SORBONNE UNIVERSITÉ

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

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From science to health

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1. Introduction

Sjögren's syndrome (SS)

A chronic and progressive autoimmune disease

→ Destruction of lacrimal & salivary glands

→ Decrease in secretion of tears & saliva [1, 2, 3].

→Lymphocytic infiltration in conjunctiva >>>> reduced goblet cell density [4].

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

Lack of validated biomarkers in Sjögren´s syndrome

→ Early diagnosis and management are challenging [6].

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

→ Lack of highly **specific** and **sensitive biomarker** in SS [7].

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

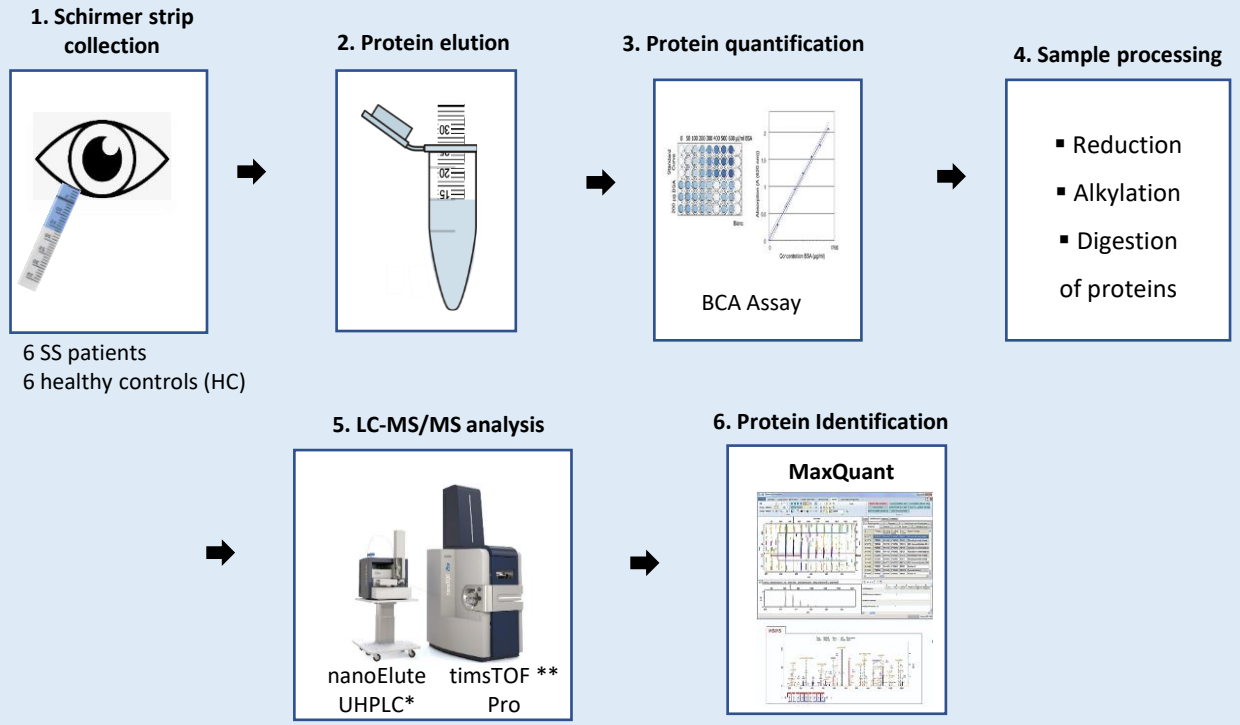
OBJECTIVE: To investigate differentially modulated tear proteins (DMTPs) in patients of Sjögren's syndrome (SSp).

References

[1] L. A. Aqrawi et al. al., PLoS One , vol. 13, no. 10, pp. 1 14, 2018
[2] L. Zhou et. al. Proteomics , vol. 13, no. 16, pp. 2469 2481, Aug. 2013
[3] L. Tong, V. Koh, and B. Y. H. Thong, J. Inflamm . Res., vol. 10, pp. 97 105, 2017
[4] S. C. Pflugfelder et al. Int. J. Mol. Sci. Sci., vol. 19, no. 9, pp. 1 9, 2018
[5] S. Brown et al. BMC Musculoskelet. Disord., vol. 15, no. 1, pp. 1 10, 2014,
[6] B. Wang et al. al., J. Autoimmun., vol. 117, no. October 2020, p. 102590, 2021
[7] W. Chen et al. Genomics, Proteomics Bioinforma., vol. 13, no. 4, pp. 219 223, 2015,

2. Methods

Tear proteins: from collection to identification



Tear sample collection with the Schirmer strips and sample preparation for nanoscale liquid chromatography coupled to tandem mass spectrometry (nano LC-MS/MS) analysis

