Autoimmune neurology is a rapidly growing field driven by the ongoing discovery of novel neural autoantibodies associated with recognizable clinical syndromes. These diseases can affect the central, peripheral and autonomic nervous systems and may co-occur with cancer. Today’s testing landscape encompasses more than 60 neural autoantibodies targeting different intracellular proteins, receptors and ion channels.

Plethora of target antigens
Most initially identified neural autoantibodies are directed against intracellular antigens expressed in the cerebellum, such as Hu, Yo, Ri, CV2, PNMA2, SOX1 and amphiphysin. These antibodies are considered non-pathogenic and are epiphenomena of tumour cells expressing neuronal antigens. It is believed that the misdirected immune response is mediated by cytotoxic T-cells, leading to the loss of neurons. The autoantibodies serve as important biomarkers for paraneoplastic neurological syndromes (PNS) and often provide the first indication of a tumour. The tumours most commonly found in patients with PNS are small-cell lung carcinoma, breast cancer, ovarian cancer, lymphoma, thymoma or seminoma.

The more recently identified autoantibodies target cell-surface antigens such as AQP4, NMDAR, LGI1, CASPR2, AMPAR1/2, GABAR, DPPX and IgLON5. These antibodies are pathogenic and frequently non-paraneoplastic. They cause inflammatory damage to the brain and nerves and can trigger seizures, impairment of visual acuity, psychosis-like symptoms and/or movement disorders. In contrast to PNS, these disorders generally respond well to immunotherapy.

Today’s research continues to identify novel autoantibodies at an unprecedented rate, leading to the definition of many
new disease phenotypes. As well as being useful for disease classification and diagnostics, the findings also increase understanding of the autoimmune pathology behind neurological syndromes. This knowledge aids implementation of suitable therapy, which is often complex and multidisciplinary.

**Identification of new autoantibodies**

Among the methods used to identify new autoantibodies, the indirect immunofluorescence assay (IFA) is one of the most versatile, especially in cases of previously unspecified target antigens. Patient sera that show a characteristic staining pattern on neural tissue, but do not react with any known antigens, are used as the starting point. To identify the antigens, immunocomplexes formed between patient antibodies and brain tissue cryosections are analysed by immunoprecipitation followed by mass spectrometry. Use of cryosections ensures authentic presentation of the antigens in their natural environment and conformation. The new discoveries are confirmed by IFA using human cells recombinantly expressing the antigen, and additionally verified by neutralization analyses. The cell-based assays (CBAs) are subsequently useful for serological diagnostics and studies on the clinical relevance of the autoantibodies.

This approach has been used by the EUROIMMUN-affiliated Institute for Experimental Immunology (https://www.euroimmun.de/en/contact/external/institute-f-exp-immunology/) in collaborative studies to identify more than twenty autoantigens in neurological diseases, among them AP3B2, ATP1A3, CLIP1, CNTN1/CASPR1, CPT1C, ERC1, flotillin-1/2, GluRδ2, GRIPAP1, hexokinase-1, Homer-3, ITPR1, KCNA2, NBCe1, neurochondrin, RG58, ROCK2, RyR2, STX1b and septin-3 [1].

The following sections detail some novel autoantibody markers to emerge in recent years from this and other research and their clinical associations observed in patients.

**Autoimmune encephalitis**

Established markers for autoimmune encephalitis include autoantibodies against NMDAR, AMPAR1/2, LGI1, CASPR2 and GABABR, DPPX, IgLON5 and mGluR5. Many of these autoantibodies are rare and about 50% of patients meeting the criteria for autoimmune encephalitis are seronegative for known neural autoantibodies. Identification of new autoantibodies helps to extend the testing spectrum. Among the new test parameters are anti-adenylate kinase 5 (AK5) antibodies, which occur in severe, non-paraneoplastic, autoimmune limbic encephalitis. Patients experience severe memory deficits which remain even with immunotherapy. Altered or loss of smell sensation is also observed in some patients [2]. Autoantibodies against gamma-aminobutyric acid receptor type A (GABAAR) are a further new marker and are associated with rapidly progressing encephalopathy and/or seizures occurring in all age groups [3,4]. There is usually clinical improvement with immunotherapy.

