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ORIGINAL

Solute Carrier Transporters in Synovial Membrane and Hoffa's Pad of Patients with Rheumatoid Arthritis

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Abstract
Rheumatoid arthritis (RA) is a complex autoimmune disease that leads to joint destruction. A number of immune cells that affect joint tissues are involved in the pathogenesis of this disease. This leads to the synthesis of many pro-inflammatory mediators. The transport of drugs, as well as many cytokines involved in the development of inflammation in RA patients, is mediated by membrane transporters. Membrane transporters are proteins that mediate the transfer of substrates across biological membranes. But to date there are no studies examining the expression of solute carrier (SLC) transporters in joint tissues. The aim of the study was to evaluate the expression of individual SLC family transporters in the synovial membranes (SMs) and infrapatellar fat pad (Hoffa's pad) of RA patients. The study included 20 patients with rheumatoid arthritis and 20 with osteoarthritis as the control group who were undergoing joint replacement surgery as a normal part of clinical care. In the SM and Hoffa's pad of RA patients the following 17 membrane transporters were defined at relevant expression levels for SLC transporter superfamily: *SLC15A2, SLC16A3, SLC19A1, SLC2A9, SLC22A1, SLC22A3, SLC22A4, SLC22A5, SLC22A18, SLC33A1, SLC47A1, SLC51A, SLC7A5, SLC7A6, SLC01C1, SLC02B1, SLC04A1*. The confirmed expression of these transporters in the SMs as well as Hoffa's pad of patients with RA and OA, and the differences in their expression between these groups, suggests the involvement of SLC transporters in both the maintenance of homeostasis under physiological conditions in the tissues of the joints, as well as in the inflammatory process in RA.

Keywords
Rheumatoid arthritis • Synovial membrane • SLC • Drug transporters • Hoffa's pad

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1. Introduction

Rheumatoid arthritis (RA) is a complex autoimmune disease that causes destruction of the small joints of the hands and feet. The pathogenesis of the disease is due to the effects of the immune system on the cells that make up joint tissues such as synovial membrane (SM), cartilage tissue, and periarticular fatty tissue (Finckh et al. 2022). Previous studies have shown that the inflammatory process takes place within all tissues that make up the joint, and they can be a source of cytokines and chemokines that cause inflammation and destruction of joint structures (Jang et al. 2022). It is also important that the drugs used in the treatment of RA reach all the tissues of the joint, thus inhibiting the development of inflammation. The transport of drugs, as well as many endogenous compounds involved in the development of inflammation in RA patients, is mediated by membrane transporters. Membrane transporters are proteins that mediate the transfer of substrates across biological membranes (Giacomini et al. 2010). The presence of membrane transporters has been found in several tissues such as liver, intestine, kidney, brain, testis, and placenta (Hediger et al. 2013; Schlessinger et al. 2013). Previous studies have shown that the expression of these transporters varies in different tissues (Hediger et al. 2013; Schlessinger et al. 2013). These studies indicate that membrane transporters exhibit tissue specificity. These transporters play a role in the delivery and exchange of various substrates and metabolites, and thus regulate cellular homeostasis. Many transporters also facilitate the transport and elimination of endogenous and xenobiotic compounds (Fotiadis et al. 2013).
The solute carrier (SLC) gene superfamily currently consists of 458 genes grouped based on the primary amino acid sequence that encodes the corresponding proteins (Hagenbuch and Stieger 2013). Some studies have demonstrated the key functions of these transporters in the transport of drugs, xenobiotics, nutrients, ions, and many metabolites (Koepsell and Endou 2004; Hagenbuch and Stieger 2013). It is recognized that the SLC family of transporters plays a key role in the process of maintaining homeostasis under physiological conditions, as well as influencing the development of some diseases and also the effectiveness of their therapy (Bröer 2008; Sanna et al. 2009; Franke et al. 2010; Jostins et al. 2012; Köttgen et al. 2013;

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Mueckler and Thorens 2013). The presence of SLC transporters has been confirmed in various tissues, but to date there are only few studies examining the expression of SLC transporters in joint tissues. To our knowledge, to date, there are no studies investigating the expression of SLC transporters in periarticular fatty tissue, which plays an important role in the development of inflammation in RA patients. This study aimed to evaluate the expression of individual SLC family transporters in the SMs and periarticular fatty tissue (Hoffa's pad) in patients with RA.

2. Materials and Methods

2.1. Study subjects

The study included 20 patients with RA and 20 with osteoarthritis (OA) as the control group who were undergoing joint replacement surgery as a normal part of clinical care. During surgery, SM and fat pad samples were collected from each patient.

The samples were snap-frozen in liquid nitrogen (stored at −80°C until RNA extraction) and placed in formalin solution for further immunohistochemistry (IHC) analysis (4% paraformaldehyde solution). The study protocol was approved by the local Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/39/17) and the study participants gave a written informed consent.

2.2. mRNA expression

Total RNA was extracted from the obtained cell homogenates using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) by following manufacturer's protocol. Quantitative real-time polymerase chain reaction (PCR) was performed using individual gene expression assays for mRNA expression analysis using the Quant Studio 7 Pro Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Prevalidated TaqMan Gene Expression Assays were used for determining the transporter genes (Applied Biosystems, catalog number 4331182) under the conditions indicated in the protocol. Totally, 35 of the SLC membrane transporters were used in this study (Supplementary Table 1). Each sample was analyzed in two technical replications, and the mean cycle threshold (CT) values were used for further analysis. The relative constitutive gene expression was calculated by the $2^{-\Delta\Delta CT}$ method with the house-keeping genes. *GUSB* and *RPS9* genes were used as reference.

2.3. IHC

Sections of the SM and Hoffa's fat pad (HFP) samples from RA and OA patients were hydrated, and heat epitope retrieval was performed in a microwave oven in citrate buffer pH = 6 (Dako Retrieval Solution, Dako Aligent Technologies, Santa Clara, CA, USA). After cooling to room temperature (RT), further procedures were performed using ImmPRESS® HRP Universal (Horse Anti-Mouse/Rabbit IgG) PLUS Polymer Kit and ImmPRESS® HRP Goat anti Rat IgG Polymer Detection kit Peroxydase (Vector Laboratories, Newark, CA, USA) and primary antibodies according to Table 1. Briefly, the activity of peroxidase was blocked with the BLOXALL Endogenous Enzyme Blocking Solution, washed twice with phosphate-buffered saline (PBS; EurX, Gdańsk, Poland), and further incubated with 2.5% Normal Horse Serum for mouse and rabbit primary antibodies and with 2.5% Normal Goat Serum for primary rat antibody. Further, slides were incubated with primary antibodies (Table 1) for 1 h in RT. After double wash in PBS, slides were incubated with ImmPRESS Universal Antibody Polymer Reagent for mouse and rabbit antibodies and goat anti-rat IgG link for rat antibody. After washing in PBS, reaction was visualized with ImmPACT DAB EqV Substrate (ImmPRESS® HRP Universal PLUS Polymer Kit, Peroxidase, Vector Laboratories). After visualization, slides were counterstained with Harris modified Hematoxilin (Sigma, Darmstadt, Germany) and mounted in Histokitt (CarlRoth, GmbH, Karlsruhe, Germany) mounting medium, and evaluated under an Olympus IX81 inverted microscope (Olympus, Hamburg, Germany). Micrographs were collected using CellSens software (Olympus).

