CNS Imaging Characteristics in Fibromyalgia Patients With and Without Peripheral Nerve Involvement

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Research Article

Keywords: fibromyalgia syndrome (FMS), CNS, fibromyalgia patients, peripheral nerve involvement, skin innervation

Posted Date: August 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-812003/v1>

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Abstract

We tested the hypothesis that reduced skin innervation in fibromyalgia syndrome is associated with specific CNS changes. This prospective case-control study included 43 women diagnosed with fibromyalgia syndrome and 40 healthy controls. We further compared the fibromyalgia subgroups with reduced (n=21) and normal (n=22) skin innervation. Brains were analysed for cortical thickness, for white matter integrity, and for functional connectivity. Compared to controls, cortical thickness was decreased in the left pars opercularis in the fibromyalgia group as a whole, and decreased in the left superior frontal and left rostral middle frontal cortex in the fibromyalgia subgroup with reduced skin innervation. Diffusion tensor imaging revealed a significant increase in fractional anisotropy in the corona radiata, the corpus callosum, cingulum and fornix in patients with fibromyalgia compared to healthy controls. Using resting-state fMRI, the fibromyalgia group as a whole showed functional hypoconnectivity between the right midfrontal gyrus and the posterior cerebellum and the right crus cerebellum, respectively. The subgroup with reduced skin innervation showed hyperconnectivity between the right cuneal cortex and the anterior parahippocampal gyrus, between the left thalamus and the lateral parietal region in the default mode network and hypoconnectivity between the left frontal lobe and the right cerebellum crus 1. Our results suggest that the subgroup of fibromyalgia patients with pronounced pathology in the peripheral nervous system shows distinctly altered morphology, structural and functional connectivity also at the level of the brain. We propose considering these subgroups when conducting clinical trials.

1. Introduction

The fibromyalgia syndrome (FMS) is a chronic pain disorder with a prevalence of approximately 2% in the general population ¹. Abnormalities in pain processing regions in the CNS, neurotransmitter levels, the autonomic nervous system, and in small fibers of the peripheral nervous system are frequent findings associated with FMS, but their causal connection to the manifestation and course of its symptoms is still unclear. Altered pain processing at the level of the CNS is regarded as a major pathophysiological factor ^{2,3}. However, structural lesions and functional deficits were also observed at the level of the PNS, where specifically small fiber pathology is a robust finding in a substantial group of patients fulfilling the established diagnostic criteria of FMS ⁴. These findings of structural and functional alterations in FMS at both CNS and PNS level were reproducible: CNS structural measurements, like voxel-based-morphometry or cortical reconstruction, have revealed atrophy of the grey matter in the left prefrontal cortex and the posterior cingulate cortex ^{5,6}. Diffusion tensor imaging (DTI) has shown changes in white matter integrity, e.g. in the corpus callosum ⁷, and functional magnetic resonance imaging (fMRI) has identified hyperactivity in many regions related to pain processing ⁸, such as the left prefrontal cortex and in the posterior cingulate cortex, the insular cortex and the cerebellum. Functional connectivity was increased in the default mode network (DMN) and pain related areas, such as the insular cortex ⁹⁻¹¹. In the PNS, we and other groups described a decrease in intraepidermal nerve fiber density (IENFD) ¹²⁻¹⁷, which was related to symptom severity ⁴.

The relative importance of CNS and PNS abnormalities for FMS pathophysiology has been a matter of debate. A continuum between peripherally driven pain at one end and centrally driven pain at the other end has been suggested³. Whether CNS and PNS abnormalities coexist in the same patients, or whether CNS and PNS pathology define two non-overlapping subgroups in FMS has never been studied and presents a particular methodological challenge. For FMS, we addressed this challenge in the following manner: We established robust differences between two cohorts of FMS patients using objective and validated criteria of injury at the PNS level (FMS with markedly reduced IENFD vs. FMS with normal IENFD). We hypothesized that structural or functional remodeling of the brain would occur differentially in these two subgroups on a global or regional level. We tested this hypothesis in these two FMS subgroups versus case matched healthy controls using MRI methods to measure brain morphometry, structural and functional connectivity

2. Materials And Methods

2.1 Subjects

Forty-three female patients with FMS were recruited at the Department of Neurology, University Hospital Würzburg, who also had taken part in a previous study investigating small fiber pathology in FMS⁴. Forty healthy female age and sex matched controls were recruited via public announcements. All patients had been diagnosed with FMS and examined by a rheumatologist and a neurologist, fulfilled the diagnostic criteria for FMS according to the guidelines released by the American College of Rheumatology¹⁸, and had been comprehensively examined in our hospital for possible differential diagnoses (see Evdokimov et al. 2019). Specifically, patients must have had widespread pain for more than three months that could not be explained by other diseases, have a Widespread Pain Index (WPI) ≥ 7 and the Symptom Severity Score ≥ 5 ¹⁸.

All patients were off their pain medication for 3 days before the examination. None of the patients and controls had been taking anticonvulsants, antihistamines, muscle relaxants or benzodiazepines within the 4 weeks before the examination. All participants in the study gave their written informed consent according to Declaration of Helsinki. The study was approved by the Ethics Committee of the University of Würzburg Medical Faculty (63/18). The exclusion criteria for patients and controls were other current autoimmune or inflammatory diseases that can cause pain, such as rheumatoid arthritis, systemic lupus erythematosus, or chronic inflammatory bowel disease, as well as neurological, cardiovascular, psychiatric diseases, such as major depression, in the past and at present, any contraindication for MRI like cardiac pacemakers, cochlear implants, vascular stents or metal splinters in the body, a history of drug abuse, a history of head trauma requiring medical attention or brains with significant structural abnormalities.

