Research article

An exploratory investigation of antibodies to NMDA-type glutamate receptor subunits in serum and cerebrospinal fluid among psychiatric patients with anti-thyroid antibodies

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A B S T R A C T

Introduction: Hashimoto's thyroiditis, which is characterized by anti-thyroid antibodies such as the anti-thyroglobulin (Tg) antibody and anti-thyroid peroxidase (TPO) antibody, is one of the autoimmune diseases associated with psychiatric illnesses. We previously reported a high prevalence of antibodies to N-terminals of N-methyl-D-aspartate (NMDA) type glutamate receptor (GluR) subunits (GluN1-NT and GluN2B-NT2) among psychiatric patients with anti-thyroid antibodies. However, it remains unclear whether the presence of anti-thyroid antibodies influences antibodies to GluN1-NT or GluN2B-NT2 among psychiatric patients. The present study aims to examine antibodies to GluN1-NT and GluN2B-NT2 in psychiatric patients with anti-thyroid antibodies (PPATs) and in those without (non-PPATs).

Material and methods: We recruited psychiatric inpatients aged 20–60 years. Patients were excluded if they had a history of neurological diseases, dementia, developmental disorders, tumors, or autoimmune diseases except autoimmune thyroiditis. The rest of the participants were divided into two groups according to the presence of serum anti-Tg and anti-TPO antibodies. We investigated serum and cerebrospinal fluid (CSF) antibodies to GluN1-NT and GluN2B-NT2 using an enzyme-linked immunosorbent assay (ELISA).

Results: We initially recruited seventy-three psychiatric inpatients. Forty-six patients were excluded because of the exclusion criteria. Eighteen PPATs and nine non-PPATs were ultimately enrolled. We also collected stored sera of eighteen healthy controls (HCs) who were age- and sex-matched with PPATs. The optical densities (ODs) of serum antibodies to GluN1-NT ($p = 0.0020$) and GluN2B-NT2 ($p = 0.039$) were significantly higher in PPATs than in HCs. The ODs of CSF antibodies to GluN1-NT ($p = 0.030$) and GluN2B-NT2 ($p = 0.017$) as well as the positive ratios of those antibodies were significantly higher in PPATs than in non-PPATs.

Conclusion: Our finding indicates that detecting anti-thyroid antibodies in psychiatric patients would be a clue to consider psychiatric conditions related to antibodies to GluN1-NT/GluN2B-NT2. Further studies focusing on the relationship between PPATs and antibodies to GluN1-NT/GluN2B-NT2 are needed.

1. Introduction

Autoimmunity in psychiatric illnesses has recently been being increasingly investigated. To date, a variety of neuronal autoantibodies have been reported to be associated with psychiatric illnesses [1, 2]. Although the diagnostic approach of antibody-associated psychiatric illnesses has not yet been established, Psychoimmunology Expert (PIE) Meeting, which is Immunology and Psychiatry Section of World Psychiatric Association, posted the position paper to propose clinical criteria

of autoimmune psychosis, for use in psychiatric practice [1]. According to the criteria, even patients with isolated psychotic presentations who have tested positive for neuronal autoantibodies such as antibodies to N-methyl-D-aspartate (NMDA) type glutamate receptor (GluR) could be diagnosed as autoimmune psychosis. Recent studies indicated the association of several types of antibodies to GluR subunit or subunits with various psychiatric illnesses, including schizophrenia, bipolar disorder, and depressive disorder [2, 3, 4]. However, studies focusing on neuronal antibodies in patients with psychiatric disorders have largely been

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restricted to serum samples, and CSF studies are rare [1]. Therefore, cumulating investigations using neuronal antibodies in CSF samples and targeting more relevant groups of psychiatric patients would add more useful evidence into this developing field.

PIE arouses the attention of features for suspicion of autoimmune encephalitis in patients with psychosis, called red flags, such as infectious prodrome, focal neurological disease, seizures, presence of a tumor, and other autoimmune diseases including autoimmune thyroid disease. Previous studies indicated a high prevalence of autoimmune diseases among patients with psychiatric illnesses [5]. Hashimoto's thyroiditis (HT), which is thyroiditis characterized by anti-thyroid antibodies such as the anti-thyroglobulin (Tg) antibody and anti-thyroid peroxidase (TPO) antibody, is one of the autoimmune diseases regularly associated with psychiatric illnesses [5, 6, 7]. Anti-thyroid antibodies are frequently positive in individuals with normal thyroid function. The prevalence rates of positive anti-Tg and anti-TPO antibodies among the disease-free population are 10 and 11%, respectively [8]. Recent studies indicated that anti-thyroid antibodies in euthyroid individuals were associated with psychiatric illnesses [6, 7]; however, the underlying mechanisms remain unclear. Cai et al. [9] detected neuroinflammation as well as psychiatric symptoms in a euthyroid HT mouse model. HT may be co-morbid with autoimmune encephalitis, called Hashimoto's encephalopathy, which exhibits various presentations, including isolated psychiatric symptoms [10]. These findings imply that not only hormonal but also neuro-immunological abnormalities contribute to the relationship between psychiatric illnesses and HT.

