Alteration of Tear Metabolomics Profiling in Infants With Retinopathy of Prematurity

Zixin Fan,[1] Shuo Yang,[1] Xiaofeng Lu,[1] Sifan Zhang,[2] Xianlu Zeng,[1] Lin Lin,[3] Duo Yuan,[1] Jian Zeng,[1] and Guoming Zhang[1]

[1]Shenzhen Eye Hospital, Shenzhen Eye Medical Center, Southern Medical University, Shenzhen, Guangdong, China
[2]New York University, New York, New York, United States
[3]Sustech Core Research Facilities, Southern University of Science and Technology, Shenzhen, Guangdong, China

Correspondence: Guoming Zhang, Shenzhen Eye Hospital, Shenzhen Eye Medical Center, Southern Medical University, 18 Zetian Rd., Futian District, Shenzhen, Guangdong 518040, China; zhang-guoming@163.com.

ZF and SY have shared first authorship.

Received: December 24, 2024
Accepted: May 28, 2025
Published: June 20, 2025

Citation: Fan Z, Yang S, Lu X, et al. Alteration of tear metabolomics profiling in infants with retinopathy of prematurity. *Invest Ophthalmol Vis Sci.* 2025;66(6):61. https://doi.org/10.1167/iovs.66.6.61

Purpose. The purpose of this study was to investigate the characteristics of metabolomic profile in the tears from infants with retinopathy of prematurity (ROP) and further define noninvasive biomarkers.

Methods. This prospective study included 94 eyes from 47 premature infants. Tear samples from 23 infants with ROP (15 treatment-requiring ones [ROP $_{(t)}$] and 8 non-treatment-requiring ones [ROP $_{(nt)}$]) and 24 infants without ROP were collected with Schirmer strips after topical anesthesia. Detailed fundus examination upon pupil dilation were conducted, with data on demographics and birth history recorded. Untargeted tear metabolomic analysis based on ultra-performance liquid chromatography-mass spectrometry was performed.

Results. Adjusted by sex, postmenstrual age, gestational age, and birth weight, among 145 metabolites quantified, 3,5-di-tert-butyl-4-hydroxybenzaldehyde, caffeine, and trehalose were identified to be upregulated in the tears from infants with ROP when compared to those from premature infants with non-ROP, whereas uric acid, dihomo-γ-linolenic acid, nootkatone, pyridoxal, ornithine, 6-keto-prostaglandin F1 alpha, adenosine, and tetrahydrocortisol were downregulated (all adjusted false discovery rate [FDR $_{adj}$] < 0.05). Nine and one dysregulated metabolites were further found in the ROP $_{(t)}$ and ROP $_{(nt)}$ subgroups, respectively. Some of these metabolites exhibited expression levels correlated with postmenstrual age, gestational age, birth weight, and ROP severity in varying degrees. The dysregulated tear metabolites were fundamentally related to vitamin B6, purine, caffeine, arginine, and starch and sucrose metabolism pathways. The 6-keto-prostaglandin F1 alpha obtained the best performance in classifying premature infants with and without ROP (area under the curve [AUC] of 0.893 [range = 0.800–0.986], $P < 0.001$).

Conclusions. Dysregulated tear metabolites in ROP exhibited abundance correlated with severity of the disease and might be noninvasive biomarkers that potentially serve as complementary screening tools.

Keywords: retinopathy of prematurity (ROP), tear fluid, metabolomics, noninvasive biomarkers

As the leading cause of blindness in childhood worldwide,[1,2] retinopathy of prematurity (ROP) is a proliferative vitreoretinopathy affecting premature or low birth weight infants. The disease is characterized by abnormal development of retinal blood vessels and neovascularization that can lead to subsequent retinal detachment and ultimately visual loss.[2,3] Approximately 101.6 thousand cases of vision impairment are due to new-onset ROP per year around the world. It is estimated that by 2050, the prevalence of moderate to severe visual loss and blindness caused by ROP will reach nearly 1%.[4]

To date, unavailable, missed, and erratic screening and unclear pathogenesis remain challenges in ROP management.[5] Early and appropriate screening is crucial for timely treatment to prevent permanent and irreversible visual loss.[3,6,7] But due to a paucity of resources, including infrastructure and experienced pediatric ophthalmologists, missed or inaccurate diagnoses occurred and ultimately led to delays in treatment or unnecessary interventions.[5] Therefore, developing objective, cost-effective, convenient, novel screening and diagnostic approaches are urgently needed. Regarding the pathogenesis, ROP is considered as a multifactorial disease that is associated with oxidative stress, inflammatory response, and angiogenesis.[8–11] Further research is required to clarify the exact pathophysiological mechanisms.

Tears are essential biological components that lubricate, protect, and nourish the ocular surface, and contain various metabolites, lipid, proteins, and electrolytes.[12] Despite the small volume for sampling, tears demonstrate unique advantages for biochemical analysis to address the current

Copyright 2025 The Authors

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Downloaded from iovs.arvojournals.org on 06/21/2025

challenges in developing novel diagnostic techniques and therapeutic targets in ocular diseases.[13] In omics studies on biomarker identification and mechanism exploration, healthy control samples are essential for comparative analysis. Tears can be easily, noninvasively, and safely obtained in healthy subjects, whereas other ocular biofluids such as aqueous and vitreous humor require invasive collection in patient cohorts during surgeries. Furthermore, the identified tear biomarker could exhibit translational potential to develop noninvasive and cost-effective diagnostic tools widely applied with minimum hazard, particularly in vulnerable populations such as infants. So, the tear is an ideal sample for biomarker discovery and mechanism elucidation of ROP.

As end-products or intermediates of processes interacted with both genetic and environmental factors, metabolites are closely related with the phenotypes,[14] especially in multifactorial diseases like ROP. The omics approach is a new field of biochemical investigations.[15] However, alteration of tear metabolomics profiling in infants with ROP remains unclear. Herein, this study aimed to investigate a global metabolomic profile in tear fluid from infants with ROP by untargeted metabolomic analysis based on ultra-performance liquid chromatography (UPLC)-mass spectrometry (MS) and to identify the tear differentially expressed metabolites in ROP, which could be potential noninvasive biomarkers and might further provide an insight into the underlying molecular mechanisms of the disease.

Materials and Methods

Study Design and Participants

This prospective study adhered to the principles of the Declaration of Helsinki[16] and was approved by the ethics committee of the Shenzhen Eye Hospital, Shenzhen Eye Medical Center, Southern Medical University, Shenzhen, Guangdong, China (k20211109-03; ChiCTR2100054263). Informed consents from the guardians of all the participants were obtained.

From May 2023 to April 2024, premature infants were recruited from outpatients seeking ROP screening in the pediatric retinal department in Shenzhen Eye Hospital. Infants with a birth weight of $\leq$ 1500 g or a gestational age (GA) of $\leq$ 30 weeks and infants with a birth weight of 1500 to 2000 g or a GA of $>$ 30 weeks that were at risk for ROP[17] were enrolled. Full-term infants or subjects with the following conditions were excluded: other vitreo-retinal diseases; other ocular diseases; a history of ocular trauma or surgery (such as laser photocoagulation, intravitreal injection, and vitrectomy); prenatal maternal infections (such as gonorrhea, syphilis, acquired immunodeficiency syndrome, etc.) or inflammatory conditions; congenital metabolic diseases; and unstable vital signs (such as sepsis, necrotizing enterocolitis, neonatal respiratory distress syndrome, and severe metabolic disorders, etc.).

Screening and Diagnosis of ROP

All the enrolled participants received standardized follow-up and screening of ROP in line with international guidelines[3,17] (Fig. 1). Diagnosis of ROP was established according to the International Classification of ROP, Third Edition,[3] based on detailed fundus examination upon pupil dilation (Supplementary Table S1). The ROP group was further divided into two subgroups as treatment-requiring ROP infants (ROP $_{(t)}$) and non-treatment-requiring ROP infants (ROP $_{(nt)}$). In addition, the severity of ROP was graded as in the previous study from 0 to 5 (see Supplementary Table S1).[12,18]

Tear Sampling

At the visits, tear samples were collected before screening for ROP to avoid the impact of ocular contact procedures, such as pediatric wide-field fundus imaging (see Fig. 1). It meant that all the samples were collected prior to any anticipated treatment. A Schirmer strip (Jingming, Tianjin, China) was applied into the inferior-temporal aspect of the conjunctival sac. By performing the Schirmer I test, with topical anesthesia, the basal tear fluids in both eyes were all collected in the morning at 9 AM to 11 AM to reduce the related bias of intra-day variability. The wetted length was recorded after 5 minutes or at completion. To prevent further metabolic changes, all the strips with tear samples were aliquoted, frozen, and stored immediately after sampling at −80°C until used, avoiding repeated freeze-thaw cycles.

