J. W. Proteomic analysis of the oxidation of cysteine residues in human age-related nuclear cataract lenses. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **2008**, *1784* (12), 1959−1964.

(6) Colligris, P.; Perez De Lara, M. J.; Colligris, B.; Pintor, J. Ocular Manifestations of Alzheimer's and Other Neurodegenerative Diseases: The Prospect of the Eye as a Tool for the Early Diagnosis of Alzheimer's Disease. *J. Ophthalmol.* **2018**, *2018*, 8538573.

(7) Ramirez, A. I.; de Hoz, R.; Salobrar-Garcia, E.; et al. The role of microglia in retinal neurodegeneration: Alzheimer's disease, Parkinson, and glaucoma. *Front Aging Neurosci.* **2017**, *9* (JUL), No. 266444.

(8) Pflugfelder, S. C.; Stern, M. E. Biological functions of tear film. *Exp. Eye Res.* **2020**, *197*, No. 108115.

(9) Johnson, M. E.; Murphy, P. J. Changes in the tear film and ocular surface from dry eye syndrome. *Prog. Retin Eye Res.* **2004**, *23* (4), 449−474.

(10) Putnam, C. M. Diagnosis and management of blepharitis: an optometrist's perspective. *Clin Optom (Auckl)* **2016**, *8*, 71−78.

(11) Ohashi, Y.; Ishida, R.; Kojima, T.; et al. Abnormal protein profiles in tears with dry eye syndrome. *Am. J. Ophthalmol.* **2003**, *136* (2), 291−299.

(12) Lam, S. M.; Tong, L.; Duan, X.; Petznick, A.; Wenk, M. R.; Shui, G. Extensive characterization of human tear fluid collected using different techniques unravels the presence of novel lipid amphiphiles. *J. Lipid Res.* **2014**, *55* (2), 289−298.

(13) Sharma, S. Diagnosis of infectious diseases of the eye. *Eye* **2011**, *26* (2), 177−184.

(14) Chung, R. H.; Kang, C. Y. A multi-omics data simulator for complex disease studies and its application to evaluate multi-omics data analysis methods for disease classification. *Gigascience.* **2019**, *8* (5), 1−12.

(15) Gika, H. G.; Theodoridis, G. A.; Plumb, R. S.; Wilson, I. D. Current practice of liquid chromatography−mass spectrometry in metabolomics and metabonomics. *J. Pharm. Biomed Anal.* **2014**, *87*, 12−25.

(16) Aparna, R.; Shanti Iyer, R. Tears and Eyewear in Forensic Investigation—A Review. *Forensic Sci. Int.* **2020**, *306*, No. 110055.

(17) Lauwen, S.; de Jong, E. K.; Lefeber, D. J.; den Hollander, A. I. Omics biomarkers in ophthalmology. *Invest Ophthalmol Vis Sci.* **2017**, *58* (6), BIO88−BIO98.

(18) Khanna, R. K.; Catanese, S.; Emond, P.; Corcia, P.; Blasco, H.; Pisella, P. J. Metabolomics and lipidomics approaches in human tears: A systematic review. *Surv Ophthalmol.* **2022**, *67* (4), 1229−1243.

(19) Teo, A. W. J.; Zhang, J.; Zhou, L.; Liu, Y. C. Metabolomics in Corneal Diseases: A Narrative Review from Clinical Aspects. *Metabolites* **2023**, *13* (3), 380.

(20) Zhang, Q.; Wang, N.; Rui, Y.; Xia, Y.; Xiong, S.; Xia, X. New insight of metabolomics in ocular diseases in the context of 3P medicine. *EPMA Journal* **2023**, *14* (1), 53−71.

(21) Cai, J.; Estes, A.; Liu, Y. Omics Analyses in Keratoconus: from Transcriptomics to Proteomics. *Curr. Ophthalmol Rep.* **2020**, *8* (4), 216−225.

(22) Grochowski, E. T.; Pietrowska, K.; Kowalczyk, T.; Mariak, Z.; Kretowski, A.; Ciborowski, M.; Dmuchowska, D. A. Omics in Myopia. *Journal of Clinical Medicine* **2020**, *9* (11), 3464.

(23) Tan, S. Z.; Begley, P.; Mullard, G.; Hollywood, K. A.; Bishop, P. N. Introduction to metabolomics and its applications in ophthalmology. *Eye (Basingstoke)* **2016**, *30* (6), 773−783.

