Insular cortex is a trait marker for pain processing in fibromyalgia syndrome – blood oxygenation level-dependent functional magnetic resonance imaging study in Korea

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ABSTRACT

Objective. *To investigate the variability in cerebral activation according to pain intensity and the association between variability in cerebral activation and clinical features in patients with fibromyalgia syndrome (FMS) using functional magnetic resonance imaging (fMRI).*

Methods. *Nineteen FMS female patients and 22 age-matched healthy female controls were enrolled in this study. Changes in cerebral activation area were measured using blood oxygenation level-dependent (BOLD) contrast fMRI after application of both medium and high pressure stimuli to the left thumbnail bed.*

Results. *We identified the insular cortex (IC) and superior temporal gyrus (STG) as regions of interest (ROIs) in this analysis. Cerebral activation at the bilateral IC in response to high pressure stimuli was significantly greater in FMS patients than it was in the controls, whereas there were no differences in BOLD signal changes in the STG regions between FMS patients and controls, irrespective of pain level. Prominent signal changes at both ROIs in FMS patients were noted between high and medium pressure ($p<0.001$ contralateral IC, $p=0.001$ for ipsilateral IC, $p=0.008$ for contralateral STG, and $p=0.049$ for ipsilateral STG). BOLD signal changes on the contralateral STG after medium stimuli were correlated with tender point count ($r=0.586$, $p=0.013$).*

Conclusion. *This study revealed more distinct signal variability in the ICs in FMS patients than in those of controls in response to high pressure stimuli. The IC can therefore be considered to be a region susceptible to pain perception in FMS patients.*

Introduction

Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterised by the presence of chronic widespread musculoskeletal pain and multiple tender points (1-3). Various hypotheses such as familial aggregation (4), disturbed regulation of pronociceptive and anti-nociceptive amines (5, 6), and neuroendocrine abnormalities (7, 8) have been suggested as causes of FMS. However, nociceptive systems involved in pain conduction have been relatively well demarcated at the peripheral nociceptive and spinal cord levels (9, 10).

Although the precise pathogenesis of FMS is not clear, central sensitisation is known to be involved in the pain perception of FMS. Functional imaging studies of the brain, including single photon emission computed tomography (SPECT), functional magnetic resonance imaging (fMRI), and magnetic resonance spectroscopy (MRS), have been performed to elucidate the functional roles of cortical and subcortical neurons in pain perception in FMS. The results of SPECT are used to infer neural activity from the pattern of regional cerebral blood flow (rCBF) (11, 12), as rCBFs in ROIs, including the thalamus and caudate nucleus, have been found to be lower in FMS patients than in healthy controls. Diverse regions of the brain including S1, S2, the inferior parietal lobule (IPL), IC, the anterior cingulated cortex (ACC), superior temporal gyrus (STG), and cerebellum were found to be activated following the application of painful pressure or subjective pain stimuli in a brain fMRI study (13). In another study, greater activations of multiple brain regions in FMS patients were noted compared to those in healthy controls after application of both non-painful and painful stimuli (14). Two

recent MRS studies also showed distinct metabolic changes in glutamate or glutamate/glutamine (Glx) within the IC, amygdale, and posterior gyrus in patients with FMS (15-17). These functional imaging studies may therefore help to clarify the roles of various regions of the brain in pain perception as well as for regulation of FMS.

However, previous fMRI studies analysed volumes of statistical activation with either lower p value (*e.g.* $p<0.05$) or no small volume correction for pain processing in FMS patients (13, 14). Therefore, research for higher statistical activation with volume correction according to painful stimulations in brain of FMS patients should be needed. In our study, we investigate changes in the blood oxygenation level-dependent (BOLD) signal in activated regions of the brain in response to application of two pain intensities (medium and high pressure) using less than corrected *p*-value of 0.005 to the left thumbnail beds of patients with FMS and in healthy controls drawn from a Korean population. Correlations between signal changes in cerebral activation following painful stimulation and clinical parameters were also analysed.

Subjects and methods

Subjects

A total of 41 female subjects (19 FMS patients and 22 healthy controls) were enrolled in this study. They were age-matched (40.2±7.3 yrs in the FMS group *vs.* 38.1±8.5 yrs in the healthy controls), and all were right-handed. The healthy controls were recruited volunteers, and all were screened for the presence of chronic widespread pain, generalised weakness, sleep disturbance, and specific tender points. All patients at the time of initial diagnosis met the classification criteria for FMS proposed by the American College of Rheumatology in 1990 (1). FMS patients were recruited consecutively from outpatient rheumatic clinics at four university-based hospitals and from one general hospital. All participants agreed to participate in our fMRI study and provided written informed consent. The protocol used for this study was approved by the Institutional Review Board.

Assessment of activity for FMS

Demographic, clinical, and psychological data including age, education, disease duration, and tender point count were obtained from reviews of medical records and an interview with each participant at the time of study enrollment. Tender points were calculated from direct palpation of 18 specific anatomical locations with a force of 4.0 kg (18). The functional abilities of FMS patients were assessed using the Korean version of the fibromyalgia impact questionnaire (FIQ) (19). Severities of fatigue and depression were evaluated using the brief fatigue inventory (BFI) (20), Beck depression inventory (BDI) (21), and Beck anxiety inventory (BAI) (22).

