# Anti-N-methyl-D-aspartate receptor antibodies are associated with fibromyalgia in patients with systemic lupus erythematosus: a case-control study

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## ABSTRACT

**Objective.** *The high concordance between systemic lupus erythematosus (SLE) and fibromyalgia (FM) suggests common underlying mechanisms related to pain and distress in both patient groups. Increasing evidence indicates that N-methyl-D-aspartate receptors (NMDARs) play a major role in the induction and maintenance of central sensitisation with chronic pain. In this study, we evaluated the role of anti-NMDAR antibodies in the development of FM in patients with SLE.*

**Methods.** *Sera from 104 patients with SLE, 112 patients with FM, and 110 healthy controls were analysed to detect antibodies to the N-terminus of the 2B subunit of NMDARs (GluN2B). Subjects underwent clinical examination and neuropsychiatric evaluation, and completed a questionnaire regarding FM and neuropsychiatric symptoms.*

**Results.** *Of the 104 patients with SLE, 18 (17.3%) had FM. The anti-GluN2B antibody titre was significantly higher in patients with SLE ($p<0.001$). Among patients with SLE, those with concomitant FM had higher anti-GluN2B antibody titres ($p<0.05$). The anti-GluN2B antibody titre was associated positively with the tender point count ($p=0.016$) and the widespread pain index ($p=0.005$), but not with other symptom measurements. Anti-GluN2B antibody–positive patients with SLE were more likely to have neuropsychiatric systemic lupus erythematosus (NPSLE) and concomitant FM ($p<0.05$). Multivariate analysis showed that the anti-GluN2B antibody was an independent predictor of concomitant FM and NPSLE.*

**Conclusion.** *To our knowledge, this report is the first to suggest that anti-NMDAR antibodies are associated with the pathogenesis of FM with SLE.*

## Introduction

Fibromyalgia (FM) is an idiopathic pain syndrome characterised by chronic widespread pain and diverse symptoms, such as fatigue, joint stiffness, sleep disturbances and various forms of psychological distress including mood or anxiety disorders (1, 2). Although the aetiology of FM is not yet understood fully, central sensitisation is currently recognised as a key factor in its pathophysiology, and it represents general over-reactivity in the function of neurons and circuits in the central nervous system (CNS) to various stimuli, including nociception (3).

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multisystemic organ involvement, and it is characterised by the presence of autoantibodies. SLE is a relatively rare disease; its overall prevalence was estimated to be <100 per 100,000 persons in a population-based study (4). In most cases, SLE can be distinguished from FM by the presence of specific autoantibodies; thus, SLE and FM are considered to be distinct diseases with different underlying pathophysiologies. However, the presence of FM symptoms in patients with SLE may pose a diagnostic challenge in clinical practice, and patients with SLE and concomitant FM have impaired quality of life compared with those without FM (5). FM is estimated to affect 1–5% of the general population, whereas it has been reported in up to 6–22% of patients with SLE, suggesting that a common pathogenesis underlies these two conditions (6, 7).

N-methyl-D-aspartate receptors (NMDARs) are receptors for glutamate, the most abundant excitatory neurotransmitter in the CNS, and are involved in synaptic plasticity, memory, and learn-

ing functions and emotional responses (8). For decades, disturbances of NMDARs have been implicated in several neurological and psychiatric conditions, such as seizures, multiple sclerosis, schizophrenia, and Alzheimer's disease (9). Likewise, different groups of researchers have shown that NMDAR systems were involved in the pathogenesis of the neuropsychiatric symptoms occurring in SLE. Anti-NMDAR antibodies have been reported in 35% of patients with SLE, and they have been suggested to be related to CNS manifestations, including seizure disorders, psychosis, cognitive dysfunction, and depressive symptoms (10, 11). In addition, multiple lines of evidence suggest that NMDARs play a major role in the induction and maintenance of central sensitation in chronic pain disorders (12). Thus, an understanding of the role of NMDARs may provide new insight to uncover a hidden link between SLE and FM.

