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The search for an autoimmune origin of psychotic disorders: Prevalence of autoantibodies against hippocampus antigens, glutamic acid decarboxylase and nuclear antigens

Carolin Hoffmann [a,1], Shenghua Zong [a], Marina Mané-Damas [a], Jo Stevens [a,2], Kishore Malyavantham [b,3], Cem İsmail Küçükali [c], Erdem Tüzün [c], Marc De Hert [d,e], Nico J.M. van Beveren [f], Emiliano González-Vioque [g,4], Celso Arango [g], Jan G.M.C. Damoiseaux [h], Bart P. Rutten [a], Peter C. Molenaar [a], Mario Losen [a], Pilar Martinez-Martinez [a,*]

[a] Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands
[b] IMMCO Diagnostics, Inc., 60 Pineview Drive, Buffalo, NY, USA
[c] Department of Neuroscience, Institute for Experimental Medical Research (DETAE), Istanbul University, Istanbul, Turkey
[d] UPC KU Leuven, KU Leuven Department of Neurosciences, Belgium
[e] Antwerp Health Law and Ethics Chair - AHLEC, University Antwerp, Antwerp, Belgium
[f] Department of Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands
[g] Child and Adolescent Psychiatry Department, Hospital General Universitario, Gregorio Marañón, School of Medicine, Universidad Complutense, IiSGM, CIBERSAM, Madrid, Spain
[h] Central Diagnostic Laboratory, Maastricht University Medical Center, Maastricht, the Netherlands

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ABSTRACT

The etiology of psychotic disorders is still unknown, but in a subgroup of patients symptoms might be caused by an autoimmune reaction. In this study, we tested patterns of autoimmune reactivity against potentially novel hippocampal antigens. Serum of a cohort of 621 individuals with psychotic disorders and 257 controls were first tested for reactivity on neuropil of rat brain sections. Brain reactive sera (67 diseased, 27 healthy) were further tested for antibody binding to glutamic acid decarboxylase (GAD) isotype 65 and 67 by cell-based assay (CBA). A sub-cohort of 199 individuals with psychotic disorders and 152 controls was tested for the prevalence of anti-nuclear antibodies (ANA) on HEp2-substrate as well as for reactivity to double-stranded DNA, ribosomal P (RPP), and cardiolipin (CL). Incubation of rat brain with serum resulted in unidentified hippocampal binding patterns in both diseased and control groups. Upon screening with GAD CBA, one of these patterns was identified as GAD65 in one individual with schizophrenia and also in one healthy individual. Two diseased and two healthy individuals had low antibody levels targeting GAD67 by CBA. Antibody reactivity on HEp-2-substrate was increased in patients with schizoaffective disorder, but only in 3 patients did antibody testing hint at a possible diagnosis of systemic lupus erythematosus. Although reactivity of serum to intracellular antigens might be increased in patients with psychotic disorder, no specific targets could be identified. GAD antibodies are very rare and do not seem increased in serum of patients with psychotic disorders.

1. Introduction

The etiology of psychotic disorders is still unknown, but in a subgroup of patients the symptoms might be caused by an autoimmune reaction (Hoffmann et al., 2016). Early suspicions on a link between autoimmunity and psychotic disorders were invigorated by the discovery of brain reactive immunoglobulins (Heath and Krupp, 1967). Also, the increased occurrence of psychosis in autoimmune disorders and vice versa suggests overlapping etiological factors (Benros et al., 2014a; Benros et al., 2014b). A shared risk factor for both diseases is the prior occurrence of infections (Benros et al.,

* Corresponding author at: Universiteitssingel 50, Room 1.108, 6229 ER Maastricht, P.O. Box 616, 6200 MD Maastricht, the Netherlands.
E-mail address: p.martinez@maastrichtuniversity.nl (P. Martinez-Martinez).
[1] Present address: Centre for Biomedical Research - CBMR, Universidade do Algarve, Portugal.
[2] Present address: Antibody Development, Centralized and Point of Care Solutions, Roche Diagnostics, Penzberg, Germany.
[3] Present address: Inova Diagnostics, San Diego, United States of America.
[4] Present address: Unit of Diagnosis and Treatment of Congenital Metabolic Diseases, Hospital Clínico Universitario de Santiago de Compostela, Health Research Institute of Santiago de Compostela (IDIS), Santiago de Compostela, Spain.

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2011). In addition, a common genetic predisposition was found for schizophrenia and six immune system-related diseases, including systemic lupus erythematosus (SLE) (Pouget et al., 2019). Recently, much attention has been drawn to the role of autoantibodies targeting neuronal surface antigens (NSAbs) in the brain of patients with autoimmune encephalitis who often present with psychosis. One of the proteins targeted is the *N*-methyl-D-aspartate receptor (NMDAR) causing patients to develop characteristic neurologic and psychiatric symptoms (Dalmau et al., 2011). It is clinically important that these diseases respond very well to immunosuppressive treatment indicating that any neuronal damage might be largely reversible (Graus et al., 2016; Varley et al., 2017; Zandi et al., 2014). Whether NSAbs can occur in purely psychotic patients, however, is less clear; their prevalence varies between 0 and 11% (Bergink et al., 2015; Endres et al., 2015; Lennox et al., 2017), possibly due to differences in the methodology of antibody detection and patient cohorts (Jezequel et al., 2017; Leypoldt et al., 2017; Pathmanandavel et al., 2015). In our own studies, autoantibodies against NMDAR and five other neuronal surface antigens in patients with a broad diagnostic spectrum of psychotic disorders were found to be absent after careful cross-validation to eliminate false positives (de Witte et al., 2015; Hoffmann et al., 2019).