Autoantibodies with a more prominent cancer association include those against inositol 1,4,5-trisphosphate receptor type 1 (ITPR1), an intracellular channel that mediates calcium signalling. These are associated with cerebellar ataxia, seizures, myelopathy and neuropathy, with around 45% of cases linked to an underlying tumour [11]. Antibodies against the regulator of G-protein signaling 8 (RGS8), a Purkinje cell protein which belongs to a class of proteins involved in the regulation of central nervous system

**Autoimmune cerebellar ataxia**

Numerous novel autoantibodies have been reported in patients presenting with idiopathic cerebellar/brainstem ataxia. Autoantibodies to neurochondrin, a neuronal cytosolic protein which plays a role in cell-surface localization of certain membrane-bound proteins, have been described in patients with non-paraneoplastic rapidly progressive rhombencephalitis with poor neurologic outcomes [5,6]. Autoantibodies to glutamate receptor δ2 (GluRδ2) have been observed in association with visual deficits and ocular motility abnormalities and appeared with young age, infectious prodromes and lyphocytic pleocytosis [7]. Autoimmunity targeting the adaptor protein 3 subunit B2 (AP3B2), a synaptic vesicle coat protein, was found in patients with subacute onset and rapidly progressive gait ataxia [8,9]. RhoGTPase-activating protein 26 (ARHGAP26) is a further novel target antigen in autoimmune cerebellar ataxia, cognitive impairment and psychosis [10].

Autoantibodies with a more prominent cancer association include those against inositol 1,4,5-trisphosphate receptor type 1 (ITPR1), an intracellular channel that mediates calcium signalling. These are associated with cerebellar ataxia, seizures, myelopathy and neuropathy, with around 45% of cases linked to an underlying tumour [11]. Antibodies against the regulator of G-protein signaling 8 (RGS8), a Purkinje cell protein which belongs to a class of proteins involved in the regulation of central nervous system

![Figure 1. Detection of anti-septin-3 autoantibodies on tissue and transfected-cell substrates (EUROIMMUN)](image-url)

**Hippocampus rat**

**HEK293 septin-3/5/6/7/11**
Clinical Laboratory International

Clinical laboratory International

CNS actions such as synaptic plasticity, memory and vision, have been described in patients with cerebellar syndrome associated with lymphoma [12]. Anti-metabotropic glutamate receptor type 1 (mGluR1) antibodies are a marker of a treatable form of cerebellar ataxia which may also be associated with lymphoma [13].

Septins have also been recently identified as target antigens of autoimmunity (Fig. 1). Septins are cytoskeletal proteins with multiple roles in cell division, cellular polarization, morphogenesis and membrane trafficking. Autoantibodies against septin-3 have been newly described in patients with paraneoplastic cerebellar ataxia [14]. Anti-septin-5 antibodies have been previously characterized in patients with non-paraneoplastic cerebellar ataxia, while anti-septin-7 antibodies were found in patients with encephalopathy with prominent neuropsychiatric features [15]. Thus, the different anti-septin antibodies appear to be associated with different clinical phenotypes.

Demyelinating diseases
Autoantibodies against aquaporin-4 (AQP4) are a highly specific, pathogenic marker for neuromyelitis optica spectrum disorders (NMOSD), a group of inflammatory demyelinating disorders of the CNS affecting the optic nerve, spinal column and brainstem. CBA is the gold standard for anti-AQP4-IgG testing and is now included in the diagnostic algorithm for NMOSD [16].

Autoantibodies against myelin oligodendrocyte glycoprotein (MOG) are a marker for MOG antibody-associated encephalomyelitis (MOG-EM), which is clinically similar to NMOSD but is now recognized as a distinct disease [17]. Recent evidence suggests that MOG-EM may be more common than NMOSD. Determination of AQP4 and MOG antibodies helps to delimit the diseases from each other and also from multiple sclerosis (MS), which can resemble NMOSD clinically in the initial stages.

