Brain, Behavior, and Immunity 108 (2023) 135–147

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Factors predisposing to humoral autoimmunity against brain-antigens in health and disease: *Analysis of 49 autoantibodies in over 7000 subjects*

Vinicius Daguano Gastaldi [a], Justus BH Wilke [a], Cosima A. Weidinger [a], Carolin Walter [a], Nadine Barnkothe [a], Bianca Teegen [b], Felix Luessi [c], Winfried Stöcker [b], Fred Lühder [d], Martin Begemann [a], Frauke Zipp [c], Klaus-Armin Nave [e], Hannelore Ehrenreich [a,*]

[a] *Clinical Neuroscience, Max Planck Institute for Multidisciplinary Sciences, City Campus, Göttingen, Germany*
[b] *Institute for Experimental Immunology, Affiliated to Euroimmun, Lübeck, Germany*
[c] *Department of Neurology, Focus Program Translational Neuroscience (FTN) and Immunotherapy (FZI), Rhine-Main Neuroscience Network (rmn2), University Medical Center of the Johannes Gutenberg University, Mainz, Germany*
[d] *Institute of Neuroimmunology and Multiple Sclerosis Research, University Medical Center, of the Georg August University, Göttingen, Germany*
[e] *Department of Neurogenetics, Max Planck Institute for Multidisciplinary Sciences, City Campus, Göttingen, Germany*

A R T I C L E I N F O

Keywords:
Age
Gender
Genetic predisposition
Immune check-point genotypes
NMDAR1-AB
GWAS
APOE4
Brain injury

A B S T R A C T

Background: Circulating autoantibodies (AB) against brain-antigens, often deemed pathological, receive increasing attention. We assessed predispositions and seroprevalence/characteristics of 49 AB in > 7000 individuals.

Methods: Exploratory cross-sectional cohort study, investigating deeply phenotyped neuropsychiatric patients and healthy individuals of GRAS Data Collection for presence/characteristics of 49 brain-directed serum-AB. Predispositions were evaluated through GWAS of NMDAR1-AB carriers, analyses of immune check-point genotypes, *APOE4* status, neurotrauma. Chi-square, Fisher's exact tests and logistic regression analyses were used.

Results: Study of N = 7025 subjects (55.8 % male; 41 ± 16 years) revealed N = 1133 (16.13 %) carriers of any AB against 49 defined brain-antigens. Overall, age dependence of seroprevalence (OR = 1.018/year; 95 % CI [1.015–1.022]) emerged, but no disease association, neither general nor with neuropsychiatric subgroups. Males had higher AB seroprevalence (OR = 1.303; 95 % CI [1.144–1.486]). Immunoglobulin class (N for IgM:462; IgA:487; IgG:477) and titers were similar. Abundant were NMDAR1-AB (7.7 %). Low seroprevalence (1.25 %-0.02 %) was seen for most AB (e.g., amphiphysin, KCNA2, ARHGAP26, GFAP, CASPR2, MOG, Homer-3, KCNA1, GLRA1b, GAD65). Non-detectable were others. GWAS of NMDAR1-AB carriers revealed three genome-wide significant SNPs, two intergenic, one in *TENM3*, previously autoimmune disease-associated. Targeted analysis of immune check-point genotypes (*CTLA4, PD1, PD-L1*) uncovered effects on humoral anti-brain autoimmunity (OR = 1.55; 95 % CI [1.058–2.271]) and disease likelihood (OR = 1.43; 95 % CI [1.032–1.985]). *APOE4* carriers (~19 %) had lower seropositivity (OR = 0.766; 95 % CI [0.625–0.933]). Neurotrauma predisposed to NMDAR1-AB seroprevalence (IgM: OR = 1.599; 95 % CI [1.022–2.468]).

Conclusions: Humoral autoimmunity against brain-antigens, frequent across health and disease, is predicted by age, gender, genetic predisposition, and brain injury. Seroprevalence, immunoglobulin class, or titers do not predict disease.

Abbreviations: APOE4, Apolipoprotein E ε4 allele; AB, Autoantibodies; BBB, Blood-brain-barrier; CTLA-4, Cytotoxic *T*-lymphocyte-associated protein 4; GWAS, Genome-wide association study; GRAS, Göttingen Research Association for Schizophrenia; Ig, Immunoglobulin; NT, Neurotrauma; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death protein ligand 1; SNP, Single nucleotide polymorphism.

* Corresponding author at: Clinical Neuroscience, Max Planck Institute for Multidisciplinary Sciences, City Campus, Hermann-Rein-Str.3, 37075 Göttingen, Germany.

E-mail address: ehrenreich@mpinat.mpg.de (H. Ehrenreich).

https://doi.org/10.1016/j.bbi.2022.10.016
Received 9 August 2022; Received in revised form 24 September 2022; Accepted 22 October 2022
Available online 30 October 2022

1. Introduction

Autoantibodies (AB) in general and brain-directed AB in particular have received increasing attention in the last decades (Diamond et al., 2009; Prüss, 2021; Ehrenreich, 2017; Giannoccaro et al., 2019b; Pollak et al., 2020; Endres et al., 2022). Human protein microarrays containing large numbers of antigens in a native conformation, identified abundant and ubiquitous natural IgG AB in human sera, many of which apparently belong to the normal autoimmune repertoire (Nagele et al., 2013; Cohen and Efroni, 2019; Ehrenreich, 2018). Brain-directed AB, dependent on the antigen, can substantially modulate brain function - provided they gain sufficient access to the brain (Hammer et al., 2014; Castillo-Gomez et al., 2016). This access is usually limited, but quite excessively possible upon intrathecal synthesis or disruption of the blood–brain-barrier (BBB), as found e.g., due to genetic predisposition (Apolipoprotein E ε4 allele [*APOE4*] genotype), after traumatic brain injury or stroke, during systemic inflammatory processes and encephalitides, or even under anesthesia (Montagne et al., 2020; Zerche et al., 2015; Wilke et al., 2021; Spieth et al., 2021; Teller et al., 2022). The *'how-when-where'* conditions of intrathecal AB synthesis are still obscure. AB against the *N*-methyl-D-aspartate-receptor subunit-NR1 (NMDAR1-AB), exhibiting the highest seroprevalence presently known for anti-brain AB (Hammer et al., 2014; Dahm et al., 2014), are particularly interesting. By acutely down-regulating NMDAR1 surface expression, they can exert a spectrum of behavioral, neurological, or psychopathological effects, resembling the pharmacology of ketamine-like agents, even including antidepressive properties (Ehrenreich, 2017; Ehrenreich, 2018; Dalmau et al., 2008; Pan et al., 2021). In case of sudden BBB leakiness, they bind in large amounts to brain tissue which virtually acts as 'targeting sponge' or 'immunoprecipitator' of these AB, massively and specifically extracting them from the circulation (Castillo-Gomez et al., 2016). Similarly, other brain-directed AB, e.g., against CASPR2 or GABA-a, can bind to respective sites in brain and exert their specific effects (Giannoccaro et al., 2019b; Kreye et al., 2021; Giannoccaro et al., 2019a; Fernandes et al., 2019; Dawes et al., 2018). Intriguingly, Lupus AB can act as positive allosteric modulators at GluN2A-containing NMDAR and impair spatial memory (Chan et al., 2020). Therefore, serological testing for brain-directed AB is an important diagnostic measure in patient care but any expected contribution to clinical syndromes has to be carefully appraised in each individual context.

Associations of e.g., NMDAR1-AB with teratoma/tumors (Dalmau et al., 2008), or with infections like Herpes (Pruss et al., 2012) or Influenza A/B (Hammer et al., 2014; Castillo-Gomez et al., 2016) were reported. However, the (patho)physiological roles of AB – e.g., those of NMDAR1-AB, highly seroprevalent across mammals (Pan et al., 2019) - are still incomprehensible. Notably, all naturally occurring NMDAR1-AB have pathogenic potential, irrespective of epitope and immunoglobulin (Ig) class (Castillo-Gómez et al., 2017). Overall, considerable mysteries have remained for both scientists and clinicians regarding syndromic or disease relevance of AB. This lack of understanding is not infrequently of disadvantage for AB-carrying patients, who may get immunosuppressive treatment too hastily (Ehrenreich et al., 2022). We have to ask under which conditions brain-directed AB in serum do gain pathological significance. Can they help distinguish between health and disease states? In other words, are carriers of these AB more likely to belong to disease groups? Are particular Ig classes more relevant than others regarding disease indication? In a nutshell, do peripheral serum titers by themselves tell us anything meaningful for diagnostic conclusions? Can we identify any solid predictors of seroprevalence?

Considering the high and ever-increasing clinical relevance of these questions, the present study was designed to investigate seroprevalence and potential predictors of 49 brain-directed AB in > 7000 subjects, healthy or suffering from neuropsychiatric diseases, i.e., schizophrenia, schizoaffective, affective, personality disorders, addiction, autism spectrum, stroke, neurodegenerative and autoimmune diseases. Importantly, we find age, gender, genetic predisposition and brain injury associated with serum AB. However, neither seroprevalence nor Ig class nor titers are solid predictors of any disease. These results question a straightforward pathogenic role of these brain-directed AB and should appeal for more fundamental research to gain a better understanding of their (patho)physiological significance.

2. Methods and materials

2.1. Subjects

Subject data collection in the scope of the extended GRAS (Göttingen Research Association for Schizophrenia) database has been approved by the ethical committee of the Georg-August-University of Göttingen (master committee) as well as by the respective local regulatories/ethical committees of collaborating centers, all in accordance with Helsinki Declaration (Ribbe et al., 2010). Individuals were recruited all across Germany and are mostly of German origin. A total of N = 7,025 participants are evaluated in this study, with N = 4,266 of them previously analyzed for 24 serum AB (Dahm et al., 2014). Of the N = 2,759 new individuals, N = 1,030 are healthy controls (mostly anonymized blood donors of Department of Transfusion Medicine, Göttingen). The remaining 1,729 individuals add to existing subgroups of the GRAS Data Collection. The newly formed autoimmune group (n = 701) and 37 individuals with neurodegenerative disorders were recruited at Department of Neurology, University Medical Center, Johannes Gutenberg University Mainz. For overview of antigens screened and AB seroprevalence in disease groups see Tables 1 and 2.

2.2. Serological analyses

Serological analyses (Table 2) were performed as described earlier (Dahm et al., 2014). Briefly, serum samples of all individuals were tested for AB presence (Table 2) using biochip mosaics (Euroimmun, Lübeck, Germany) that contained nonfixed nitrogen-frozen tissue cryosections (4 mm; rat hippocampus, monkey cerebellum) and recombinant cell substrates (formalin- or acetone-fixed transfected HEK293 cells). Recombinant protein (autoantigen) expression was validated by immunological methods employing human or commercially available monospecific animal antibodies. A total of 49 different neural antigens, previously associated with autoimmunity/autoimmune disease (Prüss, 2021; Pruss et al., 2012; Dalmau et al., 2007; Ma et al., 2017; De Camilli et al., 1993; Dalmau et al., 2017; Abboud et al., 2021; Hart et al., 1997; Jarius et al., 2010; Fang et al., 2016; Tanaka et al., 1989; Zhang et al., 2014; Irani et al., 2010; Sabatino et al., 2019; Xiao et al., 1991; Zuliani et al., 2007; Hoftberger et al., 2013; Hutchinson et al., 2008; Swayne et al., 2018; Solimena et al., 1988; Voltz et al., 1999; Jarius and Wildemann, 2015; Mathey et al., 2007; Kira et al., 2019; Newman et al., 1995; Honorat et al., 2019; Peterson et al., 1992) were evaluated: NMDAR1, AGNA, AMPAR, amphiphysin, ANNA-3, AP3B2, AQP4, ARHGAP26, AT1A3, CARPVIII, CASPR2, CNTN1, CNTN2, CV2, DPPX, DRD2, ERC1, flotillin 1/2, GABA-a, GABA-b, GAD65, GFAP, GLRA1b, GluRD2, Homer-3, Hu, IgLON5, ITPR1, KCNA1, KCNA2, LGI1, Ma2, MAG, MBP, mGluR1, mGluR5, MOG, Myelin, neurexin, neurochondrin, NF155, NF186, PCA-2, recoverin, Ri, Sez6I2, Tr/DNER, Yo, Zic-4. Table 1 lists the most frequent antigens with respective references (Prüss, 2021; Pruss et al., 2012; Dalmau et al., 2007; Ma et al., 2017; De Camilli et al., 1993; Dalmau et al., 2017; Abboud et al., 2021; Hart et al., 1997; Jarius et al., 2010; Fang et al., 2016; Tanaka et al., 1989; Zhang et al., 2014; Irani et al., 2010; Sabatino et al., 2019; Xiao et al., 1991; Zuliani et al., 2007; Hoftberger et al., 2013; Hutchinson et al., 2008; Swayne et al., 2018; Solimena et al., 1988; Voltz et al., 1999; Jarius and Wildemann, 2015; Mathey et al., 2007; Kira et al., 2019; Newman et al., 1995; Honorat et al., 2019; Peterson et al., 1992).