Scale of pain severity according to thumbnail pressure

The intensity of pain stimulation was evaluated using the method previously described by Geisser *et al*. (23). Pain stimuli were applied to the left thumbnail using a 1 cm^2-sized hard rubber probe for 5 minutes. The initial pressure applied through the rubber probe was 0.5 kg/cm^2, when was then increased gradually by 0.5 kg/cm^2 to either the maximal tolerable level or 4.5 kg/cm^2 over a 30 second time interval. The levels of pain stimulation ranged from level 1 (0.5 kg/cm^2) to level 9 (4.5 kg/cm^2). Medium intensity and high intensity pains were defined as follows: if, after the application of pain stimulation and request for pain analysis, the subject answered "tolerable," the pain intensity was considered medium intensity pain, while if they answered "painful," the pain intensity was considered to be high. 3-D T1-weighted anatomical scan was obtained for structural reference with following parameters: TR=7.8 ms, TE=3.0 ms, slice thickness 1.3mm, partition number=120, matrix size of 256x256 and FOV=22cm.

Imaging of BOLD fMRI

BOLD contrast images were collected for each subject using a 3.0 T GE EXITE (Milwaukee, WI, USA) scanner equipped with a transmit-receive body coil and a commercial eight-element head coil array. T2-weighted echo planar imaging was used for fMRI acquisition. The following acquisition parameters were used in the fMRI protocol: echo time (TE) =40 ms, repetition time (TR)=5000 ms, field of view (FOV)=19.2 cm, acquisition matrix=64 X 64. Using a midsagittal scout image, 3 mm thickness was placed along the anterior–posterior commissure (AC–PC) plane covering the entire brain. A sequence of 128 time points (brain volumes) per run was obtained, using one stimulation condition per run. For each stimulation, subjects alternately received 30 seconds of an innocuous touch and 30 seconds of painful pressure, for a total of ten one-minute cycles. Onset and offset were coincident with the beginning of a scan, and the series was initiated on the third scan. At three-second intervals, stimulating pressure was decreased for 0.3 seconds to avoid occlusion of blood flow.

Analysis of fMRI images

Whole-brain image analysis was completed using the general linear model in statistical parametric mapping [(spm5, htt://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB (MathWorks, Inc., Natick, MA, USA)]. The functional images were realigned to the first image to adjust for residual head movement. The realigned images were then spatially normalised to fit a Montreal Neurological Institute template (24) based on the standard stereotaxic coordinate system (25). Subsequently, all images were smoothed with an isotropic Gaussian kernel with a 9-mm full width at half maximum. Preprocessed MRI data were analysed statistically on a voxel-by-voxel basis using spm5. Serial correlations were corrected using an autoregressive model, and global signal changes were removed by scaling. Task-related neural activities were modelled using a boxcar function convolved with a haemodynamic response function. To identify which cerebral networks were activated during the pain stimulation, we analysed the BOLD responses; for each subject, the boxcar model convolved with the haemodynamic response function was applied to the fMRI time series at each voxel, and t-maps for the differences between contrast active pain and rest innocuous pain were computed.

The resulting statistical maps were then entered into a second-level random effects model which combined all of the single-subject data for each group, and *t*-tests were performed to assess differences between groups (one-sample *t*-test). Correction for multiple comparisons was carried out at the voxel level using small volume correction with a false discovery rate (FDR) of 0.05 (26). A one-sample *t*-test was used to calculate the main effect within each group of subjects. Finally, the resulting activation maps were created to identify anatomical correlates of the activity, and these were displayed via projection onto anatomically standardised mean T1 images for all subjects. Estimates of percent signal change during the finger tapping task were calculated from the bilateral IC and bilateral STG of each participant using MarsBaR [Marseille boîte à région d'intérêt] (27) and ROIs defined from the anatomical automatic labelling (AAL) ROI library. The average signal used in this calculation was based on all conditions and was identified as the beta value for the mean column of the regression analysis.

Statistical analysis

Data are reported as means ± standard deviations. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess whether the data had a normal distribution. These tests showed that the data were not normally distributed. Therefore, differences in sequential variables between the two groups were assessed using Mann-Whitney *U*-tests. Analysis of covariance (ANCOVA) was introduced to verify whether the differences between controls and patients were significant using covariants, and correlations between sequential variables were assessed using Spearman's rank correlation analysis. Statistical significance was determined as $p<0.05$ in the Statistical Package for the Social Sciences, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study subjects

The general characteristics of the enrolled subjects are presented in Table I. Nineteen females with FMS and 22 age-matched healthy controls were enrolled in this study. There was a difference in education duration between the two groups ($p=0.033$). The mean disease duration of FMS was 25.5±34.9 months, and the FMS patients showed an average of 11.8±5.8 tender points and average scores of 6.0±2.3 for the BFI and 54.5±17.5 for the FIQ. Both anxiety and depression scores using BAI and BDI were also assessed, with the scores of FMS patients found to be significantly different from those of the controls ($p=0.022$ and $p<0.001$, respectively).

Table I. Baseline characteristics in enrolled study subjects.

	Fibromyalgia (n=19)	Healthy controls (n=22)	*p*-value
Demographic data			
Age (years)	40.2 ± 7.3	38.1 ± 8.5	0.424
Education (years)	13.0 ± 1.5	14.1 ± 1.8	0.033
Current medications, n (%)			
Non-steroidal anti-inflammatory drugs	3 (15.8)		
Acetaminophen/tramadol	8 (42.1)		
Tricyclic anti-depressants	6 (31.6)		
Serotonin selective reuptake inhibitors	5 (26.3)		
Serotonin norepinephirine reuptake inhibitors	2 (10.5)		
Anti-convulsant	8 (42.1)		
Clinical data			
Disease duration	25.5 ± 34.9		
Tender points	11.8 ± 5.8		
FIQ	54.5 ± 17.5		
BFI	6.0 ± 2.3		
Psychological data			
BDI	19.0 ± 9.9	11.7 ± 9.4	0.022
BAI	25.6 ± 9.4	10.8 ± 10.6	<0.001

FIQ: fibromyalgia impact questionnaire; BFI: brief fatigue index; SF-36: 36-item Medical Outcomes Study Short-Form Health Survey; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory.