We previously reported a high prevalence of antibodies to N-terminals of NMDA-type GluR subunits (GluN1-NT and GluN2B-NT2) among psychiatric patients with anti-thyroid antibodies [11, 12]. However, it remains unclear whether the presence of anti-thyroid antibodies influences antibodies to GluN1-NT and GluN2B-NT2 among psychiatric patients. The present study aims to examine antibodies to GluN1-NT and GluN2B-NT2 in psychiatric patients with anti-thyroid antibodies (PPATs) and in those without (non-PPATs).

2. Materials and methods

2.1. Study setting and subjects

We recruited psychiatric inpatients aged 20–60 years admitted to the Department of Psychiatry at Yokohama City University Hospital between 2011 and 2016. All the participants have severe or treatment-resistant psychiatric symptoms, who were tested for the presence of antibodies to NMDA-type GluRs. They also underwent clinical examinations, blood tests, cerebrospinal fluid (CSF) tests, and neuroimaging studies; magnetic resonance imaging (MRI) or computed tomography (CT) of the head to differentiate organic diseases. Patients were excluded if they had a history of neurological diseases, dementia, developmental disorders, tumors, or autoimmune diseases, except autoimmune thyroiditis.

We also collected the sera of healthy controls (HCs) stored in the biobank of Yokohama City University School of Medicine, Yokohama, Japan. The health states of HCs were evaluated based on self-reported data. Additionally, we obtained the data of CSF antibodies to GluN1-NT and GluN2B-NT2 of 20–60 year-old patients with anti-NMDA receptor encephalitis (NMDAREs) from Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan. The diagnosis of NMDAREs was confirmed by the CSF anti-NMDA receptor antibodies measured by cell-based assays according to a previous study [13].

Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Ethics Committee of Yokohama City University Hospital (B140703003) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Assessment of subjects

Trained psychiatrists interviewed all psychiatric inpatients and assessed their previous and present psychiatric symptoms. A psychiatric diagnosis, the presence of catatonia and psychosis, and the Global Assessment of Functioning (GAF) were assessed based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR). All PPATs and non-PPATs underwent blood and CSF tests. The CSF/serum IgG quotient (Q_{IgG}), CSF/serum albumin quotient (Q_{alb}), and IgG index were calculated according to the following formulas: Q_{alb} = CSF albumin/serum albumin; Q_{IgG} = CSF IgG/serum IgG; IgG index = Q_{IgG}/Q_{alb}.

We analyzed the results of technetium-99 ethyl cysteinate dimer single photon emission computed tomography (ECD-SPECT) ECD-SPECT using a three-dimensional stereotaxic region-of-interest template (3DSRT) to measure regional cerebral blood flow (rCBF) [14]. 3DSRT is composed of 12 segments (callosomarginal, precentral, central, parietal, angular, temporal, posterior cerebral, pericallosal, lenticular nucleus, thalamus, hippocampus, and cerebellum) on each side.

2.3. Evaluation of antibodies to NMDA-type GluRs (ELISA)

We investigated antibodies to NMDA-type GluR subunits (GluN1-NT and GluN2B-NT2), using an enzyme-linked immunosorbent assay (ELISA) according to previous studies [15, 16]. Peptides encoding the N-terminal domains of GluN1 (GenBank accession number Q05586) and GluN2B (GenBank accession number U88963) were synthesized. Their sequences were QKRLETLLEERESK (AA177-190) for the N-terminal of GluN1 and KERKWERVGKWKDK (AA369-382) for the N-terminal of GluN2B. MaxiSorp plates (#468667, Nalge Nunc International) were coated overnight with the 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) or CSF (100 μL, undiluted) was then incubated at 37 °C for 2 h. After washing with PBST, plates were incubated with a 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. We also calculated the CSF/serum ratios of the ODs of antibodies to GluN1-NT and GluN2B-NT2.

2.4. Statistical analysis

Continuous variables were compared using a two-tailed t-test or one-way analysis of variance (ANOVA) followed by the Tukey test. Categorical variables were compared using the Fisher's exact test. P-values were corrected for multiple testing using the Benjamini-Hochberg method. The significance of differences was defined as P < 0.05. We assessed the ODs of CSF antibodies to GluN1-NT and GluN2B-NT2 using receiver operating characteristic (ROC) curve analysis. Youden index was used to identify the optimum cut-off value. The diagnostic performance was assessed by the area-under-curve (AUC).

3. Results

We initially recruited seventy-three psychiatric inpatients, whose diagnoses consisted of thirty-four mood disorders, twenty-seven schizophrenia and related disorders, eleven cognitive disorders, and one somatoform disorder. Anti-Tg and anti-TPO antibodies were positive in 31/73 (42.5%) and 30/73 (41.1%), respectively. Thirty-nine patients (53.4%) have either of the anti-thyroid antibodies. Forty-six patients were excluded because of the exclusion criteria. Most of the excluded individuals had autoimmune comorbidities, such as systemic lupus erythematosus (n = 13), Behcet's disease (n = 6), and Sjogren's syndrome (n = 3). Twenty-seven subjects, who were divided into two groups according to the presence of serum anti-Tg or anti-TPO antibodies, were

ultimately enrolled: eighteen PPATs and nine non-PPATs. None of PPATs and non-PPATs had taken immunotherapy before the present study. Five PPATs took oral levothyroxine. All PPATs and non-PPATs underwent head MRI or CT; seventeen PPATs and eight non-PPATs underwent head MRI; one PPATs and one non-PPATs underwent head CT. The results of head MRI or CT of PPATs and non-PPATs were all normal.

