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Title page

A retrospective study of autoimmune cerebellar ataxia over a 20-year period in a single institution

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Statements and Declarations

Competing Interests: The authors have no competing interests to declare that are relevant to the content of this article.

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Author contributions

AK, HY and IY contributed to the conception and design of the study. AK, HY, AK, KT and IY contributed to the acquisition and analysis of data. AK, HY, AK, KT and IY contributed to drafting the text or preparing the figures.

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Abstract

Background: It is important to differentiate autoimmune cerebellar ataxia (ACA) from neurodegenerative CA, but this is sometimes difficult. We performed a retrospective study in a single institution in Japan over a 20-year period to reveal the clinical features of ACA.

Methods: Patients with CA as the primary neurological symptom were enrolled from those admitted to the Department of Neurology, Hokkaido University Hospital between April 2002 and March 2022. ACA was diagnosed retrospectively according to the following criteria: (1) CA being the predominant symptom; (2) identification of cancer within 2 years of onset; (3) improvement in cerebellar symptoms following immunotherapy; and (4) ruling out alternative causes of CA. Patients fulfilling criteria (1), (2), and (4) were classified as paraneoplastic cerebellar degeneration (PCD), while those fulfilling (1), (3), and (4) were classified as non-PCD and enrolled as patients with ACA. Neurodegenerative diseases, e.g., multiple system atrophy (MSA), were confirmed retrospectively based on generally used diagnostic criteria and enrolled. Furthermore, the ACA diagnostic criteria proposed by Dalmau and Graus were applied retrospectively to the ACA patients to examine the validity of the diagnoses.

Results: Among the 243 patients with CA, 13 were enrolled as ACA; five were PCD and eight were non-PCD. Eight of these cases met the proposed diagnostic criteria by Dalmau and Graus. MSA was the most prevalent disease among CA patients, with 93 cases. The incidence of

cerebellar atrophy was significantly lower in ACA (3/13) than in MSA (92/92). Cerebrospinal fluid (CSF) pleocytosis was significantly more frequent in ACA than in MSA (4/13 vs. 2/55, respectively). However, there was no significant difference in the presence of oligoclonal bands, increased protein in CSF, and laterality differences in ataxia.

Conclusion: ACA was present in ~5% of Japanese CA patients. The absence of cerebellar atrophy, despite the presence of CA, strongly supports ACA over MSA. While CSF pleocytosis was observed more often in ACA, the positivity rate was only ~30%. Since ACA is treatable, further studies are needed to identify additional clinical features and accurate diagnostic biomarkers.

Introduction

Cerebellar ataxia (CA) can develop through various mechanisms including congenital conditions, neurodegenerative diseases, vascular disorders, toxin- and drug-induced mechanisms, and immune-mediated mechanisms. In clinical practice, distinguishing autoimmune cerebellar ataxia (ACA) from other types of CA is crucial as ACA can be treated with immunotherapy [1]. Nonetheless, distinguishing ACA from neurodegenerative CA, such as multiple system atrophy (MSA), can be challenging during the early stages of these diseases, e.g., there is a report summarizing 107 cases who were misdiagnosed with autoimmune encephalitis [2]. Consequently, it is important to understand the clinical features of immune-mediated diseases to distinguish them effectively from neurodegenerative and infectious diseases.

The cerebellum is the primary target in paraneoplastic neurological syndromes (PNS), and the phenotype of rapidly progressive cerebellar syndrome is indicative of PNS [3]. Consequently, paraneoplastic cerebellar degeneration (PCD) holds a significant place within the spectrum of ACA, often necessitating cancer screening in patients with ACA. However, in cases of PCD, cerebellar symptoms may appear before cancer detection, and onconeural antibodies may be observed even in the absence of malignancy. Moreover, ACA can be triggered by a variety of other conditions, including gluten ataxia and post-infectious cerebellitis. Therefore, it is crucial to diagnose ACA accurately, regardless of the presence or absence of malignancy.

Autoantibodies against neuronal antigens serve as useful biomarkers for the diagnosis of ACA, and to date, many such autoantibodies have been reported. Until approximately the year 2000, reports of ACA with positive autoantibodies predominantly featured those related to PNS, such as Hu, Yo, and Ri [4]. However, since then, there has been a notable increase in the discovery of many CA-related surface antibodies. For example, antibodies against metabotropic glutamate receptor 1 (mGluR1) and dipeptidyl peptidase-like protein-6 show pathogenicity by inhibiting the function of surface proteins [5, 6]. In addition, autoantibodies such as anti-contactin-associated protein-like 2 and anti-voltage-gated calcium channel (VGCC) antibodies, which are somewhat associated with PNS, have been detected in cases of CA, even in the absence of cancer [7, 8]. Moreover, autoantibodies targeting intracellular antigens, such as glutamic acid decarboxylase (GAD) and Rho GTPase-activating protein 26, have been reported in patients with ACA [9, 10].