Considering that FM is often seen in patients with SLE, it is surprising that NMDARs have not received more attention. We hypothesed that elevated serum levels of anti-NMDAR antibodies would be associated with FM in patients with SLE. In this study, we evaluated the roles of NMDAR antibodies in the development and symptom severity of FM with SLE.

## Patients and methods

*Patients and study design*

To evaluate the roles of anti-NMDAR antibodies, we recruited consecutive patients with SLE, those with FM, and healthy controls between January 2010 and December 2012 at Chonnam National University Hospital, a tertiary referral and academic centre in Korea. Patients with SLE fulfilled four or more of the American College of Rheumatology (ACR) 1997 revised classification criteria for SLE (13). Patients with FM and healthy controls were selected randomly, and both groups completed a tender point examination and questionnaire and patients with FM satisfied the ACR 2010 classification criteria for FM (1). Patients with SLE and those with FM were excluded if available data were incomplete or if they had infection or advanced co-morbidities, such as diabetic neuropathy or malignancies, which could complicate the assessment of SLE and FM symptoms. Three patients with SLE were excluded because inadequate stored serum samples were available for anti-NMDAR antibody detection. We randomly recruited healthy controls through unrelated friends and neighbors of the patients and hospital staff or word-of-mouth from those visiting the general health examination clinic. Healthy controls underwent screening interviews and had no history of SLE, FM diagnosis, chronic pain, or neurological or psychological disease. We evaluated a final sample of 104 patients with SLE (99 females, 5 males; median age, 34.0 years; interquartile range [IQR], 27.0–42.8 years), 112 patients with FM (102 females, 10 males; median age, 49.0 [IQR, 40.0–54.5] years), and 110 healthy controls (106 females, 4 males; median age, 36.5 [IQR, 29.0–44.6] years). This research complied with the Helsinki Declaration. All subjects provided informed consent at the time of enrolment. The Institutional Review Board of Chonnam National University Hospital approved the study (no. 2010-09-160).

*Procedures*

We interviewed patients with SLE using a structured questionnaire that documented sociodemographic data and current or past FM symptoms. Tender point counts were performed by thumb palpation according to a standarded protocol (14). Tender points were counted at 18 specific sites on the body, with counts ranging from 0 to 18. Patients were asked to complete a questionnaire based on the 2010 ACR classification criteria for FM (1) and a Korean version of the Fibromyalgia Impact Questionnaire (FIQ) (15) to assess functional disabilities in FM. We further performed comprehensive clinical and psychiatric assessments using the Beck Depression Inventory (BDI) (16) and the State-Trait Anxiety Inventory (STAI) (17) to determine the severity of depression and anxiety, respectively. Sociodemographic data, such as age, sex, lupus disease duration, education, and concomitant diseases, were obtained by interview. Clinical manifestations and laboratory findings were collected from medical records at the time of enrolment. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)-2000 (18) score was calculated to assess lupus disease activity and flare at the time of enrolment. We also measured the following serological markers: anti-double-stranded DNA (dsDNA), anti-Sm, anti-ribonucleoprotein (RNP), anti-Ro, anti-La, and antiphospholipid autoantibodies; erythrocyte sedimentation rate (ESR); and C-reactive protein (CRP) and complement (C3, C4) levels. Antinuclear antibodies were detected by indirect fluorescent antibody assay. Anti-dsDNA antibodies were detected by radioimmunoassays. Autoantibodies, such as anti-Sm, anti-RNP, anti-Ro, anti-La, immunoglobulin (Ig)M/IgG anti-cardiolipin, and IgG anti-b2 glycoprotein I, were assessed by enzyme-linked immunosorbent assays (ELISAs). Detection of lupus anticoagulant was performed using the dilute Russell's viper venom time and confirmatory tests (19).

