Glaucoma

Prediagnostic Plasma Metabolomics and the Risk of Exfoliation Glaucoma

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PURPOSE. The etiology of exfoliation glaucoma (XFG) is poorly understood. We aimed to identify a prediagnostic plasma metabolomic signature associated with XFG.

METHODS. We conducted a 1:1 matched case-control study nested within the Nurses' Health Study and Health Professionals Follow-up Study. We collected blood samples in 1989–1990 (Nurses' Health Study) and 1993–1995 (Health Professionals Follow-up Study). We identified 205 incident XFG cases through 2016 (average time to diagnosis from blood draw = 11.8 years) who self-reported glaucoma and were confirmed as XFG cases with medical records. We profiled plasma metabolites using liquid chromatography-mass spectrometry. We evaluated 379 known metabolites (transformed for normality using probit scores) using multiple conditional logistic models. Metabolite set enrichment analysis was used to identify metabolite classes associated with XFG. To adjust for multiple comparisons, we used number of effective tests (NEF) and the false discovery rate (FDR).

RESULTS. Mean age of cases ($n = 205$) at diagnosis was 71 years; 85% were women and more than 99% were Caucasian; controls ($n = 205$) reported eye examinations as of the matched cases' index date. Thirty-three metabolites were nominally significantly associated with XFG ($P < 0.05$), and 4 metabolite classes were FDR-significantly associated. We observed positive associations for lysophosphatidylcholines (FDR = 0.02) and phosphatidylethanolamine plasmalogens (FDR = 0.004) and inverse associations for triacylglycerols (FDR < 0.0001) and steroids (FDR = 0.03). In particular, the multivariable-adjusted odds ratio with each 1 standard deviation higher plasma cortisone levels was 0.49 (95% confidence interval, 0.32–0.74; NEF = 0.05).

CONCLUSIONS. In plasma from a decade before diagnosis, lysophosphatidylcholines and phosphatidylethanolamine plasmalogens were positively associated and triacylglycerols and steroids (e.g., cortisone) were inversely associated with XFG risk.

Keywords: metabolomics, glaucoma, case-control study

Exfoliation syndrome (XFS) is a systemic age-related disorder of the extracellular matrix that manifests as grey flaky deposits in the ocular anterior segment.[1] XFS is the most common identifiable cause of glaucoma (exfoliation glaucoma [XFG]) and is associated with cataract surgery complications and systemic diseases.[2,3]

XFS pathogenesis may involve a stress-induced elastosis with excessive production and abnormal cross-linking of microfibrillar components into fibrillar aggregates (exfoliation material [XFM]) in the ocular anterior segment.[1,4] XFM contains glycoproteins, proteoglycans, elastic fiber components and the cross-linking enzyme, lysyl oxidase-like 1 (LOXL1).[5] Genome-wide association studies have identified several loci, including *LOXL1*, which is a principal genetic risk factor for XFS.[6] Although the *LOXL1* variants are strongly associated with XFS, the same variants are also prevalent in unaffected populations, making XFS a complex disease involving genetics, the environment, and their interactions.[7]

Because XFS etiology is poorly understood, we conducted an untargeted plasma metabolomics study of XFG. To date, there have been two metabolomic studies of XFS/XFG[8,9]; however, they were small (<35 XFS cases) cross-sectional studies. We included 205 incident cases and 205 matched controls in a study of prediagnostic circulating plasma metabolites from approximately 10 years before XFG diagnosis.

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Investigative Ophthalmology & Visual Science

METHODS

Study Population and Blood Sample Collection

We conducted a nested case-control study in the US Nurses' Health Study (NHS) and Health Professional Follow-up Study (HPFS). In 1976, the NHS began with 121,700 female nurses aged 30 to 55 years, and in 1986, the HPFS enrolled 51,529 male health professionals aged 40 to 75 years. Participants completed biennial questionnaires that updated information on lifestyle and medical conditions, such as glaucoma.

Blood samples were collected in 1989 and 1990 among 32,826 NHS participants and in 1993, 1994, and 1995 among 18,159 HPFS participants. In the NHS, participants provided blood samples in two 15-mL sodium heparin tubes that were shipped on ice overnight to our laboratory.[10] In the HPFS, participants had blood samples placed in three 10-mL EDTA tubes, which were returned in a similar manner.[11] Returned samples were processed, and aliquots of white blood cell, red blood cell, and plasma were archived in liquid nitrogen freezers ($\leq-130°C$).

