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TEAR PROTEOME ANALYSIS OF SJOGREN'S SYNDROME PATIENTS USING TIMSTOF PRO

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Introduction

Biomarkers in Sjögren´s syndrome

Sjögren's syndrome (SjS)

- An autoimmune disease causing severe aqueous deficient dry eye [1] [2].
- Functional impairment of the lacrimal and salivary glands [1].
- Reduced goblet cell density and increased APCs* infiltration/maturation in conjunctiva[3].
- Higher prevalence in women (almost 10-fold) [1].

Diagnosis of SjS from serum

- Regularly→ **Ro52/SSA, Ro60/SSA** and **La/SSB** >>>> found only 77-90% of patients[4]
- Occasionally → Rheumatoid factor (RF), Anti-nuclear antibodies (ANA) [5]

*APCs: Antigen-presenting cells

Literature overview and the unmet needs

- SjS has 4 stages: initiation, preclinical, asymptomatic and overt stage [6].
- Early diagnosis and management is challenging [6].
- Lack of specific, sensitive biomarker in SjS [7].
- For rapid, accurate, early diagnosis and
- Stratification & treatment & follow-up of patients validated biomarkers are needed [5].

More omics studies are needed to reveal key molecular networks in SjS!!

Introduction

Tear fluid (TF), a valuable source for biomarker

Biological fluids for biomarkers exploration in SjS:

Serum and Saliva →→ Too complex composition[8]

TF →→ limited sample but less complex compared to saliva and serum[9]

- Tears reflects the physiological condition of ocular diseases [10].
- Tear proteins' concentrations show higher accuracy for diagnosis[11]

Mass Spectrometry -Proteomics Investigation

Mass spectrometry (MS) technology provides:
The largest proteomics datasets in TF and reliable quantification [12].

timsTOF Pro*
Helps to identify **differentially expressed proteins** that involved in important biological processes in SjS from a limited sample thanks to its improved **spatial resolution, sensitivity, and specificity** [13].

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

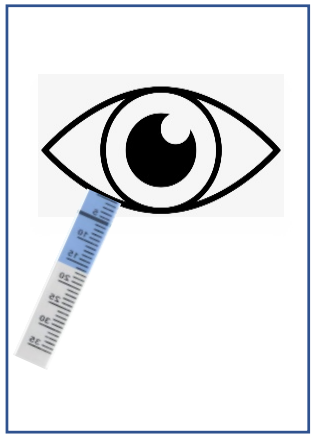
Objective

To investigate tear proteome of SjS patients using a comprehensive proteomic approach based on timsTOF Pro.

Methods

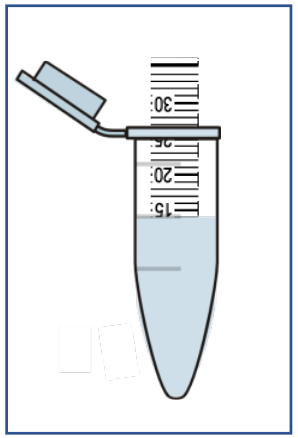
Tear proteins: from collection to identification