Data Collection

Detailed ocular examination, including fundus examination upon pupil dilation, were performed to each participant at each visit, by using a binocular indirect ophthalmoscope (Heine Optotechnik, Bavaria, Germany) and a Retcam 3 (Natus Medical, Pleasanton, CA, USA). The screening was conducted by two experienced pediatric retinal specialists independently, and the results were subsequently confirmed and recorded by both specialists.

Data on demographics (sex and postmenstrual age at the time of sampling), birth history (GA, birth weight, maternal age, multiple or single pregnancy, artificial or natural insemination, and cesarean section or spontaneous labor), and medical history (including both ophthalmic and systemic history) were collected. Postmenstrual age refers to GA

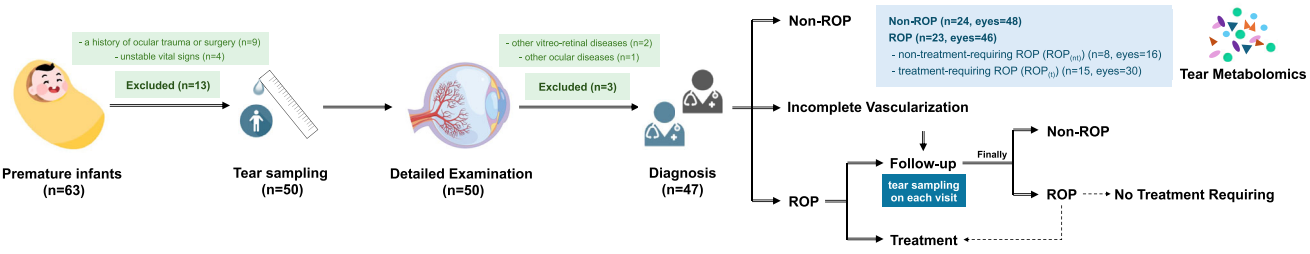


Figure 1. Study enrollment, intervention, and analysis. ROP, retinopathy of prematurity.

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

plus chronological age and can serve as a critical biological variable that standardized developmental trajectories of the preterm infants.

Sample Preparation

Schirmer strips were cut into pieces and incubated in methanol/water (1:1, v/v; 400 µL per 10 mm length of strip) to extract tear metabolites.[19] Preparations were stirred for 30 minutes and centrifuged for 10 minutes at 4000 × *g*, and the supernatant was collected. After vacuum freeze dried, each of the prepared samples were finally redissolved in 100 µL methanol/water (1:1, v/v) at equal concentrations.

Ultra-Performance Liquid Chromatography-Mass Spectrometry

An untargeted metabolomics analysis was conducted by using an UltiMate 3000 liquid chromatography system (Thermo Fisher Scientific, Waltham, MA, USA) and a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) interfaced with a heated electrospray ionization source. The prepared samples and quality controls were analyzed. A 2.1 mm × 100 mm ACQUIY UPLC BEH 1.7 µm column (Waters Corporation, Milford, MA, USA) was used for separation at a flow rate of 0.3 mL/min. The mobile phases were 0.1% formic acid in water (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The gradient was set as follows: 0 to 2 minutes, 2% B; 2 to 5 minutes, 2% to 25% B; 5 to 11 minutes, 25% to 98% B; 11 to 14 minutes, 98% B; 14 to 14.3 minutes, 98% to 2% B, and 14.3 to 17 minutes, 2% B. The injection volume was 3 µL, and the column temperature was maintained at 35°C.

The mass spectrometer was operated under positive–negative ion switching mode with the scan range of 70 to 1050 m/z at a resolution of 35,000. The spray voltages were set to 4.0 kilovolt (kV) and the capillary temperature was 320°C. Tandem mass spectroscopy (MS/MS) information was acquired using high-energy collision dissociation with stepped normalized collision energy of 15 to 45 eV. Data were acquired with Xcalibur software (Thermo Fisher Scientific, Waltham, MA, USA). The raw data were processed using Compound Discoverer 3.2 (Thermo Fisher Scientific, Waltham, MA, USA) to generate a multivariate data matrix. The super classes and species of metabolites were based on the peaks in MS data with a combination of the online database HMDB and PubChem.

Sample Size

A total of 63 subjects who met the inclusion criteria were approached. Two and one individuals were excluded owing to other vitreo-retinal diseases and other ocular diseases, respectively. Whereas nine individuals were excluded owing to a history of ocular trauma or surgery. In addition, four individuals were excluded because of unstable vital signs. Ultimately, the untargeted metabolomics analysis was performed for the remaining 47 samples, including 23 samples (46 eyes) from infants with ROP (15 samples from $ROP_{(t)}$ and 8 samples from $ROP_{(nt)}$) and 24 samples (48 eyes) from non-ROP premature infants (see Fig. 1).

Statistical Analysis

The characteristics were described as mean ± SDs for continuous data and frequency (percentage) for categorical data. Shapiro-Wilk test and histograms were used to check the normality of continuous data. Student's *t*-test and 1-way ANOVA were used to compare two and more than two continuous variables that normally distributed, correspondingly; and the Mann-Whitney *U* test and Kruskal-Wallis test were used to compare two and more than two continuous variables that non-normally distributed, correspondingly. Besides, Bonferroni correction was used for post hoc test with multiple comparison correction for different groups. Benjamini-Hochberg correction that controls the false discovery rate (FDR) was used for multiple testing for different metabolites. Categorical variables were compared using Pearson's χ^2 test and Fisher's exact test (if the sample sizes were small, that is expected frequencies were less than or equal to 5).

Orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot was established for class discrimination, with the variable importance in projection (VIP) revealing the importance of a metabolite in the OPLS-DA model.[20] According to the OPLS-DA model (VIP $\geq$ 1) together with adjusted FDR value (adjusted by sex, postmenstrual age, GA, and birth weight; $FDR_{adj} < 0.05$), differentially expressed tear metabolites were identified, with Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis further developed. Spearman rank correlation coefficient was performed to evaluate the correlation between tear metabolites and demographic characteristics and ROP severity. The predictive accuracy of the dysregulated tear metabolites for classifying ROP and non-ROP (after adjusted by sex, postmenstrual age, GA, and birth weight) was assessed by receiver operating characteristic (ROC) analysis, and the related area under the curve (AUC) was calculated. A 2-sided *P* value of less than 0.05 was considered statistically significant.

All analyses were performed with IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA), MetaboAnalyst 6.0 (www.metaboanalyst.ca; McGill University, Quebec, Canada) and R 3.4.0 (The R Foundation, Indianapolis, IN, USA), together with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and R 3.4.0 (The R Foundation, Indianapolis, IN, USA) for graphical representation.

Results

Participants

A total of 47 tear samples (94 eyes) were analyzed, including 23 samples (46 eyes) from infants with ROP and 24 samples (48 eyes) from non-ROP premature infants. For the ROP group, 15 samples were from $ROP_{(t)}$ and 8 samples were from $ROP_{(nt)}$. The sex (8 [34.78%] female infants vs. 8 [33.33%] female infants, $P = 0.917$), postmenstrual ages at the time of sampling (39.42 ± 3.41 vs. 40.93 ± 2.78, $P = 0.101$), and maternal ages (30.65 ± 2.79 vs. 31.25 ± 4.87, $P = 0.607$) were matched between the ROP and the non-ROP group. Compared to the non-ROP group, the ROP group has smaller GA (27.41 ± 2.86 vs. 30.86 ± 2.87, $P < 0.001$) and lower birth weight (1128.65 ± 687.25 vs. 1504.58 ± 532.55, $P < 0.001$), especially in the $ROP_{(t)}$ subgroup. The result of the Schirmer II test did not differ significantly between the ROP and the non-ROP infants (15.57 ± 8.14 vs. 14.67 ± 7.72,

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

Table 1. Demographic and Clinical Characteristics of Participants (n = 47; 94 Eyes)

Variables	Non-ROP (n = 24; 48 Eyes)	ROP (n = 23; 46 Eyes)			P_1 (ROP Vs. Non-ROP)	P_2 (ROP $_{(t)}$ Vs. ROP $_{(nt)}$ Vs. Non-ROP)
		Total	ROP $_{(nt)}$ (n = 8; 16 Eyes)	ROP $_{(t)}$ (n = 15; 30 Eyes)		
Female sex, n (%)	8 (33.33)	8 (34.78)	1 (12.50)	7 (46.67)	0.917*	0.268‡
Postmenstrual age at time of sampling, mean ± SD, wk	40.93 ± 2.78	39.42 ± 3.41	42.41 ± 3.46	37.82 ± 2.11	0.101	<0.001†
Gestational age, mean ± SD, wk	30.86 ± 2.87	27.41 ± 2.86	29.20 ± 3.32	26.46 ± 2.12	<0.001	<0.001†
Birth weight, mean ± SD, kg	1504.58 ± 532.55	1128.65 ± 687.25	1681.00 ± 921.11	834.07 ± 225.08	<0.001	0.001†
Maternal age, mean ± SD, y	31.25 ± 4.87	30.65 ± 2.79	30.88 ± 2.70	30.53 ± 2.92	0.607	0.863†
Multiple pregnancy, n (%)	7 (29.17)	4 (17.39)	1 (12.50)	3 (20.00)	0.341*	0.724‡
Artificial insemination, n (%)	1 (4.17)	7 (30.43)	1 (12.50)	6 (40.00)	0.023‡	0.018‡
Delivery mode (cesarean section), n (%)	12 (50.00)	5 (21.74)	3 (37.50)	2 (13.33)	0.044*	0.086‡
Schirmer I test with topical anesthesia, mean ± SD, mm	14.67 ± 7.72	15.57 ± 8.14	16.94 ± 8.52	14.83 ± 8.14	0.700	0.776†
ROP severity, median (IQR)	0.00 (0.00–0.00)	2.00 (1.00–3.00)	1.00 (1.00–1.75)	3.00 (2.00–3.00)	<0.001§	<0.001¶

ROP, retinopathy of prematurity; ROP $_{(t)}$, treatment-requiring ROP; ROP $_{(nt)}$, non-treatment-requiring ROP; SD, standard deviation; IQR, interquartile range.

