Anti-satellite glia cell IgG antibodies in fibromyalgia patients are related to symptom severity and to metabolite concentrations in thalamus and rostral anterior cingulate cortex

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A B S T R A C T

Recent translational work has shown that fibromyalgia might be an autoimmune condition with pathogenic mechanisms mediated by a peripheral, pain-inducing action of immunoglobulin G (IgG) antibodies binding to satellite glia cells (SGC) in the dorsal root ganglia. A first clinical assessment of the postulated autoimmune showed that fibromyalgia subjects (FMS) had elevated levels of antibodies against SGC (termed anti-SGC IgG) compared to healthy controls and that anti-SGC IgG were associated with a more severe disease status. The overarching aim of the current study was to determine whether the role of anti-SGC IgG in driving pain is exclusively through peripheral mechanisms, as indirectly shown so far, or could be attributed also to central mechanisms. To this end, we wanted to first confirm, in a larger cohort of FMS, the relation between anti-SGC IgG and pain-related clinical measures. Secondly, we explored the associations of these autoantibodies with brain metabolite concentrations (assessed via magnetic resonance spectroscopy, MRS) and pressure-evoked cerebral pain processing (assessed via functional magnetic resonance imaging, fMRI) in FMS. Proton MRS was performed in the thalamus and rostral anterior cingulate cortex (rACC) of FMS and concentrations of a wide spectrum of metabolites were assessed. During fMRI, FMS received individually calibrated painful pressure stimuli corresponding to low and high pain intensities. Our results confirmed a positive correlation between anti-SGC IgG and clinical measures assessing condition severity. Additionally, FMS with high anti-SGC IgG levels had higher pain intensity and a worse disease status than FMS with low anti-SGC IgG levels. Further, anti-SGC IgG levels negatively correlated with metabolites such as scyllo-inositol in thalamus and rACC as well as with total choline and macromolecule 12 in thalamus, thus linking anti-SGC IgG levels to the concentration of metabolites in the brain of FMS. However, anti-SGC IgG levels in FMS were not associated with the sensitivity to pressure pain or the cerebral processing of evoked pressure pain. Taken together, our results suggest that anti-SGC IgG might be clinically relevant for spontaneous, non-evoked pain. Our current and previous translational and clinical findings could provide a rationale to try new antibody-related treatments in FMS.

1. Introduction

As a nociceplastic pain condition (Kosek et al., 2016), fibromyalgia (FM) is a chronic pain disorder characterized by altered nociception, whose underlying causes are still to be understood. Proposed contributors to nociceplastic pain in FM are peripheral and central mechanisms, such as sensitization (Kosek et al., 2016, 2021). In the peripheral nervous system, around 50% of fibromyalgia subjects (FMS) have peripheral nerve abnormalities (Grayston et al., 2019) stemming from reduced intraepidermal nerve fiber density (IENFD) as well as enhanced excitability and spontaneous activity of small nerve fibers (Evdokimov et al., 2019; Serra et al., 2014; Üçeyler et al., 2013). Among the central
nervous system (CNS) aberrations, studies using magnetic resonance spectroscopy (MRS) and functional magnetic resonance imaging (fMRI) have revealed altered brain metabolite concentrations and cerebral pain processing in FMS (Jensen et al., 2009, 2013; Larkin et al., 2021; Peek et al., 2020; Schrepf et al., 2016). Immune-related aberrations in FM have been suggested, due to elevated levels of pro-inflammatory cytokines in serum and cerebrospinal fluid (CSF) of FMS, suggesting, respectively, the presence of systemic inflammation and neuro-inflammation (Bäckryd et al., 2017; Kadotoff et al., 2012; O’Mahony et al., 2021). Proof of neuroinflammation was also provided by a report of widespread glial activation in the brain of FMS (Albrecht et al., 2019).

Recently, an important advance in examining the role of the immune system in the FM pathophysiology was made, in that translational research provided evidence for potential autoimmune mechanisms (Goebel et al., 2021). Specifically, immunoglobulin G (IgG) from FMS, when injected in mice, induced pain-like behavior, enhanced excitability and sensitization of peripheral nociceptive afferents, as well as reduced IENFD (Goebel et al., 2021), thus mimicking the clinical manifestation of the condition. In addition, these antibodies were found to bind to satellite glia cells (SGC) in the dorsal root ganglia (DRG), suggesting a peripheral, pain-inducing action of FMS IgG (Goebel et al., 2021). Importantly, IgG from FMS were also demonstrated to bind to SGC in human DRG (Goebel et al., 2021). Furthermore, initial clinical validation of these findings was obtained by showing that increased IgG binding to murine or human (postmortem) SGC was associated with more severe FM symptoms in FMS (Krock et al., 2023).

In the current study, we profited from a larger cohort of FMS with individually determined anti-SGC IgG levels to test whether the role of IgG in driving pain is to be confined exclusively to peripheral mechanisms, as indirectly shown so far (Goebel et al., 2021; Krock et al., 2023), or could be also attributed to central mechanisms. Specifically, we investigated the potential association of anti-SGC IgG in FMS with 1) pain-related clinical measures, 2) brain metabolite concentrations (MRS), and 3) cerebral processing of evoked pain (fMRI). Due to the explorative nature of the study, we assessed the role of anti-SGC IgG in relation to a wide spectrum of metabolites in thalamus and rostral anterior cingulate cortex (rACC) via MRS, and in the whole-brain through task-based fMRI. Finding a relation between anti-SGC IgG levels and a) clinical symptoms and/or b) altered cerebral metabolite concentrations in FMS could theoretically be explained by peripheral and/or central mechanisms. However, in order to control for potential anti-SGC IgG-mediated differences in excitability of primary nociceptive afferents (peripheral mechanisms), we subjectively calibrated the intensity of pressure pain stimuli to be delivered during task-based fMRI. Thus, in this study, the assessment of the cerebral processing of evoked pain in relation to anti-SGC IgG levels reflects central mechanisms.

