



HAL
open science

Tear metabolomics highlights new potential biomarkers for differentiating between Sjögren's syndrome and other causes of dry eye

Geoffrey Urbanski, Sophie Assad, Floris Chabrun, Juan Manuel Chao de la Barca, Odile Blanchet, Gilles Simard, Guy Lenaers, Delphine Prunier-Mirebeau, Philippe Gohier, Christian Lavigne, et al.

► To cite this version:

Geoffrey Urbanski, Sophie Assad, Floris Chabrun, Juan Manuel Chao de la Barca, Odile Blanchet, et al.. Tear metabolomics highlights new potential biomarkers for differentiating between Sjögren's syndrome and other causes of dry eye. *The Ocular Surface*, 2021, 22, pp.110-116. 10.1016/j.jtos.2021.07.006 . hal-03873010

HAL Id: hal-03873010

<https://hal.science/hal-03873010v1>

Submitted on 22 Aug 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Tear metabolomics highlights new potential biomarkers for differentiating between Sjögren's syndrome and other causes of dry eye

Short title: A tear metabolomics signature of Sjögren's syndrome

Geoffrey Urbanski ^{1,2}, Sophie Assad ³, Floris Chabrun ^{2,4}, Juan Manuel Chao de la Barca ^{2,4},
Odile Blanchet ⁵, Gilles Simard ^{2,4}, Guy Lenaers ², Delphine Prunier-Mirebeau ^{2,4}, Philippe
Gohier ³, Christian Lavigne ¹, Pascal Reynier ^{2,4}

¹ Department of Internal Medicine and Clinical Immunology, University Hospital, Angers, France

² Mitolab, MitoVasc Institute, CNRS 6015, INSERM U1083, University of Angers, France

³ Department of Ophthalmology, University Hospital, Angers, France

⁴ Department of Biochemistry and Molecular Biology, University Hospital, Angers, France

⁵ Centre de Ressources Biologiques, BB-0033-00038, University Hospital, Angers, France

Corresponding author: Geoffrey Urbanski, MD, MSc. Department of Internal Medicine and Clinical Immunology, and Mitolab Team, MitoVasc Institute, CNRS 6015, INSERM U1083, University of Angers, 4 rue Larrey, 49000 Angers, France ; Phone: +33 2 41 35 40 03 ; Fax: +33 2 41 35 49 69 ; Email: urbanskigeoffrey@gmail.com

Word count: 3499

Abstract

Purpose. The lacrimal exocrinopathy of primary Sjögren's syndrome (pSS) is one of the main causes of severe dry eye syndrome and a burden for patients. Early recognition and treatment could prevent irreversible damage to lacrimal glands. The aim of this study was to find biomarkers in tears, using metabolomics and data mining approaches, in patients with newly-diagnosed pSS compared to other causes of dry eye syndrome.

Methods. A prospective cohort of 40 pSS and 40 non-pSS Sicca patients with dryness was explored through a standardized targeted metabolomic approach using liquid chromatography coupled with mass spectrometry. A metabolomic signature predictive of the pSS status was sought out using linear (logistic regression with elastic-net regularization) and non-linear (random forests) machine learning architectures, after splitting the studied population into training, validation and test sets.

Results. Among the 104 metabolites accurately measured in tears, we identified a discriminant signature composed of nine metabolites (two amino acids: serine, aspartate; one biogenic amine: dopamine; six lipids: Lysophosphatidylcholine C16:1, C18:1, C18:2, sphingomyelin C16:0 and C22:3, and the phosphatidylcholine diacyl PCaa42:4), with robust performances (ROC-AUC = 0.83) for predicting the pSS status. Adjustment for age, sex and anti-SSA antibodies did not disrupt the link between the metabolomic signature and the pSS status. The non-lipidic components also remained specific for pSS regardless of the dryness severity.

Conclusion. Our results reveal a metabolomic signature for tears that distinguishes pSS from other dry eye syndromes and further highlight nine key metabolites of potential interest for early diagnosis and therapeutics of pSS.

Keywords: biomarker; metabolomics; primary Sjögren's syndrome; sicca syndrome; tears

Funding: This work was supported by grants from the *Association Française du Gougerot-Sjögren (AFGS)* and the *Société Nationale Française de Médecine Interne (SNFMI)*.

Declarations of interest: None

1. Introduction

Eye dryness is a burden for patients, impairing their quality of life [1], especially in the case of primary Sjögren's syndrome [2], which is a systemic autoimmune disorder [3]. However, the cornerstone of pSS is an exocrinopathy leading to dry syndrome [4]. Measuring the dryness of eyes and mouth is important in pSS diagnosis, as tear and saliva production are used in the classification criteria from most consensus conferences [5–8]. The unstimulated whole saliva flow rate is the sole estimator for mouth dryness, even if its most effective threshold for pSS diagnosis remains under discussion [9]. No such gold standard exists for eye dryness among many possible tests [10]. Only the Schirmer I test and the Ocular Staining Score (OSS) are recognized criteria for pSS diagnosis in the ACR/EULAR (American College of Rheumatology / European League Against Rheumatism) 2016 classification [8]. However, the performance of the Schirmer I test between studies is fairly unreliable with quite low specificity varying from 34 to 76% and sensitivity at about 75% (42 to 90%) for pSS diagnosis [11–13]. Moreover, the Schirmer I test is limited to the aqueous-deficiency production and does not take into account the alterations in the tear film lipid layer due to Meibomian gland dysfunction [14–16]. The OSS enables a global evaluation of the ocular surface troubles [17] and shows slightly better performance than the Schirmer I test [10,18], but this test is subject to inter-operator and intra-subject variability [19,20]. Furthermore, these ocular tests measure only the consequences of the exocrinopathy, while early identification and treatment of eye dryness in pSS patients constitute a challenge. Consequently, there is a crucial need for early biomarkers that will enable clinicians to diagnose and provide perspectives to develop specific therapeutic treatments for pSS patients [21,22]. Many immunological studies reported various candidates for tear biomarkers in dry eye disease such as interleukin (IL) 1 α , IL-1 β , tumor necrosis factor (TNF) α , IL-6, IL-17, MIP-1 α , RANTES (Regulated on Activation, Normal T Cell Expressed and Secreted) and metalloproteinase (MMP) 9 [23–25]. However, these results were heterogeneous and only few studies were specifically focused on pSS [26–28].