The immunoreactive score (IRS) was calculated as described by Al-Khan et al. (2020). Briefly, each patient's sample was evaluated in five random chosen fields of view (×40 objective) by two independent researchers. IRS was calculated as IRS = estimated staining intensity (color reaction intensity 0-1-2-3-4) multiplied by the estimated proportion of cells with positive reaction (0-1-2-3-4). Where 0 is 0%–10%, 1 is 11%–25%, 2 is 26%–50%, 3 is 51%–75%, and 4 is 76%–100% positive stained cells. Values of IRS: 0–12, described as 0 negative, 1–6 positive, and 7–12 strongly positive (Al-Khan et al. 2020). Data are shown in the table with median, average, minimal, and maximal values for IRS (Beyer et al. 2021).

Table 1. Antibodies used in the IHC procedure and the IRS evaluation

Antibody	Host	Manufacturer	Rate of dilution	Time of incubation/ temperature
Anti-PEPT2 (SLC15A2)	Rabbit	Abcam, Cambridge, United Kingdom	1:75	1 h/RT
Anti-RFC1 (SLC19A1)	Mouse	Santa Cruz Biotechnology Inc., Dallas, TX, USA	1:100	1 h/RT
Anti-MATE1 (SLC47A1)	Rabbit	Sigma Prestige, Merck, Darmstadt, Germany	1:200	1 h/RT

h, hour; IHC, immunohistochemistry; IRS, immunoreactive score; RT, room temperature; SLC, solute carrier.

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2.4. Semiquantitative evaluation of IHC intensity (protein amount) in SM and Hoffa's pad sections of RA and OA patients

IHC stained sections of SM and Hoffa's pad were collected using an Olympus IX81 inverted microscope (Olympus) with Cell Sens software and used for further analysis as raw *tif* format images. The ImageJ semiquantitative method for protein expression was used according to published protocols (Crowe and Yu 2019). Microphotographs were analyzed with the use of ImageJ Fiji software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/, 1997–2018, version 1.2). After color deconvolution was performed to separate the hematoxylin and 3,3'-Diaminobenzidine (DAB) channels, a threshold was selected, and the minimum threshold value was set to 0. The maximum threshold value was chosen for removing the background signal without removing the DAB signal. Pixel intensity in ImageJ software is in the 0–255 range. The intensity of staining was measured on 30 fields of view (×40 objective magnification) for OA and RA samples.

2.5. Statistical analysis

mRNA expression data are presented as mean ± standard deviation (SD). Statistical analysis was performed using STATISTICA PL, ver. 13.3 Palo Alto, CA, USA (StatSoft, Inc. 2016, STATISTICA-data analysis software system) software. The Shapiro–Wilk normality test was used to examine the distribution of data. Statistically significant differences in mRNA expression were determined by the Mann–Whitney *U*-test. Percentage contribution of the analyzed transporters was calculated using $2^{-\Delta CT}$ values. Image data after the Shapiro–Wilk normality test were calculated using either the Student's *t*-test or the Mann–Whitney *U*-test. The correlation was calculated using Spearman rank non-parametric test. Statistically significant differences (*p*-value) were assumed at $p < 0.05$.

3. Results

3.1. The expression of SLC transporters in the SM and Hoffa's pad

In the SMs and Hoffa's pad of RA patients, the following 17 membrane transporters (out of a total of 35 total; Supplementary Table 1) were defined at relevant expression levels (CT <32 cycles, threshold 0.1), for the SLC transporter superfamily: *SLC15A2, SLC16A3, SLC19A1, SLC2A9, SLC22A1, SLC22A3, SLC22A4, SLC22A5, SLC22A18, SLC33A1, SLC47A1, SLC51A, SLC7A5, SLC7A6, SLC01C1, SLC02B1,* and *SLC04A1* (Figures 1–4).

The percentage contributions of the analyzed transporters in the analyzed samples were defined as follows ($2^{-\Delta CT}$ values, sum of all analyzed transporters): for control SM: *SLC16A3* (55.17%), *SLC22A18* (15.84%), *SLC7A6* (8.33%), *SLC33A1* (5.95%), *SLC04A1* (4.08%), *SLC02B1* (3.66%), *SLC2A9* (3.16%), *SLC22A4* (0.81%), *SLC19A1* (0.80%), *SLC47A1* (0.74%), *SLC7A5* (0.50%), *SLC22A5* (0.37%), *SLC15A2* (0.28%), *SLC22A1* (0.19%), *SLC22A3* (0.06%), *SLC01C1* (0.04%), and *SLC51A* (0.02%); for control Hoffa's pad: *SLC16A3* (35.65%), *SLC02B1* (20.41%), *SLC7A6* (12.33%), *SLC04A1* (6.61%), *SLC33A1* (5.38%), *SLC2A9* (5.36%), *SLC7A5* (4.03%), *SLC19A1* (3.11%), *SLC22A18* (2.52%), *SLC47A1* (1.73%), *SLC22A5* (0.99%), *SLC22A4* (0.94%), *SLC15A2* (0.29%), *SLC22A3* (0.29%), *SLC22A1* (0.17%), *SLC01C1* (0.14%), and *SLC51A* (0.05%); for RA SM: *SLC16A3* (53.63%), *SLC02B1* (10.92%), *SLC22A18* (7.90%), *SLC7A6* (6.58%), *SLC04A1* (4.99%), *SLC7A5* (4.67%), *SLC33A1* (3.26%), *SLC19A1* (2.55%), *SLC2A9* (2.15%), *SLC47A1* (0.91%), *SLC22A4* (0.72%), *SLC22A5* (0.54%), *SLC22A1* (0.45%), *SLC15A2* (0.33%), *SLC22A3* (0.18%), *SLC01C1* (0.16%), and *SLC51A* (0.06%); for RA Hoffa's pad: *SLC7A6* (33.57%), *SLC16A3* (11.66%), *SLC02B1* (11.39%), *SLC04A1* (9.90%), *SLC7A5* (8.68%), *SLC33A1* (6.95%), *SLC19A1* (6.20%), *SLC2A9* (2.84%), *SLC22A18* (2.40%), *SLC22A5* (1.84%), *SLC22A4* (1.21%), *SLC47A1* (1.20%), *SLC15A2* (0.72%), *SLC22A3* (0.66%), *SLC01C1* (0.37%), *SLC22A1* (0.30%), and *SLC51A* (0.11%). The study did not reveal relevant expressions of the following genes: *SLC01A2, SLC01B1, SLC01B3, SLC04C1, SLC10A1, SLC10A2, SLC15A1, SLC22A11, SLC22A12,*

