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RESEARCH ARTICLE

High prevalence of NMDA receptor IgA/IgM antibodies in different dementia types

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Abstract

Objective: To retrospectively determine the frequency of *N*-Methyl-D-Aspartate (NMDA) receptor (NMDAR) autoantibodies in patients with different forms of dementia. **Methods**: Clinical characterization of 660 patients with dementia, neurodegenerative disease without dementia, other neurological disorders and age-matched healthy controls combined with retrospective analysis of serum or cerebrospinal fluid (CSF) for the presence of NMDAR antibodies. Antibody binding to receptor mutants and the effect of immunotherapy were determined in a subgroup of patients. **Results**: Serum NMDAR antibodies of IgM, IgA, or IgG subtypes were detected in 16.1% of 286 dementia patients (9.5% IgM, 4.9% IgA, and 1.7% IgG) and in 2.8% of 217 cognitively healthy controls (1.9% IgM and 0.9% IgA). Antibodies were rarely found in CSF. The highest

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prevalence of serum antibodies was detected in patients with "unclassified dementia" followed by progressive supranuclear palsy, corticobasal syndrome, Parkinson's disease-related dementia, and primary progressive aphasia. Among the unclassified dementia group, 60% of 20 patients had NMDAR antibodies, accompanied by higher frequency of CSF abnormalities, and subacute or fluctuating disease progression. Immunotherapy in selected prospective cases resulted in clinical stabilization, loss of antibodies, and improvement of functional imaging parameters. Epitope mapping showed varied determinants in patients with NMDAR IgA-associated cognitive decline. **Interpretation**: Serum IgA/IgM NMDAR antibodies occur in a significant number of patients with dementia. Whether these antibodies result from or contribute to the neurodegenerative disorder remains unknown, but our findings reveal a subgroup of patients with high antibody levels who can potentially benefit from immunotherapy.

Introduction

The prevalence of immunotherapy-responsive autoimmune dementias remains unclear and these conditions are often misdiagnosed as primary neurodegenerative disorders,[1] suggesting that treatable etiologies are overlooked in dementia patients. Generally, autoimmunity is suspected if dementia starts subacutely, progresses rapidly, or fluctuates, or if inflammatory cerebrospinal fluid (CSF) and suggestive magnetic resonance imaging (MRI) findings are present. However, 41% of immunotherapy-responsive dementia patients of a large study had normal brain MRIs, and many patients showed normal CSF and electroencephalography (EEG).[1] Further complicating this diagnostic dilemma, autoimmune-mediated cognitive decline can progress slowly over many months, and therefore may be mistaken for a primary neurodegenerative disorder such as Alzheimer's disease (AD) or frontotemporal dementia (FTD).[2–4] A novel paradigm of how autoimmunity and neurodegeneration may interact was recently reported in a disorder with progressive sleep dysfunction, pathological findings consistent with tauopathy, and antibodies against the neuronal cell surface protein IgLON5.[5] In search of serologic clues to autoimmune dementia, the presence of N-Methyl-D-Aspartate receptor (NMDAR) antibodies of the IgA isotype has recently been described in a small cohort of patients with atypical dementia.[6] Purified IgA containing NMDAR IgA antibodies caused substantial loss of NMDARs and further synaptic proteins in primary hippocampal cultures, resulting in marked changes of NMDAR-mediated currents. In addition, immunotherapy resulted in clinical improvement of neuropsychiatric symptoms in a subgroup of patients, suggesting that NMDAR IgA is a marker of immunotherapy-responsive dementia.[6]

As neurodegenerative disorders can be clinically indistinguishable from autoimmune dementias, we systematically analyzed archived serum and CSF samples from patients

with different etiologies of dementia and controls to estimate the prevalence of NMDAR antibodies and potentially identify candidates that might respond to immunotherapy.

Methods

Patients

In all, 286 well-characterized patients with clinical diagnoses of AD (n = 100), behavioral variant FTD (43), primary progressive aphasia (PPA, 22), Lewy body dementia (LBD, 11), Creutzfeldt-Jakob disease (CJD, 10), Parkinson's disease with dementia (PDD, 25), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP, 11), Huntington's disease (HD, 14), unclassified dementia (20) and vascular dementia (30), 90 patients with neurodegenerative disease without dementia (motor neuron disease [MND, 17], Parkinson's disease without dementia [PD, 49], multiple system atrophy [MSA, 24]), 131 patients with cerebellar ataxia (spinocerebellar ataxia [SCA, 83], idiopathic sporadic ataxia [ISCA, 48]), 80 patients with other neurological disorders (such as migraine, disc prolapse, meningioma, cerebral vasculitis, paraneoplastic cerebellar degeneration, progressive multifocal leukoencephalopathy, or Fabry disease), 26 patients with psychiatric disease (schizophrenia, depression, dissociative disorders; diagnosed during clinical workup), and 47 healthy controls were recruited. Archived specimens were collected at the dementia clinics and departments of Neurology or Psychiatry at the Charité University hospital (Berlin, Germany), Massachusetts Alzheimer's Disease Research Center (Boston, USA), Harvard NeuroDiscovery Center (Boston, USA), Phillips University (Marburg, Germany), University Hospital Eppendorf (Hamburg, Germany), Paracelsus Elena Klinik (Kassel, Germany), Center for Neurology Tübingen (Tübingen, Germany), Technical University Munich (Munich, Germany), Saarland University (Homburg/Saar, Germany), University Hospital Magdeburg (Magdeburg, Germany).

Retrospective analyses were approved by the Charité University Hospital Institutional Review Board and written informed consent for material storage was obtained from patients or their representatives in the respective centers.

