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Anti-neuronal autoantibodies in the cerebrospinal fluid and serum from 106 patients with recent onset depression compared to 106 individually matched healthy controls

Nina Vindegaard Sørensen, MD, Anna Christine Nilsson, MD, Sonja Orlovska-Waast, MD, PhD, Rose Jeppesen, MD, Rune Haubo Bojesen Christensen, MSc, PhD, Michael Eriksen Benros, MD, PhD

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Title: **Anti-neuronal autoantibodies in the cerebrospinal fluid and serum from 106 patients with recent onset depression compared to 106 individually matched healthy controls**

Running title: Anti-neuronal autoantibodies in depression

Authors:
Nina Vindegaard Sørensen, MD[1,2], Anna Christine Nilsson, MD[3,4], Sonja Orlovska-Waast, MD, PhD[1], Rose Jeppesen, MD[1,2], Rune Haubo Bojesen Christensen, MSc, PhD[1], Michael Eriksen Benros, MD, PhD[1,2,*]

Affiliations:
[1]Copenhagen Research Centre for Mental Health, Copenhagen University Hospital, Hellerup, Denmark
[2]Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark
[4]Department of Clinical Research, University of Southern Denmark, Odense, Denmark

*: Corresponding author: Michael Eriksen Benros, Copenhagen Research Centre for Mental Health, Copenhagen University Hospital, Gentofte Hospitalsvej 15, 4. sal, 2900 Hellerup, Denmark, Tel: (+45) 26255239, Fax: +45 38647504, e-mail: Michael.eriksen.benros@regionh.dk

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ABSTRACT

No large studies have investigated the prevalences of cerebrospinal fluid anti-neuronal autoantibodies in isolated depression. In this case-control study comparing 106 patients with isolated depression (ICD-10: F32) to 106 healthy controls, cerebrospinal fluid and serum were tested for seven immunoglobulin G autoantibodies using commercial fixed cell-based assays. To explore validity of methods, positive samples were re-tested twice by cell-based assays and once by tissue-based assays (monkey cerebellum). The prevalences of any of the anti-neuronal autoantibodies in cerebrospinal fluid were 0.0% in both groups and the seroprevalences were 0.9% in both groups, based on consistent findings in cell-based assays. However, all were negative by tissue-based assays. Evaluation of anti-neuronal autoantibodies in cerebrospinal fluid cannot be recommended routinely for patients with isolated depression of moderate severity, and future studies of isolated depression should consider much larger sample sizes and evaluation of anti-neuronal autoantibodies by other modalities than commercial kits.

INTRODUCTION

Depressed mood as part of the initial symptomatology of autoimmune encephalitis (AE) has previously been reported (1–3) and at case report level, depression has been reported as the only phenotypical trait of anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis (4). Despite the rarity of AE (5,6), commercial kits for evaluation of several anti-neuronal autoantibodies (Abs) that can cause AE are available, including; NMDAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor 1 and 2 (AMPAR-1/2), contactin-associated protein 2 (CASPR2), leucine-rich glioma-inactivated protein 1 (LGI1), γ-Aminobutyric acid b (GABAb) receptor B1/B2 and glutamic acid decarboxylase-65 (GAD65)-directed Abs (7–9). Especially measurements of anti-neuronal autoantibodies in the cerebrospinal fluid (CSF) are of interest, as anti-neuronal Abs need to reach the brain to be pathogenic (10).

Case-control studies of unipolar depression and the prevalence of cerebrospinal fluid (CSF) anti-neuronal Abs are missing (11). A study of 39 patients with depressive syndromes, found no NMDAR, AMPAR-1/2 or GABABR Abs in CSF (12) and a study of eight patients with depression and recent suicide attempt found no CSF anti-neuronal immunoglobulin G (IgG) isotype autoantibodies (13). Seroprevalences have been more thoroughly evaluated: Among 207 patients with depression admitted to acute psychiatric care, the IgG Ab seroprevalences were estimated to 2.4% for GAD65 Abs and 0.5% for NMDAR Abs, whereas CASPR2 and AMPAR Abs were absent (14). A study of the NMDAR Ab seroprevalence found none of 99 patients with major depression to have IgG isotype NMDAR Abs, while the prevalence was 0.6% among the 357 healthy controls (15). The largest study to date of healthy individuals (N = 1 703) estimated the seroprevalence of IgG isotype NMDAR Abs to be ~1% (16).