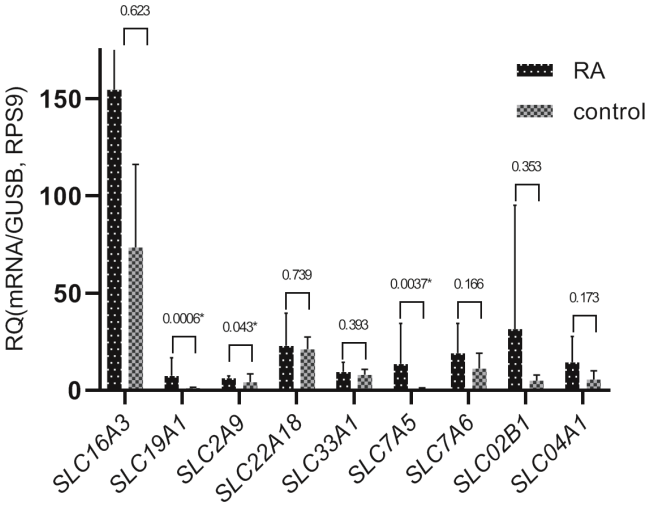

Fig 1. *Expression of SLC16A3, SLC19A1, SLC2A9, SLC22A18, SLC33A1, SLC7A5, SLC7A6, SLC02B1, and SLC04A1 membrane transporters in the SM. X: abbreviations of the examined SLC transporters superfamily. Y: RQ $2^{-\Delta CT}$ mean values (±SD); *p < 0.05. CT, cycle threshold; RA, rheumatoid arthritis; RQ, relative quantification; SD, standard deviation; SLC, solute carrier; SM, synovial membrane.*

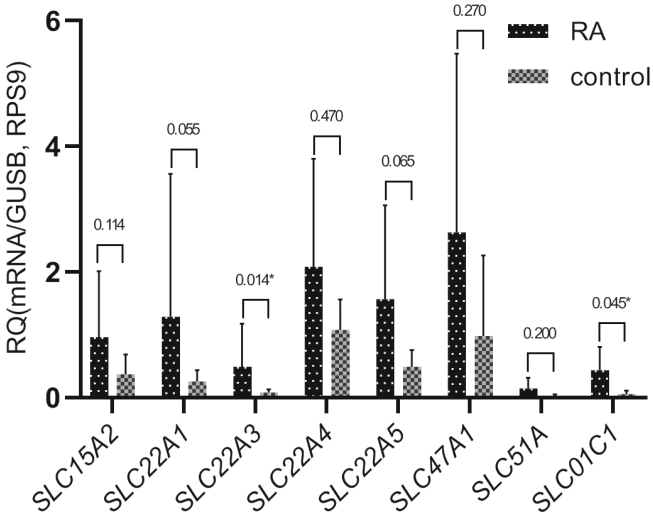

Fig 2. *Expression of SLC15A2, SLC22A1, SLC22A3, SLC22A4, SLC22A5, SLC47A1, SLC51A, and SLC01C1 membrane transporters in the SM. X: abbreviations of the examined SLC transporters superfamily. Y: RQ $2^{-\Delta CT}$ mean values (±SD); *p < 0.05. CT, cycle threshold; RA, rheumatoid arthritis; RQ, relative quantification; SD, standard deviation; SLC, solute carrier; SM, synovial membrane.*

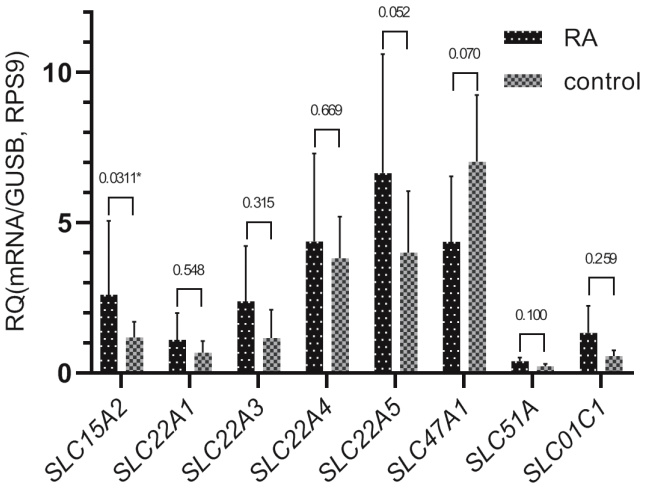

Fig 4. *Expression of SLC15A2, SLC22A1, SLC22A3, SLC22A4, SLC22A5, SLC47A1, SLC51A, and SLC01C1 membrane transporters in the Hoffa's pad. X: abbreviations of the examined SLC transporters superfamily. Y: RQ $2^{-\Delta CT}$ mean values (±SD); *p < 0.05. CT, cycle threshold; RA, Rheumatoid arthritis; RQ, relative quantification; SD, standard deviation; SLC, solute carrier.*

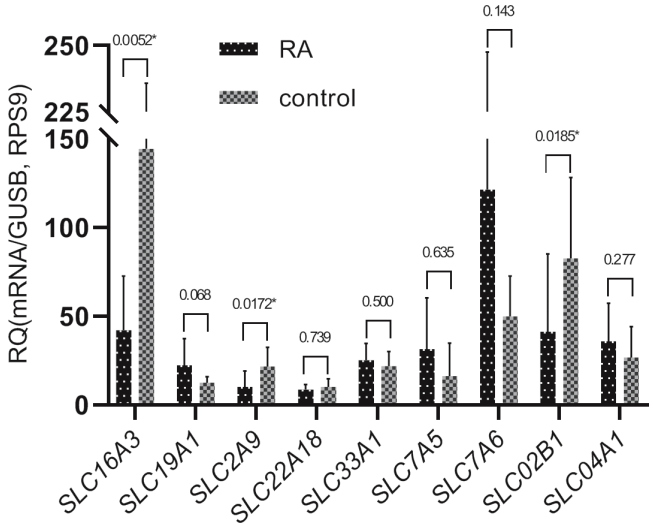

Fig 3. *Expression of SLC16A3, SLC19A1, SLC2A9, SLC22A18, SLC33A1, SLC7A5, SLC7A6, SLC02B1, and SLC04A1 membrane transporters in the Hoffa's pad. X: abbreviations of the examined SLC transporters superfamily. Y: RQ $2^{-\Delta CT}$ mean values (±SD); *p < 0.05. CT, cycle threshold; RA, rheumatoid arthritis; RQ, relative quantification; SD, standard deviation; SLC, solute carrier.*

SLC22A2, SLC22A6, SLC22A7, SLC22A8, SLC22A9, SLC28A1, SLC28A2, SLC47A2, and *SLC51B.*

Next, we compared the expression of SLC transporters between SM and Hoffa's pad in patients with RA. The expression of SLC04A1, SLC7A6, SLC15A2, SLC19A1, SLC22A3, SLC22A5, and SLC33A1 was significantly lower in SM than in Hoffa's pad, while the expression of SLC22A18 and SLC16A3 was significantly higher (Table 2).