Neuropsychiatric systemic lupus erythematosus (NPSLE), divided into diffuse CNS, focal CNS, and peripheral nervous system (PNS) forms, was diagnosed with the cooperation of psychiatrists and/or neurologists, based on several investigations and according to the ACR nomenclature and definitions (20). Other possible aetiologies of NPSLE, such as infectious causes, renal failure, and medications, were excluded. Lupus nephritis (LN) was defined according to the ACR renal disorder criteria (13) and/or renal biopsy evidence based on the 2003 International Society of Nephrology/Renal Pathology Society 2003 classification criteria (21). Concomitant affective disorder unrelated to SLE was defined in patients with SLE based on psychiatrists' diagnoses of affective disorders, such as depression and bipolar disorder.

*Evaluation of anti-NMDAR antibodies*

At the time of enrolment, peripheral blood was obtained from all subjects and stored in EDTA-coated tubes. One author (YT) used ELISA to measure serum antibody titres of the N-terminus of

**Table I.** Correlations between anti-GluN2B antibody titres and age, SLEDAI score, and FM symptoms in patients with SLE.

| | Age | Anti-dsDNA antibody | SLEDAI -2000 | TP | WPI | SSS | FIQ | STAI I | STAI II | BDI |
|---|---|---|---|---|---|---|---|---|---|---|
| Spearman's rho | -0.076 | 0.171 | 0.109 | 0.238 | 0.276 | 0.070 | 0.009 | 0.026 | 0.020 | -0.010 |
| *p*-value | 0.444 | 0.084 | 0.272 | 0.016 | 0.005 | 0.483 | 0.352 | 0.795 | 0.843 | 0.921 |

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; FM: fibromyalgia; SLE: systemic lupus erythematosus; TP: tender point; WPI: Widespread Pain Index; SSS: Symptom Severity Scale; FIQ: Fibromyalgia Impact Questionnaire; STAI: State-Trait Anxiety Inventory: BDI: Beck Depression Inventory.

the 2B subunit of NMDAR (GluN2B, also known as GluRε2 or NR2). All assays were performed without knowledge of the diagnosis or clinical presentation. The anti-GluN2B antibody was measured using the previously reported protocol of Takahashi *et al*. (22-23). Peptides were synthesed from the GluN2B sequences: amino acids 369–382 (KERKWERVGKWKDK) from the extracellular N-terminal. MaxiSorp plates (#468667; Nalge Nunc International) were coated overnight with peptide (1 μg/well) in phosphate-buffered saline (PBS; pH 7.2) and blocked with bovine serum albumin (BSA; 5% w/v) in PBS-Triton X-100 (PBST; 0.05% v/v) for 2 h. Serum (100 μL, diluted 1:10 in PBST containing 1% BSA) was then incubated at 37°C for 2 h. After washing with PBST, plates were incubated with protein A–horseradish peroxidase conjugate (1:10,000) for 2 h, and developed using the TMB Microwell Peroxidase Substrate System (#50-76-00; KPL). Optical densities (ODs; 450 nm) were measured using a microplate reader.

*Statistical analysis*

Statistical analyses were performed using the SPSS software (v. 18; SPSS Inc., Chicago, IL, USA). Values are expressed as medians with interquartile ranges (IQRs) for continuous variables and as percentages for categorical variables. Continuous variables were compared by one-way ANOVA or the Mann-Whitney U-test, and categorical variables were compared using the $\chi^2$ test. Spearman's rank correlation coefficients were calculated. Univariate and multivariate logistic regression analyses were performed to identify associations between anti-NMDAR antibodies and various organ involvement and clinical parameters. *P*-values ≤0.05 were considered to indicate statistical significance.