The current knowledge indicates that CSF and serum anti-neuronal Abs are rare among patients with depression; however, case-control studies comparing anti-neuronal Abs especially in CSF from patients with depression compared to healthy controls are absent and warranted (11,17). Thus, the aims of this study were to investigate the prevalences of anti-neuronal Abs in CSF or serum from patients with recent onset depression compared to healthy controls, including investigations of NMDAR, AMPAR1, AMPAR2, CASPR2, LGI1, GABABR and GAD65 Abs in CSF or serum, exploring the hypothesis that anti-neuronal IgG isotype Abs are rare, however more prevalent, among patients with depression compared to healthy controls. This specific anti-neuronal Ab panel was chosen for this study as the included anti-neuronal Abs have been reported to have the highest prevalence among patients with AE (18).

METHODS AND MATERIALS

This study was a prospective case-control study of patients with recent onset depression (International Classification of Diseases 10th Revision (ICD-10): F32) and individually matched (age and sex) healthy controls. The study was conducted at the facility of Biological and Precision Psychiatry, Copenhagen Research Centre for Mental Health (CORE), Mental Health Centre Copenhagen, Copenhagen, Denmark. A study protocol was published prior to this paper (19) and demographic data has previously been published (20). The study was approved by The Regional Committee on Health Research Ethics (Capital Region, j.no: H-16030985) and The Danish Data Protection Agency (j.no: RHP-2016-020, I-Suite no.: 04945).

Outcomes

The two co-primary outcomes were the prevalences of any anti-neuronal IgG Ab against NMDAR, AMPAR1, AMPAR2, CASPR2, LGI1, GABAb receptor B1/B2 or GAD65 in 1) CSF or 2) CSF or serum from patients with isolated depression compared to healthy controls.

The two secondary outcomes were the specific prevalences of each of the abovementioned anti-neuronal Abs in 1) CSF or 2) CSF or serum from patients with isolated depression compared to healthy controls.

Participants and clinical assessment

Patients were recruited from in- and outpatient clinics of the Mental Health Services of the Capital Region and Region Zealand of Denmark from October 2018 until April 2021. Inclusion criteria for patients were: 1) Patients with a first-time diagnosis of depression (according to ICD-10: F32) diagnosed within the past year. 2) Ongoing depressive symptoms. 3) Age between 18 and 50 years. 4) Obtainment of written informed consent. Age and sex matched healthy controls were recruited

mainly by internet advertisement and from the same capture area as the patients from September 2018 until July 2021. Inclusion criteria for healthy controls were: 1) Healthy individual. 2) Age between 18 and 50 years. 3) Obtainment of written informed consent. Exclusion criteria are provided in Table S1 for both groups. Clinical assessment was performed in accordance with the study protocol (19). In brief, the initial diagnosis given in the clinical setting prior to enrolment was confirmed by SCAN interview (21) at inclusion. Severity of depressive symptoms, anxiety and psychotic symptoms were assessed. Furthermore, cognitive screening was done by Mini-Mental State Examination (MMSE) (22) and Montreal Cognitive Assessment (MoCA) (23), whereas further cognitive testing was done by Brief Assessment of Cognition in Schizophrenia (BACS) (24). Prior to lumbar puncture, a neurological examination including Neurological Evaluation Scale for evaluation of Neurological Soft Signs (25) was performed.