Tears are a complex fluid composed of hundreds proteins and metabolites [29]. Recent developments in the field of omics open the way toward deeper tear phenotyping. Proteomics studies undertaken on tears identified several potential biomarkers implicated in inflammation, immunity and oxidative stress in dry eye disease [30,31] and in pSS [32–36]. However, with the exceptions of lactoferrin and lipocalin-1, these data were not confirmed, and most studies did not test their predictive diagnostic power. Due to the large diversity within tears, metabolites could also be attractive candidates for biomarker discovery, notably in immune disorders, as they are closer to the biological phenotype than proteins are [37]. Only a few metabolomic studies of tears have been reported in dry eye syndromes, but they have provided promising results using liquid chromatography (LC) coupled with mass spectrometry (MS) [38–43] or nuclear magnetic resonance spectroscopy [44–46]. Nevertheless, to our knowledge, all these metabolomic studies compared patients with dry eye syndrome to healthy controls, but none has specifically focused on pSS versus other causes of dryness, this later comparison having greater clinical relevance.

This study aimed at i) identifying a metabolomic signature of tears specific to patients with newly diagnosed pSS, compared to patients presenting a non-immune dry eye disease and ii) evaluating the variation of this metabolic signature according to age, sex, immunological status and eye dryness severity.

2. Patients and methods

2.1 Ethics

The METABOGREN study was approved by the Ethical Committees of Angers University Hospital (CPP DC2014-2224, AC2017-2993, bioethics n°2018/42) and was conducted in compliance with the declaration of Helsinki. All participants gave written informed consent.

2.2 Inclusion criteria

All patients who attended the Internal Medicine Department and who presented an objective eye and/or mouth dryness between May 2017 and May 2018 were included in this study. Objective eye dryness was defined with a Schirmer I test ≤ 5 mm / 5 min on at least one eye and/or an OSS ≥ 3 and/or a break-up time (BUT) test < 10 seconds on at least one eye. Objective mouth dryness was defined as a non-stimulated saliva flow ≤ 1.5 mL / 15 minutes. All patients were prospectively investigated for the criteria for Sjögren's syndrome and for other causes of dry syndrome as detailed above. Patients were included until two groups were formed, with 40 cases and 40 non-pSS Sicca patients. In the absence of equilibrium between the two groups, 40 patients were randomly selected among the group with more than 40 subjects. The cases represented the patients with pSS according to the ACR/EULAR 2016 criteria. The non-pSS Sicca subjects were patients without a diagnosis of pSS according to the ACR/EULAR 2016 criteria but also without anti-SSA or anti-SSB antibodies, and without a focus score ≥ 1 on minor labial gland biopsy.

2.3 Exclusion criteria

Patients with a previous diagnosis of pSS before the inclusion were not included. The other exclusion criteria were as follows: history of ocular surgery; wearers of contact lenses and scleral contact lenses; secondary Sjögren's syndrome; other systemic autoimmune disease (systemic lupus erythematosus, antiphospholipid syndrome, rheumatoid arthritis, immune-mediated myopathy, systemic scleroderma, mixed connective tissue disease, eosinophilic fasciitis, sarcoidosis, IgG4 related disease, ANCA-associated vasculitis, periarteritis nodosa, IgA vasculitis, Cogan's syndrome, Behçet disease, Still disease); inflammatory eye disease (uveitis, ocular pemphigoid); graft versus host disease; solid cancer and/or lymphoma/leukemia without remission at least for 2 years except for MALT-associated B lymphoma of parotid; severe or non-controlled arterial hypertension; chronic heart failure, defined as left ventricular ejection fraction $\leq 30\%$; non-controlled diabetes defined as glycated hemoglobin $\geq 8\%$ or diabetes with microangiopathy; chronic respiratory disease necessitating O₂ support; chronic kidney failure defined as creatinine clearance with MDRD

(Modified Diet for Renal Disease) formula $\leq 30 \text{ ml / min / } 1.73 \text{ m}^2$; known mitochondria disease or metabolic genetic disorder; psychotic disorder or unstable psychiatric disease; use of systemic immunosuppressor or immunomodulatory drugs; use of systemic corticotherapy; and use of eye drops, with the exception of artificial tears. Pregnant women and patients unable to give informed consent were not included.

2.4 Ophthalmologic assessment and data collection

Patients underwent a standardized evaluation with the same ophthalmologist, including near and distance visual acuity measurement according to logMar chart [47], a slit-lamp examination of the anterior segment of eye and eyelids and a fundus examination. In order to test the lacrimal function, patients had a Schirmer I test (GECIS, Neung sur Beuvron, France) that was recorded at 5 minutes on the strips, a break-up time test [48], a test for Meibomian gland dysfunction [49] and an OSS evaluation [17]. For those concerned, patients stopped using artificial tears 48 hours before the ophthalmologic assessment. In addition to data from ophthalmological assessment, we collected age, sex, tobacco use and use of anticholinergic drugs [50].

2.5 Analytical workflow (Fig. 1A)

2.5.1 Metabolites extraction

Tears were collected with strips for the Schirmer I test in sterile conditions and firstly during the ophthalmologic evaluation in order to avoid disturbing the metabolic composition. The Schirmer I test was recorded at 5 minutes and the strips were left in until a tear volume of 15 μL (21 mm on strips) was obtained. The lower part of the strips was cut to avoid contamination from contact with skin surface. The strips containing tears were immediately placed in Eppendorf® tubes (Eppendorf, Hamburg, Germany) and stored at -80°C . Strips were transferred in precooled 2.0 ml homogenization Precellys tubes (Bertin Technologies, Montigny-le-Bretonneux, France) prefilled with 1.4 mm diameter ceramic beads and 20 μL of cold methanol. Strips were homogenized by two grinding cycles, each at 6500 rpm for 30

seconds, spaced 20 seconds apart, using a Precellys® homogenizer kept at +4°C. The supernatant was recovered after centrifuging the homogenate at 20,000 g for 10 minutes and kept at -80°C until analysis [51]. Pretests with Schirmer's strips alone did not highlight potential interference: among the 188 metabolites measured, only methionine and spermidine were found to be measurable but at concentrations three times lower than in normal tears.

2.5.2 Metabolites analysis

We applied a targeted, quantitative metabolomic approach to the tear extracts using the Biocrates® AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria) and an AB Sciex QTRAP 5500 (Life Sciences Holdings, Villebon-sur-Yvette, France) MS as previously described [51]. More details about the metabolomic kit, the MS and LC parameters are available in Supplementary Material. All analyses were performed with the same kit to avoid batch effect.

2.5.3 Data cleaning and preprocessing

A cleaning step was performed to filter out unusable data: metabolites with more than 50% of values lying outside the quantification detection limits were removed from the datasets. For all potentially withdrawn features, a Chi-squared test was performed between the number of values within bounds and out-of-bounds in pSS and non-pSS Sicca patients, to prevent removal of discriminating features, i.e. features with a significant difference between the 2 groups. Metabolite concentrations were then normalized before further analyses as described [52].