2.2 Subgrouping according to intraepidermal nerve fiber density

Patients from the previous study ⁴ who had either normal IENFD at the lower leg (above the lower limit of normal 5.4 fibers/mm) and at the upper thigh (above the lower limit of normal 8.5 fibers/mm) or a non-length dependent abnormal IENFD, which means the IENFD was below the lower limits at both biopsy sites, were re-recruited, i.e. were contacted by H.-C. A. and invited to a follow-up appointment for MRI imaging. The first group was termed “noPNS”, the second group “PNS”. These cut-off values were determined based on skin biopsies of these two regions of 120 healthy women (median age = 50 years, range = 20–84 years) in our department. The cut-off values represent the lower limit of the standard deviation of the IENFD results of all the healthy controls investigated in our laboratory.

2.3 Fibromyalgia related symptoms

Results of the questionnaire and clinical examination data of the FMS patients have already been published ⁴. To evaluate pain severity, two pain scores were used (Graded Chronic Pain Scale (GCPS) and Neuropathic Pain Symptom Inventory (NPSI)). In order to assess the depressiveness of the patients, the “Allgemeine Depressionskala” (ADS) was used, which is a German version of the Center for Epidemiological Studies – Depression scale questionnaire ¹⁹. To evaluate catastrophizing, the Pain Catastrophizing Scale (PCS) ²⁰, which is a self-report measure, consisting of 13 items scored from 0 to 4, resulting in a total possible score of 52, was assessed. To test the anxiety level, the State-Trait Anxiety Inventory (STAI) was used ²¹, which is a commonly used measure of trait and state anxiety. In order to assess the influence of the disease on daily experience, the Fibromyalgia Impact Questionnaire (FIQ) ²² was used. Also, the Symptom Severity Scale (SSS) was used to query other FMS-associated symptoms ¹⁸. It measures three key symptoms during the past week: Fatigue, unrefreshed waking and cognitive impairment. The O’Leary-Sant Symptom and Problem Index assesses the impairment by bladder dysfunction ²³ and was selected, as FMS patients frequently report abdominal pain and problems with urination. Data collected in the context of the clinical diagnostics, such as the conduction studies of the sural nerve and the blood values, for example HbA1c and vitamin D, were also analyzed.

2.4 MR imaging and analysis

2.4.1 Data acquisition

Magnetic resonance imaging was performed on a Siemens MAGNETOM Prisma fit Scanner (Siemens Healthcare GmbH, Erlangen, Germany), operating at 3T, equipped with a 64-channel head coil at the Department of Neuroradiology, University Hospital Würzburg. For each participant we included a structural T1-weighted (T1w) sequence, diffusion weighted imaging (DWI), fieldmap data and resting-state functional MRI (rs-fMRI) series. The T1w gradient echo MPRAGE sequence (repetition time (TR) 2400 ms, echo time (TE) 3.17 ms, flip angle (FA) 8°, inversion recovery (IR) 1000 ms) contained 176 sagittal slices with an isotropic voxel size of 1 x 1 x 1 mm. The visual examination of the T1w-structural images revealed no gross morphological abnormalities for any patient or subject. DWI was obtained using multiband echo-planar imaging (EPI) with the following parameters: TR = 3100 ms, TE = 89 ms, FA = 90°, isotropic voxel size of 2 x 2 x 2 mm. Diffusion data were collected with reversed phase-encode

blips, resulting in pairs of b0-images with distortions in opposite directions for further susceptibility induced distortion correction. Resting state fMRI data was acquired using a T2*-weighted multiband EPI sequence with TR = 1610 ms, TE = 30 ms, FA = 70°, isotropic voxel size of 2 x 2 x 2 mm, 69 slices. During the 9-minute resting state fMRI acquisition period with 300 volumes the subjects were told to lie still and remain awake with their eyes open. Participants' motion was minimized using tight foam pads around the head, their physiology was monitored.

2.4.2 Structural analysis

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite v6.0.0 (Martinos Center for Biomedical Imaging, Boston, MA, USA) using the 3D T1w data. The technical details of these procedures are described in prior publications^{24,25}. Surface-based group analyses for mean differences in cortical thickness were performed for each hemisphere. Since the factor age has been shown to be associated with differences in white and grey matter volume²⁶, we decided to include this factor as a covariate. For the design of the statistical model needed for the group comparison we used a General Linear Model. Further, a Monte Carlo simulation was applied at a cluster threshold of $p < 0.05$ and vertex-wise threshold of $P < 0.001$ to control for multiple comparisons. Cluster annotation was classified according to the Desikan-Killiany Atlas²⁷.

2.4.3 Structural connectivity: Diffusion Tensor Imaging

The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain software library (FSL, Oxford, UK, www.fmrib.ox.ac.uk)²⁸ was used for DTI data analysis and preprocessing. Our diffusion data, recorded in reversed phase-encode blips, were preprocessed using the FSL tools "topup"²⁹, "eddy (correction)", "BET"³⁰, and "FNIRT". Voxelwise statistical analysis with cluster-based thresholding of the fractional anisotropy (FA) data was carried out using TBSS (Tract-Based Spatial Statistics,³¹). FA images and eigenvalue images were created by fitting a tensor model to the preprocessed diffusion data using the FSL FDT toolbox (Functional MRI of the Brain Diffusion Toolbox, DTIFIT). For ROI specific evaluation of the FA data we created a mask with the ICBM-DTI-81 white-matter labels atlas (Laboratory of Brain Anatomical MRI, Johns Hopkins University³²) in the same space and calculated the average FA value of all voxels in 48 ROIs. These data were analyzed for group comparisons with t-tests (Bonferroni-corrected significance level of 0.001, two-tailed, confidence interval 0.95) and correlated with clinical data and questionnaires using a spearman Rho correlation for non-normally-distributed z-standardized clinical data analysis (significance level of 0.01, two-tailed, confidence interval 0.95) with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA).