* Pearson's χ^2 test.

† One-way ANOVA.

‡ Fisher's exact test.

§ Mann-Whitney U test.

¶ Kruskal-Wallis test; Student's t-test was used except additional mentioned.

P = 0.700). The demographic and clinical characteristics of the participants are shown in Table 1.

Classification of Metabolites

Untargeted metabolomic analysis identified and quantified 10 super classes, 145 metabolites in the tears from the 2 groups, most of which were lipids and lipid-like molecules (34.48%), followed by organic acids and derivatives (21.38%; Fig. 2A). Organoheterocyclic compounds (13.79%), benzenoids (12.41%), organic oxygen compounds (7.59%), organic nitrogen compounds (4.14%), and other super classes were also detected.

Differentially Expressed Metabolites' Identification

As shown in Figure 2B, the OPLS-DA score plot revealed a separate clustering between premature infants with and without ROP (R2Ycum 0.750, P = 0.003; Q2 0.445, P < 0.001). According to the OPLS-DA model together with adjusted FDR value (adjusted by sex, postmenstrual age, GA, and birth weight), 11 differentially expressed tear metabolites were finally identified for the ROP group when compared to the non-ROP group. The 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO; VIP = 2.933, FDR $_{adj}$ = 0.017), caffeine (VIP = 2.647, FDR $_{adj}$ = 0.017), and trehalose (VIP = 1.789, FDR $_{adj}$ = 0.019) were upregulated, whereas uric acid (VIP = 2.521, FDR $_{adj}$ = 0.012), dihomo-γ-linolenic acid (DGLA; VIP = 2.435, FDR $_{adj}$ = 0.012), nootkatone (VIP = 2.361, FDR $_{adj}$ = 0.017), pyridoxal (VIP = 2.282, FDR $_{adj}$ = 0.024), ornithine (VIP = 2.026, FDR $_{adj}$ = 0.018), 6-keto-prostaglandin F1 alpha (6-keto-PGF1 alpha; VIP = 1.962, FDR $_{adj}$ = 0.012), adenosine (VIP = 1.900, FDR $_{adj}$ = 0.023), and tetrahydrocortisol (VIP = 1.889, FDR $_{adj}$ = 0.031) were downregulated (Fig. 2C, Table 2). Most of the dysregulated metabolites were classified as lipids and lipid-like molecules (4/11, 36.36%), followed by organoheterocyclic compounds (3/11, 27.27%) and organic oxygen compounds (2/11, 18.18%). In addition, a single dysregulated metabolite was identified within nucleosides, nucleotides, and analogues (1/11, 9.09%), whereas another is categorized into organic acids and derivatives (1/11, 9.09%; see Table 2). The principal component analysis score plots (Figs. 2D, 2E) showed that the ROP group was obviously detached from the non-ROP group based on the differentially expressed metabolites.

Metabolic Profiling of Treatment-Requiring and Non-Treatment-Requiring ROP Subgroups

Comparative metabolomic profiling revealed distinct metabolic signatures between subgroups, adjusted by sex, postmenstrual age, GA, and birth weight. When compared to non-ROP controls, nine metabolites showed alteration in the ROP $_{(t)}$ subgroup, whereas only one metabolite exhibited significant differential abundance in the ROP $_{(nt)}$ subgroup (Supplementary Tables S2, S3). The alterations of caffeine (FDR $_{adj}$ = 0.003), DGLA (FDR $_{adj}$ = 0.001), BHT-CHO (FDR $_{adj}$ = 0.012), uric acid (FDR $_{adj}$ = 0.012), pyridoxal (FDR $_{adj}$ = 0.039), nootkatone (FDR $_{adj}$ = 0.039), tetrahydrocortisol (FDR $_{adj}$ = 0.039), and 6-keto-PGF1 alpha (FDR $_{adj}$ = 0.039) that were identified in total ROP infants were also found to be remarkable in the ROP $_{(t)}$ subgroup (Fig. 3A), but not in the ROP $_{(nt)}$ subgroup (all FDR $_{adj}$ >0.05; Figs. 3B, 3C). In contrast, trehalose was downregulated in the ROP $_{(nt)}$ subgroup (FDR $_{adj}$ = 0.012), and the difference of its expression between the ROP $_{(t)}$ subgroup and the non-ROP group was not significant (FDR $_{adj}$ > 0.05; see Figs. 3B, 3C).

Correlation Analysis Between Dysregulated Tear Metabolites and Demographic Characteristics Together With Systemic Conditions

The correlation between dysregulated tear metabolites and demographic characteristics are shown as Figure 4. Postmenstrual age was positively correlated with the expression of uric acid (r = 0.501, P < 0.001), DGLA (r = 0.430, P = 0.003), adenosine (r = 0.386, P = 0.007), tetrahydrocortisol (r = 0.392, P = 0.006), pyridoxal (r = 0.344, P = 0.018), and aminocaproic acid (r = 0.338, P = 0.020), and was negatively