Detection of NMDAR antibodies

Testing for NMDAR antibodies was performed by recombinant immunofluorescence as described.[6] Briefly, plasmids encoding the NMDA receptor (using NR1a subunit homodimers and equimolar NR1a/NR2b heterodimers) were transfected into HEK293 cells, grown on cover slides, followed by acetone fixation. Slides and control-transfected cells were incubated with "blinded" patient samples at starting dilution of 1:10 (serum) or undiluted (CSF). After 30 min, slides were washed with PBS-Tween for >5 min. Bound antibodies were labeled with Fluorescein-conjugated goat anti-human IgG (DiaMed, Canton, OH; dilution 1:800), IgA (1:350), or IgM (1:500) for 30 min. Coded samples were classified by two independent blinded assessors based on immunofluorescence.

Resting-state functional MRI

Acquisition and analysis of resting-state functional MRI (fMRI) data was performed separately for subjects using independent component analysis (ICA) and dual regression as described previously.[7,8] Using temporal-concatenation ICA as implemented in FSL MELODIC,[9] the default mode network (DMN) was identified. Functional connectivity alterations of the DMN have been shown to reflect disease severity in various neuropsychiatric diseases, including patients with anti-NMDAR antibodies.[8] The treatment effect (i.e., comparison of pretherapy with posttherapy DMN functional connectivity) was assessed using the dual regression approach.[7] Statistical analysis was constrained to the individual DMN and performed using FSL's flameo with correction for multiple comparisons based on Gaussian random field theory ($z > 1.98$, $P < 0.05$, cluster corrected).

PET

Positron emission tomography (PET) analysis was performed as described.[6] Briefly, acquisition was started 40 min after IV injection of 250 MBq [F-18]-fluorodeoxyglucose (FDG). Transaxial images were reconstructed and stereotactically normalized. Follow-up PET images were coregistered to baseline prior to stereotactical normalization. Each FDG-PET image was compared with corresponding images of a group of 28 normal control subjects on a voxel-by-voxel basis. Only effects in clusters of at least 125 voxels (~1 mL) were considered. For direct visualization of changes between baseline and follow-up PETs, voxel-based subtraction was performed.[10]

Epitope mapping

Cultured HEK293 cells were transiently transfected as described previously[11] using the following constructs: wild-type NR1a, NR1a with the amino terminal domain (ATD) deleted (deletion of residues 26–382), NR1a with amino acid 368 mutated (N368Q), or a NR1a construct (ATD-TM4) with amino acids 401–792 deleted (deleting the ligand-binding domain and first 3 transmembrane domains) such that the ATD is linked directly to the fourth transmembrane domain (TM4) as described[12] (see Fig. 3G for illustration of constructs). Eighteen to 21 hours after transfection, cells were fixed with 4% parafor-

maldehyde (PFA) and immunostained[13] with anti-NR1a antibody (BD Biosciences 556308, San Jose, CA, USA; 1:1000 or, for experiments using the ATD-TM4 construct, Millipore AB1548, Billerica, MA, USA; 1:200), and patient serum was applied (subject A0 1:500, subject A6 1:100; parallel control experiments used samples from patients with NMDAR encephalitis). Coverslips were washed with PBS and secondary antibodies applied (1:500 FITC goat anti-human IgA [Invitrogen AHI0108, Carlsbad, CA, USA]; 1:1000 Alexa Fluor 488 goat anti-human IgG for control experiments; 1:1000 Alexa Fluor 568 goat anti-mouse or goat anti-rabbit). Cells were imaged on a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany).

Results

Detection of NMDAR antibodies in dementia patients

Having recently identified IgA-NMDAR antibodies in some patients with unclassified dementia (Berlin cohort), we now aimed to compare the presence of NMDAR autoantibodies with established forms of dementia and neurodegenerative disorders. For this, archived serum and CSF samples from 660 subjects were recruited from 10 dementia clinics in Germany and the United States and retrospectively analyzed for NMDAR antibodies. Subjects included 286 well-characterized patients with dementia, 90 patients with neurodegenerative disease without dementia, 80 controls with other neurological (non-neurodegenerative) disorders, 26 patients with psychiatric disease, and 47 age-matched healthy controls (mean age 66.7 years).

IgM, IgA, or IgG NMDAR antibodies were analyzed in a blinded fashion and detected in the serum of 16.1% of the 286 patients with different forms of dementia (9.5% IgM, 4.9% IgA, and 1.7% IgG), but only in 3.3% of 90 nondemented patients with neurodegenerative disorders, 1.3% of 80 patients with other neurological diseases, and 4.3% of 47 age-matched healthy controls (Fig. 1A), representing an average of 2.8% among controls (1.9% IgM, 0.9% IgA).

Antibodies were detected in routine assays of NMDAR-transfected cells (Fig. 1B, left) revealing identical results in two laboratories (Lübeck, Barcelona). However, binding to primary hippocampal neurons was only detectable when high antibody titers were present (Fig. 1B, right), while binding to rat brain sections could not be demonstrated using short-incubation routine procedures (not shown).