2.4.4 Functional connectivity: resting state BOLD fMRI

Resting state functional data were spatially preprocessed using SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, United Kingdom; <http://www.fil.ion.ucl.ac.uk/spm/>) and the CONN Toolbox v18 (www.nitrc.org/projects/conn, RRID:SCR_009550,³³) running in Matlab R2019a (The Mathworks Inc, USA). The reason for changing from FSL to the Conn Toolbox run in SPM was the

extensive ROI to ROI analysis provided by this toolbox. Functional data were realigned, slice-time corrected, spatially normalized to the Montreal Neurological Institute (MNI) space, and spatially smoothed with a FWHM Gaussian kernel of 8 mm. We collected fieldmaps and undistorted the EPI images using the Fieldmap Toolbox (SPM). Motion parameters from realignment were evaluated, and a motion artefact threshold (translation > 3 mm, rotation > 1°) was employed for exclusion). No participants displayed gross movements to require exclusion. To remove blood-oxygen-level-dependent (BOLD) signal from the cerebral white matter and ventricles, each participant's T1-weighted MPRAGE image was automatically segmented into grey matter, white matter, and cerebrospinal fluid and normalized to MNI space using the Computational Anatomy Toolbox (CAT12; <http://www.neuro.uni-jena.de/cat/>) running in SPM12. BOLD data were bandpass filtered (0.008–0.09 Hz) to reduce low-frequency drift and noise effects. We then generated seed-to-seed connectivity maps for each individual using seeds that are known to be involved in pain processing (i.e. thalamus, all default mode network seeds or cerebellum)³⁴. These seeds are provided in the CONN software³⁵. Individual correlation maps were generated. These results were subsequently used for second-level analysis of relative functional connectivity using a two-sided independent t test, implemented in the CONN toolbox, to investigate differences in seed-to-seed connectivity between groups. We applied a seed-to-seed analysis to investigate which brain areas show hyper- or hypoconnectivity between patients and controls and between subgroups. We also checked the influence of depression on the functional networks. Participant motion parameters were included as within-subject first-level covariates. To determine between-subject effects in resting-state functional networks, group association (patients and controls or PNS and noPNS), age and ADS (depression) scores were included as second-level covariates. We performed voxel-wise statistical analysis using an uncorrected level ($p < 0.001$) before a false discovery rate (FDR) correction was applied at the cluster level ($p < 0.05$).

2.5 Statistical analysis

Statistics were performed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp. Armonk, NY, USA). The data were tested for normal distribution with a Shapiro-Wilk test and then, depending on the result, examined for group differences with a two-tailed t-test or a Mann-Whitney-U test. Data are given as mean $\pm$ SD or median/range unless otherwise specified. The correlation analyses were performed with a Pearson correlation, 1000 samples of bootstrapping and a significance level of 0.01. Since the analysis of FA data contained 6 comparisons, the significance level was Bonferroni corrected to 0.001. Otherwise, the confidence interval was 95 % and the significance level at 0.05. We used the Levene test with a significance threshold of 0.05 to check the data for equivalence of variance. Voxel-wise statistics were performed within the respective neuroimaging programs and corrected with a family-wise error rate or false discovery rate correction method to reduce the probability of an alpha-error.

2.6 Data availability

The raw, skull stripped, data used to analyze the following results can be obtained upon request from the corresponding author. The processing and statistical analysis of the data was done using established

neuroimaging software, as described in the methods. The STROBE Statement-Checklist was used for the quality control of our case-control study.

3. Results

3.1 Patient population

The patient group (n = 43, mean age 53.5 ± 6.5 years, mean BMI 28.2 ± 5.0) and the healthy control group (n = 40, mean age 52.5 ± 6.7 years, mean BMI 26.6 ± 5.0) did not differ in age and BMI. The subgroups noPNS (normal IENFD) and PNS (decreased IENFD) differed in BMI, with a higher BMI in the PNS subgroup (Table 1).

Table 1
a: Clinical data compared between patients and controls

	Patients all (n = 43)	Controls (n = 40)	p-value
	Mean ± SD	Mean ± SD	
Age	53.5 ± 6.5	52.6 ± 6.7	< 0.51
BMI	28.2 ± 5	26.6 ± 5	< 0.14

Table 1
b: Clinical and questionnaire data compared between subgroups

	PNS (n = 21)	noPNS (n = 22)	p-value
	Mean $\pm$ SD / Median (Range)	Mean $\pm$ SD / Median (Range)	
Age	53.5 $\pm$ 6.7	53.4 $\pm$ 6.5	< 0.9
BMI	30.9 $\pm$ 4.2	25.5 $\pm$ 4.2	< 0.001
IENFD lower leg (fibers/mm)	3.9 $\pm$ 1.5	10 $\pm$ 2.6	< 0.001
IENFD upper thigh (fibers/mm)	5.7 $\pm$ 1.5	11.5 $\pm$ 2.8	< 0.001
Time since diagnosis (years)*	5 (1–19)	5 (0–14)	< 0.51
Duration of pain due to the disease (years)	16.8 $\pm$ 10.8	18.8 $\pm$ 12.7	< 0.71
Number of tender points*	14 (11–18)	15 (7–18)	< 0.23
WPI*	13.0 (10–19)	15 (8–18)	< 0.82
SSS*	7 (6–10)	7 (5–11)	< 0.87
HbA1c (%)	5.4 $\pm$ 0.3	5.3 $\pm$ 0.2	< 0.16
Sural nerve SNAP (μ V)	22.6 $\pm$ 7.8	25.1 $\pm$ 12.5	< 0.45
Sural nerve conduction velocity (m/s)	48.3 $\pm$ 4.05	50.4 $\pm$ 3.5	< 0.09
Serum Vitamin D (μ g/l)	30.1 $\pm$ 14.1	30.1 $\pm$ 11.1	< 0.99
Highest education level **	3 (2–5)	3 (2–5)	< 0.13
ADS: Allgemeine Depressionskala, BMI: Body Mass Index, FIQ: Fibromyalgia Impact Questionnaire, GCPS: Graded Chronic Pain Scale, IENFD: Intraepidermal nerve fibre density, NPSI: Neuropathic Pain Symptom Inventory, SNAP: Sensory nerve action potential, SSS: Symptom Severity Score, STAI: State-Trait Anxiety Inventory, WPI: Widespread Pain Index			
*These data are not normally distributed, therefore the median and the range are shown here and a Mann-Whitney U test was applied.			
** (1: Elementary school, 2: Primary school, 3: Secondary school, 4: High school, 5: University)			