2. Methods and materials

2.1. Participants

The cohort comprised 84 female FMS (mean 47.3 ± 7.8 years) and 43 age-balanced healthy controls (HC, mean 48.1 ± 7.6 years). To ensure adherence with ACR-1990 and ACR-2011 classification criteria (Wolfe et al., 1990, 2011), all FMS underwent systematic screening by a specialist in rehabilitation medicine and pain relief. Inclusion criteria for FMS also included being female, right-handed, and of working age 20–60 years. Individuals were excluded in the presence of any of the following exclusion criteria: other dominant pain conditions than FM, other autoimmune or rheumatoid diseases, other severe somatic diseases, pregnancy, self-reported claustrophobia, magnetic implants, inability to speak and understand Swedish, being unable to refrain from hypnotics, anxiolytics, or non-steroidal anti-inflammatory drugs prior to participating in the study (48 h before the first visit and 72 h before the second visit/scanning session). Healthy controls were right-handed females, without regular medications with non-steroidal anti-inflammatory drugs, sleep medication or anxiolytics, as well as free from chronic pain and from the exclusion criteria stated above for FMS.

All participants were recruited through daily press advertisement. Participants gave written informed consent before participating in the study, in accordance with the Declaration of Helsinki, and were remunerated for their time. The study was approved by the local ethical authority board (ethics permit: 2014/1604–31/1).

2.2. Experimental procedure

The current study is an extension of a larger project (study plan https://osf.io/8zqak/) and the idea for it stemmed from recent translational and clinical research that our group has performed in international collaboration providing novel evidence for potential autoimmune mechanisms in FMS (Goebel et al., 2021; Krock et al., 2023).

Here, only the experimental procedure and collection of data relevant for the current study are presented. Additional information can be found in previous publications (Ellerbrock et al., 2021a, 2021b; Fanton et al., 2022; Sandström et al., 2020).

Data were collected in two sessions on two consecutive days. Day 1 consisted of 1) administering validated questionnaires, 2) collecting serum samples for the quantification of anti-SGC IgG, 3) determining pressure pain thresholds (PPTs) to assess pain sensitivity, 4) calibrating individually painful pressures to be used during fMRI, 5) conditioning participants to associate color cues with pressure stimulations of different intensities (familiarization to pressure pain paradigm on day 2). Day 2 was the imaging session, which consisted in performing single-voxel proton MRS in bilateral thalamus and right rACC, as well as task-based fMRI.

2.2.1. Day 1

2.2.1.1. Questionnaires. All participants provided current pain intensity ratings on a scale ranging from 0 to 100 (Visual Analogue Scale, VAS), with 0 indicating no pain and 100 indicating worst imaginable pain. Only FMS filled in the Fibromyalgia Impact Questionnaire (FIQ) (Burckhardt et al., 1991), a questionnaire with 20 items assessing disability and symptoms specific to FM. The FIQ total score ranges from 0 to 100, with higher scores being indicative of a lower health status.

2.2.1.2. Blood samples for anti-SGC IgG quantification. Intravenous blood was collected in 2 × 8.5 mL BD Vacutainer® SST II Advance blood collection tubes and let stand at room temperature for 50 min, centrifuged at 2500 rpm for 10 min. The serum was collected, aliquoted and stored at − 80 °C until analysis. Satellite glia cell-enriched cultures established from mouse DRG were live-incubated with serum from FMS or HC and, after fixation, incubated with a rabbit anti-glutamine synthase antibody (marker of SGC, Abcam, Catalogue # ab75593). Cells were then incubated with fluorescently conjugated antibodies against human (ThermoFisher, A11014) and rabbit IgG (ThermoFisher, A11008). Cells were imaged with a Zeiss LSM800 confocal microscopy operated by LSM ZEN2012, segmented using an automated pipeline machine vision pipeline (Hunt et al., 2022) and analyzed in Fiji. The percentage of SGC bound by human IgG (% SGC binding) for each sample (with investigators blinded to subject identity) was determined and used as a proxy measure for serum levels of anti-SGC IgG antibodies. The procedure is described in detail in Krock et al., 2023.

In order to facilitate data analysis and based on previous findings on a smaller cohort in which FMS with severe, compared to mild, FM were shown to have over 50 % anti-SGC IgG binding (Krock et al., 2023), we...
chose to dichotomize FMS into FMS with percentage of SGC bound by IgG ≥ 50 (FMS with high anti-SGC IgG levels) and FMS with percentage of SGC bound by IgG < 50 (FMS with low anti-SGC IgG levels).