**Table II.** Patient characteristics, laboratory findings, and concomitant diseases according to the presence of anti-GluN2B antibodies.

| | Anti-GluN2B negative (n=91) | Anti-GluN2B positive (n=13) | *p*-value |
|---|---|---|---|
| Age, years | 35.1 (29.1–45.0) | 25.1 (22.1–36.0) | 0.032 |
| Women (%) | 87 (87.9) | 12 (92.3) | 0.494 |
| Education >12 years (%) | 43 (47.3) | 7 (53.8) | 0.656 |
| Disease duration, years | 8.0 (4.0–8.0) | 8.0 (1.5–10.5) | 0.457 |
| SLEDAI-2000 | 2.0 (0.0–4.0) | 5.0 (2.0–8.0) | 0.056 |
| C3, mg/dL | 79.6 (63.6–93.1) | 63.3 (49.8–83.5) | 0.062 |
| C4, mg/dL | 15.3 (11.1–18.8) | 7.47 (15.0–72.5) | <0.001 |
| ESR, mm/h | 21.0 (11.0–41.0) | 30.0 (15.0–72.5) | 0.096 |
| CRP, mm/h | 0.34 (0.10–0.34) | 0.34 (0.33–1.34) | 0.065 |
| Anti-dsDNA antibody, IU/mL | 13.5 (6.4–49.5) | 43 (15.7–102.8) | 0.058 |
| *Antibody positivity (%)* | | | |
| Anti-Sm | 30 (33.0) | 5 (38.5) | 0.757 |
| Anti-RNP | 38 (41.8) | 6 (46.2) | 0.764 |
| Anti-Ro | 52 (58.4) | 6 (46.2) | 0.404 |
| Anti-La | 23 (25.8) | 3 (23.1) | 1.000 |
| Anti-phospholipid | 12 (14.0) | 2 (16.7) | 0.680 |
| Affective disorder (%) | 5 (5.5) | 0 (0) | 1.000 |
| Lupus nephritis (%) | 42 (46.2) | 4 (30.8) | 0.378 |
| NPSLE (%) | 11 (12.1) | 5 (38.5) | 0.028 |
| Concomitant FM (%) | 13 (14.3) | 5 (38.5) | 0.047 |

Except where indicated otherwise, data are presented as medians with interquartile ranges.
Anti-GluN2B: antibodies to the N-terminus of the 2B subunit of N-methyl-D-aspartate receptors; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NPSLE: neuropsychiatric systemic lupus erythematosus; FM: fibromyalgia.

## Results

All patients with SLE were ethnically homogenous Koreans. The median age (IQR) was 34.0 (27.0–42.8) years. Most (95.2%) patients were women, and the median (IQR) duration of SLE was 8.0 (3.0–11.0) years. The median (IQR) SLEDAI score at the time of enrolment was 2.0 (0.0–5.0). The median serum titre of anti-dsDNA antibody was 18.8 IU/mL, and the frequencies of antibody positivity were: anti-Sm, 33.7%; anti-RNP, 42.3%; anti-Ro, 55.8%; anti-La, 25.8 %; and anti-phospholipid, 14.3%. Of the 104 patients with SLE, 5 (4.8%) and 16 (15.4%) patients had affective disorder and NPSLE, respectively. Forty-six (44.2%) patients with SLE had LN and 18 (17.3%) had concomitant FM. Baseline demographic and clinical characteristics, such as age, sex, disease duration, and SLEDAI-2000 score, did not differ between patients with SLE with and without FM. However, patients with SLE and concomitant FM had higher tender point counts, widespread pain indices, and symptom severity scale (SSS), FIQ, and BDI scores than did those without FM. Detailed data, including patient characteristics, laboratory findings, and FM-related symptoms according to the presence of FM, are provided in Supplementary Tables I and II.

OD values of serum antibodies to the N-terminus of GluN2B were significantly elevated in patients with SLE compared with patients with FM and healthy controls (0.50 [IQR, 0.35–0.67], 0.40 [IQR, 0.35–0.48], and 0.38 [IQR, 0.34–0.46],

respectively; $p<0.001$). These values did not differ significantly between healthy controls and patients with FM. Among patients with SLE, titres were higher in those with than in those without concomitant FM (0.58 [IQR, 0.50–0.88] and 0.36 [IQR, 0.34–0.65], respectively; $p=0.018$; Fig. 1).