Localisation and comparison of BOLD signal changes in cerebral activations of ROIs in response to pressure pain between FMS patients and healthy subjects

Anatomical locations, Talairach coordinates, and statistical Z scores for the peak voxel activation according to two pain intensities at a corrected threshold of $p<0.005$ are shown in Table II and Figure 1. Significant activations of IC and STG areas as ROIs in response to both high and medium pressure pain stimulations were identified. However, similar cerebral responses were also observed in the healthy controls (not data shown), although the activations in the healthy controls tended to be higher than those in FMS patients after both medium and high pressure stimulation (Fig. 1).

Comparison of BOLD signal changes between FMS patients and controls after cerebral activation of ROIs in response to pressure pain

For both the medium and high pain stimulation tests, the maximal tolerable pain levels of FMS patients were significantly lower than those of the healthy controls (2.08±0.35 *vs*. 2.62±0.51 kg/cm^2, $p<0.001$ for medium pain stimulation and 3.16±0.50 *vs*. 3.78±0.61 kg/cm^2, $p=0.001$ for high pain stimulation, respectively). This finding implies that the threshold or tolerance to pain stimulation of FMS patients is significantly lower than that of controls. First, we compared the degrees of signal changes in ROIs, including those of the IC and STG areas, following application of medium pain pressure to both groups. ANCOVA using covariants such as education, pain level, BDI, and BAI showed that signal changes in the bilateral IC and STG were not significantly different between FMS patients and healthy controls ($p>0.05$ for all ROIs) (Fig. 2A). Similarly, activation changes in the bilateral STG regions after high stimulation were not significantly different between the two groups

Table II. Significant increase of signal changes in fibromyalgia patients according to pain intensity (corrected $p<0.005$).

High pain pressure						Medium pain pressure					
		Talairach coordinates			Z score			Talairach coordinates			Z score
Side	Region of interest	x	y	z		Side	Region of interest	x	y	z	
Contralateral	Superior temporal gyrus	51	0	0	4.75	Contralateral	Insular cortex	36	3	12	3.70
Ipsilateral	Superior temporal gyrus	-48	-3	0	4.51	Ipsilateral	Insular cortex	-54	-33	18	4.08
Ipsilateral	Insular cortex	-45	0	0	4.48						

($p>0.05$ for bilateral STG). In contrast, activation changes in the bilateral IC of FMS patients following high pressure stimulation were significantly greater than those of the controls (2.24±0.15 *vs*. 0.07±0.07, $p=0.001$ for the contralateral IC and 0.19±0.14 *vs*. 0.08±0.07, $p=0.004$ for the ipsilateral IC, respectively) (Fig. 2B).

Next, we analysed which cerebral regions of the two studied groups were activated at the time of high stimulation compared to those activated by medium stimulation. All ROIs in FMS patients were more significantly activated after high pressure stimulation compared to those after medium stimulation ($p<0.001$ for the contralateral IC, $p=0.001$ for the ipsilateral IC, $p=0.008$ for the contralateral STG, and $p=0.049$ for the ipsilateral STG, respectively) (Fig. 3A-D). However, in healthy controls, cerebral activations in response to high pressure pain stimuli were more dominant only in the ipsilateral IC and ipsilateral STG ($p=0.009$ and $p=0.033$, respectively) (Fig. 3E-H).

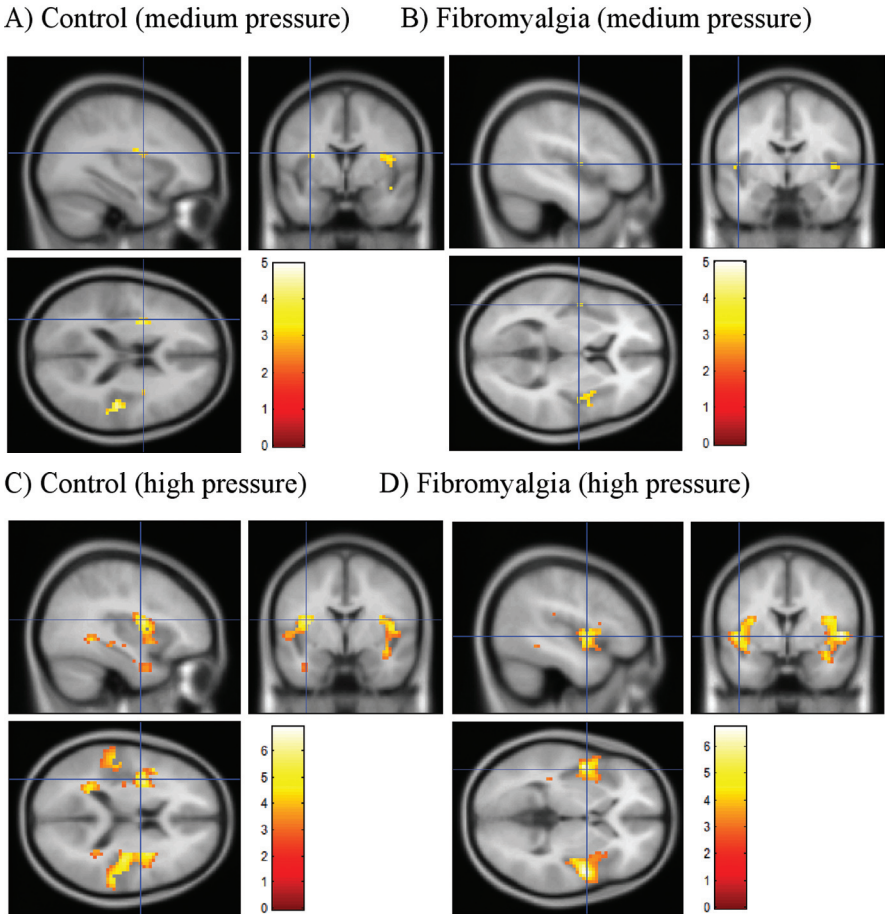

Fig. 1. Comparison of cerebral activation in region of interest between controls (**A** and **C**) and FMS patients (**B** and **D**). Cerebral activations at regions of interest in controls and FMS patients were demonstrated after application of the medium (**A** and **B**) and high (**C** and **D**) pressure stimulation.

