Neural autoantibody clusters aid diagnosis of cancer

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Running head: Clustering of Neural Autoantibodies

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Statement of Translational relevance

Analysis of 1.2 million onconeural autoantibody tests performed in approximately 80,000 patients undergoing testing for suspected paraneoplastic neurological disorders identified specific clusters of antibodies permitting individualized prediction of cancer risk and cancer type. These clusters have important implications for both the patient and the physician. They extend the clinical and neurological phenotypes beyond traditionally recognized paraneoplastic syndromes and are highly predictive of specific cancer types, thus directing a focused cancer search. These emergent autoantibody profiles represent serological ‘signatures’ that implicate multi-molecular tumor-derived antigenic complexes as initiators and targets of paraneoplastic immune responses. Extension of this novel analytical approach to larger clinical data bases involving additional validated autoantibody tests will yield greater insight into the relationship of tumor biology and autoimmunity.
Abstract

**Purpose** Clustering of neural autoantibodies in patients with paraneoplastic neurological disorders may predict tumor type. A mathematical analysis of neural autoantibody clusters was performed in 78,889 patients undergoing evaluation for a suspected paraneoplastic autoimmune neurological disorder. Tumor predictive autoantibody profiles were confirmed in sera from patients with histologically proven tonsilar cancer, thymoma, and lung cancer.

**Patients and Methods** 78,889 patient sera were tested for 15 defined neural autoantibodies (1.2 million tests). The observed and hypothesized frequencies of autoantibody clusters were compared and their tumor associations defined. A tumor validation study comprised serum from 368 patients with a variety of tumors (thymoma, lung or tonsil).

**Results** Informative oncological associations included 1) thymoma in 85% of patients with muscle striational, acetylcholine receptor antibodies plus CRMP5 autoantibodies; 2) lung carcinoma in 80% with both P/Q-type and N-type calcium channel antibodies plus SOX1-IgG; 3) in men, prostate carcinoma frequency more than doubled when striational and muscle AChR specificities were accompanied by ganglionic AChR antibody. In women, amphiphysin-IgG alone was associated commonly with breast carcinoma, but amphiphysin-IgG coexisting with ANNA-1 or CRMP5-IgG, was associated with lung cancer (p<0.0001). In the validation cohorts, many tumor-associated profiles were encountered that matched the clusters identified in the screening study (for example 15% of thymoma patients had striational, acetylcholine receptor antibodies plus CRMP5 autoantibodies).
Conclusions Neural autoantibodies commonly coexist in specific clusters that are identifiable by comprehensive screening. Signature autoantibody clusters may predict a patient’s cancer risk and type.

Funding Mayo Clinic Foundation.
Introduction

Autoimmune neurology is a rapidly evolving clinical subspecialty. Neural-specific autoantibodies serve as diagnostic and prognostic biomarkers and guide therapy.\textsuperscript{1,2} Their detection in serum or cerebrospinal fluid supports an autoimmune basis for a patient’s neurological presentation and prompts consideration of a paraneoplastic etiology. Most neural antigens with currently recognized clinical pertinence are expressed in anatomically diverse regions. Accordingly, symptoms may arise from multiple levels of the neuraxis from cerebral cortex through spinal cord, peripheral somatic and autonomic nerves and muscle.

Defined autoantigens include intracellular proteins (nuclear\textsuperscript{3-7} and cytoplasmic enzymes,\textsuperscript{8-10} transcription factors and RNA binding proteins) and plasma membrane or secreted proteins\textsuperscript{11-13} (neurotransmitter receptors, ion channels, ion channel-complex components and water channels). Molecular identification of neural autoantigens has yielded insight into pathogenic mechanisms underlying neurological autoimmunity. For example, some disorders are mediated by IgG binding to extracellular epitopes of plasmalemmal proteins and others by cytotoxic T cells specific for peptides derived from the intracellular antigens.\textsuperscript{14}