Autoantibodies against the flotillin-1/2 heterocomplex, a peripheral membrane protein that is involved in axon outgrowth and regeneration of the optic nerve, have been observed in a subset of about 1–2% of patients with bona fide MS [18], but not in patients with other neural auto-antibody-associated diseases or in healthy blood donors. This suggests that anti-flotillin antibodies may be specific for MS, although their clinical and pathological relevance has not yet been clarified.

Autoimmune nodopathies
Autoantibodies against nodal/paranodal proteins are emerging biomarkers for a novel class of neuropathies known as autoimmune nodopathies [19]. These diseases have clinical similarity to Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy (CIDP) but are pathologically distinct. The antibodies target membrane proteins located at or around the nodes of Ranvier – gaps in the myelin sheath that facilitate fast conduction of nerve signals. The target antigens include neurofascin 186 (NF186), neurofascin 155 (NF155), contactin 1 (CNTN1) and contactin-associated protein 1 (CASPR1).
The autoantibodies are considered pathogenic, and the resulting immune reactions result in slowed conduction or even complete failure of impulse transmission. Autoimmune nodopathies manifest as acute, subacute or chronic onset sensory-motor neuropathies with distinct clinical phenotypes.

**Diagnostic test systems**

The testing landscape for autoimmune neurological syndromes is continually evolving [20]. Autoantibody detection in conjunction with clinical evaluation and radiographic findings can facilitate diagnosis and prognosis of these diseases. IFA is an indispensable method for autoantibody determination. Tissue sections of nerves, cerebellum, hippocampus and intestine enable comprehensive screening of neural autoantibodies, whereas transfected-cell substrates provide easy, monospecific detection of defined autoantibodies. CBA technology is particularly suitable for neuronal cell-surface antigens, which are often conformation-dependent and fragile and thus unsuitable for the expression and purification procedures required for solid-phase methods such as ELISA or immunoblot. Further, as the antigens do not need to be obtained in purified form, the assays can be developed rapidly, enabling novel parameters to be incorporated promptly into the test repertoire. CBAs are now a core component of serological differential diagnostics for certain neurological diseases, for example anti-NMDAR encephalitis and NMOSD.

CBAs with CE mark are currently available from EUROIMMUN for the detection of autoantibodies against NMDAR, AMPAR 1/2, GABABR, LG1, CASPR2, DPPX, IgLON5, GAD65, Zic4, DNER/Tr, AQP4, MOG, AChR and MuSK. Further CBAs are commercially available for research use, for example for the detection of antibodies against NF155, NF186, CASPR1, CNTN1, GABAAR, mGluR1, mGluR5, AK5 and flotillin-1/2. Multiple antibodies can be investigated in parallel using BIOCHIP Mosaics composed of different tissue and cell substrates which are incubated simultaneously. BIOCHIP Mosaics with CE mark are available tailored to different diagnostic applications, for example autoimmune encephalitis (Fig. 2), myasthenia gravis and NMOSD.

Immunoblots are suitable for detection of antibodies against more stable antigens, including many intracellular antigens. With multiplex line blots, many different auto- bodies can be analysed in parallel. In blots of the EUROLINE range, the antigens are contained on individual membrane chips, allowing antigens with widely different properties to be combined in application-oriented profiles. Multiplex EUROLINE profiles are available for detection of up to twelve PNS-associated antibodies (Fig. 3), encompassing the antigens amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, Zic4, GAD65 and DNER/Tr, as well as for detection of different anti-ganglioside antibodies.

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### Table 1. Definitions of antigen/protein short-form names