XFG cases were diagnosed after blood draw until June 1, 2016 (NHS), or January 1, 2016 (HPFS). XFG cases (205 total; 174 women and 31 men) were first identified by self-report of glaucoma and then confirmed by medical record review. Additional XFG confirmation details are in Supplementary Methods. As of the index period of the matched cases' diagnosis date, the 205 controls reported having had eye examinations in the same period but never reported having a physician diagnosis of glaucoma (or were never confirmed to have glaucoma). Controls were matched on age, sex, ancestry (Scandinavian, Southern-European Caucasian, other Caucasian, others), month and year of blood collection, time of day of blood draw, fasting status (>8 hours or ≤8 hours), latitude and longitude of residence as of blood draw, and, among women, menopausal status and hormone therapy use at blood draw (premenopausal, postmenopausal with hormone therapy use, postmenopausal and no hormone therapy use, missing or unknown) and at diagnosis. For matching factors and covariates, we used questionnaire data collected as of the blood draw and, if not available, we used biennial questionnaire data immediately before the blood sample. The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health. Returns of self-administered questionnaires and blood samples were considered as implied consents by the institutional review boards.

Stability of Samples With Long-term Storage

Cryotubes containing plasma samples were placed in the vapor phase of liquid nitrogen freezers ($\leq-130°C$), an approach designed to minimize degradation associated with long-term storage.[12] Freezers have been alarmed and monitored 24 hours a day. The liquid nitrogen depth is 3 or more inches (enough to last 3 days), and these levels have been manually checked weekly. Importantly, samples from cases and controls, matched on date of blood collection, were processed together during aliquot preparation and metabolite profiling.

Selection of Metabolites and Pilot Study Results

Plasma metabolites were profiled using liquid chromatography tandem mass spectrometry to identify and measure endogenous, polar metabolites and lipids at the Broad Institute of MIT and Harvard University (Cambridge, MA). The platform uses triple quadrupole orbitrap mass spectrometers (two Q Exactive and one Exactive Plus MS, ThermoFisher Scientific, Waltham, MA) for high-resolution measurement of metabolites.

Selection of Metabolites. A previous pilot study[13] assaying NHS and HPFS samples using various liquid chromatography tandem mass spectrometry platforms at the Broad Institute demonstrated that metabolites on two platforms had the best reproducibility and thus, 398 metabolites were initially selected for metabolome wide analysis.

The pilot study[13] of 257 metabolites (metabolites that could be identified on three Broad platforms at the time the pilot study was initiated) from NHS and HPFS participants showed that the metabolite classes of lipids and lipid metabolites and amino acids were measured with acceptable laboratory variability (i.e., split sample reproducibility) and were reproducible over processing delays (given the 24+ hours delay in collection) and within individuals over 1 to 2 years but that metabolite assays of carbohydrates and intermediates; purines, pyrimidines, and derivatives; and specific amines were difficult. Split sample reproducibility was assessed with 60 duplicate plasma samples from participants and 20 quality control pool plasma replicates. Metabolite reproducibility over a 24- to 48-hour processing delay were assessed with 48 samples, and within-person reproducibility over 1 to 2 years was assessed with 80 samples. Overall, coefficients of variation were less than 20% for 92% of metabolites (>90% of lipids and lipid metabolites, amino acids, amines and organic acid metabolites), and approximately 75% of metabolites were reproducible over delays in blood sample processing (Spearman correlation or intraclass correlation (ICC) of 0.75 or greater comparing immediate and 24-hour delayed processing). Ninety percent of metabolites were reproducible over 1 to 2 years within individuals (Spearman correlation or ICC of ≥0.4).[13]

We also assessed the within-person stability over 10 years for 258 metabolites,[14] and we observed a median ICC of 0.4, similar to the ICC of plasma cholesterol (ICC = 0.39), which is highly predictive of coronary artery disease risk.[14–16] Results from both pilot studies indicated that one blood sample could provide a reasonable measure of longer term exposure.

Metabolite Profiling and Quality Control Measures

Metabolite Profiling for Lipids. Lipids were extracted from plasma (10 μL) using 19 volumes of 100% isopropanol. The extracts were separated using reversed phase chromatography with a C4 column and full scan MS data were acquired in the positive ion mode (Supplementary Methods).

Metabolite Profiling for Polar Metabolites. Polar metabolites that ionize in the positive ion mode were extracted from 10 μL of plasma using a mixture of acetonitrile and methanol. The extracts were separated using a hydrophilic interaction liquid chromatography and tandem mass spectrometry analyses were conducted in the positive ionization mode. The metabolites measured using this method include amino acids, amino acid derivatives,

dipeptides, and other cationic metabolites (Supplementary Methods).