In many autoimmune diseases neuropsychiatric problems are common. Within the group of systemic autoimmune rheumatic diseases (SARD), especially SLE may have neurological manifestations, the so-called neuro-lupus (Damoiseaux et al., 2015; Flower et al., 2017; Pego-Reigosa and Isenberg, 2008; Unterman et al., 2011). This warrants the testing for anti-nuclear antibodies (ANA), antibodies to double-stranded (ds) DNA, anti-extractable nuclear antigens (ENA) (including Ribosomal P protein (RPP)), and cardiolipin (CL) in patients with symptoms of psychosis. For instance, the presence of anti-RPP and anti-ds DNA cross-reacting to NMDAR subunit 2 have been associated with increased risk of neuropsychiatric symptoms in SLE (Hanly et al., 2011; Ho et al., 2016). Antibodies in general have poor or no access to intracellular antigen targets and are thus unlikely to bind to them and cause a direct pathogenic effect on the function of neurons. Yet, presence of certain autoantibodies might still be indicative for other autoimmune disease mechanisms (mediated by co-existing autoantibodies or autoreactive T cells, for example) that can be targeted by immunosuppressive treatment. Autoantibodies against the intracellularly located glutamic acid decarboxylase (GAD), which is the rate-limiting enzyme in the synthesis of gamma amino-butyric acid (GABA) occur in serum and CSF of patients with several neurological and endocrine syndromes. All these syndromes are characterized by dysfunction of the GABAergic system (Alexopoulos and Dalakas, 2013; Fouka et al., 2015; Saiz et al., 2008) suggesting that in this case the autoantibodies are a relevant biomarker for an autoimmune mechanism targeting this system. GAD autoantibodies are also a biomarker for diabetes mellitus type 1, but antibodies are reported to occur in lower titers than in neurological syndromes (Nakajima et al., 2018). Notably, some GAD antibody-positive conditions are responsive to immunosuppressive treatment, e.g. autoimmune epilepsy, even though to a lesser extent than patients with NSAbs (Daif et al., 2018; Vinke et al., 2018). There is other evidence that intracellular proteins could be autoimmune targets. In an animal model for the Stiff person syndrome, intrathecal application of antibodies against amphiphysin, an intracellular vesicular protein, leads to symptoms of reduced GABAergic transmission (Geis et al., 2010).

Here, we further characterize the serum binding patterns on rat brain of a cohort that tested negative for antibodies against neuronal surface antigens. We extend our antigen specific search for glutamic acid decarboxylase autoantibodies and tested for the occurrence of systemic autoimmune rheumatic disease-related autoantibodies in patients with psychotic disorders and healthy individuals including anti-nuclear antibodies (ANA), and antibodies to dsDNA, RPP and CL.

2. Methods

2.1. Study population

Samples and patient data were collected with written informed consent according to national and institutional ethical guidelines and the Helsinki Declaration, with additional informed consent by legal representatives for patients under age 18. The ability to provide written informed consent was evaluated by a psychiatrist by a face-to-face interview using a series of open-ended questions evaluating comprehension, reasoning, choice making and appreciation skills of the patient. The study population represents psychotic disorders and covers potential differences in diagnosis (Table 1). This is the same population used in our earlier study on the occurrence of NSAbs (Hoffmann et al., 2019). The cohorts from Rotterdam and France are part of the European network of national schizophrenia networks studying Gene-Environment Interactions (EU-GEI, http://www.eu-gei.eu).

2.2. Psychiatric diagnosis

The diagnosis was established by the treating psychiatrists based on the DSM-IV. The group of patients with psychotic disorders included schizophrenia, schizoaffective, brief psychotic disorder, schizophreniform, and other psychotic diagnoses, i.e. psychosis not otherwise specified, delusional disorder, substance induced psychosis, paranoid, and schizoid personality disorder.

2.3. Rat brain immunohistochemistry

Procedures were approved by the animal experiment committee at Maastricht University as well as the central committee of animal experiment (CCD) (WP 2016-005-001). Neuronal autoantibodies were identified by IHC on rat brain tissue following standard methods (Gresa-Arribas et al., 2014; Hoffmann et al., 2019; Titulaer et al., 2014). In brief, Lewis rat brains were fixed for 1 h in 4% paraformaldehyde and cryoprotected in 30% sucrose solution. After blocking with 0.3% H_2O_2 and 5% goat serum, sections were incubated with human serum diluted 1: 200 in 5% goat serum overnight at 4 °C. After incubating with biotinylated goat anti-human IgG Fcγ (1:3200, 109–066-008, Jackson ImmunoResearch) for 2 h at 20 °C, tissue was incubated with VECTASTAIN Elite ABC kit (Vector lab., # PK 6100) for 1 h at 20 °C and the reactivity developed using diaminobenzidine. Staining included serum samples from healthy individuals as negative controls, and from autoimmune encephalitis patients (against various autoantigens) as positive controls. Images were taken by the VENTANA iScan HT slide scanner (20× objective) and observed on the screen (Ventana Image Viewer). A grade between 0 and 3 was given based on the intensity and contrast of reactivity of sera against the hippocampal neuropil. All stainings that were scored 1–3 and inconclusive cases were repeated and validated by two independent observers of whom at least one was blinded. Those with inconsistent results were repeated at least once more and a final score (1 = borderline, 2 = weak positive, 3 = strong positive) was given according to all images of one sample.

2.4. Screening anti-GAD autoantibodies with CBA

Specific antibody screening detection was performed using an in-house CBA for glutamic acid decarboxylase isotypes 65 kDa and 67 kDa (GAD65, GAD67). The GAD plasmids expressed human GAD65 and GAD67 with the pCMV6-XL5 plasmid which was a kind gift from Dr. Francesc Graus (IDIBAPS, Barcelona) (Arino et al., 2014). HEK293 cells were plated on coverslips and transfected with 4 μg expression vectors of the respective human antigens and expression allowed for 22–26 h. Cells were fixed in 3.6% formaldehyde (#F006, TAAB) for 10 min and permeabilized with 0.3% Triton-X-100 for 10 min. After blocking with 1% bovine serum albumin (BSA) for 1 h, cells were incubated with

Table 1
Demographic description of cohorts.

	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6
Healthy, n	13	–	–	–	44	200
Psychotic disorders, n	203	23	300	95	–	–
Source	University Psychiatric Center Catholic University Leuven in Kortenberg	Public services (emergency wards, in- and out- patient clinics) and private clinics in the Paris region (Créteil)	Istanbul University, Aziz Sancar Institute of Experimental Medicine	Erasmus Medical Center (EMC) Rotterdam.	Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM)	Sanquin Maastricht
Criteria	Patients: DSM-IV diagnosis of schizophrenia, schizoaffective disorder, or bipolar disorder Healthy: No psychiatric antecedents or medication	DSM-IV diagnosis of psychotic disorder or mood disorders with psychotic features; substance-induced psychosis were excluded	DSM-IV diagnosis of schizophrenia	Consecutively admitted patients which initially presented with psychosis, and were finally diagnosed with schizophrenia, as well with a range of other mental disorders	Absence of any psychiatric diagnosis according to DSM-IV criteria No presence of a severe medical condition, and no current or past treatment with any antipsychotic drug	Blood donors, confirmed to be healthy by general indicators, tested by interviews, hemoglobin and other blood parameters, blood pressure, pulse, and body temperature as well as the absence of infectious diseases
Time-span	November 2003 to July 2007	June 2010 to May 2014	2011 to 2012.		January 2007 to December 2010	March 2014
Reference	(De Hert et al., 2006)	(Szoke et al., 2014)	–	(Schwarz et al., 2012)	(Pina-Camacho et al., 2014)	–