The demographic data of PPATs and non-PPATs are shown in Table 1. No significant differences were observed in demographic data between PPATs and non-PPATs. Seventeen PPATs and nine non-PPATs underwent electroencephalography (EEG). Two PPATs and one non-PPATs showed spike-waves, and one non-PPATs showed paroxysmal slow waves. None of those with abnormal EEG findings had any history of seizures. Fifteen PPATs and eight non-PPATs underwent ECD-SPECT. The results of ECD-SPECT are shown in Table 2. Each rCBF of PPATs was not significantly different from that of non-PPATs.

Comparisons of serum antibodies to GluN1-NT and GluN2B-NT2 are shown in Table 3. No significant differences were observed in serum antibodies to GluN1-NT and GluN2B-NT2 between PPATs and non-PPATs. We then collected eighteen sera of HCs who were age- and sex-matched with PPATs. The ODs of serum antibodies to GluN1-NT and GluN2B-NT2 in PPATs, non-PPATs, and HCs were compared using a one-way ANOVA followed by the Tukey test. The ODs of serum antibodies to GluN1-NT in PPATs, non-PPATs, and HCs were 0.518 ± 0.133, 0.475 ± 0.126, and 0.381 ± 0.077, while those of serum antibodies to GluN2B-NT2 were 0.463 ± 0.136, 0.458 ± 0.131, and 0.366 ± 0.073, respectively (means ± SD). The results of a one-way ANOVA indicated significant differences in serum antibodies to GluN1-NT (p = 0.0026) and GluN2B-NT2 (p = 0.032) among PPATs, non-PPATs, and HCs. According to the Tukey test, serum antibodies to GluN1-NT were significantly higher in PPATs than in HCs (p = 0.0020, Figure 1a). No significant differences were observed in antibodies to GluN1-NT between PPATs and non-PPATs (p = 0.61) or between non-PPATs and HCs (p = 0.11). Serum antibodies to GluN2B-NT2 were also significantly higher in PPATs than in HCs (p = 0.039, Figure 1b). There were no significant differences in serum antibodies to GluN2B-NT2 between

PPATs and non-PPATs (p = 0.99) or between non-PPATs and HCs (p = 0.13).

Serum antibodies to GluN1-NT and GluN2B-NT2 were regarded as positive when their ODs were higher than the mean+2SD of those of HCs. The positive ratios of serum antibodies to GluN1-NT in PPATs and non-PPATs were 10/18 (55.6%) and 3/9 (33.3%), while those of serum antibodies to GluN2B-NT2 were 4/18 (22.2%) and 3/9 (33.3%), respectively. No significant differences were observed in the positivity of serum antibodies to GluN1-NT and GluN2B-NT2 between PPATs and non-PPATs.

The ODs of CSF antibodies to GluN1-NT were significantly higher in PPATs than in non-PPATs (p = 0.030). The CSF/serum ratio of antibodies to GluN1-NT was also significantly higher in PPATs than in non-PPATs (p = 0.021). Similarly, CSF antibodies to GluN2B-NT2 and the CSF/serum ratio were significantly higher in PPATs than in non-PPATs (p = 0.017, 0.048, respectively).

Then, we collected the CSF data of fourteen NMDAREs (5 male, 9 female) whose mean age was 32.8 ± 9.0. The ODs of CSF antibodies to GluN1-NT and GluN2B-NT2 of NMDAREs were 1.251 ± 0.656 and 1.097 ± 0.641, respectively (means ± SD). We performed ROC curve analysis to estimate the cut-off values of ODs of CSF antibodies to GluN1-NT and GluN2B-NT2 using CSF data of NMDAREs and those of non-PPATs (Figure 2), in which NMDAREs were regarded as positive controls. The AUC for CSF antibodies to GluN1-NT and GluN2B-NT2 were 0.865 (95% CI: 0.707–1.000) and 0.905 (95% CI: 0.780–1.000), respectively. From the Youden index, the optimal cut-off values of CSF antibodies to GluN1-NT and GluN2B-NT2 were 0.537 and 0.453, respectively. According to those cut-off values, the positive ratio of CSF antibodies to GluN1-NT among PPATs was 100% (18/18), which was significantly higher than that of non-PPATs 33.3% (3/9) (p = 0.00028, Figure 3a). The positive ratios of CSF antibodies to GluN2B-NT2 of PPATs was also significantly higher than that of non-PPATs (88.9% and 33.3%, respectively, p = 0.0061, Figure 3b).

We found no significant associations between antibodies to GluN1-NT/GluN2B-NT2 and sex, catatonia, psychosis, or GAF among PPATs or non-PPATs (Table 4).

Table 1. Demographic data of PPATs and Non-PPATs.

