Cerebellar Ataxia and Glutamic Acid Decarboxylase Antibodies
Immunologic Profile and Long-term Effect of Immunotherapy

Helena Ariño, MD; Nuria Gresa-Arribas, PhD; Yolanda Blanco, MD, PhD; Eugenia Martinez-Hernández, MD, PhD; Lidia Sabater, PhD; Mar Petit-Pedrol, BS; Idoia Rouco, MD; Luis Bataller, MD; Josep O. Dalmau, MD, PhD; Albert Saiz, MD, PhD; Francesc Graus, MD, PhD

IMPORTANCE Current clinical and immunologic knowledge on cerebellar ataxia (CA) with glutamic acid decarboxylase 65 antibodies (GAD65-Abs) is based on case reports and small series with short-term follow-up data.

OBJECTIVE To report the symptoms, additional antibodies, prognostic factors, and long-term outcomes in a cohort of patients with CA and GAD65-Abs.

DESIGN, SETTING, AND PARTICIPANTS Retrospective cohort study and laboratory investigations at a center for autoimmune neurologic disorders among 34 patients with CA and GAD65-Abs, including 25 with long-term follow-up data (median, 5.4 years; interquartile range, 3.1-10.3 years).

MAIN OUTCOMES AND MEASURES Analysis of clinicoimmunologic features and predictors of response to immunotherapy. Immunohistochemistry on rat brain, cultured neurons, and human embryonic kidney cells expressing GAD65, GAD67, α1-subunit of the glycine receptor, and a repertoire of known cell surface autoantigens were used to identify additional antibodies. Twenty-eight patients with stiff person syndrome and GAD65-Abs served as controls.

RESULTS The median age of patients was 58 years (range, 33-80 years); 28 of 34 patients (82%) were women. Nine patients (26%) reported episodes of brainstem and cerebellar dysfunction or persistent vertigo several months before developing CA. The clinical presentation was subacute during a period of weeks in 13 patients (38%). Nine patients (26%) had coexisting stiff person syndrome symptoms. Systemic organ-specific autoimmunities (type 1 diabetes mellitus and others) were present in 29 patients (85%). Twenty of 25 patients with long-term follow-up data received immunotherapy (intravenous immunoglobulin in 10 and corticosteroids and intravenous immunoglobulin or other immunosuppressors in 10), and 7 of them (35%) improved. Predictors of clinical response included subacute onset of CA (odds ratio [OR], 0.50; 95% CI, 0.25-0.99; P = .047) and prompt immunotherapy (OR, 0.98; 95% CI, 0.96-0.99; P = .01). Similar frequencies of serum GAD67-Abs were found in patients with CA (24 of 34 patients [71%]) and in patients with stiff person syndrome (20 of 28 patients [71%]). However, GAD67-Abs were found in all of the cerebrospinal fluid samples examined (22 samples from patients with CA and 17 samples from patients with stiff person syndrome). Glycine receptor antibodies but not other cell surface antibodies were identified in 4 patients with CA. The presence of glycine receptor antibodies did not correlate with any specific clinical feature.

CONCLUSIONS AND RELEVANCE In patients with CA and GAD65-Abs, subacute onset of symptoms and prompt immunotherapy are associated with good outcome. Persistent vertigo or brainstem and cerebellar episodes can herald CA and should lead to GAD65-Ab testing, particularly in patients with systemic organ-specific autoimmunities.
A
toimmunity is increasingly recognized as a cause of
cerebellar dysfunction. Cerebellar ataxia (CA), associ-
ated with antibodies against the 65-kDa isoform of glu-
tamic acid decarboxylase (GAD65-Abs), is one of the best-
characterized cerebellar syndromes in which autoimmune
mechanisms probably have a relevant pathogenic role. After
stiff person syndrome (SPS), CA is the second most frequent
neurologic disorder associated with GAD65-Abs. Current
knowledge of the clinical and immunologic profile of CA with
GAD65-Abs is based on small series and single case reports, and
the long-term outcomes after immunotherapy are unknown.