2.2.1.3. Assessment of pressure pain thresholds. Pressure pain thresholds were determined, as an indicator of pain sensitivity, using a hand-held algometer (Somedic Sales AB) with a rubber probe of 1 cm². The pressure algometer was applied perpendicular to the tested body surface and manual force was exerted at a steadily increasing rate of approximately 50 kPa/s until the participants pressed a button to signal the first sensation of pain (Kosek et al., 1993). Pressure pain thresholds were measured bilaterally across four different anatomical sites: supraspinatus muscle, lateral epicondyle (elbow), gluteus muscle, and medial fat pad (knee). The average PPT, per participant, across all sites was used in the analyses (PPTmean).

2.2.2.2. Acquisition of magnetic resonance spectroscopy data. Metabolites were assessed in vivo through single-voxel proton MRS. The voxel positioning was verified by three-plane localizer images acquired before every scan. Before the acquisition of data, gradient echo shimming, frequency, and water suppression adjustments were automatically performed. The conventional point resolution spectroscopy (PRESS) method was used with the following parameters: TR/TE/TE1 = 2000/40/19 ms, spectral bandwidth 5 kHz, 4096 time-domain data points, and water suppression by 3 chemical shift selected suppression (CHESS) pre-pulses. In order to enhance voxel definition, 6 sharp outer volume suppression radio frequency pulses surrounding voxel were applied. The voxel volumes were 12.4 mL for thalamus and 5.4 mL for rACC. The metabolites in the basis set were the following: aspartate (Asp), glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA), N-acetyl aspartate (NAA), myo-inositol (Ins) and scyllo-inositol (InsS), taurine, ascorbate, glucose (Glc), creatine (Cr) and phosphocreatine (PCr), choline (PCh) and glycerophosphorylcholine (GPC), N-acetylaspartate-glutamate (NAAG), glutathione (GSH), alanine (Ala), lactate (Lac), ethanolamine, and phosphoryl-ethanolamine (PE). Besides individual metabolites, macromolecules (MM) and lipids (Lip) were simulated using generic Gaussian lineshapes. In addition, the baseline flexibility was restricted to 0.25 ppm spline distance which, in our case, provided a reasonable balance between unwanted bias due to overfitting and natural baseline smoothness. A MRS phantom (BRAINO + GABA, GE Healthcare) was used to calibrate the basis set. The quantification of the data was performed using the ratio to 1) total creatine (7mM assumed value, relative) as well as 2) total voxel water concentration (absolute). Estimation of endogenous water concentration was performed by co-registering the MRS voxel mask with an anatomical 3D T1-weighted image in native space segmented into gray matter (GM), white matter (WM), and CSF in FSL (version 5, FMRIB Software Library). The obtained tissue volumes were further masked by the voxel and partial volume estimates for each type of tissue previously used to correct the total water concentration. Refer to Fig. 1 for an overview of the acquisition and analysis of MRS data.

2.3. Statistics

Analyses were performed using IBM SPSS Statistics (version 28). Statistical significance for the reported p-values was set, conventionally, at p < 0.05. To ensure reliability in the collected pain-related measures, we first tested for potential group (FMS, HC) differences in age, current pain (VASnow), pressure pain thresholds (PPTmean) and suprathreshold pain (P10, P50) via separate one-way analysis of variance (ANOVA) tests. Additionally, groups were compared regarding anti-SGC IgG levels via a one-way ANOVA. Further, according to the study objectives, the main analyses reported below (i.e., related to anti-SGC IgG) were restricted only to FMS (excluding HC). Plots of the results were created using RStudio (R version 3.5.3) (RStudioTeam, 2016).

2.3.1. Pain-related characteristics and anti-SGC IgG: Differences between FMS with high and low anti-SGC IgG levels and correlations

Differences between FMS with high and low anti-SGC IgG levels in VASnow, PPTmean, P10, P50 as well as in FIQ, pain duration in months, and tender points were tested through separate one-way ANOVAs. Spearman’s correlations were performed to analyze the associations of anti-SGC IgG levels with, respectively, PPTmean, P10, P50, VASnow, and FIQ.

The serum samples of six FMS and three HC were missing. Thus, these individuals lacked information regarding anti-SGC IgG levels. Behavioral data from a total of 78 FMS (FMS with low anti-SGC IgG levels = 40, FMS with high anti-SGC IgG levels = 38) and 40 HC were analyzed.