human sera diluted 1:40 in 1% BSA together with an antibody targeting the respective antigen (rabbit-anti-GAD65 (clone 7309LB, gift from Christina Hampe, 1:1000); or rabbit-anti-GAD67 (clone 10266/20B, gift from Christina Hampe, 1:1000)) for 1 h at 20 °C. Reactivity was visualized after incubation with secondary antibodies goat-anti-human-IgG Fcγ-Alexa488 (#109-546-170, Jackson, 1:1000) and goat-anti-rabbit Alexa594 (#111-585-144, Jackson, 1:1000) for 1 h at 20 °C. Screenings always included a positive control from an autoantibody positive patient and a negative human serum control. Cover glasses were mounted onto 7 μl DAPI mounting medium (#H-1200, Vector Laboratories) and evaluated by two (of which one blinded) observers independently on the BX51 Olympus microscope for antibody reactivity. When positive, the staining was repeated with serial dilution (1,50 up to 1,3200).

2.5. Measuring SARD-related autoantibodies

Screening of SARD-related antibodies was performed in collaboration with IMMCO Diagnostics (Buffalo, New York, USA). Immunofluorescent analysis (IFA) for ANAs was performed using ImmuGlo™ANA HEp-2 kit (#1103, Immco Diagnostics, USA) according to manufacturer's instructions. In short, patients' sera were incubated on HEp-2 cells in a dilution of 1:40 (in serum diluent) to allow binding of antibodies. Bound antibodies were detected by incubation of the substrate with fluorescein-labeled, anti-human IgG conjugate provided by the kit. Reactions were observed under a fluorescence microscope (NikonEclipse 50i diagnostic microscope) equipped with appropriate filters. Enzyme Linked Immunosorbent Assay (ELISA) or line immune assays (LIAs) were performed to test for the presence of ENA, dsDNA, CL and RPP autoantibodies. To this end an ELISA plate was pre-coated with the respective antigen(s): ENA (collectively detects, in one well, total ENAs against dsDNA, nDNA, histones, SS-A(Ro), SS-B(La), Sm, Sm/RNP, Scl-70, Jo-1, and centrometric antigens, ImmuLisa™ Enhanced ANA Screen ELISA, catalog # 5175), dsDNA (ImmuLisa™ Double stranded DNA antibody Enhanced ELISA, #5120), RPP (IMMULisa Ribosomal P, # 4133), or CL (ImmuLisa™ Cardiolipin IgG, IgA and IgM antibody(ACA) Enhanced ELISAs, #5118G, #5118A and #5118M). Serum was incubated at a dilution of 1:100. Horseradish peroxidase (HRP) conjugated anti-human IgG was used for labelling specific antibodies. Enzyme substrate (TMB) was then added to the wells and the presence of antibodies was detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction was stopped with H_2SO_4 and the intensity of the color change read by a spectrophotometer at 450 nm. Results are expressed in ELISA Units per milliliter (EU/ml) and reported as positive or negative. Threshold for positivity was >50 for dsDNA and >20 EU/ml for all other antigens.

2.6. Statistics

To test for the difference of rat brain IHC and ANA indirect immunofluorescence scores between the groups, we performed a non-parametric Kruskal-Wallis test. All tests were done in IBM SPSS Statistics version 23.0 for Windows.

3. Results

3.1. Novel hippocampal patterns of rat brain IHC are not specific for mental disorder

In a first step, we screened 621 patients with psychotic disorders and 257 healthy individuals for the presence of NSAbs. As reported in our previous publication, we did not identify any sera with antibodies against NMDAR (GluN1 alone and GluN1/GluN2B), leucine-rich glioma-inactivated 1 (LGI1), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), or γ-aminobutyric acid receptor subunit A and B (GABAaR, GABAbR). One serum sample (from a control participant) had autoantibodies targeting contactin-associated protein-like 2 (Caspr2) at a dilution 1:100 in the live version of the CBA. Further, only 2 sera contained antibodies binding to neurons at a dilution of 1:50; one was from a patient with schizophrenia and one was from a control participant, (Hoffmann et al., 2019).

Yet, the rat brain IHC revealed some distinct hippocampal patterns, that we present here. Several sera gave unknown patterns on the hippocampal neuropil, suggesting that they target novel cell surface or synaptic antigens. Hippocampal stainings with strong binding (grading 3) could be grouped according to similarity into eight patterns (see Fig. 1, Pattern A-H). Two of these samples (indicated with * in Fig. 1) were the sera previously reported to be reactive on live neurons (Hoffmann et al., 2019). Pattern A was prominent and seen with five sera (four from patients with schizophrenia and one from a control individual). This pattern gave a gradient in the dentate gyrus and synaptic areas of the cornu ammonis (CA). With the same screening strategy for autoantibodies against neuronal surface and synaptic antigens in