Data Processing

MultiQuant 1.2 software (AB SCIEX) was used for automated peak integration, and metabolite peaks were manually reviewed for integration quality and compared against a known standard to confirm identity. Metabolites with a signal to noise ratio of less than 10 were considered unquantifiable. Metabolite signals were retained as measured liquid chromatography tandem mass spectrometry peak areas, which were proportional to metabolite concentration.[13]

Quality Control Measures

Among 398 known metabolites, we excluded 19 metabolites that did not pass quality control checks (10 that were impacted by the delay in blood processing and 9 metabolites that had coefficients of variation of >25%),[13] leaving 379 metabolites for analyses. All included metabolites had previously exhibited good within-person reproducibility over a 1-year period.[13] For metabolites with less than 10% missing across participant samples, where missingness had minimal effect on associations, missing values were imputed with one-half of the minimum value measured for that metabolite.[17,18]

Assay for Homocysteine

For homocysteine, which has been associated with XFS/XFG,[19–21] the assay from the metabolomics platform described above did not pass quality control checks. Thus, we determined the concentrations at the Boston Children's Hospital with an enzymatic assay on the Roche P Modular system (Roche Diagnostics - Indianapolis, IN), using reagents and calibrators from Catch Inc. (Seattle, WA). The coefficient of variation was less than 10%.

Statistical Analysis

Given the matched design, we used nested multivariable-adjusted conditional logistic regression models. Model 1 did not adjust for any covariates. In model 2, we more finely adjusted for matching factors as of blood draw and other major determinants of metabolite variability: age (years; matching factor), sex, month of blood draw (matching factor), year of blood draw (matching factor), time of blood draw (matching factor), fasting status (>8 hours or <=8 hours; matching factor), body mass index (kg/m^2), smoking status (never, past, current), and physical activity (metabolic equivalents of task-hours/week). In model 3, we further added established XFG risk factors: glaucoma family history, ancestry (Scandinavian ancestry, other Caucasian, others), time spent outdoors in the summer during youth,[22] history of nonmelanoma skin cancer,[23] latitude of residence,[24] and residential population density.[25] In model 4, we further added XFG risk factors of folate,[26] caffeine,[27] alcohol, and total caloric intake. In model 5, we further added comorbidities associated with XFG: stroke, heart disease, hypertension, high cholesterol, diabetes, hearing loss, and sleep duration.[28–30] In model 6, we further added use of oral or inhaled steroids as of blood draw because steroid use has been associated with glaucoma.[31]

Metabolite values were transformed to probit scores to scale to the same range and to minimize the influence of skewed distributions. For individual metabolite analyses, metabolite values were used as continuous variables (per 1 standard deviation increase). We estimated the odds ratios (OR) and 95% confidence intervals (95% CI) per 1 SD increase in metabolite levels. The number of effective tests (NEF)[32] was used to adjust for multiple comparisons given the high correlation structure of the metabolomics data; a NEF of less than 0.05 was considered statistically significant, whereas metabolites with a NEF of less than 0.2 were considered as candidates worthy of further study given the exploratory nature of the analyses.

For metabolite class analyses, the 379 metabolites were each assigned a metabolite class based on chemical taxonomy. We evaluated 17 metabolite classes: steroids and steroid derivatives; carnitines; diacylglycerols; triacylglycerols; cholesteryl esters; lysophosphatidylethanolamines (LPEs); phosphatidylethanolamines; lysophosphatidylcholines (LPCs); phosphatidylcholines; phosphatidylethanolamine plasmalogens (PEPs); phosphatidylcholine plasmalogens; organoheterocyclic compounds; ceramides; carboxylic acids and derivatives; organic acids and derivatives; nucleosides, nucleotides, and analogues; and sphingomyelins. For metabolite class analyses, a metabolite set enrichment analysis and the false discovery rate (FDR)[33] was used; an FDR of less than 0.05 was considered statistically significant. All analyses were performed with SAS 9.4 and R 3.4.1 (SAS Institute, Cary, NC).

RESULTS

Study Population

Among 205 cases, 84.9% were female, with a mean age at blood draw of 59.3 ± 6.5 years and at diagnosis of 71.1 ± 7.4 years. The mean time between blood draw to diagnosis was 11.8 years. Controls were similar to cases in matching factors (Table).