(24) Yazdani, M.; Elgstøen, K. B. P.; Rootwelt, H.; Shahdadfar, A.; Utheim, ØA; Utheim, T. P. Tear Metabolomics in Dry Eye Disease: A Review. *International Journal of Molecular Sciences* **2019**, *20* (15), 3755.

(25) Jay, N. L.; Gillies, M. Proteomic analysis of ophthalmic disease. *Clin Exp Ophthalmol.* **2012**, *40* (7), 755−763.

(26) Hou, X. W.; Wang, Y.; Wu, Q.; Ke, C.; Pan, C. W. A review of study designs and data analyses in metabolomics studies in myopia. *Anal. Biochem.* **2022**, *655*, No. 114850.

(27) Nava, A. A.; Arboleda, V. A. The omics era: a nexus of untapped potential for Mendelian chromatinopathies. *Human Genetics* **2023**, 1−21..

(28) Zanella, D.; Henin, A.; Mascrez, S.; et al. Comprehensive two-dimensional gas chromatographic platforms comparison for exhaled breath metabolites analysis. *J. Sep Sci.* **2022**, *45* (18), 3542−3555.

(29) Virgiliou, C.; Kanelis, D.; Pina, A.; et al. A targeted approach for studying the effect of sugar bee feeding on the metabolic profile of Royal Jelly. *J. Chromatogr A* **2020**, *1616*, No. 460783.
(30) Kohler, I.; Verhoeven, M.; Haselberg, R.; Gargano, A. F. G. Hydrophilic interaction chromatography − mass spectrometry for metabolomics and proteomics: state-of-the-art and current trends. *Microchemical Journal.* **2022**, *175*, No. 106986.
(31) Pirok, B. W. J.; Stoll, D. R.; Schoenmakers, P. J. Recent Developments in Two-Dimensional Liquid Chromatography: Fundamental Improvements for Practical Applications. *Anal. Chem.* **2019**, *91* (1), 240−263.
(32) Feng, J.; Zhong, Q.; Kuang, J.; Liu, J.; Huang, T.; Zhou, T. Simultaneous Analysis of the Metabolome and Lipidome Using Polarity Partition Two-Dimensional Liquid Chromatography-Mass Spectrometry. *Anal. Chem.* **2021**, *93* (45), 15192−15199.
(33) Kohler, I.; Giera, M. Recent advances in liquid-phase separations for clinical metabolomics. *J. Sep Sci.* **2017**, *40* (1), 93−108.
(34) Røberg-Larsen, H.; Lundanes, E.; Nyman, T. A.; Berven, F. S.; Wilson, S. R. Liquid chromatography, a key tool for the advancement of single-cell omics analysis. *Anal. Chim. Acta* **2021**, *1178*, No. 338551.
(35) Zaikin, V. G.; Borisov, R. S. Mass Spectrometry as a Crucial Analytical Basis for Omics Sciences. *J. Anal Chem.* **2021**, *76* (14), 1567−1587.
(36) Sivanich, M. K.; Gu, T. J.; Tabang, D. N.; Li, L. Recent advances in isobaric labeling and applications in quantitative proteomics. *Proteomics.* **2022**, *22* (19−20), No. 2100256.
(37) Dunn, W. B.; Broadhurst, D. I.; Atherton, H. J.; Goodacre, R.; Griffin, J. L. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem. Soc. Rev.* **2011**, *40* (1), 387−426.
(38) Issaq, H. J.; Van, Q. N.; Waybright, T. J.; Muschik, G. M.; Veenstra, T. D. Analytical and statistical approaches to metabolomics research. *J. Sep Sci.* **2009**, *32* (13), 2183−2199.
(39) Aebersold, R.; Mann, M. Mass-spectrometric exploration of proteome structure and function. *Nature* **2016**, *537* (7620), 347−355.