1. Schirmer strip collection

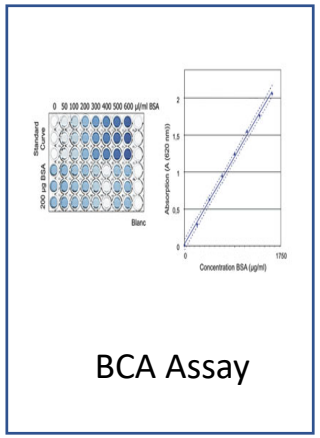


6 SjS patients
6 healthy subjects (HS)

2. Protein elution



3. Protein quantification



BCA Assay

4. Sample processing

Reduction,
Alkylation,
Digestion

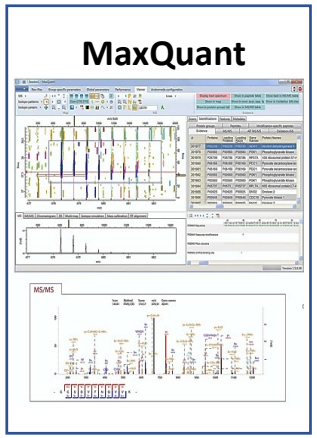
5. LC-MS/MS analysis

nanoElute UHPLC*

timsTOF Pro

6. Protein Identification

MaxQuant



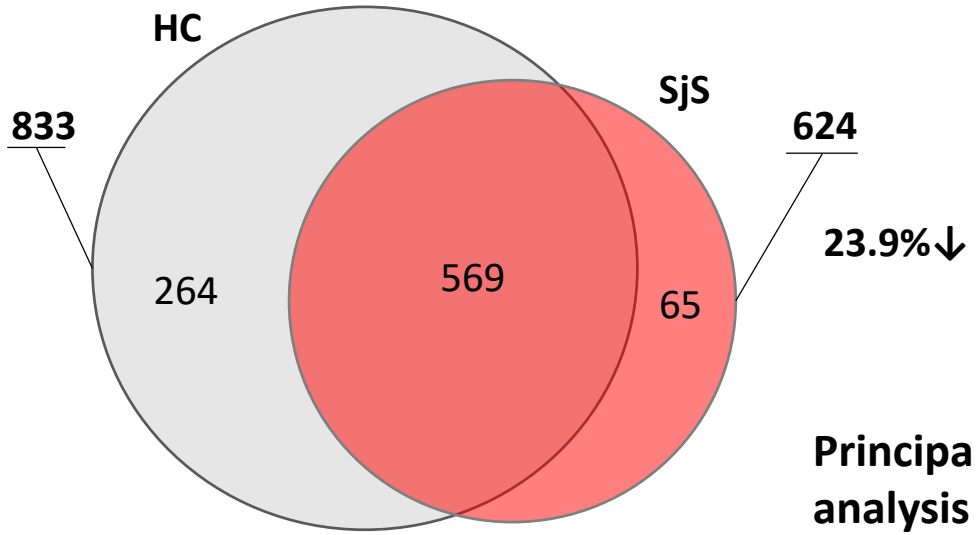
Tear sample collection with the Schirmer strips and sample preparation for LC-MS/MS analysis

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

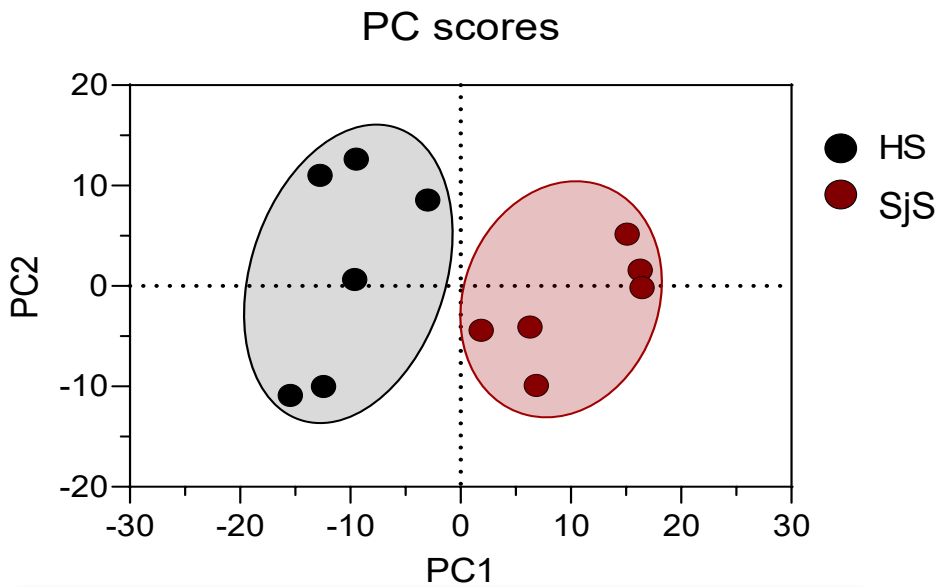
Number of common and specific proteins in HS and SjS patients.