Anti-neuronal Ab analyses

CSF and serum samples were collected as described in the protocol (19) and the samples were analyzed at the national accredited laboratory for testing of anti-neuronal Abs at the Department of Clinical Immunology, Odense University Hospital. Laboratory personnel were blinded to all clinical information, including case/control status. Anti-neuronal IgG isotype Abs were detected by indirect immunofluorescence (IIF) using commercial fixed CBAs transfected with the following antigens: NMDAR NR1 subunit, GAD65, LGI1, CASPR2, AMPAR1, AMPAR2 and GABAb receptor B1/B2 (Autoimmune Encephalitis Mosaic 1, Euroimmun, Lübeck, Germany). Serum samples were analyzed in dilution 1:10, and samples positive for anti-CASPR2 were also analyzed in dilution 1:100. CSF samples were analyzed undiluted. Analyses were performed according to the manufacturer's recommendations. Results were reported according to fluorescence intensity as negative (lack of specific fluorescence), borderline positive, weakly positive, moderately positive, and strongly

positive. TBA testing was performed according to the manufacturer's recommendations. As all TBAs were negative in screening dilution, no further titers were measured. Due to the known uncertainty regarding Abs with low titers, anti-neuronal Ab reactive samples were post-hoc re-analyzed blinded to previous results; twice by CBAs and once by a tissue-based assay (TBA) using monkey cerebellum as substrate (Euroimmun, Lübeck, Germany). Samples were considered positive in the primary analyses if consistently positive by three CBAs (borderline positive samples were not included due to the reproducibility maximum deviation of ±1 fluorescence intensity).

Follow-up

Participants with anti-neuronal Abs in CSF or serum (positive or borderline positive in ≥ 2 CBA runs) were followed up by same procedure as for inclusion. Participants, who had serum NMDAR Abs also at follow-up, had CSF and serum samples re-tested by IIF using a fixed CBA transfected with IgG NMDAR NR1 subunit antigens only (Anti-Glutamate receptor (type NMDA) IIFT, Euroimmun, Lübeck, Germany). Furthermore, their CSF and serum samples were sent blinded for a second opinion/third re-test by CBA NMDAR transfected cells at the manufacturer's expert reference laboratory (Labor Stöcker, Germany).

Statistical analyses

Pearson χ^2-tests without adjustment for continuity was applied for analyses of anti-neuronal Abs. Analyses of continuous demographic data were assessed by two-sample *t*-test and dichotomous data by Pearson's χ^2-test. Categorical variables are shown in absolute numbers and (%). Continuous variables are shown as mean (SD). Two-sided tests with $p < 0.05$ was considered significant. To further explore the seroprevalence of anti-neuronal Abs, subgroup analyses of age, sex, peripheral hs-CRP and leukocyte counts were conducted by logistic regression models. As an explorative

analysis the results of first run CBA were analyzed as well. All analyses were done in R version 4.0.5 (26).

RESULTS

The study comprised 106 patients with recent onset depression and 106 age and sex matched healthy controls (71 females and 35 males in each group) (Table S2). Average age of patients was 26.0 (7.6) years and of healthy controls 26.4 (6.8) years. Of patients, 67.0% had not initiated antidepressants and 84.0% were outpatients. Mean time from diagnosis to inclusion was 4.0 (5.5) weeks. The patients suffered on average from moderate depression (HDRS-17: 20.6 (6.3)). Details of co-morbidities are provided in Table S3.

Primary outcomes

The prevalence of any CSF anti-neuronal Ab was 0.0% and the seroprevalence was 0.9% in both groups. All CSF and serum samples were negative by confirmatory TBAs (all *p*-values > 0.05) (Table 1). A full overview of individual positive test results from CBAs and TBAs is provided in Table 2.

Secondary outcomes

The seroprevalence of NMDAR IgG Abs was 0.9% among patients and 0.0% among healthy controls and the seroprevalence of GAD65 IgG Abs was 0.0% among patients and 0.9% among healthy controls (Table 1 and Figure S1).