The association of GAD65-Abs with various syndromes
such as SPS or CA has led to several hypotheses regarding the
possible pathogenic role of these antibodies. The results of ex-
erimental investigations using monoclonal antibodies against
different GAD65 epitopes suggest that the various neurologic
syndromes could be related to the pattern of epitope recogni-
tion by human serum. Alternatively, the occurrence of CA or
SPS could result from additional mechanisms mediated by
T cells or antibodies against surface antigens such as those
described in several autoimmune encephalitis. In this regard,
γ-aminobutyric acid receptor and glycine receptor antibod-
ies (GlyR-Abs) have been described in patients with concur-
tent GAD65-Abs. We aimed to better characterize the clinical presentation, the immunologic pro-
file, the presence of additional antibodies against cell surface
antigens, and the long-term response to immunotherapy.

Methods

Patients

The study was approved by the ethics committee of the Hospi-
tal Clinic, Barcelona, Spain. Written informed consent was
obtained from all patients for the storage and use of serum and
cerebrospinal fluid (CSF) samples for research purposes. Pa-
tients with CA and GAD65-Abs who were seen at the Hospital
Clinic or whose serum or CSF samples were examined at the
Institut d’Investigacions Biomèdiques August Pi i Sunyer, Bar-
celona, Spain, between December 15, 1994, and April 4, 2013,
were included in the study if they met the following criteria:
(1) predominant or isolated cerebellar dysfunction at presen-
tation and the absence of another cause that could explain their
CA, (2) available clinical information, and (3) the presence of
high serum titers of GAD65-Abs confirmed by radioimmuno-
assay and immunohistochemistry. Evidence has shown that
positive serum immunoreactivity using rat cerebellar sec-
tions is associated with high GAD65-Ab levels on radioimmu-
noassay (usually >2000 U/mL). Overall, 49 potential study
patients were identified, 15 of whom were excluded because of
a lack of clinical information. Long-term follow-up data were
obtained in 25 of 34 patients (74%) (median, 5.4 years; inter-
quartile range, 3.1-10.3 years). Data were obtained from clinical
records, and information was collected from referring neu-
rologists using a structured questionnaire mainly focused on
the clinical presentation, the presence of neurologic symp-
toms preceding the cerebellar syndrome, concomitant symp-
toms of rigidity and spasms, and the response to immuno-
therapy. The onset of CA was defined as subacute when the
cerebellar symptoms reached their nadir or required neuro-
logic assessment within the first 3 months of symptom pre-
sentation. Ten patients were examined and followed up by 1
or more of us. Neurologic disability was measured by the modi-
fied Rankin Scale (mRS). Patients with a history of cancer who
met the criteria for definite or possible paraneoplastic neuro-
logic syndrome were excluded from the study. Serum and CSF
samples used in the study were deposited in the Neuroimmu-
нологíacollectionofbiologicalsamplesregisteredinthebio-
bank of the Institut d’Investigacions Biomèdiques August Pi i
Sunyer, Barcelona, Spain.

Autoantibody Assays

All laboratory techniques have been previously reported and
are described in detail in the eMethods in the Supplement.
These include immunohistochemistry on frozen rat brain, im-
munoblot of human GAD65 recombinant protein (Diamyd),
immunocytochemistry on cultures of rat hippocampal and cer-
ebellar granular neurons, and immunocytochemistry on human embryonic kidney 293 cells transfected with the α-1 sub-
unit of the GlyR (obtained by gift) and with GAD65 and GAD67
(OriGene).

Statistical Analysis

Nonparametric tests were used when the distribution of the
analyzed variables differed from normal using the Kolmogorov-
Smirnov test. Good outcome was defined as an mRS score of
less than 3 at the last follow-up visit, and bad outcome was de-
defined as an mRS score of 3 or higher. In treated patients,
improvement was defined as a decrease of at least 1 point on the
mRS at the last follow-up visit compared with the score at di-
agnosis. For the multivariate analysis, a generalized linear
model was used that included relevant or significant (P < .10
on univariate analysis) factors. As a dependent variable to ana-
lyze response to treatment, we used the change in mRS scores
from the onset to the last follow-up visit. Odds ratios (95% CIs)
were used to measure the effect of predictors on the exponen-
tial function of the regression coefficient. Statistical software
(SPSS Statistics version 19; IBM) was used for the analyses.