Anti-GluN2B antibody titres were associated positively with FM pain in patients with SLE. The tender point count and the widespread pain index (WPI) from the 2010 ACR classification criteria for FM were associated with antibody titres in patients with SLE (Spearman's rho = 0.238, $p=0.016$ and Spearman's rho = 0.276, $p=0.005$, respectively, Table I). No association between anti-GluN2B and anti-dsDNA antibodies was found. Anti-GluN2B antibody titres were not associated with age, SLEDAI score, or other parameters related to FM (Table I). Anti-dsDNA antibody titres were not associated with FM or FM-related symptoms (data not shown).

We compared SLE patient characteristics, laboratory findings, organ involvement, and concomitant diseases according to the presence of anti-GluN2B antibodies. Patients were considered to be positive for the antibody when the titer was greater than the mean plus two standard deviations of the controls. Thirteen (12.5%) patients were positive for anti-GluN2B antibodies and 91 (87.5%) were negative. Patients with anti-GluN2B antibody positivity were younger, had lower serum C4 levels, and were more likely to have NPSLE (38.5% *vs*. 12.1%, $p=0.028$, Table II) and concomitant FM (38.5% *vs*. 14.3%, $p=0.047$, Table II).

Three anti-GluN2B–positive patients with SLE had diffuse CNS forms (two acute confusional state and one psychosis) and four had focal CNS forms (one cerebral infarction, one demyelinating syndrome, one aseptic meningitis, and one seizure disorder); four anti-GluN2B-negative patients with SLE had diffuse CNS forms (two acute confusional state, one cognitive dysfunction, and one psychosis), six had focal CNS forms (two cerebral infarction, one demyelinating syndrome, and three seizure disorders), and one had the PNS form (polyneuropathy). One anti-GluN2B-positive patient with SLE showed an acute confusional state and had a seizure disorder, and one patient with SLE without anti-GluN2B antibodies had cerebral infarction and a seizure disorder. The two patient groups had similar SLEDAI-2000 scores, ESRs, and serum C3 and CRP levels, but not C4 levels. No significant difference was observed in anti-dsDNA antibody titres, positivity for other autoantibodies, presence of LN, or history of affective disorders, such as depression and bipolar disorder, after SLE onset (Table II).

Univariate and multivariate logistic regression analyses were performed to evaluate the associations of anti-GluN2B antibodies with FM and NP-

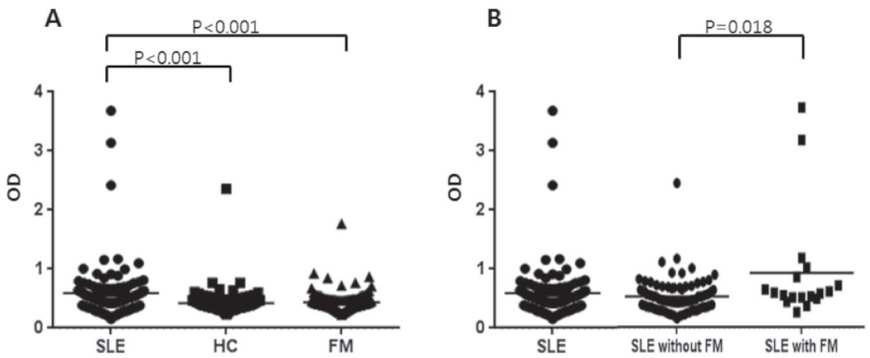


**Fig. 1.** Comparison of the measurement of serum antibody titres to synthesised peptides of the N-terminus of GluN2B.
(**A**) Increased levels of anti-GluN2B antibodies in patients with SLE with or without FM (SLE ± FM) versus healthy controls (HC) and FM alone. (**B**) Patients with SLE and FM showed increased levels of anti-GluN2B antibodies than did those without FM. Horizontal lines represent the mean values of the titres in each group. OD: optical density.