2.3.2. Analysis of magnetic resonance spectroscopy data

MRS data were quantified in LCModel (version 6.3–1 K, http://s-provencher.com/). Prior to quantification, pre-processing was performed in MATLAB (The MathWorks, Natick, MA) and included 1) the S/N2-weighted MRS signal coil combining 2) the frequency and phase correction for every trace prior to 3) the final coherent averaging of the elementary MRS traces per voxel. The quantum mechanical density formalism in MATLAB was adopted to simulate the basis set required for the LCModel by using the actual PRESS pulse sequence timing parameters as well as the chemical shifts and J-coupling constants reported in previous publications (Govind et al., 2015; Govindaraju et al., 2000). The metabolites in the basis set were the following: aspartate (Asp), glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA), N-acetyl aspartate (NAA), myo-inositol (Ins) and scyllo-inositol (InsS), taurine, ascorbate, glucose (Glc), creatine (Cr) and phosphocreatine (PCr), choline (PCh) and glycerophosphorylcholine (GPC), N-acetylaspartate-glutamate (NAAG), glutathione (GSH), alanine (Ala), lactate (Lac), ethanolamine, and phosphoryl-ethanolamine (PE). Besides individual metabolites, macromolecules (MM) and lipids (Lip) were simulated using generic Gaussian lineshapes. In addition, the baseline flexibility was restricted to 0.25 ppm spline distance which, in our case, provided a reasonable balance between unwanted bias due to overfitting and natural baseline smoothness. A MRS phantom (BRAINO + GABA, GE Healthcare) was used to calibrate the basis set. The quantification of the data was performed using the ratio to 1) total creatine (7mM assumed value, relative) as well as 2) total voxel water concentration (absolute).
Fig. 1. Overview of the acquisition and analysis of MRS data. For the acquisition of MRS data, a single MRS voxel was positioned on the bilateral thalamus (A) and on the right rACC (D). In both (A) and (D), displayed from top to bottom are, respectively, the radiological coronal, sagittal, and axial views of the respective voxel placements (adapted from Fanton et al., 2022). The voxel for thalamus was segmented in three tissue types (B): GM (red and pink), WM (green), CSF (blue). A different color coding can be seen for the segmentation in the same tissue types of the voxel for rACC (E): GM (blue), WM (green), CSF (light blue). From MRS data analysis, a representative MRS spectrum of thalamic (C) and rACC-related metabolites (F) was obtained, with the y-axis displaying each metabolite’s signal amplitude and the x-axis showing each metabolite’s resonance position on the chemical shift axis. Ins, myo-inositol; Cr, creatine; PCr, phosphocreatine; Glu, glutamate; Gln, glutamine; Glc, glucose; GSH, glutathione; Ins-S, scyllo-inositol; PCh, choline; GPC, glycerophosphorylcholine; GABA, γ-aminobutyric acid; Asp, aspartate; NAA, N-acetyl aspartate; N-acetylaspartate-glutamate; (NAAG); MM, macromolecules, 17, 14, 12, 09; Lip, lipid, 09; ppm, parts per million.
performed separately for thalamus and rACC. For thalamus, the analyses were carried out including the following metabolites, using both absolute and relative concentrations: Asp, Gln, Gln, GABA, NAA, Ins, InsS, Glc, NAAG, GSH, Ala, Lac, PE, Glu + Gln, NAA + NAAG, Cr + Pcr (only absolute), GPC + PCh, Ins + InsS, MM09 + Lip09, MM17, MM14, MM12, MM09, Lip16. For the rACC, the analyses comprised the following metabolites (absolute and relative concentrations): Asp, Gln, Gln, GABA, NAA, Ins, InsS, Glc, NAAG, GSH, PE, Gln + Gln, NAA + NAAG, Cr + Pcr (only absolute), GPC + PCh, Ins + InsS, MM18. Absolute and relative concentrations of taurine, ascorbate, and ethanolamine were excluded from the analyses since their accurate detection could not be achieved because of their low concentration and consequential peak overlap with metabolites with a stronger signal.

In FMS, a multivariate analysis of variance (MANOVA) was used to test for potential differences between FMS with high and low anti-SGC IgG levels in the thalamic and rACC-related concentration of all the metabolites reported above. In order to explore the potential relation of all these thalamic and rACC-related metabolites with anti-SGC IgG levels, non-parametric Spearman’s correlations were performed. In the presence of a significant relation between anti-SGC IgG levels and metabolite concentrations, differences between FMS and HC in the concentration of metabolites which showed to be related significantly with anti-SGC IgG levels were assessed through one-way ANOVAs. Additionally, Spearman’s correlations were performed between clinical measures and metabolite concentrations in thalamus and rACC of FMS, only for clinical measures and brain metabolite concentrations showing to be significantly related with anti-SGC IgG levels. These assessments were performed to further probe the involvement of anti-SGC IgG in brain metabolite concentration in FMS.

MRS data were collected from 116 subjects in thalamus (FMS = 74, HC = 42) and 108 in rACC (FMS = 68, HC = 40). As five FMS among the 74 FMS (thalamus dataset) and four FMS among the 68 FMS (rACC dataset) lacked serum samples for anti-SGC IgG determination, MRS data in thalamus from 69 FMS (FMS with low anti-SGC IgG levels = 33, FMS with high anti-SGC IgG levels = 36) and in rACC from 64 FMS (FMS with low anti-SGC IgG levels = 32, FMS with high anti-SGC IgG levels = 32) were analyzed.

2.3.3. Analysis of functional magnetic resonance imaging data

Functional data were processed and analyzed using statistical parametric mapping (SPM12, Wellcome Trust Centre for Neuroimaging) running under MATLAB. Firstly, anatomical and functional scans were re-oriented, manually, to the anterior commissure. In the preprocessing, volumes were spatially realigned to the mean volume via six-parameter affine transformation, the structural image was co-registered to the functional images, and functional data were then spatially normalized into the standard Montreal Neurological Institute (MNI) stereotactic space and smoothed with an isotropic Gaussian kernel of 6mm full-width at half-maximum (FWHM). Frame-wise displacement (FD) was used to quantify head movement from one frame to the next by computing the sum of the derivatives’ absolute values of the six realignment parameters (Power et al., 2012). This led to the exclusion from further analyses of four FMS due to the excessive head movement (FD > 0.5 mm in >15% of the images). The general linear model implemented in SPM12 was used for the analysis of the data. Single subject first level analysis included temporal high-pass filtering (128 s cut-off) and correction for autocorrelations using first-order autoregressive modelling. On the individual level, the modelled conditions were: the two cue or anticipation phases (green prior to P10/red prior to P50, cue for 2 s and delay of 2–6 s before the onset of the stimulus), the two pressure intensities (P10/P50, 5 s), and the rating period (8 s) following each stimulation. Six realignment-derived head motion parameters were included as regressors of no interest.