Relation Between Individual Metabolites and XFG

No significant associations were observed across the various models with homocysteine (per 1 standard deviation increase) and XFG (*P* > 0.18; model 6, OR, 1.22; 95% CI, 0.90–1.65) (Supplementary Table S1). Among 379 metabolites, 33 were nominally significant at a *P* value of less than 0.05 in any of the models (model 1 through model 6 in Fig. 1) (Supplementary Table S2). In model 1 (crude model), 20 metabolites were nominally significant, including acetaminophen, methionine sulfoxide, and C16:0 LPE, which were all positively associated (NEF < 0.2); in model 2, only 9 metabolites were nominally significant. However, in models 3 and 4, we observed that cortisone was an NEF-significant metabolite that was strongly inversely associated with XFG risk. For example, in model 4, for each standard deviation increase in cortisone level, the OR for XFG was 0.51 (95% CI, 0.36–0.73; NEF = 0.01). Also, in model 4, the OR for gabapentin, an anticonvulsant and nerve pain medication, was 1.90 (95% CI, 1.26–2.87; NEF = 0.16). In model 5, with further adjustment for comorbidities and in model 6, with further adjustment for oral/inhaled corticosteroid use, OR estimates were similar for cortisone (OR, 0.49; 95% CI, 0.32–0.74; NEF = 0.05) and gabapentin (OR, 2.21; 95% CI, 1.31–3.73; NEF = 0.21).

TABLE. Characteristics of XFG Cases and Their Matched Controls[*]

	Cases	Controls
Matching factors[†]	$n = 205$	$n = 205$
Female, n (%)	174 (84.9)	174 (84.9)
Mean age at blood draw (SD), years	59.3 (6.5)	58.7 (5.9)
Mean years from blood draw to diagnosis/index date (SD), years	11.8 (6.4)	11.8 (6.4)
Age at diagnosis/index date (SD), years	71.1 (7.4)	70.5 (7.1)
Scandinavian Caucasian, n (%)	13 (6.3)	14 (6.8)
Time of blood draw of 8:00-9:59 AM, n (%)	115 (56.1)	118 (57.6)
Season of blood draw during summer, n (%)	65 (31.7)	74 (36.1)
Fasting >8 h, n (%)	142 (69.6)	171 (83.4)[‡]
Mean latitude of residence (SD), °N	39.6 (4.3)	39.7 (4.1)
Mean longitude of residence (SD), °W	81.6 (13.0)	81.7 (12.7)
Among females: current user of postmenopausal hormones as of blood draw, n (%)	59 (37.1)	62 (38.5)
Among females: current user of postmenopausal hormones as of diagnosis/index date, n (%)	52 (32.3)	56 (34.4)
Other risk factors		
Family history of glaucoma, n (%)	45 (22.4)	39 (19.2)
6+ hours/week outdoor sunlight exposure during summers in youth, n (%)	98 (53.3)	85 (47.0)
Nonmelanoma skin cancer, n (%)	28 (13.7)	18 (8.8)
Mean caffeine intake (SD), mg/day	299.0 (246.4)	283.1 (247.1)
Mean folate intake (SD), mg/day	436.2 (229.3)	427.7 (244.5)
Mean alcohol intake (SD), g/day	6.9 (10.1)	6.4 (9.9)
Mean caloric intake (SD), kcal/day	1806.5 (528.2)	1815.3 (516.7)
Mean body mass index (SD), kg/m^2	24.9 (4.3)	24.9 (4.0)
Mean pack-years of smoking (SD), pack-years	10.4 (15.5)	10.1 (15.4)
Mean physical activity (SD), MET-hours per week	21.3 (25.2)	18.9 (20.3)
Mean sleep duration (SD), hours	6.9 (0.9)	7.0 (0.9)
Mean population density (SD) of census tract, number/km^2	1156.8 (1913.7)	1092.6 (1264.0)
Age related macular degeneration, n (%)	3 (1.5)	3 (1.5)
Hypertension, n (%)	47 (22.9)	61 (29.8)
Hyperlipidemia, n (%)	65 (31.7)	77 (37.6)
Heart disease, n (%)	3 (1.5)	8 (3.9)
Stroke, n (%)	5 (2.4)	0 (0.0) [‡]
Diabetes, n (%)	4 (2.0)	5 (2.4)
Hearing loss, n (%)	17 (8.3)	13 (6.3)
Use of oral or inhaled steroids as of blood draw, n (%)	8 (3.9)	4 (2.0)

MET, metabolic equivalent of task; SD, standard deviation.

[*] Values are means ± SD or percentages, are standardized to the age distribution of the study population and based on those with nonmissing values.

[†] The cases and controls were 1:1 matched based on age (all >40 years), menopausal status and postmenopausal hormone use at blood collection and diagnosis, month/year/time of day of blood collection, fasting status, ancestry (Scandinavian, S. European, Other Caucasian, other), latitude and longitude as of blood draw, and sample problem type. All controls reported eye examinations in the index period of the matched cases' diagnosis date.

[‡] $P < 0.05$ between cases and controls in Fisher's exact tests.