23.9%↓

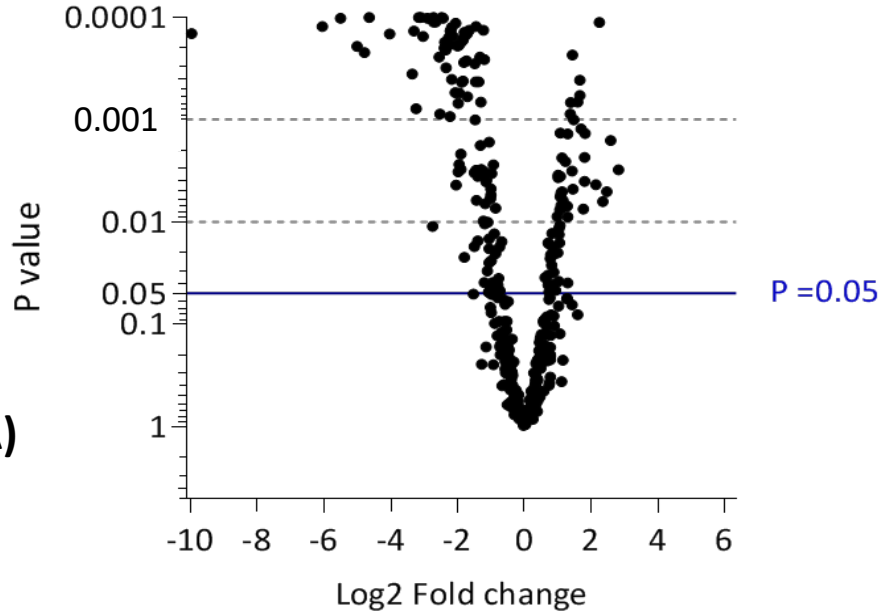
In SjS total identified proteins was decreased by 23.9% versus HC.

Principal component analysis (PCA) analysis 6 HC and 6 SjS patients



Significant proteome segregation between groups

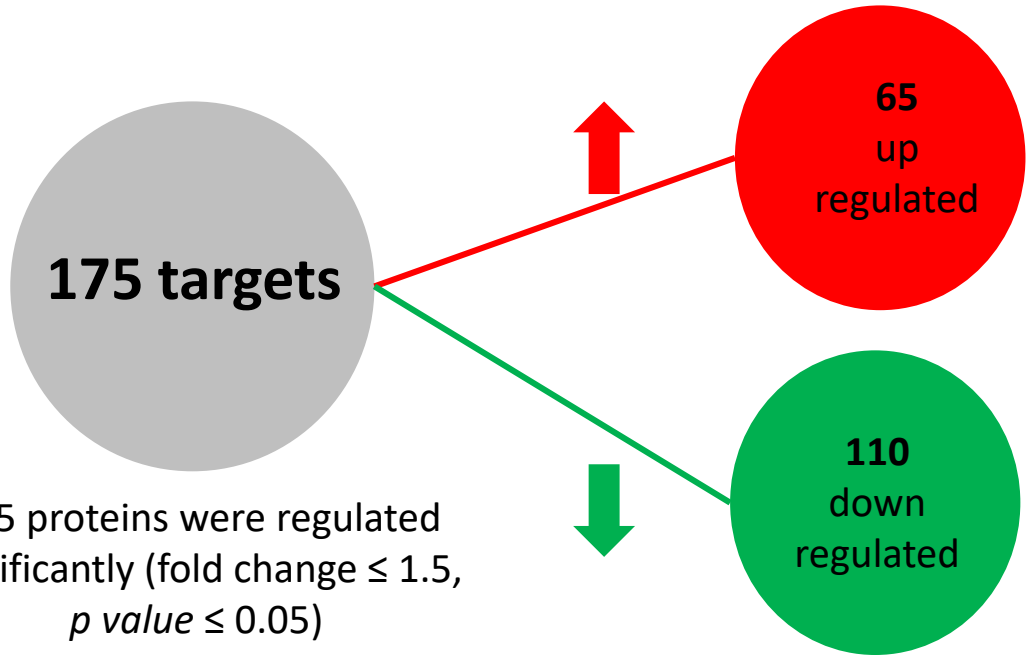
Volcano plot showing all the gene expression changes in SjS patients versus HS