Functional MRI data were acquired for 70 FMS, with a total of 10 FMS being excluded for the following reasons: structural brain anomalies (n = 1), excessive head motion (n = 4), as well as drop-outs and technical issues leading to incomplete datasets (n = 5). Further, four FMS were excluded due to lack of serum samples for anti-SGC IgG determination. Functional MRI-related results were presented for 66 FMS (FMS with low anti-SGC IgG levels = 35, FMS with high anti-SGC IgG levels = 31).

Single-subject contrast images from the individual models on the first level were taken to the second level and used in random-effects analyses to test for the effects of antibodies.

We used a whole-brain approach to inspect potential differences in pain-related cerebral processing between FMS with high and low anti-SGC IgG levels for the different painful pressure levels (P10, P50). Specifically, we performed two-sample t-tests to test for differences between FMS with high and low anti-SGC IgG levels for different evoked pain pressure levels. Further, correlations were performed in order to explore the potential relationship between anti-SGC IgG levels and evoked pain for P10 and P50 separately.

To inspect results, an initial statistical threshold of p < 0.001 was set and a cluster threshold, family-wise error corrected, of p < 0.05 was applied.

3. Results

3.1. Differences in clinical measures and anti-SGC IgG levels between FMS and HC

As expected and presented in Table 1, groups (FMS, HC) differed significantly in VASnow, PPTmean, P10 and P50, with FMS having higher current pain intensity (VASnow) and higher pain sensitivity to evoked pressure manifesting as lower pain thresholds (PPTmean) and higher sensitivity to suprathreshold pressure pain (P10, P50) than HC. In line with previous findings on a smaller cohort (Krock et al., 2023), groups did also differ in anti-SGC levels (Fig. 2A), with IgG in the serum of FMS binding a higher percentage of SGC (mean: 47.3, standard deviation: 25.8) than IgG in HC serum (mean: 34.4, standard deviation: 21.2) (F(1,116) = 7.498; p = 0.007). IgG in the serum of FMS with high anti-SGC IgG levels generated a greater immunofluorescence signal intensity in comparison to IgG in the serum of HC and FMS with low anti-SGC IgG levels (Fig. 2B).

3.2. FMS characteristics and pain-relevant measures in relation to anti-SGC IgG

FMS with high anti-SGC IgG levels had higher current pain intensity (VASnow) and more severe FM (FIQ) compared to FMS with low anti-SGC IgG levels (Fig. 3AB, Table 2). There were no differences between FMS with high and low anti-SGC IgG levels regarding PPTmean, P10, P50, pain duration, or tender points (Table 2). Further, no statistically significant correlations were found between anti-SGC IgG levels and, respectively, PPTmean, P10 and P50. However, anti-SGC IgG levels
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3.3. Magnetic resonance spectroscopy

MRS data showed excellent spectral quality, with linewidth and signal-to-noise ratio measured on Cr + Pcr peak being 70 ± 20 and 7.3 ± 1.0 Hz. Additionally, the average voxel composition was 62.9 ± 2.3% GM, 28.3 ± 2.6% WM, and 8.7 ± 2.6% CSF, indicating that the voxel predominantly contained GM. The median Cram-Rao lower bound values, missing values, and outliers for the main metabolites are displayed in Supplementary Table 3A for thalamus and Supplementary Table 3B for rACC.

Mean and standard deviation for each of the thalamic and rACC-related metabolites included in the analyses can be found in Supplementary Tables 1 and 2, respectively. There were no significant differences between FMS with high and low anti-SGC IgG levels in the concentration of any of the tested thalamic or rACC-related metabolites (Fig. 4A). As for the relationship between anti-SGC IgG levels and metabolism in thalamus, the thalamic metabolites showing a significant, although weak, negative correlation with anti-SGC IgG levels were: relative GPC + PCh (r = -0.242, p = 0.045; Fig. 4B), absolute MM12 (r = -0.238, p = 0.049; Fig. 4B), as well as absolute and relative InS (absolute: r = -0.253, p = 0.036; relative: r = -0.266, p = 0.027; Fig. 4B). In the rACC, relative InS was also found to negatively correlate with anti-SGC IgG levels (r = -0.249, p = 0.047, Fig. 4B). None of the other tested metabolites in the rACC showed a significant correlation with anti-SGC IgG levels. In thalamus, FMS and HC did not differ in the concentration of relative GPC + PCh, absolute MM12, or absolute and relative InS. However, for absolute and relative InS, the difference between groups was trending towards significance (absolute: p = 0.097; relative: p = 0.073), with FMS having lower concentrations of these metabolites (absolute InS: mean = 0.615, standard deviation = 0.369; relative InS: mean = 0.627, standard deviation = 0.364) than HC (absolute InS: mean = 0.738, standard deviation = 0.398; relative InS: mean = 0.755, standard deviation = 0.369). In the rACC, groups (FMS, HC) differed in the concentration of relative InS (F1,106 = 12.208; p < 0.001), with concentrations in HC (mean: 1.5, standard deviation: 0.7) being higher than in FMS (mean: 1.0, standard deviation: 0.6). Additionally, in FMS, thalamic relative concentrations of GPC + PCh were found to correlate negatively with VASnow (r = -0.267, p = 0.021), but not with FIQ. None of the other tested thalamic metabolites (absolute MM12, absolute and relative InS) was found to correlate with either VASnow or FIQ. Conversely, in the rACC of FMS, relative InS was found to negatively correlate with FIQ (r = -0.252, p = 0.039), but not with VASnow.