Relation Between Metabolite Classes and XFG

For analyses of 17 metabolite classes and XFG (Fig. 2), models 1 and 2 showed similar results with FDR-significant positive associations for LPEs. In model 2, LPCs, nucleosides, nucleotides, and analogues were significantly positively associated, whereas carnitines and triacylglycerols were significantly inversely associated. In models 3 and 4, the positive associations with LPE and LPCs and inverse associations with triacylglycerols were robust; cholesteryl esters, PEPs, and phosphatidylcholine plasmalogens showed FDR-significant positive associations, whereas steroids and steroid derivatives showed FDR-significant inverse associations. In models 5 and 6, associations with cholesteryl esters, LPEs, and phosphatidylcholine plasmalogens were no longer significant; in model 6, we observed four classes that were FDR significant: steroids and steroid derivatives (FDR = 0.03) and triacylglycerols (FDR < 0.0001) were inversely associated, whereas the lipid classes of LPCs (FDR = 0.02) and PEPs (FDR = 0.004) were positively associated.

Secondary Analyses

We assessed whether results varied by age (Supplementary Fig. S1), sex (Supplementary Fig. S2), latitude (Supplementary Fig. S3), time to diagnosis (Supplementary Fig. S4), and history of glaucoma (Supplementary Fig. S5); overall, the results were similar across the subgroups. Results, particularly for cortisone, did not differ by subgroups; however, there was a trend of a stronger inverse association among women (which may be due to the greater number of women) and those living in less than 41°N in latitude. In sensitivity analyses excluding those who were diagnosed within 5 years of blood draw to remove participants with possible subclinical disease (Supplementary Fig. S6), the association with cortisone was similar (although no longer NEF significant). In sensitivity analyses, (1) we excluded participants with AMD (for which ocular steroids may be prescribed) or those who have ever reported using oral or inhaled corticosteroids (Supplementary Fig. S7a), and (2) we further excluded those with conditions that would indicate treatment with
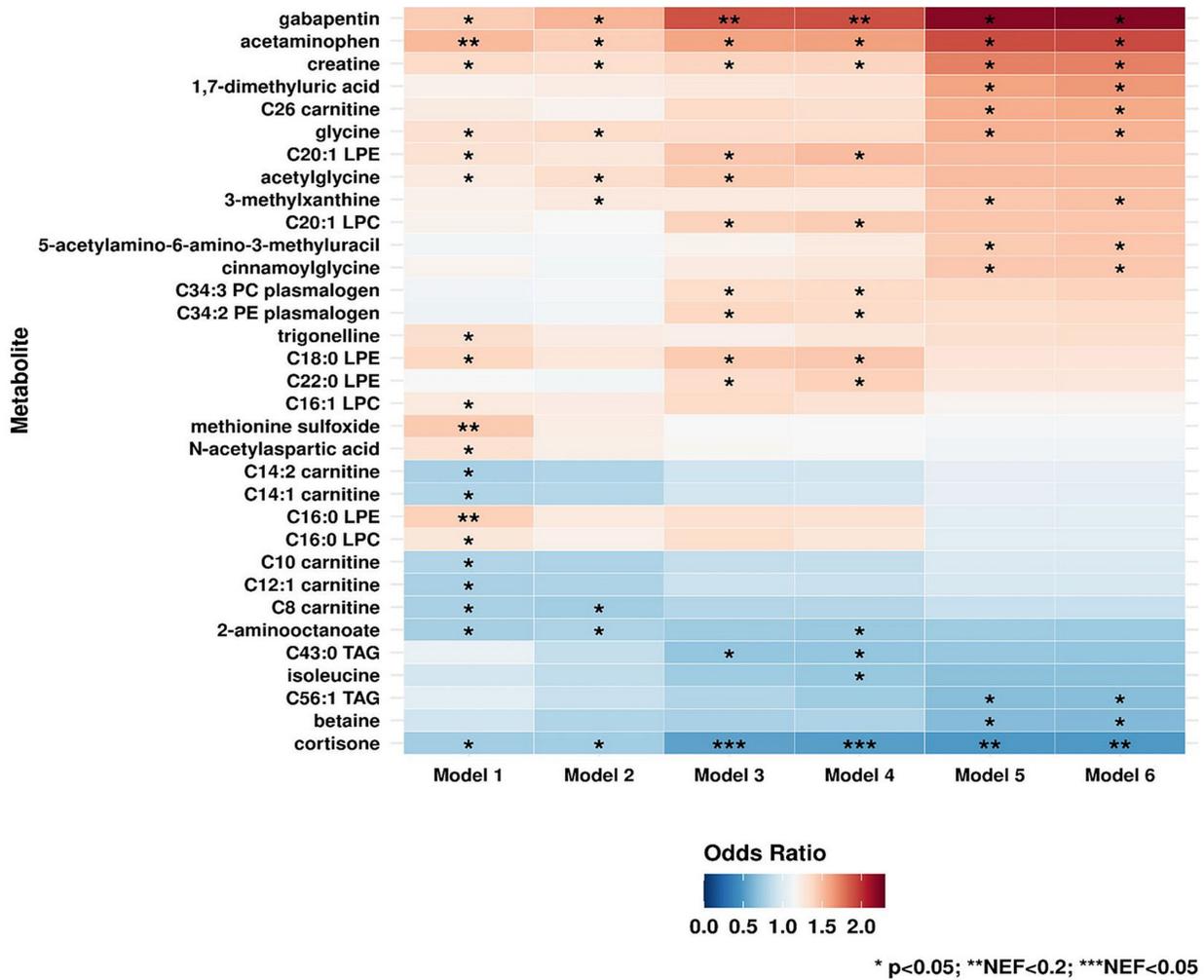
Figure 1. Individual metabolites among the 379 metabolites evaluated that were significant across the various nested multiple conditional logistic regression models of XFG (205 cases and 205 controls). Model 1: basic model, adjusting for matching factors only (see Table); model 2, factors that affect metabolite levels: model 1 + age, sex, smoking status, BMI, physical activity, time of day of blood draw, month of blood draw, fasting status; model 3, presumed XFS risk factors: model 2 + type of Caucasian, family history of glaucoma, time spent outdoors in sunlight in the summer in youth, nonmelanoma skin cancer, latitude, population density; model 4, factors that may raise homocysteine levels: model 3 + folate intake, caffeine intake, alcohol intake, caloric intake; model 5, systemic comorbidities suggested to be associated with XFS in some studies: model 4 + heart disease, stroke, diabetes, hypertension, high cholesterol, hearing loss, sleep duration; model 6, use of drugs associated with glaucoma: model 5 + steroid use. * $P < 0.05$; ** NEF < 0.2; *** NEF < 0.05. TAG, triacylglycerol.

