ANALYZING CHANGES IN TEAR PROTEINS OF SJOGREN'S SYNDROME PATIENTS WITH TIMSTOF PRO

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Introduction

Biomarkers in Sjögren´s syndrome

Sjögren's syndrome (SS)

→ A chronic, progressive autoimmune disease causing severe dry eye [1, 2].

→ Destruction of lacrimal and salivary glands >>>> reduced secretion of tears and saliva [1, 3].

→ Increased APCs* infiltration/maturation in conjunctiva & reduced goblet cell density [4].

→ Mainly affects women (9-fold higher than men)[5].

Current diagnosis of SS

Regularly→ **Ro52/SSA, Ro60/SSA** and **La/SSB** >>>> found only 77-90% of patients[6]

Occasionally → Rheumatoid factor (RF), Anti-nuclear antibodies (ANA) [7]

Unmet needs

→ SS has 4 stages: initiation, preclinical, asymptomatic and overt stage [8].

→ Early diagnosis and management is challenging [8].

→ No effective therapy exists that can halt the progress [5].

→ Lack of highly **specific** and **sensitive biomarker** in SS [9].

→ For more accurate, rapid diagnosis & stratification & treatment & follow-up of patients >>>> validated biomarkers are needed[7].

Omics studies are needed to develop new candidate biomarkers for rapid and effective diagnosis of SS.

*APCs: Antigen-presenting cells

Introduction

Tear fluid (TF), a valuable source for biomarker

Biological fluids for biomarkers exploration in SS:

Serum and Saliva →→ Too complex composition [10]

TF →→ limited sample but less complex compared to saliva and serum [11]

- TF reflects the physiological condition of ocular diseases [12].

Objective

To investigate changes in the tear proteome of SS patients using a comprehensive proteomics approach based on timsTOF Pro mass spectrometry.

Mass Spectrometry -Proteomics Investigation

Mass spectrometry (MS) technology provides:

→ The largest proteomics datasets and reliable quantification [13].

→ Proteomics technology enables analysis of biochemical changes in tear [14].

timsTOF Pro*

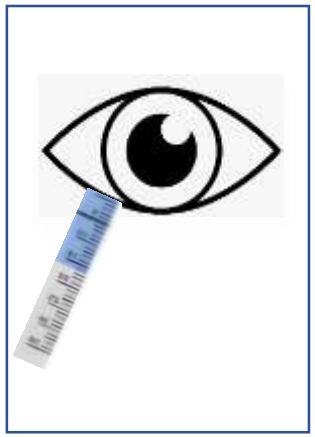
→ Helps to identify differentially expressed proteins that involved in critical signaling pathways in SS from a limited sample thanks to its improved spatial resolution, sensitivity, and specificity [15].

*trapped-ion mobility spectrometry coupled quadrupole time-of-flight

Methods

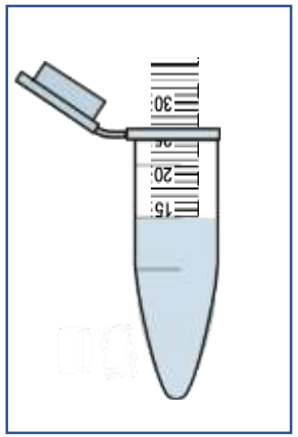
Tear proteins: from collection to identification