3.4. Functional magnetic resonance imaging

We previously demonstrated a main effect of pressure pain in brain areas related to the processing of pain, among which are the somatosensory cortices/parietal operculum and insula (Ellerbrock et al., 2021b). Whole-brain analyses in FMS revealed no differences in neural activity between FMS with high and low anti-SGC IgG levels for either of the two pressure levels (P10, P50). There was no relationship between anti-SGC IgG levels and neural activity in response to either pressure level.

4. Discussion

The current multimodal neuroimaging study provides evidence for the role of anti-SGC IgG in clinical pain and brain metabolite content, but not in cerebral pain processing. In support of the clinical relevance of anti-SGC IgG, FMS with high anti-SGC IgG levels (>50% of SGC bound by IgG), compared to FMS with low anti-SGC IgG levels (<50% of SGC bound by IgG), presented with more intense pain (VASnow) and a worse disease status (FIQ). In addition, anti-SGC IgG levels correlated
positively with VASnow and FIQ, but were not associated with pressure
pain thresholds (PPTs) nor with the sensitivity to suprathreshold pres-
sure pain (P10, P50). Furthermore, anti-SGC IgG were found to be
associated with brain metabolite concentrations (MRS) in FMS, with
data showing a negative correlation between anti-SGC IgG levels and the
concentration of metabolites such as scylo-inositol (InsS) in thalamus
and rACC as well as total choline (GPC + PCh) and macromolecule 12
(MM12) in thalamus. Finally, fMRI findings revealed no differences in
the cerebral processing of evoked pressure pain between FMS with high
and low anti-SGC IgG levels, nor a relation between anti-SGC IgG levels
and the neural response to individually calibrated evoked pressure pain.

An interesting consideration that arises from our behavioral and
fMRI-related findings is that anti-SGC IgG appear to be most likely
involved in spontaneous, rather than evoked pain in FMS. The distinc-
tion between spontaneous and evoked pain in relation to anti-SGC IgG
might be explained in light of our two previous studies in which IgG-
related pain mechanisms were explored in a subgroup of the current
FMS cohort (Goebel et al., 2021; Krock et al., 2023). Specifically, our
recent translational study demonstrated that injections of IgG from FMS,
but not HC, affected both evoked and spontaneous behaviors in mice
(Goebel et al., 2021). With regard to evoked behaviors, injections of IgG
from FMS led to increased responsiveness to cold as well as to me-
chanical stimulation by sensitizing peripheral nociceptive afferents
(Goebel et al., 2021). In detail, the receptive fields of Aδ- and C-

![Fig. 3. VASnow and FIQ differ between FMS with high and low anti-SGC levels and are related with anti-SGC levels. FMS with high anti-SGC IgG levels displayed higher current pain intensity (VASnow, A) and a worse FM condition (FIQ, B). In addition, anti-SGC IgG levels were found to positively correlate with the samepain-related measures, VASnow (C) and FIQ (D). Figures (A) and (B) show raincloud plots with raw data and data distribution. For completeness, boxplots are presented, with the black line being the median, the white square and number indicating the mean, the lower and upper boxes showing the 25th (Q1) and 75th (Q3) percentile [equivalent to the interquartile range (IQR)], and whiskers representing Q1–1.5*IQR and Q3 + 1.5*IQR. Figures (C) and (D) display a scatter plot per pain measure (VASnow and FIQ), each with its best-fitting regression line surrounded by a shaded area indicating the 95% confidence interval for the line. Both scatter plots have the same x-axis indicating anti-SGC IgG levels, defined as the percentage (0–100) of satellite glia cells bound by IgG. The y-axis represents ratings of VASnow and FIQ, respectively. The statistics related to computing Spearman’s correlations is displayed on the bottom right corner of each scatter plot, with r being the correlation coefficient and p the related p-value. VASnow, current pain on visual analogue scale; FIQ, fibromyalgia impact questionnaire; FMS, fibromyalgia subjects; low anti-SGC IgG levels, percentage of satellite glia cells bound by IgG < 50; high anti-SGC IgG levels, percentage of satellite glia cells bound by IgG ≥ 50.