**Table III.** Univariate and multivariate logistic regression analyses of predictors of the presence of FM and NPSLE in patients with SLE.

| | FM | | NPSLE | |
|---|---|---|---|---|
| | Odds ratio (95% CI) | *p*-value | Odds ratio (95% CI) | *p*-value |
| *Anti-GluN2B antibody* | | | | |
| Unadjusted | 3.750 (1.061–13.250) | 0.040 | 4.545 (1.260–16.395) | 0.021 |
| Age-, sex-adjusted | 3.749 (1.022–13.703) | 0.046 | 4.284(1.147–16.003) | 0.031 |
| Age-, sex-, SLEDAI-2000–adjusted | 4.183 (1.552–11.272) | 0.047 | 4.362 (1.156–16.455) | 0.030 |

FM: fibromyalgia; NPSLE: neuropsychiatric systemic lupus erythematosus; Anti-GluN2B: antibodies to the N-terminus of the 2B subunit of N-methyl-D-aspartate receptors; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

**Table IV.** Comparison of FM-related symptoms according to the presence of anti-GluN2B antibodies in patients with SLE.

| | Anti-GluN2B negative (n=91) | Anti-GluN2B positive (n=13) | *p*-value |
|---|---|---|---|
| Tender point count | 2.0 (2.0-5.0) | 3.00 (2.0–6.0) | 0.106 |
| Widespread Pain Index | 2.0 (0.0–4.0) | 4.0 (2.0–6.5) | 0.017 |
| Symptom Severity Scale | 3.0 (2.0–5.0) | 4.0 (2.0–6.5) | 0.346 |
| FIQ | 13.0 (5.17–32.5) | 20.3 (5.33–40.67) | 0.555 |
| STAI I | 44.0 (41.0–49.0) | 44.0 (41.0–48.5) | 0.760 |
| STAI II | 46.0 (41.0–50.0) | 47.0 (45.0–51.5) | 0.322 |
| BDI | 8.0 (3.0–15.0) | 17.0 (4.50–19.5) | 0.159 |

Data are presented as medians with interquartile ranges.
FM: fibromyalgia; Anti-GluN2B: antibodies to the N-terminus of the 2B subunit of N-methyl-D-aspartate receptors; FIQ: Fibromyalgia Impact Questionnaire; STAI: State-Trait Anxiety Inventory; BDI: Beck Depression Inventory.

SLE (Table III). Anti-GluN2B antibodies were independent predictors of FM in an age-, sex-, and SLEDAI-2000 score–adjusted model (odds ratio [OR]=4.183, 95% confidence interval [CI]=1.552–11.272, $p$=0.047). Anti-GluN2B antibodies were also independent predictors of NPSLE in an age-, sex-, and SLEDAI-2000 score–adjusted model (OR = 4.362, 95% CI =1.156–16.455, $p$=0.030). Although not shown, when anti-phospholipid antibody was added to the age-, sex, and SLEDAI-2000 score-adjusted model, anti-GluN2B antibodies remained significant predictors of FM (OR=4.161, 95% CI=1.088–15.909, $p$=0.037) and NPSLE (OR=5.667, 95% CI=1.358–23.633, $p$=0.017). In that case, anti-phospholipid antibody was also a predictor of NPSLE (OR=7.720, 95% CI=1.911–31.189, $p$=0.004), but not FM. Anti-dsDNA antibody was not a predictor of FM or NPSLE, and anti-GluN2B antibody was not associated with LN or affective disorders.