Manifestations of neurological autoimmunity are often protean, extending beyond the scope of textbook “syndromic” descriptions. For example, the antineuronal nuclear autoantibody-type 1 (ANNA-1 or “anti-Hu”) is not restricted to sensory neuronopathy (the “Hu syndrome”) or limbic encephalitis. ANNA-1 is commonly associated with gastrointestinal dysmotility, sensorimotor neuropathy or other neurological manifestations.\textsuperscript{3} It is unusual for paraneoplastic neurological autoimmunity to target a single autoantigen. As a neoplasm evolves, neoantigens may appear as part of the mutagenic process. Upregulated self proteins expressing "foreign" epitopes could bypass self-tolerized T cells when exposed to the immune system as
peptides bound to Class II MHC. Thus paraneoplastic autoimmunity is likely to be driven by multiple tumor-derived onconeural antigens.

This study addresses 3 questions: 1) Do neural autoantibodies cluster more than would be hypothesized by chance? 2) What clusters are most commonly encountered? 3) Do autoantibody clusters add diagnostic value in a patient’s clinical and oncologic evaluation? To answer these questions we performed a mathematical analysis of neural autoantibody clusters in 78,889 patients undergoing comprehensive autoantibody evaluation for a suspected paraneoplastic autoimmune neurological disorder, and analyzed clinical and oncological associations in patients with commonly-encountered clusters. We then validated the tumor predictive profiles identified in the screening phase in sera from patients with histologically proven tumors.

Methods

This study was approved by the institutional Review Board, Mayo Clinic, Rochester, Minnesota (IRB 11-004305). It involved analyzing results of a standardized neural autoantibody evaluation performed in the Mayo Clinic Neuroimmunology Laboratory (2008-2011) on sera from 78,889 patients undergoing testing for suspected paraneoplastic autoimmune neurological disorders. A detailed review of seropositive patients’ records was performed. To validate the tumor associated profiles identified in the first phase analysis, we investigated neural autoantibody frequencies and clusters in sera of 368 patients with known cancer (thymoma [98 patients; 33 without neurological disease], lung cancer [240; all without clinical evidence of neurological disease] and tonsillar cancer [30; all without evidence of neurological disease]).

Serological evaluation
All sera were tested for autoantibodies specific for: neuronal and glial nuclear proteins (anti-neuronal nuclear antibody-type 1 [anti-Hu or ANNA-1], anti-neuronal nuclear antibody-type 2 [anti–Ri or ANNA-2], anti-neuronal nuclear antibody-type 3 [ANNA-3] and anti-glial nuclear antibody [AGNA or SOX16,7]), neuronal cytoplasmic proteins (Purkinje cell antibody-type 1 [PCA-1 or anti-Yo], PCA-2, PCA-Tr, amphiphysin, collapsin response-mediator protein-5 [CRMP5]), muscle cytoplasmic proteins (striational antibodies [Str]) and plasma membrane proteins (neuronal voltage-gated-channels: Kv1.1 potassium channel-complexes [VGKC-complex], P/Q-type and N-type calcium channels [N-type VGCC and P/Q-type VGCC] and skeletal muscle-type and neuronal ganglionic-type nicotinic acetylcholine receptors [muscle AChR and ganglionic AChR]).

**Statistical methods**

The observed frequencies of antibody clusters were compared to hypothesized frequencies. Hypothesized frequencies for clusters of two or three autoantibodies were calculated under a conditional independence assumption (as if the cluster occurred by chance) and were compared with the observed frequencies (appendix). Data for 15 antibodies of interest were available for analysis in all the 78,889 patients with. We reviewed the clinical records of patients with clusters whose observed frequencies far outnumbered hypothesized frequencies. We restricted the clinical analysis to clusters for which clinical information was available for at least five patients. In the validation study, the frequencies of individual neural autoantibodies and their clusters in each cancer type were compared with tumor-specific profiles identified in the initial mathematical phase of analysis.
Comparisons of clinical variables between groups were made using Chi-square and Fisher’s exact test where appropriate. All analyses were conducted using SAS version 9 (Cary, NC) and JMP 9.0.1.