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mechanosensitive nociceptors in skin-saphenous nerve preparations were found to respond to mechanical stimulation at a reduced force compared to preparations from HC IgG-treated mice (Goebel et al., 2021). As an example of spontaneous behavior, FMS IgG-injected mice showed less spontaneous locomotor activity at peak activity hours compared to HC IgG-injected mice (Goebel et al., 2021). While this translational research provided evidence for the presence of IgG in FMS inducing pain-like behavior (evoked and spontaneous) in mice, it is important to note that the injected antibodies were not exclusively IgG against SGC, but rather the total serum IgG fraction. Speculatively, this might potentially indicate that other pathogenetic autoantibodies might be involved in evoked pain in FMS. However, due to the fact that IgG from FMS were found to bind mouse and human SGC (Goebel et al., 2021), anti-SGC IgG became the focus of a recent clinical assessment, in which we demonstrated that high levels of anti-SGC IgG were associated with more intense pain, more severe FM (higher FIQ scores) and, although weakly, also with increased sensitivity to pressure pain in a subgroup of the current cohort of FMS (Krock et al., 2023). In the larger FMS cohort examined in the present study, we confirmed the positive correlations found between anti-SGC IgG levels and a clinical measure of spontaneous pain (VASnow) as well as disease severity (FIQ). However, anti-SGC IgG levels were unrelated to evoked pain measures assessing sensitivity to pressure pain, i.e., PPTmean, P10, and P50. Thus, our behavioral results, obtained from testing a larger cohort of FMS, suggest that anti-SGC IgG may not be related to evoked pain processes. However, given the large interindividual variability in the assessment of pressure pain sensitivity even in healthy subjects (Kosek et al., 1993) and the conflicting data presented above, further investigations are required into the potential involvement of, specifically, anti-SGC IgG in the sensitivity to evoked pain and the potential clinical relevance thereof.

However, further support to the assumption that anti-SGC IgG are not related to the processing of evoked pain comes from our fMRI data showing no difference between FMS with high and low anti-SGC IgG levels in the cerebral processing of painful pressure, and no relation between anti-SGC IgG and the neural response to painful pressure. The understanding of the potential effects of anti-SGC IgG on the CNS is very limited. However, in our recent clinical assessment, anti-SGC IgG levels did not relate to conditioned pain modulation (CPM) (Krock et al., 2023), with CPM being a measure of descending pain inhibition found to be dysfunctional in FMS (Kosek & Hansson, 1997; Potvin & Marchand, 2016). Prior to this finding, in our recent translational study, we also demonstrated that IgG bound to SGC in the DRG, whereas no binding to any cell was seen in the spinal cord or brain of IgG-injected mice (Goebel et al., 2021). Taken together, these findings point to anti-SGC IgG not being involved in central mechanisms of evoked pain. However, as reasoned above, this does not preclude the possibility that antibodies against non-SGC antigens impact central mechanisms of evoked pain.

Conversely, our findings regarding the relation between brain metabolite concentrations and anti-SGC IgG levels offer an initial observation, which certainly necessitates further testing and validation, into the potential association between anti-SGC IgG and a CNS-derived measurement of metabolite concentrations. The use of proton MRS allowed us to obtain, non-invasively, information regarding the metabolic concentration of neuronal and glial markers in the thalamus and rACC of FMS, and to relate these with anti-SGC IgG. Although there were no significant differences between FMS with high and low anti-SGC IgG levels in the concentration of metabolites in thalamus or rACC, we found statistically significant, but weak, negative correlations between anti-SGC IgG levels and InsS in thalamus and rACC, as well as GPC + PCh and MM12 in thalamus. Additionally, we found a negative correlation between relative InsS in rACC and disease severity (FIQ) as well as between GPC + PCh in thalamus and pain intensity (VASnow), linking low levels of these metabolites to more severe FM symptoms within the FM group. In support of the relevance of these findings, further analyses revealed that FMS, compared to HC, had lower relative InsS in rACC and thalamus, although, in thalamus, this difference failed to reach statistical significance.

The highest concentrations of inositol are found in the brain, where myo-inositol (Ins) reversibly epimerizes to form its stereoisomer scyllo-inositol (InsS) that normally presents in much lower concentrations (Fenili et al., 2007; Kaiser et al., 2005). Given its abundance in astrocytes, myo-inositol has been regarded as a marker of glia, particularly astroglia, activity (Glavin et al., 1989; Hattingen et al., 2008). Altered concentrations of myo-inositol (Fayed et al., 2010), as well as positive and negative associations between myo-inositol in various brain regions with pain and FM-related measures have been reported in FMS (Ferracini et al., 2011; Lee et al., 2021; Valdes et al., 2010). However, to our knowledge, no previous study has focused on scyllo-inositol in FMS, or in the context of chronic pain. Elevated cerebral concentrations of scyllo-inositol have been reported in patients with Alzheimer’s disease and in individuals with amnestic mild cognitive impairment (Griffith et al., 2007), as well as in patients suffering from chronic alcoholism (Viola et al., 2004). On the contrary, decreased cerebral concentrations of scyllo-inositol, which we found to feature FMS in our study, have also been observed in the supplementary motor area of patients with progressive supranuclear palsy, with low concentrations of scyllo-inositol being associated to poor working memory and attention deficits (Barbagallo et al., 2019).