Figure 1: Analytical (A) and Statistical (B) workflow of the study. Footnotes: LC: liquid chromatography; MS: mass spectrometry; MS/MS: tandem mass spectrometry; FIA: flow injection analysis.

2.6 Statistical workflow (Fig. 1.B)

2.6.1 General statistics

Quantitative data were presented with medians, and 25th and 75th quartiles. Qualitative data were presented as absolute values and as percentages. Quantitative data were compared using a Student's t-test or a Mann-Whitney test as appropriate. Qualitative data were compared using a Chi-squared test or a Fisher's test as appropriate. The type I error was 5%. Image manipulation and formatting were performed using GNU Image Manipulation Program (GIMP) software (version 2.10). The analyses were carried out using R software (version 4.0.1, R-project-org, Vienna, Austria) and SPSS (v23.0, IBM Corp, NY, USA). R packages are listed hereafter: FactoMineR for principal component analysis (PCA) [53]; Rpart for stratified partitioning [54]; glmnet for logistic regression with or without regularization [55] based on λ with 1 standard error (SE) [58]; randomForest [56]; and pROC to evaluate the area under curves for receiver operating characteristic curves - area under the curve (ROC-AUC) [57].

2.6.2 Determination of the metabolomic signature and influence of main confounders

Full statistical workflow is detailed in the Supplementary Material. In brief, we randomly partitioned the study population into three subsets with stratification on case-control status: training set (n = 58), validation set (n = 10) and test set (n = 12). To identify the best predictive model, we carried out a feature selection using elastic-net regularized logistic regression and we compared ROC-AUC from linear (elastic-net regularized logistic regression) and non-linear (random forests) machine learning architectures in the training and validation sets. The model with the highest performance was finally evaluated on the test set to determine the metabolomic signature. The main components of this signature were extracted by means of PCA and the influences of main confounders (age, sex, presence of anti-SSA antibody, tobacco use, and use of anticholinergic drugs and intensity of ocular dryness) were tested on them using linear regression.

3. Results

3.1 Characteristics of the study population

Among 100 patients explored for dryness, 90 were included in the cohort. The study population was composed of 40 patients with pSS in whom 20 showed anti-SSA antibodies, and 40 non-pSS Sicca patients randomly selected from 50 other patients. Median age for the whole studied population was 60.5 [46.8-67] years with sex ratio (W/M) of 7.9. General characteristics are summarized in Table 1. pSS patients only differed from non-pSS participants for ocular objective assessment for dryness, with more altered Schirmer I tests, break-up time, and OSS in pSS patients, with more frequent Meibomian gland dysfunction in patients without pSS. In non-pSS sicca patients, anticholinergic drugs (45%), tobacco use (55%), post-menopause (57.5%) and/or Meibomian gland dysfunction (77.5%) represented the risk factors, sometimes associated, for developing a dry syndrome.

	pSS	Non-pSS Sicca	p-value
Number of patients	40	40	
Women n (%)	36 (90%)	35 (87.5%)	> 0.99
Age (years)	63 [51.8 - 67]	58.5 [46.8 - 66.3]	0.45
Distance visual acuity (LogMar)	0 [0-0.05]	0 [0-0]	0.28
Near visual acuity (LogMar)	0.18 [0.18-0.18]	0.18 [0.18-0.18]	0.29
Tobacco use	17 (42.5%)	22 (55%)	0.37
Anticholinergic drugs use	17 (42.5%)	18 (45%)	> 0.99
Dry syndrome features			
Subjective dry eye syndrome n (%)	36 (90%)	35 (87.5%)	> 0.99
Delay from first symptoms of eye dryness to diagnosis (months)	42 [16.5 - 108.5]	42 [14 - 126.5]	0.66
Schirmer I test (mm / 5 min) (mean for both eyes)	2.3 [0 - 4.5]	3.25 [0.8 - 7.4]	0.03
Break-up time (sec) (mean for both eyes)	2 [1.5-3]	3 [2-4]	< 0.0001

Ocular staining score (highest value)	4 [2 - 5]	2 [1 - 3]	< 0.0001
Meibomian gland dysfunction	21 (52.5%)	31 (77.5%)	0.03
Subjective dry mouth syndrome n (%)	36 (90%)	36 (90%)	> 0.99
Delay from first symptoms of mouth dryness to diagnosis (months)	39 [14 - 81.8]	32 [13.5 - 122]	0.60
UWS flow rate (1.5 mL / 15 min)	3.2 [1.6-4.8]	4 [1.8-6.6]	0.12
ACR/EULAR 2016 criteria for pSS			
Schirmer I test ≤ 5 mm / 5 min n (%)	39 (97.5%)	30 (75%)	0.007
OSS ≥ 5 n (%)	15 (37.5%)	5 (12.5%)	0.02
UWS flow rate ≤ 1.5 mL / 15 min n (%)	10 (25%)	10 (25%)	> 0.99
Anti-SSA antibodies n (%)	20 (50%)	0	< 0.0001
Anti-SSB antibodies n (%)	12 (30%)	0	0.0002
Focus score ≥ 1 on Minor Salivary Gland Biopsy n (%)	35 (87.5%)	0	< 0.0001
Immunological features			
Antinuclear antibodies titer ≥ 1/320 n (%)	18 (45%)	5 (12.5%)	0.003
Presence of rheumatoid factors n (%)	15 (37.5%)	6 (15%)	0.04
Extra-glandular involvement of pSS n (%)	15 (37.5%)	-	

Table 1: General and immunological characteristics of the 2 groups. Footnotes: pSS: primary Sjögren's syndrome.

Footnotes: UWS unstimulated whole saliva

3.2 Metabolomic signature of tears distinguishing pSS from other causes of dry eye syndrome (Figure 1B)

Among the 188 measured metabolites, 84 were excluded as they were beyond the limits of detection. Unsupervised analysis did not reveal spontaneous clustering on principal component analysis. For the step of feature selection, the logistic regression with elastic-net regularization isolated 19 models with the best AUC (0.84) and, among them, five consistent models (alpha coefficients: 0.72; 0.74; 0.77; 0.79; 0.80) with the same nine metabolites of interest: serine, aspartate, dopamine, lysophosphatidylcholines (LysoPC) C16:1, C18:1, C18:2, sphingomyelin (SM) C16:0 and C22:3, and phosphatidylcholine diacyl (PCaa) 42:4. After training models with only the metabolites of interest, the best models obtained using logistic regression disclosed AUCs from 0.84 (nine models) to 0.88 (two models) on the validation set (and of 0.81 to 0.82 on the training set). Due to the lowest number of models with an AUC of 0.88 and higher gap between AUCs on the training and the validation sets for those models, highlighting a potential overfitting, the two models with an AUC of 0.88 were discarded and the optimal model was chosen from those with an AUC of 0.84 and with the lowest alpha value to retain the maximum information among the metabolites of interest. Non-linear models, using Random Forest, with the selected nine metabolites and with whole metabolites, were discarded as there was a huge gap of AUC between training (1.00) and validation (0.68) sets for both. The final prediction on the test set showed an AUC of 0.83, which was homogenous according to the two other sets: training set (0.81) and validation set (0.84). In the final selected metabolomics signature, serine, aspartate and dopamine showed decreased concentrations in pSS, whereas the six phospholipids (LysoPC C18:1, C18:2, C16:1, SM C16:0, C22:3, and PCaa 42:4) had increased concentrations in pSS, compared to the non-pSS Sicca group (Figure 2 and Supplementary table).