Results

Of the 78,889 patients tested, 9,183 (12%) had one or more neural autoantibodies: 7,592 (83%) had only one, 1,316 (14%) had two, 213 (2.3%) had three, 52 (0.57%) had four, 9 (0.1%) had five and 1 (0.01%) had six. These observed frequencies exceed the frequencies hypothesized if clustering of autoantibodies occurred only by chance: 1,316 patients were observed with 2 neural autoantibodies, compared with 365 expected by chance; 213 patients were observed with 3 neural autoantibodies, compared with 10 expected by chance; 62 patients were observed four or more autoantibodies, compared with 0 expected by chance. For each neural autoantibody tested, its frequency and the frequency of the 3 most common coexisting autoantibodies (first, second and third) are shown in table 1.

Clusters of 2 neural autoantibodies (“duo clusters”):

The observed versus hypothesized frequencies for clusters of two neural autoantibodies are illustrated in figure 1a. Duo clusters in which the observed frequencies most exceeded the hypothesized frequencies were: muscle AChR and striational (535 vs 46), N-type VGCC and P/Q-type VGCC (150 vs 6) and VGKC-complex and striational (129 vs 67). Figure 1b illustrates, for each neural autoantibody, the observed and hypothesized frequency of co-occurrence of each of the 14 other autoantibodies. For some neural autoantibodies, there were striking differences in the observed vs hypothesized cluster distributions. Examples include muscle AChR and striational (86% vs 55% of all clusters in which muscle AChR is one of the duo), ANNA-1 and
P/Q-type VGCC (17% vs 1% of all clusters in which ANNA-1 is one of the duo), ANNA-1 and CRMP5 (34% vs 1% of all clusters in which ANNA-1 is one of the duo), SOX1 and P/Q-type VGCC (60% vs 8% of all clusters in which SOX1 is one of the duo), SOX1 and N-type VGCC (30% vs 8% of all clusters in which SOX1 is one of the duo), amphiphysin and P/Q-type VGCC (27% vs 7% of all clusters in which amphiphysin is one of the duo), amphiphysin and ANNA-1 (36% vs 3% of all clusters in which amphiphysin is one of the duo), amphiphysin and CRMP5 (9% vs 1% of all clusters in which amphiphysin is one of the duo), PCA-2 and ANNA-1 (27% vs 3% of all clusters in which PCA-2 is one of the duo), PCA-2 and CRMP5 (27% vs 1% of all clusters in which PCA-2 is one of the duo), N-type VGCC and P/Q-type VGCC (60% vs 10% of all clusters in which N-type VGCC is one of the duo).

**Clusters of 3 neural autoantibodies (“trio clusters”) or more:**

The observed versus hypothesized frequencies for clusters of three neural autoantibodies are illustrated in figure 2. The observed frequencies most exceeded the hypothesized frequencies for the following trio clusters: muscle AChR, striational plus ganglionic AChR (83 vs 1); muscle AChR, striational plus VGKC-complex (20 vs 1); VGKC-complex, N-type VGCC plus P/Q-type VGCC (18 vs 0); striational, N-type VGCC plus P/Q-type VGCC (13 vs 0); muscle AChR, striational plus CRMP5 (7 vs 0); VGKC-complex, striational plus P/Q-type VGCC (6 vs 1); muscle AChR, N-type VGCC plus P/Q-type VGCC (6 vs 0); SOX1, N-type VGCC plus P/Q-type VGCC (5 vs 0). Interestingly, all of these trio clusters arose from the addition of a third autoantibody to the duo clusters that we identified as having an observed frequency exceeding the hypothesized frequency.
Results for clusters of four (52 patients) and five (9 patients) neural autoantibodies are shown in supplemental tables 1 and 2 (Appendix).

**Clinical implications of neural autoantibody clusters:**

This analysis was restricted to the clusters identified in Figures 1 and 2.

**Muscle AChR and striational autoantibodies (Table 2):** This duo cluster was associated with a tumor diagnosis in 45% of patients. The association with cancer was more frequent and more type-specific when this duo was combined with a third neural autoantibody. For a trio cluster with ganglionic AChR, the cancer frequency was 67%; with CRMP5 or VGKC-complex autoantibodies the cancer frequency was 81%. As an illustration of tumor specificity, with VGKC-complex or CRMP5 as the third autoantibody, the frequency of thymoma as the associated cancer rose from 36% to 85%, (p<0.004). Male sex predominated when ganglionic AChR was the third autoantibody (p = 0.0004 compared to 31267 seronegative patients), and the frequency of prostate cancer rose by 30% (p=0.0443). Prostate carcinoma was the most common cancer associated with this trio. Lung cancer also was encountered with the muscle AChR and striational duo cluster but not when a third autoantibody was associated.