(40) Sneekes, E. J. Nano LC: Principles, Evolution, and State-of-the-Art of the Technique. *LCGC North America* **2011**, *29* (10), 926−934.
(41) Dupree, E. J.; Jayathirtha, M.; Yorkey, H.; Mihasan, M.; Petre, B. A.; Darie, C. C. A Critical Review of Bottom-Up Proteomics: The Good, the Bad, and the Future of This Field. *Proteomes* **2020**, *8* (3), 14.
(42) Wu, S.; Ma, H.; Prytkova, I.; Stenoien, D.; Paša-Tolić, L. Proteomics, Top-Down. *Encyclopedia of Spectroscopy and Spectrometry* **2017**, 774−778.
(43) Du, C. X.; Huang, Z. Analysis of amino acids in human tears by hydrophilic interaction liquid chromatography and quadrupole orbitrap mass spectrometry. *RSC Adv.* **2019**, *9* (63), 36539−36545.
(44) Wishart, D. S.; Jewison, T.; Guo, A. C.; et al. HMDB 3.0—The Human Metabolome Database in 2013. *Nucleic Acids Res.* **2012**, *41* (D1), D801−D807.
(45) Wilson, I. D.; Plumb, R.; Granger, J.; Major, H.; Williams, R.; Lenz, E. M. HPLC-MS-based methods for the study of metabonomics. *Journal of Chromatography B* **2005**, *817* (1), 67−76.
(46) Tolstikov, V. V.; Fiehn, O. Analysis of highly polar compounds of plant origin: Combination of hydrophilic interaction chromatography and electrospray ion trap mass spectrometry. *Anal. Biochem.* **2002**, *301* (2), 298−307.
(47) Tang, D. Q.; Zou, L.; Yin, X. X.; Ong, C. N. HILIC-MS for metabolomics: An attractive and complementary approach to RPLC-MS. *Mass Spectrom Rev.* **2016**, *35* (5), 574−600.
(48) Han, X. *Lipidomics: Comprehensive Mass Spectrometry of Lipids*; John Wiley & Sons, Inc., 2016; pp 1−466.
(49) Macabuhay, A.; Arsova, B.; Walker, R.; Johnson, A.; Watt, M.; Roessner, U. Modulators or facilitators? Roles of lipids in plant root−microbe interactions. *Trends Plant Sci.* **2022**, *27* (2), 180−190.
(50) Butovich, I. A. On the Lipid Composition of Human Meibum and Tears: Comparative Analysis of Nonpolar Lipids. *Invest Ophthalmol Vis Sci.* **2008**, *49* (9), 3779−3789.
(51) Rantamäki, A. H.; Seppänen-Laakso, T.; Oresic, M.; Jauhiainen, M.; Holopainen, J. M. Human Tear Fluid Lipidome: From Composition to Function. *PLoS One* **2011**, *6* (5), No. e19553.
(52) Kaluzny, M. A.; Duncan, L. A.; Merritt, M. V.; Epps, D. E. Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J. Lipid Res.* **1985**, *26* (1), 135−140.
(53) Ruiz-Gutiérrez, V.; Pérez-Camino, M. C. Update on solid-phase extraction for the analysis of lipid classes and related compounds. *J. Chromatogr A* **2000**, *885* (1−2), 321−341.
(54) Malavolta, M.; Bocci, F.; Boselli, E.; Frega, N. G. Normal phase liquid chromatography−electrospray ionization tandem mass spectrometry analysis of phospholipid molecular species in blood mononuclear cells: application to cystic fibrosis. *J. Chromatogr B* **2004**, *810* (2), 173−186.
