

PROFILING MODULATED ENZYMES IN THE OCULAR SURFACE OF SJÖGREN'S SYNDROME PATIENTS

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Sjögren's Syndrome (SjS)

- Systemic **autoimmune** disease characterized by a complex pathogenesis.
- Higher prevalence in women (10-fold)
- Functional impairment of the lacrimal and salivary glands
→ severe aqueous deficient **dry eye** and **dry mouth**
- Reduced goblet cell density and increased antigen presenting cells (APC) infiltration/maturation in conjunctiva
- Major biomarkers of SjS are anti-SSA and anti-SSB.

Pathophysiology of SjS

Both T cells and B cells involves in the pathology of SjS.

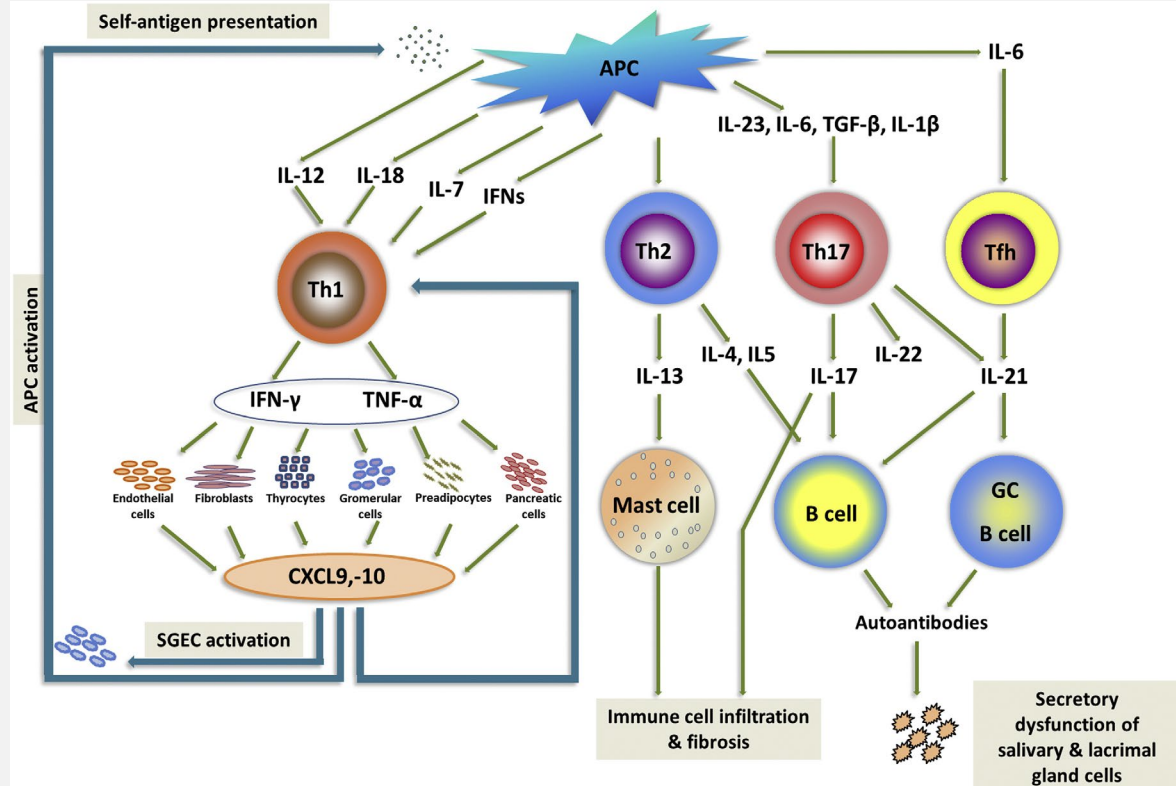


Figure 1. Immune system involvement in SjS pathogenesis. SGECs; salivary gland epithelial cells. Adapted from K. Psianou *et al.*, *Autoimmun. Rev.*, 2018

Introduction

Role of Enzymes in Redox Balance on Ocular Surface

- Oxidative stress (OS) disrupts the lacrimal functional unit (LFU) and cause dry eye disease.
- Antioxidant enzymes cleave reactive oxygen species (ROS) in LFU and also have antimicrobial activities.
- Peroxidase enzymes such as LPO and GPx are markers of the secretory activity of the LFU.