**N-type VGCC and P/Q-type VGCC, Table 2:** The duo cluster N-type VGCC and P/Q-type VGCC was associated with Lambert-Eaton syndrome most commonly in a trio combination with SOX1 (6% vs 30%). This trio was associated more highly with lung cancer than the cluster N-type VGCC and P/Q-type VGCC alone (p=0.002) or with trio clusters that included VGKC-complex, striational plus muscle AChR.

**Striational and VGKC-complex, Table 2:** The duo cluster striational and VGKC-complex, with or without P/Q-type VGCC, did not associate with myasthenia gravis or Lambert-Eaton syndrome.
syndrome and cancer was found in less than 25% of cases. In contrast, when muscle AChR coexisted with the duo cluster striational and VGKC-complex, 78% of patients had myasthenia gravis (p<0.0001). This trio cluster was highly associated with thymoma (p=0.008).

Amphiphysin-IgG, Table 3, Figure 1b: Amphiphysin autoantibody was associated with ANNA-1 or CRMP5 more frequently than hypothesized. It is recognized, for men, that small-cell lung carcinoma is the cancer encountered most frequently with amphiphysin autoantibody, regardless of coexisting autoantibodies. In this study, amphiphysin-IgG was detected in 7 male patients; the 4 for whom histories were available all had small-cell lung carcinoma regardless of coexisting autoantibodies. Cancer was diagnosed in 31 of 37 women who had amphiphysin-IgG without a coexisting autoantibody, 31 had cancer (84%). Lung cancer accounted for only 26% of those cancers; 61% had breast cancer. However, when amphiphysin-IgG was accompanied by ANNA-1 or CRMP5 the frequency of lung cancer was 94% (p < 0.0001); only 1 patient had breast cancer (6%, p = 0.0004).

ANNA-1-IgG, Table 3: Among 155 ANNA-1-seropositive patients for whom clinical information was available, cancer was identified more frequently when ANNA-1-IgG coexisted with P/Q-type VGCC or CRMP5-IgG than when ANNA-1 occurred alone (79% vs 58%, p = 0.02, Table 3). The coexistence of P/Q-type VGCC or CRMP5-IgG with ANNA-1 increased the likelihood of small-cell carcinoma from 83% (with ANNA-1 alone, 55 of 65 patients) to 100% (34 of 34 patients with accompanying P/Q-type VGCC or CRMP5, p = 0.01). Other tumor types encountered in 10 patients with ANNA-1 alone included breast carcinoma (1), neuroblastoma (4), non-Hodgkin lymphoma (1), uterine carcinoma (1), basal cell skin carcinoma (2), neuroendocrine (non-specified, 1).
**Tumor Validation**

This phase of the study comprised patients with a histologically proven tumor without neurological symptoms: 33 with thymoma, 240 with lung carcinoma (30 small-cell and 210 non-small-cell) and 30 with tonsil carcinoma. We additionally tested 61 patients who had thymoma and a paraneoplastic neurological syndrome. All were tested for the same 15 autoantibodies as in the screening phase of the study. The frequency of the most commonly encountered neural autoantibodies is shown for each group in table 4. The tonsil carcinoma group lacked neural autoantibodies except for a single patient seropositive for striational antibody. However, neural autoantibodies were frequent in sera of the thymoma and lung carcinoma groups, and even more frequent in thymoma patients with a neurological paraneoplastic syndrome. As observed in the screening phase, striational, muscle AChR, VGKC and CRMP5 IgGs were commonly encountered in thymoma (≥10%). Muscle AChR and striational antibodies were commonly observed in both thymoma groups but were more frequent in patients with thymoma and neurological syndromes than patients with thymoma and non neurological syndromes (p<0.002). VGCC (N or P/Q type) antibodies were encountered in 6% of lung carcinoma patients. ANNA-1 was encountered in 10% of patients with small-cell lung carcinoma but in 0% of those with non-small-cell carcinoma (p=0.007).