Follow-up investigations of the individuals tested positive for anti-neuronal autoantibodies

One patient and four healthy controls were followed up within a mean time of 17.6 (7.9) months. The patient and healthy control, who were seropositive of NMDAR Abs at inclusion, remained seropositive at follow-up; both when tested by the mosaic and the specific NMDAR IgG kit. The

healthy control, who additionally was CSF positive/borderline positive of NMDAR Abs at inclusion, were negative by the CBA from the reference laboratory. The healthy control had a normal neurological examination and no psychiatric symptoms neither at inclusion nor at follow-up visit. The seropositive patient suffered from moderate depression (HDRS-17 score of 23), had no neurological complaints at inclusion and had a normal neurological examination except for hyperreflexia. A full characteristic of the participants with NMDAR Abs in CSF and/or serum is found in Table S4 and Figure S1. The three remaining participants did not have serum Abs at follow-up (Table 2) and the healthy control, who had GAD65 Abs in serum, did not have diabetes mellitus.

Explorative analyses

We found no significant subgroup differences regarding sex, age, hs-CRP levels or peripheral leukocyte levels related to the seroprevalences of anti-neuronal Abs (Table S5) nor regarding serum CBA positive or borderline positive results of first run CBA (Table S6).

DISCUSSION

Comprising 212 individually matched participants (106 patients with recent onset depression and 106 healthy controls), this study evaluate anti-neuronal CSF Abs in isolated, unipolar depression. The anti-neuronal IgG isotype Abs were evaluated by a commercially available kit, and five participants were followed up due to potential abnormal test results at first visit. In both groups, the prevalences of any CSF anti-neuronal Ab were 0.0% and the seroprevalences were 0.9%. One patient was consistently seropositive of NMDAR Abs and one healthy control was consistently seropositive of GAD65 Abs in three CBAs. At follow-up, two participants were still positive in serum. However, our study emphasizes the uncertainties related to the current commercially available measurements of anti-neuronal IgG isotype Abs.

As no previous case-control studies have systematically investigated CSF anti-neuronal Abs in a cohort of patients with isolated depression compared to age and sex matched healthy controls, such study has been warranted to explore the significance of anti-neuronal Abs in depression (11). An informed consent was necessary for participation, why patients with decreased or altered level of consciousness—a criterion for possible AE (27)—were not included. Furthermore, patients were excluded if they had seizures within the past 10 years. Only one eligible participant met the second diagnostic criterion for possible AE (27), namely a patient with CSF pleocytosis, detected also as part of the present study (28). The patient underwent thorough neurological examination including re-lumbar puncture and broad evaluation of CSF anti-neuronal Abs, but no cause was found (28). Additionally, the patient had no anti-neuronal Abs in CSF and serum when re-tested for the present study. Thus, in this study of patients with recent onset depression, who were mainly outpatients and who did not fulfill the criteria for possible AE, CSF anti-neuronal Abs were absent, indicating that anti-neuronal Abs are very rare contributors to depression pathophysiology among patients with

isolated depression of moderate severity. Furthermore, regardless of symptomatology, AE is a very rare condition with low prevalence and incidence rates (5,6) indicating that estimates of the prevalence of AE presenting as isolated depression will most likely require a much larger sample size than of our study.

The seroprevalence of any IgG anti-neuronal Ab was estimated to be 0.9% among patients with depression. Previous studies have reported that one of 207 (14) and none of 99 (15) patients with depression had NMDAR IgG isotype Abs in serum, corroborating the low seroprevalence found in our study and emphasizing that serum IgG Abs are rare among patients with depression. Furthermore, we estimated the seroprevalence of anti-neuronal Abs to be 0.9% among the healthy controls, as one healthy control had consistent GAD65 Abs in serum. The finding of anti-neuronal Abs in serum from healthy individuals is in line with the largest previous study of IgG type anti-neuronal Abs among healthy individuals to date (N = 1 703), revealing prevalences of IgG NMDAR, CASPR2 and GAD65 Abs to be approximately 1.2%, 0.3% and 0.3%, respectively (16). Thus, the prevalence of anti-neuronal Abs in serum are approximately the same in healthy individuals as in patients, questioning the relevance of serum IgG anti-neuronal Abs, when there is no clinical suspicion of AE. This has previously been addressed by others (29), and anti-neuronal Abs as minimum need to reach the brain to be pathogenic (10); thus, mainly the prevalence of anti-neuronal Abs in the CSF is of clinical relevance. Thus, based on the findings from our study, we do not recommend routine CSF and serum investigation for AE among patients with isolated depression of moderate severity. However, AE should still be considered for patients with signs of possible AE or atypical presentation of depression.