(55) Nakamura, T.; Bratton, D. L.; Murphy, R. C. Analysis of Epoxyeicosatrienoic and Monohydroxyeicosatetraenoic Acids EsteriÐed to Phospholipids in Human Red Blood Cells by Electrospray Tandem Mass Spectrometry. *J. Mass Spec.* **1997**, *32*, 888−896.
(56) Fernández, A. S.; Rodríguez-González, P.; Álvarez, L.; García, M.; Iglesias, H. G.; García Alonso, J. I. Multiple heart-cutting two dimensional liquid chromatography and isotope dilution tandem mass spectrometry for the absolute quantification of proteins in human serum. *Anal. Chim. Acta* **2021**, *1184*, No. 339022.
(57) Gilar, M.; Olivova, P.; Daly, A. E.; Gebler, J. C. Two-dimensional separation of peptides using RP-RP-HPLC system with different pH in first and second separation dimensions. *J. Sep Sci.* **2005**, *28* (14), 1694−1703.
(58) Yazdani, M.; Elgstøen, K. B. P.; Rootwelt, H.; Shahdadfar, A.; Utheim, ØA; Utheim, T. P. Tear Metabolomics in Dry Eye Disease: A Review. *Int. J. Mol. Sci.* **2019**, *20* (15), 3755.
(59) Wróbel-Dudzińska, D.; Kubik-Komar, A.; Rykwa, D.; Kosior-Jarecka, E.; Żarnowski, T.; Chałas, R. The use of Schirmer strips to measure salivary and lacrimal flow in non-Sjögren patients. *Clin Oral Investig.* **2021**, *25* (6), 4107−4114.
(60) Koduri, M. A.; Prasad, D.; Pingali, T.; et al. Optimization and evaluation of tear protein elution from Schirmer's strips in dry eye disease. *Indian J. Ophthalmol.* **2023**, *71* (4), 1413−1419.
(61) Pandolfi, M.; Astrup, T. A histochemical study of the fibrinolytic activity. Cornea, conjunctiva, and lacrimal gland. *Arch Ophthalmol.* **1967**, *77* (2), 258−264.
(62) Small, D.; Hevy, J.; Tang-Liu, D. Comparison of tear sampling techniques for pharmacokinetics analysis: ofloxacin concentrations in rabbit tears after sampling with schirmer tear strips, capillary tubes, or surgical sponges. *J. Ocul Pharmacol Ther.* **2000**, *16* (5), 439−446.
(63) Mann, A.; Tighe, B. J. Wound healing studies and interfacial phenomena: use and relevance of the corneal model. *Advanced Wound Repair Therapies* **2011**, 284−320.
(64) Chen, J.; Nichols, K. K.; Wilson, L.; Barnes, S.; Nichols, J. J. Untargeted lipidomic analysis of human tears: A new approach for quantification of O-acyl-omega hydroxy fatty acids. *Ocul Surf.* **2019**, *17* (2), 347−355.
(65) Ngo, W.; Chen, J.; Panthi, S.; Nichols, K. K.; Nichols, J. J. Comparison of Collection Methods for the Measure of Human Meibum and Tear Film-Derived Lipids Using Mass Spectrometry. *Curr. Eye Res.* **2018**, *43* (10), 1244−1252.
(66) Rossi, C.; Cicalini, I.; Cufaro, M. C. Multi-omics approach for studying tears in treatment-naïve glaucoma patients. *Int. J. Mol. Sci.* **2019**, *20* (16), 4029.
(67) Balaiya, S.; Zhou, Z.; Chalam, K. V. Characterization of vitreous and aqueous proteome in humans with proliferative diabetic retinopathy and its clinical correlation. *Proteomics Insights.* **2017**, *8* (1), 1−10.
(68) Brownridge, P.; Beynon, R. J. The importance of the digest: proteolysis and absolute quantification in proteomics. *Methods* **2011**, *54* (4), 351−360.