Correlations between signal changes at individual cerebral regions and clinical parameters

We found that signal changes in the contralateral STG following moderate stimulation were closely related to tender point counts (r=0.586, $p=0.013$) (Fig. 4). However, other parameters, including disease duration, FIQ, and BFI, did not show significant associations with cerebral signal responses to the pain stimuli.

Discussion

In this study, we investigated signal changes in cerebral responses after application of two different painful stimuli, medium and high pressure pain, to the left thumbnail pads of FMS patients and healthy controls using BOLD fMRI. Our fMRI study focused on two ROIs, namely the IC and STG, using a threshold of $p<0.005$ to heighten the accuracy of our analysis of associations between pain perception and localisation of cerebral activation, although diverse regions of cerebral activation were detected at the lower threshold of $p<0.01$ (Supplementary Tables I and II). We confirmed that FMS patients have a much lower tolerance for painful pressure stimuli than do healthy controls. Our study showed that both medium and high pain stimuli evoked greater spatial variability (signal changes) in both the IC and STG in FMS patients compared to those of healthy controls. However, this difference in signal variability between the two groups was only significant for the IC area after application of a high pressure stimulus. FMS patients showed higher BOLD signal variability to high

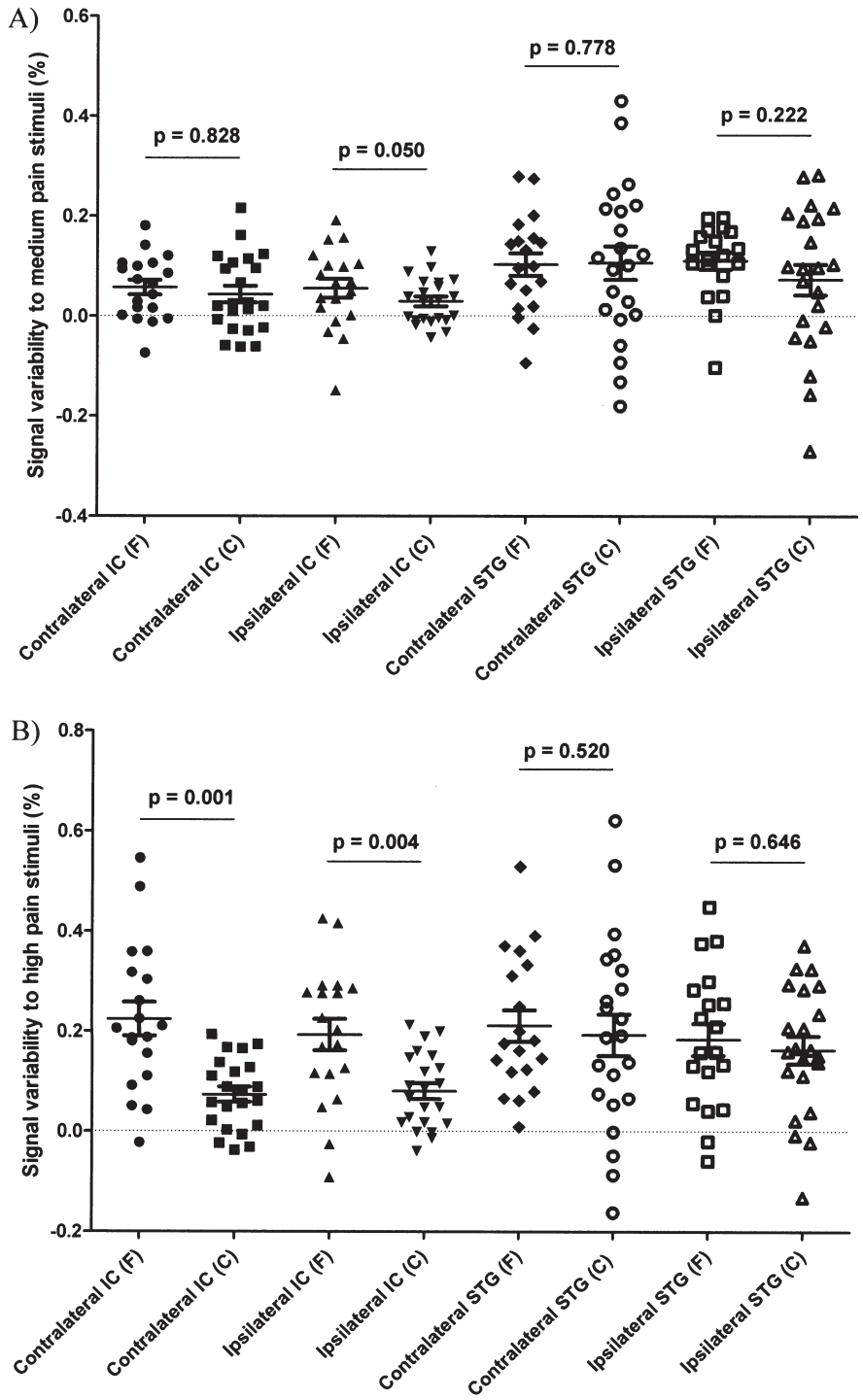

Fig. 2. Comparison of signal changes for medium or high pressure stimuli to non-stimuli between FMS patients and healthy controls. **A**. No significant differences of bilateral IC and bilateral STG between two groups. **B**. Greater activation at bilateral IC of FMS patients after application of high pressure stimuli was noted, compared to those of healthy controls (p=0.001 for contralateal IC and p=0.004 for ipsilateral IC). However, activation at bilateral STG regions was not different between two groups. p value were assessed after correction using education, pain intensity, BDI, and BAI.
Abbreviations: F: fibromyalgia, C: control, IC: insular cortex, STG: superior temporal gyrus.

stimuli than to medium stimuli in the two ROIs. In addition, tender point counts were significantly associated with signal changes in cerebral activation in the contralateral STG. Our BOLD fMRI findings therefore suggest that the IC may be one of the regulatory ROIs involved in pain perception in FMS patients.