There are cases of immune-mediated CA of unknown etiology, often referred to as primary ACA (PACA). In 2020, the International Task Force on Immune-Mediated Cerebellar Ataxias released diagnostic criteria for PACA [11]. Within these diagnostic criteria, some autoantibodies detected in ACA are listed, despite their unclear pathogenicity, and their presence is incorporated as supportive evidence for diagnosis. However, these autoantibodies do not include newly recognized ones, such as anti-glutamate kainate receptor subunit 2 and anti-seizure-related 6 homolog-like 2 (Sez6l2) antibodies, which have been increasingly reported in recent years [12-

14].

In 2022, Dalmau and Graus proposed a more comprehensive set of diagnostic criteria for ACA, encompassing PCD, gluten ataxia, and post-infectious cerebellitis in their textbook [1]. Furthermore, these proposed diagnostic criteria emphasize the presence of onconeural, anti-GAD, and anti-mGluR1 antibodies, and incorporate other antibodies, for which information is limited or preliminary, as supportive evidence for the diagnosis of ACA. However, these proposed diagnostic criteria are not yet publicly available.

In this study, we conducted a retrospective review of clinically diagnosed cases of ACA over a 20-year period in a single institution in Japan. We examined these cases in relation to the diagnostic criteria for ACA proposed by Dalmau and Graus [1], despite the limitations associated with autoantibody measurements. Furthermore, we investigated the clinical data and frequency of ACA in comparison to MSA, with the aim to reveal the clinical features of ACA.

Methods

Study design and ethics

We retrospectively reviewed the medical records at the Department of Neurology, Hokkaido University Hospital between April 1, 2002 and March 31, 2022.

The enrollment process for cases with CA is illustrated in Figure 1. Initially, we selected cases

presenting with neurological findings suggestive of CA from all hospital admissions. We excluded patients at this stage based on the following criteria: (1) lack of neurological findings supporting CA upon initial admission; (2) initial admission for treatment of infections such as aspiration pneumonia or urinary tract infections; and (3) brief admission solely for a clinical trial or the initiation of a medication regimen**.** Subsequently, we selected patients with CA as the predominant symptom. At this stage, patients were excluded if: (1) they exhibited sensory ataxia rather than CA, e.g., Miller Fisher syndrome; and (2) their primary neurological symptoms were not related to CA, such as parkinsonism, e.g., MSA-P. Finally, we conducted a retrospective review of diagnoses based on the clinical data from the last visit and selected those who received a definitive diagnosis of CA. At this stage, we excluded cases (n = 37) with ambiguous or indeterminate diagnoses. In addition, we examined the clinical characteristics of the enrolled cases with CA.

This clinical study was approved by the Ethics Committee of Hokkaido University Hospital (protocol number: 022-0209).

Classification

We classified the diagnoses of CA into five categories: (1) immune-mediated diseases; (2) neurodegenerative diseases; (3) infectious diseases; (4) toxin- and drug-induced diseases; and (5) other diseases. The “other diseases” category included conditions such as mitochondrial diseases,

tumors, vascular diseases, among others.

Diagnostic methods

We established the retrospective diagnoses of cases with ACA, MSA, autosomal dominant spinocerebellar degeneration (AD-SCD), idiopathic CA (IDCA), and progressive supranuclear palsy with predominant CA (PSP-C) using the following methods.

ACA

We categorized ACA into PCD and non-PCD using the following criteria: (1) CA being the predominant symptom; (2) identification of a cancer within 2 years of symptom onset; (3) improvement in cerebellar symptoms following immunotherapy; and (4) elimination of alternative causes of CA. A diagnosis of PCD required the fulfillment of criteria (1), (2), and (4), whereas a diagnosis of non-PCD required the fulfillment of criteria (1), (3), and (4). One limitation to consider is that we evaluated the response to immunotherapy (3) primarily based on our clinical judgement, with the Scale for the Assessment and Rating of Ataxia (SARA) supplementing this evaluation in some cases. Finally, we retrospectively diagnosed patients meeting these definitions with ACA based on the clinical data available at the last visit.