Table 1
Characteristics of the top 15 most frequent anti-brain antigens for autoantibody seroprevalence screening.

Antigen	Full name	Location	Function	Associated diseases/syndromes	First description of autoimmune association
NMDAR1	Glutamate ionotropic receptor NMDA type subunit 1	Extracellular	Obligatory subunit of tetrameric NMDA-receptors, which have a major role in excitatory neurotransmission	Anti-NMDAR-encephalitis (Dalmau et al., 2007), Herpes simplex encephalitis (Pruss et al., 2012), Japanese encephalitis (Ma et al., 2017)	*Ann Neurol* (Dalmau et al., 2007)
Amphiphysin	Amphiphysin	Intracellular/synaptic	Presynaptic vesicle protein, involved in vesicle endocytosis	Stiff-person syndrome (De Camilli et al., 1993; Dalmau et al., 2017), breast cancer (De Camilli et al., 1993), SCLC (Prüss, 2021), (limbic) encephalomyelitis (Prüss, 2021), neuropathy (Abboud et al., 2021)	*J Exp Med* (De Camilli et al., 1993)
KCNA2	Potassium voltage-gated channel subfamily A member 2	Extracellular	Voltage gated potassium channel, neuronal excitability	Neuromyotonia (Hart et al., 1997)	*Ann Neurol* (Hart et al., 1997)
ARHGAP26	Rho GTPase activating protein 26	Intracellular/somata/ neuropil	Clathrin independent endocytosis	Subacute inflammatory cerebellar ataxia (Jarius et al., 2010)	*J Neuroinflamm* (Jarius et al., 2010)
GFAP	Glial fibrillary acidic protein	Intracellular	Part of cytoskeleton, maintenance of astrocytic structure	Autoimmune GFAP astrocytopathy (Fang et al., 2016); meningoencephalitis (Abboud et al., 2021); Alzheimer's disease (Tanaka et al., 1989), traumatic brain injury (Zhang et al., 2014)	*JAMA Neurol* (Fang et al., 2016)
CASPR2	Contactin-associated protein 2	Extracellular/neuropil	Cell adhesion protein, antigen is VGKC associated protein	Neuromyotonia (Prüss, 2021; Irani et al., 2010), Morvan's syndrome (Prüss, 2021; Irani et al., 2010), neuropathic pain (Prüss, 2021), limbic encephalitis (Abboud et al., 2021; Irani et al., 2010), cerebellitis/cerebellar degeneration (Abboud et al., 2021)	*Brain* (Irani et al., 2010)
MOG	Myelin oligodendrocyte glycoprotein	Extracellular/outer myelin sheet	Cell adhesion molecule in oligodendrocytes, maintenance of myelin structure	Acute disseminated encephalomyelitis (Prüss, 2021; Sabatino et al., 2019); multiple sclerosis (Sabatino et al., 2019; Xiao et al., 1991); neuromyelitis optica spectrum disorder (Sabatino et al., 2019), cortical/subcortical encephalitis (Abboud et al., 2021), brainstem encephalitis (Abboud et al., 2021)	*J Neuroimmunol* (Xiao et al., 1991)
Homer-3	Homer protein homolog 3	Intracellular/cytoplasm	Postsynaptic calcium responses in dendritic spines of Purkinje cells, modulating activity of metabotropic glutamate-receptors	Cerebellar ataxia (Zuliani et al., 2007), cerebellitis (Hoftberger et al., 2013)	*Neurology* (Zuliani et al., 2007)
KCNA1	Potassium voltage-gated channel subfamily A member 1	Extracellular	Voltage gated potassium channel, neuronal excitability	Neuromyotonia (Hart et al., 1997)	*Ann Neurol* (Hart et al., 1997)
GLRA1b	Glycine receptor alpha 1 isoform b	Extracellular	Alpha1 subunit of inhibitory glycine receptor	Progressive encephalopathy with rigidity and myoclonus (Hutchinson et al., 2008; Swayne et al., 2018), epilepsy (Swayne et al., 2018)	*Neurology* (Hutchinson et al., 2008)
GAD65	Glutamate decarboxylase 2	Intracellular/cytoplasm	Intracellular/presynaptic protein involved in neurotransmitter synthesis	Stiff-person syndrome (Dalmau et al., 2017; Solimena et al., 1988), limbic encephalitis (Abboud et al., 2021), cerebellitis/cerebellar degeneration (Abboud et al., 2021), encephalomyelitis (Abboud et al., 2021)	*N Eng J Med* (Solimena et al., 1988)
Ma2	PNMA family member 2	Intracellular/nuclear	Possibly involved in positive regulation of the apoptotic process	Limbic encephalitis (Abboud et al., 2021; Voltz et al., 1999), brainstem encephalitis (Abboud et al., 2021; Voltz et al., 1999), diencephalic encephalitis (Abboud et al., 2021)	*New Eng J Med* (Voltz et al., 1999)
Yo	Cerebellar degeneration related protein 2	Intracellular/nuclear	DNA binding protein	Paraneoplastic cerebellar degeneration (Jarius and Wildemann, 2015; Peterson et al., 1992), cerebellitis/ cerebellar degeneration (Abboud et al., 2021)	*Neurology* (Peterson et al., 1992)
NF155	Neurofascin 155 kD isoform	Extracellular	Glial cell adhesion, expressed in cell bodies of oligodendrocytes	Multiple sclerosis (Mathey et al., 2007; Kira et al., 2019); chronic inflammatory demyelinating polyneuropathy (Kira et al., 2019)	*J Exp Med* (Mathey et al., 2007)
AP3B2	Adaptor Related Protein Complex 3 Subunit Beta 2	Intracellular/Golgi apparatus	Neuron specific vesicle coat protein, controlling levels of selected membrane proteins in synaptic vesicles	Paraneoplastic cerebellar degeneration (Jarius and Wildemann, 2015; Newman et al., 1995), autoimmune cerebellar ataxia (Honorat et al., 2019)	*Cell* (Newman et al., 1995)

Table 2
Overview of the top 15 most frequent brain autoantibodies according to identified seroprevalence.

Disorders/diseases	**Schizophrenia Schizoaffective**	**Affective**	**Personality & Addiction**	**Neuro-developmental**	**Stroke**	**Neuro-degenerative**	**Autoimmune**	**ALL Diseases**	**Healthy Controls**	**ALL Subjects**
No. Individuals[†]	**2043** (1818–2043)	**267** (264–267)	**334** (193–333)	**141**	**442**	**349** (310–349)	**701**	**4277** (3909–4265)	**2748** (2391–2735)	**7025** (6300–7000)
Male, %	**64.90 %**	**50.56 %**	**56.29 %**	**65.25 %**	**54.75 %**	**59.89 %**	**27.39 %**	**55.74 %**	**55.93 %**	**55.81 %**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
Any AB Total No.	2043	267	334	141	442	349	701	4277	2748	7025
Seropositive No. (%)	346 (16.94)	62 (23.13)	34 (10.18)	12 (8.51)	147 (33.26)	41 (11.75)	91 (12.98)	733 (17.14)	400 (14.56)	1133 (16.13)
Seropositive, males	230	38	25	7	87	27	34	448	240	688
IgM/IgA/IgG #	134/148/141	21/26/25	7/20/13	6/5/5	69/58/49	31/25/17	39/39/47	307/321/297	155/166/180	462/487/477
NMDAR-1 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	158 (7.73)	31 (11.74)	12 (3.6)	3 (2.13)	84 (19)	31 (9.09)	34 (4.85)	353 (8.28)	183 (6.69)	536 (7.66)
Seropositives, males	94	17	7	1	46	22	12	199	117	316
IgM/IgA/IgG No.	87/91/12	15/17/4	3/10/0	2/2/0	56/39/4	28/20/15	22/23/3	213/202/38	105/107/17	318/309/55
Titer range IgM	1:10–1:3200	1:10–1:320	1:32–1:320	1:10–1:100	1:10–1:1000	1:10–1:1000	1:10–1:1000	1:10–1:3200	1:10–1:1000	1:10–1:3200
Titer range IgA	1:10–1:3200	1:10–1:100	1:10–1:100	1:32–1:32	1:10–1:1000	1:10–1:1000	1:10–1:1000	1:10–1:3200	1:10–1:1000	1:10–1:3200
Titer range IgG	1:10–1:320	1:32–1:100	–	–	1:10–1:32	1:32–1:1000	1:32–1:100	1:10–1:1000	1:10–1:320	1:10–1:1000
IgM/IgA/IgG median	1:32/1:32/1:32	1:100/1:32/ 1:66	1:32/1:32/–	1:32/1:32/–	1:32/1:32/1:10	1:32/1:100/ 1:100	1:32/1:100/ 1:32	1:32/1:32/1:32	1:32/1:32/1:32	1:32/1:32/1:32
KCNA2 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	26 (1.43)	5 (1.87)	3 (1.55)	1 (0.71)	8 (1.81)	4 (1.15)	4 (0.57)	51 (1.3)	35 (1.46)	86 (1.37)
Seropositives, males	24	4	3	1	6	2	1	41	24	65
IgM/IgA/IgG, No.	1/6/20	1/0/4	1/0/2	0/0/1	0/2/6	0/3/1	0/2/2	3/13/36	3/8/26	6/21/62
Titer range IgM	1:32	1:10	1:32	–	–	–	–	1:10–1:32	1:10–1:32	1:10–1:32
Titer range IgA	1:32–1:320	–	–	–	1:10–1:320	1:100–1:320	1:10–1:100	1:10–1:320	1:10–1:100	1:10–1:320
Titer range IgG	1:10–1:1000	1:32–1:32	1:100–1:320	1:100	1:10–1:3200	1:32	1:10–1:10	1:10–1:3200	1:10–1:1000	1:10–1:3200
IgM/IgA/IgG median	1:32/1:100/1:66	1:10/–/1:32	1:32/–/1:210	–/–/1:100	-/1:100/1:66	-/1:320/1:32	-/1:32/1:10	1:32/1:100/ 1:32	1:10/1:32/1:66	1:21/1:32/1:32
Amphiphysin Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	28 (1.37)	6 (2.27)	3 (0.9)	0 (0)	10 (2.26)	1 (0.32)	0 (0)	48 (1.13)	39 (1.43)	87 (1.25)
Seropositives, males	21	3	2	0	6	1	0	33	22	55
IgM/IgA/IgG, No.	1/7/23	1/1/5	2/3/3	0/0/0	0/6/6	0/1/0	0/0/0	4/18/37	4/14/29	8/32/66
Titer range IgM	1:320	1:100	1:32	–	–	–	–	1:32–1:320	1:10–1:320	1:10–1:320
Titer range IgA	1:10–1:32	1:32	1:10–1:100	–	1:10–1:100	1:32	–	1:10–1:100	1:10–1:100	1:10–1:100
Titer range IgG	1:10–1:100	1:10–1:100	1:10–1:100	–	1:10–1:32	–	–	1:10–1:100	1:10–1:100	1:10–1:100
IgM/IgA/IgG median	1:320/1:32/1:32	1:100/1:32/ 1:32	1:32/1:100/1:10	–	-/1:32/1:21	-/1:32/–	–	1:100/1:32/ 1:32	1:100/1:32/ 1:32	1:100/1:32/ 1:32
GFAP Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	22 (1.21)	4 (1.5)	1 (0.52)	0 (0)	9 (2.04)	0 (0)	1 (0.14)	37 (0.95)	21 (0.88)	58 (0.92)
Seropositives, males	14	3	0	0	6	0	1	24	13	37
IgM/IgA/IgG, No.	5/7/16	1/2/2	0/0/1	0/0/0	1/2/8	0/0/0	0/0/1	7/11/28	1/2/20	8/13/48
Titer range IgM	1:320–1:1000	1:320	–	–	1:100	–	–	1:100–1:1000	1:100	1:100–1:1000
Titer range IgA	1:100–1:1000	1:100–1:320	–	–	1:100–1:100	–	–	1:100–1:1000	1:320–1:1000	1:100–1:1000
Titer range IgG	1:100–1:3200	1:100–1:320	1:320	–	1:100–1:1000	–	1:320	1:100–1:3200	1:100–1:1000	1:100–1:3200
IgM/IgA/IgG median	1:320/1:320/1:320	1:320/1:210/ 1:210	–/–/1:320	–/–/–	1:100/1:100/ 1:320	–/–/–	–/–/1:320	1:320/1:100/ 1:320	1:100/1:660/ 1:320	1:320/1:320/ 1:320