corticosteroids (Supplementary Fig. S7b). The OR estimates for cortisone remained similar in model 5 in both sensitivity analyses: OR, 0.54 (95% CI, 0.39–0.75) (Supplementary Fig. S7a) and OR, 0.55 (95% CI, 0.28–1.08) (Supplementary Fig. S7b). These analyses suggest that the lower cortisone levels in XFG cases compared with controls was unlikely to be explained by corticosteroid use.

Discussion

In this nested case-control study of prediagnostic plasma metabolites in relation to XFG ($n = 410$), with a mean 11.8 years between blood draw and diagnosis, the metabolite cortisone and the metabolite class of triacylglycerols were inversely associated with XFG, whereas the lipid classes of LPCs and PEPs were positively associated with XFG (Supplementary Fig. S8). These findings should be confirmed in future studies.

Two previous studies have investigated the metabolic signature of XFS. Leruez et al.[8] found that, compared with controls with cataracts ($n = 18$), XFS cases ($n = 16$) had elevated plasma levels of amino acids and two carnitine metabolites but lower levels of spermine and spermidine polyamines, diacyl PC phospholipids (C38:0 and C38:1), and sphingomyelin (C26:1). Myer et al.[9] evaluated aqueous humor metabolites in those with XFS ($n = 31$; of whom 29 had cataracts) versus controls ($n = 25$; all with cataracts) and observed that while creatine and 5-hydroxypentanoate were positively associated, propylene glycol, N(6)-acetonyllysine, and five amino acids were inversely associated with XFS. Our study results are not directly comparable as in the prior studies, the designs were cross-sectional studies and metabolites in postdiagnosis samples were compared from XFS cases and controls with cataracts; in contrast, our larger study evaluated prediagnostic plasma and those with XFG were matched to those without XFG (regardless of cataract status).
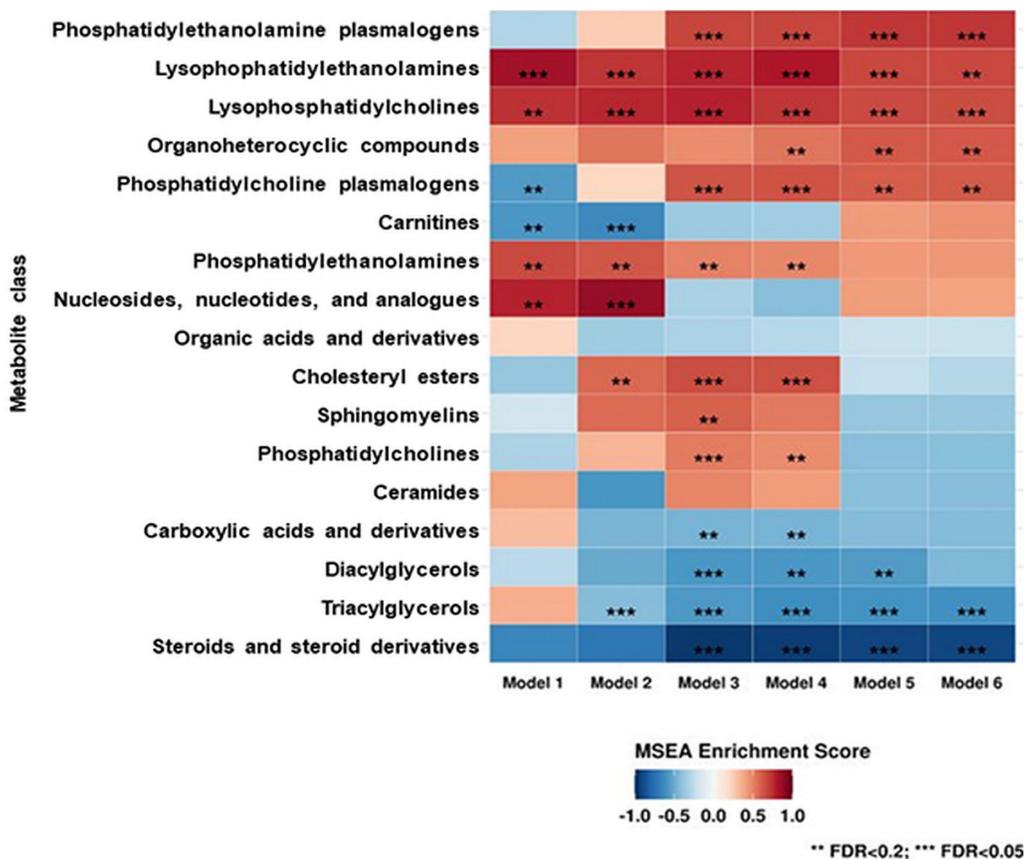
FIGURE 2. Metabolite classes ($n = 17$) evaluated in various nested multiple conditional logistic regression models of XFG (205 cases and 205 controls). Model 1, basic model, adjusting for matching factors only (see Table); model 2, factors that affect metabolite levels: model 1 + age, sex, smoking status, BMI, physical activity, time of day of blood draw, month of blood draw, fasting status; model 3, presumed XFS risk factors: model 2 + type of Caucasian, family history of glaucoma, time spent outdoors in sunlight in the summer in youth, nonmelanoma skin cancer, latitude, population density; model 4, factors that may raise