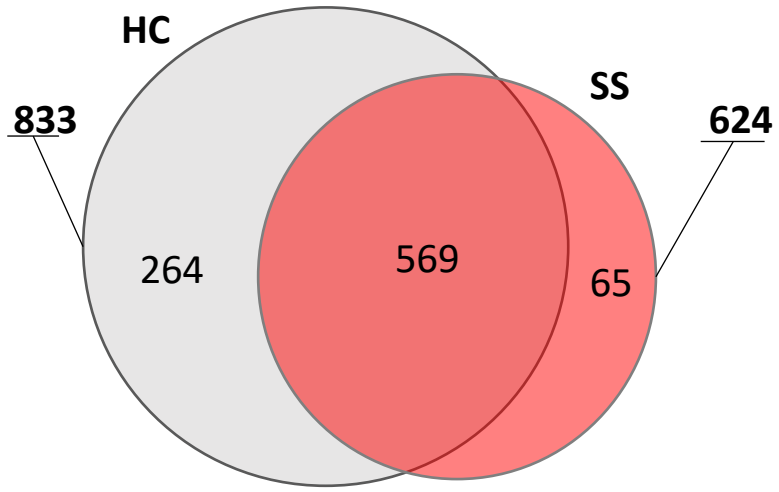
MS/MS data was processed using MaxQuant software for protein identification. Protein Gene Ontology classification was performed by using Panther.

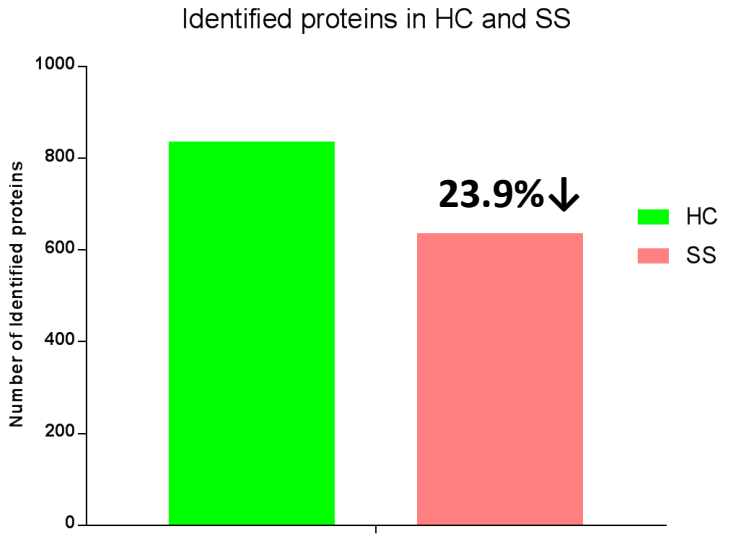
*UHPLC: ultrahigh-pressure liquid chromatography
**timsTOF: Trapped ion mobility quadrupole time of flight

➡ Nano LC-MS/MS technology is a powerful tool to reveal DMTPs in SSp.

3. Results

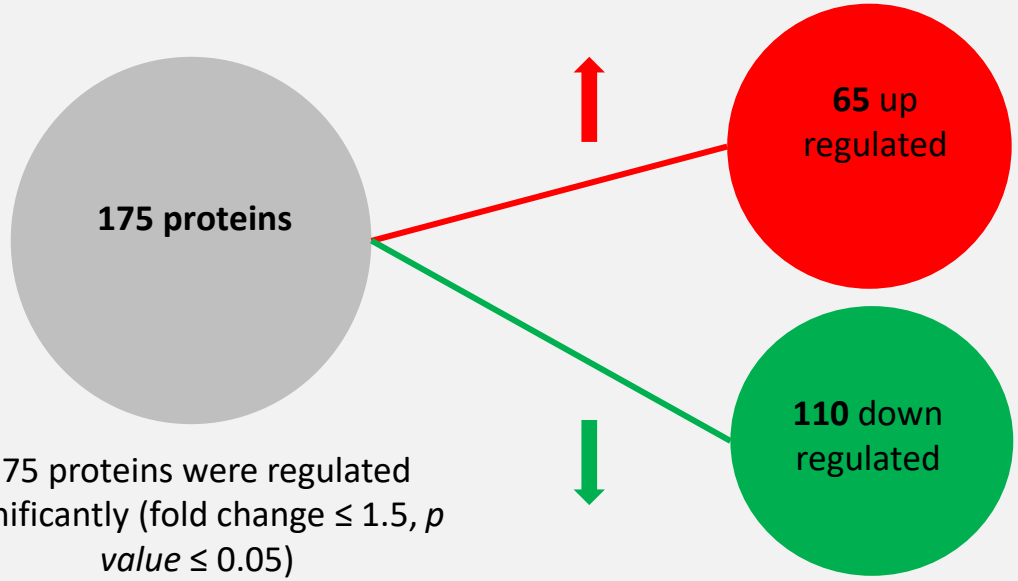
1. Common and Unique Proteins between Healthy Controls and SS patients