In addition, we retrospectively applied the diagnostic criteria for ACA proposed by Dalmau and Graus [1]. These criteria consist of four main components: (A) predominant or isolated cerebellar

syndrome with normal brain magnetic resonance imaging (MRI) or minor cerebellar atrophy at presentation; (B) presence of onconeural, anti-GAD, or anti-mGluR1 antibodies; and (C) at least two additional criteria: (a) subacute onset of symptoms (rapid progression ≤ 3 months); (b) history of other autoimmune disorders; (c) cerebrospinal fluid (CSF) pleocytosis (>5 white blood cells/mm^3) or positive IgG oligoclonal bands (OCBs); (d) MRI features suggestive of inflammation highly restricted to the cerebellum; (e) presence of anti-gliadin antibodies or autoantibodies against neural antigens with limited/preliminary information; and (f) infectious episode in the previous 3 weeks or recent cancer diagnosis (<2 years); and (D) reasonable exclusion of alternative causes. A diagnosis of definite ACA required (A), (B), and (D), while a diagnosis of probable ACA required (A), (C), and (D).

MSA

We applied the 2022 Movement Disorder Society (MDS) criteria for the diagnosis of MSA retrospectively to cases with MSA [15]. We included cases primarily presenting with CA, suggestive of MSA-C. We excluded cases suspected of having MSA-P, with parkinsonism as the primary symptom, even with CA, at initial admission. We did not exclude cases of MSA with CA as the primary symptom at initial admission, but parkinsonism became the main symptom at the last visit.

AD-SCD

AD-SCD constitutes the majority of cases of hereditary SCD and is caused by genetic mutations. We defined patients with definite AD-SCD as those with confirmed genetic mutations either in themselves or their first-degree relatives. We defined patients with probable AD-SCD as those whose first-degree relatives had been diagnosed with SCD, irrespective of whether they were negative in genetic tests or had not been tested. The primary phenotypes of AD-SCD that underwent genetic testing included spinocerebellar ataxia (SCA) 1, 2, 3/Machado-Joseph disease, 6, 8, 17, and 31 and dentatorubral-pallidoluysian atrophy. These phenotypes are commonly found in Japanese patients with AD-SCD [16, 17].

IDCA

A group of neurodegenerative diseases that present with slowly progressive cerebellar atrophy and CA with adult-onset are known as sporadic SCD, cortical cerebellar atrophy, late cortical cerebellar atrophy, and IDCA. When diagnosing this group, it is important to exclude MSA, hereditary SCD, and secondary CA including ACA. In Japan, the diagnostic criteria for IDCA were published in 2018 [18]. We retrospectively examined cases, including those previously diagnosed with sporadic SCD, cortical cerebellar atrophy, and late cortical cerebellar atrophy, using the diagnostic criteria for IDCA, which consist of three mandatory criteria and three exclusion criteria. The mandatory criteria require that: (1) the case is sporadic; (2) the onset of slowly progressive CA occurs at or after 30 years of age; and (3) bilateral cerebellar hemisphere

atrophy is observed on brain computed tomography or MRI. Meanwhile, the exclusion criteria require the exclusion of: (1) MSA using the 2008 second consensus statement [19]; (2) major AD-SCD in Japanese patients, or SCA1, 2, 3/Machado-Joseph disease, 6, 8, 17, and 31 and dentatorubral-pallidoluysian atrophy based on genetic testing; and (3) secondary CA. Cases meeting all of these criteria are diagnosed as probable IDCA, and those fulfilling the exclusion criteria excepting (2) without genetic testing are diagnosed as possible IDCA.

These diagnostic criteria are extremely important for excluding other pathologies. However, there were significant limitations in this study. For instance, only a few patients with sporadic CA received genetic testing for AD-SCD. Furthermore, in the Japanese population, there are no reports of Friedreich's ataxia and autosomal recessive SCD is rare (1.8%) [17], so genetic testing for autosomal recessive SCD is conducted infrequently. Among the patients who were initially suspected of IDCA, those who required further clinical investigation underwent exercise testing and muscle biopsy to check for mitochondrial abnormalities. Additionally, we performed genetic analysis, including mitochondrial-related genes [20]. We excluded any patients who showed abnormalities in these tests from IDCA.

PSP-C

PSP presents with several clinical subtypes, including typical Richardson's syndrome. The MDS-PSP diagnostic criteria exclude prominent appendicular ataxia [21]. Conversely, although

rare, cases of PSP-C presenting with CA have been reported [22], and diagnostic criteria for PSP-C have been proposed [23], which require the fulfillment of five clinical items: (A) slowly progressive course; (B) onset > 40 years of age; (C) supranuclear palsy; (D) truncal and limb ataxia within 2 years after symptom onset; and (E) postural instability with a fall within 2 years after symptom onset. The criteria also demand the exclusion of MSA by requiring the absence of autonomic dysfunction and a hot cross bun sign on brain MRI. If all criteria are met, the diagnosis is probable PSP-C; if all criteria except for (C) are met, the diagnosis is possible PSP-C. We retrospectively applied these diagnostic criteria for PSP-C to cases who were diagnosed with PSP and presented with CA, and selected cases of PSP-C.