homocysteine levels: model 3 + folate intake, caffeine intake, alcohol intake, caloric intake; model 5, systemic comorbidities suggested to be associated with XFS in some studies: model 4 + heart disease, stroke, diabetes, hypertension, high cholesterol, hearing loss, sleep duration; model 6, use of drugs associated with glaucoma: model 5 + steroid use. ** FDR < 0.2; *** FDR < 0.05.

Therefore, our results are notable in their large sample size and in evaluating prediagnostic plasma that may reflect metabolic changes occurring earlier in the XFS/XFG disease process.

Cortisone and Cortisol

The strong inverse association between XFG and steroids or steroid derivatives, especially cortisone, was notable. Lower levels of cortisone in cases may reflect lower systemic endogenous anti-inflammatory corticosteroid levels, which may prevent the disruption of the uveal tract blood–ocular barrier integrity and thereby also prevent subsequent XFM formation.[34] Alternatively, although the absolute cortisone levels are unknown, relatively lower levels may reflect adrenal insufficiency; this association may be plausible, given that body aches and joint pain are common symptoms,[35] and gabapentin and acetaminophen were metabolites that were nominally associated with XFG. Adrenal insufficiency has multiple causes[35]; one cause may be the adrenal suppression owing to exogenous corticosteroid use.[36] Indeed, XFG cases had a higher prevalence of corticosteroid use than controls (3.9% vs 2.0%). However, we observed similar associations in sensitivity analyses where we excluded participants who self-reported ever taking oral or inhaled corticosteroids or had indications for them, and our XFG cases did not include those with corticosteroid-induced secondary glaucoma, making it unlikely that corticosteroid use may explain the cortisone association. Some studies,[37–39] although not all,[37,40] have observed that low cortisone or cortisol may indicate greater UV exposure, a strong XFS and XFG risk factor.[41] Indeed, inverse cortisone associations were stronger in the subgroup residing in 41° N latitude or lower, where cortisone levels may be stronger markers of UV exposure. Finally, cortisone/cortisol are glucocorticoids, and higher levels in controls versus cases may indicate that controls may have had more frequent hyperglycemia. Several studies[42–49] have reported inverse associations between diabetes and XFG; it has been hypothesized that greater glycation of basement membrane components may prevent XFM aggregation.[45,50]