Table 2. *SLC transporters expression in SM and HFP*

		SM (mean ± SD)	HFP (mean ± SD)	*p*-value*
SLC0C1	ΔΔCT	0.437 ± 0.37	1.332 ± 0.90	0.088
SLC02B1		31.462 ± 63.66	41.233 ± 43.92	0.070
SLC04A1		14.388 ± 13.31	35.838 ± 21.45	**0.036**
SLC2A9		6.197 ± 1.22	10.295 ± 8.95	0.895
SLC7A5		13.45 ± 20.90	31.441 ± 28.92	0.239
SLC7A6		18.971 ± 15.31	121.553 ± 125.98	**0.0005**
SLC15A2		0.963 ± 1.05	2.6 ± 2.47	**0.039**
SLC16A3		154.528 ± 206.39	42.223 ± 30.48	**0.041**
SLC19A1		7.35 ± 9.30	22.44 ± 15.00	**0.006**
SLC22A1		1.289 ± 2.28	1.101 ± 0.89	0.307
SLC22A3		0.491 ± 0.69	2.386 ± 1.84	**0.010**
SLC22A4		2.081 ± 1.72	4.371 ± 2.93	0.064
SLC22A5		1.569 ± 1.49	6.656 ± 3.96	**0.0008**
SLC22A18		22.775 ± 16.89	8.694 ± 2.87	**0.005**
SLC33A1		9.382 ± 5.01	25.173 ± 9.62	**0.0008**
SLC47A1		2.632 ± 2.84	4.3612 ± 2.18	0.225
SLC51A		0.146 ± 0.17	0.397 ± 0.12	0.275

HFP, Hoffa's fat pad; SD, standard deviation; SLC, solute carrier; SM, synovial membrane.
Bold values indicate *p*-value <0.05.
*Mann–Whitney *U*-test.

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3.2. The correlation between the expression of SLC transporters in SM and Hoffa's pad and clinical parameters of RA patients

Additionally, we analyzed the correlation between the expression of SLC transporters in SM and Hoffa's pad and clinical parameters of RA patients.

The expression of SLC2A9 in SM correlated significantly negatively with C-reactive protein (CRP) and disease activity score (DAS28) values, while SLC22A4 correlated positively with disease duration (Table 3).

The expression of SLC22A5 and SLC04A1 in Hoffa's pad correlated significantly negatively with CRP values. The expression of SLC04A1 correlated significantly positively with CRP values. The expression of SLC22A3 correlated significantly negatively with WBC values and positively with age of patients. The expression of SLC33A1 correlated positively with disease duration (Table 4).

Table 3. *Correlation between the expression of SLC transporters in SM and clinical parameters*

		CRP	WBC	Age (years)	Disease duration	DAS28
SLC15A2	*R*	−0.267	0.517	−0.385	0.017	−0.217
	p-value	0.488	0.154	0.306	0.965	0.576
SLC22A18	*R*	0.188	−0.200	0.261	−0.118	0.176
	p-value	0.603	0.580	0.466	0.746	0.627
SLC16A3	*R*	0.006	−0.091	−0.176	0.285	−0.018
	p-value	0.987	0.803	0.626	0.425	0.960
SLC33A1	*R*	0.261	−0.382	0.122	0.415	0.188
	p-value	0.467	0.276	0.738	0.233	0.603
SLC19A1	*R*	−0.357	0.548	0.190	0.400	−0.333
	p-value	0.385	0.160	0.651	0.326	0.420
SLC47A1	*R*	−0.321	0.214	−0.500	0.436	−0.250
	p-value	0.482	0.645	0.253	0.328	0.589
SLC2A9	*R*	**−0.783**	0.100	−0.393	−0.339	**−0.767**
	p-value	**0.013**	0.798	0.295	0.372	**0.016**
SLC51A	*R*	1.000	0.500	−0.500	−0.866	1.000
	p-value	–	0.667	0.667	0.333	–
SLC22A1	*R*	−0.238	0.595	−0.132	0.146	−0.238
	p-value	0.570	0.120	0.756	0.729	0.570
SLC22A3	*R*	−0.464	0.429	0.214	0.436	−0.429
	p-value	0.294	0.337	0.645	0.328	0.337
SLC7A5	*R*	−0.050	0.333	−0.519	0.373	−0.033
	p-value	0.898	0.381	0.152	0.323	0.932
SLC22A4	*R*	0.036	0.036	−0.286	**0.764**	0.036
	p-value	0.939	0.939	0.535	**0.046**	0.939
SLC7A6	*R*	0.200	0.455	0.195	0.415	0.212
	p-value	0.580	0.187	0.590	0.233	0.556
SLC22A5	*R*	−0.350	−0.033	0.259	0.602	−0.333
	p-value	0.356	0.932	0.500	0.086	0.381
SLC01C1	*R*	−0.700	−0.200	0.600	0.500	−0.700
	p-value	0.188	0.747	0.285	0.391	0.188
SLC02B1	*R*	−0.491	0.503	0.255	0.223	−0.503
	p-value	0.150	0.138	0.476	0.536	0.138
SLC04A1	*R*	0.143	0.095	−0.048	0.424	0.024
	p-value	0.736	0.823	0.911	0.295	0.955

CRP, C-reactive protein; DAS28, disease activity score; SLC, solute carrier; WBC, white blood cells.
Bold values indicate *p*-value <0.05.

Table 4. *Correlation between the expression of SLC transporters in Hoffa's pad and clinical parameters*

		CRP	WBC	Age (years)	Disease duration	DAS28
SLC15A2	*r*	0.256	0.250	−0.357	0.382	0.255
	p-value	0.579	0.589	0.432	0.398	0.582
SLC22A18	*r*	−0.106	−0.261	0.292	0.344	0.085
	p-value	0.770	0.467	0.413	0.331	0.815
SLC16A3	*r*	0.400	0.552	−0.195	0.368	0.530
	p-value	0.252	0.098	0.590	0.295	0.115
SLC33A1	*r*	−0.052	0.017	0.393	**0.703**	0.286
	p-value	0.894	0.966	0.295	**0.034**	0.456
SLC19A1	*r*	−0.355	−0.024	0.357	0.194	0.217
	p-value	0.388	0.955	0.385	0.645	0.606
SLC47A1	*r*	0.473	**0.821**	−0.643	0.273	0.691
	p-value	0.284	**0.023**	0.119	0.554	0.086
SLC2A9	*r*	−0.279	−0.283	0.117	−0.177	0.185
	p-value	0.468	0.460	0.765	0.648	0.634
SLC51A	*r*	0.500	1.000	−0.500	0.866	1.000
	p-value	0.667	–	0.667	0.333	–
SLC22A1	*r*	−0.866	−0.500	0.500	−0.866	−0.866
	p-value	0.333	0.667	0.667	0.333	0.333
SLC22A3	*r*	−0.540	**−0.667**	**0.733**	0.203	−0.445
	p-value	0.134	**0.050**	**0.025**	0.601	0.230
SLC7A5	*r*	0.516	0.486	0.200	0.088	0.530
	p-value	0.295	0.329	0.704	0.868	0.280
SLC22A4	*r*	−0.185	−0.357	**0.821**	0.327	−0.182
	p-value	0.691	0.432	**0.023**	0.474	0.696
SLC7A6	*r*	−0.256	−0.188	0.213	0.491	0.110
	p-value	0.475	0.603	0.555	0.150	0.763
SLC22A5	*r*	**−0.669**	−0.224	0.018	−0.153	−0.189
	p-value	**0.034**	0.533	0.960	0.672	0.601
SLC01C1	*r*	**−0.906**	−0.250	0.393	0.255	−0.436
	p-value	**0.005**	0.589	0.383	0.582	0.328
SLC02B1	*r*	−0.344	−0.030	−0.188	0.436	−0.268
	p-value	0.331	0.934	0.602	0.208	0.454
SLC04A1	*r*	**0.732**	0.143	−0.119	−0.590	0.395
	p-value	**0.039**	0.736	0.779	0.123	0.333

CRP, C-reactive protein; DAS28, disease activity score; SLC, solute carrier; WBC, white blood cells.
Bold values indicate *p*-value <0.05.