	PPATs (n = 18)	Non-PPATs (n = 9)	P
Age, mean ± SD	42.7 ± 11.8	34.7 ± 12.7	0.12
Male (%)	3/18 (16.7)	4/9 (44.4)	0.18
Female (%)	15/18 (83.3)	5/9 (55.6)	0.18
Duration of illness, mean ± SD	12.6 ± 10.1	6.9 ± 6.7	0.14
GAF, mean ± SD	24.9 ± 12.8	27.9 ± 8.9	0.53
Psychiatric symptoms			
Psychosis (%)	13/18 (72.2)	7/9 (77.8)	1.00
Catatonic features (%)	1/18 (5.6)	2/9 (22.2)	0.25
Thyroid function			
TSH (μIU/mL), mean ± SD	4.72 ± 4.37	3.00 ± 3.45	0.31
FT3 (pg/mL), mean ± SD	2.48 ± 0.52	2.69 ± 0.65	0.37
FT4 (ng/dL), mean ± SD	1.22 ± 0.27	1.09 ± 0.18	0.18
CRP (mg/dL)	0.68 ± 1.51	0.13 ± 0.17	0.29
EEG abnormalities (%)	2/17 (11.8)	2/9 (22.2)	0.59
CSF Findings			
Cells (/μL)	2.4 ± 3.2	2.9 ± 3.3	0.74
Total Protein (mg/dL)	43.7 ± 25.5	36.6 ± 12.0	0.44
Glucose (mg/dL)	62.1 ± 10.6	59.0 ± 5.0	0.41
IgG (mg/dL)	4.2 ± 2.5	3.6 ± 2.0	0.59
Q_{alb} (x10^{-3})	5.9 ± 3.4	4.9 ± 1.8	0.42
Q_{IgG} (x10^{-3})	3.8 ± 2.7	3.3 ± 1.8	0.65
IgG index	0.61 ± 0.12	0.68 ± 0.33	0.39

Age and duration of illness are expressed in years.
Abbreviations: PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies; GAF, global assessment of functioning; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; CRP, C-reactive protein; EEG, electroencephalography.

Table 2. Regional cerebral blood flow of PPATs and Non-PPATs.

Region	PPATs (n = 15)	Non-PPATs (n = 9)	P
Left Callosomarginal	47.1 ± 6.6	45.6 ± 6.7	0.60
Right Callosomarginal	47.4 ± 6.8	45.7 ± 6.9	0.56
Left Precentral	47.2 ± 7.1	44.7 ± 6.3	0.39
Right Precentral	48.0 ± 7.4	45.2 ± 6.4	0.35
Left Central	47.7 ± 6.8	46.3 ± 5.0	0.60
Right Central	47.4 ± 6.6	45.3 ± 5.3	0.41
Left Parietal	49.3 ± 8.4	45.9 ± 5.6	0.29
Right Parietal	50.3 ± 8.5	46.4 ± 5.7	0.24
Left Angular	53.9 ± 9.4	48.4 ± 5.8	0.12
Right Angular	54.4 ± 9.0	47.4 ± 6.8	0.06
Left Temporal	46.1 ± 6.4	43.6 ± 5.2	0.34
Right Temporal	47.3 ± 6.6	44.4 ± 6.0	0.30
Left Posterior Cerebral	57.0 ± 7.2	52.9 ± 6.0	0.16
Right Posterior Cerebral	57.5 ± 7.7	53.3 ± 6.2	0.18
Left Pericallosal	56.0 ± 8.5	52.8 ± 7.3	0.36
Right Pericallosal	56.4 ± 8.6	53.4 ± 7.0	0.38
Left Lenticular Nucleus	56.0 ± 6.8	52.6 ± 8.3	0.29
Right Lenticular Nucleus	56.7 ± 8.1	52.8 ± 9.0	0.29
Left Thalamus	51.0 ± 6.3	47.6 ± 7.4	0.24
Right Thalamus	52.8 ± 7.2	48.9 ± 4.6	0.16
Left Hippocampus	43.3 ± 4.8	42.0 ± 5.6	0.56
Right Hippocampus	42.8 ± 5.3	41.3 ± 5.6	0.52
Left Cerebellum	63.5 ± 8.4	57.5 ± 8.3	0.10
Right Cerebellum	64.9 ± 8.6	58.6 ± 8.8	0.10

Data are expressed as means ± SD (mL/100 g/min).
Abbreviations: PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies.

Table 3. Comparison of antibodies to GluN1-NT and GluN2B-NT2 (Means ± SD) between PPATs and Non-PPATs.

	PPATs (n = 18)	Non-PPATs (n = 9)	P	Pcorr
Serum GluN1-NT	0.518 ± 0.133	0.475 ± 0.126	0.42	0.51
Serum GluN2B-NT2	0.463 ± 0.136	0.458 ± 0.131	0.93	0.93
CSF GluN1-NT	0.867 ± 0.267	0.558 ± 0.277	0.0099	0.030*
CSF GluN2B-NT2	0.749 ± 0.312	0.429 ± 0.224	0.011	0.017*
CSF/serum GluN1-NT	1.751 ± 0.578	1.162 ± 0.373	0.010	0.021*
CSF/serum GluN2B-NT2	1.732 ± 0.832	0.907 ± 0.261	0.0080	0.048*

Abbreviations: PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies; pcorr, p-values corrected for multiple testing using the Benjamini-Hochberg method.
* Significantly different.

4. Discussion

The results of the present study showed significantly higher ODs of serum antibodies to GluN1-NT and GluN2B-NT2 in PPATs than in HCs. Additionally, ODs and the positive ratios of CSF antibodies to GluN1-NT and GluN2B-NT2 were significantly higher in PPATs than in non-PPATs. Siegmann et al. revealed an increased prevalence of depression and anxiety disorders among patients with autoimmune thyroiditis [7]. They insisted on the importance of a test for not only thyroid hormone but also anti-thyroid antibodies among psychiatric patients, not to miss autoimmune thyroiditis. Our finding suggests an additional meaning of detecting anti-thyroid antibodies in psychiatric patients. Recent studies figured out a high positivity of several types of antibodies to GluR among psychiatric patients, which might indicate that their psychiatric conditions are due to the antibodies to GluR [2, 3, 4]. However, the specific features to suspect the presence of antibodies to GluR among psychiatric patients have not yet been found. Our finding suggests that the presence of anti-thyroid antibodies in psychiatric patients may help clinicians consider psychiatric conditions related to antibodies to GluR.