➢ Fewer proteins were identified in SS patients

2. Significantly modulated proteins



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

3. Functional classification of modulated proteins

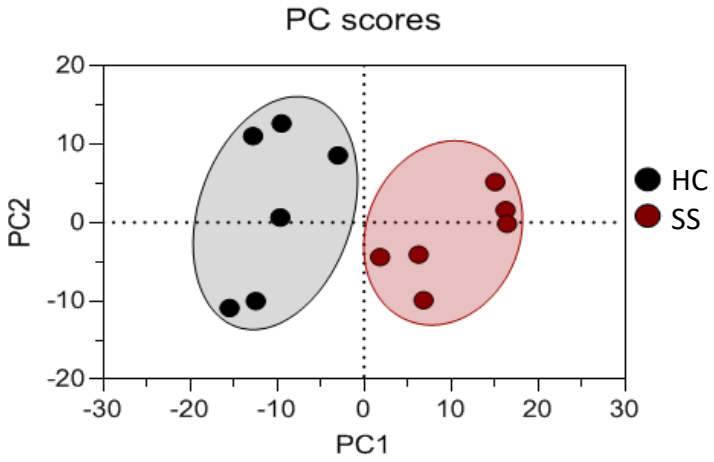
Molecular Function	Number of regulated proteins ↓	↑
Binding (%38.5)	38	17
Catalytic activity (%46.9)	31	36
Molecular function regulator (7%)	8	2

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 subgroups of biological process were decreased**

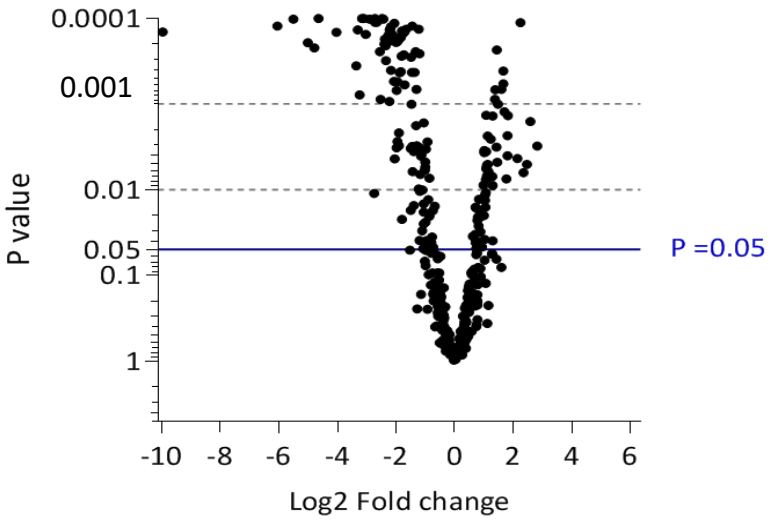
3. Results

Principal component analysis (PCA) analysis



➢ Significant proteome segregation between 6 HC and 6 SSp

Changes in protein expression of SS patients versus HC



➢ 175 proteins significantly modulated in SS vs HC

Significantly Modulated Protein Families

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

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
- Tubulin alpha-1B chain
- Tubulin beta-4B chain
- Cofilin-1
- Destrin
- Gelsolin
- Plastin-3 , Profilin-1
- Transgelin-2
- Myosin-9 , -14

Significantly up-regulated proteins

Enzymes

- Caspase-3
- Glutathione synthetase
- Transketolase

Calcium-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

4. Conclusions

❑ Several proteins were not detected or significantly modulated in SS patients vs. healthy control.

❑ Cytoskeleton/actin-binding proteins were down-regulated.

❑Caspase-3, 12 proteasomes were up regulated significantly.

➢Increased apoptotic and catalytic activity

➢Alteration in actin cytoskeleton

❑S100A8, S100A9 and calmodulin-3 were up regulated.

❑Peroxiredoxin-1,-5,-6, sulfhydryl oxidase-1, lactoperoxidase and glutathione peroxidase-3 were down regulated.

➢Alteration in oxidoreductase activity and calcium binding

❑<u>Nano LC-MS/MS allows us to:</u>

➢Profiling the tear proteome of SS patients to understand better the disease mechanism.

➢Detect the target of interests for candidate biomarkers.