Results

Clinical Characteristics at Diagnosis

The median age of patients was 58 years (range, 33-80 years); 28
of 34 patients (82%) were women. Twenty-nine patients
(85%) had concomitant systemic organ-specific autoimmune
disorders. Type 1 diabetes mellitus was present at diagnosis
in 13 patients (38%), and the incidence increased to 17 of 25 pa-
tients (68%) during the follow-up period. Twenty-one pa-
tients (62%) had other organ-specific autoimmune disorders,
mainly thyroiditis and pernicious anemia (Table 1). Thirteen
patients (38%) had subacute presentation of CA lasting for
weeks, while the other 21 patients (62%) had a chronic course
progressing during months or years. Overall, the demo-
graphic features and autoimmune clinical associations of 34 patients with CA were similar to those of 28 patients with SPS and GAD65-Abs.

Gait ataxia was the most common clinical presentation (31 patients), followed by limb ataxia (25 patients) that was asymmetric in 20, dysarthria (24 patients), and nystagmus (20 patients). Muscle rigidity and spasms were identified in 9 patients (26%), and 4 of them manifested electromyographic features of SPS (Table 2). Muscle rigidity and spasms occurred at the time of their CA in 5 patients and 2 to 5 years later in 3 patients. In 1 patient, leg spasms triggered by emotional stimuli or anxiety were present 2 years before the onset of her CA. Four patients also had epilepsy. In 3 of them, their epilepsy antedated the diagnosis of CA by 15, 13, and 2 years. Two of these patients met the criteria for refractory temporal lobe epilepsy. The fourth patient developed 2 generalized seizures 18 months after the onset of CA.

Neurologic symptoms antedating the diagnosis of CA were reported in 9 patients (26%), with 2 different profiles. Six patients reported fluctuating vertigo 7 to 26 months before developing CA. All manifested exacerbations that lasted days to weeks. During this period, the neurologic examinations showed no signs of CA. The remaining 3 patients had at least 1 episode of transient neurologic deficit, suggesting brainstem or cerebellar involvement, 2 to 24 months before the diagnosis of CA. The first patient had an episode of isolated vertical diplopia that lasted 10 days. The second patient had 2 episodes of dysarthria and gait ataxia that lasted a few days. The third patient developed dysarthria and right arm ataxia that lasted for 2 months and resolved spontaneously. The presence of prodromal symptoms was 3 times more common in men (P = .07).

### Long-term Outcomes
Long-term follow-up data were available for 25 patients. Figure 1 shows the type and duration of treatment during the course of the disease. Five patients received no immunotherapy, and 2 patients were untreated for more than 2 years. None of them improved, and the condition in 3 patients slowly deteriorated. Twenty patients received immunotherapy, which in 10 patients included intravenous immunoglobulin (IVIg), while 9 patients received intravenous methylprednisolone alone (4 patients) or in combination with IVIg (4 patients) or rituximab (1 patient). One patient was treated with oral prednisone (1 mg/kg/d). Among treated patients, 17 received various types of maintenance therapy: 6 were treated with IVIg during a median of 56.2 months (interquartile range, 24.4-121.5 months), and 11 received 1 or more regimens of oral corticosteroids, azathioprine, or mycophenolate mofetil.

The eFigure in the Supplement shows the degree of disability at diagnosis, at the end of the first treatment (up to 6 months), and at the last follow-up visit. At the last follow-up visit, 11 patients had an mRS score of less than 3 (good out-
come), and 14 patients had an mRS score of 3 or higher (bad outcome). Patients with good outcome had a better mRS score at diagnosis than patients with bad outcome (median, 2 vs 3; \( P = .01 \)) and responded to immunotherapy more frequently (6 patients [55%] vs 1 patient [7%]; \( P = .01 \)) (Table 3).