Table IV shows differences in FM-related symptoms according to the presence of anti-GluN2B antibodies. Patients with SLE and anti-GluN2B antibodies had higher WPI scores (4.0 [IQR, 2.0–6.5] *vs*. 2.0 [IQR, 0.0–4.0]; $p$=0.017) than did those without anti-GluN2B antibodies. The tender point count was higher in patients with anti-GluN2B antibodies, but this difference was not significant. Other symptom severity scales for FM, such as the SSS, FIQ, STAI, and BDI, did not differ significantly between groups.

## Discussion

To our knowledge, this is the first reported study to show that NMDAR (anti-GluN2B) antibodies are significantly elevated in patients with SLE and concomitant FM, and that anti-GluN2B antibody titres were associated positively with SLE pain symptoms. Furthermore, we have also showed that NMDAR systems may also be related to SLE neuropsychiatric symptoms. These findings suggest that anti-GluN2B antibodies are involved in the pathogenesis of NPSLE as well as FM in patients with SLE.

In the current study, anti-GluN2B antibody titres were associated with FM pain severity in patients with SLE in terms of tender point count and WPI. Furthermore, patients with SLE and anti-GluN2B antibodies were more likely to have concomitant FM and had higher WPI scores. Considering that FM is more prevalent in patients with SLE than in the general population and that the NMDAR system is involved in chronic pain disorders, including FM, an association between anti-GluN2 antibodies and FM in these patients seems plausible. One hypothesis is that anti-GluN2B antibodies stimulate NMDARs, causing pain and eventually FM in patients with SLE. The mechanism of central sensitisation in nociception is mediated mainly by NMDAR activation, and abnormal NMDAR activation results in the prolongation of nociceptive transmission to the CNS, which is inhibited by γ-aminobutyric acid (GABA)-A under physiological conditions (12). Multiple lines of evidence have implicated the GluN2B subunit of NMDAR in pain mechanisms. Mouse studies have suggested that GluN2B-containing NMDARs are particularly important for the regulation of nociception in pain transmission (24-25). Human studies have demonstrated that selective GluN2B antagonists have effective anti-nociceptive activity, with reduced side effects, compared with non-selective NMDA antagonists, which emphasises the participation of the GluN2B subunit in chronic pain (26-27). In support of these data, low-dose ketamine, which is a relatively selective and potent antagonist of GluN2B, has an analgesic effect in several pain disorders, reducing central sensitisation and the blockade of the peripheral NMDAR contribution to pain (28). Based on these findings, although understanding of the exact mechanisms underlying our observations requires further investigation, anti-GluN2B antibodies may be associated with direct stimulation of NMDARs in relation to the occurrence of FM in SLE.

Other possible explanations of the role(s) of anti-GluN2B antibodies include the following. Anti-GluN2B antibodies may be related to a disinhibition phenomenon in a way that antagonises NMDARs of presynaptic GABA-containing neurons. NMDARs have also been implicated in the modulation of GABA release in the CNS (29). Thus, anti-GluN2B antibody–mediated suppression of inhibitory GABAergic inputs, which constitute the descending analgesic pathway, may eventually cause pain hypersensitivity (30). Additionally, this blockade of GABA inhibitory mechanisms may lead to direct release of glutamate, causing central pain sensitisation (31). A recent study showed that GABA levels in the right anterior insula of the brain are significantly lower in patients with FM (32). In this context, anti-GluN2B antibodies may be associated indirectly with FM in patients with SLE through blockade of GABA inhibition.

In the present study, serum titres of anti-GluN2B antibody did not differ significantly between patients with FM and healthy controls. Although FM is generally regarded as a non-autoimmune disease, affected patients have several symptoms, such as morning stiffness, arthralgia, and Raynaud's phenomenon, that are observed frequently in patients with autoimmune diseases. Thus, many investigations have been performed to discover immunological markers for the detection of FM or assessment of its symptom severity. However, those studies did not produce consistent results (33). Likewise, in contrast to the occurrence of FM in SLE, anti-GluN2B antibody was not a reliable marker of pure FM without SLE in our study. These findings suggest that different pathophysiologies are involved in pain hypersensitivity in FM and SLE, although similar clinical phenotypes appear in patients with FM with and without SLE. The pathogenesis of FM occurring in SLE may be related to an antibody-mediated autoimmune mechanism. These findings may provide a scientific basis explaining the higher prevalence of FM in patients with SLE.