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259

(69) Aass, C.; Norheim, I.; Eriksen, E. F.; Thorsby, P. M.; Pepaj, M. Single unit filter-aided method for fast proteomic analysis of tear fluid. *Anal. Biochem.* **2015**, *480*, 1−5.
(70) Jones, G.; Lee, T. J.; Glass, J.; Rountree, G.; Ulrich, L.; Estes, A.; Sezer, M.; Zhi, W.; Sharma, S.; Sharma, A. Comparison of Different Mass Spectrometry Workflows for the Proteomic Analysis of Tear Fluid. *Int. J. Mol. Sci. 2022, Vol 23, Page 2307* **2022**, *23* (4), 2307.
(71) Chen, L.; Zhou, L.; Chan, E. C. Y.; Neo, J.; Beuerman, R. W. Characterization of the human tear metabolome by LC-MS/MS. *J. Proteome Res.* **2011**, *10* (10), 4876−4882.
(72) Ó Fearghail, F.; Behan, P.; Engström, N.; Scheers, N. A LCMS Metabolomic Workflow to Investigate Metabolic Patterns in Human Intestinal Cells Exposed to Hydrolyzed Crab Waste Materials. *Front Bioeng Biotechnol.* **2021**, *9*, No. 629083.
(73) Folch, J.; Lees, M.; Stanley, G. H. S. A SIMPLE METHOD FOR THE ISOLATION AND PURIFICATION OF TOTAL LIPIDES FROM ANIMAL TISSUES. *J. Biol. Chem.* **1957**, *226* (1), 497−509.
(74) Wong, M. W. K.; Braidy, N.; Pickford, R.; Sachdev, P. S.; Poljak, A. Comparison of single phase and biphasic extraction protocols for lipidomic studies using human plasma. *Front Neurol.* **2019**, *10* (AUG), No. 458366.
(75) Thulasi, P.; Djalilian, A. R. Update in Current Diagnostics and Therapeutics of Dry Eye Disease. *Ophthalmology.* **2017**, *124* (11), S27−S33.
(76) Lam, S. M.; Tong, L.; Duan, X.; Petznick, A.; Wenk, M. R.; Shui, G. Extensive characterization of human tear fluid collected using different techniques unravels the presence of novel lipid amphiphiles. *J. Lipid Res.* **2014**, *55* (2), 289−298.
(77) Chhadva, P.; Goldhardt, R.; Galor, A. Meibomian Gland Disease: The Role of Gland Dysfunction in Dry Eye Disease. *Ophthalmology.* **2017**, *124* (11), S20−S26.
(78) Baird, P. N.; Saw, S.-M.; Lanca, C.; Guggenheim, J. A.; Smith III, E. L.; Zhou, X.; Matsui, K.-O.; Wu, P.-C.; Sankaridurg, P.; Chia, A.; et al. Myopia. *Nature Reviews Disease Primers 2020 6:1* **2020**, *6* (1), 1−20.
(79) Chauhan, M. Z.; Valencia, A. K.; Piqueras, M. C.; Enriquez-Algeciras, M.; Bhattacharya, S. K. Optic Nerve Lipidomics Reveal Impaired Glucosylsphingosine Lipids Pathway in Glaucoma. *Invest Ophthalmol Vis Sci.* **2019**, *60* (5), 1789−1798.
(80) Weinreb, R. N.; Aung, T.; Medeiros, F. A. The pathophysiology and treatment of glaucoma: a review. *JAMA* **2014**, *311* (18), 1901−1911.
(81) Anderson, J. R.; Phelan, M. M.; Clegg, P. D.; Peffers, M. J.; Rubio-Martinez, L. M. Synovial Fluid Metabolites Differentiate between Septic and Nonseptic Joint Pathologies. *J. Proteome Res.* **2018**, *17* (8), 2735−2743.
(82) Davidson, A. E.; Hayes, S.; Hardcastle, A. J.; Tuft, S. J. The pathogenesis of keratoconus. *Eye 2014 28:2* **2014**, *28* (2), 189−195.
(83) Canamary, A. M.; Takahashi, W. Y.; Sallum, J. M. F. Autoimmune retinopathy: A Review. *Int. J. Retina Vitreous.* **2018**, *4* (1), 1−6.
(84) Soria, J.; Acera, A.; Merayo-LLoves, J.; Duran, J. A.; Gonzalez, N.; Rodriguez, S.; Bistolas, N.; Schumacher, S.; Bier, F. F.; Peter, H.; et al. Tear proteome analysis in ocular surface diseases using label-free LC-MS/MS and multiplexed-microarray biomarker validation. *Scientific Reports 2017 7:1* **2017**, *7* (1), 1−15.
(85) Aretz, S.; Krohne, T. U; Kammerer, K.; Warnken, U.; Hotz-Wagenblatt, A.; Bergmann, M.; Stanzel, B. V; Kempf, T.; Holz, F. G; Schnolzer, M.; Kopitz, J. In-depth mass spectrometric mapping of the human vitreous proteome. *Proteome Sci.* **2013**, *11* (1), 1−10.
(86) Mohanty, V.; Pinto, S. M.; Subbannayya, Y.; et al. Digging Deeper for the Eye Proteome in Vitreous Substructures: A High-Resolution Proteome Map of the Normal Human Vitreous Base. *OMICS* **2020**, *24* (6), 379−389.
(87) Perumal, N.; Funke, S.; Wolters, D.; Pfeiffer, N.; Grus, F. H. Characterization of human reflex tear proteome reveals high expression of lacrimal proline-rich protein 4 (PRR4). *Proteomics* **2015**, *15* (19), 3370−3381.