(*continued on next page*)

Table 2 (*continued*)

Disorders/diseases	**Schizophrenia Schizoaffective**	**Affective**	**Personality & Addiction**	**Neuro-developmental**	**Stroke**	**Neuro-degenerative**	**Autoimmune**	**ALL Diseases**	**Healthy Controls**	**ALL Subjects**
No. Individuals†	**2043** (1818–2043)	**267** (264–267)	**334** (193–333)	**141**	**442**	**349** (310–349)	**701**	**4277** (3909–4265)	**2748** (2391–2735)	**7025** (6300–7000)
Male, %	**64.90 %**	**50.56 %**	**56.29 %**	**65.25 %**	**54.75 %**	**59.89 %**	**27.39 %**	**55.74 %**	**55.93 %**	**55.81 %**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
ARHGAP26 Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	16 (0.78)	6 (2.27)	3 (0.9)	0 (0)	10 (2.26)	0 (0)	0 (0)	35 (0.83)	24 (0.88)	59 (0.85)
Seropositives, males	14	4	3	0	8	0	0	29	18	47
IgM/IgA/IgG, No.	0/5/15	0/1/5	0/1/2	0/0/0	0/2/9	0/0/0	0/0/0	0/9/31	0/9/19	0/18/50
Titer range IgM	–	–	–	–	–	–	–	–	–	–
Titer range IgA	1:10–1:320	1:10	1:320	–	1:32–1:100	–	–	1:10–1:320	1:10–1:100	1:10–1:320
Titer range IgG	1:10–1:320	1:10–1:320	1:32	–	1:10–1:100	–	–	1:10–1:320	1:10–1:100	1:10–1:320
IgM/IgA/IgG median	-/1:32/1:32	-/1:10/1:100	-/1:320/1:32	–/–/–	-/1:66/1:32	–/–/–	–/–/–	-/1:32/1:32	-/1:32/1:32	-/1:32/1:32
CASPR2 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	24 (1.17)	3 (1.14)	0 (0)	2 (1.42)	2 (0.45)	0 (0)	9 (1.28)	40 (0.94)	13 (0.48)	53 (0.76)
Seropositives, males	19	1	0	0	0	0	5	25	4	29
IgM/IgA/IgG, No.	12/3/9	2/1/1	0/0/0	1/1/0	1/0/1	0/0/0	4/1/6	20/6/17	7/0/6	27/6/23
Titer range IgM	1:10–1:32	1:32	–	1:10	1:10	–	1:10–1:320	1:10–1:320	1:10–1:100	1:10–1:320
Titer range IgA	1:10–1:32	1:10	–	1:32	–	–	1:100	1:10–1:100	–	1:10–1:100
Titer range IgG	1:10–1:100	1:32	–	–	1:10	–	1:10–1:1000	1:10–1:1000	1:10–1:32	1:10–1:1000
IgM/IgA/IgG median	1:10/1:10/1:10	1:32/1:10/1:32	–/–/–	1:10/1:32/–	1:10/–/1:10	–/–/–	1:32/1:100/ 1:100	1:10/1:32/1:32	1:10/–/1:21	1:10/1:32/1:32
MOG Total No.	2043	265	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	17 (0.83)	0 (0)	1 (0.3)	0 (0)	8 (1.81)	3 (0.97)	1 (0.14)	30 (0.71)	11 (0.4)	41 (0.59)
Seropositives, males	9	0	1	0	3	1	1	15	8	23
IgM/IgA/IgG, No.	11/5/2	0/0/0	0/1/0	0/0/0	6/2/1	3/0/0	0/0/1	20/8/4	8/2/1	28/10/5
Titer range IgM	1:10–1:320	–	–	–	1:10–1:100	1:10–1:100	–	1:10–1:320	1:10–1:320	1:10–1:320
Titer range IgA	1:10–1:32	–	1:10–1:10	–	1:10–1:10	–	–	1:10–1:32	1:10–1:100	1:10–1:100
Titer range IgG	1:10–1:32	–	–	–	1:10	–	1:100	1:10–1:100	1:32	1:10–1:100
IgM/IgA/IgG median	1:32/1:10/1:21	–/–/–	-/1:10/–	–/–/–	1:32/1:10/1:10	1:32/–/–	–/–/1:100	1:32/1:10/1:32	1:66/1:32/1:32	1:32/1:10/1:32
Homer-3 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	12 (0.66)	1 (0.37)	1 (0.52)	1 (0.71)	2 (0.45)	0 (0)	1 (0.14)	18 (0.46)	13 (0.54)	31 (0.49)
Seropositives, males	11	1	1	0	1	0	0	14	8	22
IgM/IgA/IgG, No.	0/8/7	0/0/1	0/0/1	0/1/0	0/2/0	0/0/0	0/1/0	0/12/9	3/6/7	3/18/16
Titer range IgM	–	–	–	–	–	–	–	–	1:100–1:320	1:100–1:320
Titer range IgA	1:100–1:1000	–	–	1:1000	1:320–1:3200	–	1:320	1:100–1:3200	1:100–1:320	1:100–1:3200
Titer range IgG	1:10–1:320	1:1000	1:100	–	–	–	–	1:10–1:1000	1:32–1:1000	1:10–1:1000
IgM/IgA/IgG median	-/1:320/1:100	–/–/1:1000	–/–/1:100	-/1:1000/–	-/1:1000/–	–/–/–	-/1:320/–	-/1:320/1:100	1:100/1:210/ 1:100	1:100/1:320/ 1:100
KCNA1 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	8 (0.44)	2 (0.75)	1 (0.52)	0 (0)	4 (0.9)	0 (0)	2 (0.29)	17 (0.43)	12 (0.5)	29 (0.46)
Seropositives, males	8	2	1	0	2	0	0	13	8	21
IgM/IgA/IgG, No.	1/3/4	0/0/2	0/0/1	0/0/0	0/0/4	0/0/0	0/2/0	1/5/11	2/4/8	3/9/19
Titer range IgM	1:100	–	–	–	–	–	–	1:100	1:10–1:1000	1:10–1:1000
Titer range IgA	1:32–1:100	–	–	–	–	–	1:32–1:100	1:32–1:100	1:10–1:100	1:10–1:100
Titer range IgG	1:10–1:100	1:320–1:1000	1:320	–	1:10–1:1000	–	–	1:10–1:1000	1:10–1:320	1:10–1:1000
IgM/IgA/IgG median	1:100/1:32/1:32	–/–/1:660	–/–/1:320	–/–/–	–/–/1:32	–/–/–	-/1:66/–	1:100/1:32/ 1:100	1:320/1:10/ 1:100	1:100/1:32/ 1:100

(*continued on next page*)

Table 2 (*continued*)

Disorders/diseases	**Schizophrenia Schizoaffective**	**Affective**	**Personality & Addiction**	**Neuro-developmental**	**Stroke**	**Neuro-degenerative**	**Autoimmune**	**ALL Diseases**	**Healthy Controls**	**ALL Subjects**
No. Individuals†	**2043** (1818–2043)	**267** (264–267)	**334** (193–333)	**141**	**442**	**349** (310–349)	**701**	**4277** (3909–4265)	**2748** (2391–2735)	**7025** (6300–7000)
Male, %	**64.90 %**	**50.56 %**	**56.29 %**	**65.25 %**	**54.75 %**	**59.89 %**	**27.39 %**	**55.74 %**	**55.93 %**	**55.81 %**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
GLRA1b Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	5 (0.24)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (1.43)	15 (0.35)	13 (0.48)	28 (0.4)
Seropositives, males	2	0	0	0	0	0	4	6	6	12
IgM/IgA/IgG, No.	2/2/1	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/9	2/3/10	2/1/10	4/4/20
Titer range IgM	1:32–1:32	–	–	–	–	–	–	1:32–1:32	1:10–1:32	1:10–1:32
Titer range IgA	1:10–1:32	–	–	–	–	–	1:10	1:10–1:32	1:10	1:10–1:32
Titer range IgG	1:100	–	–	–	–	–	1:10–1:100	1:10–1:100	1:10–1:320	1:10–1:320
IgM/IgA/IgG median	1:32/1:21/1:100	–/–/–	–/–/–	–/–/–	–/–/–	–/–/–	-/1:10/1:32	1:32/1:10/1:32	1:21/1:10/1:32	1:32/1:10/1:32
GAD65 Total No.	2043	264	333	141	442	341	701	4265	2735	7000
Seropositives, No. (%)	9 (0.44)	1 (0.38)	1 (0.3)	0 (0)	3 (0.68)	0 (0)	3 (0.43)	17 (0.4)	9 (0.33)	26 (0.37)
Seropositives, males	8	1	1	0	2	0	0	12	7	19
IgM/IgA/IgG, No.	0/1/8	0/0/1	0/0/1	0/0/0	0/0/3	0/0/0	0/1/3	0/2/16	1/4/7	1/6/23
Titer range IgM	–	–	–	–	–	–	–	–	1:32	1:32
Titer range IgA	1:100	–	–	–	–	–	1:100	1:100–1:100	1:32–1:100	1:32–1:100
Titer range IgG	1:10–1:100	1:320	1:320	–	1:32–1:100	–	1:100–1:3200	1:10–1:3200	1:10–1:320	1:10–1:3200
IgM/IgA/IgG median	-/1:100/1:32	–/–/1:320	–/–/1:320	–/–/–	–/–/1:32	–/–/–	-/1:100/1:1000	-/1:100/1:32	1:32/1:100/ 1:32	1:32/1:100/ 1:32
Ma2 Total No.	2043	264	333	141	442	310	701	4234	2726	6960
Seropositives, No. (%)	7 (0.34)	1 (0.38)	4 (1.2)	0 (0)	2 (0.45)	0 (0)	0 (0)	14 (0.33)	8 (0.29)	22 (0.32)
Seropositives, males	4	1	3	0	1	0	0	9	2	11
IgM/IgA/IgG, No.	3/2/2	0/1/0	0/4/0	0/0/0	1/0/1	0/0/0	0/0/0	4/7/3	3/2/4	7/9/7
Titer range IgM	1:10–1:320	–	–	–	1:32	–	–	1:10–1:320	1:32–1:100	1:10–1:320
Titer range IgA	1:10–1:32	1:32	1:10–1:32	–	–	–	–	1:10–1:32	1:32–1:320	1:10–1:320
Titer range IgG	1:32–1:32	–	–	–	1:10	–	–	1:10–1:32	1:10–1:100	1:10–1:100
IgM/IgA/IgG median	1:320/1:21/1:32	-/1:32/–	-/1:32/–	–/–/–	1:32/–/1:10	–/–/–	–/–/–	1:100/1:32/ 1:32	1:32/1:100/ 1:100	1:32/1:32/1:32
Yo Total No.	2043	264	333	141	442	310	701	4234	2725	6959
Seropositives, No. (%)	5 (0.24)	1 (0.38)	0 (0)	1 (0.71)	1 (0.23)	1 (0.32)	1 (0.14)	10 (0.24)	10 (0.37)	20 (0.29)
Seropositives, males	4	1	0	1	1	1	0	8	6	14
IgM/IgA/IgG, No.	0/2/3	0/1/0	0/0/0	0/0/1	0/0/1	0/1/0	0/0/1	0/4/6	1/4/7	1/8/13
Titer range IgM	–	–	–	–	–	–	–	–	1:10	1:10
Titer range IgA	1:10–1:100	1:10	–	–	–	1:32–1:32	–	1:10–1:100	1:10–1:32	1:10–1:100
Titer range IgG	1:10–1:100	–	–	1:100	1:32	–	1:10000	1:10–1:10000	1:10–1:100	1:10–1:10000
IgM/IgA/IgG median	-/1:32/1:10	-/1:10/–	–/–/–	–/–/1:100	–/–/1:32	-/1:32/–	–/–/1:10000	-/1:32/1:66	1:10/1:21/1:10	1:10/1:32/1:32
NF155 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	5 (0.28)	0 (0)	0 (0)	0 (0)	3 (0.68)	0 (0)	6 (0.86)	14 (0.36)	3 (0.13)	17 (0.27)
Seropositives, males	3	0	0	0	2	0	1	6	1	7
IgM/IgA/IgG, No.	2/0/3	0/0/0	0/0/0	0/0/0	2/0/1	0/0/0	5/1/2	9/1/6	2/0/1	11/1/7
Titer range IgM	1:10–1:10	–	–	–	1:10–1:32	–	1:32–1:32	1:10–1:32	1:10–1:32	1:10–1:32
Titer range IgA	–	–	–	–	–	–	1:100	1:100	–	1:100
Titer range IgG	1:10–1:100	–	–	–	1:10	–	1:10–1:32	1:10–1:100	1:32	1:10–1:100
IgM/IgA/IgG median	1:10/–/1:32	–/–/–	–/–/–	–/–/–	1:21/–/1:10	–/–/–	1:32/1:100/ 1:21	1:32/1:100/ 1:32	1:21/–/1:32	1:32/1:100/ 1:32