Clinical examinations and findings

SARA

The SARA has been used to assess cerebellar symptoms in many patients with CA [24]. Laterality in patients with CA can be evaluated by assessing four items: finger chase, nose-finger test, fast alternating hand movements, and heal-shin test. We defined the laterality score as the absolute value of the score that is summed for each difference between the score on the right side minus the score on the left side. We defined laterality as a total laterality score ≥ 1, while we defined major laterality as a total laterality score ≥ 2. We only reviewed the SARA scores of cases

who were assessed at initial admission.

Testing for autoantibodies

The autoantibodies measured were determined by the attending physician, and no additional measurements were undertaken for this study. As such, the range of autoantibodies measured varied considerably from case to case (Table 1). In cases of non-ACA, autoantibodies were measured, but not many, and the attending physician chose which autoantibodies were assessed.

Unless stated otherwise, autoantibodies were tested in serum. Examination of anti-Sez6l2 antibodies was performed using a cell-based assay at the Department of Neurology, Hokkaido University Graduate School of Medicine (Sapporo, Japan) [25]. Examination of anti-contactin-associated protein-like 2 antibodies was performed in serum and CSF at the Brain Research Institute, Niigata University Graduate School of Medicine (Niigata, Japan). Examination of anti-mGluR1 antibodies was performed at the Department of Neurology, Gifu University Graduate School of Medicine (Gifu, Japan). Examination of anti-VGCC antibodies was by radioimmunoassays at SRL, Inc. (Tokyo, Japan). Commercial line blot kits for PNS (PNS-kits) could measure 12 onconeural antibodies (amphiphysin, CV2, Ma2/Ta, Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65, and delta/notch-like epidermal growth factor-related receptor), and measurements were performed at BML, Inc. (Tokyo, Japan).

Statistical analysis

Analysis was performed using R-4.2.2. Statistical significance was set at $p < 0.05$. The age of onset and the duration from onset to initial admission were analyzed for two groups of patients, those with ACA and those with MSA, using the Wilcoxon signed-rank test. Clinical findings including complications of cancer, cerebellar atrophy, hot cross bun sign on brain MRI, pleocytosis (>5 white blood cells/mm^3), increased protein (>50 mg/dL), and presence of OCBs in CSF, decreased cerebellar blood flow in ^{123}I-N-isopropyl-p-iodoamphetamine single-photon emission computed tomography (^{123}I-IMP SPECT), and laterality evaluated by the SARA were analyzed in both groups using Fisher's exact test.

Results

Proportion of patients with different diseases

We reviewed the medical records of 3,312 patients and initially screened 335 patients who were admitted with neurological symptoms suggestive of CA at initial admission. After we excluded patients with sensory ataxia and those in whom CA was not the primary symptom, we identified 280 patients with predominant CA for further screening. Ultimately, we excluded 37 patients due to ambiguous or indeterminate diagnoses, leaving a final cohort of 243 patients enrolled as patients with CA (Figure 1).

The proportions of diagnoses of patients with CA, based on the clinical information at the final visit, are shown in Figure 2a. There were 25 patients (10.3%) with immune-mediated diseases consisting of 13 with ACA, four with neurosarcoidosis, three with neuro-Behçet's disease, three with multiple sclerosis, one with myelin oligodendrocyte glycoprotein antibody disease, and one with clinically isolated syndrome. There were 176 patients (72.4%) with neurodegenerative diseases consisting of 93 with MSA, 51 with AD-SCD, 22 with IDCA, five with PSP-C, two with episodic ataxia, two with neuronal intranuclear inclusion diseases, one with Wilson's disease, and one with adrenoleukodystrophy. There were 10 patients (4.1%) with infectious diseases consisting of six with Creutzfeldt-Jakob disease and four with progressive multifocal leukoencephalopathy. There were seven patients (2.9%) with toxin- or drug-induced diseases consisting of five with alcoholic cerebellar degeneration and two with phenytoin toxicity. There were 24 patients (9.9%) with other diseases consisting of 10 with mitochondrial diseases, four with tumors, two with vascular diseases, two with cerebellar hypoplasia, and one each with posterior reversible encephalopathy syndrome, superficial siderosis, cerebral amyloid angiopathy, adult-onset type 2 citrullinemia, sequela of encephalitis, and sequela of cerebellitis.

The 51 patients with AD-SCD consisted of 32 with definite AD-SCD and 19 with probable AD-SCD. The patients with definite AD-SCD consisted of three with SCA1 (5.9%), three with SCA2 (5.9%), nine with SCA3/Machado-Joseph disease (17.6%), 10 with SCA6 (19.6%), three with

SCA31 (5.9%), and four with dentatorubral-pallidoluysian atrophy (7.8%). The 22 patients with IDCA consisted of five with probable IDCA, who were excluded from major AD-SCD by genetic testing, and 17 with possible IDCA, who did not undergo genetic testing. The five patients with PSP-C consisted of four with probable PSP-C and one with possible PSP-C.