(88) de Souza, G. A.; Godoy, L. M. F.; Mann, M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. *Genome Biol.* **2006**, *7* (8), R72.
(89) Byambajav, M.; Arroyo-del Arroyo, C.; Enriquez-de-Salamanca, A.; Fernandez, I.; Martin, E.; Hagan, S. Enríquez-de-salamanca A, Fernández I, Martin E, Hagan S. A pilot proteomic study of normal human tears: Leptin as a potential biomarker of metabolic disorders. *Appl. Sci.* **2021**, *11* (12), 5755.
(90) Al-Moujahed, A.; Velez, G.; Vu, J. T.; Lima de Carvalho, J. R.; Levi, S. R.; Bassuk, A. G.; Sepah, Y. J.; Tsang, S. H.; Mahajan, V. B. Proteomic analysis of autoimmune retinopathy implicates NrCAM as a potential biomarker. *Ophthalmol Sci.* **2022**, *2* (2), 100131.
(91) Liu, H.; Anders, F.; Funke, S.; Mercieca, K.; Grus, F.; Prokosch, V. Proteome alterations in aqueous humour of primary open angle glaucoma patients. *Int. J. Ophthalmol.* **2020**, *13* (1), 176−179.
(92) Ma, J.; Sze, Y.; Bian, J.; Lam, T. Critical role of mass spectrometry proteomics in tear biomarker discovery for multifactorial ocular diseases (Review). *Int. J. Mol. Med.* **2021**, *47* (5), 1−15.
(93) Azkargorta, M.; Soria, J.; Acera, A.; Iloro, I.; Elortza, F. Human tear proteomics and peptidomics in ophthalmology: Toward the translation of proteomic biomarkers into clinical practice. *J. Proteomics.* **2017**, *150*, 359−367.
(94) Sambursky, R.; Davitt, W. F.; Friedberg, M.; Tauber, S. Prospective, multicenter, clinical evaluation of point-of-care matrix metalloproteinase-9 test for confirming dry eye disease. *Cornea.* **2014**, *33* (8), 812−818.
(95) Ji, Y. W.; Kim, H. M.; Ryu, S. Y.; et al. Changes in Human Tear Proteome Following Topical Treatment of Dry Eye Disease: Cyclosporine A Versus Diquafosol Tetrasodium. *Invest Ophthalmol Vis Sci.* **2019**, *60* (15), 5035−5044.
(96) Nättinen, J.; Jylhä, A.; Aapola, U.; et al. Topical fluorometholone treatment and desiccating stress change inflammatory protein expression in tears. *Ocul Surf.* **2018**, *16* (1), 84−92.
(97) Zou, C.; Han, C.; Zhao, M.; et al. Change of ranibizumab-induced human vitreous protein profile in patients with proliferative diabetic retinopathy based on proteomics analysis. *Clin Proteomics.* **2018**, *15* (1), 1−10.
(98) Nicholson, J. K.; Holmes, E.; Kinross, J. M.; Darzi, A. W.; Takats, Z.; Lindon, J. C. Metabolic phenotyping in clinical and surgical environments. *Nature.* **2012**, *491* (7424), 384−392.
(99) Dammeier, S.; Martus, P.; Klose, F.; et al. Combined Targeted Analysis of Metabolites and Proteins in Tear Fluid With Regard to Clinical Applications. *Transl Vis Sci. Technol.* **2018**, *7* (6), 22−22.
(100) Zhang, W.; Li, H.; Ogando, D. G.; et al. Glutaminolysis is Essential for Energy Production and Ion Transport in Human Corneal Endothelium. *EBioMedicine.* **2017**, *16*, 292−301.
(101) Craig, J. P.; Nichols, K. K.; Akpek, E. K.; et al. TFOS DEWS II Definition and Classification Report. *Ocul Surf.* **2017**, *15* (3), 276−283.
(102) Chen, X.; Rao, J.; Zheng, Z.; et al. Integrated Tear Proteome and Metabolome Reveal Panels of Inflammatory-Related Molecules via Key Regulatory Pathways in Dry Eye Syndrome. *J. Proteome Res.* **2019**, *18* (5), 2321−2330.
(103) Dai, L.; Yang, W.; Qin, X.; et al. Serum metabolomics profiling and potential biomarkers of myopia using LC-QTOF/MS. *Exp. Eye Res.* **2019**, *186*, No. 107737.
(104) Barbas-Bernardos, C.; Armitage, E. G.; García, A.; et al. Looking into aqueous humor through metabolomics spectacles − exploring its metabolic characteristics in relation to myopia. *J. Pharm. Biomed Anal.* **2016**, *127*, 18−25.
(105) Tang, Y.; Pan, Y.; Chen, Y.; et al. Metabolomic Profiling of Aqueous Humor and Plasma in Primary Open Angle Glaucoma Patients Points Towards Novel Diagnostic and Therapeutic Strategy. *Front Pharmacol.* **2021**, *12*, No. 621146.
(106) Buisset, A.; Gohier, P.; Leruez, S.; et al. Metabolomic Profiling of Aqueous Humor in Glaucoma Points to Taurine and Spermine