1. Schirmer strip collection

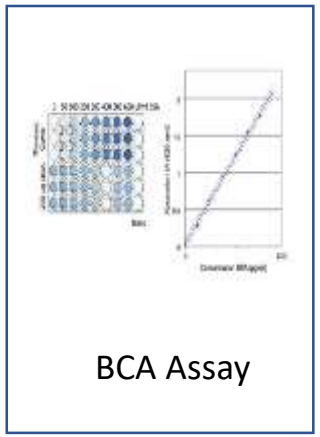


6 SS patients
6 healthy controls (HC)

2. Protein elution



3. Protein quantification



BCA Assay

4. Sample processing

- Reduction
- Alkylation
- Digestion

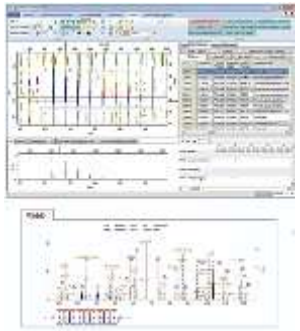
of proteins

5. LC-MS/MS analysis

nanoElute UHPLC* timsTOF Pro

6. Protein Identification

MaxQuant



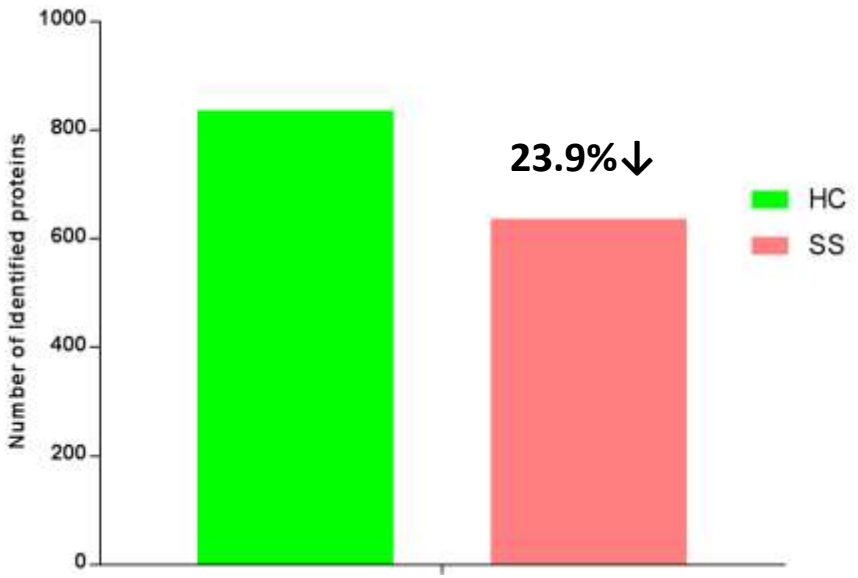
Tear sample collection with the Schirmer strips and sample preparation for LC-MS/MS analysis

MS/MS data was processed using MaxQuant software for protein identification. Protein Gene Ontology classification was performed by using Panther.

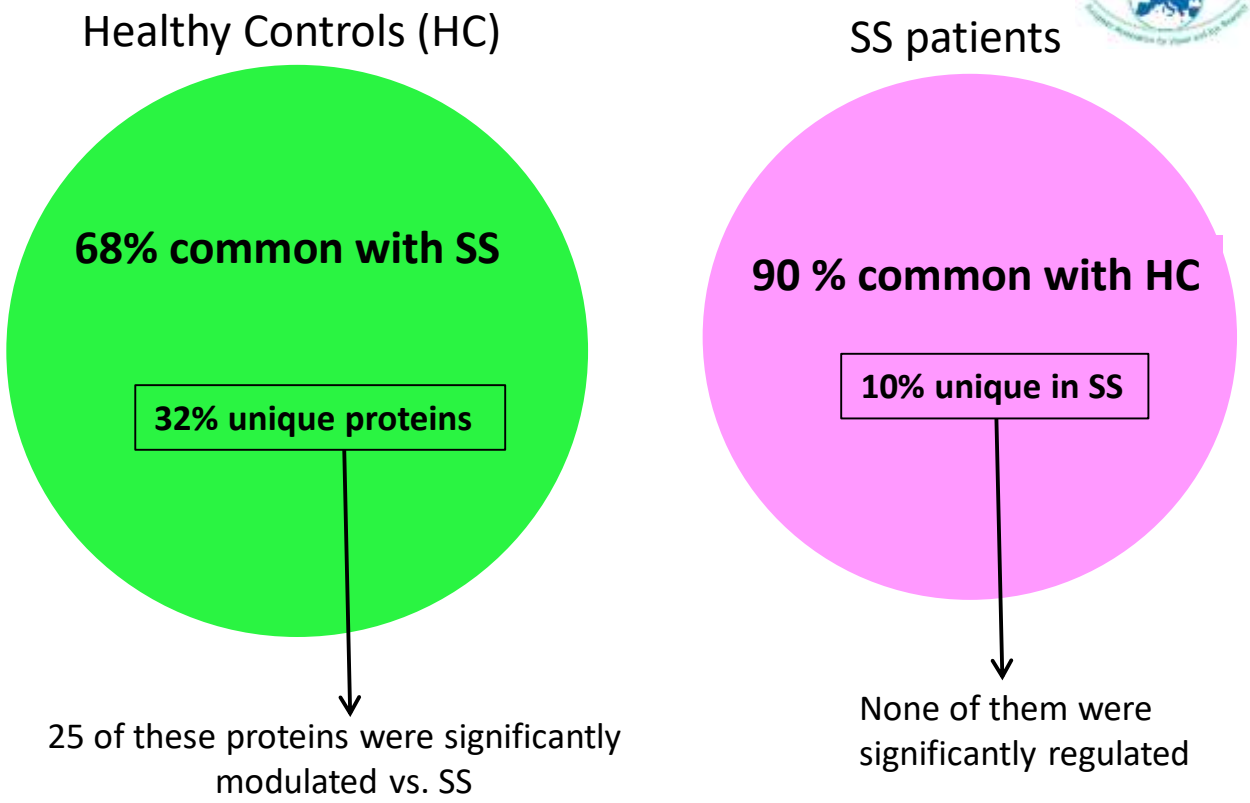
*UHPLC: ultrahigh-pressure liquid chromatography