Profile of the patients with ACA

Of the initial 335 patients screened, 19 were diagnosed with ACA upon initial admission based on the attending physician's judgement. However, the diagnosis changed during the disease course for three of these patients. One patient, who was initially diagnosed with PCD due to concurrent CA and laryngeal cancer, was re-diagnosed with MSA after the symptoms continued to progress after treatment for laryngeal cancer, with the patient also showing autonomic dysfunction and a hot cross bun sign on MRI. Another patient who responded to initial immunotherapy was diagnosed with ACA (non-PCD) during the first admission. This diagnosis was supported by the presence of anti-CV2 antibodies in serum and positive OCBs in CSF. However, this patient had a hot cross bun sign on MRI. After multiple courses of immunotherapy including intravenous methylprednisolone, oral prednisolone, and intravenous immunoglobulin, the response to treatment waned, and the symptoms progressed with apparent autonomic dysfunction, leading to a final diagnosis of MSA. The last three patients had a temporary

improvement in response to initial immunotherapy, but subsequently showed disease progression. Given the presence of a hot cross bun sign on MRI, MSA was suspected, but the diagnosis did not meet the MDS criteria for MSA. This patient, whose diagnosis remained unclear, was excluded from the final group of 243 CA patients. Of the remaining 16 patients, three were diagnosed with ACA (non-PCD) based on the attending physician's judgement at the last visit. However, two of these patients did not respond to immunotherapy, and their symptoms progressed, and the remaining patient was not treated by immunotherapy. One patient received treatment with intravenous methylprednisolone, oral prednisolone, intravenous immunoglobulin, and plasma exchange, while the other was administered intravenous methylprednisolone, oral prednisolone, and intravenous immunoglobulin, but neither received potent immunosuppressants or B-cell therapy such as rituximab. While the lack of a response to immunotherapy does not rule out ACA, we had clearly defined criteria for ACA in this study, and since these patients did not meet these criteria and their diagnoses remained unclear, we excluded them from the final group of 243 patients with CA.

Finally, we diagnosed 13 patients retrospectively with ACA (five men and eight women; Table 1). Among these ACA patients, five were diagnosed with PCD (38.5%) and eight were diagnosed with non-PCD (61.5%) (Figure 2b). The clinical features of the 13 patients with ACA are shown in Table 2. Two patients were positive for autoantibodies associated with ACA (anti-VGCC in

Patient #6, anti-Sez6l2 in Patient #7). Upon retrospectively applying the proposed diagnostic criteria for ACA [1] to the 13 patients diagnosed with ACA, we classified one as definite ACA, seven as probable ACA, and five did not meet the proposed criteria. Conversely, only one case (#8) was positive according to the diagnostic criteria for PACA [11] . The primary explanations for this are that PCD and CA with well-characterized autoantibodies were excluded in the PACA criteria and most of the recommended autoantibodies are difficult to measure in Japan. Moreover, the cases in this study were acquired over a 20-year period, resulting in a large number of patients lacking measurements for autoantibodies, especially the recently identified ones.

Profiles of the patients with MSA

There were 93 patients with MSA at the final diagnosis according to the MDS criteria [15] (Table 1) consisting of 90 with MSA-C and three with MSA-P. The three patients diagnosed with MSA-P were initially admitted with CA as the predominant symptom in the early stages of the disease. The MSA cases consisted of one neuropathologically established case (1.1%), 49 clinically established cases (52.7%), 30 clinically probable cases (32.3%), and 13 possible prodromal cases (14.0%).

Clinical features of ACA

The clinical features of ACA compared with those of MSA are shown in Table 3. There was no difference in the age of onset between patients with ACA (61.2 years) and MSA (58.9 years; p = 0.171). The mean duration from onset to initial admission was shorter in patients with ACA (6.2 months) than in patients with MSA (39.0 months; $p < 0.01$). The proportion of patients with cancer complications was larger in patients with ACA (5/13, 38.4%) than in patients with MSA (9/93, 7.8%; p = 0.01353). The proportion of patients with cerebellar atrophy on brain MRI was smaller in patients with ACA (3/13, 23.1%) than in patients with MSA (92/92, 100.0%; $p < 0.01$). The proportion of patients with CSF pleocytosis was larger in patients with ACA (4/13, 30.8%) than in patients with MSA (2/55, 3.6%; p = 0.01036). However, there was no difference in the proportion of patients with increased protein (4/13, 30.8% vs. 13/55, 23.6%, respectively; p = 0.723) and OCBs (2/12; 16.7% vs. 3/36, 8.3%, respectively; p = 0.5872) in CSF between patients with ACA and those with MSA. The proportion of patients with decreased cerebellar blood flow on ^{123}I-IMP SPECT was smaller in patients with ACA (4/13, 30.8%) than in patients with MSA (84/88, 95.5%; $p < 0.01$).