Varying prevalence of NMDAR antibodies among dementia groups

The presence of NMDAR antibodies was not uniformly distributed among the cohorts of patients with neurodegenerative disorders (Fig. 2). Most prominently, patients with neurodegenerative disease but without dementia (MND, PD, MSA) had antibody frequencies in the range of controls. Specifically, PD patients had a significantly lower percentage compared with PDD (2% vs. 20%, $P = 0.0067$, Fisher's exact test). Serum frequency was between 14% and 55% in patients with PPA, LBD, and PSP/CBS, 9–12% in FTD, AD, HD, CJD, and ataxias, and not detected in vascular dementia patients (Fig. 2A and B). NMDAR antibodies in CSF were detected only in 11 of 334 available samples (Fig. S1). Interestingly, in all CSF cases, low titers (≤1:10) of IgA isotype antibodies were

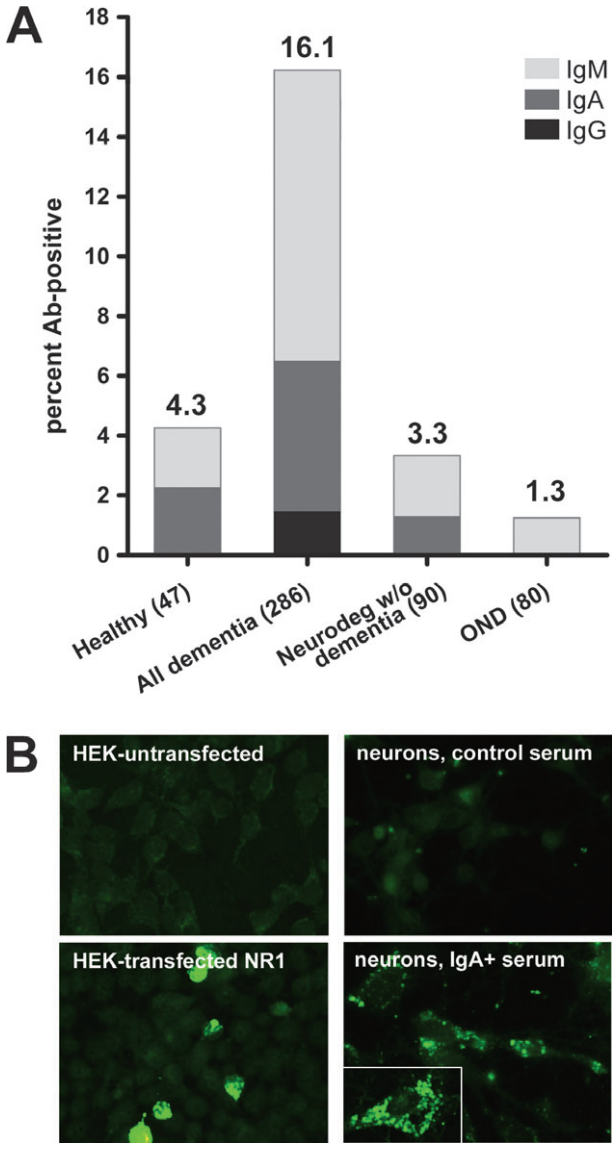

Figure 1. High frequency of *N*-Methyl-D-Aspartate receptor (NMDAR) antibodies in dementia. Percentage of NMDAR antibody-positive patients (serum, A) and detection of NMDAR antibodies using transfected HEK cells and primary hippocampal neuronal cultures (B).

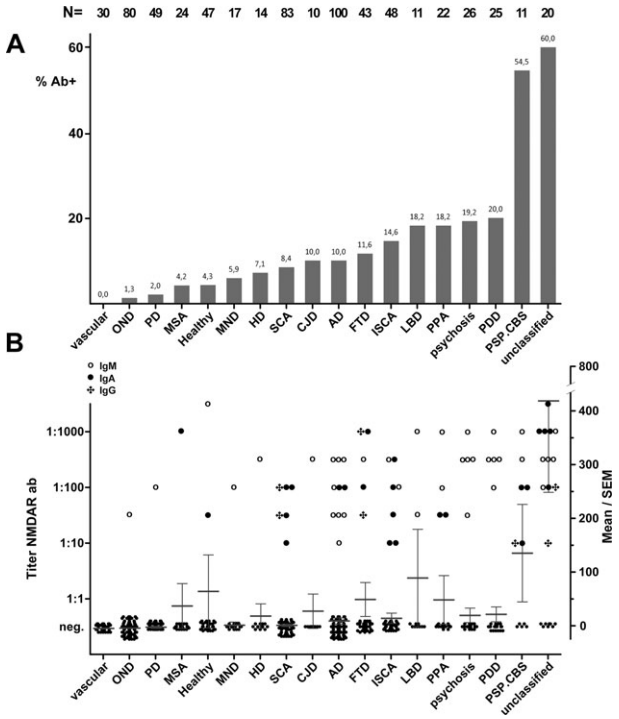

Figure 2. Frequency distribution, isotypes, and titers of serum *N*-Methyl-D-Aspartate receptor (NMDAR) antibodies in study subjects. Number of subjects per group, percentage of dementia patients and controls with positive NMDAR antibodies (IgM, IgA, or IgG) in serum (A). Antibody isotypes and serum titers across dementia groups and controls (B).

present, seen in patients with unclassified dementia (n = 4), FTD (3), vascular (1), other neurological disorders (OND) (1), AD (1), and psychosis (1).

Among all dementia patients, those with progressive cognitive dysfunction of unclear etiology from the Berlin cohort showed the highest frequency of NMDAR antibodies (60%, Fig. 2A). The term "unclassified" describes the difficulties to assign the patients to one of the established dementia groups. In fact, the distinct clinical characteristics (see Table 1 for details) define a subgroup of dementia patients in which there is a higher frequency of CSF abnormalities (69%), rapid onset or fluctuating disease course (95%), immune challenges (current infection, cancer, or concomitant autoimmune disease, 63%), and psychiatric abnormalities (75%) (Table 1). Difficulties in establishing a clear dementia diagnosis in several cases also resulted from imaging abnormalities, such as leukoencephalopathy, focal or rapidly developing atrophy, or heterogeneously reduced glucose uptake without underlying atrophy (Fig. 3A–D).