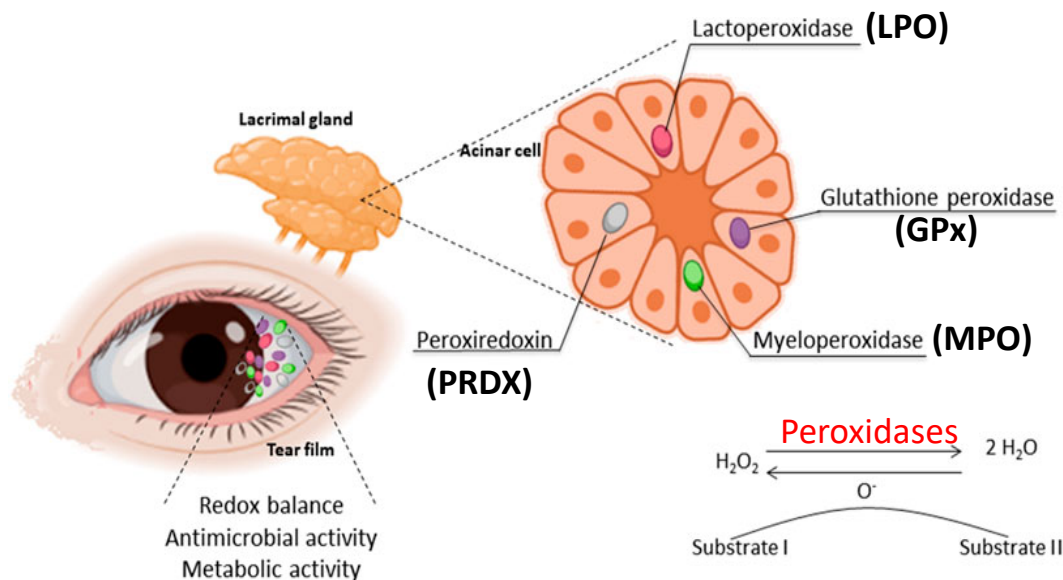


Figure 2. Production of peroxidases in Lacrimal gland. Adapted from C. N. Lemos *et al.*, *Front. Cell Dev. Biol.*, 2022

- Excess ROS **oxidizes** DNA, protein, intracellular molecules and cause uncontrolled **cell death**