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

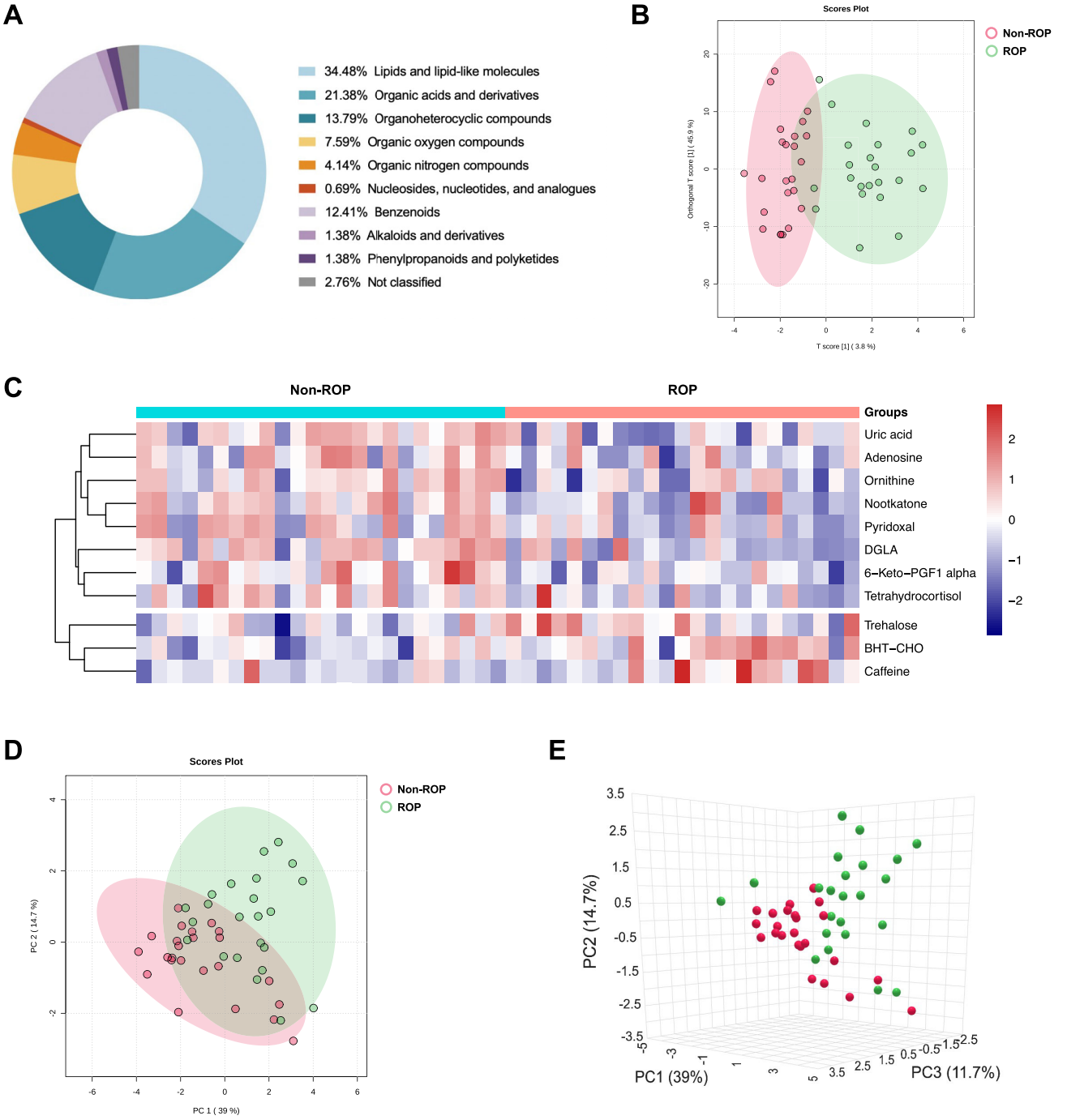


Figure 2. Classification of metabolites and differentially expressed metabolites in the tears from infants wth ROP. (**A**). All tear metabolites super classes detected in the premature infants. (**B**) OPLS-DA score plot of tear samples from the ROP group and the non-ROP group. The *green dots* represent ROP (n = 23), and the *red dots* represent non-ROP (n = 24). OPLS-DA, orthogonal partial least squares-discriminant analysis. (**C**) Heat map of the tear differentially expressed metabolites for ROP (n = 23) compared to non-ROP (n = 24). The color bar indicates the relative expression level. *Red* indicates high expression and *blue* indicates low expression. DGLA, dihomo-γ-linolenic acid; 6-Keto-PGF1 alpha, 6-keto-prostaglandin F1 alpha; BHT-CHO, 3,5-Di-tert-butyl-4-hydroxybenzaldehyde. (**D**) Principal component analysis of the metabolite profiles. The *green dots* represent ROP (n = 23), and the *red dots* represent non-ROP (n = 24). (**E**) Three-dimensional plot of principal component analysis of the metabolite profiles. The *green dots* represent ROP (n = 23), and the *red dots* represent non-ROP (n = 24).

correlated with caffeine intensity ($r = -0.488$, $P < 0.001$). Besides, GA was negatively correlated with the expression of caffeine ($r = -0.422$, $P = 0.003$) and BHT-CHO ($r = -0.351$, $P = 0.016$) and was positively correlated with that of nootkatone ($r = 0.333$, $P = 0.022$). Caffeine ($r = -0.372$, $P = 0.010$) and BHT-CHO ($r = -0.303$, $P = 0.039$) intensities were also identified to be negatively correlated with birth weight. However, all the identified dysregulated tear

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

Table 2. Differentially Expressed Tear Metabolites Identified for ROP to Non-ROP

Metabolites	VIP	Adjusted *P* Value*	Adjusted FDR*	Dysregulated in ROP	Super Class
BHT-CHO	2.933	0.006	0.017	Upregulated	Organic oxygen compounds
Caffeine	2.647	0.007	0.017	Upregulated	Organoheterocyclic compounds
Trehalose	1.789	0.010	0.019	Upregulated	Organic oxygen compounds
Uric acid	2.521	0.002	0.012	Downregulated	Organoheterocyclic compounds
DGLA	2.435	<0.001	0.012	Downregulated	Lipids and lipid-like molecules
Nootkatone	2.361	0.005	0.017	Downregulated	Lipids and lipid-like molecules
Pyridoxal	2.282	0.016	0.024	Downregulated	Organoheterocyclic compounds
Ornithine	2.026	0.008	0.018	Downregulated	Organic acids and derivatives
6-Keto-PGF1 alpha	1.962	0.002	0.012	Downregulated	Lipids and lipid-like molecules
Adenosine	1.900	0.014	0.023	Downregulated	Nucleosides, nucleotides, and analogues
Tetrahydrocortisol	1.889	0.023	0.031	Downregulated	Lipids and lipid-like molecules

BHT-CHO, 3,5-Di-tert-butyl-4-hydroxybenzaldehyde; DGLA, dihomo-γ-linolenic acid;FDR, false discovery rate; ROP, retinopathy of prematurity; VIP, variable importance in projection; 6-Keto-PGF1 alpha, 6-keto-prostaglandin F1 alpha.
*Adjusted by sex, postmenstrual age, gestational age, and birth weight.

metabolites exhibited expression levels that were not significantly associated with sex (all $P > 0.05$). Moreover, correlations were further found among some of the differentially expressed metabolites.

We further investigated the clinical correlations between the identified tear dysregulated metabolites and bronchopulmonary dysplasia (BPD), a systemic complication prevalent in preterm infants. A significant elevation in tear BHT-CHO ($r = 0.477$, $P = 0.001$) and caffeine ($r = 0.434$, $P = 0.002$) concentrations and a reduction in tear ornithine ($r = -0.384$, $P = 0.008$), tetrahydrocortisol ($r = -0.365$, $P = 0.012$), DGLA ($r = -0.362$, $P = 0.012$), aminocaproic acid ($r = -0.325$, $P = 0.026$), and uric acid ($r = -0.303$, $P = 0.039$) abundances were observed in infants with BPD than those without BPD.

Correlation Analysis Between Dysregulated Tear Metabolites and ROP Severity

The expression of dysregulated tear metabolites was associated with ROP severity expect for that of trehalose ($P = 0.200$; Figs. 5A, 5B). To be specific, caffeine ($r = 0.425$, $P = 0.003$) and BHT-CHO ($r = 0.421$, $P = 0.003$) were expressed in positive correlation with ROP severity. In addition, the expression of DGLA ($r = -0.504$, $P < 0.001$), uric acid ($r = -0.502$, $P < 0.001$), nootkatone ($r = -0.468$, $P < 0.001$), 6-keto-PGF1 alpha ($r = -0.445$, $P = 0.002$), tetrahydrocortisol ($r = -0.413$, $P = 0.004$), pyridoxal ($r = -0.399$, $P = 0.006$), adenosine ($r = -0.395$, $P = 0.006$), aminocaproic acid ($r = -0.351$, $P = 0.015$), and ornithine ($r = -0.336$, $P = 0.021$) were negatively correlated with ROP severity.

Functional Enrichment Analysis

Functional annotation of the KEGG pathway for the dysregulated tear metabolites indicated involvement of vitamin B6 metabolism, purine metabolism, caffeine metabolism, arginine biosynthesis, starch and sucrose metabolism, glutathione metabolism, arginine and proline metabolism, biosynthesis of unsaturated fatty acids, and steroid hormone biosynthesis (Fig. 6A).

Potential Biomarkers Selection

In Figure 6B, the ROC curves were shown for each of the identified differentially expressed tear metabolites for ROP to non-ROP (after adjusted by sex, postmenstrual age, GA, and birth weight). The best classification performance was obtained by 6-keto-PGF1 alpha in discriminating premature infants with ROP from that without ROP (AUC of 0.893 [range = 0.800–0.986], $P < 0.001$). Other tear metabolites additionally had good performance in classifying ROP and non-ROP with AUCs of more than 0.8 (all $P < 0.05$).

Discussion

To our best knowledge, this is the first study that carries out a comprehensive analysis of the tear metabolic signature for ROP with untargeted metabolomics. After adjusted by sex, postmenstrual age, GA, and birth weight, 11 differentially expressed tear metabolites were identified for ROP to non-ROP, with 9 and 1 dysregulated metabolites further found in the ROP $_{(t)}$ and ROP $_{(nt)}$ subgroups, respectively. Some of these metabolites exhibited expression levels correlated with postmenstrual age, GA, birth weight, and ROP severity in varying degrees. The dysregulated tear metabolites were involved in several metabolism pathways, such as vitamin B6, purine, caffeine, arginine, and starch and sucrose metabolism, and so on.