Association of clinical signs and NMDAR antibodies

Irrespective of the type of dementia, the presence of NMDAR antibodies was associated with certain clinical features (Table 2). Compared to NMDAR antibody-negative patients, antibody-positive patients more often

Table 1. Clinical characteristics of patients with "unclassified dementia" compared to other forms of dementia.

	Unclassified ("Unclassified")	AD	PD	FTD	CBS/PSP	LBD
Irregular progression[1]	95% (20)	13% (31)	14% (14)	27% (11)	27% (11)	17% (6)
Cognitive deficits	100% (20)	100% (31)	57% (14)	100% (11)	100% (11)	100% (6)
Aphasia	68% (19)	58% (31)	21% (14)	91% (11)	63% (11)	80% (5)
Psychiatric symptoms[2]	75% (20)	32% (31)	14% (14)	64% (11)	36% (11)	83% (6)
Epileptic seizures	23% (17)	13% (31)	0% (14)	0% (11)	0% (11)	0% (6)
Further CNS signs	68% (19)	32% (31)	100% (14)	45% (11)	100% (11)	100% (6)
Extrapyramidal	47% (19)	26% (31)	100% (14)	36% (11)	100% (11)	100% (6)
Cerebellar	47% (19)	3% (31)	0% (14)	0% (11)	0% (11)	0% (6)
CSF abnormalities	69% (16)	32% (28)	0% (11)	27% (11)	30% (10)	0% (6)
Pleocytosis[3]	12% (16)	0% (28)	0% (11)	0% (11)	10% (10)	0% (6)
BBB dysfunction	50% (16)	32% (28)	0% (11)	27% (11)	20% (10)	0% (6)
OCB[3]	38% (16)	4% (28)	0% (10)	0% (11)	0% (8)	0% (4)
Immune challenges[4]	63% (19)	32% (31)	21% (14)	36% (11)	20% (10)	17% (6)
Positive family history (dementia)	17% (18)	16% (31)	14% (14)	27% (11)	0% (10)	33% (6)

AD, Alzheimer's disease; PD, Parkinson's disease; FTD, frontotemporal dementia; CBS, corticobasal syndrome; PSP, progressive supranuclear palsy; LBD, Lewy body dementia; CSF, cerebrospinal fluid; CNS, central nervous system.

[1]Subacute onset, partial regression, or plateau.

[2]Affective symptoms, irritability, aggression, delusions, hallucinations.

[3]OCB, oligoclonal bands in the CSF. Pleocytosis >4 white blood cells per μL CSF.

[4]Current infection, cancer, or other autoimmunity.