Deficiency: Findings from the Eye-D Study. *J. Proteome Res.* **2019**, *18* (3), 1307−1315.
(107) Kouassi Nzoughet, J.; Guehlouz, K.; Leruez, S.; Gohier, P.; Bocca, C.; Muller, J.; Blanchet, O.; Bonneau, D.; Simard, G.; Milea, D.; et al. A Data Mining Metabolomics Exploration of Glaucoma. *Metabolites* **2020**, *10* (2), 49.
(108) Javadiyan, S.; Burdon, K. P.; Whiting, M. J.; et al. Elevation of Serum Asymmetrical and Symmetrical Dimethylarginine in Patients with Advanced Glaucoma. *Invest Ophthalmol Vis Sci.* **2012**, *53* (4), 1923−1927.
(109) Yoshikawa, T.; Obayashi, K.; Miyata, K.; Saeki, K.; Ogata, N. Association Between the Asymmetric Dimethylarginine Levels and Glaucoma Severity: A Cross-Sectional Analysis of the LIGHT Study. *Invest Ophthalmol Vis Sci.* **2021**, *62* (4), 7−7.
(110) Firat, P. G.; Demirel, E. E.; Demirel, S.; Dikci, S.; Turkoz, Y.; Ozyalın, F. Increased Aqueous Humor Symmetric Dimethylarginine Level in Patients with Primary Open Angle Glaucoma. *Curr. Eye Res.* **2019**, *44* (6), 619−622.
(111) Haines, N. R.; Manoharan, N.; Olson, J. L.; D'Alessandro, A.; Reisz, J. A. Metabolomics Analysis of Human Vitreous in Diabetic Retinopathy and Rhegmatogenous Retinal Detachment. *J. Proteome Res.* **2018**, *17* (7), 2421−2427.
(112) Yumnamcha, T.; Guerra, M.; Singh, L. P.; Ibrahim, A. S. Metabolic Dysregulation and Neurovascular Dysfunction in Diabetic Retinopathy. *Antioxidants* **2020**, *9* (12), 1244.
(113) Narayanan, S. P.; Rojas, M.; Suwanpradid, J.; Toque, H. A.; Caldwell, R. W.; Caldwell, R. B. Arginase in retinopathy. *Prog. Retin Eye Res.* **2013**, *36*, 260−280.
(114) Al-Shabrawey, M.; Smith, S. Prediction of diabetic retinopathy: Role of oxidative stress and relevance of apoptotic biomarkers. *EPMA Journal.* **2010**, *1* (1), 56−72.
(115) Karamichos, D.; Zieske, J. D.; Sejersen, H.; Sarker-Nag, A.; Asara, J. M.; Hjortdal, J. Tear metabolite changes in keratoconus. *Exp. Eye Res.* **2015**, *132*, 1−8.
(116) Nishtala, K.; Pahuja, N.; Shetty, R.; Nuijts, R. M. M. A.; Ghosh, A. Nuijts RMMA, Ghosh A. Tear biomarkers for keratoconus. *Eye and Vision.* **2016**, *3* (1), 1−7.
(117) Rantamäki, A. H.; Seppänen-Laakso, T.; Oresic, M.; Jauhiainen, M.; Holopainen, J. M. Human Tear Fluid Lipidome: From Composition to Function. *PLoS One.* **2011**, *6* (5), No. e19553.
(118) Lam, S. M.; Tong, L.; Reux, B.; Lear, M. J.; Wenk, M. R.; Shui, G. Rapid and sensitive profiling of tear wax ester species using high performance liquid chromatography coupled with tandem mass spectrometry. *J. Chromatogr A* **2013**, *1308*, 166−171.
(119) Cwiklik, L. Tear film lipid layer: A molecular level view. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2016**, *1858* (10), 2421−2430.
(120) Chen, J.; Nichols, K. K.; Wilson, L.; Barnes, S.; Nichols, J. J. Untargeted lipidomic analysis of human tears: A new approach for quantification of O-acyl-omega hydroxy fatty acids. *Ocul Surf.* **2019**, *17* (2), 347−355.