Interestingly, scyllo-inositol has been shown to potentially have beneficial effects in animal models of Alzheimer disease, improving cognitive functions (McLaurin et al., 2006), and drug trials aiming at increasing scyllo-inositol levels have been conducted in Alzheimer’s patients (Salloway et al., 2011). FMS typically complain of cognitive problems (Bell et al., 2018). In this perspective, we speculate that elevations of scyllo-inositol can form part of a protective mechanism, and that the negative correlation between anti-SGC IgG levels and scyllo-inositol (the higher the anti-SGC IgG levels, the lower the
concentration of InsS in FMS could, hypothetically, indicate a negative cerebral involvement of anti-SGC IgG, either directly with anti-SGC IgG passing the blood–brain barrier (BBB), or indirectly through prolonged peripheral sensitization. Regarding the penetration of the BBB as a potential mechanism of action of these autoantibodies, the crossing of anti-SGC IgG may occur given the changes in the permeability of BBB associated with chronic pain (DosSantos et al., 2014). With regard to peripheral sensitization as an alternative mechanism of action of anti-SGC IgG, it may be that these autoantibodies increase spontaneous activity of primary nociceptive afferents. Evidence of increased spontaneous activity and sensitization of C nociceptors has been provided in FMS (Serra et al., 2014). Further abnormalities in peripheral sensory afferents have also been documented, with FMS having reduced IENFD (Evdkimov et al., 2019; Üçeyler et al., 2013). Interestingly, this relates to our previously translational findings showing a reduction in IENFD in FMS IgG-injected mice (Goebel et al., 2021).

Choline compounds (i.e., GPC and PCh) are involved in phospholipid metabolism, cellular turnover, osmotic regulation (Chang et al., 2013; Fayed et al., 2010) and have been associated with glial activation and neuroinflammation (Chang et al., 2013; Jung et al., 2020). Previous studies present a complex picture regarding the role of choline in FMS, with no statistically significant differences between FMS and HC being reported in the concentration of choline compounds in thalamus (Fayed et al., 2010; Feraco et al., 2011; Valdès et al., 2010), but with elevated as well as reduced concentrations in other areas of the brain being found (Fayed et al., 2010; Jung et al., 2020). Furthermore, positive as well as negative correlations between choline compounds and pain/clinical measures have been reported in FMS (Jung et al., 2020; Valdès et al., 2010). Here, we found a negative correlation between anti-SGC IgG levels and the concentration of thalamic total choline, as well as between thalamic total choline concentrations and pain intensity in FMS. In agreement with previous studies (Fayed et al., 2010; Feraco et al., 2011; Valdès et al., 2010), we did not find significant differences between FMS and HC in the concentration of this metabolite in thalamus.

The inverse relation between anti-SGC IgG levels and thalamic choline (the higher the anti-SGC IgG levels, the lower the concentration of total choline) is hard to interpret, but could be hypothetically explained by a reduction in activation of protective glia in FMS with high anti-SGC IgG levels or, instead, be due to the possibility that choline is implicated in metabolic rather than neuroinflammatory processes. While the former speculation might find support in previous findings from our group on patients suffering from rheumatoid arthritis (Forsberg et al., 2019), the latter was provided as a potential explanation by the authors of a study showing reduced concentration of choline in the thalamus of patients with painful osteoarthritis compared to HC (Weerasekera et al., 2021). However, given the multiple physiological actions of choline, these interpretations must be regarded as highly speculative.

Regarding macromolecule 12, we refrain from speculating regarding its role, as the exact biochemical composition of macromolecules is largely unknown, thus making their potential involvement in pathologies too speculative to interpret (Choi et al., 2007).

4.1. Limitations

The acquisition of MRS data at rest provides information regarding baseline metabolites. However, in order to better understand the potential role of anti-SGC IgG in evoked pain, it would have been beneficial to also measure the concentration of metabolites during brain activation via functional MRS. Additionally, calibrating pressure pain stimuli to have them correspond to each individual’s pain ratings of 10 mm and 50 mm on a 0–100 mm VAS scale was performed in order to compensate for possible differences in pain sensitivity between FMS with high and low anti-SGC IgG levels. However, this might have leveled out the potential pressure-evoked cerebral processing differences between these two groups and in relation to anti-SGC IgG levels. Furthermore, although patients taking gabapentinoids and antidepressants were excluded and analgesics as well as sleep medications were stopped 72 h before the MRS/MRI session, it is important to be mindful regarding the potential consequences of medication use (refer to Supplementary Table 4 for a detailed account of the medications taken by each patient of our study cohort). Finally, our quantification of autoantibodies in FMS is limited by not having yet identified the precise autoantigens. Testing FMS serum for autoantibodies against specific antigens, rather than a cell type, will likely improve specificity as non-specific IgG could be binding SGC.

5. Conclusions

Our findings showing more intense current pain and a higher impact of FM in FMS with high anti-SGC IgG levels support the clinical relevance of anti-SGC IgG for spontaneous pain in FMS. Conversely, evoked pain might not be related to anti-SGC IgG, as suggested by our findings failing to show differences between FMS with high and low anti-SGC IgG levels, or an association of anti-SGC IgG with pressure pain thresholds, sensitivity to suprathreshold pressure pain, or pressure-evoked cerebral pain processing. Our results regarding the negative correlation between anti-SGC IgG levels and, specifically, the brain metabolites scylo-inositol and total choline merit further investigation, particularly as low levels of these metabolites in FMS correlated with more severe FM symptoms and higher pain intensity, respectively. Altogether, our study indicates that anti-SGC IgG levels are associated with the intensity of spontaneous pain and are related with brain metabolite concentrations, but not with the sensitivity to and cerebral processing of evoked pressure pain. In the future, analysis of anti-SGC IgG may become a valuable tool to subgroup FMS. This may open up new approaches for individualized treatment of FMS, including decreasing the IgG concentrations via, for example, injection of anti-FcRn antibodies or plasmapheresis, or performing pharmacological immunomodulation (e.g., abatacept or rituximab) in these patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.