3.3. Semi-quantitative immunohistochemical determination of SLC transporters protein expression in SMs and Hoffa's pad sections of RA and OA patients analyzed with the use of ImageJ Fiji software

To confirm the expression of SLC transporters at the protein level the immunohistochemical analysis was performed. We performed IRS estimation for expression of SLC transporters in SM and Hoffa's pad in RA and OA patients. The results of IRS estimation are presented in Tables 5 and 6. The data are presented as mean values of the IRS and minimal and maximal values for each antibody in SM and Hoffa's pad in RA and OA patients. The expression of SLC transporters was semi-quantitatively analyzed using ImageJ Fiji software, was positive reaction intensity, and was calculated in the range of 0–255 pixel intensity (Figure 5).

A positive reaction was found in the cell membrane and cytoplasm of epithelial cells in the SM of RA and OA samples, as indicated by the green arrows (Figure 6) and in adipocytes' membranes in HFP samples (Figure 7). Some patients

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Table 5. *IRS values for transportation proteins expression in SM samples in RA and OA patients*

Protein	RA				OA			
	Median	Average	Min.	Max.	Median	Average	Min.	Max
PEPT2	8	8.13	4	12	6	5.25	0	9
RFC1	8	7.93	4	12	6	5.63	1	12
MATE1	6	6.43	2	9	6	5.85	0	12

IRS values: 0 negative, 1–6 positive, 7–12 strongly positive.
IRS, immunoreactive score; OA, osteoarthritis; RA, rheumatoid arthritis; SM, synovial membrane.

Table 6. *IRS values for transportation proteins expression in HFP in RA and OA patients*

Protein	RA				OA			
	Median	Average	Min.	Max.	Median	Average	Min.	Max.
PEPT2	8	9.47	4	12	8	7.17	2	12
RFC1	12	9.06	1	12	4	4.93	0	12
MATE1	8	7.47	2	12	8	6.5	1	12

IRS values: 0 negative, 1–6 positive, 7–12 strongly positive.
HFP, Hoffa's fat pad; IRS, immunoreactive score; OA, osteoarthritis; RA, rheumatoid arthritis.

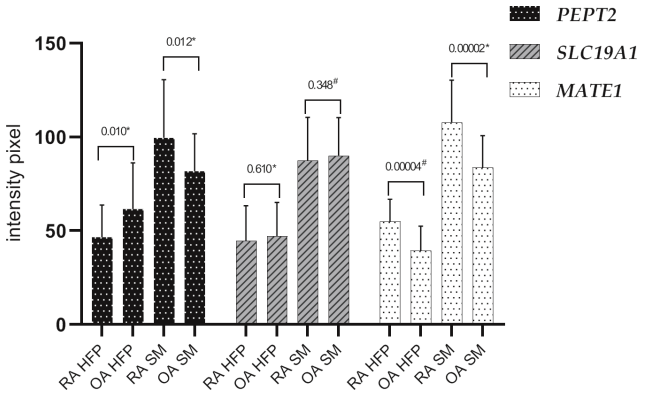

Fig 5. *Transporter protein levels in SM and HFPs samples from RA and OA patients for PEPT2, SLC19A1, and MATE1 proteins. Pixel intensity acquired by ImageJ software. *Student's t-test; #Mann–Whitney U-test. HFPs, Hoffa's fat pads; OA, osteoarthritis; RA, rheumatoid arthritis; SLC, solute carrier; SM, synovial membrane.*

also showed a positive reaction in the SM stroma cells—a cytoplasmic reaction in synoviocytes, as indicated by red arrows. There was also positive reaction for transportation proteins in the blood vessel endothelium, indicated with red asterisks (Figure 6 (a and c)). There were strong positive reactions for PEPT2, MATE1 transporters in HFP in both tested groups (OA and RA) with higher average IRS in RA patients (Table 6 and Figure 7). Strong reaction was observed also for the RFC1 transporter protein in RA patients, but reaction was noted in OA patients. In SMs, a strong positive reaction was observed for PEPT2 and RFC1 in RA patients and a positive reaction for MATE1 in this group of patients (Table 5 and Figure 6). Positive reaction was noted for all transportation proteins in OA patients. It was also noted that the pixel intensity average for all tested transporters was lower in HFP than in the SMs of patients in both studied groups, although these differences were not reflecting the differences found in mRNA expressions. Differences between IRS and ImageJ results may be a result of IRS methodology: subjective estimation on the microphotographs and result description. In IRS, results <6 are described as positive but >7 as strong positive using the image with double Hematoxilin and DAB staining, while the ImageJ estimation of intensity is performed after channel (color) separation and only DAB intensity is calculated by software in the given range. There were also strong individual differences in the expression of tested proteins shown as minimal and maximal values of IRS for each protein (Tables 5 and 6). Figure 8 shows the molecules transported by this family of transporters.

4. Discussion

This study aimed to examine the expression of SLC family transporters in joint tissues: SMs and periarticular fatty tissue (Hoffa's pad). To date, there are only few studies that have confirmed the constitutive expression of some SLC family transporters in SMs; however, according to our knowledge, there are no studies examining the expression of SLC transporters in periarticular fatty tissue, which plays an important role in the inflammatory response in RA patients. SLC transporters have been shown to play an important role in a number of physiological processes, but their expression has also been investigated to influence the concentration of various metabolites and drugs in cells and their involvement in the pathogenesis of various diseases, including RA and OA (Beckmann et al. 2015; Seki et al. 2017; Sohn et al. 2021). Figure 8 shows the molecules transported by this family of transporters. Previous studies indicated that the expression of some transporters may be increased in RA patients as compared with OA patients, suggesting the involvement of these