Antibodies to thyroid and GluR have both been implicated in multiple illnesses. HT may be concurrent with autoimmune diseases, such as Sjogren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis [17, 18]. Antibodies to GluR may be present in patients with tumors [19], autoimmune diseases, including Sjogren's syndrome and systemic lupus erythematosus [20], and neurodegenerative diseases [15]. The present study excluded patients with tumors, neurological diseases, and autoimmune diseases, except for autoimmune thyroiditis, to rule out the influence of these illnesses. Therefore, the present results suggest that the presence of anti-thyroid antibodies in psychiatric patients is independently linked to antibodies to GluN1-NT and GluN2B-NT2.

Although the positive ratios of CSF antibodies to GluN1-NT and GluN2B-NT2 significantly differed between PPATs and non-PPATs, those of serum antibodies to GluN1-NT and GluN2B-NT2 did not. This discrepancy might be attributed to the small sample size and/or lower prevalence of both antibodies in the serum than in the CSF. We previously reported that the prevalence of antibodies to GluN2B-NT2 was significantly higher in the CSF than in the serum of PPATs [11]. In the
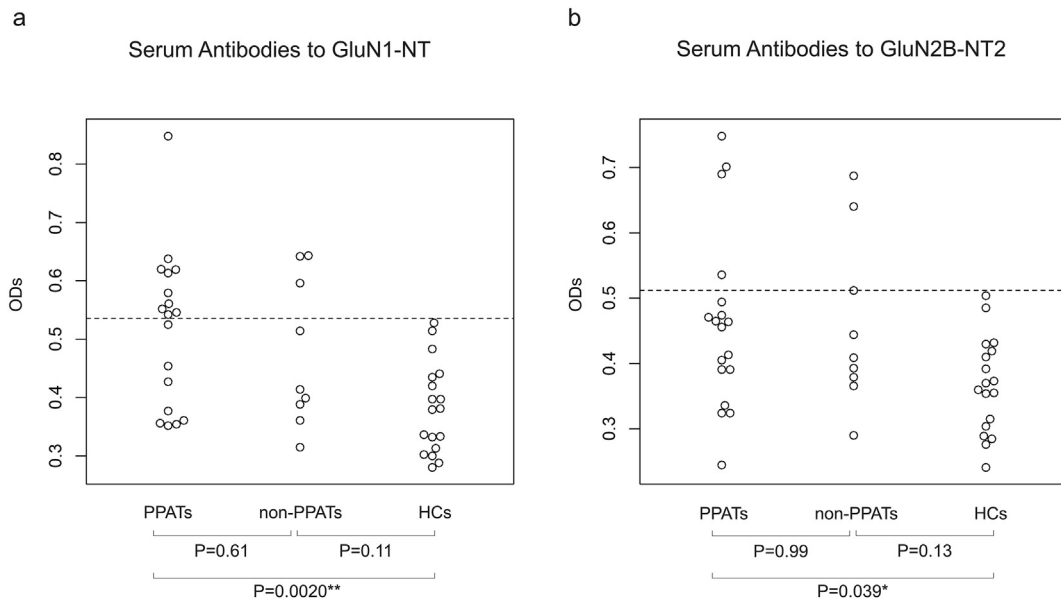
a

Serum Antibodies to GluN1-NT

b

Serum Antibodies to GluN2B-NT2

Figure 1. Comparison of Serum Antibodies to GluN1-NT and GluN2B-NT2. The ODs of serum antibodies to GluN1-NT and GluN2B-NT2 were compared using a one-way analysis of variance (ANOVA) followed by the Tukey test. The ODs of serum antibodies to GluN1-NT (a) and GluN2B-NT2 (b) were significantly higher in PPATs than in HCs. The dashed lines indicate cut-off values. Abbreviations: ODs, Optical densities; PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies; HCs, healthy controls. *p < 0.05, **p < 0.01.