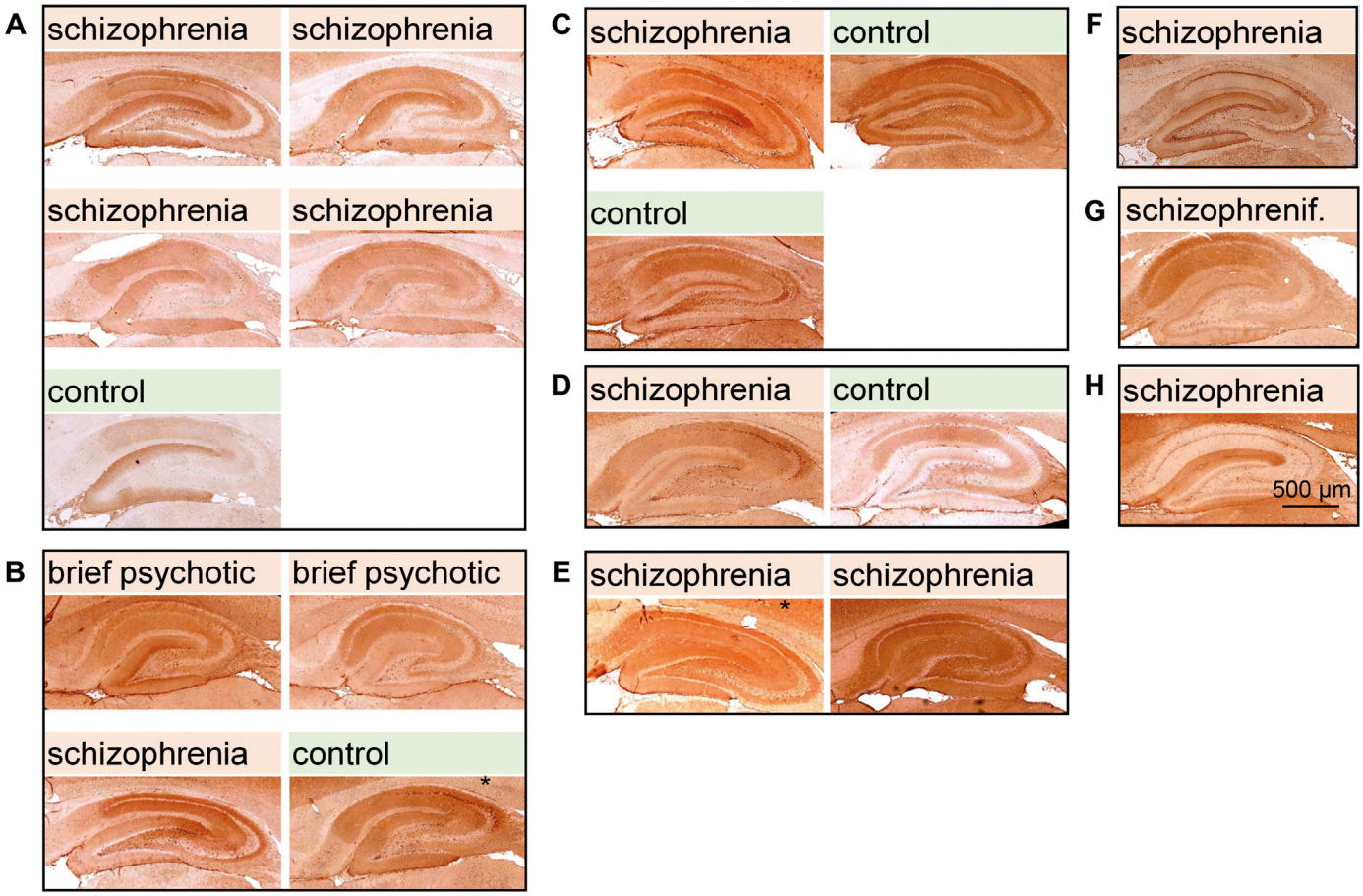

Fig. 1. Microscope images from sera positive by rat brain immunohistochemistry (IHC). Hippocampal IHC patterns graded 3 were sorted according to similarity into eight groups. Each image represents reactivity of one serum sample. Each box represents sera with similar hippocampal immunoreactivity patterns. Images labeled with * are from sera that were also reactive on live hippocampal neurons. Pattern F was identified as GAD65 antibody positive.

patients with depression and/or anxiety we also found some novel staining patterns on the hippocampus (Zong et al., 2020). Some of these were overlapping with the here reported reactivity, e.g. the here reported pattern A has similarity with the pattern C reported for 4 patients in the study by Zong et al. Interestingly, two samples resulting in this pattern that had higher antibody titers in that study also tested positive on live neurons. Further the here reported pattern C (2 schizophrenia patients, 1 healthy individual) was also reported for 2 healthy controls labeled as pattern K by Zong et al.. Pattern D (1 schizophrenia patient, 1 control individual) was similar to that in a previously published staining by Bergink et al. (2015) with serum from a patient with post-partum psychosis, which was also reactive to live neurons.

3.2. Autoantibodies against GAD65 and GAD67 are rare and do not differ between healthy controls and disease groups

Some of the IHC positive sera might also have autoantibodies targeting intracellular neuronal antigens. As GAD is an antigen that is related to neurological syndromes with GABA disturbances, we extended our antigen-specific screening of all sera that were graded 1–3 and with CBA testing for GAD65 and GAD67 reactivity. An overview of all the autoantibody screening results is shown in Table 2. Two sera were found to be reactive to GAD65 and GAD67; one of a schizophrenia patient (Fig. 1, Pattern F) and one of a healthy participant. We further identified 4 sera reactive to GAD67 alone (at a max. dilution 1:100 or 1:200). Most GAD67 antibodies cross-react to GAD65 as they bind to a common epitope. This has been observed for antibodies occurring in patients with diabetes as well as in neurological conditions (Gresa-Arribas et al., 2015; Jayakrishnan et al., 2011). However, a case of cerebellar ataxia presented with antibodies restricted to the GAD67 isotype, indicating the possibility that there is another type of GAD67 antibodies (Guasp et al., 2016). As there is not enough research on GAD65/67 antibody reactivity in psychiatric disorders, we prefer not to make the assumption of cross-reactivity.

Those sera reactive to GAD65 and GAD67 showed hippocampal reactivity pattern specific to GAD65, which is the same pattern in e.g. GAD antibody-positive epilepsy (Niehusmann et al., 2009). Those sera reactive to GAD67 alone had each a different hippocampal binding pattern and therefore were considered to contain neuronal autoantibodies or autoantibodies against unknown antigens (Fig. 2). In conclusion, we identified 2 sera reactive to GAD65 and GAD67 (Table 3).

3.3. Serum reactivity to HEp-2 substrate is increased in individuals with schizoaffective disorder, but specific autoantibodies against dsDNA, RPP, and CL are not

We investigated the prevalence of SARD-related autoantibodies in sera of a sub-cohort consisting of 199 patients with psychotic disorders, and 152 healthy individuals (Table 2). The reactivity of sera to ANAs on HEp-2 cells was increased in individuals with schizoaffective disordercompared to healthy individuals (Kruskal Wallis, $p = 0.011$). However, the number of sera testing positive for specific antigens (dsDNA, RPP, and CL) here was too low for relevant statistical analysis. We found that 3 sera (2 schizophrenia, 1 schizoaffective disorder) were consistently positive in different diagnostic assays (Table A.1), so it might be relevant to examine for clinical signs of SLE in these patients.