Figure 2: Word cloud showing the metabolomic signature of tears for pSS.

Legend: the increased metabolites in pSS are presented in red and decreased ones in blue. The importance of the metabolites in the signature is proportional in size to the beta coefficient in the logistic regression equation of the final model.

Footnotes: LysoPC: lysophosphatidylcholine; PCaa: phosphatidylcholine diacyl; SM: sphingomyelin.

3.3 Analysis of the lipidic component of the metabolomic signature

The ratio of the sum of the LysoPC to the sum of PC, an indicator of the activity of the enzyme phospholipase A2, was increased in the pSS group compared to the non-pSS Sicca group (77.3 [50.2 – 188.3] versus 55.3 [34.8 – 80.7], $p = 0.004$). The ratio of the sum of the SM to the sum of the PC representing the sphingomyelinase activity was also increased in the tears from pSS patients (174.8 [23.9 – 431.3] vs 24.4 [19.3 – 73.0], $p = 0.0004$).

3.4 Influence of age, sex, presence of anti-SSA antibodies and ocular dryness severity on the metabolomic signature

Dimensional reduction of the metabolomic signature revealed that two first principal components (PC) explained 74.5% of total variance. Eight of the nine metabolites were mainly distributed in one of those components: the six lipids in the PC 1 and the aminoacids in the second one (Figure 3).

Figure 3: Distribution of the metabolites from the metabolomic signature in the two main components. Legend: the two main components were extracted after dimensional reduction with principal component analysis. Footnotes: LysoPC: lysophosphatidylcholine; PC: principal component; PCaa: phosphatidylcholine diacyl; SM: sphingomyelin.

The association between the metabolomic signature and the pSS status was not altered by age, sex, use of anticholinergic drugs or presence of anti-SSA antibodies for the two first principal components (Table 2). The use of tobacco was significantly associated with the lipid

component but not with the PC2. The link between PC1 and pSS status disappeared with adjustment for the dryness severity measurement, i.e. Schirmer I test and OSS. Moreover, the results of OSS were associated with the PC1. None of the parameters for adjustment disrupted the link between PC2 and pSS status.

	Principal component 1 (mainly lipids)		Principal component 2 (mainly aminoacids)	
	Beta regressor	p-value	Beta regressor	p-value
<i>Model 1</i>				
pSS status	0.57	0.03	-0.66	0.02
Sex (male)	0.19	0.60	0.20	0.60
Age	-0.01	0.57	0.006	0.53
Tobacco use	-0.54	0.02	0.20	0.40
Anticholinergic drugs use	-0.07	0.78	-0.10	0.68
Anti-SSA antibodies	0.02	0.95	0.51	0.11
<i>Model 2</i>				
pSS status	0.51	0.06	-0.64	0.03
Sex (male)	0.19	0.60	0.20	0.60
Age	-0.01	0.47	0.007	0.50
Tobacco use	-0.51	0.03	0.19	0.44
Anticholinergic drug use	-0.03	0.88	-0.11	0.65
Anti-SSA antibodies	0.02	0.95	0.51	0.12
Schirmer I test	-0.02	0.33	0.009	0.72
<i>Model 3</i>				
pSS status	0.43	0.10	-0.76	0.007
Sex (male)	0.35	0.32	0.31	0.41
Age	-0.01	0.33	0.004	0.72
Tobacco use	-0.58	0.01	0.18	0.45
Anticholinergic drug use	-0.02	0.92	-0.07	0.78
Anti-SSA antibodies	-0.27	0.39	0.31	0.36

Ocular Staining Score	0.19	0.008	0.13	0.07
<i>Model 4</i>				
pSS status	0.53	0.05	-0.69	0.02
Sex (male)	0.23	0.52	0.23	0.55
Age	-0.01	0.51	0.005	0.58
Tobacco use	-0.58	0.02	0.18	0.47
Anticholinergic drug use	-0.04	0.88	-0.08	0.75
Anti-SSA antibodies	0.001	0.99	0.50	0.13
Meibomian glands dysfunction	-0.22	0.36	-0.16	0.53

Table 2: Influence of age, sex, presence of anti-SSA antibodies and severity of dry eye syndrome on the link between the primary Sjögren's syndrome and the metabolomic signature.

Footnotes: The 4 models are independent. The value of Beta regressor is indicative for the direction of the association. Age, Schirmer I test and OSS are quantitative variables. pSS status, sex, tobacco use, anticholinergic drugs use, anti-SSA antibodies and Meibomian gland dysfunction are dummy variables.

4. Discussion

In this study, we demonstrated that the metabolic signature of tears composed of nine metabolites could differentiate newly diagnosed pSS patients from patients suffering from others causes of dryness syndrome. The signature was established through a machine-learning architecture disclosing highly homogenous predictive values in 3 different data sets. This indicates the robustness of the metabolomic signature in predicting pSS without overfitting. The signature was composed of two amino acids (serine and aspartate), one biogenic amine (dopamine) and six phospholipids: two LysoPC (C18:1, C18:2, C16:1), two SM (C16:0, C22:3), and one PC (aa42:4). Few metabolomic studies of tear composition were

reported in dry eye disease [38–45] and, to the best of our knowledge, none specifically compared pSS patients to other causes of dry eye syndrome, which is clinically more relevant.

The characteristics of the study population were similar to other pSS populations described in the literature with a median age of 60 years old, a sex ratio of approximately 1:7 and the presence of anti-SSA antibodies in 50% of patients [44,58].

