eMethods

All serum and CSF samples were examined using rat brain immunohistochemistry (serum diluted 1:200 and CSF 1:2) optimized to detect antibodies against most neuronal surface antigens and glutamic acid decarboxylase 65 (GAD65).[1,2] If positive with a pattern of neuropil immunostaining, samples were examined with an extensive array of cell-based assays (CBAs) (serum 1:40, CSF 1:2 for fixed and live cells) that included all known cell-surface antigens.[3,4] If negative for known cell-surface autoantigens, samples were then examined with cultures of rat dissociated hippocampal neurons (serum 1:40, CSF 1:2) in order to determine whether the antibodies were directed against proteins expressed on the cell surface.[2] For myelin oligodendrocyte glycoprotein (MOG) antibodies, live CBAs were conducted with dilations of 1:160 for serum and 1:2 for CSF.

If the brain immunohistochemistry demonstrated intracellular staining, samples were subsequently examined with an array of intracellular antigens using immunoblot (i.e., EUROLINE including Hu, Yo, Ri, CRMP5, Ma1, Ma2) or CBA (i.e., GAD, adenylate kinase 5 [AK5], Sox1, and Kelch-like protein 11 [KLHL11]).

The repertoire of neuronal and glial (neural) antigens tested by CBAs included (1) Euroimmune mosaics containing 6 antigens: N-methyl-D-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), γ-aminobutyric acid B receptor ($GABA_BR$), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), and dipeptidyl-peptidase-like protein 6 (DPPX), *and* (2) in-house CBAs that in addition to all the above included: γ-aminobutyric acid A receptor ($GABA_AR$), glycine receptor (GlyR), dopamine 2 receptor (D2R), metabotropic glutamate receptor 1, 2, 3,and 5 (mGluR1, mGluR2, mGluR3, and mGluR5), glutamate kainate receptor subunit 2 (GluK2), neurexin-3α, delta/notch-like epidermal growth factor-related receptor (DNER), immunoglobulin-like cell adhesion molecule 5 (IgLON5), neural cell adhesion molecule 2 (NCAM2), MOG, aquaporin 4 (AQP4), and glial fibrillary acidic protein (GFAP).

CBAs were done with fixed HEK293 cells except those for $GABA_AR$,[5] GlyR,[6] mGluR1,[7] mGluR2,[8] mGluR3,[3] mGluR5,[9] GluK2,[10] DNER,[11] IgLON5,[12] MOG and AQP4,[13,14] that were done with live HEK cells.

eTable 1. Clinical manifestations of pediatric autoimmune encephalitis associated with autoantibodies against unknown antigens

Location of antigen by staining pattern; number of patients	**Age in years, median (range); male/female**	**Syndrome/symptoms**
Unknown neuronal surface antigen* 11	7 (1 - 16) 6/5	AE with psychiatric/cognitive symptoms, seizures, and altered consciousness (3/11) Plus movement disorder (3/11) Plus movement disorder and autonomic dysfunction (1/11) Brainstem/cerebellar encephalitis (2/11) Limbic encephalitis (1/11) Encephalomyelitis (1/11)
Unknown intracellular membrane antigen** 29	9 (1 - 17) 16/13	AE with psychiatric/cognitive symptoms, seizures, and altered consciousness (9/26) Plus autonomic dysfunction (5/26) Plus movement disorder (3/26) Plus movement disorder and autonomic dysfunction (1/26) Limbic encephalitis (4/26) Brainstem/cerebellar encephalitis (3/26) Encephalomyelitis (1/26) Clinical information not available in 3 patients
Unknown intracellular cytoplasmic/nuclear antigen*** 13	13 (2 - 17) 3/10	AE with psychiatric/cognitive symptoms, seizures, and altered consciousness (5/12) Plus movement disorder (1/12) Plus movement disorder and autonomic dysfunction (1/12) Brainstem/cerebellar encephalitis (2/12) Encephalomyelitis (2/12) Basal ganglia encephalitis (1/12) Clinical information not available in 1 patient

*Neuropil pattern of tissue immunohistochemistry and positive cell-surface live neuron staining; **Neuropil pattern of tissue immunohistochemistry staining but negative cell-surface live neuron staining; ***Intracellular pattern of tissue immunohistochemistry staining.
AE: autoimmune encephalitis.

eTable 2: Literature review of neural antibody frequencies in pediatric autoimmune encephalitis

Study, duration	Number of cases	Methods		Antibodies other than NMDAR and MOG	Comments
		Tissue-based	Other		
Mayo Clinic;[15] 2010 – 2017	13,319	No	RIA for VGKC complex; Commercial CBAs	CSF: LGI1 (0), CASPR2 (0) Serum: LGI1 (10) > CASPR2 (6)	Study focused on LGI1 and CASPR2
Epilepsy Center Bethel,[16] Germany; 2011 – 2015	1,426	No	Commercial CBAs	GAD (10) > LGI1 (2) = CASPR2 (2); serum or CSF N/A	-
Danish national cohort;[17] 2011 – 2017	375	No	Commercial CBAs; RIA	CSF: GAD (9) Serum: GAD (3) > CASPR2 (1)	$GABA_AR$ not included
Switzerland and Germany multicenter study;[18] 2011 – 2018	2,513	No	Commercial CBAs	CSF: CASPR2 (4) Serum: CASPR2 (8)	Study focused on CASPR2
France national cohort;[19] 2016 – 2018	N/A	Indirect immunofluorescence	In-house CBAs; Immunoblot	In children: LGI1 (0), Hu (0); other Ab frequency N/A	Frequency of other antibodies not reported
Mayo Clinic;[20] 2018 – 2019	5,649	Indirect immunofluorescence	CBAs; Immunoblot; RIA	CSF: GAD (24) > other* Serum: GAD (17) > other**	$GABA_AR$ not included
Our cohort; 2011 – 2022	2,750	Indirect immuno-peroxidase	In-house and commercial CBAs; live neuron immunofluorescence; immunoblot	GAD (10) > $GABA_AR$ (8) > other***	-

CBAs: cell-based assays; RIA: radioimmunoassay; VGKC: voltage-gated potassium channel.

*GFAP (5), Hu (2), LGI1 (2).

**CASPR2 (8), LGI1 (5), $GABA_BR$ (3), Hu (2), AMPAR (1).

***AQP4 (5), Hu (4), GlyR (3), mGluR5 (3), LGI1 (2), GluK2 (2), Neurexin (1), DNER (1), mGluR2 (1), NCAM2 (1), AMPAR & $GABA_BR$ (1), GFAP (1), D2R (1), Ma2 (1), Yo (1), KLHL11 (1).

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