The SARA scores at initial admission were available for eight patients with ACA and 45 patients with MSA. There was no difference in the average total SARA score between patients with ACA (13.3) and patients with MSA (15.5; p = 0.1766). There was also no difference in the proportion of whole (≥ 1) (7/8, 87.5% vs. 26/45, 57.8%, respectively; p = 0.2336) and major (≥ 2) laterality

(4/8, 50.0% vs. 12/45, 26.7%, respectively; $p = 0.224$) between ACA and MSA.

Discussion

There were three important findings in this study regarding ACA. First, we identified 13 patients with ACA among 243 patients with CA, and patients with ACA accounted for 5.3% of the patients. Second, in the 13 patients with ACA, the number of patients with non-PCD (eight patients) was larger than the number of patients with PCD (five patients). Third, the absence of cerebellar atrophy was a supportive clinical finding for the differentiation of ACA from MSA. CSF pleocytosis was significantly higher in patients with ACA than in patients with MSA, but the positive rate in patients with ACA was only 30.8%. In addition, we investigated the usefulness and limitations of the diagnostic criteria for ACA proposed by Dalmau and Graus [1] .

In a prospective study conducted in the UK involving 1,500 cases of CA over a 20-year period, immune-mediated ataxias were common, accounting for 25% of cases [26]. The main difference between that study and our study is that we did not recruit any patients with gluten ataxia, because gluten ataxia is a common form of CA in Europe and the United States, but there have been few reports in Japan. Measurements of anti-deamidated gliadin peptide and anti-gliadin antibodies are important for the diagnosis of gluten ataxia, and it is possible that patients with gluten ataxia have been underreported in Japan due to the low frequency of measurements for these autoantibodies

[27].

In a retrospective study of 127 cases of ACA in China, the proportion of PCD was 31% [28]. Similarly, in the present study, PCD accounted for 38.5% of cases, with non-PCD cases being the majority. Therefore, it is necessary to consider autoimmune pathogenicity in patients with CA regardless of the presence or absence of cancer.

We analyzed the clinical features of ACA and MSA, and investigated the characteristics that can be useful in distinguishing between them. Most of the patients with ACA (10/13, 76.9%) exhibited no cerebellar atrophy, two patients (15.4%) displayed mild cerebellar atrophy, and the remaining patient (7.7%) showed moderate cerebellar atrophy. Despite predominant cerebellar symptoms at the early stages, the absence of cerebellar atrophy is a supportive finding for the diagnosis of ACA compared to MSA. Pleocytosis and OCBs in CSF have been found frequently in patients with ACA [29]. In this study, CSF pleocytosis was more common in patients with ACA (30.8%) than in those with MSA (3.6%). However, it is important to note that even among patients with ACA, the positivity rate was relatively low; therefore, the possibility of ACA should not be ruled out even in cases without pleocytosis. The presence of OCBs and increased protein in CSF tended to be more prevalent in patients with ACA than in those with MSA, but the difference was not statistically significant, and these markers could not be considered as distinguishing clinical features between ACA and MSA. The SARA is the gold standard method for the assessment of

CA, and it is a useful tool for evaluating treatment efficacy and severity in patients with ACA[30]. Some cases of ACA associated with anti-GAD antibodies present with predominant cerebellar atrophy and ataxia on one side [31]; therefore, a unilateral manifestation is considered to be an important finding for suspecting ACA. In this study, there was a tendency for laterality in patients with ACA compared to those with MSA, but the difference was not statistically significant. However, one patient with ACA (#8) presented with marked left predominant hemiataxia with a laterality score of six. Obvious hemiataxia may be a highly specific finding in patients with ACA.

Of the 13 patients with ACA, eight (61.5%) met the diagnostic criteria for ACA proposed by Dalmau and Graus [1], with one being classified as definite (#6) and seven as probable (#1, #3, #4, #5, #7, #8, and #12). Conversely, only one (7.7%) met the diagnostic criteria for PACA proposed by the International Task Force on Immune-Mediated Cerebellar Ataxias [11]. Two patients (#2 and #13) had abnormal signals outside of the cerebellum on MRI, and one patient (#10) presented with moderate cerebellar atrophy; therefore, they did not meet the ACA diagnostic criteria [1]. The remaining two patients (#9 and #11) exhibited only subacute onset and did not fulfill the third item; thus, they did not meet the ACA diagnostic criteria [1]. In this study, patients without cancer complications and who responded to immunotherapy were enrolled retrospectively as ACA. Five of the eight non-PCD patients (62.5%) who did not meet the ACA diagnostic criteria [1] all responded to immunotherapy, making it difficult to exclude ACA