(*continued on next page*)

Table 2 (*continued*)

Disorders/diseases	Schizophrenia Schizoaffective	Affective	Personality & Addiction	Neuro-developmental	Stroke	Neuro-degenerative	Autoimmune	ALL Diseases	Healthy Controls	ALL Subjects
No. Individuals[†]	**2043** (1818–2043)	**267** (264–267)	**334** (193–333)	**141**	**442**	**349** (310–349)	**701**	**4277** (3909–4265)	**2748** (2391–2735)	**7025** (6300–7000)
Male, %	**64.90 %**	**50.56 %**	**56.29 %**	**65.25 %**	**54.75 %**	**59.89 %**	**27.39 %**	**55.74 %**	**55.93 %**	**55.81 %**
Age, yr ± SD	**40.3 ± 13.1**	**47.7 ± 15.4**	**35 ± 12.9**	**29.7 ± 9.9**	**68.3 ± 12.5**	**61.6 ± 14.8**	**41.7 ± 13.7**	**44.8 ± 16.9**	**35 ± 13.1**	**41 ± 16.2**
AP3B2 Total No.	1816	267	193	141	442	349	701	3909	2391	6300
Seropositives, No. (%)	9 (0.5)	2 (0.75)	0 (0)	0 (0)	3 (0.68)	0 (0)	1 (0.14)	15 (0.38)	2 (0.08)	17 (0.27)
Seropositives, males	5	2	0	0	2	0	1	10	2	12
IgM/IgA/IgG, No.	1/6/3	0/2/0	0/0/0	0/0/0	0/3/0	0/0/0	0/1/1	1/12/4	0/1/1	1/13/5
Titer range IgM	1:32	–	–	–	–	–	–	1:32	–	1:32
Titer range IgA	1:10–1:100	1:10–1:100	–	–	1:10–1:100	–	1:10	1:10–1:100	1:10	1:10–1:100
Titer range IgG	1:10–1:320	–	–	–	–	–	1:100	1:10–1:320	1:32	1:10–1:320
IgM/IgA/IgG median	1:32/1:32/1:32	-/1:32/–	–/–/–	–/–/–	-/1:32/–	–/–/–	-/1:10/1:100	1:32/1:32/1:66	-/1:10/1:32	1:32/1:32/1:32

[†]Range accounts for missing determinations; Ig class numbers do not always add up to the total number of seropositives, due to double and triple positives; No. = number; yr = years; SD = standard deviation; Ig = immunoglobulin.

2.3. Genotyping

A semi-custom Axiom® myDesignTM genotyping array (Affymetrix, Santa Clara, CA, USA), based on a CEU (Caucasian residents of European ancestry from Utah, USA) marker backbone including 518,722 single nucleotide polymorphisms (SNPs), and a custom marker set including 102,537 SNPs was used for genotyping detailed description (Hammer et al., 2014). A total of 493,925 variants passed quality control, had minor allele frequency > 0.05, were in Hardy–Weinberg equilibrium ($p > 0.001$) and therefore included in genetic analyses.

2.4. Genetic association analyses

PLINK v1.90 (Purcell et al., 2007) was used for all genetic association analyses, including calculations of relatedness, principal components, and LD-based clumping (index variant *p* value threshold = 0.01). A total of 254,250 variants and 5,393 individuals were available after these steps. For related individuals, one was randomly excluded in each pair (second to third-degree relatives, PIHAT > 0.185). We executed two different approaches: (ii) A genome-wide association study (GWAS) with NMDAR1-AB seropositivity as target phenotype (254,250 SNPs – Bonferroni threshold 1.97e-07) and (i) a hypothesis-driven strategy, including all 9 directly genotyped SNPs available in our array of immune-checkpoint genes (*cytotoxic T-lymphocyte-associated protein 4 [CTLA-4]*: rs231777, rs3087243, rs11571316; *programmed cell death protein 1 [PD-1]*: rs28680420; *programmed cell death protein ligand 1 [PD-L1]*: rs1411262, rs2890658, rs2297137, rs2297136, rs4143815; Bonferroni threshold 0.0055). All *p* values adjusted using "p.adjust" from R (R Core Team, 2021).

2.5. APOE genotyping

APOE genotyping was done using KASP by Design assay (LGC/Biosearch^{TM} Technologies, Berlin, Germany), targeting *APOE* SNPs rs7412 and rs429358, as described earlier (He et al., 2014). Plates were run using LightCycler® 480 II (Roche Diagnostics ltd., Rotkreuz, Switzerland) and the measured values exported using LightCycler® 480 software (v1.5.0.39). Final genotype assignment was done using R (R Core Team, 2021).

2.6. Neurotrauma evaluation

Neurotrauma (NT) information was available for a subset of GRAS individuals (N = 2061), all based on semi-standardized interviews that were additionally complemented by medical/discharge letters (Table 3). Based on conditions underlying categories, an **overall severity score** was calculated. For each individual, the NT with highest severity gave an initial rank. Any preceding or repeated NT, provoking potential accumulation of consequences, received additional NT scores, dependent on category, all summed up with initial rank (Table 3). Subjects were then dichotomously divided into 2 groups: Individuals with severity score of ≥ 2.5 considered as severe head injury (NT+) and < 2.5 as not having had severe head injury (NT-).

Table 3
Severity Categories for Neurotrauma (NT).

Category	*Conditions*	*Initial rank*	*Additional NT(s)*
Mild	Head bump, nausea, laceration, or unconsciousness for < 15 s	1	+ 0.5
Moderate	Hematoma, hospitalization, or unconsciousness between 15 s – 1 h	2	+ 2
Severe	Concussion, coma, fracture, bleeding/edema, or unconsciousness for > 1 h	3	+ 3

2.7. Statistical analysis

Chi-square tests and Fisher's exact tests were used to compare categorical variables between groups, with odds ratios and Wald confidence intervals calculated using R package "epitools" (Aragon, 2020). Logistic regression analysis was employed to assess effects of disease status, age, gender, neurotrauma, *APOE4* genotype on seropositivity. Titer values were compared by Wilcoxon Rank Sum test with healthy controls as reference, using R package "e1071" (Meyer et al., 2021). Statistical tests were conducted using R (R Core Team, 2021) with RStudio (RStudio Team, 2020). Statistical significance was set to 0.05 after Bonferroni correction where indicated. Figures were plotted using R package "ggplot2" (Wickham, 2016) or GraphPad Prism version 9.3.1 for Windows (GraphPad Software, San Diego, California USA).

3. Results

3.1. Distribution of 15 most seroprevalent brain-directed AB across disease & health

A comprehensive overview of the 15 most seroprevalent of our selected 49 brain-directed AB across disease groups and health, i.e., NMDAR1-AB, amphiphysin, KCNA2, ARHGAP26, GFAP, CASPR2, MOG, Homer-3, KCNA1, GLRA1b, GAD65, Ma2, Yo, NF115, and AP3B2, is presented in Table 2. Information includes number of seropositive subjects, gender, mean age, Ig classes, and titer range. AB below cutoff of ≤ 0.25 % were NF186, CNTN1, myelin, neurochondrin, flotillin1/2, CNTN2, IgLON5, AMPA, Sez6l2, recoverin, neurexin, mGluR1, ITPR1, GABA-b, LGI1, mGluR5, DRD2, CV2, Hu, Tr/DNER, AQP4, GluDR2, Zic-4, ERC1 *(data not shown)*. Non-detectable in N = 7025 subjects were GABA-a, MBP, AT1A3, Ri, AGNA, CARPVIII, PCA-2, ANNA-3, DPPX, MAG, all expected to be extremely rare (Sæther et al., 2017; Mantere et al., 2018). For brief biological description of the 15 most seroprevalent brain-directed AB see Table 1. Analysis of 49 defined anti-brain AB in serum of > 7000 subjects, healthy or diagnosed with neuropsychiatric diseases, revealed > 16 % carriers of one or more of these selected AB.

3.2. Impact of disease status, age or gender on seroprevalence of brain-directed AB

Using logistic regression with any seroprevalence of all 49 brain-directed AB (no matter which Ig class or titer) as dependent variable, we first evaluated the overall impact of age, gender and disease versus health. Neither general disease status nor individual disease groups predicted AB seropositivity (all p >.05). The same negative result was obtained when only NMDAR1-AB, with highest seroprevalence, or all non-NMDAR1-AB together (all p >.05) were checked analogously. Age is strongly predictive of seropositivity when all 49 AB (OR = 1.018/year, 95 % CI [1.015–1.022], p = 2.95e-21) or only NMDAR1-AB (OR = 1.029/year, 95 % CI [1.023–1.034], p = 2.2e-27) are considered. However, age is just weakly predictive for non-NMDAR1-AB (OR = 1.007/year, 95 % CI [1.002–1.012], p =.0028) (Fig. 1 A, B). In contrast, gender is not (yet) significantly associated with seropositivity when considering just NMDAR1-AB (p =.052). Here, significance is driven by the heterogeneous non-NMDAR1-AB 'bag' – with higher seroprevalence of males (OR = 1.365, 95 % CI [1.155–1.615], p =.0003) (Fig. 1 A, B). Interestingly, but still unexplained, IgM seropositivity is not associated with gender in any of the tested scenarios (all p >.05).