a

CSF Antibodies to GluN1-NT
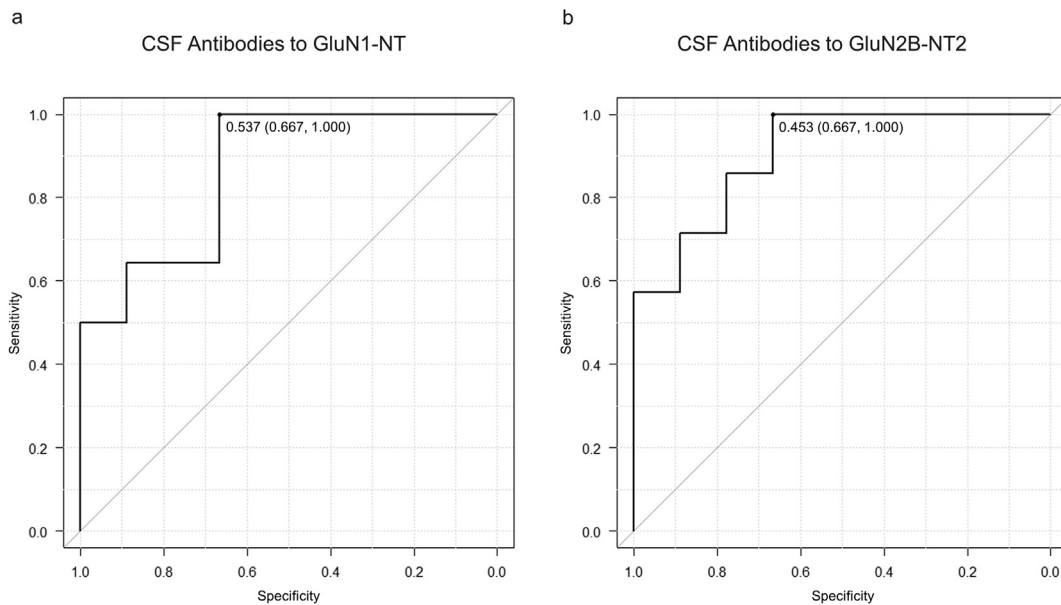
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CSF Antibodies to GluN2B-NT2

Figure 2. ROC Curves for CSF Antibodies to GluN1-NT and GluN2B-NT2. ROC curves for CSF antibodies to GluN1-NT (a) and GluN2B-NT2 (b) for discriminating NMDAREs from non-PPATs. A cut-off values of CSF antibodies of GluN1-NT and GluN2B-NT2 are 0.537 (specificity: 66.7%, sensitivity: 100.0%) and 0.453 (specificity: 66.7%, sensitivity: 100.0%), respectively. Abbreviations: ROC, Receiver-operating characteristic; non-PPATs, psychiatric patients without anti-thyroid antibodies; NMDAREs; anti-NMDA receptor encephalitis patients.

case of anti-NMDA receptor encephalitis, antibodies to GluR are more prevalent in the CSF than in the serum, which indicated the intrathecal synthesis of these antibodies [19]. On the other hand, antibodies to GluR in the CSF may be derived from the blood due to a dysfunctional blood-brain barrier (BBB) [21]. We evaluated BBB dysfunctions using Q_{alb} [22], and the results obtained showed no significant difference between PPATs and non-PPATs. The intrathecal synthesis of total IgG was estimated based on Q_{IgG} and the IgG index [22], which did not significantly differ between the groups. The CSF/serum ratios of antibodies to GluN1-NT and GluN2B-NT2 were significantly higher in PPATs than in non-PPATs, which indicates that these antibodies were elevated more

predominantly in the CSF than in the serum of PPATs. These results suggest that the presence of anti-thyroid antibodies is associated with the intrathecal synthesis of antibodies to GluN1-NT and GluN2B-NT2 rather than BBB dysfunctions in psychiatric patients.

We measured cerebral perfusion in PPATs and non-PPATs to evaluate brain function. Abnormalities in cerebral perfusion were frequently detected, even in euthyroid HT patients [23]. According to the study by Hardoy et al., patients with HT and depressive disorder may have a specific abnormality in CBF [24]. Conversely, the results of ECD-SPECT showed no significant differences in rCBF between PPATs and non-PPATs in the present study. We included multiple psychiatric
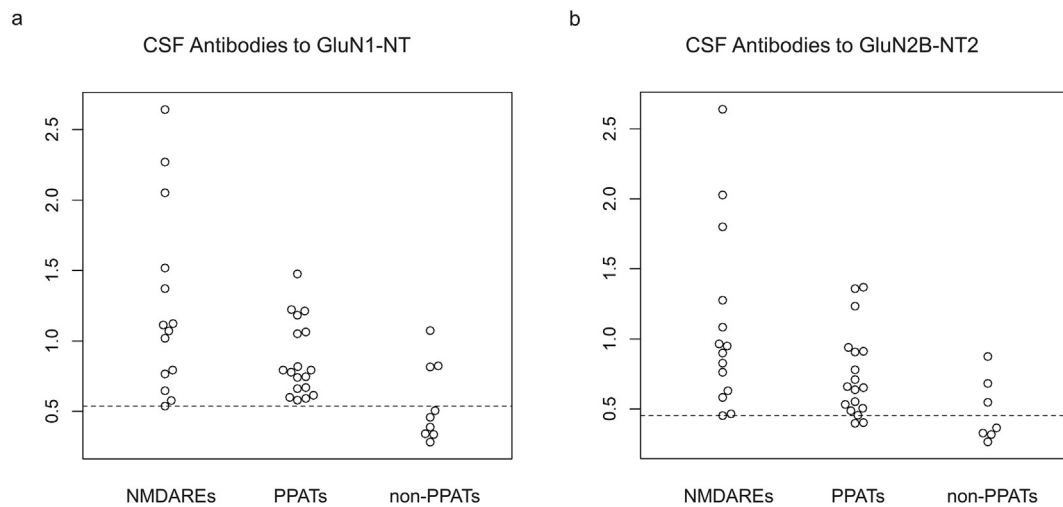
a

CSF Antibodies to GluN1-NT

b

CSF Antibodies to GluN2B-NT2

Figure 3. CSF Antibodies to GluN1-NT and GluN2B-NT2. The ODs of CSF antibodies to GluN1-NT (a) and GluN2B-NT2 (b) among NMDAREs, PPATs, and non-PPATs, with dashed lines indicating cut-off values. Abbreviations: ODs, Optical densities; NMDAREs, anti-NMDA receptor encephalitis patients; PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies.

illnesses, while Hardoy et al. exclusively researched depressive disorder. This difference may have influenced the results obtained herein.