175 proteins modulated in SjS

Results

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

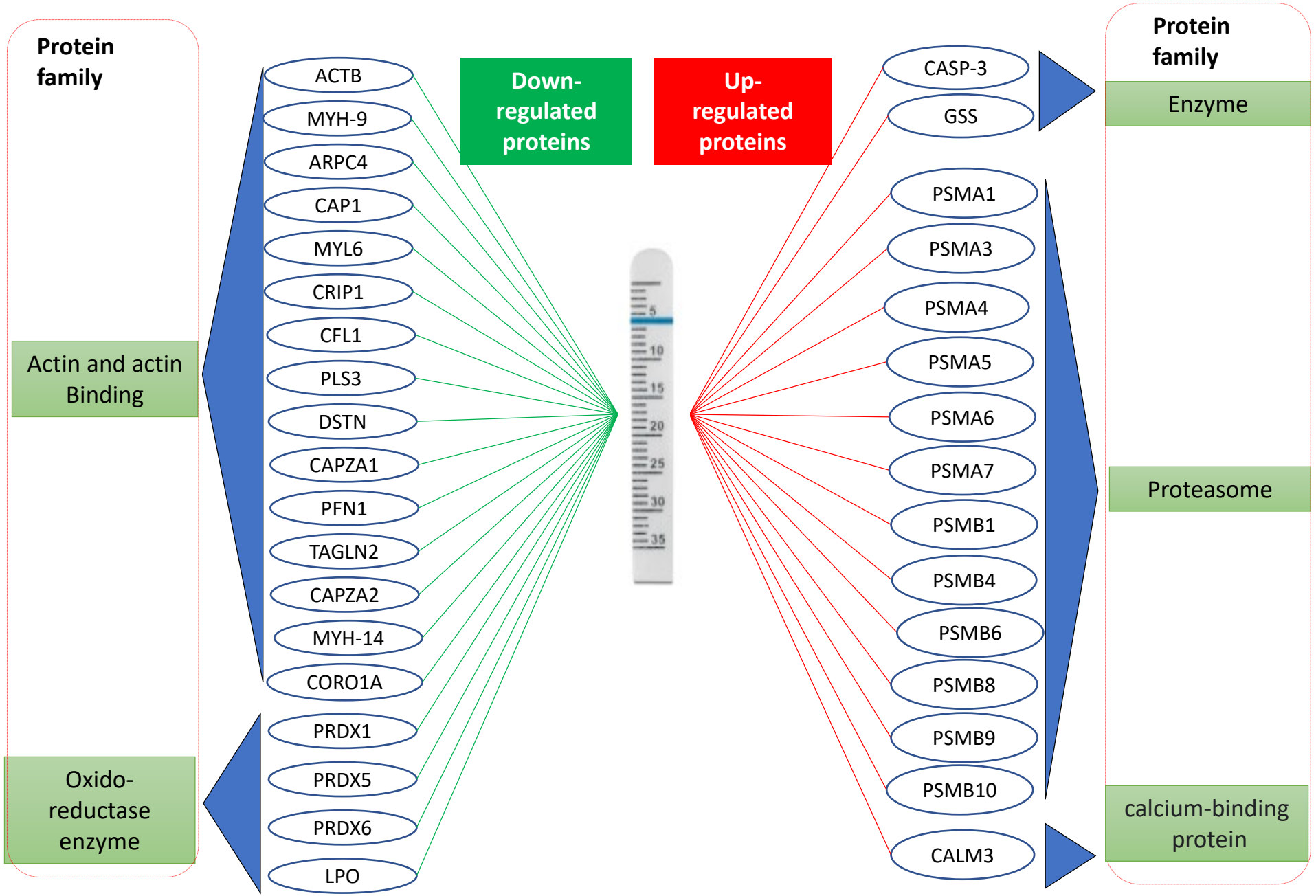
	Number of regulated proteins	
Binding	**38**	**17**
Catalytic activity	**31**	**36**
Molecular function regulator	**8**	**2**

Biological Process

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

✓ **Proteins involved in biological process and binding protein were decreased**

Results



- Proteins involved in apoptosis are upregulated in SjS ($p<0.05$)
- Glutathione synthase and Calmodulin-3 were also up-regulated.
- Peroxiredoxin-1,-5,-6 and lactoperoxidase were down-regulated.

These results suggest an imbalance in antioxidase activity.

Conclusions

- More proteins were down-regulated and fewer were up-regulated in SjS patients.
  - Actin and numerous 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 SjS patients.
- Profiling tear proteome of SjS patients using advanced mass spectrometry can help understanding better the disease mechanism.
- This study should be supported and validated by more studies and different techniques.

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