Precise locations responsible for pain regulation and perception in FMS have not been determined. Early SPECT studies performed during the resting state have demonstrated lower rCBF, an indication of reduced neural activity, in FMS patients compared to those of healthy controls (11, 12). Although different studies have reported different results, ROIs such as the thalamus, caudate nucleus, inferior pontine tegementum, and surrounding lentiform nucleus have been suggested to be regions involved in pain regulation or perception in FMS patients. However, assessment of real-time functional status following pain stimuli has some limitations. Gracely *et al*. attempted to demonstrate regions of cerebral activation following the application of various painful pressure stimuli or subjective pain stimuli using fMRI (13). Although low pressure stimuli were applied to both patients and healthy controls, the FMS patients showed greater activations of cerebral regions including the contralateral S1, contralateral IPL, contralateral IC, contralateral ACC, contralateral PCC, ipsilateral S2, bilateral STG, and bilateral cerebellum in comparison to those of the controls. In addition, more enhanced cerebral activity was demonstrated in FMS patients than in the controls (14). Harris *et al*. suggested that changes in glutamate levels in the IC may be related to pain perception (15), and another MRS study showed that the ratios of inositol and glutamate/glutamine(Glx) compounds to creatine were significantly associated with the right amygdala (16). Recently, Fayed *et al*. demonstrated that glutamate/glutamine(Glx) within the posterior gyrus might play a pathologic role in FM (17). Our study also demonstrated activations of diverse cerebral areas including the ACC, IPL, STG, IC, and precental gyrus after painful pressure stimulation (Supplementary Tables I and II), consistent with previous studies (13, 14). Thus diverse cerebral regions, including the IC, STG, and ACC, could be considered to be major domains in the network of FMS-related pain.

We focused on two cerebral regions, the IC and STG, as distinct signal changes at a threshold of p<0.005 were observed in these two regions. Signal