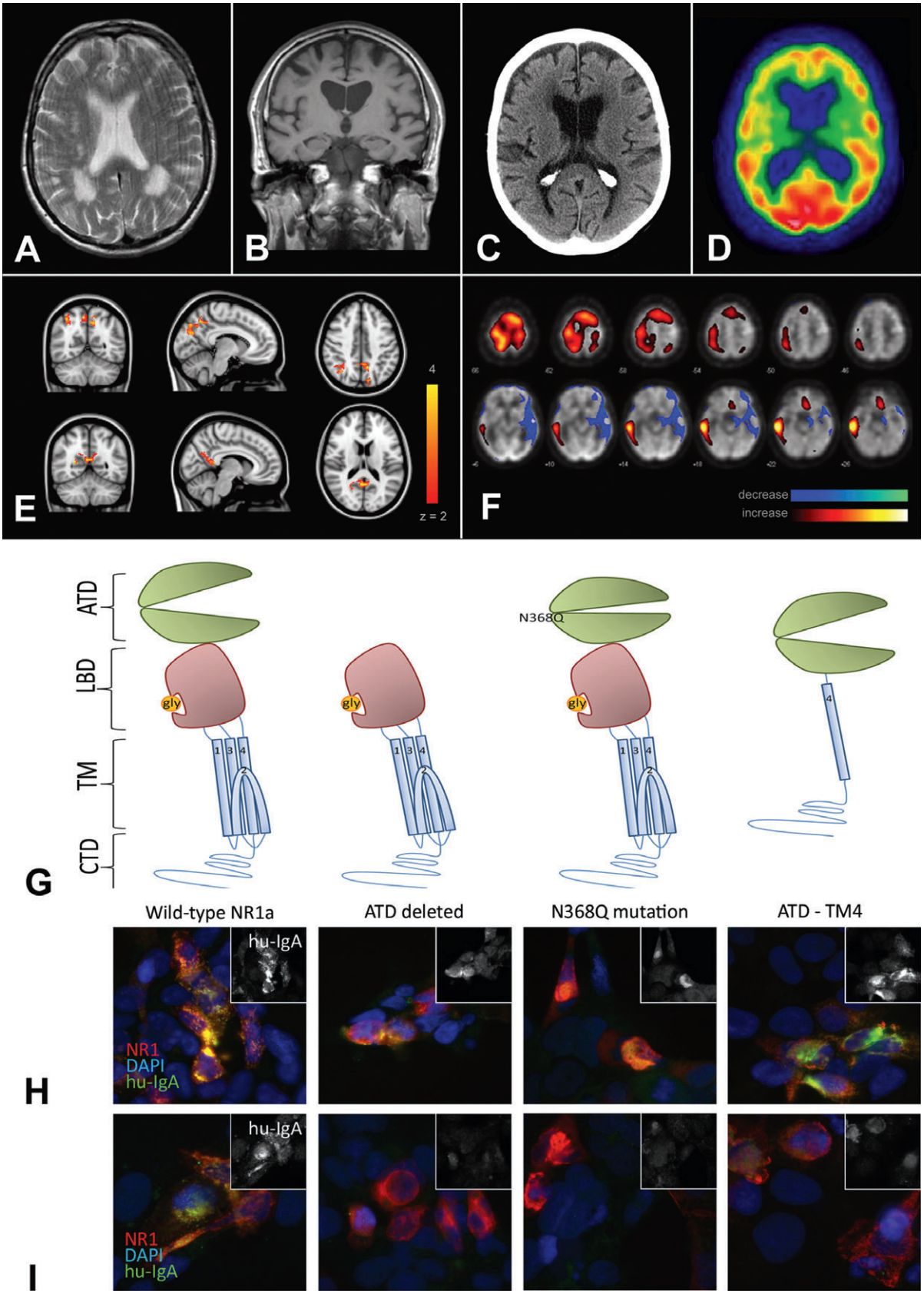

Figure 3. Imaging findings in dementia patients with NMDAR antibodies and epitope mapping with IgA-positive serum. Several patients with unclassified dementia and IgA/IgM NMDAR antibodies showed MRI and PET abnormalities that were not typical of primary neurodegenerative disorders. These included patients with otherwise unexplained marked bilateral leukoencephalopathy (A), global atrophy associated with very rapidly developing dementia (B and C), or patchy FDG uptake with reduction in paraventricular and cortical areas (D). NMDAR antibody levels in these patients were IgA 1:100 in serum and 1:10 in CSF (A), IgM 1:3200 in serum (B), IgA 1:1000 in serum (C and D). Imaging demonstrates treatment effects following immunotherapy with plasma exchange using fMRI (E) and PET (F). Functional connectivity of the default mode network (a set of brain regions with strongly correlated neural activity) was significantly decreased with the posterior cingulate cortex, the precuneus, and the superior parietal cortex in posttreatment scans in comparison with pretreatment scans (E). PET studies in a patient with unclassified dementia and IgA antibodies documented significant improvement of cerebral metabolism in cortical brain areas after plasma exchange (F). HEK cells were transfected with wild-type NR1a, or with NR1a mutants lacking the amino terminal domain (ATD-deleted), with amino acid 368 mutated (N368Q), or lacking the ligand-binding domain and first 3 transmembrane domains (ATD-TM4) (G). Subject A0 had IgA antibodies that strongly recognized NR1a. ATD deletion and N368Q mutation both only mildly reduced antibody binding while binding to the ATD-TM4 construct was increased (H; NR1a commercial antibody staining in red, human IgA antibodies in green; insert – corresponding grayscale images of human IgA). Subject A6 had IgA antibodies that recognized NR1a. For this subject, ATD deletion and N368Q mutation nearly eliminated staining of the NR1a construct and the antibodies had reduced binding to the ATD-TM4 construct (I). NMDAR, *N*-Methyl-D-Aspartate receptor; FDG, [F-18]-fluorodeoxyglucose; fMRI, functional magnetic resonance imaging; CSF, cerebrospinal fluid.

showed subacute disease onset or fluctuating disease progression (21.1% vs. 52.8%, $P = 0.003$, Fisher's exact test), aphasia (50% vs. 77.1%, $P = 0.014$), and CSF abnormalities, in particular blood–brain barrier dysfunction as defined by increased CSF/serum albumin quotients (17.0% vs. 44.1%, $P = 0.012$) (Table 2). In contrast, psychiatric symptoms, extrapyramidal movement disorders, or frequency of accompanying cancer were rather equally distributed between both groups (Table 2). NMDAR antibodies were usually not present in cases with a positive family history of dementia (24.5% vs. 2.9%, $P = 0.0067$). The analysis was intended as an exploratory statistical analysis to generate hypotheses for further discussions and planning of prospective trials, thus no adjustments for multiple testing have been made.

Table 2. Clinical characteristics of antibody-positive versus -negative patients (all dementia groups).

	NMDAR ab-positive	NMDAR ab-negative	P[1]
Irregular progression	19/36 (52.8%)	12/57 (21.1%)	0.003[2]
Aphasia	27/35 (77.1%)	28/56 (50.0%)	0.014[2]
Psychiatric symptoms[3]	20/36 (55.6%)	26/56 (46.4%)	0.52
Further CNS signs	24/34 (70.6%)	34/56 (60.7%)	0.37
Extrapyramidal	20/34 (58.8%)	30/56 (53.6%)	
Cerebellar	5/34 (14.7%)	5/56 (8.9%)	
Blood–brain barrier dysfunction	15/34 (44.1%)	8/47 (17.0%)	0.012[2]
OCB/pleocytosis[4]	6/34 (17.6%)	5/47 (10.6%)	0.51
Cancer[5]	4/33 (12.1%)	6/57 (10.5%)	0.99
Positive family history (dementia)	1/35 (2.9%)	13/53 (24.5%)	0.0067[2]

NMDAR, *N*-Methyl-D-Aspartate receptor.
[1]Fisher's exact test (exploratory, e.g., uncorrected for multiple comparisons).
[2]$P < 0.05$ is considered significant.
[3]Affective symptoms, irritability, aggression, delusions, hallucinations.
[4]OCB, oligoclonal bands in the CSF only. Pleocytosis >4 white blood cells per μL CSF.
[5]Cancer within $\pm$1 year of presentation.