Table 2
Autoantibody screening results.

			Controls[a]	Psychotic disorders	Schizophrenia	Schizoaffective	Brief psychotic disorder	Schizophreniform	Other psychotic diagnoses
	No.		**257**	**621**	**476**	**44**	**38**	**47**	**16**
	av. age		44.1	34.4	36.6	31.4	26.7	22.9	31.5
	% female		47.5	39.9	39.5	61.4	34.2	36.1	18.8
Methods:									
IHC rat brain[b]	Tested, no.		**257**	**621**	**476**	**44**	**38**	**47**	**16**
	Grade = 1, no. (%)		14 (5.4)	40 (6.4)	29 (6.1)	4 (9.1)	2 (5.3)	3 (6.4)	2 (12.5)
	Grade = 2, no. (%)		8 (3.1)	13 (2.1)	10 (2.1)	2 (4.5)	1 (2.6)	0 (0)	0 (0)
	Grade = 3, no. (%)		5 (1.9)	14 (2.3)	10 (2.1)	1 (2.3)	2 (5.3)	1 (2.2)	0 (0)
CBA (IHC^+cohort)	Tested, no.		**27**	**67**	**49**	**7**	**5**	**3**	**3**
	Identified antigen		1× GAD65; 2× GAD67	1× GAD65; 2× GAD67	1xGAD65; 1× GAD67				1× GAD67
SARD-related antibodies	Tested, no.		**152**	**199**	**114**	**40**	**0**	**43**	**2**
	av. age		41.9	28.4	29.7	31.3		22.3	27.5
	% female		49.3	41.2	37.7	57.5		37.2	66.7
ANA IFA	Borderline, no. (%)	Nucleus	21 (13.8)	18 (9.0)	8 (7.0)	6 (15.0)		3 (7.0)	1 (50.0)
		Cytoplasm	1 (0.7)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)
	Positive, no. (%)	Nuclear	2 (1.4)	17 (8.5)	10 (8.8)	5 (12.5)		2 (4.7)	0 (0)
		Cytoplasm	0 (0)	7 (3.5)	4 (3.5)	3 (7.5)		0 (0)	0 (0)
ENA ELISA	Positive, no. (%)		2 (1.3)	4 (2)	2 (1.8)	2 (5.1)		0 (0)	
dsDNA ELISA	Positive, no. (%)		1 (0.7)	7 (3.5)	4 (3.5)	3 (7.7)		0 (0)	
RPP ELISA/LIA	Positive, no. (%)		9 (5.9)	7 (3.5)	5 (4.5)	2 (5.1)		0 (0)	
CL ELISA	Positive, no. (%)		12 (7.9)	9 (4.5)	6 (5.5)	2 (5.1)		1 (2.3)	
	No. (IgA/IgG/IgM)		(1/5/0)	(3/1/1)	(1/1/1)	(1/0/0)		(1/0/0)	

[a] Controls consist of 200 blood donors and 57 individuals without psychiatric diagnosis.
[b] Data taken from Hoffmann et al. (2019).

4. Discussion

In our cohort of patients with psychotic disorders only two samples were considered positive for GAD autoantibodies; one schizophrenia patient and one control. This suggests that serum GAD autoantibodies at high titers are very rare (2 out of 621) in schizophrenia-spectrum disorders. A previous meta-analysis on the prevalence of GAD autoantibodies in patients with psychosis reported that patients were more likely to have GAD65 antibodies than controls (odds ratio [OR], 2.24; 95%CI: 1.28–3.92%; $P = 0.005$; eight studies; $I2 = 0\%$) with a pooled prevalence of 5.8% (Grain et al., 2017). However, previous studies were using the more sensitive radio immuno assay (RIA), radio immunoprecipitation