ROP is the leading cause of potentially preventable childhood blindness worldwide.[3,7] Early screening is crucial because ROP could be successfully treated if in a timely manner.[3,7] In this instance, as ophthalmic biofluid that can be easily and noninvasively obtained, the tear is an ideal alternative for ROP screening sampling. Our study identified tear metabolites that differentially expressed in ROP. With AUCs of more than 0.8, these metabolites had good performance in discriminating premature infants with ROP from that without ROP, suggesting tear dysregulated metabolites might be potential biomarkers contributing to ROP screening. The dysregulated tear metabolites as promising noninvasive biomarkers could be applied in developing complementary screening tools for establishing decentralized ROP diagnostic systems, especially in low-resource settings where fundus photography and experienced pediatric ophthalmologists are unavailable. Besides, the utility of the identified biomarkers for predicting subsequent ROP at birth remains uncertain. This study was conducted in a tertiary ophthalmic center by recruiting infants from outpatients that were seeking ROP screening. Therefore, there is a lack of tear samples at the time of birth for assessing the predictive value of these tear metabolites. Further inves-

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

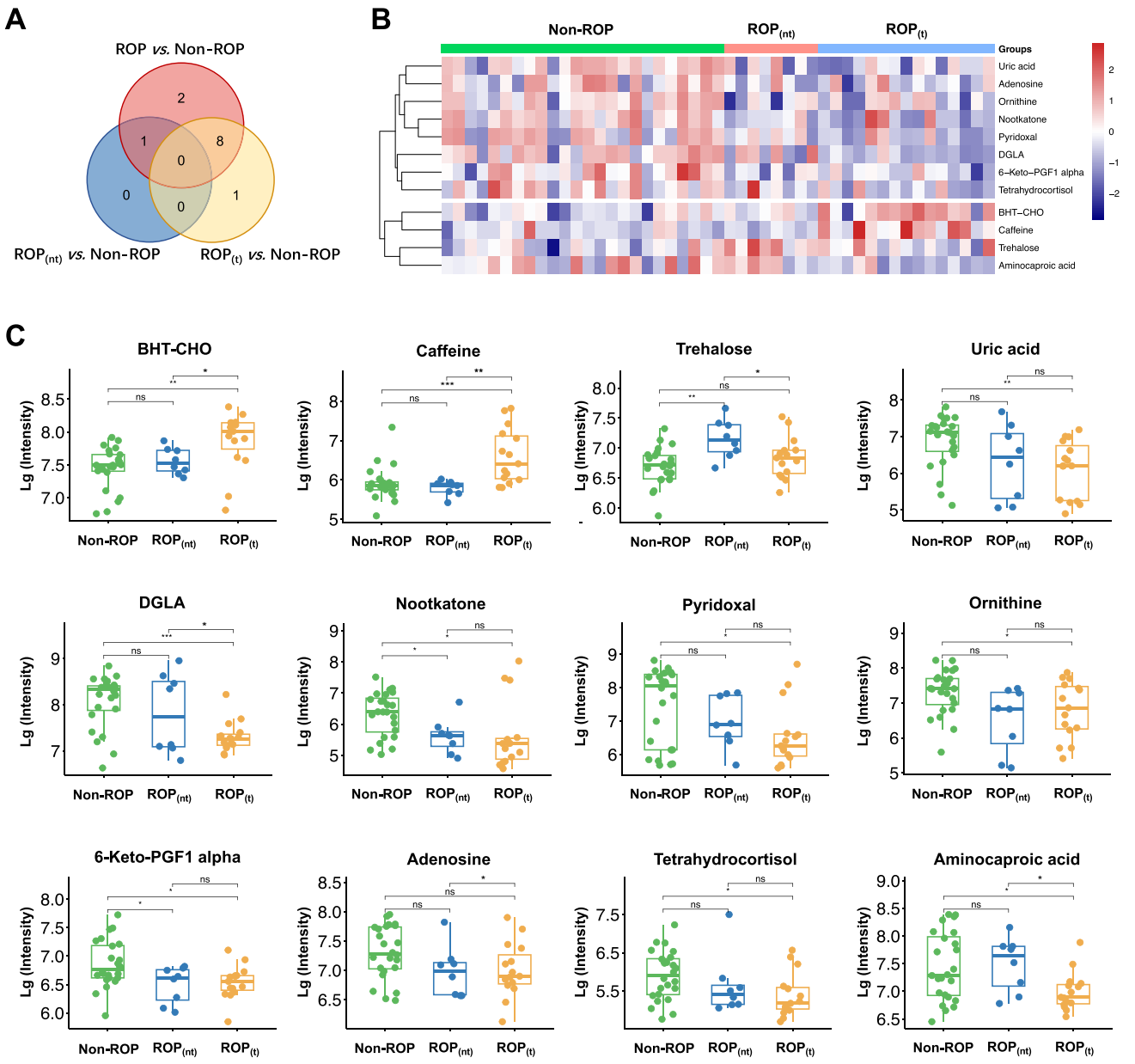


Figure 3. Metabolic profiling of the ROP (t) and the ROP (nt) subgroups. ROP (t), treatment-requiring ROP; ROP (nt), non-treatment-requiring ROP. (**A**) Venn diagram of differentially expressed proteins when compared the ROP (t) ($n = 15$) and the ROP (nt) ($n = 8$) subgroups with the non-ROP group ($n = 24$), respectively. (**B**) Heat map of all the tear differentially expressed metabolites. The color bar indicates the relative expression level. *Red* indicates high expression and *blue* indicates low expression. (**C**) Boxplots for the UPLC-MS quantitative data (Lg(intensity), median) for the tear differentially expressed metabolites (ROP (t), $n = 15$; ROP (nt), $n = 8$; and non-ROP, $n = 24$). *P* value was adjusted by sex, postmenstrual age, GA, and birth weight. *** $P_{adj} < 0.001$, ** $P_{adj} < 0.01$, * $P_{adj} < 0.5$.

tigation in large-scale prospective clinical cohorts involving neonatology is needed, to explore their potential as biomarkers for early prediction.

It is noteworthy that sex, postmenstrual age, GA, and birth weight were adjusted in this study when making comparisons. The enrolled ROP group, especially the ROP (t) subgroup, has smaller GA and lower birth weight than the enrolled non-ROP group, which is consistent with epidemiological characteristics of ROP.[7] The evidence from previous studies,[21,22] including ours,[23] revealed that tear components are associated with demographic data. Thus, an adjustment was made to get more reliable results. Interestingly, our findings of further correlation analysis in turn validated the necessity of adjustment. Some of the top dysregulated tear metabolites that identified in ROP exhibited intensities correlated with postmenstrual age, GA, or birth weight. However, all the identified dysregulated tear metabolites exhibited expression levels that were not significantly associated with sex. According to previous studies, the impact of sex on tear metabolites remains contentious.[21] It has been reported that sex-related differences of the tear lipid profile were observed in the older age groups,[24] although other studies failed to detect any sex-related variability on tear metabolites.[25,26] This phenomenon might be associated

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

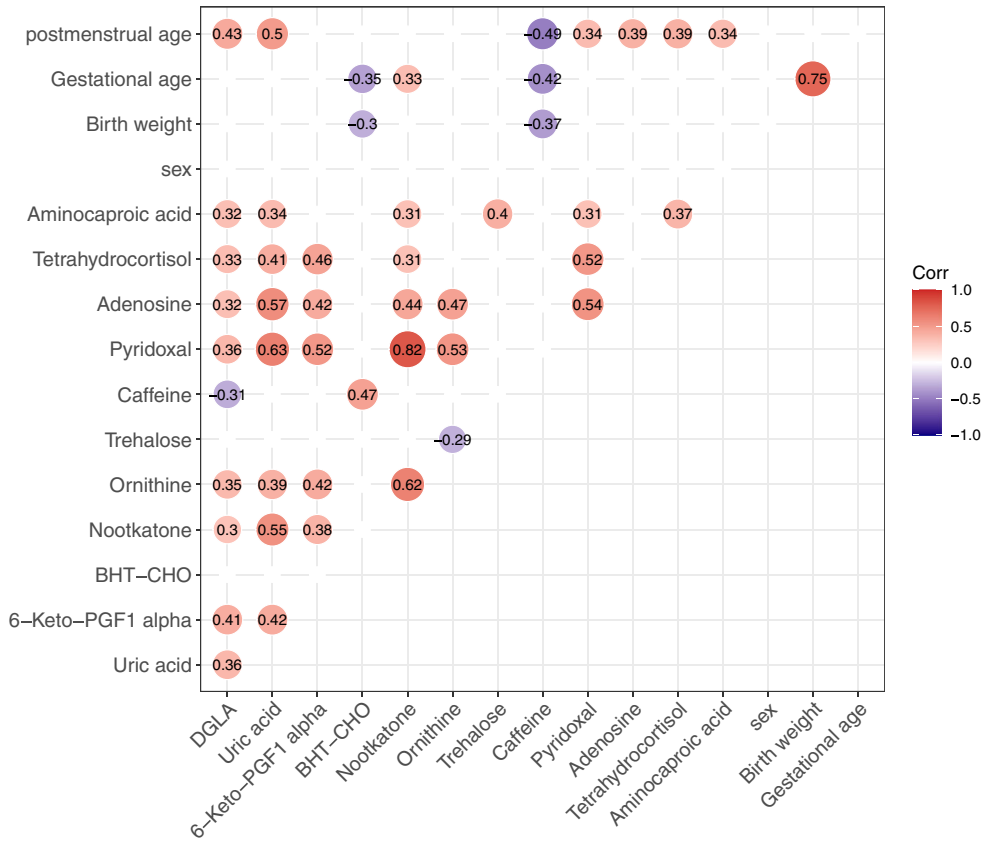


Figure 4. Correlation analysis between dysregulated tear metabolites and demographic characteristics. The color bar indicates the relative correlation coefficient. *Red* indicates high correlation coefficient and *blue* indicates low correlation coefficient.

with different targeted metabolites and different enrolled subjects.