	PNS (n = 21)	noPNS (n = 22)	p-value
	Mean $\pm$ SD / Median (Range)	Mean $\pm$ SD / Median (Range)	
NPSI sum score	31.1 $\pm$ 4.2	25.5 $\pm$ 4.2	< 0.09
GCPS pain intensity	73.6 $\pm$ 10.8	64 $\pm$ 15.1	< 0.02
GCPS disability due to pain*	66.7 (10-83.3)	53.3 (16.6–86.6)	< 0.09
Pain Catastrophizing Scale	26.7 $\pm$ 10.1	20.7 $\pm$ 10.3	< 0.06
ADS	27.8 $\pm$ 11.8	21.2 $\pm$ 11.4	< 0.07
FIQ	51.9 $\pm$ 12	42.3 $\pm$ 13.2	< 0.01
The O’Leary-Sant Symptom Index and Problem Index*	12 (0–33)	9 (1–22)	< 0.28
STAI	47.1 $\pm$ 11.6	44.3 $\pm$ 13.3	< 0.48
ADS: Allgemeine Depressionskala, BMI: Body Mass Index, FIQ: Fibromyalgia Impact Questionnaire, GCPS: Graded Chronic Pain Scale, IENFD: Intraepidermal nerve fibre density, NPSI: Neuropathic Pain Symptom Inventory, SNAP: Sensory nerve action potential, SSS: Symptom Severity Score, STAI: State-Trait Anxiety Inventory, WPI: Widespread Pain Index			
*These data are not normally distributed, therefore the median and the range are shown here and a Mann-Whitney U test was applied.			
** (1: Elementary school, 2: Primary school, 3: Secondary school, 4: High school, 5: University)			

3.2 Clinical data and questionnaires

We included patients with normal skin innervation and patients with reduced IENFD both at the lower leg and the upper thigh from the cohort described in ⁴. In patients with reduced distal and proximal IENFD (PNS group), FMS symptoms were more severe ($p = 0.02$) and quality of life was lower compared to FMS patients with normal distal and proximal IENFD ($p = 0.01$) as reflected by the values of the GCPS pain intensity and the FIQ questionnaire (Table 1).

3.3 Structural analysis

With the values of the cortical thickness calculated by Freesurfer, we performed four group comparisons: Patients ($n = 43$) versus controls ($n = 40$), PNS ($n = 21$) versus noPNS ($n = 22$), PNS versus controls, and noPNS versus controls. The comparison between the FMS patients and controls showed one significant

cluster, with a lower cortical thickness in the patient group in the left pars opercularis of the inferior frontal gyrus with a cluster wise probability (CWP) of 0.032 (Fig. 1a). The comparison between the PNS and control groups (FMS-PNS versus healthy controls) showed two clusters with a lower cortical thickness of PNS patients in the left superior frontal (CWP = 0.036) and in the left rostral middle frontal (CWP = 0.048) cortex (Fig. 1b). These two group comparisons showed overlapping areas with decreased cortex thickness in the left pars opercularis. All clusters were located in the left hemisphere (Table 2). The direct comparison between the FMS-PNS and FMS-noPNS subgroups revealed no differences after correction for multiple comparisons. The comparison between the noPNS and the control group revealed no clusters. Our correlation analysis, which we restricted a-priori to the left prefrontal cortex based on the cluster results, showed that the severity of classic FMS symptoms, as reported in the FIQ, of depression and of pain catastrophizing, correlated negatively with cortical thickness in the same regions that were significant in our cluster analysis, namely the left lateral and medial orbitofrontal cortex (Table 3).

Table 2

Results of cortical thickness analysis after Monte-Carlo simulation

	Cortex area	MNI (x,y,z)	CWP	CWP-Low	CWP-High
Patients vs. controls	Pars opercularis	-47.3, 15.0, 8.2	0.032	0.029	0.036
	Superior frontal	-7.7, 58.1, 18.2	0.137	0.131	0.143
PNS vs. controls	Superior frontal	-7.8, 57.1, 19.7	0.036	0.033	0.038
	Rostral middle frontal	-38.8, 44.4, 4.1	0.048	0.045	0.051
CWP = Cluster wise probability of p-value; MNI = Montreal Neurological Institute space coordinates					
CWP-Low/High = 90% confidence interval for CWP					

Table 3
Correlations ($p \leq 0.01$) between grey matter thickness in the left frontal lobe and symptom questionnaires (Bootstrapping ns = 1000)

	Region of interest	r-value
Pain Catastrophizing Scale	Left frontal pole	-0.45
ADS	Left lateral orbitofrontal cortex	-0.42
	Left frontal pole	-0.53
FIQ	Left lateral orbitofrontal cortex	-0.48
	Left medial orbitofrontal cortex	-0.49
The O'Leary-Sant Symptom Index	Left pars orbitalis	0.41
ADS: Allgemeine Depressionskala, FIQ: Fibromyalgia Impact Questionnaire		

3.4 Diffusion tensor imaging

In the ROI-based analysis comparing patients and controls, a significant increase in FA was found in 6 out of 48 ROIs in FMS patients. This was evident in corticospinal pathways such as the corona radiata, but also in regions of the limbic systems such as the fornix and cingulum. A detailed list of these regions with the respective FA values can be found in the Supplementary table 1. The voxel-based analysis, corrected for multiple comparisons, revealed the same clusters (Fig. 2). The ROI-based comparison of the two subgroups PNS and noPNS showed no differences in FA after setting the accepted significance level to less than 0.001 for Bonferroni correction.

The Spearman correlation analysis, a-priori restricted to the 6 regions that revealed differences in the group comparison, showed that these areas were affected to different degrees depending on the severity of different clinical parameters (Table 4). As patient-related parameters for the correlation analysis, we included the z-scores of the NPSI sum score, ADS, FIQ, STAI, time since diagnosis, duration of pain due to the disease, age, BMI, and the IENFD at both biopsy sites. A positive correlation, i.e. a positive r-value, means that a higher value in the patient-related parameter was associated with a higher FA value in the respective ROI. With a negative correlation, higher scores indicate a decrease in the FA value. The negative correlation of the anxiety questionnaire with the FA of several regions such as the corona radiata was most prominent.