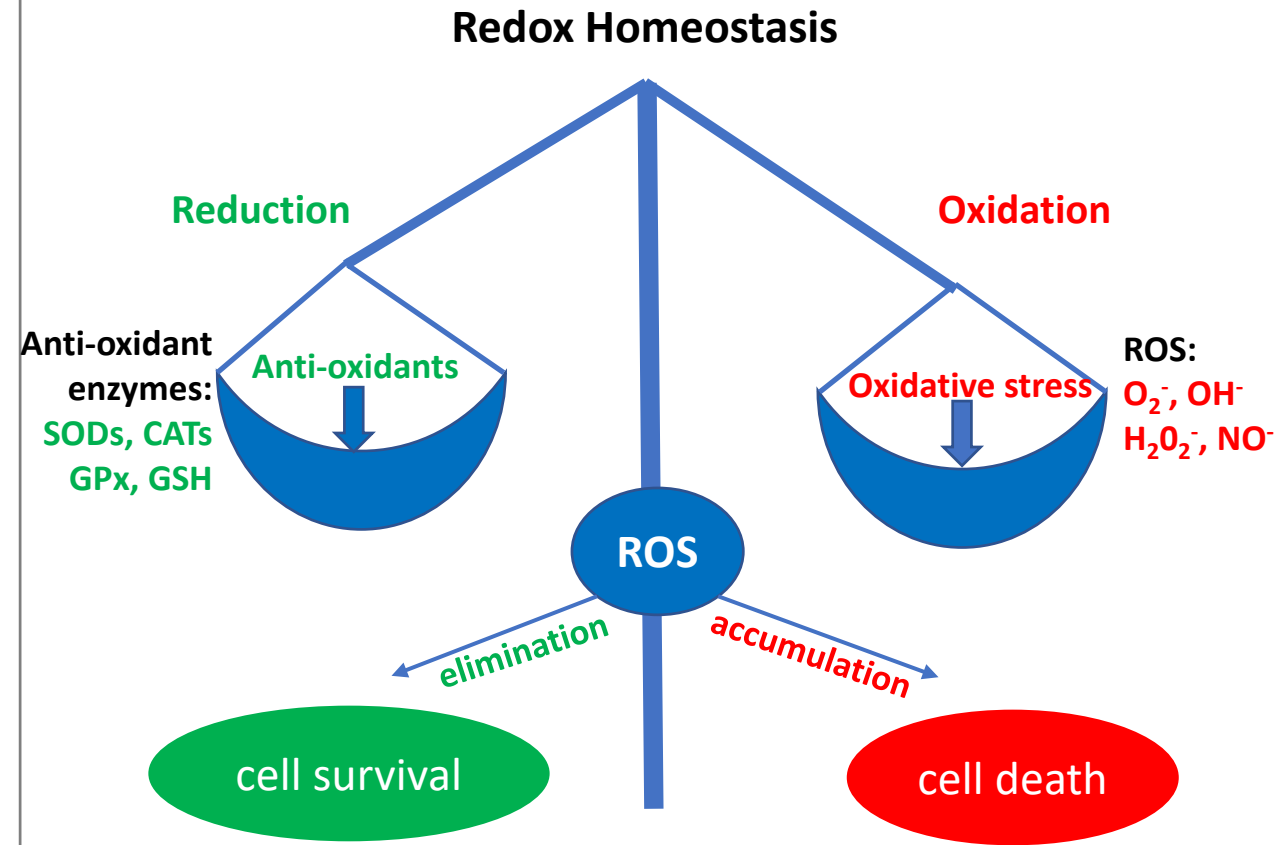
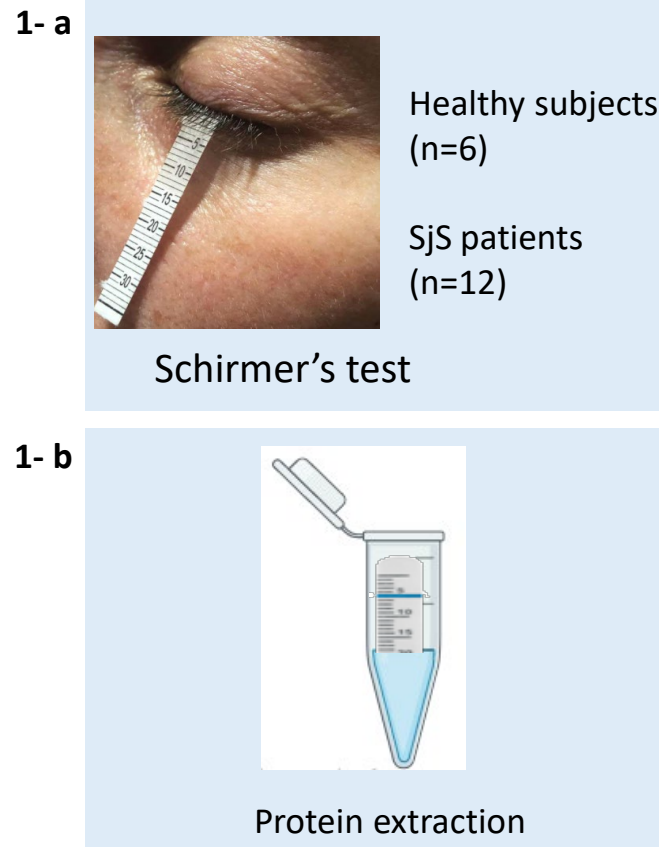