Results - 1

Comparison of total identified proteins



➢ In SS, total identified proteins was decreased by 23.9% versus HC.



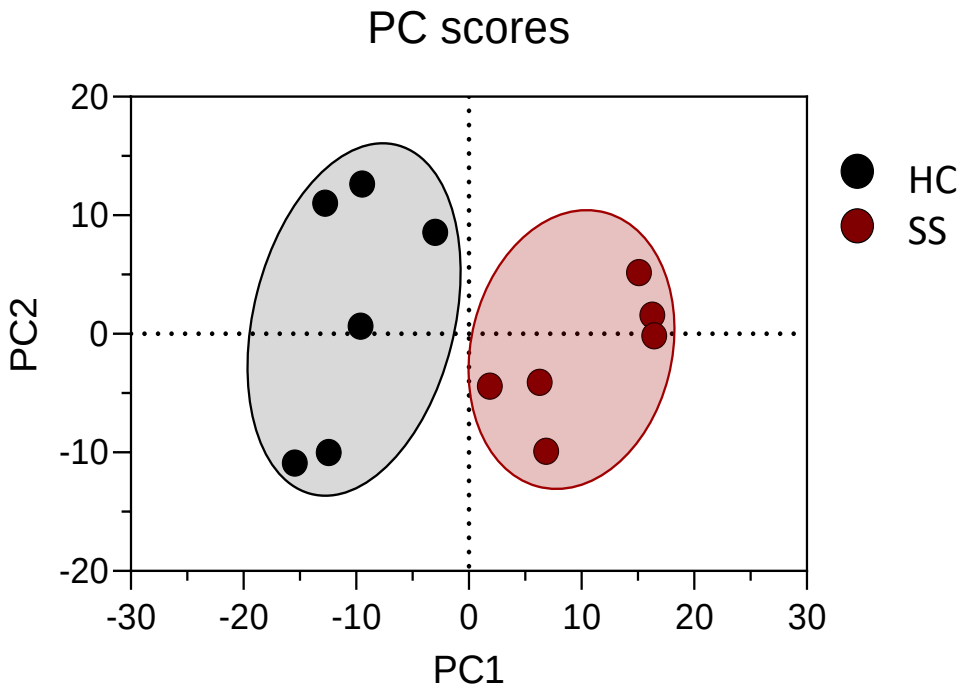
➢ 150 proteins were significantly modulated among common proteins between HC and SS .