The metabolic signature included six phospholipids that were all increased in the tears from pSS patients. Lam et al. also reported increased concentrations of LysoPC C18:1, C18:2 and SM C16:0 in the meibum of patients suffering from dry eye syndrome compared to healthy controls [59]. However, the causes of sicca syndrome in this study were not precisely described. The authors hypothesized that the increased concentrations of LysoPC were related to the activity of the enzyme phospholipase A2. In our study, we noticed that the ratio of LysoPC to PC was increased in pSS patients and correlated with this assessment. Some authors recently demonstrated that phospholipase A2 is linked to dry eye disease through prostaglandin E2 and pro-inflammatory cytokines (TNF- α and IL-1 β) [60], known to be implicated in pSS (27–29). Lipids are important in tears, as they represent the outer part of the film secreted by the Meibomian glands [61]. However, some authors have hypothesized that the lacrimal gland is the origin of many tear lipids, as they have observed positive correlations between the tear levels of cholesteryl sulfates and glycosphingolipids with tear secretion [62]. These authors noticed reduced concentrations of LysoPC with decreased tear production. In our study, we did not find a significant link between the outcome of the Schirmer I test and the lipid component of the metabolomic signature. However, the six lipids of the signature had increased concentrations in tears from pSS patients, having more severe eye dryness, compared to non-pSS Sicca patients. The sole test of our study significantly correlated to the lipid component was the OSS, meaning that this component was partially influenced by eye dryness severity. Indeed, the results of the Schirmer I test and the OSS varied according to the association between the pSS status and the principal component related to the six lipids.

The reduction of dopamine, a biogenic amine which is well known for its implication in Parkinson's disease [63], correlated with decreased frequency of eye blinks [64], inducing a dry eye syndrome via a decreased lipid secretion by the Meibomian glands that are stimulated by blinks [65]. Interestingly, some authors have reported an unexplained increasing risk of Parkinson's disease in pSS patients [66]. From another perspective, a possible lacrimal secretion of dopamine has been described [67], which could link Parkinson's disease and pSS through Meibomian gland dysfunction. In pSS, we can hypothesize that the primary involvement of the lacrimal gland induces a decreased local secretion of dopamine responsible for a reduced frequency of eye blinks, and consequently lead to Meibomian gland dysfunction frequently noted in pSS patients [68].

The reduction of serine has already been demonstrated in a study including patients with dry eye disease without defined causes [69]. The serine is a non-essential aminoacid which can be converted in glycine, a rate limiting factor for the synthesis of glutathione [70]. Aspartate is also linked to glutathione production through glutamate [71]. Glutathione and the enzyme glutathione peroxidase are known to provide protection from oxidative stress. A decreased activity of glutathione peroxidase was reported in pSS patients which could result in an increased oxidative stress and contributing to the development of anterior eye surface injuries [72]. This would explain the particular severity of dry eye in pSS patients. Moreover, this mechanism could be particular to pSS, since in our study, the signature component linked to aminoacids was always associated with pSS status, even after adjustment for markers of dry eye severity.

Our metabolomic study is limited by its targeted approach. Untargeted metabolomic studies would cover a wider part of the tear metabolome. However, the untargeted approaches are also limited by their lesser level of standardization, with larger coefficients of variation of metabolite concentrations, which make them difficult to use in the discovery of clinical

biomarkers. Our results are limited by the absence of healthy control group. However, several studies already compared the tear metabolome of patients suffering from dry eye disease with those of healthy controls [38,39,44,45,40,58,69]. To the best of our knowledge, our study is the first specifically focusing on the comparison between pSS and other causes of dry eye syndrome, this latter comparison being more relevant in clinical practice where physicians have to identify pSS patients among other causes of dry syndromes. Finally, we were not able to include patients at the very first steps of pSS evolution, before the consequences of exocrinopathy, as the pSS status was determined according the current ACR/EULAR 2016 criteria [8]. However, to reduce this limitation, we prospectively included patients suffering from dryness without knowing the cause, which is a strength of this study.

5. Conclusions

The metabolomic signature identified here could be helpful as an early biomarker of pSS. This signature has high diagnostic performance with a simple method to collect the tears that can be easily reproduced in clinics. It would be interesting to test whether one or more of the nine metabolites identified here could be validated as a clinical relevant tear biomarker of pSS in a larger multicentric study.

Acknowledgments: The authors warmly thank all the individuals who participated to this study.

References

- [1] Mertzanis P, Abetz L, Rajagopalan K, Espindle D, Chalmers R, Snyder C, et al. The relative burden of dry eye in patients' lives: comparisons to a U.S. normative sample. *Invest Ophthalmol Vis Sci* 2005;46:46–50. <https://doi.org/10.1167/iovs.03-0915>.

- [2] Saldanha IJ, Bunya VY, McCoy SS, Makara M, Baer AN, Akpek EK. Ocular Manifestations and Burden Related to Sjögren Syndrome: Results of a Patient Survey. *Am J Ophthalmol* 2020;219:40–8. <https://doi.org/10.1016/j.ajo.2020.05.043>.
- [3] Mariette X, Criswell LA. Primary Sjögren's Syndrome. *N Engl J Med* 2018;378:931–9. <https://doi.org/10.1056/NEJMcp1702514>.
- [4] Akpek EK, Bunya VY, Saldanha IJ. Sjögren's Syndrome: More Than Just Dry Eye. *Cornea* 2019;38:658–61. <https://doi.org/10.1097/ICO.0000000000001865>.
- [5] Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, et al. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum* 1993;36:340–7. <https://doi.org/10.1002/art.1780360309>.
- [6] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
- [7] Shiboski SC, Shiboski CH, Criswell LA, Baer AN, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res* 2012;64:475–87. <https://doi.org/10.1002/acr.21591>.
- [8] Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol* Hoboken NJ 2017;69:35–45. <https://doi.org/10.1002/art.39859>.
- [9] Lacombe V, Lacout C, Lozac'h P, Ghali A, Gury A, Lavigne C, et al. Unstimulated whole saliva flow for diagnosis of primary Sjögren's syndrome: time to revisit the threshold? *Arthritis Res Ther* 2020;22:38. <https://doi.org/10.1186/s13075-020-2132-3>.

