

Supplementary Material

The integrated stress response is activated in the salivary glands of Sjögren's syndrome patients

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1 Supplementary Table 1. Demographic and serological characteristics of SS-patients and control subjects.

Parameters	Control subjects	Patients with primary Sjögren's syndrome
Numbers of individuals	34	41
Sex, n° . female / n° . male	31/3	39/2
Age, mean (range), years	39.35 (18-68)	45.49 (21-71)
Focus score ^a		
1	0	16
2	0	8
≥3	0	17
USWSF, mL/15 minutes mean (range)	4.16 (0.2-18.5)	1.76 (0-5.8)
Ro antibodies n° (%)	0 % 80.48 %	
La antibodies n° (%)	0 %	48.78 %
Antinuclear antibodies $n^\circ \left(\%\right)$	11.7%	92.68 %
Rheumatoid factor n° (%)	0 %	48.78 %
ESSDAI mean-median; IQR [25- 75]	-	7.88 -6 [4-9.5]

n°, number; USWSF, Unstimulated whole salivary flow; %, percentage; ESSDAI, EULAR SS disease activity index; EULAR, European League against Rheumatism; IQR, interquartile range.

^a Number of foci per 4 mm² of tissue.

Gene	Accession number	Primer sequences	
	NM_002759.3	F: 5'-CGACCCTGAGGGTGAATTTCAACT-3'	
PKR	NM_001135651.3 NM_001135652.2	R: 5'-CAGATGCTGGTGCCATGTTTCTTG-3'	
PERK	NM_001313915.2	F: 5'-AGGGAATTGGCTCGGGAAAA-3'	
	NM_004836.7	R: 5'-TGGCCAGTCTGTGCTTTCAT-3'	
HRI	NM_182810.2 NM_001134335.1	F: 5'-GAGTGGGTACTTGTCTGTACGCTT-3'	
		R: 5'-TTCCGGCAACTGACCAGTTCTT-3'	
GCN2	NM_001013703.4	F: 5'-TCGAGAAGGAAAGGCAGACAGAGA-3'	
		R: 5'-TAGCACACTCACAATGGGAACCAC-3'	
1120	NM_004094.5	F: 5'-AGCCCTAAGAGCAGGTTTGA-3'	
elF2α		R: 5'-ACACACCCCTCTTTTCCTCA-3'	
554	NM 002710.4	F: 5'-AGAGGGTCCAAGCCTGGTAAGAAT-3'	
PP1c	NM_001244974.2	R: 5'-TGACTGCTTTCCCCTGTCCACATA-3'	
CREP		F: 5'-GGGAGCCAAGAAAGTGAATGTCCA-3'	
	NM_032833.5	R: 5'-ACCTGCATCCATCCCTTGCAAA-3'	
GADD34	NM_014330.5	F: 5'-TTCCGAGTGGCCATCTATGTACCT-3'	
		R: 5'-AAGCGCACCTTTCTGGCCTTTA-3'	
	NM_001675.4 NM_182810.2	F: 5'-TGGTGAGTGCAAAGAGCTGGAA-3'	
ATF4		R: 5'-ACAAGCACATTGACGCTCCTGA-3'	
	S707992*	F: 5'-ATAAAAATTTTTCGTCGGAAAA-3'	
ATF4 met		R: 5'-TAATCAAAATCCGTTCTTTCC-3'	
CIT.	NM_014331.4	F: 5'-TGGGGAGAGTTCTGGTACTGCAAT-3'	
xCT		R: 5'-GAAGCAACTAGAAGCGTGACAGGT-3'	
	NM_001012662.3 NM_001012664.3 NM_001013251.3 NM_002394.6	F: 5'-CCTCATTCTTGGCTGAGTGGCAAA-3'	
4F2hc		R: 5'-TGCTCCCCAGTAGAACCAGAATCA-3'	
~ · ·	NM_194397.3	F: 5'-CGAACCCCAGACCTGTTTGTATCA-3'	
Survivin		R: 5'-GAGTACAGAGGCTGGAGTGCATTT-3'	
	NM_001195053.1 NM_001195054.1 NM_001195055.1 NM_001195056.1 NM_001195057.1 NM_004083.6	F: 5'-AACGGCTCAAGCAGGAAATC-3'	
СНОР		R: 5'-TAGCCACTTCTGGGAAAGGT-3'	
NRF2	 NM_001145412.3	F: 5'-ATGCCCTCACCTGCTACTTT-3'	
		R: 5'-TGTTCTGGTGATGCCACACT-3'	
h18S	NM_022551.2	F: 5'-GATATGCTCATGTGGTGTTG-3'	
		R: 5'-AATCTTCTTCAGTCGCTCCA-3'	