➢ 25 of the unique proteins to HC were also significantly modulated versus SS

(fold-change ≥ 1.5, p-value ≤0.05)

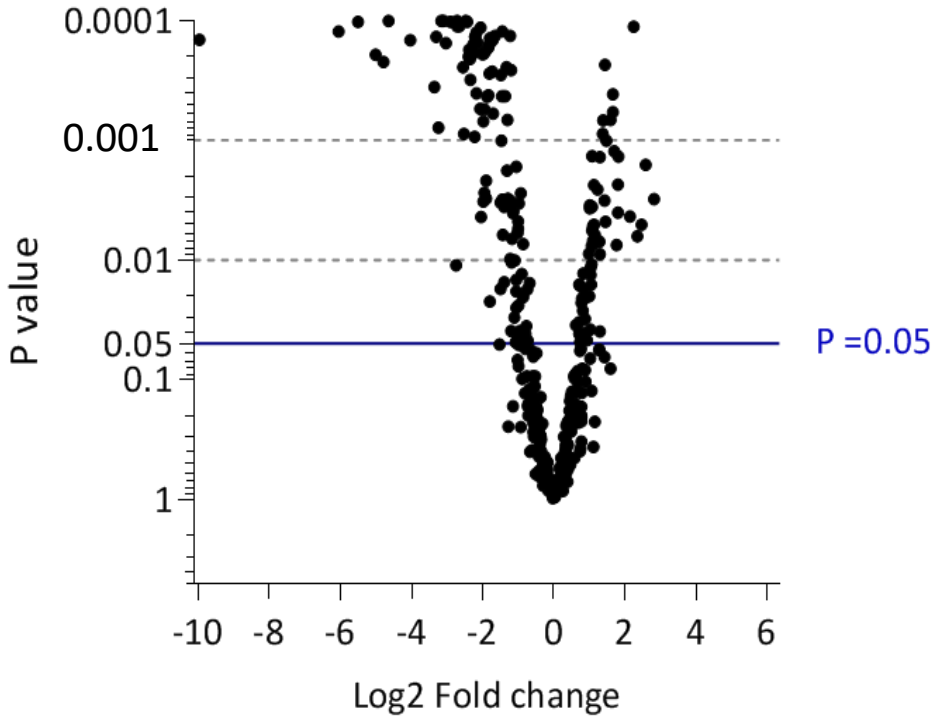
Results - 2

Principal component analysis (PCA) analysis of 6 HC and 6 SS patients



➢ Significant proteome segregation between HC and SS

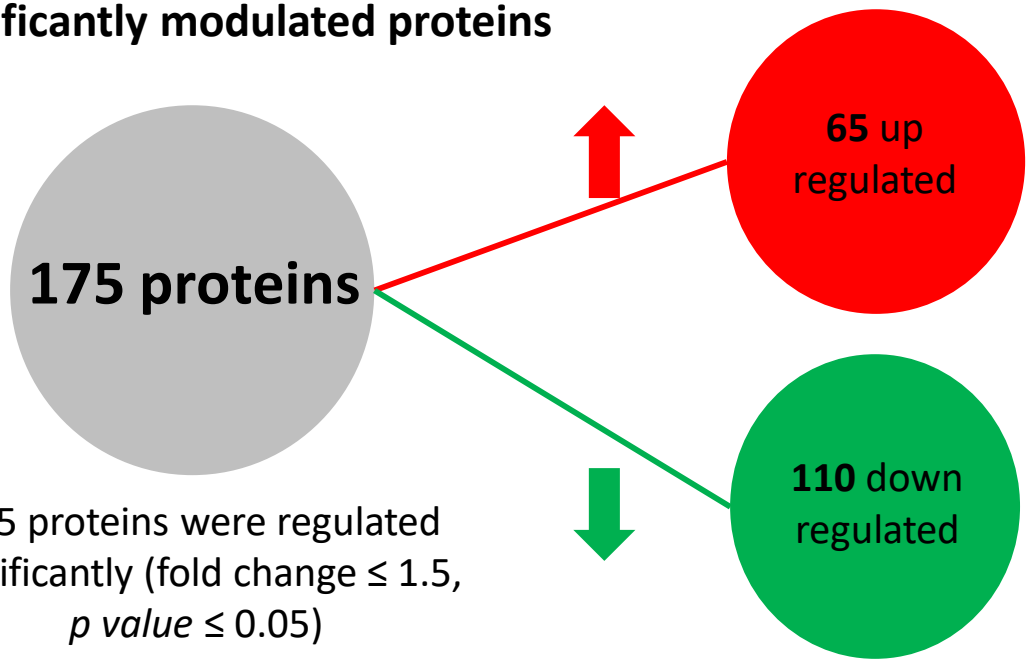
Volcano plot showing all the gene expression changes in SS patients versus HC