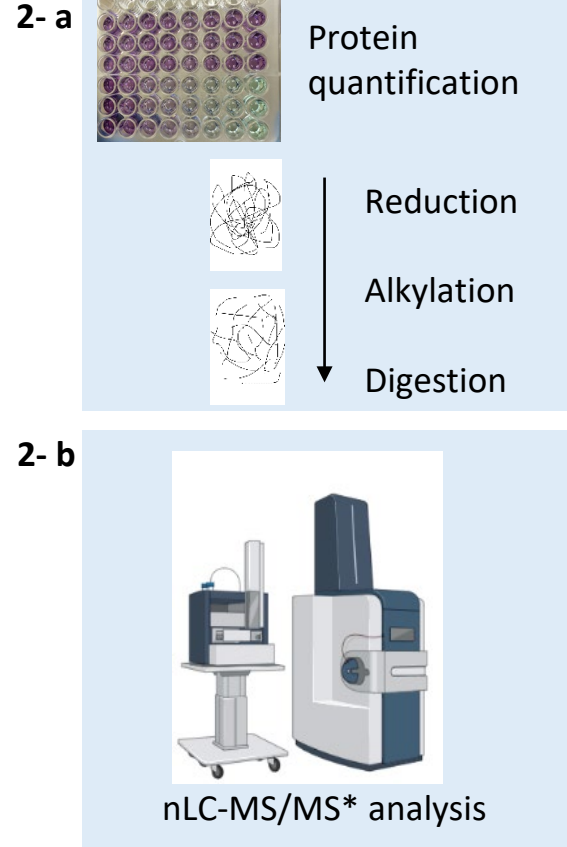
Figure 3. Redox homeostasis and involved antioxidant enzymes. Adapted from C. Zhang *et al.* *Toxicology*, pp. 215–224, Jan. 2021

Methodology