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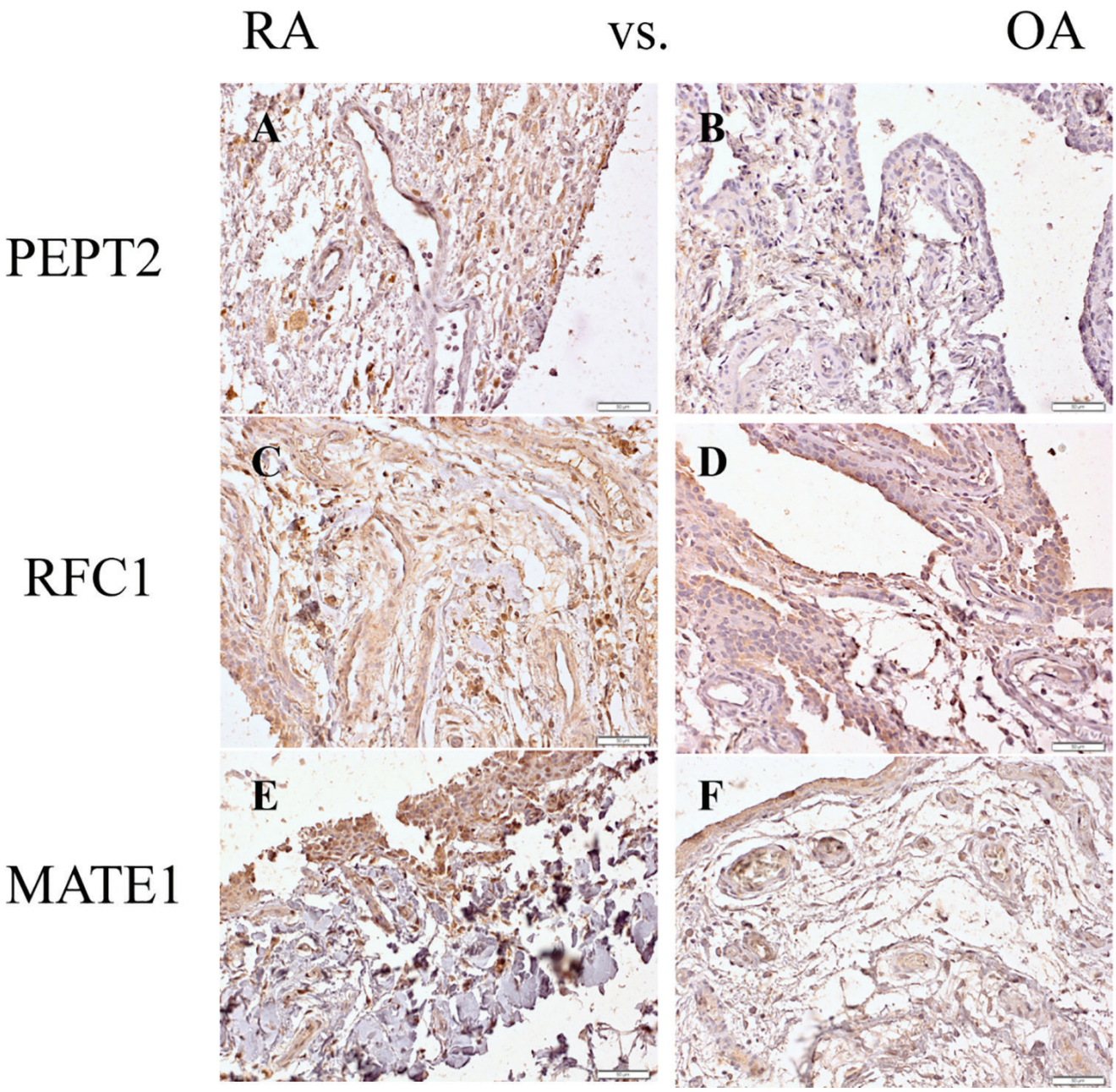

Fig 6. *Transporter protein expressions in SM samples from RA and OA patients. Transporters expression in RA patients panels (a, c, and e); transporters expressions in OA SM samples panels: (b, d, and f); green arrows—positive reaction of epithelial cells, red arrows—positive reaction of synoviocytes, red star—positive reaction in blood vessels; scale bar 50 μm; only representative images are presented. OA, osteoarthritis; RA, rheumatoid arthritis; SM, synovial membrane.*

transporters in transport of inflammatory mediators in RA (Xu et al. 2020).

We demonstrated the expression of 17 membrane transporters of the SLC family in the SM and periarticular fatty tissue (Hoff's pad) of RA and OA patients. We showed differences in the expression of these transporters between RA and OA patients, as well as between the SM and periarticular fatty tissue. Moreover, the expression of some SLC transporters correlated significantly with disease activity parameters. These findings suggest that the inflammation present in RA patients may affect the expression of SLC transporters, which likely influences disease activity and progression. Differences in the expression of SLC transporters between SM and periarticular adipose tissue were also

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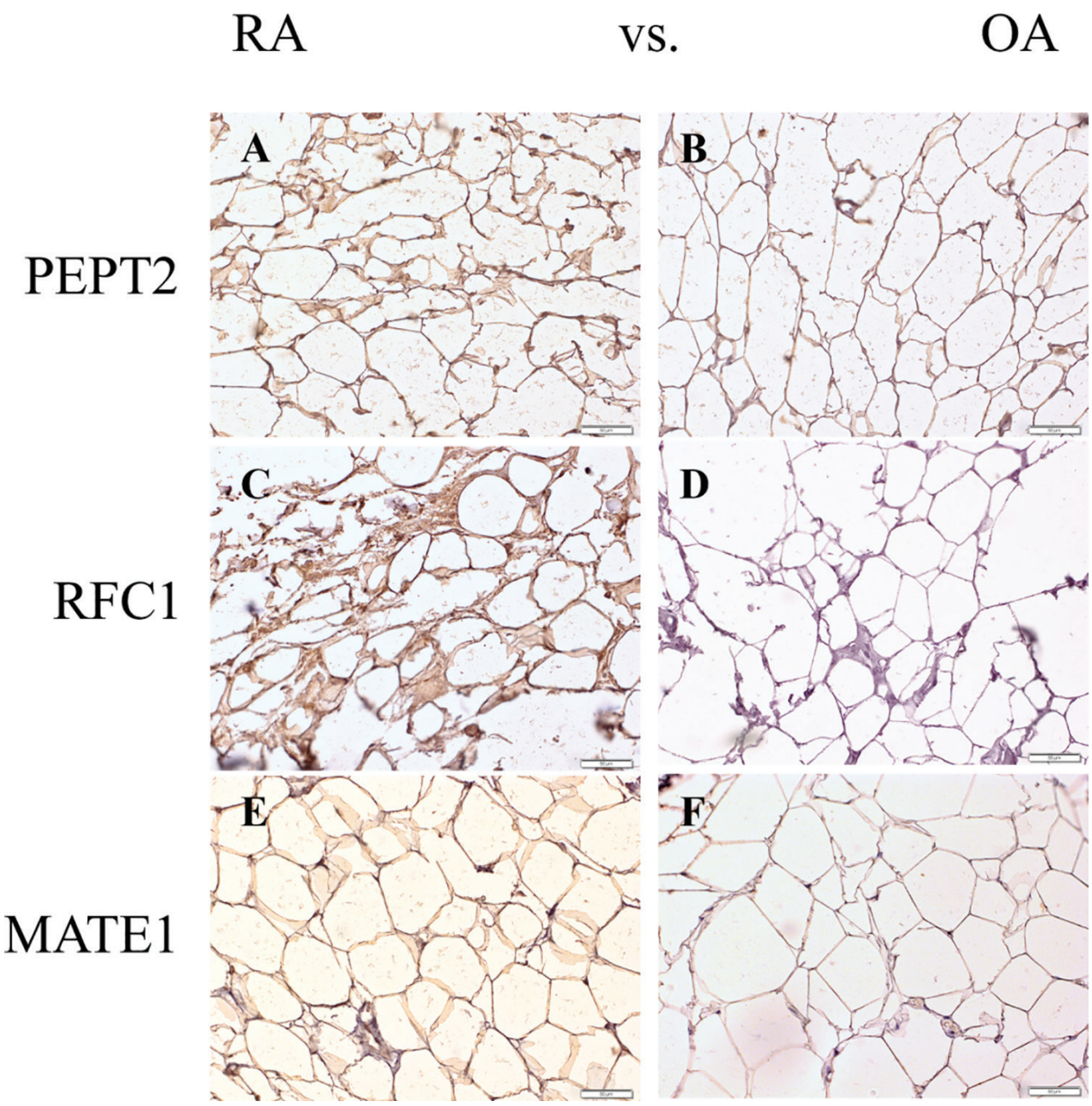

Fig 7. *Transporter protein expressions in HFPs samples from RA and OA patients. Transporters expression in RA patients panels (a, c, and e); transporters expressions in OA HFP samples panels: (b, d, and f); scale bar 50 µm; only representative images are presented. HFPs, Hoffa's fat pads; OA, osteoarthritis; RA, rheumatoid arthritis.*

demonstrated. This confirms the results of previous studies showing that the expression of SLC transporters varies in different tissues and is tissue-specific (Hediger et al. 2013; Schlessinger et al. 2013).