Table 4

Correlations ($p \leq 0.001$, Bootstrapping $ns = 1000$) between white matter integrity, measured by fractional anisotropy (FA), and clinical parameters

	Region of interest	r-value
NPSI	Fornix	0.43
STAI	Right post. corona radiata	-0.41
	Left post. corona radiata	-0.48
	Fornix	-0.42
Age	Cingulum	0.48
NPSI: Neuropathic Pain Symptom Inventory, STAI: State-Trait Anxiety Inventory		

3.5 Functional resting state imaging

Seed-to-seed analysis between patients and controls showed significant hypoconnectivity of the right midfrontal gyrus to the posterior cerebellum ($p\text{-FDR} = 0.048$) and to the right crus cerebelli 1 ($p\text{-FDR} = 0.048$) in FMS patients. Seed-to-seed analysis between the subgroups noPNS and PNS (Fig. 3) showed hyperconnectivity between the right cuneal cortex and the anterior parahippocampal gyrus ($p\text{-FDR} = 0.017$) and between the left thalamus and the lateral parietal region of the default mode network ($p\text{-FDR} = 0.036$) in the PNS subgroup (Fig. 3). Hypoconnectivity was observed between the left frontal lobe and the right cerebellum crus 1 ($p\text{-FDR} = 0.025$) in the PNS subgroup compared to the noPNS group. Parallels to our structural results can be seen here, as a decrease of the cortex thickness of the left frontal lobe can also be seen in the PNS group compared to controls. These subgroup results of the functional connectivity analysis are robust in the sense that a direct comparison of the FMS-PNS group with the healthy controls showed significantly more pathological connectivity than the direct comparison of the FMS-noPNS group with the healthy controls or the direct comparison between the subgroups (Fig. 3).

The degree of depression affected connections of two regions in the posterior lobe: The functional connectivity of the left occipital fusiform gyrus to the bilateral cuneal cortices ($p\text{-FDR} = 0.044$), as well as the connectivity of the right occipital lobe to the right insular cortex ($p\text{-FDR} = 0.011$) and to the left insular cortex ($p\text{-FDR} = 0.042$), parts of the salience network, was enhanced with increasing ADS values in FMS patients.

4. Discussion

In this study, two group comparisons were conducted using structural, DWI and functional MRI data: Firstly, we compared FMS patients to healthy controls, secondly, we divided the FMS group into two subgroups with and without PNS pathology (PNS and noPNS groups) and compared these subgroups

with each other. In the structural analysis, we separately compared the two subgroups with the healthy control group.

We show that in FMS 1) cortical thickness is decreased in the left pars opercularis of the inferior frontal gyrus, 2) FA is generally increased in corticospinal tracts and regions of the limbic system and 3) functional connectivity is reduced between the right midfrontal gyrus and the posterior cerebellum as well as the right crus cerebelli.

Importantly, we demonstrate that structural and functional brain changes are more prominent in those patients with objective evidence of small fiber impairment. 1) A detailed subgroup analysis of cortical thickness revealed two clusters with reduced thickness in the left frontal cortex in the PNS group compared to the control group, but no differences in the subgroup of patients without reduced skin innervation (noPNS subgroup). 2) The PNS subgroup, compared to the noPNS subgroup, showed hypoconnectivities between several brain regions that process and evaluate sensory information.

4.1 Comparison of the present findings with published data

Our cortical thickness data are in line with the results of a recent meta-analysis on voxel-based morphometry studies, which compared FMS patients to healthy controls, regarding the left rostral middle frontal cortex⁵, and it has also been shown that the left superior frontal cortex of FMS patients decreases in thickness with increasing duration of the disease³⁶. The literature concerning the left pars opercularis in FMS is not congruent. In our study, this part of the inferior frontal gyrus shows a decrease in cortical thickness in the FMS group compared to controls. One previous study showed a correlation of pars opercularis cortical volume loss with duration of disease³⁶, and a SPECT study showed symptom improvement and stronger activation of the pars opercularis in FMS patients after therapy with hyperbaric oxygen³⁷.

Other studies that have evaluated the relationship between clinical parameters and structural measurements of individual pain processing brain regions showed correlations in areas of the prefrontal cortex, the anterior cingulum or the cerebellum^{38,39}. In our study the severity of symptoms, such as disability caused by pain or depressiveness, also correlated negatively with the cortex thickness of regions in the left prefrontal cortex (left medial/lateral orbitofrontal cortex and frontal pole). However, these regions of the correlation analysis do not exactly correspond to the clusters where decreased volume was found in the group comparisons (pars opercularis and the left rostral middle/ superior frontal cortices). Thus, because the cortex areas correlating with depression or pain are not the same as those with reduced cortex thickness in our group comparisons, the hypothesis that the CNS changes are a consequence of symptom severity is not supported by this finding. The prefrontal cortex is a known site of pain modulation. Indeed, a dual role has been described including antinociceptive effects by modulating sensory afferent influx, as well as the furthering of chronic pain via corticostriatal projections. Interestingly, decline of prefrontal cortex volume in chronic pain can be reversed with successful

biopsychosocial therapy, be it cognitive behavioral therapy, exercise or transcranial magnetic stimulation
40.

Regarding FA, a marker for the integrity of the white matter, we found an increase in FA in the corona radiata and regions of the limbic system (e.g. fornix and cingulate cortex) in the FMS group compared to controls. Some DTI studies with a smaller number of FMS patients found, consistent with our outcomes, increased FA in right and left ACC³⁹ or in the anterior thalamic radiation, anterior limb of internal capsule up to the putamen⁴¹. Other studies found a decrease in FA in FMS, especially in the corpus callosum⁷. It is assumed that the increase in FA is related to an increased number of oligodendrocytes, the myelin-producing cells of the CNS^{42,43}. There is evidence that these play a role in the modulation of pain mechanisms by producing and modulating chemokines and cytokines and interacting with microglia^{44,45}. A PET study in FMS patients found increased microglial activity in the medial and lateral areas of the temporal and frontal lobes overlapping with our areas of FA increase⁴⁶. Our results might therefore indicate an inflammatory process in the white matter in FMS patients, particularly in those without peripheral nerve pathology. Further studies combining FA, PET, and possibly CSF measures of inflammation are needed to confirm this. It has also been shown specifically in FMS patients that white matter pathways, whose FA increases after a prolonged period of increased activity⁴⁷, in this case in pain processing regions, decrease again after pain chronification and show lower values than healthy controls
41.