Table 4 illustrates the frequency of neural antibodies occurring alone, or in duos, trios and groups of 4 or more for each cancer group. Clustering of neural autoantibodies was common with thymoma patients (69% had clusters of 2 or more antibodies, no significant difference between those with and without neurological symptoms). For lung carcinoma patients, clustering of 2 or more antibodies was observed in 22% of sera.
The most common duo in both thymoma groups was muscle AChR and striational antibodies (accounting for 89% of duos encountered in that group). This duo was the most commonly observed cluster in the screening phase and was more strongly associated with thymoma than lung cancer. Muscle AChR with striational and CRMP5 followed by muscle AChR with striational and VGKC were the most commonly encountered trios in both thymoma cohorts (none were observed in the other cancer cohorts). These trios were identified in the screening phase of the project as having a much higher observed than hypothesized frequency. In the screening phase, thymoma accounted for 85% of tumors identified in patients seropositive for these trios.

Two duos were more frequent in lung cancer: N–type VGCC and P/Q-type VGCC; N-type VGCC and ganglionic AChR. The most common trio in the lung cancer cohort was N-type VGCC, P/Q-type VGCC plus CRMP5. Neither of these duos or trios were observed in the other cancer cohorts. In the screening phase, lung carcinoma accounted for 13% of tumors identified in patients seropositive for the duo N-type VGCC and P/Q-type VGCC and none of the patients with this duo or combinations of VGCC and CRMP5 had thymoma.

**Discussion/Conclusion**

The clustering of onconeural autoantibodies revealed in the mathematical phase of this study was confirmed in the separate cancer validation study, and supports and extends previous clinical observations. These results implicate multi-molecular tumor-derived antigenic complexes as initiators and targets of paraneoplastic immune responses. The autoantibody profiles described here are a measurable immunobiological outcome of cancer that aids both neurological and oncologic diagnosis. Broad screening with a comprehensive neural
autoantibody evaluation rather than testing for single antibodies permits the identification of such clusters.

Recognition of autoantibody clusters increases the likelihood of cancer detection. In our study we found that the frequency of cancer was higher when ANNA-1-IgG coexisted with CRMP5-IgG or VGKC-complex-IgG than when occurring alone. Similar findings were reported by a Honnorat et al. who reported that the frequency of cancer with ANNA-1 and CRMP-5-IgG alone was 77% and 86%, respectively, but > 90% when occurring together. In our cancer validation study we found that clusters of 2 or more neural autoantibodies occur in a majority of patients with thymoma and are not uncommon in lung carcinoma patients (especially small-cell carcinoma).

These clusters have important implications for both the patient and the physician. They extend the clinical and neurological phenotypes beyond traditionally recognized paraneoplastic syndromes and are highly predictive of specific cancer types, thus directing a focused cancer search. The diagnostic value of antibody clustering is exemplified by the detection of amphiphysin-IgG in women. Amphiphysin-IgG alone is highly associated with breast cancer, but when amphiphysin-IgG is accompanied by ANNA-1-IgG or CRMP5-IgG the cancer search is redirected toward lung cancer. Another example is the addition of CRMP5-IgG or VGKC-complex-IgG to the duo cluster of muscle AChR and striational autoantibodies. These clusters focus the cancer search on thymoma.

Autoantibodies that are recognized accompaniments of lung carcinoma tended to cluster more frequently than hypothesized both as a duo (N-type VGCC and P/Q-type VGCC) and as a trio (N-type VGCC, P/Q-type VGCC and SOX1). The addition of SOX1 antibodies to the duo N-type VGCC and P/Q-type VGCC increased the frequency of small-cell carcinoma from
13% to 86%. Similarly, autoantibodies recognized as accompaniments of thymoma tended to cluster as the duo muscle AChR and striational and as the trio muscle AChR, striational and CRMP5. 9,16,17

The findings of this study do not support the traditional concept that P/Q-type VGCC coexisting with N-type VGCC autoantibodies is necessarily associated with Lambert-Eaton syndrome, and muscle AChR and striational antibodies are synonymous with myasthenia gravis. When tested in the context of a paraneoplastic evaluation, cancer was found in one third of 47 patients with both P/Q-type VGCC and N-type VGCC (6% lung cancer); only three of those 47 patients had evidence of Lambert-Eaton syndrome. Similarly less than 70% of patients with muscle AChR and striational antibodies had a diagnosis of myasthenia gravis.