According to the principal component analysis score plot, the first 2 principal components explained 53.7% of total variance, indicating substantial structural heterogeneity within the multidimensional tear metabolic signatures in preterm infants. The overlap between ROP and non-ROP groups might be a result from phenotypic continuum. In particular, although without ROP, the enrolled individuals in the non-ROP group were also preterm infants at high risk of the disease, but not full-term infants. The overlap implied that ROP progression might follow a pathophysiological continuum, rather than a discrete dichotomy of "affected" versus "unaffected" patients.

The alterations of uric acid,[27] ornithine,[18] adenosine,[27] and caffeine[18] of tears in the present study were consistent with that of plasma in previous metabolomics studies. Ornithine is an amino acid converted from arginine and is also a precursor of citrullines and arginine. The arginine and proline metabolism pathway was identified to be an important pathway in the occurrence and development of rat oxygen-induced retinopathy.[28] As a metabolic intermediary, adenosine is distributed ubiquitously throughout the body and can regulate vessel growth of various tissues, including the retina in a complicated way.[29] It is reported that adenosine was an endogenous protective regulator in oxygen-induced retinopathy.[30] Caffeine is a non-selective adenosine receptor antagonist that can modulate angiogenesis.[29] Recently, treatment of apnea of prematurity with caffeine has been found to additionally reduce ROP-related problems.[31] But caffeine was identified to be upregulated in the tears (this study) and plasma[18] from ROP infants. This might be because more participants had been treated with caffeine for comorbidities, such as BPD, in the ROP group than that in the non-ROP group. In addition, the positive correlation between tear caffeine concentration and BPD in this study could support this hypothesis to some extent.

Vitamin B6 metabolism was the top involved pathway of the dysregulated metabolites. Pyridoxal, one of the major forms of vitamin B6, was identified to be downregulated in the tears from infants with ROP and its expression was negatively correlated with ROP severity. It has been revealed that pyridoxal plays a crucial role in protecting cells from oxidative stress as effective lipid glycation inhibitors and exhibits beneficial effects on diabetic complications, including diabetic retinopathy, which is another retinopathy characterized by retinal neovascularization that is similar to ROP.[32,33]

The inflammatory process was reported as an important contributor to ROP.[9] Our results indicated that as anti-inflammatory factors, DGLA and 6-keto-PGF1 alpha might be associated with ROP onset. DGLA can be enzymatically converted to several metabolites with anti-inflammatory effects, including 6-keto-PGF1 alpha.[34,35] Decreased level of plasma DGLA has been reported in diabetic complications, atopic dermatitis, cardiac disease, etc.[34] In addition, it is known that in response to hypoxia, which plays a critical role in the nature of ROP, reduced production of

Downloaded from iovs.arvojournals.org on 06/21/2025

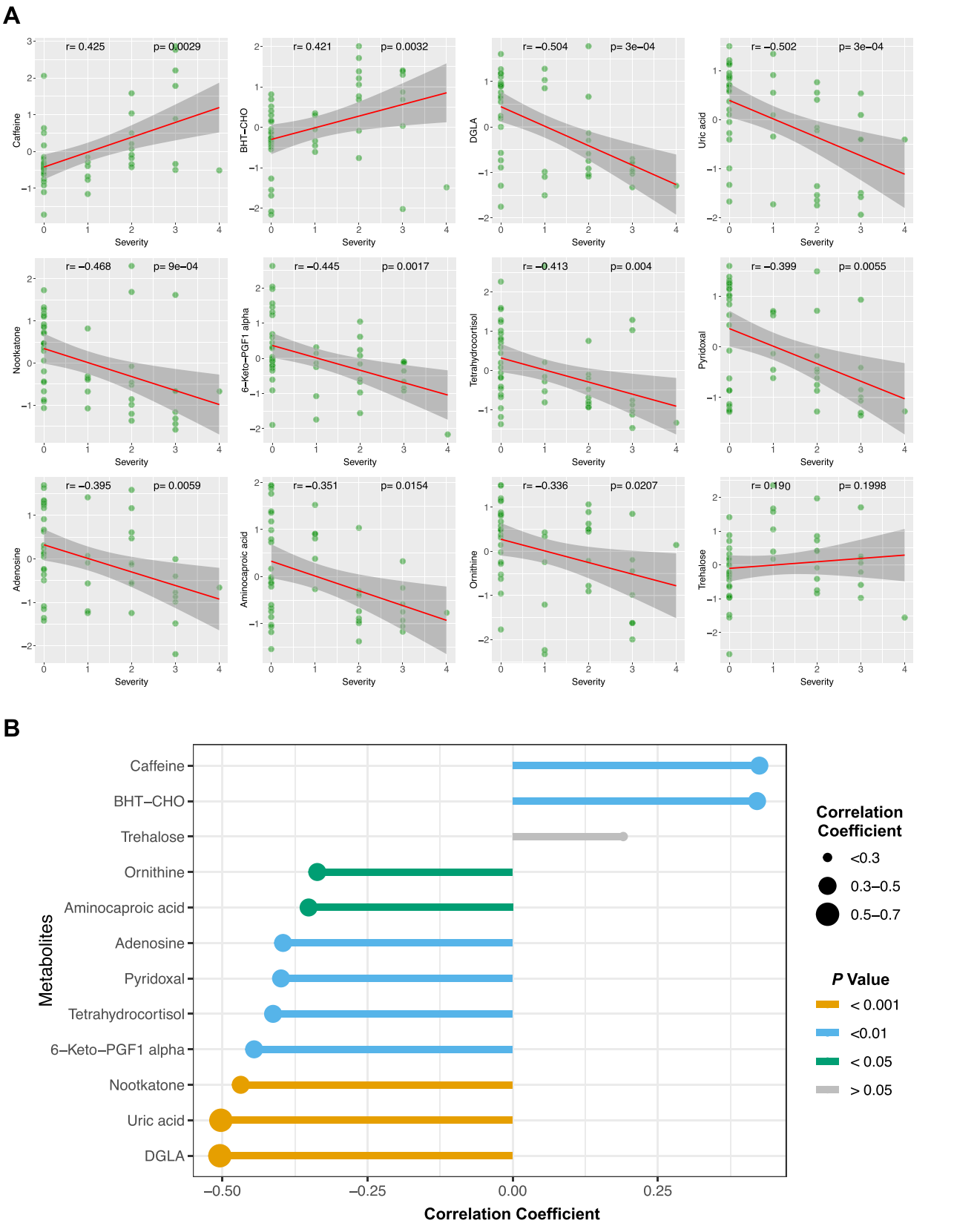


Figure 5. Correlation analysis between dysregulated tear metabolites and ROP severity. (**A**) Scatter plots for correlation analysis between the expression of tear differentially expressed metabolites and severity of ROP ($n = 47$). (**B**) Correlation coefficient of the expression of tear differentially expressed metabolites and severity of ROP ($n = 47$).

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025

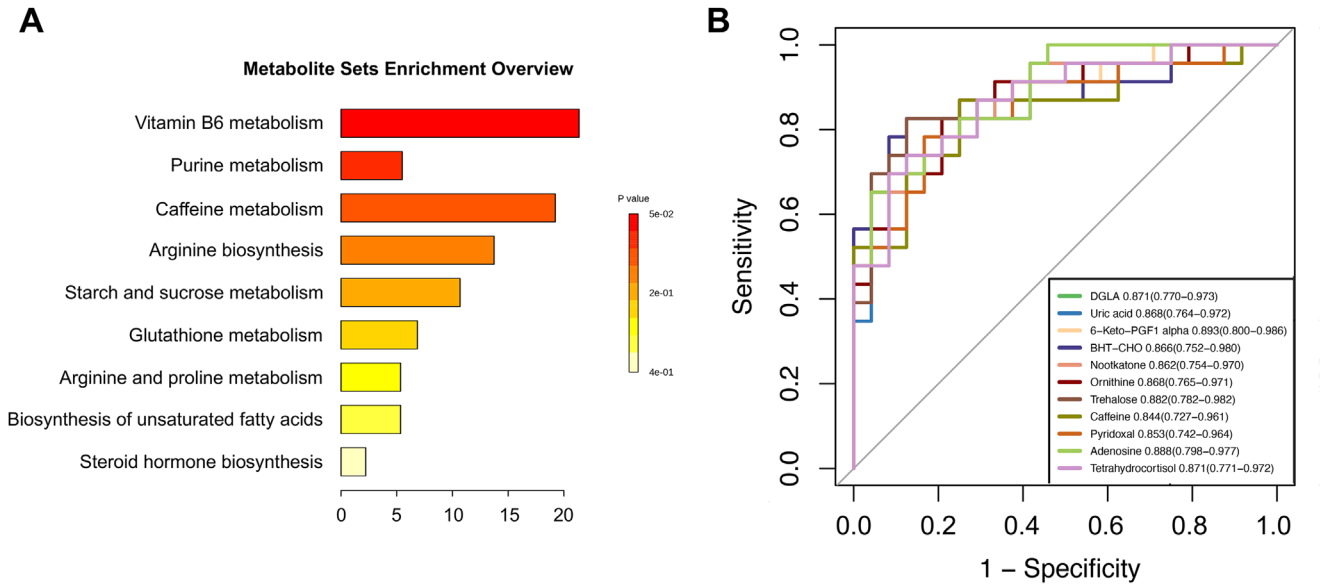


Figure 6. Enrichment analysis and biomarker selection. (**A**) The enriched KEGG pathway from the identified tear differentially expressed metabolites. (**B**) ROC curves of the identified tear differentially expressed metabolites for discriminating ROP from non-ROP premature infants ($n = 47$).