- [10] Gonzales JA, Shiboski SC, Bunya VY, Akpek EK, Rose-Nussbaumer J, Seitzman GD, et al. Ocular Clinical Signs and Diagnostic Tests Most Compatible With Keratoconjunctivitis Sicca: A Latent Class Approach. *Cornea* 2020;39:1013–6. <https://doi.org/10.1097/ICO.0000000000002311>.
- [11] Vissink A, Kalk WWI, Mansour K, Spijkervet FKL, Bootsma H, Roodenburg JLN, et al. Comparison of lacrimal and salivary gland involvement in Sjögren's syndrome. *Arch Otolaryngol Head Neck Surg* 2003;129:966–71. <https://doi.org/10.1001/archotol.129.9.966>.
- [12] Versura P, Frigato M, Mulé R, Malavolta N, Campos EC. A proposal of new ocular items in Sjögren's syndrome classification criteria. *Clin Exp Rheumatol* 2006;24:567–72.
- [13] Versura P, Frigato M, Cellini M, Mulè R, Malavolta N, Campos EC. Diagnostic performance of tear function tests in Sjogren's syndrome patients. *Eye Lond Engl* 2007;21:229–37. <https://doi.org/10.1038/sj.eye.6702204>.
- [14] Chen X, Utheim ØA, Xiao J, Adil MY, Stojanovic A, Tashbayev B, et al. Meibomian gland features in a Norwegian cohort of patients with primary Sjögren's syndrome. *PloS One* 2017;12:e0184284. <https://doi.org/10.1371/journal.pone.0184284>.
- [15] Wang Y, Qin Q, Liu B, Fu Y, Lin L, Huang X, et al. Clinical Analysis: Aqueous-Deficient and Meibomian Gland Dysfunction in Patients With Primary Sjogren's Syndrome. *Front Med* 2019;6:291. <https://doi.org/10.3389/fmed.2019.00291>.
- [16] Daniel E, Pistilli M, Ying G-S, Bunya VY, Massaro-Giordano M, Asbell PA, et al. Association of meibomian gland morphology with symptoms and signs of dry eye disease in the Dry Eye Assessment and Management (DREAM) study. *Ocul Surf* 2020;18:761–9. <https://doi.org/10.1016/j.jtos.2020.07.014>.
- [17] Whitcher JP, Shiboski CH, Shiboski SC, Heidenreich AM, Kitagawa K, Zhang S, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's Syndrome International Registry. *Am J Ophthalmol* 2010;149:405–15. <https://doi.org/10.1016/j.ajo.2009.09.013>.

- [18] Kim M, Kim HS, Na K-S. Correlation between Tear Osmolarity and Other Ocular Surface Parameters in Primary Sjögren's Syndrome. *Korean J Ophthalmol KJO* 2017;31:25–31. <https://doi.org/10.3341/kjo.2017.31.1.25>.
- [19] Rose-Nussbaumer J, Lietman TM, Shiboski CH, Shiboski SC, Bunya VY, Akpek EK, et al. Inter-grader Agreement of the Ocular Staining Score in the Sjögren's International Clinical Collaborative Alliance (SICCA) Registry. *Am J Ophthalmol* 2015;160:1150-1153.e3. <https://doi.org/10.1016/j.ajo.2015.08.021>.
- [20] Rasmussen A, Stone DU, Kaufman CE, Hefner KS, Fram NR, Siatkowski RL, et al. Reproducibility of Ocular Surface Staining in the Assessment of Sjögren Syndrome-Related Keratoconjunctivitis Sicca: Implications on Disease Classification. *ACR Open Rheumatol* 2019;1:292–302. <https://doi.org/10.1002/acr2.1033>.
- [21] Baldini C, Ferro F, Elefante E, Bombardieri S. Biomarkers for Sjögren's syndrome. *Biomark Med* 2018;12:275–86. <https://doi.org/10.2217/bmm-2017-0297>.
- [22] Simsek C, Dogru M. Ocular Surface Biomarkers. *Eye Contact Lens* 2020. <https://doi.org/10.1097/ICL.0000000000000767>.
- [23] Lam H, Bleiden L, de Paiva CS, Farley W, Stern ME, Pflugfelder SC. Tear cytokine profiles in dysfunctional tear syndrome. *Am J Ophthalmol* 2009;147:198-205. e1. <https://doi.org/10.1016/j.ajo.2008.08.032>.
- [24] Chotikavanich S, de Paiva CS, Li DQ, Chen JJ, Bian F, Farley WJ, et al. Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome. *Invest Ophthalmol Vis Sci* 2009;50:3203–9. <https://doi.org/10.1167/iovs.08-2476>.
- [25] Boehm N, Riechardt AI, Wiegand M, Pfeiffer N, Grus FH. Proinflammatory cytokine profiling of tears from dry eye patients by means of antibody microarrays. *Invest Ophthalmol Vis Sci* 2011;52:7725–30. <https://doi.org/10.1167/iovs.11-7266>.
- [26] Yang S, Lee HJ, Kim D-Y, Shin S, Barabino S, Chung S-H. The Use of Conjunctival Staining to Measure Ocular Surface Inflammation in Patients With Dry Eye. *Cornea* 2019;38:698–705. <https://doi.org/10.1097/ICO.0000000000001916>.

- [27] Akpek EK, Bunya VY, Saldanha IJ. Sjögren's Syndrome: More Than Just Dry Eye. *Cornea* 2019;38:658–61. <https://doi.org/10.1097/ICO.0000000000001865>.
- [28] Willems B, Tong L, Minh TDT, Pham ND, Nguyen XH, Zumbansen M. Novel Cytokine Multiplex Assay for Tear Fluid Analysis in Sjogren's Syndrome. *Ocul Immunol Inflamm* 2020;1–6. <https://doi.org/10.1080/09273948.2020.1767792>.
- [29] de Souza GA, Godoy LMF, Mann M. Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. *Genome Biol* 2006;7:R72. <https://doi.org/10.1186/gb-2006-7-8-R72>.
- [30] Saijyothi AV, Angayarkanni N, Syama C, Utpal T, Shweta A, Bhaskar S, et al. Two dimensional electrophoretic analysis of human tears: collection method in dry eye syndrome. *Electrophoresis* 2010;31:3420–7. <https://doi.org/10.1002/elps.201000271>.
- [31] Aluru SV, Agarwal S, Srinivasan B, Iyer GK, Rajappa SM, Tatu U, et al. Lacrimal proline rich 4 (LPRR4) protein in the tear fluid is a potential biomarker of dry eye syndrome. *PloS One* 2012;7:e51979. <https://doi.org/10.1371/journal.pone.0051979>.
- [32] Aqrabi LA, Galtung HK, Vestad B, Øvstebø R, Thiede B, Rusthen S, et al. Identification of potential saliva and tear biomarkers in primary Sjögren's syndrome, utilising the extraction of extracellular vesicles and proteomics analysis. *Arthritis Res Ther* 2017;19. <https://doi.org/10.1186/s13075-017-1228-x>.
- [33] Versura P, Giannaccare G, Vukatana G, Mulè R, Malavolta N, Campos EC. Predictive role of tear protein expression in the early diagnosis of Sjögren's syndrome. *Ann Clin Biochem* 2018;55:561–70. <https://doi.org/10.1177/0004563217750679>.
- [34] Aqrabi LA, Galtung HK, Guerreiro EM, Øvstebø R, Thiede B, Utheim TP, et al. Proteomic and histopathological characterisation of sicca subjects and primary Sjögren's syndrome patients reveals promising tear, saliva and extracellular vesicle disease biomarkers. *Arthritis Res Ther* 2019;21:181. <https://doi.org/10.1186/s13075-019-1961-4>.