responsive to treatment solely based on the ACA diagnostic criteria [1]. The ACA diagnostic criteria [1] and the PACA diagnostic criteria [11] do not apply when lesions are found outside of the cerebellum, such as in the brainstem. In cases involving autoantibodies, it is possible that antigens are expressed outside of the cerebellum, so the presence of non-cerebellar lesions or symptoms does not necessarily rule out ACA. For example, IgLON5 disease presents with a variety of neurological symptoms such as sleep disorders and bulbar palsy, but also includes gait disturbances due to CA [32]. As discussed below in the *Limitations* section, the range of autoantibodies measured was limited and not all known important autoantibodies related to ACA, such as anti-VGCC and anti-IgLON5 antibodies, were measured. Only two patients (2/13, 15.4%) tested positive for autoantibodies, and if a wider range of autoantibodies was measured, the three cases that did not meet the ACA diagnostic criteria [1] may be included.

The clinical features of the patient (#6) who was positive for anti-VGCC antibodies have been reported in detail [33]. The patient (#7) in our study who was positive for anti-Sez6l2 antibodies was reported as the first such patient in the world [25] and we showed that the pathogenic mechanism of anti-Sez6l2 antibodies was via the disruption of the direct binding between Sez6l2 and GluR1, both of which are membrane proteins [34]. Measuring more autoantibodies may increase the sensitivity and specificity of the diagnosis for ACA. Conversely, it is difficult to measure them comprehensively in clinical practice. To solve this problem, we are trying to

establish a system to measure all of the autoantibodies described in the proposed diagnostic criteria for ACA [1], including anti-Sez6l2 antibodies. While these autoantibodies can serve as diagnostic biomarkers, caution is needed regarding the risk of false positives potentially confounding the diagnosis. In this study, the presence of anti-CV2 antibodies, which have been reported in patients with CA, especially in those with CA associated with PCD [35], was detected using a commercial line blot kit for PNS in one patient who received a final diagnosis of MSA-C. Although the presence of autoantibodies is important for the diagnosis of immune-mediated diseases, the false positive rate in misdiagnosed autoimmune encephalitis cases is high (48/105, 46%)[2].

In the diagnostic criteria for autoimmune encephalitis, in addition to established disease types such as NMDA receptor encephalitis, the detection of specific neuronal autoantibodies such as anti-AMPA and anti-leucine-rich glioma-inactivated 1 antibodies is essential for diagnosing definite autoimmune limbic encephalitis [36]. In addition, the profile of these autoantibodies, appropriate methods for their measurement, and the diagnostic process for autoantibody-negative, but probable, autoimmune encephalitis have been described. These diagnostic criteria are internationally recognized and hold a crucial position in diagnosing autoimmune encephalitis and conducting clinical trials. In the context of ACA, the diagnostic criteria for PACA were published in 2020 [11], and their widespread application is anticipated. However, the diagnostic criteria for

PACA exclude cases with recognized established diseases such as PCD and gluten ataxia or those exhibiting well-characterized neuronal autoantibodies, making it challenging to diagnose ACA accurately. In the future, the diagnostic criteria for ACA may need to evolve from those for PACA, based on a wider range of biomarkers such as autoantibodies, similar to the diagnostic criteria for autoimmune encephalitis. Comprehensive diagnostic criteria could potentially aid in distinguishing ACA from other neurodegenerative diseases such as MSA, thereby avoiding misdiagnoses and oversight. In this study, we referred to the diagnostic criteria for ACA proposed by Dalmau and Graus [1], which are expected to become significant in the future. ACA is less common than autoimmune encephalitis, requiring a more extensive accumulation of cases to establish firm diagnostic criteria.

In conclusion, we identified 13 patients with ACA (5.3%) among 243 patients with CA over a 20-year period in a single institution in Japan. Although it is difficult to distinguish ACA from other neurodegenerative diseases, the absence of cerebellar atrophy on MRI further supports the diagnosis of ACA, despite the presence of CA. CSF pleocytosis was more common in patients with ACA compared to those with MSA, but the positivity rate was limited to 30.8%. Similarly, the presence of OCBs and increased protein in CSF and laterality differences in ataxia according to the SARA were not found to be strong diagnostic biomarkers for ACA in this study. Autoantibodies are highly expected to become biomarkers for ACA, but in this study, the number

of cases with data for autoantibodies was limited. ACA is a treatable disease, and further studies are needed to establish the diagnostic criteria for ACA and methods for measuring autoantibodies with broad sensitivity and high specificity should be included in the criteria.