3.3. Influence of anti-brain AB Ig class and titer regarding health or disease status

Evaluating the above obtained negative results on disease versus health status separately for the different Ig classes of brain-directed AB, including IgG, did not result in any appreciable association with disease (all p >.05). We then tested whether the distribution of AB titers was different between health and disease using Wilcoxon Rank Sum test. Positive individuals for either any disease or for each disease group separately were paired with matching healthy controls regarding age and gender to the maximum possible extent. For each individual, the highest titer (independent of Ig class) for each AB was selected, i.e., one individual could contribute to the comparison with 2 or 3 titers if

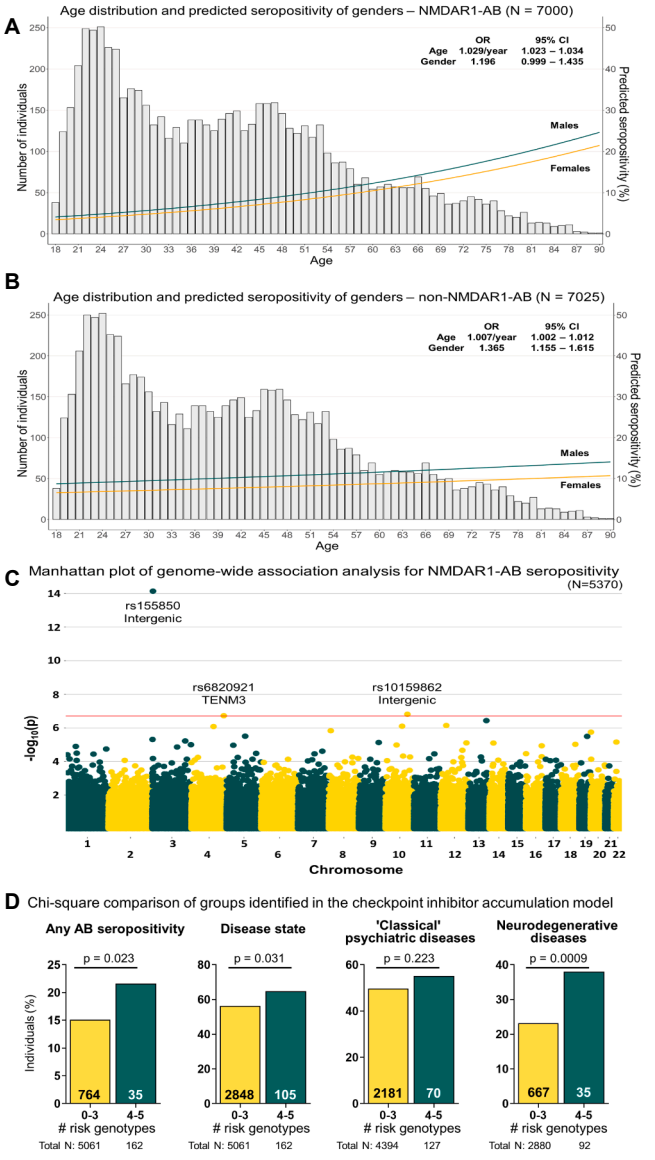

Fig. 1. A. Predicted NMDAR1-AB seropositivity per age group and gender. Note the strong association between predicted seropositivity and age. Males are more likely to be NMDAR1-AB seropositive, although the lower limit of the CI goes slightly below 1. B: Predicted non-NMDAR1-AB seropositivity per age group and gender. Males are more likely to be non-NMDAR-1 AB seropositive, but the association between predicted seropositivity and age is weaker compared to NMDAR1-AB. *Note that we are considering a 'mixed bag' of AB which may not all follow the same laws.* C. Manhattan plot of genome-wide association analysis for NMDAR1-AB seropositivity. The × axis represents chromosomal position and the y axis gives the significance (–log10(P); 2-tailed) of association as calculated by PLINK's Genotypic (2df) test. D. Chi-square comparison between dichotomously divided groups of individuals with 0–3 versus 4–5 immune-checkpoint risk genotypes in the accumulation model. Individuals with 4–5 risk genotypes are more likely to be AB seropositive and to have a disease diagnosis. Dividing the latter into classical psychiatric and neurodegenerative disease diagnoses reveals that the disease association of the accumulation model is mainly driven by neurodegeneration.

seropositive for different AB. Importantly, no differences in titer distribution were found regardless of disease group (all p >.05).

3.4. Genome-wide association study of NMDAR1-AB carriers versus non-carriers

We next started a number of approaches to identify potential genetically predisposing factors for seroprevalence of AB against brain-antigens. An ideal readout for GWAS are NMDAR1-AB, most abundant for still unknown reasons, whereas low seroprevalence is seen for most other screened AB, some even being non-detectable. A Manhattan plot illustrating the GAS of NMDAR1-AB carriers is presented in Fig. 1C. GWAS revealed three genome-wide significant SNPs, two intergenic (rs155850 – risk allele T on chromosome 3 and rs10159862 – risk allele G on chromosome 10), and one intronic in *TENM3* (rs6820921 – risk allele C on chromosome 4), a gene associated with childhood autoimmune diseases (Li et al., 2015).

3.5. Targeted analysis of immune check-point genotypes (CTLA4, PD1, PD-L1)

Subsequently, we performed a hypothesis-driven analysis on 9 SNPs of immune-checkpoint genes, *CTLA-4*, *PD-1*, or its ligand, *PD-L1*. We had previously shown in a smaller sample that *CTLA4* genotypes predispose to serum NMDAR1-AB in humans (Pan et al., 2021). Now, we also included SNPs in *PD-1* and *PD-L1* in our analyses of 49 brain-directed AB in a population of N = 5223 individuals with information available on all originally screened 9 SNPs, 5 of which were found here to be risk SNPs (*CTLA-4*–2 SNPs, *PD-1*–1 SNP, *PD-L1*–2 SNPs; Table 4). A model, accumulating these 5 risk SNPs, and analyzing in a dichotomous fashion the presence of 0–3 versus 4–5 risk genotypes, uncovered effects not only on humoral anti-brain autoimmunity (OR = 1.55; 95 % CI [1.058–2.271]), but very interestingly also on disease likelihood (OR = 1.43; 95 % CI [1.032–1.985]). Subdivision of disease entities revealed that an association with 'classical' psychiatric diseases does not reach significance (p = 0.223), whereas an association is observed between number of immune-checkpoint risk genotypes and probability of neurodegenerative disease (OR = 2.04; 95 % CI [1.326–3.131]; p =.0009; Fig. 1 D).

3.6. Potential roles of APOE4 genotypes for seroprevalence of brain-directed AB

Determination of *APOE4* genotypes in our population resulted in the expected range of around 20 % (heterozygous 17.41 %; homozygous 1.24 %). Surprising at first view, however, regression analysis revealed that *APOE4* carriers with their known 'leaky' BBB (Montagne et al., 2020; Zerche et al., 2015; Pendlebury et al., 2020; McFadyen et al., 2021; Knox et al., 2022) have a lower chance of being AB seropositive (OR = 0.766, 95 % CI [0.625–0.933], p =.009). Due to effects of brain-bound AB, seropositive compared to seronegative *APOE4* carriers might have a higher prevalence or severity of neuropsychiatric phenotypes. However, evaluating just presence/absence of a disease as readouts did not yet support this idea (p >.05 for all chi-squares).

3.7. Influence of previous neurotrauma on seroprevalence of brain-directed AB

As an environmental risk factor, we evaluated neurotrauma in a dichotomous fashion, dependent on symptom severity (Table 3). Indeed, neurotrauma was associated with a higher chance of carrying serum NMDAR1-AB of the IgM class (OR = 1.599; 95 % CI [1.022–2.468], p =.036).

4. Discussion

The present work has been designed to provide a thus far lacking, comprehensive investigation of brain-directed serum AB which should assist clinicians as well as basic researchers in putting AB findings in more solid perspective. We investigated seroprevalence and potential predictors of 49 selected, brain-directed AB in > 7000 subjects, healthy or suffering from neuropsychiatric diseases, a number never analyzed and reported before. In fact, thousands of different AB, likely belonging to the physiological autoimmune repertoire of individual mammals, circulate in blood (Nagele et al., 2013). Brain-directed AB may gain pathophysiological significance as they can substantially modulate brain function when crossing the BBB in sufficient amounts or upon their intrathecal production (Diamond et al., 2009). Here, we report humoral autoimmunity against brain-antigens equally frequent across health and disease, with overall > 16 % carriers of one or more of these selected AB, and with age, gender, genetic predisposition and brain injury as predictors. We note that our present AB selection represents only a small

Table 4
SNPs in checkpoint inhibitor genes predispose to AB seropositivity.

		AAB Positive		AAB Negative				AAB Positive		AAB Negative				
SNP	**Allele**	**N**	**%**	**N**	**%**	**P**	**Genotype**	**N**	**%**	**N**	**%**	**P**	**Gene**	**Seropositivity**
	A	283	52.21 %	4604	45.08 %		AA	80	29.52 %	1056	20.67 %			
rs3087243	G	259	47.79 %	5608	54.92 %	**0.010**	AG	123	45.39 %	2495	48.83 %	**0.016**	**CTLA-4**	**Extracellular IgA**
							GG	68	25.09 %	1558	30.50 %			
	A	260	48.33 %	4228	41.65 %		AA	66	24.53 %	889	17.51 %			
rs11571316	G	278	51.67 %	5924	58.35 %	**0.020**	AG	128	47.59 %	2450	48.27 %	0.058	**CTLA-4**	**Extracellular IgA**
							GG	75	27.88 %	1737	34.22 %			
	T	163	36.88 %	3046	30.15 %		TT	33	14.93 %	494	9.78 %			
rs28680420	C	279	63.12 %	7056	69.85 %	**0.024**	TC	97	43.89 %	2058	40.75 %	0.095	**PD-1**	**IgM NMDAR-1**
							CC	91	41.18 %	2499	49.47 %			
	A	31	34.44 %	2381	22.39 %		AA	9	20.00 %	269	5.06 %			
rs2297137	G	59	65.56 %	8251	77.61 %	0.058	AG	13	28.89 %	1843	34.68 %	**0.0004**	**PD-L1**	**IgG NMDAR-1**
							GG	23	51.11 %	3202	60.26 %			
	G	30	33.33 %	5246	49.34 %		GG	6	13.33 %	1311	24.66 %			
rs2297136	A	60	66.67 %	5386	50.66 %	**0.022**	AG	18	40.00 %	2624	49.36 %	**0.049**	**PD-L1**	**IgG NMDAR-1**
							AA	21	46.67 %	1381	25.98 %			

part of circulating AB (Nagele et al., 2013), but findings obtained with them may be widely representative.

The global age association of AB seroprevalence seemed mainly driven by NMDAR1-AB. We note, however, that grouping all non-NMDAR1-AB, due to their overall low seroprevalence, essentially generates a 'mixed bag', i.e., not all AB may follow the same rules. Nevertheless, there seems to be a general tendency of age association at least for many of these AB. Significance of gender association in turn is driven by the heterogeneous non-NMDAR1-AB 'bag' – with higher seroprevalence of males. This is somewhat unexpected, since females are more affected by autoimmune disorders (Gleicher and Barad, 2007; Hayter and Cook, 2012; Ngo et al., 2014; Roberts and Erdei, 2020; Voskuhl, 2020; Huang et al., 2022). Together, the observed age and gender dependence of seroprevalence of our selected 49 anti-brain AB, their apparent lack of disease association, both general and with neuropsychiatric subgroups, and their similar overall Ig class distribution and titer ranges may represent a more general picture to be expected from thousands of serum AB (Nagele et al., 2013; Cohen and Efroni, 2019).

As NMDAR1-AB of the IgG class are often connected to autoimmune encephalitis, we screened the literature to compare the titer values found here with those of patients with confirmed anti-NMDAR encephalitis. In a recently published Dutch cohort of anti-NMDAR encephalitis patients (n = 104), two sets of NMDAR-1 IgG titer ranges, also determined by commercial cell-based assays from Euroimmun, were reported: (i) Subjects < 45 years with titer median 1:800 (range 1:100–1:6,400) and (ii) subjects > 45 years with titer median of 1:200 (range 1:100–1:12,800). Highest median in the present work was 1:100 (overall median 1:32, ranges 1:10–1:1,000), which we expected, as no individuals in our cohort were diagnosed with anti-NMDAR encephalitis. Notably, however, out of 55 individuals, positive for NMDAR-1 IgG, 18 (32.73 %) had titers of 1:100 or higher, thus were comparable to numbers presented in the Dutch study (Bastiaansen et al., 2022).