From the findings of this mathematical analysis we anticipate that continued investigation of the clinical and oncological phenotypes of larger numbers of cases will reveal serological signatures permitting individualized prediction of cancer risk and cancer type.
Acknowledgments

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Author contributions

ESH acquired and analyzed the data and wrote the first draft of the manuscript. VAL provided scientific input, critically revised the manuscript and acquired funding. SMJ and CYS acquired and analyzed the data. AM provided scientific input and critically revised the manuscript. DHL acquired and analyzed the data, and critically revised the manuscript. CK provided scientific input and critically revised the manuscript. SJP designed the study, acquired and analyzed the data, wrote the manuscript and acquired funding.
**Conflicts of interest**

Drs. Horta and McKeon, and S. Jenkins report no disclosures. Ms. Smith works on research funded by AbbVie.

Dr. Pittock has received no royalties to date but may accrue revenue for patents relating to AQP4 antibodies for diagnosis of neuromyelitis optica and AQP4 autoantibody as a cancer marker. He has received research support from the Guthy-Jackson Charitable Foundation, Alexion Pharmaceuticals, Inc. and the National Institutes of Health (R01-NS065829). Dr. Pittock has provided consultation to Alexion Pharmaceuticals, Medimmune and Chugai Pharma USA but has received no personal fees or personal compensation for these consulting activities. All compensation for consulting activities is paid directly to Mayo Clinic.

Dr. Lennon is a named inventor on a patent (#7101679 issued 2006) relating to aquaporin-4 antibodies for diagnosis of neuromyelitis optica and receives royalties for this technology; is a named inventor on patents (#12/678,350 filed 2010 and #12/573,942 filed 2008) that relate to functional AQP4/NMO-IgG assays and NMO-IgG as a cancer marker; receives research support from the National Institutes of Health (NS065829).
References


Autoantibody specificities: ANNA-1, ANNA-2 and ANNA-3 = anti-neuronal nuclear, types 1, 2 and 3; CRMP5 = collapsin response-mediator protein-5; gAChR and mAChR = ganglionic-type and muscle-type nicotinic acetylcholine receptors; PCA-1, PCA-2, PCA-Tr = Purkinje cell cytoplasmic, types 1, 2 and Tr; VGCC\textsubscript{N} and VGCC\textsubscript{P/Q} = voltage-gated N-type and P/Q-type calcium channels; VGKC = voltage-gated...
potassium channel-complex.

Table 1: Frequency of coexisting autoantibodies among 78889 sera (15 antibodies tested)
<table>
<thead>
<tr>
<th>mACHR and Str</th>
<th>VGCC\textsubscript{P/Q} and VGCC\textsubscript{N}</th>
<th>Str and VGKC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duo</td>
<td>Trio</td>
<td>Duo</td>
</tr>
<tr>
<td>gAChR or CRMP5</td>
<td>VGKC</td>
<td>or striational or mACHR</td>
</tr>
<tr>
<td>Number with histories available</td>
<td>122</td>
<td>12</td>
</tr>
<tr>
<td>Patients with tumor, n (%)</td>
<td>55 (45)</td>
<td>8 (67)</td>
</tr>
</tbody>
</table>

**Tumor type**

| | Thymoma, n (%) | Prostate, n (% of men with cancer) | Lung, n (%) |
| | 20 (36) | 9 (20) | 6 (11) |
| | 0 | 4 (50) | 0 |
| | 11 (85) | 1 (13) | 0 |
| p | <0.004** | NS | NS |