Anti-GluN2B antibodies were associated significantly with NPSLE. In our study, 16 (15.4%) patients with SLE had neuropsychiatric symptoms, and 5 (38.5%) of these patients had elevated serum levels of anti-GluN2B antibodies. To date, the association between anti-NMDAR antibodies and NPSLE

remains unclear. Anti-NMDAR antibodies, including anti-GluN2B, have been evaluated in the pathogenesis of NPSLE (10, 11, 34). Although such an association with NPSLE was shown more strongly in cerebrospinal fluid (CSF) anti-NMDAR antibodies than in serum antibodies(10, 11), researchers have shown that serum anti-NMDAR antibodies can also be associated with neuropsychiatric complications in SLE (35-36). Gono *et al*. (35) showed that the frequency of NPSLE was significantly higher ($p$=0.002) in patients with SLE with serum anti-NMDAR antibodies. We also found that serum anti-GluN2B antibodies were associated independently with NPSLE. However, we should consider that anti-NMDAR antibodies, in our study, were some different types of anti-NMDAR antibodies in the previous researches regarding NPSLE. Those antibodies are a subset of anti-dsDNA antibodies that cross react with peptide sequences (DWEYSVWLSN) that is also found in the NMDAR GluN2 subunits (10, 11, 35, 36). As mentioned in the methods, we evaluated anti-NMDAR antibody which binds to peptide sequence from the GluN2B subunit; KERKWERVGKWKDK (22, 23), which have not been previously evaluated in the pathogenesis of NPSLE. Nevertheless, our study provides further evidence supporting the involvement of NMDAR systems in the pathogenesis of SLE neuropsychiatric symptoms.

Our study had several strengths. A large number of subjects were included in each group to establish a clear association between anti-NMDAR antibodies and FM. A comprehensive and systematic evaluation of FM and neuropsychiatric symptoms was used to establish a relationship with anti-GluN2B antibodies. However, this study also had several limitations. First, the association between anti-GluN2B antibodies and FM pain severity was relatively weak. This finding indicates that other mechanisms are also involved in the pathogenesis of FM in patients with SLE. Nonetheless, we believe that this does not weaken the value of our findings. However, more research is needed to fully understand the mechanism of pain hypersensitivity in patients with SLE. Second, anti-GluN2B antibodies were not measured in the CSF. Based on previous reports of intrathecal IgG production (37-38), anti-NMDAR antibodies may be synthesised in the CSF. GluN2B antibodies from the CSF are likely to have a strong relationship with FM and NPSLE symptom severity. However, serum anti-NMDAR antibodies produced extrathecally may also influence brain function. Yoshiro *et al*. (11) demonstrated a positive relationship between CSF and serum anti-NMDAR antibody titres. This finding supports the possibility that anti-NMDAR antibodies cross the blood–brain barrier (BBB) into the CSF. A murine-model study showed that anti-NMDAR antibodies could cross a BBB compromised by lipopolysaccharides. Anti-NMDAR antibodies outside the brain are capable of causing neuronal damage and cognitive dysfunction (39).

In conclusion, our study provided important insights into NMDAR involvement in the pathogenesis of FM occurring in SLE. Patients with SLE and anti-GluN2B antibodies were more sensitive to pain and were more likely to have concomitant FM. Based on our findings, inhibition of serum anti-GluN2B antibodies might alleviate pain symptoms related to FM in SLE. However, our findings call for further studies to explore the cellular and synaptic mechanisms of anti-GluN2B antibodies in the development of FM with SLE.

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