1. Sample collection and Protein extraction



2. Sample preparation and MS analysis



3. Bioinformatics Analysis

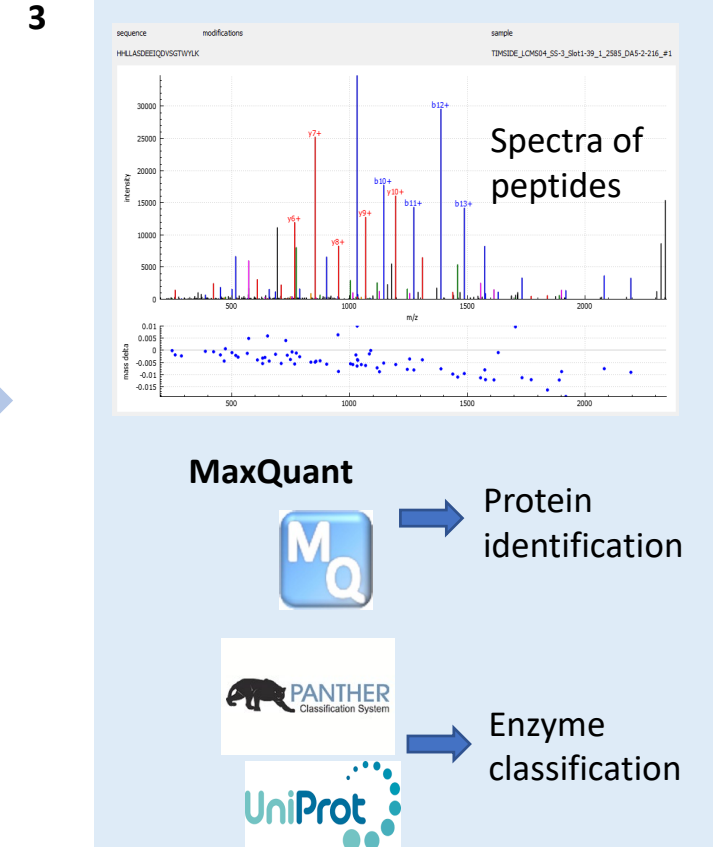
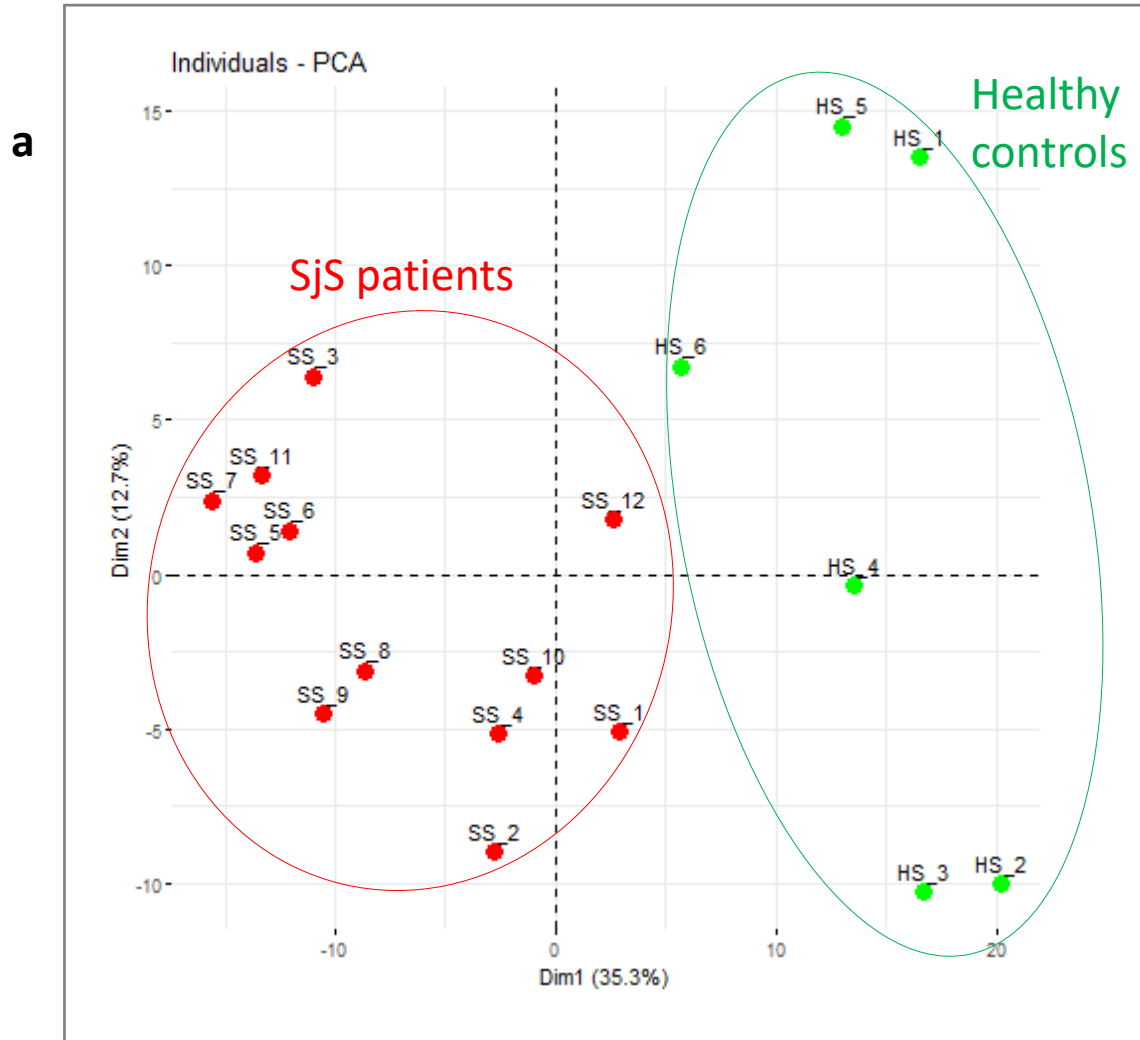