LPCs

LPCs are proinflammatory and may be involved in glaucoma via LPCs' role in the autotaxin–lysophophatidic acid pathway in intraocular pressure regulation, although the data have been inconsistent.[8,51–55] Our results are consistent

with other targeted lipidomics studies that have revealed higher total LPCs in the aqueous humor of glaucomatous eyes, including XFG[51,52]; however, two POAG aqueous humor studies observed null associations with LPCs,[53,54] and Leruez et al.[8] and another study of LPCs in the optic nerve tissue observed inverse associations.[55] These discrepancies may be related to LPCs with inflammatory properties being limited to those with shorter and unsaturated fatty acids,[56,57] whereas LPCs with longer and more saturated fatty acids have been inversely associated with type 2 diabetes[58] and heart disease.[59] We observed stronger associations with longer chain length (C20 and C22) LPCs; thus, it is also possible that higher levels of these long chain LPCs may be markers of healthier cardiometabolic profiles in cases than controls and thus less frequent hyperglycemia that may be associated with less XFM aggregation.[45,50]

PEPs

Plasmalogens have been little studied in glaucoma.[60] Higher PEPs have been observed with greater physical activity,[61] which may be a marker for greater UV exposure that in turn would increase XFG risk. In addition, higher PEPs have been associated with a lower quality diet,[62–65] characterized by fewer folate sources; lower folate intake may lead to higher homocysteine levels, which has been observed in XFS.[20,21,66] Although we observed nonsignificant modest positive associations with homocysteine itself, given that other metabolites such as lower phosphatidylcholines,[67] lower betaine,[68] and higher levels of coffee-related metabolites (i.e., 3-methylxanthine and trigonelline)[69,70] that increase homocysteine were near significant, the homocysteine pathway may be relevant in the disease process.

Triacylglycerols

Higher triacylglycerols as a metabolite class were significantly inversely associated with XFG, which is in contrast to findings from multiple cross-sectional studies that observed positive associations with XFG.[71–75] Although we adjusted for caffeine intake and recreational physical activity, lower triacylglycerols in cases may be capturing residual information about greater coffee consumption[76] or lower sedentary behavior and greater physical activity,[77,78] which are likely associated with higher homocysteine levels and greater UV exposure,[79] respectively, as well as a higher XFG risk. In addition, because higher triacylglycerol levels are a component of the metabolic syndrome, it could also be a proxy for greater hyperglycemia, which may have protective effects against the accumulation of XFM.[45,50]

A limitation of our data is that our study population was relatively homogenous, with mostly White health professionals; therefore, our findings may not be generalizable to other populations with different race and ethnicity compositions. Our blood samples were collected at one timepoint; however, we have only included metabolites with good correlations for within-person stability over at least 1 year.[13] Also, there may have been residual confounding by other unmeasured factors. Furthermore, because we did not confirm with medical records that the selected controls did not have XFG, we may have had some minor nondifferential misclassification of the outcome (XFS/XFG prevalence is 2.6% in the United States for those aged 52–64 years),[80] which would have biased results toward the null.[81] Owing to the use of samples that have been stored for many

years, we may have had degradation of samples and artifacts from metabolite–other molecule interactions or from thawing. However, because cases and controls were matched on date of blood collection and were processed together for aliquoting and metabolomic analyses, any biases would likely have been toward the null. Previously, using NHS and HPFS samples stored long term, metabolites have been identified that were significantly associated with multiple health outcomes (e.g., pancreatic cancer,[82] breast cancer,[83,84] prostate cancer,[85] rheumatoid arthritis,[86] and cardiovascular disease[87]), and these findings have been replicated in other studies,[88–91] suggesting that storage time does not substantially impact biomarker-disease associations.

Our study had several strengths. To our knowledge, this study is the first to assess the associations of prediagnostic metabolites and XFG. The sample size was relatively large with 205 cases and 205 matched controls. Also, we had detailed covariate information and a long time between blood draw and diagnosis date (mean, 11.8 years).

In plasma from a decade before diagnosis, LPCs and PEPs were positively associated and triacylglycerols and steroids (e.g., cortisone) were inversely associated with XFG risk.

Disclosure: **J.H. Kang**, None; **O. Zeleznik**, None; **L. Frueh**, None; **J. Lasky-Su**, None; **A.H. Eliassen**, None; **C. Clish**, None; **B.A. Rosner**, None; **L.R. Pasquale**, Eyenovia (C), Twenty Twenty (C), Skye Biosciences (C); **J.L. Wiggs**, Allergan (C), Avellino (C), Editas (C), Maze (C), Regenxbio (C), Aerpio (F)

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