2 Supplementary Table 2. Sequences of RT-qPCR and MS-HRM primers

*Accession number http://switchgeargenomics.com/

Antibodies	Source	Dilution	Company	
Anti-PKR, polyclonal, 3072, IgG. Rabbit WB: 1: IF: 1:		WB: 1:1.000	Cell Signaling technology®, Inc.	
		IF: 1:200		
Anti-pPKR (T446), monoclonal, ab32036, I&G	Rabbit	WB: 1:1.000	Abcam plc.	
		IF: 1:200		
Anti-PERK, monoclonal, C33E10, IgG.	Rabbit	WB 1:1.000	Cell Signaling technology®, Inc.	
I		IF: 1:75		
Anti-pPERK(Thr981), polyclonal.sc-32577.	Rabbit	WB 1:1.000	Santa Cruz Biotechnology, Inc.	
		II ¹ . 1.200		
Anti-HRI, polyclonal, ab28530, IgG	Rabbit	WB: 1:1.000	Abcam plc.	
Anti-GCN2, polyclonal, 3302.	Rabbit	WB: 1:1.000	Cell Signaling technology®, Inc.	
Anti-pGCN2 (T899), monoclonal, ab75836 IgG	Rabbit	WB: 1:1.000	Abcam plc.	
		WB 1:1.000	Cell Signaling technology®, Inc.	
Anti-elf 2α, polycional, 9722	Rabbit	IF: 1:75		
Anti-peIF2a (Ser51) monoclonal 3398	Rabbit	WB 1:1.000	Call Signaling tashnology. Inc	
	Rabbit	IF 1:100	Cen Signaning technology®, inc.	
Anti-PPP1CC, polyclonal, NBP1-32858, IgG	Rabbit	WB: 1:1.000	Novus Biologicals, EEUU.	
Anti-CReP, polyclonal, 14634-1-AP, IgG	Rabbit	WB: 1:1.000	Proteintech Group, Inc.	
Anti-Gadd34, polyclonal, NB100-778, IgG	Goat	WB: 1:1.000	Novus Biologicals, EEUU.	
Anti-ATF4, monoclonal, D4B8, IgG.	Rabbit	WB 1:1.000	Cell Signaling technology®, Inc.	
Anti-CREB2 (ATF4) (C20), c-200. Polyclonal.	Rabbit	IF 1:100	Santa Cruz Biotechnology, Inc.	
Arti - CT ND200 219 lust l	WB 1:1.000			
Anti-xC1, NB300-518, polycional.	Kaddit	IF: 1:200	Novus Biologicals, EEUU.	
Anti-human survivin, polyclonal.	Rabbit	WB 1:1.000	R&D Systems, Inc.	
		WB 1:1.000		
And-CHOP, monocional, LOSF /, 1902a.	Mouse	IF: 1:100	Cen Signaling technology®, Inc.	
Anti-NRF2, polyclonal, bs-1074R, IgG	Rabbit	WB: 1:1.000	Bioss	
Anti-NRF2 (D1C9), monoclonal, IgG.	Rabbit	IF: 1:100	Cell Signaling technology®, Inc.	
Anti-pNRF2 (Ser40), polyclonal, bs-2013R, IgG	Rabbit	WB: 1:1.000	Bioss	
Anti-KEAP1,monoclonal, 8047S	Rabbit	WB: 1:1.000	Cell Signaling technology®, Inc.	
Anti-β-actin, monoclonal (BA3R)	Mouse	WB 1:20.000	Invitrogen	