In our genetic approaches to identify potential predisposing factors for seroprevalence of AB against brain-antigens, we performed a GWAS of NMDAR1-AB carriers versus non-carriers. Even though the obtained genome-wide significant hits do not allow deeper mechanistic insight at this point (as with most GWAS studies), they underline a genetic influence at least on NMDAR1-AB carrier status. An interesting find, however, may be *TENM3,* a gene previously associated with childhood autoimmune diseases (Li et al., 2015), which encodes a large transmembrane protein expressed in neurons, possibly involved in the regulation of neuronal development (Tucker and Chiquet-Ehrismann, 2006).

Next, we conducted a hypothesis-driven analysis of immune-checkpoint genotypes (SNPs) for *CTLA-4*, *PD-1*, or its ligand, *PD-L1,* that resulted in 5 SNPs associated with AB seroprevalence. These genes are expressed by *T*-cells and serve as control elements of their immune response. Immune checkpoint inhibitors block these molecules and enhance antitumor *T*-cell activity (Yao et al., 2013; Pardoll, 2012). While providing clinical benefits in a percentage of patients with advanced cancers, they are usually associated with a remarkable spectrum of immune-related adverse events, including autoimmunity (Luhder et al., 1998; Poto et al., 2022). CTLA-4 for example is an important regulator of the immune response, i.e., reactivity to foreign and self-antigens. Allelic variation of *CTLA-4* or CTLA-4 blockade by anti-CTLA4 treatment influences the signaling threshold of CD4 *T*-cells (Luhder et al., 1998; Maier et al., 2007), thereby augmenting antitumor immunity but also exacerbating or inducing autoimmune disease. We had previously shown in a smaller sample that *CTLA4* genotypes predispose to serum NMDAR1-AB in humans (Pan et al., 2021). Accumulating 5 risk SNPs of immune-checkpoint genes revealed effects not only on humoral anti-brain autoimmunity, but also on likelihood of neurodegenerative disease. Provided replication in independent samples, this would indicate a role of the genetic immune checkpoint constellation also for neurodegeneration.

APOE4 genotypes are known to be risk factors for various diseases, e.g., Morbus Alzheimer, or to predict unfavorable outcomes, e.g., of stroke or brain injury. These risks may be related to their negative influence on BBB integrity (Montagne et al., 2020; Zerche et al., 2015; Pendlebury et al., 2020; McFadyen et al., 2021; Knox et al., 2022). We thus wondered whether such genetically induced higher accessibility of brain tissue to the immune system would be reflected in humans by enhanced seroprevalence of AB directed against brain-antigens, as hypothesized by some authors for ischemic stroke or brain injury (Javidi and Magnus, 2019). Determination of *APOE4* genotypes in our population resulted in the expected range of around 20 %. Surprising at first view, however, *APOE4* carriers have a lower chance of being AB seropositive. This phenomenon is in good agreement with our earlier findings in *ApoE* KO mice (Pan et al., 2021) and may well be explained by the chronically leaky BBB. Brain-directed AB can under these circumstances readily cross the dysfunctional BBB and specifically bind to brain tissue, which acts as 'immunoprecipitator', as demonstrated in experimental work in *ApoE* KO mice (Castillo-Gomez et al., 2016). This finding spontaneously suggested that seropositive compared to seronegative *APOE4* carriers might have a higher prevalence or severity of neuropsychiatric phenotypes due to the effects of bound AB. However, just appraising presence of a disease did not back this assumption. We note as important limitations, that we did not consider in this study the severity of disease symptoms, and that the immunoprecipitator role of the brain, efficiently extracting AB from the circulation, may lead to false negative seroprevalence data.

A recognized inducer of acute BBB breakdown is traumatic brain injury. In addition, multiple, mechanistically widely unexplained, late downstream sequelae of neurotrauma are known, e.g., various organ dysfunctions and increased risk of mental health problems (Izzy et al., 2022; Krishnamoorthy and Vavilala, 2022; Ledoux et al., 2022). Some of these consequences might be autoimmune-mediated and autoimmunity in turn a corollary of BBB disruption (Javidi and Magnus, 2019). In fact, a standardized small brain lesion in mice led to BBB breakdown and months later to increased NMDAR1-AB seroprevalence (Pan et al., 2021). Here we show also in humans first exploratory signals that neurotrauma, as environmental risk factor, was associated with a higher chance of carrying serum NMDAR1-AB of the IgM class. As after ischemic stroke, this finding may indicate potential long-term consequences of ongoing presence of circulating AB, e.g., years later neuropsychiatric symptoms including cognitive dysfunction and fatigue (Deutsch et al., 2021).

4.1. Limitations

Despite evaluating > 7000 subjects with > 16 % seropositive individuals among them, total N numbers can never be large enough, particularly when subgroups are to be assessed. In addition to some limitations already mentioned above, appraising a ‘mixed bag of AB’, put together to compare with the highly frequent NMDAR1-AB, has its clear confines, as not all AB may follow the same rules. But it certainly is a worthwhile start in our struggle to understand their roles and functions. In contrast, NMDAR1-AB can be analyzed separately, and GWAS even resulted in genome-wide significant hits, however, of still unknown function. Certainly, a replication GWAS would be desirable to confirm our findings. Also, detection of autoantibodies was limited here to commercially available *in vitro* diagnostic assays, as we focused on clinical relevance. However, in some cases, the use of live cell-based assays for NMDAR1-AB may be desirable which are believed to be more sensitive (Thouin et al., 2021). The fact that the 49 AB are just a selection and ‘represent’ probably thousands of others may be seen as another limitation. Here, use of tissue-based assays can be useful for the identification of novel CNS-directed autoantibodies (Endres et al., 2022), but this was clearly not the purpose of the present study. Another limitation is that not all potential predisposing factors could be investigated here. Viral infections are among them, and also other influences, e.g., the microbiome, have been suggested, but could not be included in the present study.

5. Conclusions

Humoral autoimmunity against brain-antigens is frequent across health and disease, and predicted by age, gender, genetic predisposition, and brain injury. Important for clinical practice, seroprevalence, Ig class or titers alone do not predict disease. Nevertheless, serological testing of brain-directed AB is of high diagnostic and therapeutic importance once their syndrome relevance is carefully considered in full context using multimodal approaches (including cerebrospinal fluid analysis, magnetic resonance imaging and electroencephalography). Much work needs to be done to better understand the physiological significance of circulating AB and to clearly identify situations that lead to their pathological consequences.

6. Ethical approval

The GRAS data collection has been approved by the ethical committee of the Georg-August-University of Göttingen (master committee) as well as by the respective local regulatories/ethical committees of all collaborating centers.

Funding

This work was supported by the Max Planck Society, the Max Planck Förderstiftung, the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), via DFG-Center for Nanoscale Microscopy & Molecular Physiology of the Brain (CNMPB), DFG-TRR 274/1 2020 – 408885537, as well as CRC-TR128 and CRC-TR1292. VDG received support from the IMPRS-Genome Science PhD program. The funders of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the article for publication. All authors had full access to the data in the study and had final responsibility for the decision to submit for publication.

Declaration of Competing Interest

Winfried Stöcker is head and Bianca Teegen full-time employee of a diagnostic reference laboratory, integrated into patient care, collaborating with the company *Euroimmun*, nowadays PerkinElmer. All other authors declare no subject-related conflict of interest.

Data availability

The authors do not have permission to share data.

Acknowledgements

The authors thank all subjects for participating in the study, and the many colleagues who have contributed over the past decades to the extended GRAS Data Collection.

References

Abboud, H., Probasco, J.C., Irani, S., Ances, B., Benavides, D.R., Bradshaw, M., Christo, P.P., Dale, R.C., Fernandez-Fournier, M., Flanagan, E.P., Gadoth, A., George, P., Grebenciucova, E., Jammoul, A., Lee, S.-T., Li, Y., Matiello, M., Morse, A. M., Rae-Grant, A., Rojas, G., Rossman, I., Schmitt, S., Venkatesan, A., Vernino, S., Pittock, S.J., Titulaer, M.J., 2021. Autoimmune encephalitis: proposed best practice recommendations for diagnosis and acute management. J. Neurol. Neurosurg. Psychiatry 92 (7), 757–768.

Aragon T.J. 2020. epitools: Epidemiology Tools. 0.5-10.1 ed. https://cran.r-project.org/web/packages/epitools/index.html.

Bastiaansen, A.E.M., de Bruijn, M.A.A.M., Schuller, S.L., Martinez-Hernandez, E., Brenner, J., Paunovic, M., Crijnen, Y.S., Mulder, M.J.H.L., Schreurs, M.W.J., de Graaff, E., Smitt, P.A.E., Neuteboom, R.F., de Vries, J.M., Titulaer, M.J., 2022. Anti-NMDAR encephalitis in the netherlands, focusing on late-onset patients and antibody test accuracy. Neurol. Neuroimmunol. Neuroinflammat. 9 (2), e1127.

Castillo-Gomez, E., Kästner, A., Steiner, J., Schneider, A., Hettling, B., Poggi, G., Ostehr, K., Uhr, M., Asif, A.R., Matzke, M., Schmidt, U., Pfander, V., Hammer, C., Schulz, T.F., Binder, L., Stöcker, W., Weber, F., Ehrenreich, H., 2016. The brain as immunoprecipitator of serum autoantibodies against N-Methyl-D-aspartate receptor subunit NR1. Ann. Neurol. 79 (1), 144–151.

Castillo-Gómez, E., Oliveira, B., Tapken, D., Bertrand, S., Klein-Schmidt, C., Pan, H., Zafeiriou, P., Steiner, J., Jurek, B., Trippe, R., Prüss, H., Zimmermann, W.-H., Bertrand, D., Ehrenreich, H., Hollmann, M., 2017. All naturally occurring autoantibodies against the NMDA receptor subunit NR1 have pathogenic potential irrespective of epitope and immunoglobulin class. Mol. Psychiatry 22 (12), 1776–1784.

Chan, K., Nestor, J., Huerta, T.S., Certain, N., Moody, G., Kowal, C., Huerta, P.T., Volpe, B.T., Diamond, B., Wollmuth, L.P., 2020. Lupus autoantibodies act as positive allosteric modulators at GluN2A-containing NMDA receptors and impair spatial memory. Nat. Commun. 11 (1).

Cohen, I.R., Efroni, S., 2019. The immune system computes the state of the body: crowd wisdom, machine learning, and immune cell reference repertoires help manage inflammation. Front. Immunol. 10, 10.

Dahm, L., Ott, C., Steiner, J., Stepniak, B., Teegen, B., Saschenbrecker, S., Hammer, C., Borowski, K., Begemann, M., Lemke, S., Rentzsch, K., Probst, C., Martens, H., Wienands, J., Spalletta, G., Weissenborn, K., Stöcker, W., Ehrenreich, H., 2014. Seroprevalence of autoantibodies against brain antigens in health and disease. Ann. Neurol. 76 (1), 82–94.

Dalmau, J., Tüzün, E., Wu, H.-Y., Masjuan, J., Rossi, J.E., Voloschin, A., Baehring, J.M., Shimazaki, H., Koide, R., King, D., Mason, W., Sansing, L.H., Dichter, M.A., Rosenfeld, M.R., Lynch, D.R., 2007. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann. Neurol. 61 (1), 25–36.

Dalmau, J., Gleichman, A.J., Hughes, E.G., Rossi, J.E., Peng, X., Lai, M., Dessain, S.K., Rosenfeld, M.R., Balice-Gordon, R., Lynch, D.R., 2008. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol. 7 (12), 1091–1098.

Dalmau, J., Geis, C., Graus, F., 2017. Autoantibodies to synaptic receptors and neuronal cell surface proteins in autoimmune diseases of the central nervous system. Physiol. Rev. 97 (2), 839–887.

Dawes, J.M., Weir, G.A., Middleton, S.J., Patel, R., Chisholm, K.I., Pettingill, P., Peck, L. J., Sheridan, J., Shakir, A., Jacobson, L., Gutierrez-Mecinas, M., Galino, J., Walcher, J., Kühnemund, J., Kuehn, H., Sanna, M.D., Lang, B., Clark, A.J., Themistocleous, A.C., Iwagaki, N., West, S.J., Werynska, K., Carroll, L., Trendafilova, T., Menassa, D.A., Giannoccaro, M.P., Coutinho, E., Cervellini, I., Tewari, D., Buckley, C., Leite, M.I., Wildner, H., Zeilhofer, H.U., Peles, E., Todd, A.J., McMahon, S.B., Dickenson, A.H., Lewin, G.R., Vincent, A., Bennett, D.L., 2018. Immune or genetic-mediated disruption of CASPR2 causes pain hypersensitivity due to enhanced primary afferent excitability. Neuron 97 (4), 806–822.