Figure 4. Workflow outlining the each step from sample collection to identification and functional analysis of modulated enzymes. Panther and Uniprot software were used for enzyme classification.

*, nano liquid chromatography (nLC) tandem mass spectrometry (MS/MS)

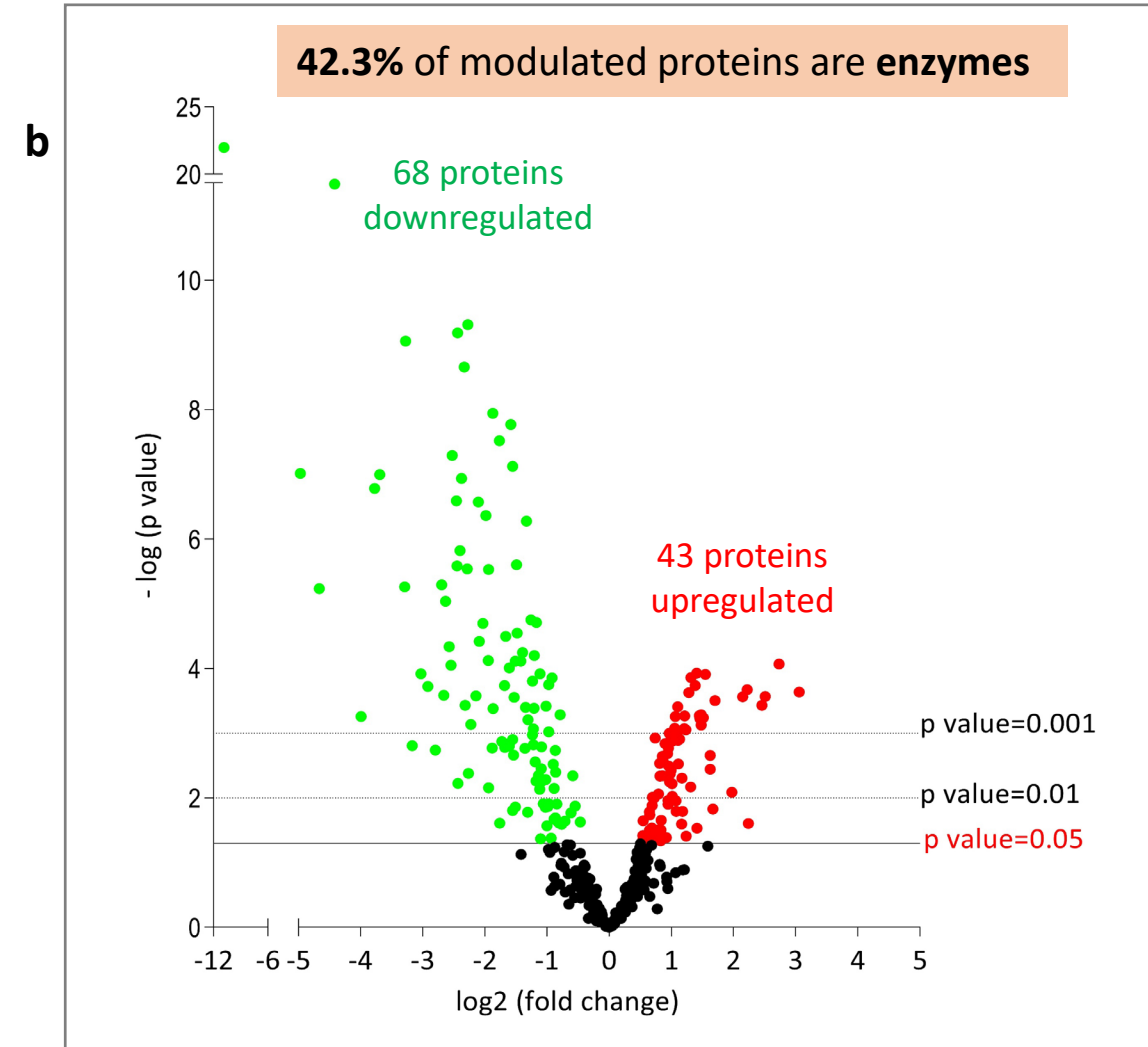
Results

➤ Significant proteome segregation between two groups



Principal component analysis (PCA) analysis displays a significant protein segregation between two groups (red color; Sjs patients and green color; healthy controls).

➤ 111 proteins were modulated in Sjs patients versus healthy controls



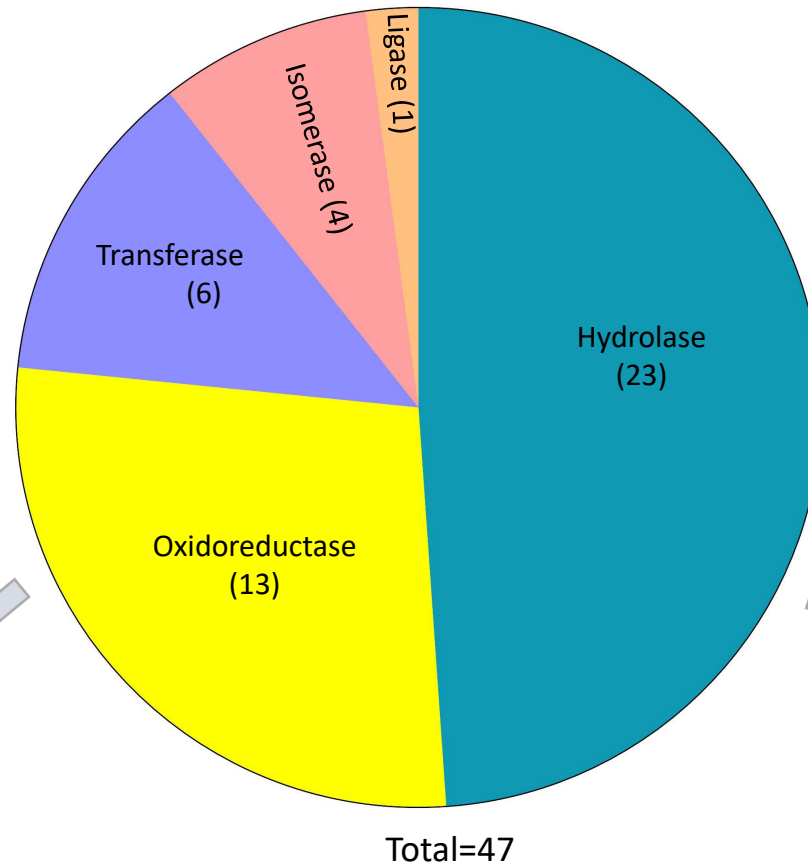
Volcano plot showing distribution of the modulated proteins. Green color symbolizes downregulated, red color symbolizes upregulated proteins.