**Clinical**

| MG or LES, n (% of patients) | MG 69 (57) | MG 10 (78) | MG 11 (69) | NS | LES 3 (6) | LES 2 (14) | LES 3 (30) | NS | 0 | MG 7 (78) | 0 | <0.001**** |

CRMP5 = collapsin response-mediator protein-5; gAChR and mACHR = ganglionic-type and muscle-type nicotinic acetylcholine receptors; Str = striational; VGCC\textsubscript{N} and VGCC\textsubscript{P/Q} = N-type and P/Q-type voltage-gated calcium channels; VGKC = voltage-gated potassium channels; MG = myasthenia gravis; LES = Lambert-Eaton myasthenic syndrome; NS = Not statistically significant.

* p value for comparison between the duo mACHR/Str cluster with the trio clusters in gray.

** p value for comparison between the duo mACHR/Str cluster with either trio cluster in gray, or comparison between trio clusters in gray.
*** p value for comparison between the duo cluster VGCC_{P/Q}/VGCC_N and the trio cluster VGCC_{P/Q}, VGCC_N and SOX1.

**** p value for comparison between the trio cluster mAChR, Str and VGKC and the other clusters in green.

Table 2: Clinical and oncological associations of the most common duos and trios autoantibody clusters.
<table>
<thead>
<tr>
<th></th>
<th>ANNA-1</th>
<th>Amphiphysin (women)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>Coexisting autoantibody, VGCC&lt;sub&gt;P/Q&lt;/sub&gt; or CRMP5</td>
</tr>
<tr>
<td>Histories available, n</td>
<td>112</td>
<td>43</td>
</tr>
<tr>
<td>Patients with tumor (% of patients)</td>
<td>65 (58)*</td>
<td>34 (79)</td>
</tr>
</tbody>
</table>

**Tumor Type**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast, n (%)</td>
<td>1 (2)</td>
<td>1 (3) NS</td>
</tr>
<tr>
<td>Lung cancer, n (%)</td>
<td>55 (83)</td>
<td>34 (100)**</td>
</tr>
</tbody>
</table>

ANNA-1 = anti-neuronal nuclear autoantibody, type 1; CRMP5 = collapsin response-mediator protein-5; VGCC<sub>P/Q</sub> = P/Q-type voltage-gated calcium channel.

* limited follow-up

** Small-cell lung carcinoma proven, 27 patients; non-small-cell lung carcinoma, 1 patient; histological type unknown, 5 patients; extrapulmonary small-cell carcinoma, 1 patient.

*Table 3: Influence of coexisting autoantibodies on tumor type prediction.*
Neurological symptoms in the thymoma group were: MG in 80%, encephalopathy in 10%, cramp fasciculation syndrome in 3%, other in 7%.

* When compared to lung cancer patients, the overall number of patients seropositive for 1 or more neural autoantibodies, $p < 0.0001$; mAChR antibody, $p < 0.0001$; striational antibody $p < 0.0001$; 

<table>
<thead>
<tr>
<th>Autoantibody (frequency %)</th>
<th>Neuro (61)</th>
<th>Non neuro (33)*</th>
<th>P value</th>
<th>SCLC (30)</th>
<th>NSCLC (210)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striational</td>
<td>1 (3%)</td>
<td>51 (84)</td>
<td>17 (52)</td>
<td>0.0009</td>
<td>3 (10)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>VGKC</td>
<td>0</td>
<td>10 (16)</td>
<td>2 (6)</td>
<td>NS</td>
<td>2 (7)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>gAChR</td>
<td>0</td>
<td>5 (8)</td>
<td>4 (12)</td>
<td>NS</td>
<td>2 (7)</td>
<td>19 (9)</td>
</tr>
<tr>
<td>mAChR</td>
<td>0</td>
<td>54 (89)</td>
<td>14 (42)</td>
<td>&lt;0.0001</td>
<td>0</td>
<td>3 (1)</td>
</tr>
<tr>
<td>VGCCN</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (3)</td>
<td>NS</td>
<td>2 (7)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>VGCCP/Q</td>
<td>0</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>NS</td>
<td>2 (7)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>ANNA-1</td>
<td>0</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>NS</td>
<td>3 (10)</td>
<td>0</td>
</tr>
<tr>
<td>CRMP5</td>
<td>0</td>
<td>13 (21)</td>
<td>6 (18)</td>
<td>NS</td>
<td>3 (10)</td>
<td>7 (3)</td>
</tr>
</tbody>
</table>

+ Clinical history unavailable in 4 patients.
0.0001; CRMP5-IgG, p = 0.0013 were more common in patients with thymoma (with or without neurological symptoms).