RA is an autoimmune chronic inflammatory disease leading to the destruction of joints and the development of a number of extra-articular complications such as sicca syndrome and amyloidosis. The ongoing inflammatory process primarily involves the small joints of the hands and feet leading to their destruction. The inflammatory process has been shown to affect all joint structures, including the periarticular adipose tissue, which can be a source of many mediators that enhance and sustain the

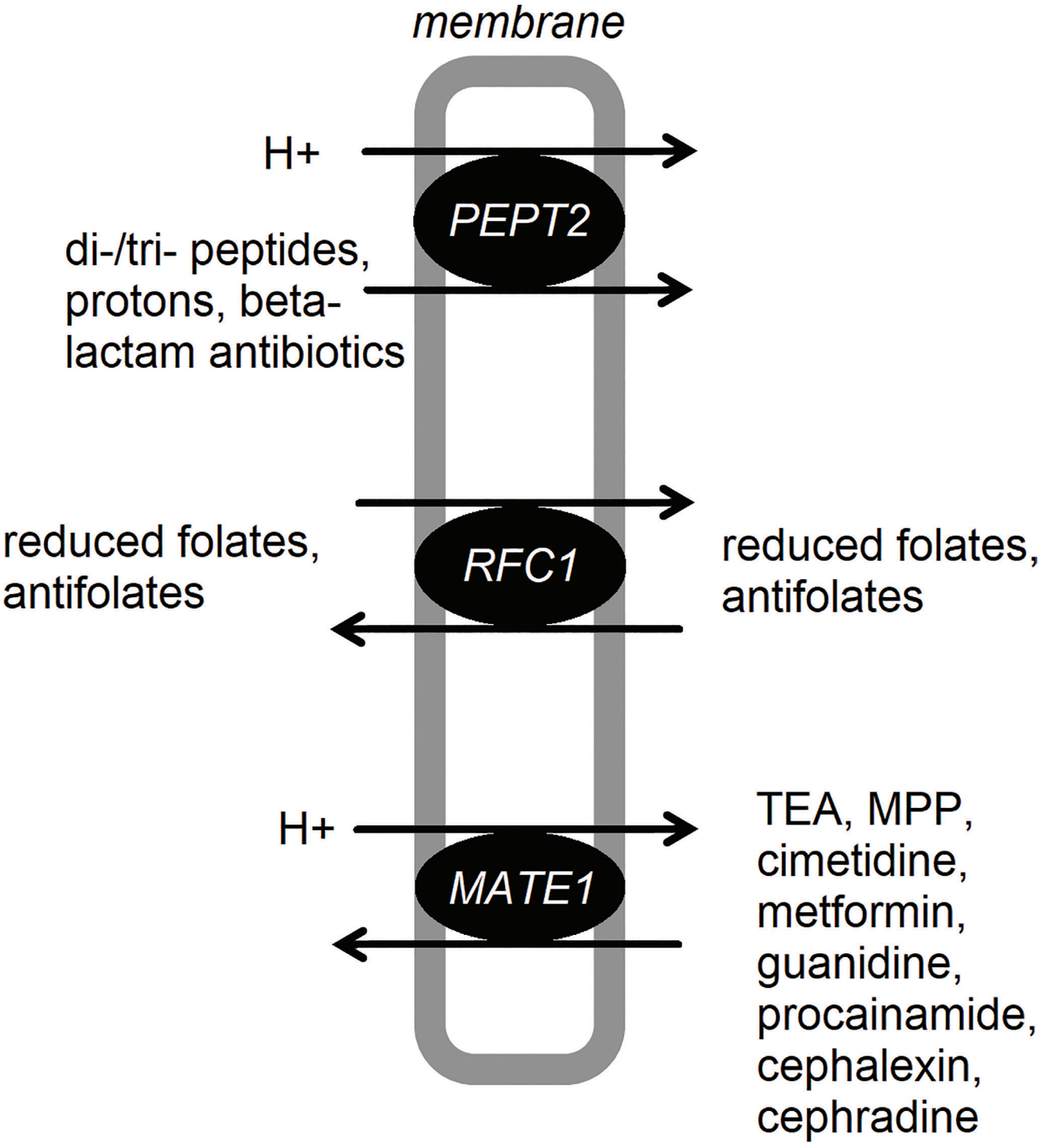

Fig 8. *SLC transporters: transport type of PEPT2 (cotransporter), RFC1 (exchanger) and MATE1 (exchanger) and their substrates. MPP, 1-methyl-4-phenylpyridinium; SLC, solute carrier; TEA, tetraethylammonium.*

development of the inflammatory process and the destruction of joint structures (Kuca-Warnawin et al. 2011; Komatsu and Takayanagi 2022). However, it seems that the key site of ongoing inflammation is the SM. The presence of protein transporters in these joint structures is very important due to the transport of many mediators involved in the development of inflammation, the transport of various metabolites as well as drugs used in the treatment of RA. It is very important that the drugs used

reach all tissues of the joint involved in inflammation. Previous studies have confirmed the presence of SLC family transporters in many tissues such as the liver, kidney, intestine, and brain tissue (Koepsell and Endou 2004). Knowing the localization of these transporters in synovial and periarticular adipose tissue can be important in selecting therapies for RA patients, as well as in understanding the processes that occur within these joint structures and lead to inflammation and joint destruction.

The important role of SLC transporters in the pathogenesis of RA, as well as the efficacy of RA therapy, seems to be indirectly confirmed by studies of the polymorphism of these genes in RA patients (Tokuhiro et al. 2003; Martínez et al. 2006; Lima et al. 2014; Eektimmerman et al. 2018; Pawlik et al. 2019; Wang et al. 2020). The results of our previous study suggest that the *SLC22A5* polymorphism may be associated with the development of extra-articular manifestations in RA (Pawlik et al. 2019). Lima et al. (2014) have shown that *SLC19A1*, *SLC46A1*, and *SLCO1B1* genotypes were associated with increased risk of MTX-related overall toxicity, whereas *SLC19A1* and *SLCO1B1* genotypes and *SLC19A1* haplotypes were associated with increased risk of MTX-related gastrointestinal toxicity. Eektimmerman et al. (2018) have found the significant association between Methotrexate (MTX) toxicity and rs624249, and rs1060896. However, this association has not been confirmed in other study groups, in which no association was observed between methotrexate efficacy and *SLC04A1*, *SLC22A2*, or *SLC28A2* variants (Eektimmerman et al. 2018). Wang et al. (2020) examined the association between the *SLCO1A2* gene (G550A, G553A, A775C, and G862A) polymorphisms and MTX-related toxicity in RA patients. The A775C and G862A polymorphisms were not detected in RA patients, but the 550AA genotype was associated with a high risk of MTX adverse effects (Wang et al. 2020). In our study, we demonstrated the expression of 17 membrane transporters from the SLC family in the SMs and Hoffa's pad of RA and OA patients. In addition, we showed the different expressions of some transporters in the SM and Hoffa's pad of RA patients compared to OA. It has been indicated that the SLC transporters play an important role in regulating cellular metabolism, especially in the SM, affecting the development of inflammation in RA patients (Torres et al. 2022a, b). Differences in epigenetic factors between RA and OA patients have been shown to affect the expression of SLC transporters in the synovial fibroblasts (Torres et al. 2022a, b). This affects changes in cellular metabolism, which may also affect the immune response and development of inflammation in RA.