Patients with high NMDAR antibody titers can benefit from immunotherapy

Due to the retrospective nature of this study, a systematic analysis of the effect of immunotherapies is not possible. However, from five prospectively included patients with unexplained dementia and highly positive NMDAR IgA antibodies (titers $\geq$1:1000), two patients were selected for an immunotherapy attempt. Both received high-dose methylprednisolone and plasma exchange, in one case followed by cyclophosphamide. One patient markedly improved in the first weeks (cognitive testing, alertness, aphasia, and motivation) and both patients did not progress during the follow-up of 8–12 months. The clinical improvement was supported by imaging findings. Both patients underwent resting-state fMRI before and after plasma exchange which showed a significantly increased DMN functional connectivity in pretreatment scans (Fig. 3E). This is in line with correlations of increased DMN synchronization with severity of cognitive dysfunction in neuropsychiatric diseases, including AD, multiple sclerosis, and schizophrenia.[14–16] Available in one of the two cases, PET studies documented significant improvement of cerebral metabolism in cortical brain areas after plasma exchange (Fig. 3F).

IgA antibodies bind to different epitopes of the NMDA receptor

HEK cells were transfected with different NR1 mutants (Fig. 3G). Immunostaining was performed with serum of two patients with the highest titers (Fig. 3H and I). Parallel control experiments using samples from patients with NMDAR-IgG encephalitis were also performed (data not

shown). Subject A0, who improved markedly after immunotherapy, had IgA antibodies that strongly recognized NR1a. ATD deletion and N368Q mutation both only partially reduced antibody binding while binding to the ATD-TM4 construct was increased (Fig. 3H). Subject A6, who had a milder response to immunotherapy, had IgA antibodies that recognized NR1a. For this subject, ATD deletion and N368Q mutation nearly eliminated NR1a staining and the antibodies had reduced binding to the ATD-TM4 construct (Fig. 3I). Taken together, epitope specificity varies between patients. The results suggest that the ligand-binding domain and first 3 transmembrane domains are not necessary for recognition by subject A0's antibodies, and that the ATD, TM4, or intracellular c-terminal tail are sufficient for recognition. In subject A6, the ATD, and amino acid 368 within the ATD, seem to be important for antibody recognition; however, the ligand-binding domain or TM1-3 may also contribute to epitope formation. For neither subject were the ATD or amino acid 368 as crucial as they are for epitope recognition of IgG antibodies from patients with anti-NMDAR encephalitis (data not shown, see also[12]).

Discussion

Contribution of autoantibodies to neurodegeneration has been suspected for a long time and correlations of increased antibody titers with dementia have been shown for very diverse autoantibodies ranging from anti-GM1,[17] anti-adrenergic receptors,[18] to antibodies against tau protein, neurofilaments,[19] β-amyloid,[20] GFAP[21], or neurotransmitters.[22] However, selection of dementia patients that could potentially benefit from immunotherapy is challenging, mainly because of limited understanding of the pathogenic role of individual antibodies and the lack of systematic data showing clinical improvement of autoantibody-positive dementia patients with immunotherapy.

The finding of high-titer IgA/IgM NMDAR antibodies in a subgroup of patients with dementia, the association with immunotherapy-responsive clinical entities, and the profound in vitro effects of patient IgA/IgM on hippocampal neurons[6,23] led to the question how frequent these antibodies are in different forms of dementia. For this, archived serum and CSF samples from different dementia cohorts in Germany and the United States were retrospectively analyzed. Although present in 16.1% of all dementia patients in the participating tertiary referral centers, NMDAR IgA/IgM antibodies were disproportionately distributed with highest frequency of 60% in "unclassified" dementia not fulfilling routine criteria for the established dementia forms. These patients frequently showed CSF abnormalities, a fluctuating disease course and psychiatric symptoms. NMDAR antibodies were also frequently detected in FTD, PPA, LBD, and PSP/CBS. Antibodies were detected much less often in patients with a family history of dementia or in patients with nondementing neurodegenerative disorders such as PD. With some exceptions, antibodies were primarily present in serum, but not CSF, supporting a peripheral origin of the autoimmune response.

The frequency of NMDAR antibodies was much lower in healthy controls and in patients with other neurological disorders. Mean ages of healthy controls, AD, PD, and PPA patients were 66.7, 70.4, 65.2, and 63.5 years, respectively, thus excluding that the low antibody frequency results from a younger cohort.[24] Frequencies of NMDAR antibody-positive cases vary in the literature. They range from the absence of IgG-positive cases in >8000 controls with various disorders[25] to the absence in more than 500 healthy control subjects,[23,26–29] to the presence of IgG and further isotypes in 7% of controls, none of them solely tested against the NR1 subunit of the NMDAR.[30] Another study found NMDAR IgM (but not IgA and IgG) in two of 21 healthy controls.[31] A large recent study used blood donors as controls and found a ~10% seroprevalence of NMDAR antibodies, mostly IgM and IgA subtypes.[32] One explanation might be the naturally limited information about the psychiatric and cognitive status of compensated blood donors, while the control subjects in the present cohort were thoroughly investigated for comorbidities and memory performance.

It remains unclear whether the presence of NMDAR antibodies in neurodegenerative and dementia patients simply reflects a biomarker for progressive brain disease or whether NMDAR antibodies directly participate in the disease process. It is possible that neuronal degeneration results in the presentation of neo-autoantigens to the immune system, in some cases mounting an immune response with synaptic antibodies that could potentially lead to synaptic dysfunction and accelerate cognitive decline. This hypothesis is supported by the documented effects of patient material on primary hippocampal neurons[6,24] and the clinical and radiological improvement in selected patients receiving immunotherapy. Serum antibodies might find facilitated conditions to penetrate into the brain in demented patients as the blood–brain barrier is not preserved at older ages or inflammation.[33] In this way, serum immunoglobulins have been shown to cause severe neuronal dysfunction in experimental models.[34] Also, the clinical differences between NMDAR antibody-positive and -negative patients (such as more frequent disease fluctuations, aphasia, and blood–brain barrier dysfunction) support a contribution of NMDAR antibodies to the clinical phenotype.