We estimated the specific CSF prevalence of anti-neuronal Abs from healthy controls to be 0.0% defined by three consistently positive results CBAs (borderline positives not included). However, in the first CBA run, the CSF prevalence was 0.9% in the healthy group. Only two previous studies investigated CSF anti-neuronal Abs among 40 (30) and 48 (31) healthy individuals respectively and found no anti-neuronal Abs in CSF from the healthy controls. Thus—to our knowledge—CSF anti-neuronal Abs among healthy individuals have not previously been reported in the peer-reviewed scientific literature. However, according to the manufacturer's instruction, in one of their cohorts one (1.7%) of 60 healthy individuals had CSF NMDAR Abs (9). When followed up 6 months after inclusion, the healthy control in our cohort was still borderline positive for NMDAR Abs in CSF and weakly seropositive. Nevertheless, the healthy control was continuously healthy at re-examination and therefore the CSF was sent for re-test in the reference laboratory and the results here from were negative. As CSF analysis is considered the most important test in evaluation of AE (32), the finding of false positive NMDAR Abs in CSF from a healthy individual highlights the unconditional need for a clinical context when interpreting Ab results, as also addressed by others (33). Furthermore, our results emphasize the importance of a healthy control group when investigating the contribution of anti-neuronal Abs in diseases.

This study was strengthened by a large sample size, including a large control group and a well-characterized population. It is an additional strength that positive or borderline positive results by first CBA were re-run twice by CBA and once by TBA. However, the generalizability of the study was limited by design, as patients, who met most of the criteria for possible AE (27), were not included. Likewise, the age criterium for participation (18-50 years) is a limitation, since e.g. anti-LGI1 encephalitis is more prevalent above 50 years of age (34) and as the prevalence of anti-neuronal Abs in general increases with age (35). Furthermore, anti-NMDAR encephalitis has been reported

among individuals younger than 18 years of age (34) and our findings do not apply to a pediatric population. Many other anti-neuronal Abs have been identified (8), including paraneoplastic Abs and novel Abs like neurexin 3α that has also been associated with depression at case report level (36). Thus, the results of our study are limited by the Ab panel chosen. Additionally, it might be a limitation that we used fixed CBA instead of live CBA, as live CBA has been shown to reveal more serum IgG Abs against NMDAR among patients with psychosis as compared to fixed CBA (37). It is also a potential limitation that TBA was only used confirmatory and not consistently as this might have revealed reactivity not found by CBA (38).

Despite our study revealing a low prevalence of the seven most commonly occurring anti-neuronal Abs among patients with isolated depression, depression is a highly heterogenous disorder (39) estimated to affect more than 260 million people worldwide (40). Thus, we cannot rule out that specific subgroups of patients with symptoms mimicking depression can have anti-neuronal Abs contributing to the symptomatology. However, in individuals with isolated depression symptoms, we conclude that anti-neuronal Abs are very rare in the CSF and should mainly be investigated in individuals with specific symptoms of probable AE or CSF alterations. Commercial fixed CBAs are more sensitive than TBAs using monkey cerebellum, and borderline or weak Abs can only rarely be confirmed by a positive reaction on the tissue. Thus, when designing future studies, the methods (e.g. fixed CBA, live CBA, TBA, a combination of modalities or even other modalities (41)) and management of reproducibility with re-testing of positive results should be considered.

Conclusion

This is the first large, systematic, prospective case-control study to estimate the prevalence of anti-neuronal Abs in CSF and serum from patients with isolated depression compared to healthy controls. Anti-neuronal Abs were not present in CSF from this group of mainly outpatients with recent onset depression of moderate severity and without signs of AE. Thus, much larger sample sizes will be needed to estimate the prevalence and other modalities for anti-neuronal Ab evaluation could be considered. The study emphasizes the importance of a healthy control group and highlights the need of a clinical evaluation when interpreting—especially weak—Ab results. The results supported the hypothesis that anti-neuronal Abs are rare among patients with depression without symptoms of potential AE. Therefore, we do not recommend evaluating CSF or serum anti-neuronal Abs as part of the routine examination of patients with isolated unipolar depression of moderate severity in the clinical setting, unless there is an abnormal CSF test or a clinical suspicion of AE.