Among 20 patients who were treated, 10 showed clinical improvement (±1 point on the mRS during the first 6 months of treatment), which in 7 patients (35%) persisted at the last follow-up visit. Four of them were treated with pulses of intravenous immunoglobulin; M, male; PE, plasma exchange; SPS, stiff person syndrome. Continuous motor unit potentials firing at rest and during contraction of antagonist muscles in needle electromyographic recordings.

The patient experienced leg spasms triggered by emotional stimuli and prominent anxiety. She was diagnosed as having conversion disorder until she developed CA.

### Immunologic Studies

All serum and CSF samples from patients with CA and SPS included in the study showed reactivity to human embryonic kidney cells transfected with GAD65. Similarly, all but one CA serum samples were positive in immunoblots of GAD65 recombinant protein, suggesting that the antibodies from patients with CA and SPS recognize linear epitopes. The assessment of GAD65-Abs by immunohistochemistry revealed that in all cases the predominant IgG isotype was IgG1. Additional isotypes were detected in a small proportion of patients, including IgG3 in 2 of 34 patients with CA and 2 of 28 patients with SPS and IgG2 in 2 of 34 patients with CA and 6 of 28 patients with SPS.

The frequencies of serum GAD67-Abs were similar in patients with CA (24 of 34 patients [71%]) and in patients with SPS (20 of 28 patients [71%]). GAD67-Abs were found in all CSF samples available from 22 patients with CA and from 17 patients with SPS. GAD67-Abs were present in CSF samples but not in serum samples of 7 patients with CA and 4 patients with SPS having paired serum and CSF samples (Figure 2). Serum and CSF samples from patients with CA did not immunoreact with live hippocampal and granular cerebellar neurons, and none manifested antibodies against N-methyl-D-aspartate, \( \gamma \)-aminobutyric acid B, and \( \alpha \)-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors or leucine-rich glioma-inactivated protein 1, dipeptidyl peptidase–like protein 6, and contactin-associated protein 2 antigens. In contrast, 4 patients with CA had GlyR-Abs in serum samples but not in the 2 CSF samples available. The presence of GlyR-Abs did not correlate with any specific clinical feature, no patient