It is important to note that IgM/IgA-NMDAR antibodies do not define anti-NMDAR encephalitis, a

well-known severe immunotherapy-responsive encephalitis.[13,27,35] Although patients with anti-NMDAR encephalitis additionally had IgA-NMDAR antibodies in 31%,[6] the disease associates with IgG antibodies to a distinct NR1a amino terminal epitope; furthermore, amino acid N368 within this domain is crucial for epitope recognition.[12] Impaired binding of IgM/IgA-NMDAR antibody-positive serum to rat brain sections (using routine procedures for IgG detection) further suggests that disease mechanisms, antibody affinity, and epitopes are different between IgG-positive anti-NMDAR encephalitis and IgM/IgA-associated cognitive decline. Indeed, our epitope mapping pilot studies with two IgA-positive patient sera already demonstrate that epitopic determinants vary between patients with anti-NMDAR IgA-associated cognitive decline. Deletion of the ATD reduces antibody binding, but does not eliminate it, and the presence of this domain is not sufficient to fully preserve binding in both patients. Additionally, N368 is not absolutely required. Given that deletion of the ATD does not completely eliminate binding, additional parts of the receptor must also be targeted by IgA antibodies. The relationship between response to immunotherapy, NMDAR epitope, and functional assays of receptor downregulation should be explored in further detail; specific epitopes may be associated with distinct disease pathophysiology, neurologic symptoms, or treatment response.

Based on the present (principally retrospective) study, it is too early to give definite treatment recommendations in cases where NMDAR IgA/IgM antibodies are present. The imaging and in vitro data as well as analyses of the few cases that received immunotherapy suggest that the presence of NMDAR-IgA could possibly predict partial reversibility of the disease with immunotherapy. As the antibody levels likely play a role in pathogenicity, in the Charité hospital center, we consider immunotherapy if repeated NMDAR IgA/IgM antibody titers are ≥1:1000. Preliminary data showing clinical stabilization or improvement are encouraging, even more so as patients always presented late in the disease when irreversible neurodegeneration has already occurred. It remains unclear whether treatment with steroids is sufficient to define immunotherapy-(non)responsive dementia or whether further therapy (including plasma exchange, IVIg, rituximab, cyclophosphamide) and longer treatment duration are required in patients with high-level NMDAR antibodies. One should keep in mind that in a related case of a patient with VGKC complex (likely LGI1) antibody-associated dementia mimicking FTD, the patient improved from steroids only after a few weeks with further improvement occurring after several months of therapy.[4]

The current findings suggest that (1) IgA/IgM NMDAR serum antibodies are associated with dementia and could help to identify patients with cognitive decline who might benefit from immunotherapy, (2) patients with subacute or fluctuating dementia, CSF abnormalities, further autoimmune diseases, or atypical forms of dementia should be tested for IgA/IgM NMDAR antibodies, (3) as most patients in this constellation have no other treatment options, repeated IgA-NMDAR antibody titers ≥1:1000 in our opinion justify an immunotherapy attempt if the cognitive impairment is not explained otherwise, in particular if little irreversible brain damage has occurred and the patient is in good physical shape. Future studies should determine whether the high frequency of NMDAR antibodies in some dementia groups (such as PPA) is related to the specific pathogenesis or whether the antibodies define subgroups of the disorder (such as Progressive Nonfluent Aphasia [PNFA]). In addition, prospective analyses should determine which patients respond to immunotherapy, whether antibodies against additional targets (e.g., other synaptic proteins) are involved, and how antibody levels, epitope binding, and in vitro receptor downregulation using patient serum help to predict the clinical response.

Conflict of Interest

S. D. received financial support for a research project, travel, and speakers' honoraria from Actelion, and financial support for a research project from teva. B. M. has received grants from TEVA-Pharma, Desitin, Boehringer Ingelheim, GE Healthcare and honoraria for consultancy from Bayer Schering Pharma AG, AbbVie, TEVA-Pharma, for presentations from GlaxoSmithKline, Orion Pharma, TEVA-Pharma. B. M. is a member of the executive steering committee of the Parkinson Progression Marker Initiative of the Michael J. Fox Foundation for Parkinson's Research and has received grants from the BMBF, EU, Deutsche Parkinson Vereinigung, Michael J. Fox Foundation for Parkinson's Research, Stifterverband für die deutsche Wissenschaft, and has scientific collaborations with Roche, Ely Lilly, Covance. F. P. has received research support and speaker honoraria from Biogen, Bayer, MerckSerono, Teva, Sanofi and Novartis. K. R. received research support from Novartis as well as speaking fees and travel grants from Bayer Healthcare, Biogen Idec, Merck Serono, Sanofi/Genzyme, Teva, and Novartis. J. P. is an advisor to Actelion and Neuroimmune. C. P., B. T., and L. K. are employees of EUROIMMUN AG. C. P. and W. S. are shareholders of EUROIMMUN AG. W. S. is member of the Board of EUROIMMUN AG. J. D. and D. R. L. hold a patent for the use of NMDAR as antibody test and have a research grant from Euroimmun. The other authors report no disclosures.