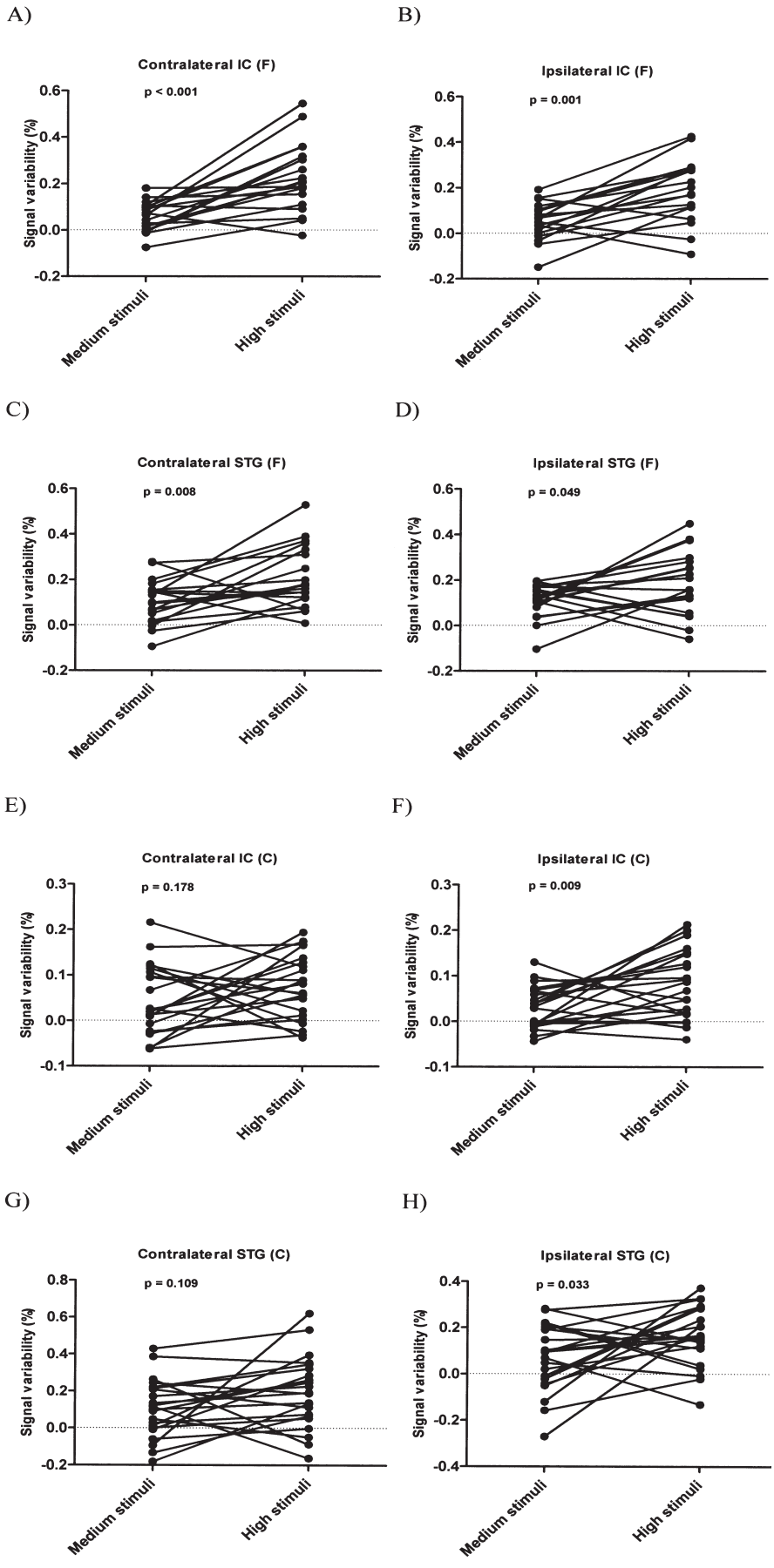

Fig. 3. Comparisons of differences for signal activations according to painful pressure intensity in each study group.
A-D. Significantly increased activation of bilateral insular cortices and bilateral STG at high pressure stimuli in FMS patients compared to medium stimuli (p<0.001 for contralateral IC, p=0.001 for ipsilateral IC, p=0.008 for contralateral STG, and p=0.049 for ipsilateral STG, respectively).
E-H. Signal changes at ipsilateral IC and ipsilateral STG regions at high stimuli in healthy controls were increased, compared to medium stimuli (p=0.009 for ipsilateral IC and p=0.033 for ipsilateral STG, respectively), whereas contralateral IC and STG did not show difference according to pain intensities.
Abbreviations: F: fibromyalgia, C: control, IC: insular cortex, STG: superior temporal gyrus.

variability in response to both high and medium pressure pain on the left thumb was investigated at these two ROIs. No significant difference in BOLD signal variability (cerebral activity) was noted between FMS patients and healthy controls when responses to medium pain stimuli were analysed, although the signal was slightly higher in FMS patients than it was in the controls. In contrast, signal changes in the bilateral IC were significantly increased in FMS patients after stimulation with high intensity pain in comparison to those of healthy controls; however, this was not true for the STG regions. Prominent activation of the contralateral IC is consistent with previous data (13, 14). However, there are discrepancies between our study and previous studies with respect to activation of the ipsilateral IC and the absence of activation of the STG regions. Activation of the ipsilateral IC area in our study population can be explained as follows. First, the IC and the ACC are major components of the limbic system and are known to be involved in pain-related affective processing (28). In addition to contralateral activation of the IC, activation of secondary affective components in response to pain stimuli might be involved in bilateral activation. Second, one recent study demonstrated that glutamate levels before and after acupuncture were closely associated with pain changes in FMS patients (15). Interestingly, BOLD activation at the left posterior IC was significantly correlated with the glutamate/creatinine ratio at the right poste-

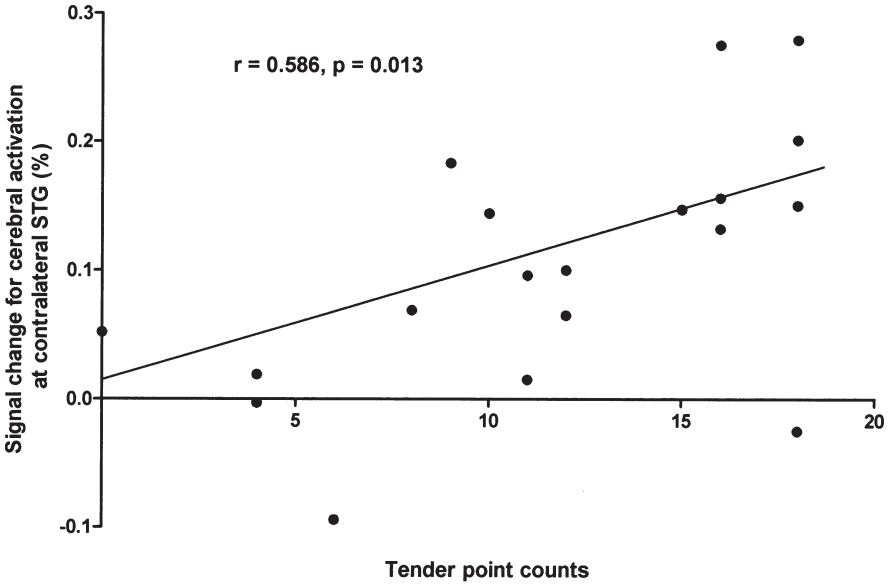

Fig. 4. Correlation between cerebral activation of regions assessed atender point counts at the time of enrollment in FMS. Tender point counts were closely associated with cerebral activation at contralateral STG in FMS patients after moderate pressure stimuli (r=0.586, p=0.013).

rior IC, suggesting close interaction between the bilateral cerebral regions. It remains to be determined whether the STG is involved in pain processing of tactile or painful stimuli. We could not confirm differential involvement of the STG in pain perception between FMS patients and controls, although the signal variability in the STG region was more prominent in FMS patients than it was in the controls. Gracely *et al*. demonstrated that the STG contributed to high pain perception in FMS patients when low pressure was applied (13). In addition, a voxel-based morphometry study showed altered brain morphology in the STG region with a decrease in gray matter (29). These two studies suggest that the STG might play a role in pain perception in FMS. However, other studies have not found the STG region to be a meaningful cerebral region associated with pain (14-16).

Cerebral signal variability could be dependent on the intensity of painful stimulation. We found greater signal changes in the bilateral IC and STG regions following high pain pressure compared to those due to medium pain pressure. However, contralateral activations of the IC and STG were only significantly greater after high pain stimulation. The STG region may therefore be considered to be susceptible to changes in pain intensity in both FMS patients and healthy controls.