The present study has some limitations. First, the subjects in the present study have heterogeneous psychiatric disorders. We considered it difficult to focus on specific mental disorders because antibodies to GluR could be detected among patients with multiple psychiatric disorders such as schizophrenia, depressive disorder, bipolar disorder, and neurotic disorder [2, 3, 4]. Second, the assessment method of cut-off values of CSF antibodies to GluN1-NT and GluN2B-NT2 were different from those of serum antibodies to GluN1-NT and GluN2B-NT2. Due to the

Table 4. Patient characteristics and positivity of antibodies to GluN1-NT and GluN2B-NT2 (serum/CSF).

No.	Sex/Age	Psychiatric Diagnosis	GAF	Psy.	Cat.	GluN1-NT	GluN2B-NT2
PPATs							
1	F/27	Schizophrenia	30	+	-	+/+	+/-
2	F/28	Major depressive disorder	30	-	-	+/+	+/+
3	F/30	Major depressive disorder	55	+	-	+/+	−/+
4	F/30	Major depressive disorder with anorexia nervosa	20	-	-	−/+	−/+
5	M/30	Schizophrenia	5	+	-	+/+	+/+
6	F/33	Schizophrenia	20	+	-	+/+	−/+
7	F/38	Schizophrenia	30	+	-	−/+	−/+
8	F/39	Major depressive disorder	10	+	-	+/+	−/+
9	F/42	Schizoaffective disorder	40	+	-	+/+	+/+
10	F/42	Schizophrenia	30	+	-	−/+	−/+
11	F/44	Schizophrenia	5	+	-	+/+	−/+
12	F/46	Schizophrenia	21	+	-	+/+	−/+
13	M/48	Major depressive disorder	30	+	-	−/+	−/+
14	F/57	Major depressive disorder	40	-	-	−/+	−/+
15	F/58	Major depressive disorder	21	-	-	−/+	−/+
16	F/59	Major depressive disorder	25	-	-	−/+	−/+
17	F/59	Schizophrenia	25	+	-	−/+	−/+
18	M/59	Schizophrenia	11	+	+	+/+	−/−
Non-PPATs							
19	M/22	Schizophrenia	35	+	-	−/−	−/−
20	F/23	Schizophrenia	21	+	+	+/-	−/−
21	F/25	Schizophrenia	30	+	-	+/+	+/+
22	F/28	Schizophrenia	11	+	-	−/+	+/+
23	F/30	Schizophrenia	31	+	-	−/−	−/−
24	F/37	Bipolar disorder (depressive phase)	31	-	-	−/−	−/−
25	M/38	Schizophrenia	21	+	+	+/+	+/+
26	M/50	Major depressive disorder	41	-	-	−/−	−/−
27	M/59	Major depressive disorder	30	+	-	−/−	−/−

Abbreviations: PPATs, psychiatric patients with anti-thyroid antibodies; non-PPATs, psychiatric patients without anti-thyroid antibodies; Psy., Psychosis; Cat., Catatonic features.

lack of normal CSF samples, we estimated the cut-off values of CSF antibodies to GluN1-NT and GluN2B-NT2 using the CSF results of non-PPATs as negative controls. Although CSF anti-NMDA receptor antibodies of NMDAREs were detected by cell-based assays, those of non-PPATs were not investigated in the same way. The CSF data of normal healthy individuals who tested negative for anti-NMDA receptor antibodies by cell-based assays are needed to assess their cut-off values precisely. Third, we only examined antibodies to GluR, although multiple anti-neuronal antibodies are considered to be associated with psychiatric illnesses. Schou et al. detected not only antibodies to GluR but also other anti-neuronal antibodies, including contactin-associated protein-like 2 antibodies, glutamic acid decarboxylase-65 antibodies, and α-amino-3--hydroxy-5-methyl-4-isoxazolepropionic acid receptor antibodies among psychiatric patients [2]. These anti-neuronal antibodies may be associated with PPATs. Future studies should focus on the co-occurrence of PPATs and anti-GluR antibodies and/or the other anti-neuronal antibodies in the serum and CSF.

5. Conclusion

The present results revealed higher titers of serum antibodies to GluN1-NT and GluN2B-NT2 in PPATs than in HCs. The titers and the positive ratios of CSF antibodies to GluN1-NT and GluN2B-NT2 were higher in PPATs than in non-PPATs. Our finding indicates that detecting anti-thyroid antibodies in psychiatric patients would be a clue to consider psychiatric conditions related to antibodies to GluN1-NT/GluN2B-NT2. The relationship between antibodies to GluN1-NT/GluN2B-NT2, anti-thyroid antibodies, and psychiatric illnesses should be further researched.

Declarations

Author contribution statement

T. Saito and Y. Chiba: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

K. Abe, S. Hattori, O. Katsuse and A. Suda: Conceived and designed the experiments; Performed the experiments.

Y. Takahashi: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] T.A. Pollak, B.R. Lennox, S. Müller, et al., Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin, The Lancet Psychiatry 7 (2020) 93–108.

[2] M. Schou, S.G. Sæther, K. Borowski, et al., Prevalence of serum anti-neuronal autoantibodies in patients admitted to acute psychiatric care, Psychol. Med. 46 (2016) 3303–3313.