### Table 2. Symptoms of SPS in 9 Patients With CA and GAD65-Abs Having Muscle Rigidity and Spasms

<table>
<thead>
<tr>
<th>Sex/Age at Diagnosis, y</th>
<th>Distribution of Stiffness</th>
<th>Other SPS Symptoms</th>
<th>Neurophysiological Findings</th>
<th>Temporal Relationship With CA</th>
<th>Treatment</th>
<th>Clinical Course of SPS and CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/53</td>
<td>Legs</td>
<td>Frequent falls</td>
<td>Compatible with SPS</td>
<td>Same time</td>
<td>IVIg</td>
<td>No change with SPS and CA</td>
</tr>
<tr>
<td>F/77</td>
<td>Legs, trunk</td>
<td>Leg spasms, lumbar pain</td>
<td>Compatible with SPS</td>
<td>2 y Later</td>
<td>Oral IS, rituximab</td>
<td>SPS improved and later relapsed, CA improved and then progressed</td>
</tr>
<tr>
<td>F/76</td>
<td>Right leg</td>
<td>Leg spasms</td>
<td>Compatible with SPS</td>
<td>Same time</td>
<td>IVIg, rituximab, chronic IS</td>
<td>Remission of SPS after first-line treatment and relapsing at last visit (5 y), slow partial improvement of CA</td>
</tr>
<tr>
<td>F/50</td>
<td>Legs, first, then trunk</td>
<td>Leg spasms, lumbar hyperlordosis</td>
<td>Compatible with SPS</td>
<td>2 y Later</td>
<td>PE, oral IS, rituximab</td>
<td>SPS improved, CA stable</td>
</tr>
<tr>
<td>M/74</td>
<td>Leg, trunk</td>
<td>Leg spasms</td>
<td>Sensorimotor polyneuropathy</td>
<td>Same time</td>
<td>IVIg, oral IS</td>
<td>Improvement of both syndromes after 1 y</td>
</tr>
<tr>
<td>F/40</td>
<td>Right leg</td>
<td>Leg spasms</td>
<td>Signs of denervation</td>
<td>Same time</td>
<td>IVIg, oral IS</td>
<td>Both syndromes stable</td>
</tr>
<tr>
<td>F/59</td>
<td>Legs, trunk</td>
<td>Lumbar pain, hyperlordosis</td>
<td>Normal</td>
<td>Same time</td>
<td>IVIg, oral IS</td>
<td>No response after 4 mo, patient lost to follow-up data</td>
</tr>
<tr>
<td>F/52</td>
<td>Legs, first asymmetric</td>
<td>Leg spasms</td>
<td>Not done</td>
<td>5 y Later</td>
<td>IVIg, PE</td>
<td>Mild improvement of SPS spams, CA stable</td>
</tr>
<tr>
<td>F/52</td>
<td>Legs</td>
<td>Leg spasms, agoraphobia</td>
<td>Normal under benzodiazepine treatment</td>
<td>2 y Before(^b)</td>
<td>IVIg</td>
<td>Clear improvement of SPS after 1 cycle, no CA immediate effect, patient lost to follow-up data</td>
</tr>
</tbody>
</table>

Abbreviations: CA, cerebellar ataxia; F, female; GAD65-Abs, glutamic acid decarboxylase 65 antibodies; IS, immunosuppression; IVIg, intravenous immunoglobulin; M, male; PE, plasma exchange; SPS, stiff person syndrome.

\(^*\) Continuous motor unit potentials firing at rest and during contraction of antagonist muscles in needle electromyographic recordings.

\(^b\) The patient experienced leg spasms triggered by emotional stimuli and prominent anxiety. She was diagnosed as having conversion disorder until she developed CA.
Discussion

To our knowledge, this is the first study to describe the long-term outcomes in a series of patients with CA and GAD65-Abs. Our findings indicate that patients with subacute presentation of CA are more likely to respond to immunotherapy and achieve good functional status (mRS score, <3) and confirm that a shorter delay in the initiation of immunotherapy predicts clinical improvement.\(^4\)

Previous autopsy studies\(^{15,16}\) of patients with CA and GAD65-Abs revealed selective loss of Purkinje cells. However, the clinical improvement observed in some of our patients indicates that part of the cerebellar dysfunction at the time of diagnosis may be due to functional impairment that can be reversed by early onset of immunotherapy. Although the pathogenic role of GAD65-Abs is unclear, studies\(^{17,18}\) have shown that they interfere with the \(\gamma\)-aminobutyric acid–ergic synaptic transmission in tissue culture systems and that these effects are reversible after removing the GAD65-Abs. In addition, intracerebellar injection of GAD65-Abs induces an increase in glutamate levels that may lead to glutamate excitotoxic effects.\(^4\) These neurochemical and neurophysiological
abnormalities could initially affect the function of Purkinje cells without causing irreversible damage but may cause their death in the long run.

The retrospective design of this study and the multicenter locations of the patients make it difficult to recommend a particular type of immunotherapy. The effect of various immunotherapies has been described in single case reports (summarized in the eTable in the Supplement). As in the present series, the most common treatments used were cycles of IVIg or methylprednisolone. Most of the described patients had a chronic presentation of symptoms, and treatment was started after a median delay of 12 months (range, 2-120 months). Improvement was reported in 13 of 16 patients (81%) compared with 7 of 20 patients (35%) in this series. However, the figures are not comparable because the degree of improvement reported in many of these case reports would not fulfill the required decrease of at least 1 point on the mRS as used herein (eTable in the Supplement). As in SPS, we observed that patients with CA who responded to immunotherapy did so early, during the first 6 months. A lack of improvement in this short period should be an indication for switching to a second-line immunotherapy or stopping treatment. Our study does not clarify whether maintenance immunotherapy is useful: 3 of 7 patients who improved with the initial treatment remained stable without subsequent immunotherapy.