Few studies have evaluated the clinical significance of fMRI with regard to the FMS-related clinical features of FMS patients. One study showed that pain intensity was dependent on activations of the bilateral IC, contralateral ACC, and prefrontal cortex in FMS patients with depression and/or major depression disorders (30). A diffusion-tensor and volumetric imaging study revealed a positive correlation between high fractional anisotropy values of the right superior temporal gyrus and higher pain scores in FMS patients (31). In addition, Schmidt-Wilcke *et al*. described a positive correlation between the pain

Supplementary Table I. Significant increase of signal in fibromyalgia patients after stimulation with high pressure (uncorrected p<0.01).

Fibromyalgia (high pressure)							Controls (high pressure)						
			Coordinate (mm)						Coordinate (mm)			
Region of interest		Cluster size	x	y	z	Peak T	Region of interest		Cluster size	x	y	z	Peak T
IFG	L	42	-36	21	9	3.62	SFG	L	123	-9	30	48	4.57
	R	47	39	36	9	3.04	MFG	L	93	-42	21	48	4.34
IPL	L	134	-42	-48	39	4.77		R	125	36	39	-3	5.15
	R	131	60	-28	21	4.49	IFG	L	129	-45	12	12	4.99
ACC	L	72	-15	30	24	3.50		R	89	42	42	12	4.67
Precentral Gyrus	L	68	-57	3	12	4.29	IPL	L	322	-48	-39	27	4.92
	R	49	51	-3	6	5.71		R	394	54	-27	24	5.00
Insula	L	256	-45	0	0	6.16	Postcentral Gyrus	L	96	-51	-27	18	4.81
	R	328	51	0	0	6.71		R	189	51	-21	18	5.30
Caudate	L	82	-12	-3	18	3.61	Thalamus	L	74	-9	-9	18	4.79
	R	57	15	0	18	3.45		R	193	9	-9	18	4.18
STG	L	115	-48	-3	0	6.16	Insula	L	304	-36	-3	15	6.00
	R	229	51	-3	0	6.71		R	472	42	-21	18	6.89
Hippocampus	R	47	40	-5	-15	3.39	Caudate	L	87	-6	3	12	5.89
								R	79	15	0	21	5.93
							STG	L	256	-51	6	0	6.88
								R	284	48	3	3	5.84
							Hippocampus	R	132	39	-9	-18	4.54

SFG: Superior Frontal Gyrus; MFG: Middle Frontal Gyrus; IFG: Inferior Frontal Gyrus; IPL: Inferior Parietal Lobule; STG: Superior Temporal Gyrus; ACC: Anterior Cingulate Cortex; L: ipsilateral side; R: contralateral side.

Suppl. Table II. Significant increase of signal in fibromyalgia patients after stimulation with medium pressure (uncorrected $p<0.01$).

Fibromyalgia medium pressure							Controls medium pressure						
			Coordinate (mm)						Coordinate (mm)				
Region of interest		Cluster size	x	y	z	Peak T	Region of interest		Cluster size	x	y	z	Peak T
Insula	R	80	36	3	12	4.38	IPL	L	62	-57	-54	45	3.59
STG	L	20	-54	33	18	5.01		R	86	12	15	15	5.08
	R	36	57	-33	15	4.51	Postcentral Gyrus	R	40	45	-24	24	4.81
							Insula	R	85	45	-24	21	4.98
							Caudate	L	57	-15	6	21	3.77
								R	86	12	15	15	5.08
							STG	R	45	57	9	-3	3.83

IPL: Inferior Parietal Lobule; STG: Superior Temporal Gyrus; L: ipsilateral side; R: contralateral side.

experience scale score and the gray matter value (29). In the current study, we found a close relationship between signal changes in the right STG and tender point counts (r=0.586, p=0.013), but no other significant associations were observed between clinical parameters and the signal variability of cerebral responses. Further studies to identify potent imaging markers that reflect the pain status of FMS patients are required.

This study had some limitations. First, a potential disadvantage of fMRI BOLD is that there is repeated switching between stimulus 'on' and 'off' conditions, complicating the imaging of static or long-lasting drug effects (for example, before and after treatment). To minimise the risk of false positive results, we performed an ROI-analysis in which we only analysed brain areas of a prior expected importance. Second, there may also have been differences between the two groups with respect to anxiety and depression. However, to rule out the possibility that our results are an epiphenomenon of affective factors, we included the depression score and an anxiety score as nuisance variables in our model. Third, small sample size (n=19 of FMS) might be a major limitation in this study. It was exploratory study in FMS patients. It needs to perform fMRI study in larger study population. Finally, we analysed BOLD changes in FMS only in a cross-sectional manner. Longitudinal changes in the brain activities of ROIs should therefore be investigated in future studies.

In summary, we measured BOLD signal variability in cerebral responses to medium and high intensity painful pressure stimuli. Furthermore, we investigated signal variability between FMS patients and controls for two intensities of painful stimuli and investigated the associations among clinical parameters and signal variability. The bilateral IC and STG of FMS patients were significantly activated in response to high intensity pain compared to those brain regions of the controls. Furthermore, FMS patients were more susceptible to signal changes in the two ROIs than were the controls, and the contralateral STG signal was also associated with the tender point count in these patients. Our study results strongly suggest that the IC plays a role in pain perception in FMS.