[3] J. Steiner, M. Walter, W. Glanz, et al., Increased prevalence of diverse N-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from N-methyl-D-aspartate glutamate receptor encephalitis, JAMA Psychiatry. 70 (2013) 271–278.

[4] T.A. Pollak, R. McCormack, M. Peakman, et al., Prevalence of anti-N-methyl-d-aspartate (NMDA) receptor antibodies in patients with schizophrenia and related psychoses: a systematic review and meta-analysis, Psychol. Med. 44 (2014) 2475–2487.

[5] L. Cremaschi, M. Kardell, V. Johansson, et al., Prevalences of autoimmune diseases in schizophrenia, bipolar I and II disorder, and controls, Psychiatry. Res. 258 (2017) 9–14.

[6] D. Degner, M. Haust, J. Meller, et al., Association between autoimmune thyroiditis and depressive disorder in psychiatric outpatients, Eur Arch Psychiatry. Clin. Neurosci. 265 (2015) 67–72.

[7] E.M. Siegmann, H.H.O. Müller, C. Luecke, et al., Association of depression and anxiety disorders with autoimmune thyroiditis: a systematic review and meta-analysis, JAMA Psychiatry. 75 (2018) 577–584.

[8] J.G. Hollowell, N.W. Staehling, W. Dana Flanders, et al., Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): national health and nutrition examination survey (NHANES III), J. Clin. Endocrinol. Metab. 87 (2002) 489–499.

[9] Y.J. Cai, F. Wang, Z.X. Chen, et al., Hashimoto's thyroiditis induces neuroinflammation and emotional alterations in euthyroid mice, J. Neuroinflammation 15 (2018) 299.

[10] C. Laurent, J. Capron, B. Quillerou, et al., Steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT): characteristics, treatment and outcome in 251 cases from the literature, Autoimmun. Rev. 15 (2016) 1129–1133.

[11] Y. Chiba, O. Katsuse, Y. Takahashi, et al., Anti-glutamate receptor ε2 antibodies in psychiatric patients with anti-thyroid autoantibodies - a prevalence study in Japan, Neurosci. Lett. 534 (2013) 217–222.

[12] T. Ikura, O. Katsuse, Y. Chiba, et al., Evaluation of titers of antibodies against peptides of subunits NR1 and NR2B of glutamate receptor by enzyme-linked immunosorbent assay in psychiatric patients with anti-thyroid antibodies, Neurosci. Lett. 628 (2016) 201–206.

[13] S. Takano, Y. Takahashi, H. Kishi, et al., Detection of autoantibody against extracellular epitopes of N-methyl-d-aspartate receptor by cell-based assay, Neurosci. Res. 71 (2011) 294–302.

[14] R. Takeuchi, H. Matsuda, K. Yoshioka, Y. Yonekura, Cerebral blood flow SPET in transient global amnesia with automated ROI analysis by 3DSRT, Eur. J. Nucl. Med. Mol. Imaging 31 (2004) 578–589.

[15] K. Fujita, T. Yuasa, Y. Takahashi, et al., Antibodies to N-methyl-D-aspartate glutamate receptors in Creutzfeldt-Jakob disease patients, J. Neuroimmunol. 251 (2012) 90–93.

[16] T. Fukuyama, Y. Takahashi, Y. Kubota, et al., Semi-quantitative analyses of antibodies to N-methyl-d-aspartate type glutamate receptor subunits (GluN2B & GluN1) in the clinical course of Rasmussen syndrome, Epilepsy. Res. 113 (2015) 34–43.

[17] A. Antonelli, S.M. Ferrari, A. Corrado, et al., Autoimmune thyroid disorders, Autoimmun. Rev. 14 (2015) 174–180.

[18] R.M. Ruggeri, F. Trimarchi, G. Giuffrida, et al., Autoimmune comorbidities in Hashimoto's thyroiditis: different patterns of association in adulthood and childhood/adolescence, Eur. J. Endocrinol. 176 (2017) 133–141.

[19] M.J. Titulaer, L. McCracken, I. Gabilondo, et al., Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study, Lancet Neurol. 12 (2013) 157–165.

[20] S.H. Tay, A.M. Fairhurst, A. Mak, Clinical utility of circulating anti-N-methyl-D-aspartate receptor subunits NR2A/B antibody for the diagnosis of neuropsychiatric syndromes in systemic lupus erythematosus and Sjögren's syndrome: an updated meta-analysis, Autoimmun. Rev. 16 (2017) 114–122.

[21] C. Hammer, B. Stepniak, A. Schneider, et al., Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity, Mol. Psychiatry. 19 (2014) 1143–1149.

[22] M. Bonnan, Intrathecal IgG synthesis: a resistant and valuable target for future multiple sclerosis treatments, Mult. Scler. Int. 2015 (2015) 1–15.

[23] M. Piga, A. Serra, L. Deiana, et al., Brain perfusion abnormalities in patients with euthyroid autoimmune thyroiditis, Eur. J. Nucl. Med. Mol. Imaging 31 (2004) 1639–1644.

[24] M.C. Hardoy, M. Cadeddu, A. Serra, et al., A pattern of cerebral perfusion anomalies between major depressive disorder and Hashimoto thyroiditis, BMC Psychiatry. 11 (2011) 148.