Regarding the functional connectivity, the posterior cingulate cortex, as the central hub of the DMN, has been identified in several FMS studies as the area for processing pain catastrophizing^{48,49}. Thalamic hyperconnectivity to parts of the DMN, e.g. the posterior cingulate cortex or the precuneus cortex, was shown after application of acute pain stimuli in FMS patients⁵⁰. It has been hypothesized that these enhanced connections are associated with increased activity of the hippocampus, which results in increased alertness and anxiety of patients in response to pain⁵¹. Other preliminary work on connectivity analysis also finds changes in structural connectivity analysis (DTI) in regions such as the parahippocampal gyrus or the supramarginal gyrus, which showed changes in our fMRI connectivity analysis⁵². Similar findings to those in our present study have been published using other methods to quantify connectivity measurements, such as electroencephalography and magnetoencephalography^{53,54}. In our subgroup comparison, we showed greater differences in functional connectivity between PNS and noPNS patients than between patients and controls, however, the question of cause and effect cannot be answered by our data. Intriguingly, a recent study, using DTI, found reduced connectivity between the thalamus and pain-related areas in patients with reduced skin innervation due to small fiber neuropathy⁵⁵, in contrast to the hyperconnectivity found in our PNS subgroup. Thus, the relation of small fiber pathology and CNS connectivity seems to be disease specific.

4.2 Are the findings specific for FMS?

Some of the findings we report here have also been observed in other chronic pain disorders⁵⁶. Changes of connectivity in the DMN were also found in patients with chronic back pain, complex regional pain syndrome and knee osteoarthritis⁵⁷. It has already been suggested that a lower activity of the prefrontal cortex, a well-known pain modulation area, could lead to a failure in the elimination of subcortically driven fear behaviors, thereby resulting in pain chronification⁵⁸. It is currently unclear whether these processes are adaptive, maladaptive or cause some of the symptoms. In order to better understand the pathophysiology of FMS, it is therefore important to first understand the role of brain neuroplasticity in chronic pain, as a brain signature of pain appears to be found across various pain syndromes⁵⁹.

4.3 Limitations of our study

Our study has some limitations. Because the PNS subgroup with damage to the small nerve fibers also had more severe neuropathic pain and fibromyalgia-specific disease symptoms compared with the noPNS subgroup, it is not clearly distinguishable whether the subgroup effects were driven by disease severity or neuropathic changes in the small nerve fibers.

The healthy controls in our study did not receive a skin biopsy, so we cannot rule out that some persons with reduced IENFD might have been in this group. However, in our previous study⁴, only 2% of normal controls had reduced IENFD at the lower and upper leg, so that it is highly unlikely that a large number of our present controls would have had this finding.

5. Conclusions

Our FMS subgroup with small fiber pathology showed more extensive CNS pathology than the subgroup without. These findings have several implications for the investigation of the pathophysiology of FMS and also for the diagnosis of FMS subgroups. While most clinical trials on FMS therapy included only patients diagnosed according to current diagnostic criteria, one has to consider that FMS is a heterogeneous condition with potentially different underlying pathophysiological processes within subgroups. These subgroups might respond differentially to specific treatments. One hypothesis would be that lesions of small nerve fibers lead to sensitization in the CNS with consecutive changes in its structure and function. Psychiatric comorbidities, such as depression and anxiety, also affect the brain structure in FMS and thus influence the overall picture in MRI imaging. We therefore advocate that future studies should take into account the different subgroups of patients both on the basis of small nerve fiber pathology, symptom severity, and psychiatric comorbidities.

6. Declarations

Acknowledgements

The authors thank Professor Vania Apkarian, Chicago, USA, for encouragement to engage in this project and for valuable discussion in its planning phase. The authors would like to thank the medical staff of

the Outpatient Clinic of the Neurological University Hospital Würzburg, who actively helped with the clinical examinations of the patients.

This work was supported by a grant from the Interdisciplinary Clinical Research Center of the University Hospital Würzburg (FN376) to MP and CS, and by the “Evangelisches Studienwerk Villigst” (German Federal Ministry of Education and Research, stipend to HCA). The study was in part funded by Else Kröner-Fresenius-Stiftung (EKFS, N.Ü.: 2014_A129).

The authors report no conflict of interest.

7. References

1. Heidari, F., Afshari, M. & Moosazadeh, M. Prevalence of fibromyalgia in general population and patients, a systematic review and meta-analysis. *Rheumatol Int* **37**, 1527-1539, doi:10.1007/s00296-017-3725-2 (2017).
2. Staud, R. Brain imaging in fibromyalgia syndrome. *Clin Exp Rheumatol* **29**, S109-117 (2011).
3. Sluka, K. A. & Clauw, D. J. Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience* **338**, 114-129, doi:10.1016/j.neuroscience.2016.06.006 (2016).
4. Evdokimov, D. *et al.* Reduction of skin innervation is associated with a severe fibromyalgia phenotype. *Ann Neurol* **86**, 504-516, doi:10.1002/ana.25565 (2019).
5. Lin, C., Lee, S. H. & Weng, H. H. Gray Matter Atrophy within the Default Mode Network of Fibromyalgia: A Meta-Analysis of Voxel-Based Morphometry Studies. *Biomed Res Int* **2016**, 7296125, doi:10.1155/2016/7296125 (2016).
6. Diaz-Piedra, C., Guzman, M. A., Buena-Casal, G. & Catena, A. The impact of fibromyalgia symptoms on brain morphometry. *Brain Imaging Behav* **10**, 1184-1197, doi:10.1007/s11682-015-9485-2 (2016).
7. Kim, D. J. *et al.* Altered white matter integrity in the corpus callosum in fibromyalgia patients identified by tract-based spatial statistical analysis. *Arthritis Rheumatol* **66**, 3190-3199, doi:10.1002/art.38771 (2014).
8. Sawaddiruk, P., Paiboonworachit, S., Chattipakorn, N. & Chattipakorn, S. C. Alterations of brain activity in fibromyalgia patients. *J Clin Neurosci* **38**, 13-22, doi:10.1016/j.jocn.2016.12.014 (2017).
9. Schmidt-Wilcke, T. & Diers, M. New Insights into the Pathophysiology and Treatment of Fibromyalgia. *Biomedicines* **5**, doi:10.3390/biomedicines5020022 (2017).
10. Fallon, N., Chiu, Y., Nurmikko, T. & Stancak, A. Functional connectivity with the default mode network is altered in fibromyalgia patients. *PloS one* **11**, e0159198, doi:10.1371/journal.pone.0159198 (2016).
11. Napadow, V. *et al.* Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum* **62**, 2545-2555, doi:10.1002/art.27497 (2010).
12. Üçeyler, N. *et al.* Small fibre pathology in patients with fibromyalgia syndrome. *Brain* **136**, 1857-1867, doi:10.1093/brain/awt053 (2013).