References

1. WOLFE F, SMYTHE HA, YUNUS MB *et al*.: The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. *Arthritis Rheum* 1990; 33: 160-72.
2. MALEMUD CJ: Focus on pain mechanisms and pharmacotherapy in the treatment of fibromyalgia syndrome. *Clin Exp Rheumatol* 2009; 27 (Suppl. 56): S86-91.
3. SALAFFI F, SARZI-PUTTINI P, CIAPETTI A, ATZENI F: Assessment instruments for patients with fibromyalgia: properties, applications and interpretation. *Clin Exp Rheumatol* 2009; 27 (Suppl. 56): S92-105.
4. ARNOLD LM, HUDSON JI, HESS EV *et al*.: Family study of fibromyalgia. *Arthritis Rheum* 2004; 50: 944-52.
5. OFFENBAECHER M, BONDY B, DE JONG S *et al*.: Possible association of fibromyalgia with a polymorphism in the regulatory region. *Arthritis Rheum* 1999; 42: 2482-8.
6. ZUBIETA JK, HEITZEG M, SMITH YR: COMT Val 158 Met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 2003; 5610: 1240-3.
7. CROFFORD LJ, PILLEMER SR, KALOGERAS KT *et al*.: Hypothalamicpituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis Rheum* 1994; 37: 1583-92.
8. MCLEAN SA, WILLIAMS DA, STEIN PK *et al*.: Cerebrospinal fluid corticotropin-releasing factor concentration is associated with pain but not fatigue symptoms in patients with fibromyalgia. *Neuropsychopharmacology* 2006; 31: 2776-82.
9. WILLIAMS DA, GRACELY RH: Biology and therapy of fibromyalgia. Functional magnetic resonance imaging findings in fibromyalgia. *Arthritis Res Ther* 2006; 8: 224.
10. APKARIAN AV, BUSHNELL MC, TREEDE RD, ZUBIETA JK: Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain* 2005; 9: 463-84.
11. MOUNTZ JM, BRADLEY LA, MODELL JG *et al*.: Fibromyalgia in women. Abnormalities of regional cerebral blood flow in the thalamus and the caudate nucleus are associated with low pain threshold levels. *Arthritis Rheum* 1995; 38: 926-38.
12. KWIATEK R, BARNDEN L, TEDMAN R *et al*.: Regional cerebral blood flow in fibromyalgia: singlephoton-emission computed tomography evidence of reduction in the pontine tegmentum and thalami. *Arthritis Rheum* 2000; 43: 2823-33.
13. GRACELY RH, PETZKE F, WOLF JM, CLAUW DJ: Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum* 2002; 46: 1333-43.
14. COOK DB, LANGE G, CICCONE DS, LIU WC, STEFFENER J, NATELSON BH: Functional imaging of pain in patients with primary fibromyalgia. *J Rheumatol* 2004; 31: 364-78.
15. HARRIS RE, SUNDGREN PC, PANG Y *et al*.: Dynamic levels of glutamate within the insula are associated with improvements in multiple pain domains in fibromyalgia. *Arthritis Rheum* 2008; 58: 903-7.
16. VALDÉS M, COLLADO A, BARGALLÓ N *et al*.: Increased glutamate-glutamine compounds (Glx) in the brain of patients with fibromyalgia: A MR spectroscopy study. *Arthritis Rheum* 2010; 62: 1829-36.
17. FAYED N, GARCIA-CAMPAYO J, MAGALLÓN R *et al*.: Localized 1H-NMR spectroscopy in patients with fibromyalgia: a controlled study of changes in cerebral glutamate/glutamine, inositol, choline, and N-acetylaspartate. *Arthritis Res Ther* 2010; 12: R134.
18. OKIFUJI A, TURK DC, SINCLAIR JD, STARZ TW, MARCUS DA: A standardized manual tender point survey. I. Development and determination of a threshold point for the identifica-

tion of positive tender points in fibromyalgia syndrome. *J Rheumatol* 1997; 24: 377-83.
19. KIM YA, LEE SS, PARK K: Validation of a Korean version of the Fibromyalgia Impact Questionnaire. *J Korean Med Sci* 2002; 17: 220-4.
20. YUN YH, WANG XS, LEE JS *et al*.: Validation study of the Korean version of the brief fatigue inventory. *J Pain Symptom Manage* 2005; 29: 165-72.
21. JO SA, PARK MH, JO I, RYU SH, HAN C: Usefulness of Beck Depression Inventory (BDI) in the Korean elderly population. *Int J Geriatr Psychiatry* 2007; 22: 218-23.
22. YOOK SP, KIM ZS: A clinical study on the Korean version of Beck anxiety inventory: comparative study of patient and non-patient. *Korean J Clin Psychol* 1997; 16: 185-97.
23. GEISSER ME, GLASS JM, RAJCEVSKA LD *et al*.: A psychophysical study of auditory and pressure sensitivity in patients with fibromyalgia and healthy controls. *J Pain* 2008; 9: 417-22.
24. EVANS AC, COLLINS DL, MILLS SR, BROWN ED, KELLY RL, PETERS TM: 3D statistical neuroanatomical models from 305 MRI volumes. *In* Proceedings of the IEEE Nuclear Science Symposium and Medical Imaging Conference. 1993; 3: 1813-7.
25. TALAIRACH J, TOURNOUX P: Co-Planar Stereotaxic Atlas of the Human Brain (Thieme, Stuttgart, Germany, 1988).
26. GENOVESE CR, LAZAR NA, NICHOLS T: Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 2002; 15: 870-8.
27. BRETT M, ANTON JL, VALABREGUE R, POLINE JB: Region of interest analysis using an SPM toolbox [abstract]. *NeuroImage* 2002; 16: S497.
28. FULBRIGHT RK, TROCHE CJ, SKUDLARSKI P, GORE JC, WEXLER BE: Functional MR imaging of regional brain activation associated with the affective experience of pain. *Am J Roentgenol* 2001; 177: 1205-10.
29. SCHMIDT-WILCKE T, LUERDING R, WEIGAND *et al*.: Striatal grey matter increase in patients suffering from fibromyalgia - a voxel-based morphometry study. *Pain* 2007; 132 (Suppl. 1): S109-16.
30. GIESECKE T, GRACELY RH, WILLIAMS DA, GEISSER ME, PETZKE FW, CLAUW DJ: The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. *Arthritis Rheum* 2005; 52: 1577-84.
31. LUTZ J, JÄGER L, DE QUERVAIN D *et al*.: White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis Rheum* 2008; 58: 3960-9.