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DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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Table 1 | Prevalences of anti-neuronal autoantibodies (Abs) in CSF and serum

	Depression N (%)		Healthy controls N (%)		*p*-values
	CBA[1]	Confirmatory TBA[2]	CBA[1]	Confirmatory TBA[2]	CBA[1]
Primary outcomes					
Any anti-neuronal Ab in CSF	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Any anti-neuronal Ab in serum	1 (0.9)	0 (0.0)	1 (0.9)	0 (0.0)	1.000
Secondary outcomes					
CSF anti-neuronal Abs					
NMDAR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
AMPAR1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
AMPAR2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
CASPR2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
LGI1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
GABAbR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
GAD65	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Serum anti-neuronal Abs					
NMDAR	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0.316
AMPAR1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
AMPAR2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
CASPR2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
LGI1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
GABAbRR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
GAD65	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)	0.316

[1]The samples were considered positive for an anti-neuronal autoantibody if three independent tests by CBA were consistently positive (borderline positives not included). [2]Confirmatory TBA tests were only assessed on samples positive in first CBA.
p-values are based on Pearson's χ^2 test.

Abbreviations: Ab: autoantibody. CBA: cell-based assay. TBA: tissue-based assay. CSF: cerebrospinal fluid. NMDAR: N-methyl-D-aspartate-receptor. AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor. CASPR2: contactin-associated protein 2. LGI1: leucine-rich glioma-inactivated protein 1. GABA: γ-Aminobutyric acid-receptor. GAD65: glutamic acid decarboxylase-65.

Table 2 | Anti-neuronal autoantibodies in CSF and serum by CBA 1, CBA 2, CBA 3, TBA and CBA at follow-up

	CBA tests					
Individuals with questionable or positive test results	CBA test 1	CBA test 2	CBA test 3	Positive in all 3 CBA tests	TBA test (Monkey cerebellum)	Follow-up[1]
CSF						
Healthy control 1	NMDAR +	NMDAR ++	NMDAR (+)	No	Negative	Negative[2]
Serum						
Patient 1	NMDAR +	NMDAR ++	NMDAR ++	NMDAR +/++	Negative	NMDAR +[2]
Healthy control 1	NMDAR (+)	NMDAR (+)	NMDAR ++	No	Negative	NMDAR +[2]
Healthy control 2	GAD65 ++	GAD65 ++	GAD65 ++	GAD65 ++	Negative	Negative
Healthy control 3	GAD65 ++	Negative	Negative	No	Negative	No follow-up
Healthy control 4	GAD65 (+)	Negative	Negative	No	Negative	No follow-up
Healthy control 5	CASPR2 (+)[3]	CASPR2 +	CASPR2 +	No	Negative	Negative
Healthy control 6	CASPR2 (+)[3]	CASPR2 (+)	CASPR2 (+)	No	Negative	Negative
Healthy control 7	CASPR2 (+)[3]	Negative	Negative	No	Negative	No follow-up
Healthy control 8	CASPR2 (+)[3]	Negative	Negative	No	Negative	No follow-up

(+): borderline positive. +: weakly positive. ++: moderately positive. +++: strongly positive.

[1]Mean follow-up time was 17.6 months. All samples were negative by TBA at follow-up. [2]CBA of transfected cells (Euroimmun) by reference laboratory (Labor Stöcker, Germany). Further information is found in Table S4. [3]All CASPR2 samples were negative when analyzed in dilution 1:100.

Abbreviations: CSF: cerebrospinal fluid. CBA: cell-based assay. TBA: tissue-based assay. NMDAR: N-methyl-D-aspartate-receptor. CASPR2: contactin-associated protein 2. GAD65: glutamic acid decarboxylase-65.