6-keto-PGF1 alpha was noted.[35] In this study, expression of DGLA and 6-keto-PGF1 alpha were both found to be decreased in the tears from infants with ROP, especially in those that required treatment. Besides, among all the identified dysregulated tear metabolites, 6-keto-PGF1 alpha exhibited the best performance in discriminating premature infants with ROP from that without ROP, with an AUC of 0.893. Therefore, the reduction of tear 6-keto-PGF1 alpha may potentially serve as a complementary screening biomarker for high-risk ROP infants, necessitating confirmatory wide-field fundus photography. Targeted metabolomics and investigation in an independent cohort will be conducted in our subsequent research to validate the hypothesis.

Metabolites related to angiogenesis were also recognized as dysregulated tear metabolites for ROP, such as tetrahydrocortisol and aminocaproic acid. Tetrahydrocortisol, which was previously believed to be an inactive metabolite of cortisol,[36] has been currently reported to be one of the most potent naturally antiangiogenic steroids.[37,38] The detection of tetrahydrocortisol in neonatal tears may be attributed to three pathways: (1) transplacental transfer of maternal corticosteroid administered during preterm deliveries where glucocorticoid therapy is implemented to accelerate fetal lung maturation; (2) metabolism of cortisol generated by the neonatal hypothalamic-pituitary-adrenal axis, characterized by dysregulated cortisol synthesis and metabolism; and (3) exposure to maternal hormones via breast milk.[39] There is a lack of study on the correlation between serum or urine tetrahydrocortisol and ROP. However, the correlation between serum cortisol is controversial. It has been reported that low cortisol, in the presence of dopamine-resistant hypotension, had the greatest magnitude of association with severe ROP.[40] Whereas another study found that serum cortisol levels showed a negative but weak correlation with the severity of ROP.[41] Tough tear tetrahydrocortisol holds promise as a candidate biomarker for ROP with AUC of more than 0.8, its clinical applicability requires validation, particularly regarding whether there is potential confounding by exogenous therapies or breastfeeding-derived steroids remain unclear. Further investigation in large-scale prospective clinical cohorts and targeted metabolomics is needed. Aminocaproic acid is an antifibrinolytic agent. For numerous studies that have revealed the involvement of fibrinogen in angiogenesis and inflammatory response,[42–45] aminocaproic acid might play a role in inhibiting angiogenesis and inflammation. As antiangiogenic factors, both of tetrahydrocortisol and aminocaproic acid were downregulated in the tears from ROP infants, and the underlying mechanisms need further studies.

Moreover, for the first time, BHT-CHO and nootkatone were detected in tear fluids. BHT-CHO is one of the typical metabolites of synthetic phenolic antioxidants (SPAs), which have been widely used in foodstuffs, cosmetics, and plastics to lengthen product shelf life.[46] Evidence from previous studies showed that SPAs and the related metabolites could be detected in various environmental matrices and in human samples, such as serum, urine, breast milk, ovarian follicular fluid, nails, and placenta.[47,48] Our study additionally revealed that metabolites of SPAs could also be observed in tear fluids, especially in those from infants with ROP. SPAs and their metabolites might induce DNA damage and cell apoptosis,[46] which were related to the underlying mechanisms of ROP.[9] Nootkatone was originally separated from plants and commercially used in food, pharmaceutical, and cosmetic industries.[49,50] Currently, nootkatone has been reported to exert antioxidant and anti-inflammatory activities and thus might be a promising candidate for treatment of diseases such as Parkinson's disease.[50,51] Further studies are needed for elucidating why BHT-CHO and nootkatone could be found in tear fluids from infants and how they are associated with the development of ROP. Maybe an environmental element could be a probable factor that contributes to ROP.

The present study has limitations. First, whether there is any direct, cause-effect relationship of the alteration of

Downloaded from iovs.arvojournals.org on 06/21/2025

tear metabolites and retinal lesion remains unclear. The possible mechanisms are as follows: (1) tear metabolic dysregulation could be the consequence of vascular damage and inflammatory response caused by prematurity, which leads to systemic abnormal metabolism apart from retinopathy; (2) retina and tissues that secrete tears, including lacrimal gland, accessory lacrimal gland, and conjunctival epithelium, were all originated from the ectoderm, and thus potentially establishing intercellular communication. Further studies such as animal experiments are needed. Second, microbiota on the ocular surface might affect metabolism in tear fluids. Conducting studies on the ocular microbiota of ROP would be helpful to clarify it. Third, targeted metabolomics and analyses using specific kits are warranted for validating the performance of the identified tear dysregulated metabolites in developing ROP screening tools. Further investigation in an independent cohort will be conducted in our subsequent research. Fourth, subjects with unstable vital signs were excluded due to ethical considerations. So, correlation between the identified tear metabolites and sepsis or necrotizing enterocolitis was not analyzed and clarified in the present study.

Although there were no retinal cell specific metabolites observed in the tear samples, the correlation between the tear metabolite profile changes and ROP was demonstrated. Directly contacts to the retina, vitreous humor serves as a reservoir for retinal neuron-derived metabolites, which enables a more intuitive visualization of pathological alterations and underlying molecular mechanisms in retinal diseases. Currently, there is still a paucity of published data on vitreous humor metabolomics profiling in ROP infants, which we would like to explore in the future. Whether tear metabolites correlate with vitreous humor metabolites remains unknown. Studies will be conducted to simultaneously profile the tear and vitreous humor metabolomic signatures of ROP and to further figure out their correlation.

In brief, our findings showed alteration of tear metabolites in ROP, which could be potential noninvasive biomarkers contributing to ROP screening, such as 6-keto-PGF1 alpha. The differentially expressed metabolites were associated with inflammatory response, oxidative stress, angiogenesis, and even environmental factors, furnishing potential indication into the disease pathophysiology.

Acknowledgments

The authors thank the support of colleagues from Sustech Core Research Facilities of Southern University of Science and Technology.

Supported by National Natural Science Foundation of China (No. 82271103), Shenzhen Medical Research Fund (No. C2301005), Sanming Project of Medicine in Shenzhen (No. SZSM202311018), and Lumitin Vision to Brightness Research Funding for the Young and middle-aged Ophthalmologists (No. BCF-KH-YK-20230803-08).

Clinical Trial Registration: Chinese Clinical Trial Registry Identifier: ChiCTR2100054263. http://www.chictr.org.cn/edit.aspx?pid=143177&htm=4. Date of registration: 2021/12/12.

Disclosure: **Z. Fan**, None; **S. Yang**, None; **X. Lu**, None; **S. Zhang**, None; **X. Zeng**, None; **L. Lin**, None; **D. Yuan**, None; **J. Zeng**, None; **G. Zhang**, None