Abbreviations used: seropos = seropositive; neuro = patients with neurological syndromes, non neuro: patients without neurological syndromes; ANNA-1 = anti-neuronal nuclear types 1; CRMP5 = collapsin response-mediator protein-5; gAChR and mAChR = ganglionic-type and muscle-type nicotinic acetylcholine receptors; VGCC\textsubscript{N} and VGCC\textsubscript{P/Q} = voltage-gated N-type and P/Q-type calcium channels; VGKC = voltage-gated potassium channel-complex.

**Table 4: Frequency of neural autoantibodies in each cancer cohort**
Figure Legends

Figure 1. Duo clusters of neural autoantibodies

These graphs illustrate autoantibody pairs that were encountered more frequently than expected by chance.

(A) Observed versus hypothesized frequencies for each cluster of two autoantibodies. The line illustrates the distribution expected if the observed frequency was equal to the hypothesized frequency (identity line). The distance of a designated cluster from the identity line reflects the magnitude of difference between the observed and hypothesized frequencies (i.e., anticipated by chance). The red cluster (muscle AChR and striational autoantibodies) is more than two standard deviations away from the identity line. Green points were assigned to clusters that were more than one standard deviation from the identity line.

(B) Bar graph comparing the observed and hypothesized frequency distribution of all duo clusters involving each of 15 tested autoantibodies (all are color coded).

Abbreviations: ANNA-1, ANNA-2 and ANNA-3 = anti-neuronal nuclear autoantibody, types 1, 2 and 3; CRMP5 = collapsin response-mediator protein-5; gAChR and mAChR = ganglionic-type and muscle-type nicotinic acetylcholine receptors; PCA-1, PCA-2, PCA-Tr = Purkinje cell cytoplasmic antibodies types 1, 2 and Tr; Str = striational antibodies; VGCC\textsubscript{N} and VGCC\textsubscript{P/Q} = voltage-gated N-type and P/Q-type calcium channels; VGKC = voltage-gated potassium channel complex.
Figure 2. Trio Clusters of neural autoantibodies

Observed versus hypothesized frequencies for each cluster of three autoantibodies. The line illustrates the distribution expected if the observed frequency were equal to the hypothesized frequency (identity line). The distance of a designated cluster from the identity line reflects the magnitude of difference between the observed and hypothesized frequencies (i.e., anticipated by chance). The red clusters are more than two standard deviations away from the identity line. The green clusters are more than one standard deviation from the identity line.

Abbreviations: ANNA-1, ANNA-2 and ANNA-3 = anti-neuronal nuclear autoantibody, types 1, 2 and 3; CRMP5 = collapsin response-mediator protein-5; gAChR and mAChR = ganglionic-type and muscle-type nicotinic acetylcholine receptors; PCA-1, PCA-2, PCA-Tr = Purkinje cell cytoplasmic antibodies types 1, 2 and Tr; Str = striational antibodies; VGCC\textsubscript{N} and VGCC\textsubscript{P/Q} = voltage-gated N-type and P/Q-type calcium channels; VGKC = voltage-gated potassium channel-complex.
Figure 2

- Observed (number of patients): mAChR, Str, gAChR, mAChR, Str, VGKC, VGCC\textsubscript{P/Q}, VGCC\textsubscript{N}, VGKC
- Hypothesized (number of patients): VGCC\textsubscript{P/Q}, VGCC\textsubscript{N}, SOX1, VGCC\textsubscript{P/Q}, VGCC\textsubscript{N}, mAChR, VGCC\textsubscript{P/Q}, Str, VGKC, VGCC\textsubscript{P/Q}, VGCC\textsubscript{N}, mAChR
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