- [35] Kuo M-T, Fang P-C, Chao T-L, Chen A, Lai Y-H, Huang Y-T, et al. Tear Proteomics Approach to Monitoring Sjögren Syndrome or Dry Eye Disease. *Int J Mol Sci* 2019;20. <https://doi.org/10.3390/ijms20081932>.
- [36] Li B, Sheng M, Li J, Yan G, Lin A, Li M, et al. Tear proteomic analysis of Sjögren syndrome patients with dry eye syndrome by two-dimensional-nano-liquid chromatography coupled with tandem mass spectrometry. *Sci Rep* 2014;4:5772. <https://doi.org/10.1038/srep05772>.
- [37] Yazdani M, Elgstøen KBP, Rootwelt H, Shahdadfar A, Utheim ØA, Utheim TP. Tear Metabolomics in Dry Eye Disease: A Review. *Int J Mol Sci* 2019;20. <https://doi.org/10.3390/ijms20153755>.
- [38] Peral A, Carracedo G, Acosta MC, Gallar J, Pintor J. Increased levels of diadenosine polyphosphates in dry eye. *Invest Ophthalmol Vis Sci* 2006;47:4053–8. <https://doi.org/10.1167/iovs.05-0980>.
- [39] Pescosolido N, Imperatrice B, Koverech A, Messano M. L-carnitine and short chain ester in tears from patients with dry eye. *Optom Vis Sci Off Publ Am Acad Optom* 2009;86:E132-138. <https://doi.org/10.1097/OPX.0b013e318194e767>.
- [40] Pieragostino D, Agnifili L, Cicalini I, Calienno R, Zucchelli M, Mastropasqua L, et al. Tear Film Steroid Profiling in Dry Eye Disease by Liquid Chromatography Tandem Mass Spectrometry. *Int J Mol Sci* 2017;18. <https://doi.org/10.3390/ijms18071349>.
- [41] English JT, Norris PC, Hodges RR, Dartt DA, Serhan CN. Identification and Profiling of Specialized Pro-Resolving Mediators in Human Tears by Lipid Mediator Metabolomics. *Prostaglandins Leukot Essent Fatty Acids* 2017;117:17–27. <https://doi.org/10.1016/j.plefa.2017.01.004>.
- [42] Chen X, Rao J, Zheng Z, Yu Y, Lou S, Liu L, 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;18:2321–30. <https://doi.org/10.1021/acs.jproteome.9b00149>.

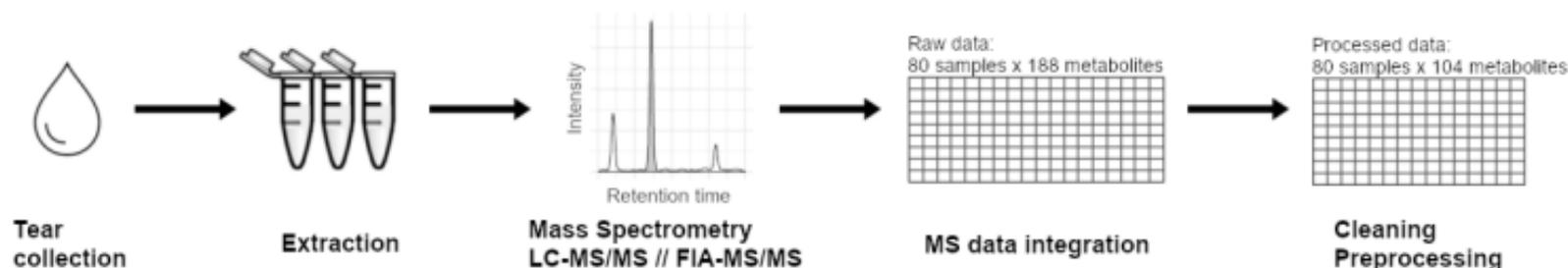
- [43] Jiang Y, Yang C, Zheng Y, Liu Y, Chen Y. A Set of Global Metabolomic Biomarker Candidates to Predict the Risk of Dry Eye Disease. *Front Cell Dev Biol* 2020;8:344. <https://doi.org/10.3389/fcell.2020.00344>.
- [44] Galbis-Estrada C, Martinez-Castillo S, Morales JM, Vivar-Llopis B, Monleón D, Díaz-Llopis M, et al. Differential effects of dry eye disorders on metabolomic profile by ¹H nuclear magnetic resonance spectroscopy. *BioMed Res Int* 2014;2014:542549. <https://doi.org/10.1155/2014/542549>.
- [45] Galbis-Estrada C, Pinazo-Durán MD, Martínez-Castillo S, Morales JM, Monleón D, Zanon-Moreno V. A metabolomic approach to dry eye disorders. The role of oral supplements with antioxidants and omega 3 fatty acids. *Mol Vis* 2015;21:555–67.
- [46] Benítez Del Castillo JM, Pinazo-Duran MD, Sanz-González S, Muñoz-Hernández AM, Garcia-Medina JJ, Zanón-Moreno V. Tear ¹H NMR-based metabolomics application to the molecular diagnosis of aqueous tear deficiency and Meibomian gland dysfunction. *Ophthalmic Res* 2020. <https://doi.org/10.1159/000510211>.
- [47] Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Am J Optom Physiol Opt* 1976;53:740–5. <https://doi.org/10.1097/00006324-197611000-00006>.
- [48] Abelson MB, Ousler GW, Nally LA, Welch D, Krenzer K. Alternative reference values for tear film break up time in normal and dry eye populations. *Adv Exp Med Biol* 2002;506:1121–5. https://doi.org/10.1007/978-1-4615-0717-8_157.
- [49] Asiedu K, Kyei S, Dzasimatu SK, Morny EKA. Meibomian Gland Dysfunction in a Youthful Clinical Sample in Ghana. *Optom Vis Sci Off Publ Am Acad Optom* 2018;95:349–53. <https://doi.org/10.1097/OPX.0000000000001192>.
- [50] Durán CE, Azermai M, Vander Stichele RH. Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol* 2013;69:1485–96. <https://doi.org/10.1007/s00228-013-1499-3>.
- [51] Chao de la Barca JM, Simard G, Amati-Bonneau P, Safiedeen Z, Prunier-Mirebeau D, Chupin S, et al. The metabolomic signature of Leber's hereditary optic neuropathy