13. Caro, X. J. & Winter, E. F. Evidence of abnormal epidermal nerve fiber density in fibromyalgia: clinical and immunologic implications. *Arthritis Rheumatol* **66**, 1945-1954, doi:10.1002/art.38662 (2014).
14. Kosmidis, M. L. *et al.* Reduction of Intraepidermal Nerve Fiber Density (IENFD) in the skin biopsies of patients with fibromyalgia: a controlled study. *J Neurol Sci* **347**, 143-147, doi:10.1016/j.jns.2014.09.035 (2014).
15. Giannoccaro, M. P., Donadio, V., Incensi, A., Avoni, P. & Liguori, R. Small nerve fiber involvement in patients referred for fibromyalgia. *Muscle Nerve* **49**, 757-759, doi:10.1002/mus.24156 (2014).
16. Oaklander, A. L., Herzog, Z. D., Downs, H. M. & Klein, M. M. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain* **154**, 2310-2316, doi:10.1016/j.pain.2013.06.001 (2013).
17. de Tommaso, M. *et al.* Update on laser-evoked potential findings in fibromyalgia patients in light of clinical and skin biopsy features. *J Neurol* **261**, 461-472, doi:10.1007/s00415-013-7211-9 (2014).
18. Wolfe, F. *et al.* The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res (Hoboken)* **62**, 600-610, doi:10.1002/acr.20140 (2010).
19. Radloff, L. S. A Self-Report Depression Scale for Research in the General Population. *Applied Psychological Measurement* **1**(3), 385-401 (1977).
20. Sullivan, M. B., S; Pivik, J The Pain Catastrophizing Scale: development and validation. *Psychol Assess* **7**, 524-532 (1995).
21. Spielberger, C., Gorsuch, RL, Lushene, R, Vagg, PR, Jacobs, GA. Manual for the State-Trait Anxiety Inventory. *Consulting Psychologists Press* (1983).
22. Burckhardt, C. S., Clark, S. R. & Bennett, R. M. The fibromyalgia impact questionnaire: development and validation. *J Rheumatol* **18**, 728-733 (1991).
23. Lubeck, D. P., Whitmore, K., Sant, G. R., Alvarez-Horine, S. & Lai, C. Psychometric validation of the O'leary-Sant interstitial cystitis symptom index in a clinical trial of pentosan polysulfate sodium. *Urology* **57**, 62-66, doi:10.1016/s0090-4295(01)01126-8 (2001).
24. Dale, A. M., Fischl, B. & Sereno, M. I. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* **9**, 179-194, doi:10.1006/nimg.1998.0395 (1999).
25. Fischl, B. & Dale, A. M. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A* **97**, 11050-11055, doi:10.1073/pnas.200033797 (2000).
26. Farokhian, F., Yang, C., Beheshti, I., Matsuda, H. & Wu, S. Age-related gray and white matter changes in normal adult brains. *Aging Dis* **8**, 899-909, doi:10.14336/AD.2017.0502 (2017).
27. Desikan, R. S. *et al.* An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* **31**, 968-980, doi:10.1016/j.neuroimage.2006.01.021 (2006).
28. Smith, S. M. *et al.* Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* **23 Suppl 1**, S208-219, doi:10.1016/j.neuroimage.2004.07.051 (2004).

29. Andersson, J. L., Skare, S. & Ashburner, J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. *Neuroimage* **20**, 870-888, doi:10.1016/S1053-8119(03)00336-7 (2003).
30. Smith, S. M. Fast robust automated brain extraction. *Human brain mapping* **17**, 143-155, doi:10.1002/hbm.10062 (2002).
31. Smith, S. M. *et al.* Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* **31**, 1487-1505, doi:10.1016/j.neuroimage.2006.02.024 (2006).
32. Hua, K. *et al.* Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. *Neuroimage* **39**, 336-347, doi:10.1016/j.neuroimage.2007.07.053 (2008).
33. Whitfield-Gabrieli, S. & Nieto-Castanon, A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* **2**, 125-141, doi:10.1089/brain.2012.0073 (2012).
34. Apkarian, A. V., Hashmi, J. A. & Baliki, M. N. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. *Pain* **152**, S49-64, doi:10.1016/j.pain.2010.11.010 (2011).
35. Whitfield-Gabrieli, S. *et al.* Associations and dissociations between default and self-reference networks in the human brain. *Neuroimage* **55**, 225-232, doi:10.1016/j.neuroimage.2010.11.048 (2011).
36. Jensen, K. B. *et al.* Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis Rheum* **65**, 3293-3303, doi:10.1002/art.38170 (2013).
37. Hadanny, A. *et al.* Hyperbaric oxygen therapy can induce neuroplasticity and significant clinical improvement in patients suffering from fibromyalgia with a history of childhood sexual abuse-randomized controlled trial. *Front Psychol* **9**, 2495, doi:10.3389/fpsyg.2018.02495 (2018).
38. Luerding, R., Weigand, T., Bogdahn, U. & Schmidt-Wilcke, T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. *Brain* **131**, 3222-3231, doi:10.1093/brain/awn229 (2008).
39. Lutz, J. *et al.* White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis Rheum* **58**, 3960-3969, doi:10.1002/art.24070 (2008).
40. Ong, W. Y., Stohler, C. S. & Herr, D. R. Role of the prefrontal cortex in pain processing. *Mol Neurobiol* **56**, 1137-1166, doi:10.1007/s12035-018-1130-9 (2019).
41. Ceko, M., Bushnell, M. C., Fitzcharles, M. A. & Schweinhardt, P. Fibromyalgia interacts with age to change the brain. *Neuroimage Clin* **3**, 249-260, doi:10.1016/j.nicl.2013.08.015 (2013).
42. Mandl, R. C. *et al.* Functional diffusion tensor imaging: measuring task-related fractional anisotropy changes in the human brain along white matter tracts. *PloS one* **3**, e3631, doi:10.1371/journal.pone.0003631 (2008).
43. Yi, S. Y. *et al.* Detecting microglial density with quantitative multi-compartment diffusion MRI. *Front Neurosci* **13**, 81, doi:10.3389/fnins.2019.00081 (2019).