<table>
<thead>
<tr>
<th>Short-form name</th>
<th>Definition (bold, defined in text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChR</td>
<td>acetylcholine receptors</td>
</tr>
<tr>
<td>AK5</td>
<td>adenylate kinase 5</td>
</tr>
<tr>
<td>AMPAR1/2</td>
<td>glutamate receptors (type AMPA1/2)</td>
</tr>
<tr>
<td>AP3B2</td>
<td>adaptor protein 3 subunit B2</td>
</tr>
<tr>
<td>AQP4</td>
<td>aquaporin-4</td>
</tr>
<tr>
<td>ARHGAP26</td>
<td>RhoGTPase-activating protein 26</td>
</tr>
<tr>
<td>ATP1A3</td>
<td>sodium/potassium-transporting ATPase subunit alpha-3</td>
</tr>
<tr>
<td>CASPR1</td>
<td>contactin-associated protein 1</td>
</tr>
<tr>
<td>CASPR2</td>
<td>contactin-associated protein 2</td>
</tr>
<tr>
<td>CLIP1</td>
<td>CAP-Gly domain-containing linker protein 1</td>
</tr>
<tr>
<td>CNTN1</td>
<td>contactin 1</td>
</tr>
<tr>
<td>CPT1C</td>
<td>carnitine O-palmitoyltransferase 1, brain isoform</td>
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<td>CV2</td>
<td>CV2/collapsin response mediator protein 5 (CRMP5)</td>
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<tr>
<td>DNER/Tr</td>
<td>delta/notch-like epidermal growth factor-related receptor</td>
</tr>
<tr>
<td>DPPX</td>
<td>dipeptidyl aminopeptidase-like protein 6</td>
</tr>
<tr>
<td>ERC1</td>
<td>ELKS/RAB6-interacting/CAST family member 1</td>
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<tr>
<td>GABAAR</td>
<td>gamma-aminobutyric acid receptor type A</td>
</tr>
<tr>
<td>GABABR</td>
<td>gamma-aminobutyric acid receptor type B</td>
</tr>
<tr>
<td>GABAR</td>
<td>gamma-aminobutyric acid receptor</td>
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<tr>
<td>GAD65</td>
<td>glutamic acid decarboxylase 65 kDa isoform</td>
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<td>GluRδ2</td>
<td>glutamate receptor δ2</td>
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<td>GRIPAP1</td>
<td>GRIP1-associated protein 1</td>
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<td>IgLON5</td>
<td>immunoglobulin-like cell adhesion molecule 5</td>
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<td>ITPR1</td>
<td>inositol 1,4,5-triphosphate receptor type 1</td>
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<td>KCNA2</td>
<td>potassium voltage-gated channel subfamily A member 2</td>
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<td>LGI1</td>
<td>leucine-rich glioma-inactivated protein 1</td>
</tr>
<tr>
<td>mGluR1</td>
<td>metabotropic glutamate receptor type 1</td>
</tr>
<tr>
<td>mGluR5</td>
<td>metabotropic glutamate receptor type 5</td>
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<tr>
<td>MOG</td>
<td>myelin oligodendrocyte glycoprotein</td>
</tr>
<tr>
<td>MuSK</td>
<td>muscle-specific kinase</td>
</tr>
<tr>
<td>NBCe1</td>
<td>electrogenic sodium-bicarbonate cotransporter 1</td>
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<tr>
<td>NF155</td>
<td>neurofascin 155</td>
</tr>
<tr>
<td>NF186</td>
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<td>NMDAR</td>
<td>glutamate receptor (type NMDA)</td>
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<td>PNMA2 (Ma2/Ta)</td>
<td>paraneoplastic antigen Ma2</td>
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<td>regulator of G-protein signaling 8</td>
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<td>ROCK2</td>
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<td>RyR2</td>
<td>ryanodine receptor 2</td>
</tr>
<tr>
<td>SOX1</td>
<td>SRY-box transcription factor 1</td>
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<tr>
<td>STX1b</td>
<td>syntaxin-1B</td>
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<tr>
<td>Yo</td>
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<tr>
<td>Zic4</td>
<td>Zic family member 4</td>
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differentiating potentially immuno-
therapy-responsive syndromes from
those that are unlikely to benefit from
this line of treatment. Autoantibody
detection can also guide cancer screening
to detect tumours at an early and highly
treatable stage. Continued identification
of novel autoantibodies will help to
expand the testing repertoire further and
close diagnostic gaps. Additional
research will probe the underlying
immune mechanisms and patho-
physiology with the aim of developing
new therapeutic strategies.

Note
For readability, predominantly the short
form names are used for the antigens/
proteins. However, for completeness,
the full names have been provided in Table 1.

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