➢ 175 proteins significantly modulated in SS vs HC

Results - 3

Significantly modulated proteins



175 proteins were regulated significantly (fold change ≤ 1.5, *p value* ≤ 0.05)

Most Up-regulated Proteins	Fold change
1. Serotransferrin	7
2. Albumin	6
3. Protein S100-A9	5.5
4. Protein S100-A8	5
5. Aldehyde dehydrogenase 1-A3	4.4

Most Down-regulated Proteins	Fold change
1. Proline-rich protein 3	1703
2. Proline-rich protein 27	66
3. Perlecan	46
4. Mammaglobin-B	32
5. Proline-rich protein 1	27

Molecular Function

	Number of regulated proteins	
Binding (%38.5)	**38**	**17**
Catalytic activity (%46.9)	**31**	**36**
Molecular function regulator (7%)	**8**	**2**

Biological Process

	Number of regulated proteins	
Cellular process (25.7%)	**53**	**29**
Metabolic process (15%)	**29**	**24**
Biological regulation (15%)	**31**	**9**
Response to stimulus (9%)	**25**	**6**

✓ **Proteins involved in binding activity and entire groups of biological process were decreased**

Results - 4

Down regulated oxidoreductases

Significantly down regulated in SS:

- Sulfhydryl oxidase 1
- Lactoperoxidase
- Peroxiredoxin-1, -2, -5, -6
- Aldehyde dehydrogenase family 1 member A3

Only detected in HC

- Ketimine reductase mu-crystallin
- Peptidyl-glycine alpha-amidating monooxygenase
- Superoxide dismutase
- Thioredoxin-dependent peroxide reductase
- Aldehyde dehydrogenase family 16 member A1
- Glutathione peroxidase 3

Down regulated cytosketon/actin-binding proteins

Unique to HC

- Plastin-2 , -3
- Coronin-1A
- Twinfilin-1
- Adseverin
- Tubulin beta chain
- Tubulin alpha-4A chain
- Tubulin alpha-1C chain
- Desmoplakin
- Septin-2
- Filaggrin-2

Significantly down regulated in SS

- Actin, cytoplasmic 1
- Cysteine-rich protein 1
- Myosin light polypeptide 6
- Tubulin alpha-1B chain
- Tubulin beta-4B chain
- Cofilin-1, Coronin-1A
- Destrin, Gelsolin
- Plastin-3 , Profilin-1
- Transgelin-2
- Myosin-9 , -14

Significantly up-regulated proteins

Enzymes

- **Caspase-3**
- Glutathione synthetase
- Transketolase

C alcium-binding protein

- Calmodulin-3
- Protein S100-A8
- Protein S100-A9

Proteasomes

- Proteasome subunit alpha type-1, -3, -4, -5, -6, -7
- Proteasome subunit beta type-1, -4, -6, -8, -9, -10

Conclusions

❑ **In SS patients 23.9% less proteins** were detected and in total 175 were differentially regulated versus HC (% UR DR) .

- Cytoskeleton/actin-binding proteins, Peroxiredoxin-1,-5,-6 and Lactoperoxidase were down-regulated.
- Caspase-3, 12 Proteasomes, Glutathione synthase and Calmodulin-3 were up regulated significantly.
- Apoptotic and catalytic activity were increased.
- Balance in antioxidant activity and calcium binding was altered in SS patients.

❑ Advanced mass spectrometry technologies allow us profiling the tear proteome of SS patients to understand better the disease mechanism.

❑ This study should be supported and validated by more studies and different techniques.

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