44. Peferoen, L., Kipp, M., van der Valk, P., van Noort, J. M. & Amor, S. Oligodendrocyte-microglia cross-talk in the central nervous system. *Immunology* **141**, 302-313, doi:10.1111/imm.12163 (2014).
45. Skaper, S. D., Facci, L., Zusso, M. & Giusti, P. An inflammation-centric view of neurological disease: Beyond the neuron. *Front Cell Neurosci* **12**, 72, doi:10.3389/fncel.2018.00072 (2018).
46. Albrecht, D. S. *et al.* Brain glial activation in fibromyalgia - A multi-site positron emission tomography investigation. *Brain Behav Immun* **75**, 72-83, doi:10.1016/j.bbi.2018.09.018 (2019).
47. Olesen, P. J., Nagy, Z., Westerberg, H. & Klingberg, T. Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in a fronto-parietal network. *Brain Res Cogn Brain Res* **18**, 48-57, doi:10.1016/j.cogbrainres.2003.09.003 (2003).
48. Lee, J. *et al.* Encoding of self-referential pain catastrophizing in the posterior cingulate cortex in fibromyalgia. *Arthritis Rheumatol* **70**, 1308-1318, doi:10.1002/art.40507 (2018).
49. Gracely, R. H. *et al.* Pain catastrophizing and neural responses to pain among persons with fibromyalgia. *Brain* **127**, 835-843, doi:10.1093/brain/awh098 (2004).
50. Ichesco, E. *et al.* Altered fMRI resting-state connectivity in individuals with fibromyalgia on acute pain stimulation. *European journal of pain (London, England)* **20**, 1079-1089, doi:10.1002/ejp.832 (2016).
51. Gonzalez-Roldan, A. M. *et al.* Controllability and hippocampal activation during pain expectation in fibromyalgia syndrome. *Biol Psychol* **121**, 39-48, doi:10.1016/j.biopsycho.2016.09.007 (2016).
52. Kim, H. *et al.* Fibromyalgia is characterized by altered frontal and cerebellar structural covariance brain networks. *Neuroimage Clin* **7**, 667-677, doi:10.1016/j.nicl.2015.02.022 (2015).
53. Vanneste, S., Ost, J., Van Havenbergh, T. & De Ridder, D. Resting state electrical brain activity and connectivity in fibromyalgia. *PloS one* **12**, e0178516, doi:10.1371/journal.pone.0178516 (2017).
54. Choe, M. K., Lim, M., Kim, J. S., Lee, D. S. & Chung, C. K. Disrupted resting state network of fibromyalgia in theta frequency. *Sci Rep* **8**, 2064, doi:10.1038/s41598-017-18999-z (2018).
55. Chao, C. C. *et al.* Brain imaging signature of neuropathic pain phenotypes in small-fiber neuropathy: altered thalamic connectome and its associations with skin nerve degeneration. *Pain*, doi:10.1097/j.pain.0000000000002155 (2020).
56. Yang, S. & Chang, M. C. Chronic Pain: Structural and Functional Changes in Brain Structures and Associated Negative Affective States. *Int J Mol Sci* **20**, doi:10.3390/ijms20133130 (2019).
57. Baliki, M. N., Mansour, A. R., Baria, A. T. & Apkarian, A. V. Functional reorganization of the default mode network across chronic pain conditions. *PloS one* **9**, e106133, doi:10.1371/journal.pone.0106133 (2014).
58. Thompson, J. M. & Neugebauer, V. Cortico-limbic pain mechanisms. *Neurosci Lett* **702**, 15-23, doi:10.1016/j.neulet.2018.11.037 (2019).
59. Newman, E., Moulton, E., Becerra, L. & Borsook, D. in *Chronic Pain and Brain Abnormalities* (ed C. Saab) 15 - 40 (Academic Press, 2014).

Figures

Figure 1

Group differences of cortical thickness Cortical surface reconstruction showing a significant decrease in cortical thickness in the left pars opercularis of the inferior frontal gyrus in FMS patients compared to healthy controls (A) and a significant decrease in cortical thickness in the superior frontal and rostral middle frontal cortex in the PNS group compared to healthy controls (B). Color bar indicates the strength of the statistical difference (p-value, corrected for multiple comparisons) per voxel.

Figure 2

Group differences of white matter integrity Increases of fractional anisotropy in the FMS group compared to controls (MNI x = 83, y = 148, z = 88). Color bar indicates the strength of the statistical difference (p-value, corrected for multiple comparisons) per voxel.

Figure 3

Group differences of functional seed-to-seed connectivity Differences in functional connectivity between the subgroups (PNS > noPNS, seed-to-seed), red connections indicate hyperconnectivity, blue connections indicate hypoconnectivity. Corrected for multiple comparisons.

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