References

1. Flanagan EP, McKeon A, Lennon VA, et al. Autoimmune dementia: clinical course and predictors of immunotherapy response. Mayo Clin Proc 2010;85:881–897.
2. Flanagan EP, Caselli RJ. Autoimmune encephalopathy. Semin Neurol 2011;31:144–157.
3. Lyons MK, Caselli RJ, Parisi JE. Nonvasculitic autoimmune inflammatory meningoencephalitis as a cause of potentially reversible dementia: report of 4 cases. J Neurosurg 2008;108:1024–1027.
4. McKeon A, Marnane M, O'connell M, et al. Potassium channel antibody associated encephalopathy presenting with a frontotemporal dementia like syndrome. Arch Neurol 2007;64:1528–1530.
5. Sabater L, Gaig C, Gelpi E, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. Lancet Neurol 2014;13:575–586.
6. Prüss H, Höltje M, Maier N, et al. IgA NMDA receptor antibodies are markers of synaptic immunity in slow cognitive impairment. Neurology 2012;78:1743–1753.
7. Filippini N, MacIntosh BJ, Hough MG, et al. Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci USA 2009;106:7209–7214.
8. Finke C, Kopp UA, Scheel M, et al. Functional and structural brain changes in anti-*N*-methyl-D-aspartate receptor encephalitis. Ann Neurol 2013;74:284–296.
9. Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 2005;360:1001–1013.
10. Apostolova I, Lindenau M, Fiehler J, et al. Detection of a possible epilepsy focus in the residual mesial temporal lobe in a patient operated on a ganglioglioma by ictal and interictal perfusion SPECT only after computer-aided sub-traction analysis: a case report. Nuklearmedizin 2008;47:N65–N68.
11. Wu HY, Hsu FC, Gleichman AJ, et al. Fyn-mediated phosphorylation of NR2B Tyr-1336 controls calpain-mediated NR2B cleavage in neurons and heterologous systems. J Biol Chem 2007;282:20075–20087.
12. Gleichman AJ, Spruce LA, Dalmau J, et al. Anti-NMDA receptor encephalitis antibody binding is dependent on amino acid identity of a small region within the GluN1 amino terminal domain. J Neurosci 2012;32:11082–11094.
13. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091–1098.
14. Westlye ET, Lundervold A, Rootwelt H, et al. Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE epsilon4 carriers: relationships with memory performance. J Neurosci 2011;31:7775–7783.
15. Hawellek DJ, Hipp JF, Lewis CM, et al. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. Proc Natl Acad Sci USA 2011;108:19066–19071.
16. Whitfield-Gabrieli S, Thermenos HW, Milanovic S, et al. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proc Natl Acad Sci USA 2009;106:1279–1284.
17. Hatzifilippou E, Koutsouraki E, Banaki T, et al. Antibodies against GM1 in demented patients. Am J Alzheimers Dis Other Demen 2008;23:274–279.
18. Karczewski P, Pohlmann A, Wagenhaus B, et al. Antibodies to the alpha1-adrenergic receptor cause vascular impairments in rat brain as demonstrated by magnetic resonance angiography. PLoS One 2012;7:e41602.
19. Bartos A, Fialova L, Svarcova J, Ripova D. Patients with Alzheimer disease have elevated intrathecal synthesis of antibodies against tau protein and heavy neurofilament. J Neuroimmunol 2012;252:100–105.
20. Taguchi H, Planque S, Nishiyama Y, et al. Catalytic antibodies to amyloid beta peptide in defense against Alzheimer disease. Autoimmun Rev 2008;7:391–397.
21. Petzold A, Keir G, Lim D, et al. Cerebrospinal fluid (CSF) and serum S100B: release and wash-out pattern. Brain Res Bull 2003;61:281–285.
22. Colasanti T, Barbati C, Rosano G, et al. Autoantibodies in patients with Alzheimer's disease: pathogenetic role and potential use as biomarkers of disease progression. Autoimmun Rev 2010;9:807–811.
23. Prüss H, Finke C, Höltje M, et al. *N*-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. Ann Neurol 2012;72:902–911.
24. Hammer C, Stepniak B, Schneider A, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. Mol Psychiatry 2013; [Epub ahead of print]. doi: 10.1038/mp.2013.110.
25. Titulaer MJ, Kayser MS, Dalmau J. Authors' reply. Lancet Neurol 2013;12:425–426.
26. Irani SR, Bera K, Waters P, et al. *N*-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. Brain 2010;133:1655–1667.
27. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol 2013;12:157–165.
28. Wandinger KP, Saschenbrecker S, Stoecker W, Dalmau J. Anti-NMDA-receptor encephalitis: a severe, multistage,

treatable disorder presenting with psychosis. J Neuroimmunol 2010;231:86–91.
29. Prüss H, Dalmau J, Harms L, et al. Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin. Neurology 2010;75:1735–1739.
30. Steiner J, Teegen B, Schiltz K, et al. Prevalence of *N*-methyl-D-aspartate receptor autoantibodies in the peripheral blood: healthy control samples revisited. JAMA Psychiatry 2014;71:838–839.
31. Busse S, Brix B, Kunschmann R, et al. *N*-methyl-D-aspartate glutamate receptor (NMDA-R) antibodies in mild cognitive impairment and dementias. Neurosci Res 2014; pii: S0168-0102(14)00113-8. doi: 10.1016/j.neures.2014.06.002. [Epub ahead of print]
32. Dahm L, Ott C, Steiner J, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. Ann Neurol 2014;76:82–94.
33. Galea I, Bechmann I, Perry VH. What is immune privilege (not)? Trends Immunol 2007;28:12–18.
34. Kowal C, DeGiorgio LA, Nakaoka T, et al. Cognition and immunity; antibody impairs memory. Immunity 2004;21:179–188.
35. Peery HE, Day GS, Dunn S, et al. Anti-NMDA receptor encephalitis. The disorder, the diagnosis and the immunobiology. Autoimmun Rev 2012;11:863–872.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Frequency distribution, isotypes, and titers of CSF NMDAR antibodies in study subjects. Number of subjects per group, percentage of dementia patients and controls with positive NMDAR antibodies (IgM, IgA, or IgG) in CSF (top). Antibody isotypes and CSF titers across dementia groups and controls (bottom).