De Camilli, P., Thomas, A., Cofiell, R., Folli, F., Lichte, B., Piccolo, G., Meinck, H.M., Austoni, M., Fassetta, G., Bottazzo, G., 1993. The synaptic vesicle-associated protein amphiphysin is the 128-kD autoantigen of Stiff-Man syndrome with breast cancer. J. Exp. Med. 178 (6), 2219–2223.

Deutsch, N.R., Worthmann, H., Steixner-Kumar, A.A., Schuppner, R., Grosse, G.M., Pan, H., Gabriel, M.M., Hasse, I., van Gemmeren, T., Lichtinghagen, R., Ehrenreich, H., Weissenborn, K., 2021. Autoantibodies against the NMDAR subunit NR1 are associated with neuropsychiatric outcome after ischemic stroke. Brain Behav. Immun. 96, 73–79.

Diamond, B., Huerta, P.T., Mina-Osorio, P., Kowal, C., Volpe, B.T., 2009. Losing your nerves? Maybe it's the antibodies. Nat. Rev. Immunol. 9 (6), 449–456.

Ehrenreich, H., 2017. Autoantibodies against the N-methyl-d-aspartate receptor subunit NR1: untangling apparent inconsistencies for clinical practice. Front. Immunol. 8, 181.

Ehrenreich, H., 2018. Autoantibodies against N-methyl-d-aspartate receptor 1 in health and disease. Curr. Opin. Neurol. 31 (3), 306–312.

Ehrenreich, H., Gastaldi, V.D., Wilke, J.B.H., 2022. Quo vaditis anti-brain autoantibodies: causes, consequences, or epiphenomena? Biol. Psychiatry 92, 254–255.

Endres, D., Lüngen, E., Hasan, A., Kluge, M., Fröhlich, S., Lewerenz, J., Bschor, T., Haußleiter, I.S., Juckel, G., Then Bergh, F., Ettrich, B., Kertzscher, L., Oviedo-Salcedo, T., Handreka, R., Lauer, M., Winter, K., Zumdick, N., Drews, A., Obrocki, J., Yalachkov, Y., Bubl, A., von Podewils, F., Schneider, U., Szabo, K., Mattern, M., Philipsen, A., Domschke, K., Wandinger, K.-P., Neyazi, A., Stich, O., Prüss, H., Leypoldt, F., Tebartz van Elst, L., 2022. Clinical manifestations and immunomodulatory treatment experiences in psychiatric patients with suspected autoimmune encephalitis: a case series of 91 patients from Germany. Mol. Psychiatry 27 (3), 1479–1489.

Endres, D., von Zedtwitz, K., Matteit, I., Bünger, I., Foverskov-Rasmussen, H., Runge, K., Feige, B., Schlump, A., Maier, S., Nickel, K., Berger, B., Schiele, M.A., Cunningham, J.L., Domschke, K., Prüss, H., Tebartz van Elst, L., 2022. Spectrum of novel anti-central nervous system autoantibodies in the cerebrospinal fluid of 119 patients with schizophreniform and affective disorders. Biol. Psychiatry 92 (4), 261–274.

Fang, B., McKeon, A., Hinson, S.R., Kryzer, T.J., Pittock, S.J., Aksamit, A.J., Lennon, V. A., 2016. Autoimmune glial fibrillary acidic protein astrocytopathy: A novel meningoencephalomyelitis. JAMA Neurol. 73 (11), 1297.

Fernandes, D., Santos, S.D., Coutinho, E., Whitt, J.L., Beltrão, N., Rondão, T., Leite, M.I., Buckley, C., Lee, H.-K., Carvalho, A.L., 2019. Disrupted AMPA receptor function upon genetic- or antibody-mediated loss of autism-associated CASPR2. Cereb. Cortex 29 (12), 4919–4931.

Giannoccaro, M.P., Menassa, D.A., Jacobson, L., Coutinho, E., Prota, G., Lang, B., Leite, M.I., Cerundolo, V., Liguori, R., Vincent, A., 2019a. Behaviour and neuropathology in mice injected with human contactin-associated protein 2 antibodies. Brain 142 (7), 2000–2012.