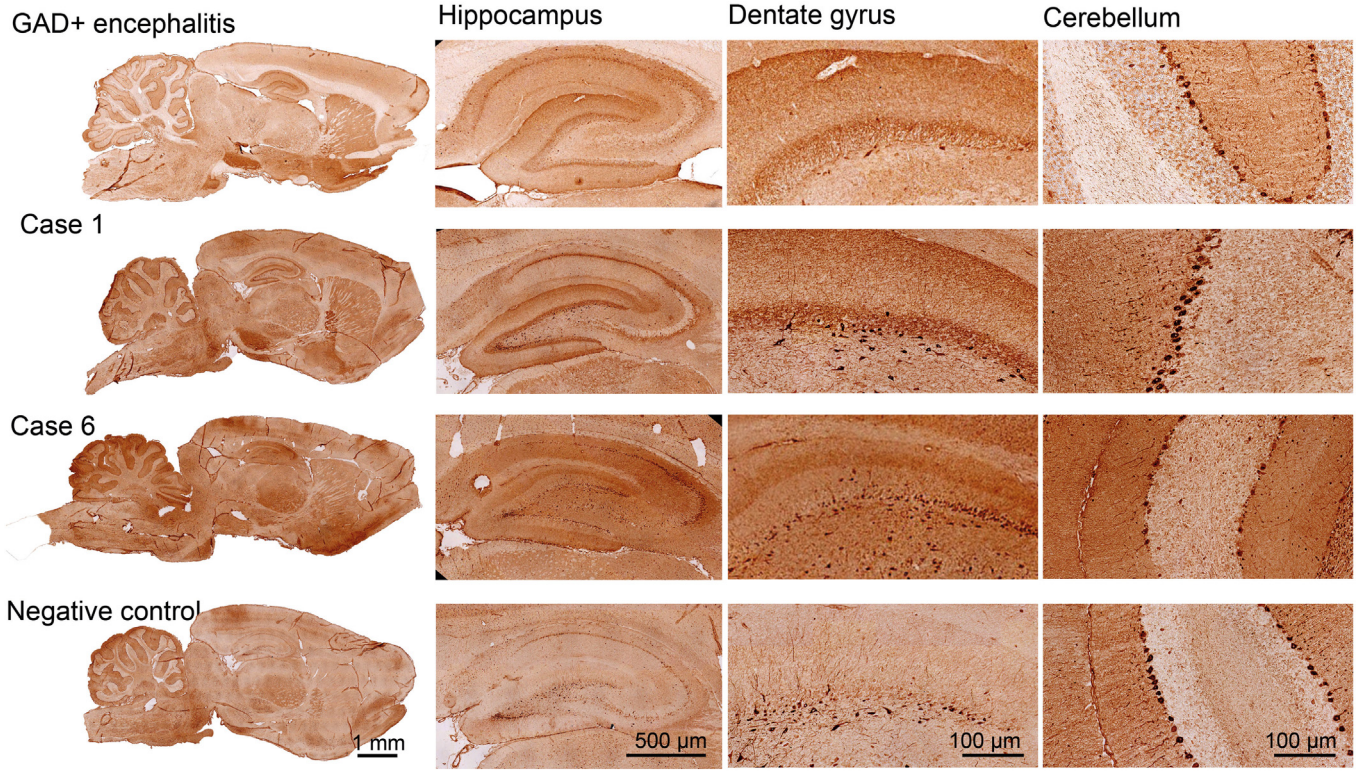

Fig. 2. Rat brain immunohistochemistry (IHC) for GAD+ cases. Case 1 shows a typical GAD65+/67+ antibody binding pattern similar to serum from a patient with GAD+ epilepsy. Case 6 shows unknown binding pattern of serum with GAD67 autoantibodies. More details of cases can be found in Table 3.

Table 3
Characteristics of patients with positive CBA results.

	CBA result[a]	Conc.	Diagnoses	Age	Sex	IHC grade[b] (UM/IDIBAPS)	Correlating pattern[c]	Combined conclusion[d]
Case 1	GAD65/67	1:6400	Schizophrenia	43	f	3/pos.	Yes	GAD65/67
Case 2	GAD65/67	1:3200	Control[e]	59	m	2/NA	Yes	GAD65/67
Case 3	GAD67	1:100	Schizophrenia	31	f	1/neg.	No	ND
Case 4	GAD67	1:100	Control[e]	55	f	1/neg.	No	ND
Case 5	GAD67	1:200	Psychosis NOS	32	m	1/neg.	No	ND
Case 6	GAD67	1:100	Control[e]	68	m	2/pos.	No	ND

Conc. = highest still positive serum concentration, UM = Maastricht University, IDIBAPS = Institut d'investigacions Biomèdiques August Pi i Sunyer, ND = not determined.
[a] CBA for GAD was performed on fixed cells.
[b] IHC at UM was graded 0–3 and at IDIBAPS positive/negative.
[c] Indicates whether the IHC hippocampal pattern resembled the typical pattern of the antigen identified by CBA.
[d] A conclusion was drawn based on the combination of different methods. Only, if a sera was tested positive by CBA and the IHC pattern correlated with the CBA results, it was considered positive.
[e] Controls consisted of blood donors and individuals without psychiatric diagnosis.

assay (RIPA), or enzyme-linked immunosorbent assay (ELISA). Therefore, reported results likely included also the lower antibody titers. From our unpublished studies, we know that the CBA and IHC detect GAD antibodies of higher titer and we expected higher titers to be more relevant for autoimmune-related neurological diseases while low titers are usually a diagnostic marker for diabetes mellitus type 1. A diagnosis of diabetes, however, was not excluded, which is a confounding factor, especially because patients with schizophrenia have a higher risk for comorbid diabetes mellitus type 1 (Benros et al., 2014b).

None of the patients in our study had a registered diagnosis of SLE, though we detected autoantibodies to specific SLE related antigens in 3 patients. Further clinical assessment of these patients would be necessary to determine a possible diagnosis of SLE. SARD-related autoantibodies, particularly those in SLE, have been associated with the occurrence of neuropsychiatric symptoms, also referred to as neuropsychiatric lupus (NPSLE) (Tay and Mak, 2017) whereby the most common symptoms are depression, cognitive dysfunction and anxiety. The reported prevalence of psychosis in SLE varies greatly depending on the population with 2.3% in an English population (Pego-Reigosa and Isenberg, 2008), and up to 11% in a black Caribbean study population (Flower et al., 2017). Little has been reported on the prevalence of SLE in patients with schizophrenia spectrum disorders. In the overall population of Europe the prevalence is estimated to be 35/100000 and 110/100000 in Afro-Caribbean people (Rees et al., 2017). In a large Danish National Register study of 7704 schizophrenia patients, none was reported with a diagnosis of SLE (Eaton et al., 2006). Yet, cases of patients presenting with a psychotic disorder or atypical SLE with predominant psychiatric manifestations have been described before (Lungen et al., 2019; Mack et al., 2017) and raise the question whether atypical presentation of SLE might hamper the diagnosis in patients presenting with psychotic disorders. In these cases, psychiatric symptoms might represent a prodromal stage of the disease or a subtype of SLE with isolated CNS involvement. Marrie et al. found that the incidence of psychiatric comorbidity is elevated in the immune-mediated inflammatory diseases (IMID) population (such as inflammatory bowel disease, multiple sclerosis and rheumatoid arthritis) as compared with a matched population as early as 5 years before diagnosis (Marrie et al., 2019). Whether this reflects shared risk factors for psychiatric disorders and IMID, a shared final common inflammatory pathway or other etiology is still matter of debate.

In the last 10 years, 16 novel autoimmune diseases of the CNS have been identified, leading to new treatment strategies for neurological and psychiatric syndromes (Dalmau et al., 2017). However, our previous studies (de Witte et al., 2015; Hoffmann et al., 2019) indicate that the prevalence of NSAbs is very low in patients with psychotic disorders and not different from the prevalence in control populations. It should be mentioned however, that the percentage of patients with an autoimmune origin of the disease might still be larger, but the tested autoimmune antibodies are signs of specific disorders that only affect a small number of individuals. It was our strategy to select a very heterogeneous population to represent a wide spectrum of patients. Treatment-resistant and acute/recent onset patients have been reported to have a higher prevalence of known autoimmune encephalitis-related autoantibodies (Pollak et al., 2019). Whether this is also the case for potentially novel antigens or SARD-related autoimmunity remains to be shown.