- reveals endoplasmic reticulum stress. *Brain J Neurol* 2016;139:2864–76. <https://doi.org/10.1093/brain/aww222>.
- [52] Chabrun F, Dieu X, Rousseau G, Chupin S, Letournel F, Procaccio V, et al. Metabolomics reveals highly regional specificity of cerebral sexual dimorphism in mice. *Prog Neurobiol* 2020;184:101698. <https://doi.org/10.1016/j.pneurobio.2019.101698>.
- [53] Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. *J Stat Softw* 2008;25:1–18. <https://doi.org/10.18637/jss.v025.i01>.
- [54] Therneau T, Atkinson B, port BR (producer of the initial R, maintainer 1999-2017). *rpart: Recursive Partitioning and Regression Trees*. 2019.
- [55] Friedman JH, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw* 2010;33:1–22. <https://doi.org/10.18637/jss.v033.i01>.
- [56] Liaw, Wiener. *Classification and Regression by randomForest* Andy Liaw and Matthew Wiener. *R news* 2002;2:18–22.
- [57] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12:77. <https://doi.org/10.1186/1471-2105-12-77>.
- [58] Chen X, Rao J, Zheng Z, Yu Y, Lou S, Liu L, 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;18:2321–30. <https://doi.org/10.1021/acs.jproteome.9b00149>.
- [59] Lam SM, Tong L, Yong SS, Li B, Chaurasia SS, Shui G, et al. Meibum lipid composition in Asians with dry eye disease. *PloS One* 2011;6:e24339. <https://doi.org/10.1371/journal.pone.0024339>.
- [60] Wei Y, Asbell PA. sPLA2-IIa participates in ocular surface inflammation in humans with dry eye disease. *Exp Eye Res* 2020;201:108209. <https://doi.org/10.1016/j.exer.2020.108209>.

- [61] DEBBASCH C, POULIQUEN P. Compte rendu de 3rd International conference on the lacrymal gland tear film and dry eye syndromes, congrès de Maui, novembre 2000. Revue Sicca n°15, eds La librairie Médicale, Laboratoires Théa. n.d.
- [62] Lam SM, Tong L, Reux B, Duan X, Petznick A, Yong SS, et al. Lipidomic analysis of human tear fluid reveals structure-specific lipid alterations in dry eye syndrome. *J Lipid Res* 2014;55:299–306. <https://doi.org/10.1194/jlr.P041780>.
- [63] Miller DB, O'Callaghan JP. Biomarkers of Parkinson's disease: present and future. *Metabolism* 2015;64:S40-46. <https://doi.org/10.1016/j.metabol.2014.10.030>.
- [64] Taylor JR, Elsworth JD, Lawrence MS, Sladek JR, Roth RH, Redmond DE. Spontaneous blink rates correlate with dopamine levels in the caudate nucleus of MPTP-treated monkeys. *Exp Neurol* 1999;158:214–20. <https://doi.org/10.1006/exnr.1999.7093>.
- [65] Définition et classification de la sécheresse oculaire : rapport du sous-comité de définition et classification du DEWS (Dry Eye workshop) international (2007) 2007.
- [66] Ju U-H, Liu F-C, Lin C-S, Huang W-Y, Lin T-Y, Shen C-H, et al. Risk of Parkinson disease in Sjögren syndrome administered ineffective immunosuppressant therapies: A nationwide population-based study. *Medicine (Baltimore)* 2019;98:e14984. <https://doi.org/10.1097/MD.00000000000014984>.
- [67] Sharma NS, Acharya SK, Nair AP, Matalia J, Shetty R, Ghosh A, et al. Dopamine levels in human tear fluid. *Indian J Ophthalmol* 2019;67:38–41. https://doi.org/10.4103/ijo.IJO_568_18.
- [68] Daniel E, Pistilli M, Ying G-S, Bunya VY, Massaro-Giordano M, Asbell PA, et al. Association of meibomian gland morphology with symptoms and signs of dry eye disease in the Dry Eye Assessment and Management (DREAM) study. *Ocul Surf* 2020. <https://doi.org/10.1016/j.jtos.2020.07.014>.
- [69] Jiang Y, Yang C, Zheng Y, Liu Y, Chen Y. A Set of Global Metabolomic Biomarker Candidates to Predict the Risk of Dry Eye Disease. *Front Cell Dev Biol* 2020;8:344. <https://doi.org/10.3389/fcell.2020.00344>.

- [70] Gao X, Lee K, Reid MA, Sanderson SM, Qiu C, Li S, et al. Serine Availability Influences Mitochondrial Dynamics and Function through Lipid Metabolism. *Cell Rep* 2018;22:3507–20. <https://doi.org/10.1016/j.celrep.2018.03.017>.
- [71] Rebholz CM, Yu B, Zheng Z, Chang P, Tin A, Köttgen A, et al. Serum metabolomic profile of incident diabetes. *Diabetologia* 2018;61:1046–54. <https://doi.org/10.1007/s00125-018-4573-7>.
- [72] Cejková J, Ardan T, Simonová Z, Cejka C, Malec J, Dotrelová D, et al. Decreased expression of antioxidant enzymes in the conjunctival epithelium of dry eye (Sjögren's syndrome) and its possible contribution to the development of ocular surface oxidative injuries. *Histol Histopathol* 2008;23:1477–83. <https://doi.org/10.14670/HH-23.1477>.

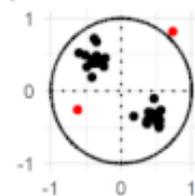
A. Analytical pipeline



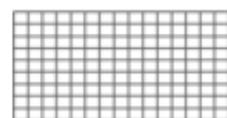
B. Statistical pipeline

Unsupervised analysis

Check absence of outliers and spontaneous clustering

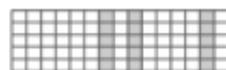


Stratified partitioning

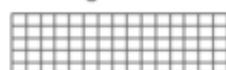


Feature selection

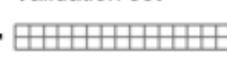
Select discriminant features for Sjögren's Syndrome prediction



Training set



Validation set

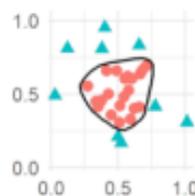
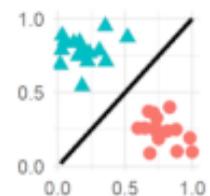


Test set



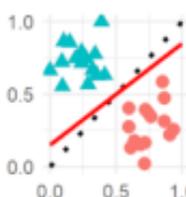
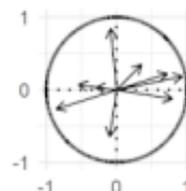
Linear and non-linear predictive models

Confirm predictive value of selected features



Unsupervised analysis with selected features

Adjust for covariates



LysoPCaC18:2

LysoPCaC18:1

Serine

PCaaC4,2:4

SMC22:3

Aspartate

Dopamine

SMC16:0

LysoPCaC16:1