Giannoccaro, M.P., Wright, S.K., Vincent, A., 2019b. In vivo mechanisms of antibody-mediated neurological disorders: animal models and potential implications. Front. Neurol. 10, 1394.
Gleicher, N., Barad, D.H., 2007. Gender as risk factor for autoimmune diseases. J. Autoimmun. 28 (1), 1–6.
Hammer, C., Stepniak, B., Schneider, A., Papiol, S., Tantra, M., Begemann, M., Sirén, A.-L., Pardo, L.A., Sperling, S., Mohd Jofrry, S., Gurvich, A., Jensen, N., Ostmeier, K., Lühder, F., Probst, C., Martens, H., Gillis, M., Saher, G., Assogna, F., Spalletta, G., Stöcker, W., Schulz, T.F., Nave, K.-A., Ehrenreich, H., 2014. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. Mol. Psychiatry 19 (10), 1143–1149.
Hart, I.K., Waters, C., Vincent, A., Newland, C., Beeson, D., Pongs, O., Morris, C., Newsom-Davis, J., 1997. Autoantibodies detected to expressed K+ channels are implicated in neuromyotonia. Ann. Neurol. 41 (2), 238–246.
Hayter, S.M., Cook, M.C., 2012. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. Autoimmun. Rev. 11 (10), 754–765.
He C, Holme J, Anthony J (2014): SNP Genotyping: The KASP Assay. In: Fleury D., Whitford R., editors. *Crop Breeding: Methods and Protocols*. New York, NY: Springer New York, pp 75-86. https://link.springer.com/protocol/10.1007/978-1-4939-0446-4_7.
Hoftberger, R., Sabater, L., Ortega, A., Dalmau, J., Graus, F., 2013. Patient with homer-3 antibodies and cerebellitis. JAMA Neurol. 70, 506–509.
Honorat, J.A., Lopez-Chiriboga, A.S., Kryzer, T.J., Komorowski, L., Scharf, M., Hinson, S. R., Lennon, V.A., Pittock, S.J., Klein, C.J., McKeon, A., 2019. Autoimmune gait disturbance accompanying adaptor protein-3B2-IgG. Neurology 93 (10), e954–e963.
Huang, X., Zhang, Q., Zhang, H., Lu, Q., 2022. A contemporary update on the diagnosis of systemic lupus erythematosus. Clin. Rev. Allergy Immunol.
Hutchinson, M., Waters, P., McHugh, J., Gorman, G., O'Riordan, S., Connolly, S., Hager, H., Yu, P., Becker, C.-M., Vincent, A., 2008. Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. Neurology 71 (16), 1291–1292.
Irani, S.R., Alexander, S., Waters, P., Kleopa, K.A., Pettingill, P., Zuliani, L., Peles, E., Buckley, C., Lang, B., Vincent, A., 2010. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain 133 (9), 2734–2748.
Izzy, S., Chen, P.M., Tahir, Z., Grashow, R., Radmanesh, F., Cote, D.J., Yahya, T., Dhand, A., Taylor, H., Shih, S.L., Albastaki, O., Rovito, C., Snider, S.B., Whalen, M., Nathan, D.M., Miller, K.K., Speizer, F.E., Baggish, A., Weisskopf, M.G., Zafonte, R., 2022. Association of traumatic brain injury with the risk of developing chronic cardiovascular, endocrine, neurological, and psychiatric disorders. JAMA Netw. Open 5 (4), e229478.
Jarius, S., Wildemann, B., 2015. 'Medusa head ataxia': the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 3: Anti-Yo/CDR2, anti-Nb/AP3B2, PCA-2, anti-Tr/DNER, other antibodies, diagnostic pitfalls, summary and outlook. J. Neuroinflammation 12, 168.
Jarius, S., Wandinger, K.P., Horn, S., Heuer, H., Wildemann, B., 2010. A new Purkinje cell antibody (anti-Ca) associated with subacute cerebellar ataxia: immunological characterization. J. Neuroinflammation 7 (1), 21.
Javidi, E., Magnus, T., 2019. Autoimmunity after ischemic stroke and brain injury. Front. Immunol. 10, 686.
Kira, J.-I., Yamasaki, R., Ogata, H., 2019. Anti-neurofascin autoantibody and demyelination. Neurochem. Int. 130, 104360.
Knox, E.G., Aburto, M.R., Clarke, G., Cryan, J.F., O'Driscoll, C.M., 2022. The blood-brain barrier in aging and neurodegeneration. Mol. Psychiatry 27 (6), 2659–2673.
Kreye, J., Wright, S.K., van Casteren, A., Stoffler, L., Machule, M.L., Reincke, S.M., et al., 2021. Encephalitis patient-derived monoclonal GABAA receptor antibodies cause epileptic seizures. J. Exp. Med. 218.
Krishnamoorthy, V., Vavilala, M.S., 2022. Traumatic brain injury and chronic implications beyond the brain. JAMA Netw. Open 5 (4), e229486.
Ledoux, A.-A., Webster, R.J., Clarke, A.E., Fell, D.B., Knight, B.D., Gardner, W., Cloutier, P., Gray, C., Tuna, M., Zemek, R., 2022. Risk of mental health problems in children and youths following concussion. JAMA Netw. Open 5 (3), e221235.
Li, Y.R., Li, J., Zhao, S.D., Bradfield, J.P., Mentch, F.D., Maggadottir, S.M., Hou, C., Abrams, D.J., Chang, D., Gao, F., Guo, Y., Wei, Z., Connolly, J.J., Cardinale, C.J., Bakay, M., Glessner, J.T., Li, D., Kao, C., Thomas, K.A., Qiu, H., Chiavacci, R.M., Kim, C.E., Wang, F., Snyder, J., Richie, M.D., Flatø, B., Førre, Ø., Denson, L.A., Thompson, S.D., Becker, M.L., Guthery, S.L., Latiano, A., Perez, E., Resnick, E., Russell, R.K., Wilson, D.C., Silverberg, M.S., Annese, V., Lie, B.A., Punaro, M., Dubinsky, M.C., Monos, D.S., Strisciuglio, C., Staiano, A., Miele, E., Kugathasan, S., Ellis, J.A., Munro, J.E., Sullivan, K.E., Wise, C.A., Chapel, H., Cunningham-Rundles, C., Grant, S.F.A., Orange, J.S., Sleiman, P.M.A., Behrens, E.M., Griffiths, A. M., Satsangi, J., Finkel, T.H., Keinan, A., Prak, E.T.L., Polychronakos, C., Baldassano, R.N., Li, H., Keating, B.J., Hakonarson, H., 2015. Meta-analysis of shared genetic architecture across ten pediatric autoimmune diseases. Nat. Med. 21 (9), 1018–1027.
Luhder, F., Hoglund, P., Allison, J.P., Benoist, C., Mathis, D., 1998. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) regulates the unfolding of autoimmune diabetes. J. Exp. Med. 187, 427–432.
Ma, J., Zhang, T., Jiang, L., 2017. Japanese encephalitis can trigger anti-N-methyl-d-aspartate receptor encephalitis. J. Neurol. 264 (6), 1127–1131.
Maier, L.M., Anderson, D.E., De Jager, P.L., Wicker, L.S., Hafler, D.A., 2007. Allelic variant in CTLA4 alters T cell phosphorylation patterns. Proc. Natl. Acad. Sci. U. S. A. 104 (47), 18607–18612.
Mantere, O., Saarela, M., Kieseppä, T., Raij, T., Mäntylä, T., Lindgren, M., Rikandi, E., Stoecker, W., Teegen, B., Suvisaari, J., 2018. Anti-neuronal anti-bodies in patients with early psychosis. Schizophr. Res. 192, 404–407.
Mathey, E.K., Derfuss, T., Storch, M.K., Williams, K.R., Hales, K., Woolley, D.R., Al-Hayani, A., Davies, S.N., Rasband, M.N., Olsson, T., Moldenhauer, A., Velhin, S., Hohlfeld, R., Meinl, E., Linington, C., 2007. Neurofascin as a novel target for autoantibody-mediated axonal injury. J. Exp. Med. 204 (10), 2363–2372.
McFadyen, C.A., Zeiler, F.A., Newcombe, V., Synnot, A., Steyerberg, E., Gruen, R.L., Rosand, J., Palotie, A., Maas, A.I.R., Menon, D.K., 2021. Apolipoprotein E4 polymorphism and outcomes from traumatic brain injury: a living systematic review and meta-analysis. J. Neurotrauma 38 (8), 1124–1136.
Meyer D., Dimitriadou E., Hornik K., Weingessel A., Leisch F. 2021: e1071: Misc Functions of the Department of Statistics, Probability Theory Group, TU Wien. 1.7-9 ed. https://cran.r-project.org/web/packages/e1071/index.html.
Montagne, A., Nation, D.A., Sagare, A.P., Barisano, G., Sweeney, M.D., Chakhoyan, A., Pachicano, M., Joe, E., Nelson, A.R., D'Orazio, L.M., Buennagel, D.P., Harrington, M. G., Benzinger, T.L.S., Fagan, A.M., Ringman, J.M., Schneider, L.S., Morris, J.C., Reiman, E.M., Caselli, R.J., Chui, H.C., Tcw, J., Chen, Y., Pa, J., Conti, P.S., Law, M., Toga, A.W., Zlokovic, B.V., 2020. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature 581 (7806), 71–76.
Nagele, E.P., Han, M., Acharya, N.K., DeMarshall, C., Kosciuk, M.C., Nagele, R.G., Tsokos, G.C., 2013. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. PLoS One 8 (4), e60726.
Newman, L.S., McKeever, M.O., Okano, H.J., Darnell, R.B., 1995. β-NAP, a cerebellar degeneration antigen, is a neuron-specific vesicle coat protein. Cell 82 (5), 773–783.
Ngo, S.T., Steyn, F.J., McCombe, P.A., 2014. Gender differences in autoimmune disease. Front. Neuroendocrinol. 35 (3), 347–369.
Pan, H., Oliveira, B., Saher, G., Dere, E., Tapken, D., Mitjans, M., Seidel, J., Wesolowski, J., Wakhloo, D., Klein-Schmidt, C., Ronnenberg, A., Schwabe, K., Trippe, R., Mätz-Rensing, K., Berghoff, S., Al-Krinawe, Y., Martens, H., Begemann, M., Stöcker, W., Kaup, F.-J., Mischke, R., Boretius, S., Nave, K.-A., Krauss, J.K., Hollmann, M., Lühder, F., Ehrenreich, H., 2019. Uncoupling the widespread occurrence of anti-NMDAR1 autoantibodies from neuropsychiatric disease in a novel autoimmune model. Mol. Psychiatry 24 (10), 1489–1501.
Pan, H., Steixner-Kumar, A.A., Seelbach, A., Deutsch, N., Ronnenberg, A., Tapken, D., von Ahsen, N., Mitjans, M., Worthmann, H., Trippe, R., Klein-Schmidt, C., Schopf, N., Rentzsch, K., Begemann, M., Wienands, J., Stöcker, W., Weissenborn, K., Hollmann, M., Nave, K.-A., Lühder, F., Ehrenreich, H., 2021. Multiple inducers and novel roles of autoantibodies against the obligatory NMDAR subunit NR1: a translational study from chronic life stress to brain injury. Mol. Psychiatry 26 (6), 2471–2482.
Pardoll, D.M., 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 12 (4), 252–264.
Pendlebury, S.T., Poole, D., Burgess, A., Duerden, J., Rothwell, P.M., Oxford Vascular, S., 2020. APOE-epsilon4 genotype and dementia before and after transient ischemic attack and stroke: population-based cohort study. Stroke 51, 751–758.
Peterson, K., Rosenblum, M.K., Kotanides, H., Posner, J.B., 1992. Paraneoplastic cerebellar degeneration. I. A clinical analysis of 55 anti-Yo antibody-positive patients. Neurology 42 (10), 1931.
Pollak, T.A., Lennox, B.R., Müller, S., Benros, M.E., Prüss, H., Tebartz van Elst, L., Klein, H., Steiner, J., Frodl, T., Bogerts, B., Tian, L.i., Groc, L., Hasan, A., Baune, B.T., Endres, D., Haroon, E., Yolken, R., Benedetti, F., Halaris, A., Meyer, J.H., Stassen, H., Leboyer, M., Fuchs, D., Otto, M., Brown, D.A., Vincent, A., Najjar, S., Bechter, K., 2020. Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin. Lancet Psychiatry 7 (1), 93–108.
Poto, R., Troiani, T., Criscuolo, G., Marone, G., Ciardiello, F., Tocchetti, C.G., et al., 2022. Holistic approach to immune checkpoint inhibitor-related adverse events. Front. Immunol. 13, 804597.
Prüss, H., 2021. Autoantibodies in neurological disease. Nat. Rev. Immunol. 21 (12), 798–813.
Pruss, H., Finke, C., Holtje, M., Hofmann, J., Klingbeil, C., Probst, C., et al., 2012. N-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. Ann. Neurol. 72, 902–911.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575.
R Core Team, 2021. R: A Language and Environment for Statistical Computing, 4.1.0 ed: R Foundation for Statistical Computing.
Ribbe, K., Friedrichs, H., Begemann, M., Grube, S., Papiol, S., Kästner, A., Gerchen, M.F., Ackermann, V., Tarami, A., Treitz, A., Flögel, M., Adler, L., Aldenhoff, J.B., Becker-Emner, M., Becker, T., Czernik, A., Dose, M., Folkerts, H., Freese, R., Günther, R., Herpertz, S., Hesse, D., Kruse, G., Kunze, H., Franz, M., Löhrer, F., Maier, W., Mielke, A., Müller-Isberner, R., Oestereich, C., Pajonk, F.-G., Pollmächer, T., Schneider, U., Schwarz, H.-J., Kröner-Herwig, B., Havemann-Reinecke, U., Frahm, J., Stühmer, W., Falkai, P., Brose, N., Nave, K.-A., Ehrenreich, H., 2010. The cross-sectional GRAS sample: a comprehensive phenotypical data collection of schizophrenic patients. BMC Psychiatry 10 (1).
Roberts, M.H., Erdei, E., 2020. Comparative United States autoimmune disease rates for 2010–2016 by sex, geographic region, and race. Autoimmun. Rev. 19 (1), 102423.
RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Sabatino, J.J., Pröbstel, A.-K., Zamvil, S.S., 2019. B cells in autoimmune and neurodegenerative central nervous system diseases. Nat. Rev. Neurosci. 20 (12), 728–745.
Sæther, S.G., Schou, M., Stoecker, W., Teegen, B., Borowski, K., Vaaler, A., Kondziella, D., Reitan, S.K., 2017. Onconeural antibodies in acute psychiatric inpatient care. J. Neuropsychiatry Clin. Neurosci. 29 (1), 74–76.
Solimena, M., Folli, F., Denis-Donini, S., Comi, G.C., Pozza, G., De Camilli, P., Vicari, A. M., 1988. Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. N. Engl. J. Med. 318 (16), 1012–1020.
Spieth, L., Berghoff, S.A., Stumpf, S.K., Winchenbach, J., Michaelis, T., Watanabe, T., Gerndt, N., Düking, T., Hofer, S., Ruhwedel, T., Shaib, A.H., Willig, K., Kronenberg, K., Karst, U., Frahm, J., Rhee, J.S., Minguet, S., Möbius, W., Kruse, N., von der Brelie, C., Michels, P., Stadelmann, C., Hülper, P., Saher, G., 2021. Anesthesia triggers drug delivery to experimental glioma in mice by hijacking caveolar transport. Neurooncol. Adv. 3 (1).
Swayne, A., Tjoa, L., Broadley, S., Dionisio, S., Gillis, D., Jacobson, L., Woodhall, M.R., McNabb, A., Schweitzer, D., Tsang, B., Vincent, A., Irani, S.R., Wong, R., Waters, P., Blum, S., 2018. Antiglycine receptor antibody related disease: a case series and literature review. Eur. J. Neurol. 25 (10), 1290–1298.
Tanaka, J., Nakamura, K., Takeda, M., Tada, K., Suzuki, H., Morita, H., Okado, T., Hariguchi, S., Nishimura, T., 1989. Enzyme-linked immunosorbent assay for human autoantibody to glial fibrillary acidic protein: higher titer of the antibody is detected in serum of patients with Alzheimer's disease. Acta Neurol. Scand. 80 (6), 554–560.
Teller, J., Jung, C., Wilke, J.B.H., Schimmelpfennig, S.-D., Hindermann, M., Hinken, L., Gabriel, M.M., Fegbeutel, C., Schäfer, A., Laser, H., Lichtinghagen, R., Worthmann, H., Weissenborn, K., Ehrenreich, H., 2022. Autoantibodies against NMDAR subunit NR1 disappear from blood upon anesthesia. Brain Behav. Immunity – Health 24, 100494.
Thouin, A., Gastaldi, M., Woodhall, M., Jacobson, L., Vincent, A., 2021. Comparison of N-methyl-d-aspartate receptor antibody assays using live or fixed substrates. J. Neurol. 268 (5), 1818–1826.
Tucker, R.P., Chiquet-Ehrismann, R., 2006. Teneurins: A conserved family of transmembrane proteins involved in intercellular signaling during development. Dev. Biol. 290 (2), 237–245.
Voltz, R., Gultekin, S.H., Rosenfeld, M.R., Gerstner, E., Eichen, J., Posner, J.B., Dalmau, J., 1999. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. N. Engl. J. Med. 340 (23), 1788–1795.
Voskuhl, R.R., 2020. The effect of sex on multiple sclerosis risk and disease progression. Mult. Scler. J. 26 (5), 554–560.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Wilke, J.B.H., Hindermann, M., Berghoff, S.A., Zihsler, S., Arinrad, S., Ronnenberg, A., Barnkothe, N., Steixner-Kumar, A.A., Röglin, S., Stöcker, W., Hollmann, M., Nave, K.-A., Lühder, F., Ehrenreich, H., 2021. Autoantibodies against NMDA receptor 1 modify rather than cause encephalitis. Mol. Psychiatry 26 (12), 7746–7759.
Xiao, B.-G., Linington, C., Link, H., 1991. Antibodies to myelin-oligodendrocyte glycoprotein in cerebrospinal fluid from patients with multiple sclerosis and controls. J. Neuroimmunol. 31 (2), 91–96.
Yao, S., Zhu, Y., Chen, L., 2013. Advances in targeting cell surface signalling molecules for immune modulation. Nat. Rev. Drug Discov. 12 (2), 130–146.
Zerche, M., Weissenborn, K., Ott, C., Dere, E., Asif, A.R., Worthmann, H., Hassouna, I., Rentzsch, K., Tryc, A.B., Dahm, L., Steiner, J., Binder, L., Wiltfang, J., Sirén, A.-L., Stöcker, W., Ehrenreich, H., 2015. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in acute ischemic stroke. Stroke 46 (5), 1180–1186.
Zhang, Z., Zoltewicz, J.S., Mondello, S., Newsom, K.J., Yang, Z., Yang, B., Kobeissy, F., Guingab, J., Glushakova, O., Robicsek, S., Heaton, S., Buki, A., Hannay, J., Gold, M. S., Rubenstein, R., Lu, X.-C., Dave, J.R., Schmid, K., Tortella, F., Robertson, C.S., Wang, K.K.W., Cohen, I.R., 2014. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. PLoS One 9 (3), e92698.
Zuliani, L., Sabater, L., Saiz, A., Baiges, J.J., Giometto, B., Graus, F., 2007. Homer 3 autoimmunity in subacute idiopathic cerebellar ataxia. Neurology 68 (3), 239–240.