Results

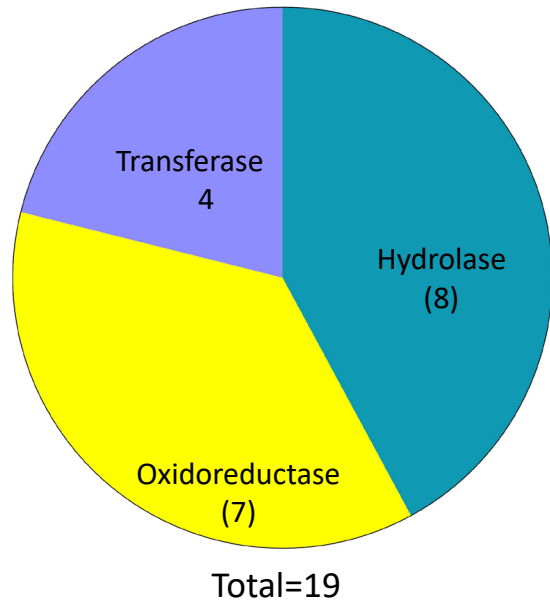
All Modulated Enzymes

Modulated enzyme classes

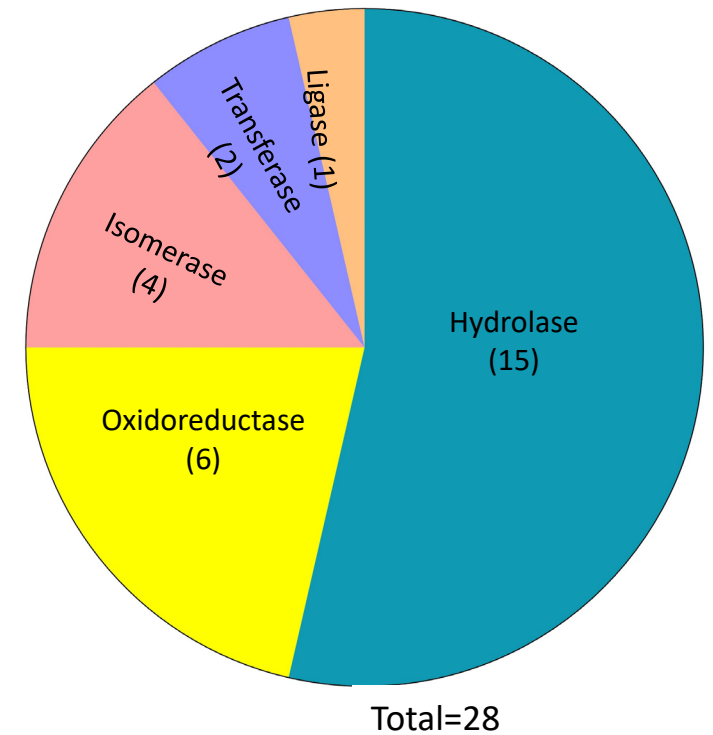
- EC 1- Oxidoreductases (13)
- EC 2- Transferases (6)
- EC 3- Hydrolases (23)
- EC 5- Isomerases (4)
- EC 6- Ligases(1)