3 Supplementary Table 3. Antibodies used for Western blot and immunofluorescence

Parameters	R	р
USWSF - p-PERK/PERK ratio	-0.466	0.017
USWSF - PP1c protein	0.648	< 0.001
USWSF - CREP protein	0.481	0.023
USWSF - xCT protein	-0.788	0.001
Ro antibodies - p-PERK/PERK ratio	0.569	< 0.001
Ro antibodies - PKR mRNA	0.590	0.003
Ro antibodies - PP1c protein	-0.682	< 0.001
Ro antibodies - ATF4 protein	0.751	< 0.001
Ro antibodies - xCT protein	0.500	0.021
La antibodies - p-PERK/PERK ratio	0.431	0.014
La antibodies - PP1c protein	-0.524	0.010
La antibodies - ATF4 protein	0.561	< 0.001
La antibodies - xCT protein	0.557	0.009
Rheumatoid Factor - p-PERK/PERK ratio	0.395	0.025
Rheumatoid Factor - CREP protein	-0.504	0.017
Rheumatoid Factor - ATF4 protein	0.436	0.011
Antinuclear antibodies - p-PERK/PERK ratio	0.624	< 0.001
Antinuclear antibodies - PP1c mRNA	-0.482	0.020
Antinuclear antibodies - PP1c protein	-0.564	< 0.001
Antinuclear antibodies - ATF4 protein	0.684	< 0.001
Focus score - p-PERK/PERK ratio	0.583	< 0.001
Focus score - PKR mRNA	0.496	0.016
Focus score - PP1c mRNA	-0.565	0.005
Focus score - PP1c protein	-0.755	< 0.001
Focus score - CREP protein	-0.444	0.039
Focus score - ATF4 protein	0.690	< 0.001
Focus score - xCT protein	0.475	0.030
ESSDAI - p-PERK/PERK ratio	0.482	0.023
ESSDAI - PP1c protein	-0.609	0.003
ESSDAI - ATF4 protein	0.661	< 0.001
p-PERK/PERK ratio - PP1c protein	-0.539	0.041
PKR protein - eIF2α protein	0.676	0.003
PKR protein - p-eIF2α protein	0.538	0.023
p-PKR protein - p-eIF2α protein	0.616	0.008
p-PKR protein – PERK protein	0.745	0.003
PP1c protein - ATF4 protein	-0.621	0.016
ATF4 mRNA - ATF4 promoter methylation	-0.566	0.022

4 **Supplementary Table 4.** Spearman's rank correlation coefficients between clinical parameters and molecular determinations of SS-patients and control subjects.

*p<0.05. USWSF: unstimulated whole salivary flow.



Supplementary Figure 1. Expression of HRI in LSGs from control and SS-patients. A, dot plot showing HRI transcript levels relative to h18S in controls (C) and SS-patients (P) (n= 11C, 12P). B, representative images of HRI immunoblots from control and SS-patients using β -actin as a loading control. C, dot plot showing densitometric analysis of HRI (n= 11C, 12C). These experiments were repeated at least three times (*). P values lower than 0.05 were considered significant.



Supplementary Figure 2. Expression and activation of GCN2 in LSGs from control and SSpatients. A, dot plot showing GCN2 transcript levels relative to h18S in controls (C) and SS-patients (P) (n= 11C, 12P). B, representative images of p-GCN2 and GCN2 immunoblots from control and SSpatients using β -actin as a loading control. C, dot plot showing densitometric analysis of GCN2 (n= 10C, 10P). D, dot plot showing densitometric analysis of p-GCN2 (n=10C, 10P). E, dot plot showing the p-GCN2/GCN2 ratio (n= 10C, 10P). F, Spearman's correlation between p-GCN2 and GCN2 protein levels. These experiments were repeated at least three times (*). P values lower than 0.05 were considered significant.

xCT / Nuclei



Supplementary Figure 3. Localization of xCT in LSGs from control and SS-patients. A-C, G, xCT (green) staining was mainly observed in the cytoplasm of epithelial cells in LSGs from control subjects. **D-F**, **H**, stronger xCT (green) staining was observed in the cytoplasm of epithelial and plasma cells (yellow arrows) in LSGs from SS-patients. Differences in xCT staining intensity were observed between neighbour acinar cells. **A** and **D** higher magnifications of regions surrounded by broken lines in G and H, respectively. **B-C** and **E-F** higher magnifications of regions surrounded by broken lines in A and B, respectively. Nuclei (red) were counterstained with Hoechst-33342. a: acini; d: duct; f: focus of inflammatory cells. Bars A, B, D and E: 10 μm; C and F: 5 μm; G and H: 40 μm.



CHOP / Nuclei

Supplementary Figure 4. Localization of CHOP in LSGs from control and SS-patients. A-C, G, CHOP (green) staining was almost undetectable in epithelial cells in LSG from control subjects. D-F, H, stronger CHOP (green) staining was observed in the nuclei and cytoplasm of epithelial and inflammatory cells (yellow arrows) in LSGs from SS-patients. A and D higher magnifications of regions surrounded by broken lines in G and H, respectively. B-C and E-F higher magnifications of regions surrounded by broken lines in A and B, respectively. Nuclei (red) were counterstained with Hoechst-33342. a: acini; d: duct; f: focus of inflammatory cells. Bars A, B, D and E: 10 μ m; C and F: 5 μ m; G and H: 40 μ m.