We have identified a previously unrecognized feature to date of patients with CA and GAD65-Abs. Three patients (9%)...
reported episodes of diplopia or combinations of dysarthria and ataxia of unclear etiology months before the development of full-blown CA. In addition, 6 patients (18%) reported isolated vertigo in the absence of other symptoms of cerebellar dysfunction. We consider that vertigo in these patients was heralding the development of more widespread involvement of the cerebellum. This feature has been observed in other cerebellar syndromes. In patients with spinocerebellar ataxia, episodic vertigo antedated the development of gait ataxia by several years in 4% of patients.33 Investigations of selective ischemic infarcts of the cerebellum indicate that patients who were seen with isolated vertigo more frequently had had the infarct in the caudal vermis.34 Taken together, these data suggest that in some patients GAD65 autoimmunity may result in subtle, focal, or transient symptoms, likely representing involvement of selective brainstem or cerebellar regions. In some patients, particularly those with diseases that are associated with GAD autoimmunity (eg, type 1 diabetes mellitus), the development of persistent vertigo of unknown etiology or episodes of diplopia, dysarthria, or ataxia, should lead to GAD65-Ab testing. In that clinical scenario, the detection of high-titer GAD65-Ab (usually >2000 U/mL) should raise concern about impending CA.

In our patients with CA, the immunologic response against GAD did not differ from that observed in patients with SPS. We found no significant increase in intrathecal production of GAD65-Ab in patients having CA compared with those having SPS, as previously suggested.35 A relevant observation was that all patients in whom CSF samples could be analyzed manifested GAD67-Ab, despite that some of them did not have these antibodies in serum samples. The presence of GAD67-Ab only in CSF, along with previous demonstration of intrathecal synthesis of GAD65-Ab35 and a different epitope repertoire noted between paired serum and CSF samples,36 strongly supports the presence of GAD-specific B cells in the central nervous system and emphasizes the importance of examining the CSF in autoimmune disorders of the central nervous system.

Except for 4 patients with CA who had concomitant GlyR-Abs in serum samples, we did not find (as suggested in SPS37) other antibodies against neuronal surface antigens. Glycine receptor antibodies were initially described in patients having progressive encephalomyelitis with rigidity and myoclonus.38 More recently, GlyR-Abs were reported in 12% to 15% of patients with SPS (with or without GAD65-Ab) and in 3% of patients with epilepsy.39,40 In our patients, the significance of GlyR-Abs is unknown because the clinical course and outcome did not differ in patients without this antibody. In 2 of these 4 patients, CSF samples were available and were negative for GlyR-Abs in both of them. Although earlier described patients having progressive encephalomyelitis with rigidity and myoclonus showed GlyR-Abs in serum and CSF samples,41,42 in patients with SPS or epilepsy GlyR-Abs were usually studied based on serum samples only.39,40 Therefore, it is unclear whether the presence or absence of GlyR-Abs in CSF is associated with different clinical phenotypes as recently reported in other autoimmune encephalitides.43 Future studies should determine the degree of syndrome specificity of GlyR-Abs, comparing paired serum and CSF samples in larger groups of patients and control subjects.

Conclusions

Our findings reveal that patients with CA and GAD65-Ab may respond to immunotherapy and maintain good functional status for many years. The retrospective design of the study prevents us from making definite recommendations, but in our experience the use of IVIg or corticosteroids should be considered in all patients with CA and GAD65-Ab, particularly those with subacute presentation. Early initiation of treatment likely offers a greater chance of improvement.


Ye BS, Kim YD, Nam HS, Lee HS, Nam CM, Heo JH. Clinical manifestations of cerebellar infarction according to specific lobular involvement. Cerebellum. 2010;9(4):571-579.