Downregulated enzymes

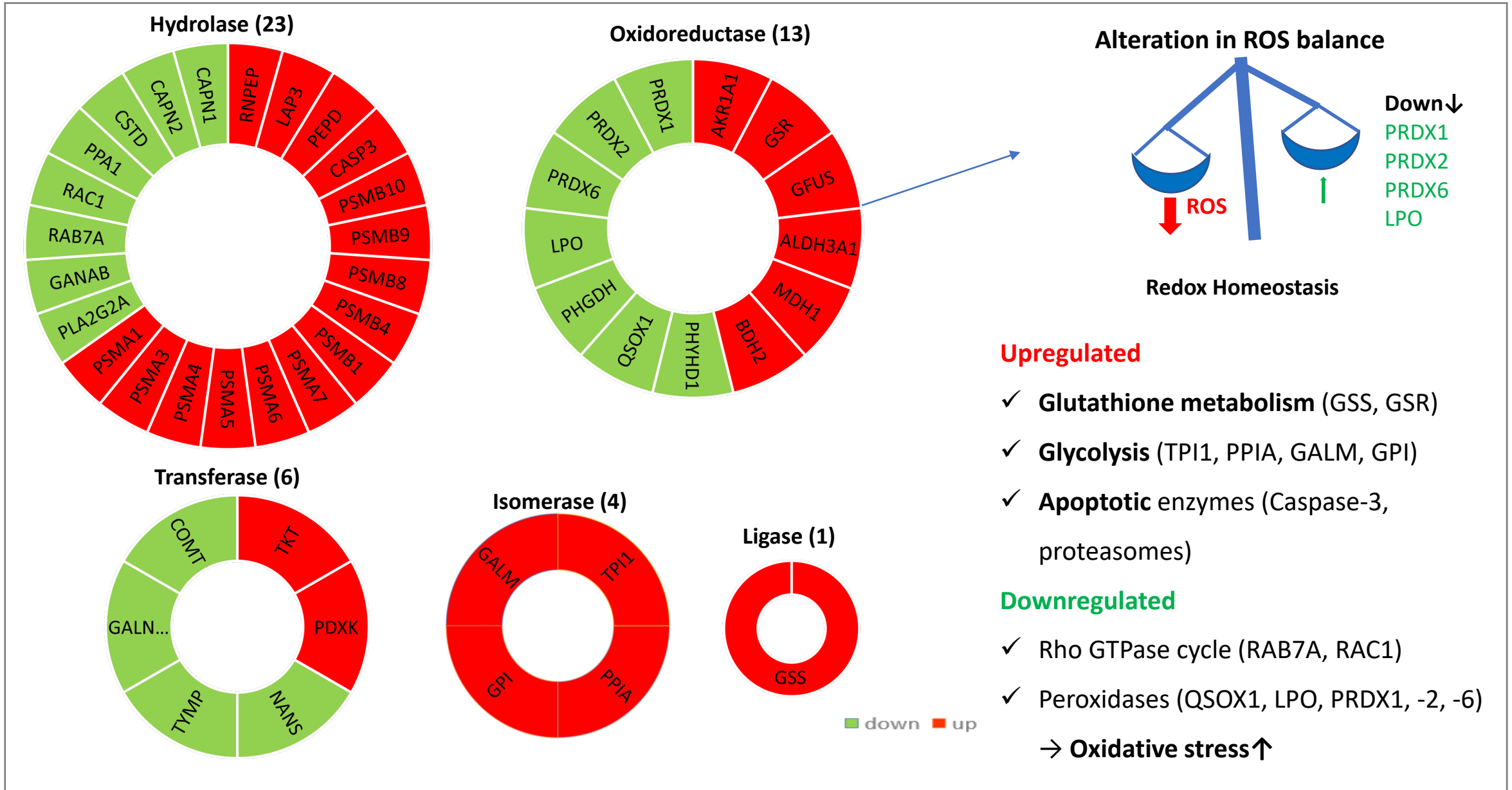


Upregulated enzymes



Results

Dysregulated enzymes involve in various biological processes in SjS patients



Conclusions

- ✓ Main conclusions of the proteomic investigation of Schirmer strips from SjS patients
 - Majority of modulated proteins formed by enzymes.
 - Balance in antioxidant activity was altered.
 - Apoptotic enzymes involved in cell death were overexpressed.
- ✓ Altered redox metabolism might play an important role in the initiation and progression of DED.
- ✓ Monitoring the enzymes involved in ROS balance could inform about disease progress.
- ✓ These modulated enzymes can be potential biomarkers and druggable targets for the treatment of SjS disease.

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