In our study, the presence of SLC transporters in RA and OA patients was detected at the mRNA level, as well as confirmed at the protein level by IHC. We have detected statistically significant differences in the expression of some transporters between RA and OA patients. The observed differences may be primarily due to the different pathogenesis of the two diseases or the influence of different epigenetic factors. Previous studies have also shown that the expression of SLC transporters can be modulated by a number of mediators and metabolites (Bonaventura et al. 2016; Bustamante et al. 2018; Amrhein et al. 2020; Gallagher et al. 2020). Regardless of the reason, however, the differences we observed in the expression of SLC transporters between patients with RA and OA suggest the involvement of these transporters in their pathogenesis, in metabolic processes, transport of pro-inflammatory mediators, or transport of drugs.

We also observed correlations between certain transporters and CRP, WBC, and DAS28 values, suggesting that the inflammation present in RA patients may both increase and decrease the expression of some SLC transporters. RA is a complex autoimmune disease that results from interactions between immune cells such as T and B lymphocytes and macrophages with cells in joint tissues (synovial cells, chondrocytes, or periarticular fatty tissue). This leads to the enhanced synthesis of a number of pro-inflammatory mediators, such as cytokines and chemokines, which increase inflammation within the joint tissues (Song et al. 2020). SLC transporters may be involved in the transport of these mediators, thus influencing the development of inflammation (Diao et al. 2010; Foster et al. 2013; Liu et al. 2015; Song et al. 2020; Mirdamadi et al. 2021). Pro-inflammatory mediators can affect the expression of some membrane transporters. This regulatory effect may be related to the influence of various inflammatory mediators, such as pro-inflammatory cytokines and chemokines (Bonaventura et al. 2016; Bustamante et al. 2018; Amrhein et al. 2020; Gallagher et al. 2020). Pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ affect the expression of membrane transporters (Bonaventura et al. 2016; Bustamante et al. 2018; Amrhein et al. 2020; Gallagher et al. 2020). On the other hand, it has been shown that upregulated SLC7A5 activates mTOR-P70S6K signaling and enhances MMP3 and MMP13 expression in fibroblast-like synoviocytes from RA patients (Xu et al. 2020).

In our study, we demonstrated the expression of SLC transporters in the SMs and periarticular fatty tissue of patients with RA and OA. Our results confirmed the presence of SLC transporters not only in SMs, but also in periarticular fatty tissue, which plays an important role in the development of the inflammatory state in RA. We showed differences in the expression of these transporters between RA and OA patients. Regardless of the reasons for these differences, this suggests the possible involvement of certain transporters in the pathogenesis of these diseases, whether by affecting the transport of metabolites, pro-inflammatory mediators, or drugs. We also showed an association between the expression of some transporters and the parameters of clinical activity in RA. This may indicate the involvement of these transporters in the course of the inflammatory process and the influence of pro-inflammatory mediators on the

expression of some transporters. However, conclusive confirmation of the involvement of SLC transporters in processes leading to the development of RA requires studies in cellular or animal models to assess signaling pathways and metabolic processes in which SLC transporters may be involved.
A limitation of our study is the lack of determination of their expression in healthy subjects and the lack of explanation of the role of SLC transporters in RA pathogenesis. SLC transporters are involved in numerous processes that may underlie the development of RA and affect the efficacy of treatment. However, explaining their involvement in RA pathogenesis requires numerous studies, especially in cellular models.
We hope that the confirmation of the expression of a number of SLC transporters in the SM of joints and periarticular adipose tissue will contribute to further studies aimed at explaining the role of particular SLC transporters in the pathogenesis of RA.
In conclusion, our results show the expression of 17 membrane transporters from the SLC family in the SMs and the periarticular fatty tissue (Hoffa's pad) of RA and OA patients. To our knowledge, no studies have been conducted to date on the presence of expression of these transporters in periarticular fatty tissue. The confirmed expression of these transporters in the SMs as well as Hoffa's pad of patients with RA and OA, and the differences in their expression between these groups, suggest the involvement of SLC transporters in both the maintenance of homeostasis under physiological conditions in the tissues of the joints, as well as in the inflammatory process in RA. It is likely that these transporters may also play a role in the efficacy of RA treatment. We hope that our results will prompt further studies aimed at understanding the role of SLC transporters in joint tissues under physiological conditions as well as in the pathogenesis of RA.

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Author Contributions

D.M., methodology, investigation, visualization, gene expression, manuscript preparation, statistical analysis; K.P., histological preparation, visualization, IHC, ImageJ analysis; M.D., validation; A.P., conceptualization, formal analysis, supervision, manuscript preparation. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was approved by the Ethics Committee of Pomeranian Medical University, Szczecin, Poland (KB-0012/39/17).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Original data as well as calculations are available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Materials

Supplementary Table 1. *TaqMan gene expression assays*

SLC transporters		Housekeeping genes	
Gene symbol	TaqMan assay ID	Gene symbol	TaqMan assay ID
SLC01A2	Hs00366488_m1	*GUSB*	Hs99999908_m1
SLC01B1	Hs00272374_m1	*RPS9*	Hs02339424_g1
SLC01B3	Hs00351987_m1		
SLC01C1	Hs00213714_m1		
SLC02B1	Hs01030353_m1		
SLC04A1	Hs00983988_m1		
SLC04C1	Hs00698884_m1		
SLC10A1	Hs00161820_m1		
SLC10A2	Hs01001557_m1		
SLC15A1	Hs00192639_m1		
SLC15A2	Hs01113665_m1		
SLC16A3	Hs00358829_m1		
SLC19A1	Hs00953344_m1		
SLC22A1	Hs00427552_m1		
SLC22A11	Hs00945829_m1		
SLC22A12	Hs00375985_m1		
SLC22A18	Hs00180039_m1		
SLC22A2	Hs01010726_m1		
SLC22A3	Hs00222691_m1		
SLC22A4	Hs01548718_m1		
SLC22A5	Hs00929869_m1		
SLC22A6	Hs00537914_m1		
SLC22A7	Hs00198527_m1		
SLC22A8	Hs00188599_m1		
SLC22A9	Hs00375768_m1		
SLC28A1	Hs00984403_m1		
SLC28A2	Hs00188407_m1		
SLC2A9	Hs00417125_m1		
SLC33A1	Hs00270469_m1		
SLC47A1	Hs00217320_m1		
SLC47A2	Hs00945650_m1		
SLC51A	Hs00380895_m1		
SLC51B	Hs01057182_m1		
SLC7A5	Hs01001183_m1		
SLC7A6	Hs00187727_m1		

SLC, solute carrier.