Our finding of reactive autoantibodies to rat brain warrants further research for the identification of the antigen as their target. As most of the sera were not reactive to live neurons, they potentially target intracellular or glial antigens. Neuronal surface receptors and channels have a high homology between rat and human so rat tissue can be used for detection of most human antibodies against these antigens. Some antigens however, e.g. dopamine receptor 2 are not detected by rat brain IHC. Especially when considering that novel antigens might be targeted in psychotic patients, one has to be aware that these might be missed when using the rat brain IHC. Also, we confirmed neuronal specificity on primary cultured life neurons, but we cannot exclude that (i) some neuronal antigens are expressed at a very low level in the cultured environment, or (ii) some antibodies target other cell types, e.g. microglia or astrocytes. Finally, a combination of serum analysis with cerebrospinal fluid (CSF) is always desirable as the presence of intrathecal antibodies increases the likelihood for disease relevance. Some antibodies, such as anti-NMDAR are better detectable in CSF, whereas others, such as those targeting LGI-1 and AQP4 might only be detectable in serum (Graus et al., 2016). General CSF analysis including white blood cell count, oligoclonal bands, and protein concentration can give further indications of increased intrathecal inflammation. CSF and serum analysis in 456 patients with schizophreniform syndromes also found that established anti-neuronal IgG antibodies are rare in serum (Endres et al., 2020). The study further reports, that antibodies are even rarer in the CSF but CSF alterations revealed a substantial subgroup with neuroinflammatory signs.

5. Conclusion

Autoantibodies against nuclear antigens, cardiolipin and GAD are rare in psychotic disorders. Serum reactivity on brain tissue indicates antibodies binding to unidentified antigens and future studies should thus focus on the identification of potentially novel antigens, including those targeting microglia and astrocytes as well as their disease relevance. The occurrence of SLE-related autoantibodies in 3 patients with schizophrenia-spectrum disorders warrants further research into the prevalence of atypical (undiagnosed) SLE in psychiatric disorders as well as whether psychotic symptoms are increased in the years preceding a diagnosis of SLE. The role of T-cell autoimmunity has not been addressed in our study but might represent an important pathogenic mechanism. Additionally, a better characterization of the patient cohort including neurological and general CSF examination is desirable to increase the chance of identifying individuals with an autoimmune origin of the disease.

Role of the funding source

The funding sources were not involved in study design; in the collection, analysis or interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declaration of competing interest

None.

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Appendix A

Table A.1
Three cases with comorbid anti-nuclear autoantibodies specific to systemic lupus erythematosus.

Diagnose	Age	Sex	Illness duration (yrs.)	ELISA					
				ANA IFA	ANA	dsDNA	RPP	CL	
Schizophrenia (paranoid type)	39	Male	0.5	+	+	−	+	+	(IgG)
Schizoaffective disorder	23	Female	8	+	+	+	−	+	
Schizophrenia (paranoid type)	21	Female	4.9	+	−	−	+	+	(IgA)

ELISA = enzyme-linked immunosorbent assay, RPP = ribosomal protein P, dsDNA = double-stranded deoxyribonucleic acid, ANA = anti-nuclear antibodies, CL = cardiolipin, ANA IFA = immunofluorescence on HEp-2 substrate for detection of antinuclear antibodies.

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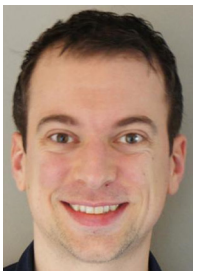
Carolin Hoffmann performed her PhD at Maastricht University, where she set-up the research line of autoantibodies in psychotic disorders under supervision of Prof. Pilar Martinez. Since receiving her PhD degree in 2018, she continues her research line in Maastricht as a PostDoc. In 2019 she stayed 3 months in the Neuroscience department of Biodonostia (San Sebastian, Spain). She recently received a 6-year fellowship from the Portuguese Foundation for Science and Technology (FCT) to work on the role of complement in schizophrenia. In the end of 2020, she joins the Molecular Neuroscience and Gene Therapy Group at the Center for Biomedical Research in Faro (Portugal).

Shenghua Zong received a Master degree on clinical medicine in 2012 from the Zhengzhou University. He was licensed as a medical doctor and worked one year as a resident (on the training to become a neurosurgeon) in Nanjing Benq hospital, China. In October 2014, he came to Prof. Pilar Martinez's lab, Maastricht University and worked as a research assistant. From 2015 till 2019, he performed his PhD study on the project of detecting neuronal autoantibodies in neuropsychiatric disorders at Maastricht University supported by a scholarship from China Scholarship Council. Since 2019, he continues his research under the Kootstra Talent Fellowship as a postdoctoral researcher.

Marina Mané-Damas is a PhD-student at the Department of Psychiatry and Psychology at Maastricht University. In 2012 she received her Bachelor degree in Biotechnology at Rovira I Virgili University (Spain) and subsequently performed her Master degree education in Neuroscience at University of Barcelona (Spain) where she graduated in 2013. She worked as a research assistant in Immune receptors of the innate and adaptive system in Institut d-Invertigacions Biomededicques Agust Pi I Sunyer (IDIBAPS). In December 2014 she started her PhD fellowship to study the prevalence and mechanisms of action of autoantibodies of patients with early onset psychotic disorders. She is also involved in the study of pathogenicity and drug mechanisms in other antibody mediated neuromuscular disorders.



Jo Stevens performed his PhD research 2011–2016 at Maastricht University focusing on Advanced diagnostics and therapeutics for Alzheimer's disease. During this time, he successfully developed a novel PET diagnostic for Alzheimer's disease based on antibodies, investigated effects of immunotherapy in Alzheimer's using antibody engineering techniques, setup a recombinant antibody production technique in an international collaboration, and discovered a novel role of lipid metabolism in an AD mouse model using AAV overexpression. After his graduation, he joined Roche (Munich, Germany) as postdoctoral research involved in antibody discovery and generation, where he was promoted to group leader in 2018.

Kishore Malyavantham is currently the Director of Research and Development at Inova Diagnostics, a Werfen Company. Kishore has a Master's degree in Biotechnology and Genetic Engineering from India and a Doctorate in Cell and Molecular Biology from University at Buffalo, New York, USA. Kishore's doctoral research focused on the structure and function of the mammalian cell nucleus and genomic organization. Prior to joining Inova, Kishore served as the Director of R&D at Immco Diagnostics and a Research Assistant Professor at the School of Medicine, University at Buffalo, NY. While at Immco, Kishore lead the development of a new family of multiplex diagnostic assays in line blot format for autoimmune and infectious disease areas. Kishore also pioneered the development of an FDA cleared HEp-2 substrate composed of cells knocked out for DFS70 gene product. Kishore's research has resulted in 30 peer reviewed publications and active patents in varied disciplines. Kishore's current focus is on clinical biomarker discovery as well as development of diagnostic reagents and technologies.