(121) Rohit, A.; Stapleton, F.; Brown, S. H. J.; Mitchell, T. W.; Willcox, M. D. P. Comparison of tear lipid profile among basal, reflex, and flush tear samples. *Optom Vis Sci.* **2014**, *91* (12), 1391−1395.
(122) Catanese, S.; Khanna, R. K.; Lefevre, A. Validation of metabolomic and lipidomic analyses of human tears using ultra-high-performance liquid chromatography tandem mass spectrometry. *Talanta* **2023**, *253*, 123932.
(123) Brown, S. H. J.; Kunnen, C. M. E.; Papas, E. B.; et al. Intersubject and Interday Variability in Human Tear and Meibum Lipidomes: A Pilot Study. *Ocul Surf.* **2016**, *14* (1), 43−48.
(124) Panthi, S.; Chen, J.; Wilson, L.; Nichols, J. J. Detection of Lipid Mediators of Inflammation in the Human Tear Film. *Eye Contact Lens* **2019**, *45* (3), 171−181.
(125) Shim, J.; Park, C.; Lee, H. S.; et al. Change in Prostaglandin Expression Levels and Synthesizing Activities in Dry Eye Disease. *Ophthalmology.* **2012**, *119* (11), 2211−2219.
(126) Pieragostino, D.; Agnifili, L.; Cicalini, I.; Calienno, R.; Zucchelli, M.; Mastropasqua, L.; Sacchetta, P.; Del Boccio, P.; Rossi, C. Tear Film Steroid Profiling in Dry Eye Disease by Liquid Chromatography Tandem Mass Spectrometry. *Int. J. Mol. Sci.* **2017**, *18* (7), 1349.
(127) Lam, S. M.; Tong, L.; Reux, B.; et al. Lipidomic analysis of human tear fluid reveals structure-specific lipid alterations in dry eye syndrome. *J. Lipid Res.* **2014**, *55* (2), 299−306.
(128) Chhadva, P.; Goldhardt, R.; Galor, A. Meibomian Gland Disease: The Role of Gland Dysfunction in Dry Eye Disease. *Ophthalmology* **2017**, *124* (11), S20−S26.
(129) Amano, S.; Inoue, K. Estimation of Prevalence of Meibomian Gland Dysfunction in Japan. *Cornea.* **2017**, *36* (6), 684−688.
(130) Kuriakose, R. K.; Braich, P. S. Dyslipidemia and its Association with Meibomian Gland Dysfunction: A Systematic Review. *Int. Ophthalmol.* **2018**, *38* (4), 1809−1816.
(131) Suzuki, T.; Kitazawa, K.; Cho, Y.; et al. Alteration in meibum lipid composition and subjective symptoms due to aging and meibomian gland dysfunction. *Ocul Surf.* **2022**, *26*, 310−317.
(132) Cabrerizo, J.; Urcola, J. A.; Vecino, E. Changes in the Lipidomic Profile of Aqueous Humor in Open-Angle Glaucoma. *J. Glaucoma* **2017**, *26* (4), 349−355.
(133) BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem Physiol.* **1959**, *37* (8), 911−917.
(134) Azbukina, N. V.; Chistyakov, D. V.; Goriainov, S. V.; Kotelin, V. I.; Fedoseeva, E. V.; Petrov, S. Y.; Sergeeva, M. G.; Iomdina, E. N.; Zernii, E. Y.. Targeted lipidomic analysis of aqueous humor reveals signaling lipid-mediated pathways in primary open-angle glaucoma. *Biology (Basel)* **2021**, *10* (7), 658.
(135) Umeno, A.; Tanito, M.; Kaidzu, S.; Takai, Y.; Horie, M.; Yoshida, Y. Comprehensive measurements of hydroxylinoleate and hydroxyarachidonate isomers in blood samples from primary open-angle glaucoma patients and controls. *Scientific Reports* **2019**, *9* (1), 1−11.

https://doi.org/10.1021/acsmeasuresciau.3c00067
ACS Meas. Sci. Au 2024, 4, 247−259