References

1. Stahl A, Sukgen EA, Wu WC, et al. Effect of intravitreal aflibercept vs laser photocoagulation on treatment success of retinopathy of prematurity: the FIREFLEYE Randomized Clinical Trial. *JAMA*. 2022;328(4):348–359.
2. Kim SJ, Port AD, Swan R, et al. Retinopathy of prematurity: a review of risk factors and their clinical significance. *Surv Ophthalmol*. 2018;63(5):618–637.
3. Chiang MF, Quinn GE, Fielder AR, et al. International Classification of Retinopathy of Prematurity, Third Edition. *Ophthalmology*. 2021;128(10):e51–e68.
4. Zhang RH, Liu YM, Dong L, et al. Prevalence, years lived with disability, and time trends for 16 causes of blindness and vision impairment: findings highlight retinopathy of prematurity. *Front Pediatr*. 2022;10:735335.
5. Hoyek S, Cruz N, Patel NA, et al. Identification of novel biomarkers for retinopathy of prematurity in preterm infants by use of innovative technologies and artificial intelligence. *Prog Retin Eye Res*. 2023;97:101208.
6. Hansen ED, Hartnett ME. A review of treatment for retinopathy of prematurity. *Expert Rev Ophthalmol*. 2019;14(2):73–87.
7. Sabri K, Ells AL, Lee EY, Dutta S, Vinekar A. Retinopathy of prematurity: a global perspective and recent developments. *Pediatrics*. 2022;150(3):e2021053924.
8. Agrawal G, Dutta S, Prasad R, Dogra MR. Fetal oxidative stress, micronutrient deficiency and risk of retinopathy of prematurity: a nested case-control study. *Eur J Pediatr*. 2021;180(5):1487–1496.
9. Fevereiro-Martins M, Marques-Neves C, Guimarães H, Bicho M. Retinopathy of prematurity: a review of pathophysiology and signaling pathways. *Surv Ophthalmol*. 2023;68(2):175–210.
10. Green EA, Garrick SP, Peterson B, et al. The role of the interleukin-1 family in complications of prematurity. *Int J Mol Sci*. 2023;24(3):2795.
11. Wu PY, Fu YK, Lien RI, et al. Systemic cytokines in retinopathy of prematurity. *J Pers Med*. 2023;13(2):291.
12. Zhan X, Li J, Guo Y, Golubnitschaja O. Mass spectrometry analysis of human tear fluid biomarkers specific for ocular and systemic diseases in the context of 3P medicine. *Epma J*. 2021;12(4):449–475.
13. Khanna RK, Catanese S, Emond P, et al. Metabolomics and lipidomics approaches in human tears: a systematic review. *Surv Ophthalmol*. 2022;67(4):1229–1243.
14. Qiu S, Cai Y, Yao H, et al. Small molecule metabolites: discovery of biomarkers and therapeutic targets. *Signal Transduct Target Ther*. 2023;8(1):132.
15. Chen L, Gao Y, Wang LZ, et al. Recent advances in the applications of metabolomics in eye research. *Anal Chim Acta*. 2018;1037:28–40.
16. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191–2194.
17. Fierson WM. American Academy of Pediatrics Section on Ophthalmology; American Academy of Ophthalmology; *et al*. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics*. 2018;142(6):e20183061.
18. Yang Y, Yang Q, Luo S, et al. Comparative analysis reveals novel changes in plasma metabolites and metabolomic networks of infants with retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2022;63(1):28.
19. Catanese S, Khanna RK, Lefevre A, et al. Validation of metabolomic and lipidomic analyses of human tears using

Downloaded from iovs.arvojournals.org on 06/21/2025

ultra-high-performance liquid chromatography tandem mass spectrometry. *Talanta*. 2023;253:123932.
20. Anwardeen NR, Diboun I, Mokrab Y, Althani AA, Elrayess MA. Statistical methods and resources for biomarker discovery using metabolomics. *BMC Bioinformatics*. 2023;24(1):250.
21. Ponzini E. Tear biomarkers. *Adv Clin Chem*. 2024;120:69–115.
22. Chao C, Lattery L, Qin G, et al. Tear proteomics of children and young adult soft contact lens, orthokeratology and spectacle wearers - a pilot study. *Curr Eye Res*. 2022;47(6):832–842.
23. Fan Z, Hu Y, Chen L, et al. Multiplatform tear proteomic profiling reveals novel non-invasive biomarkers for diabetic retinopathy. *Eye (Lond)*. 2024;38(8):1509–1517.
24. Brown SH, Kunnen CM, Papas EB, et al. Intersubject and interday variability in human tear and meibum lipidomes: a pilot study. *Ocul Surf*. 2016;14(1):43–48.
25. Nakatsukasa M, Sotozono C, Shimbo K, et al. Amino acid profiles in human tear fluids analyzed by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *Am J Ophthalmol*. 2011;151(5):799–808.e1.
26. Ambaw YA, Chao C, Ji S, et al. Tear eicosanoids in healthy people and ocular surface disease. *Sci Rep*. 2018;8(1):11296.
27. Zhou Y, Xu Y, Zhang X, et al. Plasma metabolites in treatment-requiring retinopathy of prematurity: potential biomarkers identified by metabolomics. *Exp Eye Res*. 2020;199:108198.
28. Lu F, Liu Y, Guo Y, et al. Metabolomic changes of blood plasma associated with two phases of rat OIR. *Exp Eye Res*. 2020;190:107855.
29. Chen JF, Zhang S, Zhou R, et al. Adenosine receptors and caffeine in retinopathy of prematurity. *Mol Aspects Med*. 2017;55:118–125.
30. Zhang S, Li B, Tang L, et al. Disruption of CD73-derived and equilibrative nucleoside transporter 1-mediated adenosine signaling exacerbates oxygen-induced retinopathy. *Am J Pathol*. 2022;192(11):1633–1646.
31. Schmidt B, Roberts RS, Davis P, et al. Long-term effects of caffeine therapy for apnea of prematurity. *N Engl J Med*. 2007;357(19):1893–1902.
32. Miyazawa T, Nakagawa K, Shimasaki S, Nagai R. Lipid glycation and protein glycation in diabetes and atherosclerosis. *Amino Acids*. 2012;42(4):1163–1170.
33. Liu H, Tang J, Lee CA, Kern TS. Metanx and early stages of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2015;56(1):647–653.
34. Mustonen AM, Nieminen P. Dihomo-γ-linolenic acid (20:3n-6)-metabolism, derivatives, and potential significance in chronic inflammation. *Int J Mol Sci*. 2023;24(3):2116.
35. Rabinovitch M, Boudreau N, Vella G, Coceani F, Olley PM. Oxygen-related prostaglandin synthesis in ductus arteriosus and other vascular cells. *Pediatr Res*. 1989;26(4):330–335.
36. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci USA*. 1992;89(21):9991–9995.
37. Folkman J, Ingber DE. Angiostatic steroids. Method of discovery and mechanism of action. *Ann Surg*. 1987;206(3):374–383.
38. Teicher BA, Sotomayor EA, Huang ZD. Antiangiogenic agents potentiate cytotoxic cancer therapies against primary and metastatic disease. *Cancer Res*. 1992;52(23):6702–6704.
39. Vacaru SV, Brett BE, Eckermann H, De Weerth C. Determinants of maternal breast milk cortisol increase: examining dispositional and situational factors. *Psychoneuroendocrinology*. 2023;158:106385.
40. Catenacci M, Miyagi S, Wickremasinghe AC, et al. Dopamine-resistant hypotension and severe retinopathy of prematurity. *J Pediatr*. 2013;163(2):400–405.
41. Ng PC, Kwok AK, Lee CH, et al. Early pituitary-adrenal responses and retinopathy of prematurity in very low birth weight infants. *Pediatr Res*. 2004;55(1):114–119.
42. Yakovlev S, Strickland DK, Medved L. Current view on the molecular mechanisms underlying fibrin(ogen)-dependent inflammation. *Thromb Haemost*. 2022;122(11):1858–1868.
43. Chung E, Rytlewski JA, Merchant AG, et al. Fibrin-based 3D matrices induce angiogenic behavior of adipose-derived stem cells. *Acta Biomater*. 2015;17:78–88.
44. Zhao N, Suzuki A, Zhang X, et al. Dual aptamer-functionalized in situ injectable fibrin hydrogel for promotion of angiogenesis via codelivery of vascular endothelial growth factor and platelet-derived growth factor-BB. *ACS Appl Mater Interfaces*. 2019;11(20):18123–18132.
45. Jing H, Wu X, Xiang M, Wang C, Novakovic VA, Shi J. Microparticle phosphatidylserine mediates coagulation: involvement in tumor progression and metastasis. *Cancers (Basel)*. 2023;15(7):1957.
46. Xu X, Liu A, Hu S, et al. Synthetic phenolic antioxidants: metabolism, hazards and mechanism of action. *Food Chem*. 2021;353:129488.
47. Hao Y, Wang Y, Yan L, et al. Synthetic phenolic antioxidants and their metabolites in follicular fluid and association with diminished ovarian reserve: a case-control study. *Environ Health Perspect*. 2023;131(6):67005.
48. Wang W, Xiong P, Zhang H, et al. Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: a review. *Environ Res*. 2021;201:111531.
49. Li X, Ren JN, Fan G, Zhang LL, Pan SY. Advances on (+)-nootkatone microbial biosynthesis and its related enzymes. *J Ind Microbiol Biotechnol*. 2021;48(7-8):kuab046.
50. Zhang LL, Chen Y, Li ZJ, Fan G, Li X. Production, function, and applications of the sesquiterpenes valencene and nootkatone: a comprehensive review. *J Agric Food Chem*. 2023;71(1):121–142.
51. Yao Z, Li J, Bian L, et al. Nootkatone alleviates rotenone-induced Parkinson's disease symptoms through activation of the PI3K/Akt signaling pathway. *Phytother Res*. 2022;36(11):4183–4200.

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 06/21/2025