Cem İsmail Küçükali after graduating from the Faculty of Medicine in 1988, he worked as a research assistant in the neurology department of Ghent University, Belgium between 1990 and 1994. He has been working as a lecturer at Istanbul University, Aziz Sancar Experimental Research Institute, Neuroscience Department since 2010. Following his neurology expertise, he completed his master's and doctorate in neuroscience. He has received the title of associate professor in Neurology since 2018. Psychiatric diseases (Psychosis, Depression, Bipolar Affective Disorder and Obsessive Compulsive Disorder), Neuroimmunology, Animal Modeling, and Neurogenetic research fields. He has 86 articles and 135 papers in the field of Psychiatry and Neuroscience.

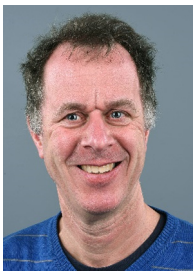
Erdem Tüzün received his medical doctor and neurologist titles in Istanbul University and then worked as a postdoctoral researcher at Oxford, Texas and Pennsylvania Universities. He is currently the chairman of Neuroscience Department at Istanbul University. His scientific studies are mainly focused on clinical neuroimmunology.

Marc De Hert is a psychiatrist and psychotherapist at the University Psychiatric Centre (UPC) KU Leuven. He does ambulatory clinical work in the psychosis care program. He is also a member of the medical council of the UPC KU Leuven. He studied medicine at the University of Antwerp and holds a PhD in biomedical sciences (PhD) from KU Leuven. He is a professor at KU Leuven, in the neuroscience department. Both his clinical expertise and his research area are in the domain of psychotic disorders. His current research includes: epidemiology, long-term outcomes, physical comorbidity and side effects of antipsychotics, genetic and environmental interactions and psychosocial interventions. He is currently a PhD law student at the University Antwerp.

Nico J.M. van Beveren currently works at Parnnassia Antes Center for Metal Health Care, and is affiliated with the Erasmus MC, departments of psychiatry and neuroscience. He does research in psychosis His current projects are about finding clinically relevant subgroups of psychotic disorders, focussing on immune and metabolic abnormalities.

Emiliano González-Vioque is at present the Head of Genetics Laboratory of the Unit of Diagnosis and Treatment of Congenital Metabolic Diseases, Hospital Clínico Universitario de Santiago de Compostela, Health Research Institute of Santiago de Compostela (IDIS). After his training as specialist in biochemical chemistry in 12 de Octubre Hospital, Madrid, he obtained a Ph.D. in Biochemistry and Molecular Biology at Universidad Autónoma de Madrid School of Medicine focused on mitochondrial disorders. During the next years he continued his research career as a postdoctoral researcher in the fields of mitochondrial disorders in the Vall d'Hebron Research Institute, Barcelona, and molecular basis of neuropsychiatric diseases in the University of Cambridge (UK) and Gregorio Marañón Research Institute, Madrid.

Dr Celso Arango, MD, PhD, is currently Chair of the Child and Adolescent Department of Psychiatry at Hospital General Universitario Gregorio Marañón, Complutense University in Madrid, Spain, as well as Director of the Gregorio Marañón Psychiatric and Mental Health Institute, Professor of Psychiatry at the Maryland Psychiatric Research Center of the University of Maryland in Baltimore, Adjunct Professor of Psychiatry at UCSF in San Francisco, Visiting Professor of Psychiatry at Kings College London, and Tenured Full Professor at Complutense University in Madrid. Past President of the ECNP, has served on many executive committees of international societies and is currently President of the Spanish Psychiatry Society since 2019.



Jan G.M.C. Damoiseaux (PhD) is medical immunologist and as such involved in diagnostic testing for immune-mediated diseases. His career has started in basic immunology research and evolved, via research in animal models for autoimmune diseases, towards clinical immunology research. The research has been focussed on immune regulation and on autoantibody testing. He is an active member of the European Autoantibody Standardisation Initiative (EASI) and the International Consensus on ANA Patterns (ICAP) working party. He has published more than 250 scientific papers in peer-reviewed journals. Many of these papers were the result of close collaboration with renowned national and international scientists.

Bart Rutten is an internationally renowned academic scientist and clinician in translational neuroscience on gene-environment interplay and epigenetics in mental disorders. He graduated as medical doctor with first class honours in 2000. During his MD and PhD period, Bart performed research at RWTH University in Aachen, the University of California in San Francisico, Emory University (Atlanta), and at Maastricht University, receiving his PhD degree in 2005. Since 2009, he has been active as a certified clinical psychiatrist, combining his clinical activities with teaching, management and particularly research on translational psychiatry. From 2013 – 2017, Bart Rutten has chaired the division of Neuroscience within the school for Mental Health and Neuroscience, and since 2017 he has become the chair of the department of Psychiatry and Neuropsychology as well as the chair of the clinical department of Psychiatry at MUMC+.

Peter C. Molenaar is senior scientist at the department of Psychiatry and Psychology Division Neuroscience at the School for Mental Health and Neuroscience at Maastricht University. He is trained as a pharmacologist and he has a special interest in diseases with dysfunctional neuronal ion channels (channelopathies). As an associate professor he was formerly heading a neuromuscular research group at the Leiden University Medical Centre.

Mario Losen trained in molecular biology, biochemistry and neuroimmunology. He is an Assistant Professor at the Department of Psychiatry and Psychology Division Neuroscience at the School for Mental Health and Neuroscience at Maastricht University. His work has mainly been focused on the development of novel experimental therapies for the treatment of myasthenia gravis, e.g. with recombinant anti-inflammatory IgG4 antibodies, leading to the serendipitous discovery of the Fab-arm exchange reaction of human IgG4 in rhesus monkeys. He is PI in the Research Group Nervous System Neuroinflammation and Autoimmunity.

Pilar Martinez-Martinez is trained in molecular biology, biochemistry and neuroimmunology. She is Professor of Neuroinflammation in neuropsychiatric disorders at the department of Psychiatry and Psychology Division Neuroscience at the School for Mental Health and Neuroscience at Maastricht University. She is the PI of several European grants examining neuroinflammation with special focus in neurodegenerative diseases and its relationship with the sphingolipid metabolism. Specifically, she has studied CERT and developed the technology to measure and to modulate CERT expression levels. Additionally, she has long lasting expertise in translational neurosciences, and her research focus on understanding the molecular pathogenic mechanisms and uses new therapeutic approaches in animal models with peripheral nervous system and central nervous system disease.