Limitations

There are five main limitations of this study. First, there was a selection bias since this was a retrospective cohort study conducted in a single center. Second, patients with ACA were not proven to be immune-mediated by examinations such as an indirect immunofluorescence assay using rodent brain tissue. Third, some clinical features were not documented in the medical records, and some cases with suspected MSA could not be evaluated properly when applying the new diagnostic criteria retrospectively. Fourth, the proposed diagnostic criteria for ACA [1] used in this study are only described in a textbook, have not been reviewed for validity, and are not publicly available. Fifth, we were not able to measure many different autoantibodies, including those associated with gluten ataxia, because many autoantibodies have only been reported in the past decade and the costs for measuring most of them are high.

Figure legends

Figure 1 Flowchart for the enrollment of patients with CA.

This flowchart illustrates the process leading up to the enrollment of 243 patients with CA whose diagnoses were eventually confirmed. Firstly, we screened patients with neurological symptoms suggestive of CA from 3,312 hospitalized patients. We then excluded cases who were ultimately diagnosed as sensory ataxia and patients who had other dominant neurological symptoms. Finally, we retrospectively confirmed the diagnoses and excluded patients with unclear diagnoses, resulting in the registration of 243 patients with CA. CA, cerebellar ataxia; MSA, multiple system atrophy.

Figure 2 Prevalence of ACA among patients.

(a) The distribution of different diagnoses among patients with CA. (b) The number of patients with PCD and non-PCD in the final diagnosis of ACA. ACA, autoimmune cerebellar ataxia; AD-SCD, autosomal dominant spinocerebellar degeneration; CA, cerebellar ataxia; IDCA, idiopathic cerebellar ataxia; MSA, multiple system atrophy; PCD, paraneoplastic cerebellar degeneration.

Table 1 Profiles of patients with ACA and MSA.

ACA, autoimmune cerebellar ataxia; MSA, multiple system atrophy.

Table 2 Clinical features of ACA.

CSF, cerebrospinal fluid; GA, gastric adenocarcinoma; GAD, glutamic acid decarboxylase; mGluR1, metabotropic glutamate receptor type 1; IVIg, intravenous immunoglobulin; IVMP, intravenous methylprednisolone; MTX-LPDs, methotrexate-associated lymphoproliferative disorder; OCBs, oligoclonal bands; PCD, paraneoplastic cerebellar degeneration; PE, plasma exchange; PNS, paraneoplastic neurological syndrome; PSL, prednisolone; SCC, squamous cell carcinoma; Sez6l2, seizure-related 6 homolog-like 2; ULDs, upper limb disorders; VGCC, voltage-gated calcium channel.

Table 3 Comparison of clinical features between ACA and MSA.

Significant *p*-values are highlighted in bold font. ^{123}I-IMP SPECT, ^{123}I-N-isopropyl-p-iodoamphetamine single-photon emission computed tomography; ACA, autoimmune cerebellar ataxia; CSF, cerebrospinal fluid; MSA, multiple system atrophy; OCBs, oligoclonal bands; SARA, Scale for the Assessment and Rating of Ataxia; WBCs, white blood cells.

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Table 1 Profiles of patients with ACA and MSA.

	n	Male:Female	Mean age of onset, years
ACA	13	5:8	61.2 (range, 22–87)
PCD	5 (38.5%)	4:1	71.0 (range, 59–87)
Non-PCD	8 (61.5%)	1:7	55.0 (range, 22–70)
MSA	93	47:46	58.9 (range, 36–75)
Neuropathologically established	1 (1.1%)	1:0	54
Clinically established	49 (52.7%)	24:25	58.6 (range, 36–73)
Clinically probable	30 (32.3%)	15:15	59.5 y (range, 40–74)
Possible prodromal	13 (14.0%)	8:5	58.9 (range, 45–75)

Table 2 Clinical features of ACA.

No.	Age at onset (years)	Sex	Onset to admission, months	Final diagnosis	Symptoms	Brain MRI	Positive autoantibodies	Negative autoantibodies	Subacute onset	Previous infectious episode	Cancer	Other autoimmune disorders	CSF findings	Immuno-therapy	Oncological therapy	Clinical outcome	Proposed diagnostic criteria [1]
1	70	M	8	PCD	Gait ataxia	Normal	No	PNS-kit, GAD	Yes	No	Unknown primary	No	Pleocytosis; increased protein	No	Surgery	Good	Probable
2	87	M	2	PCD	Dysarthria, diplopia, gait ataxia	Abnormal signals in brainstem	No	PNS-kit	Yes	No	Lung; unknown pathology	No	Increased protein	No	No	No change	Negative
3	67	M	26	PCD	Gait ataxia	Normal	No	Hu, Yo, Ri, GAD	No	No	GA	Hashimoto's disease	Pleocytosis	No	No	No change	Probable
4	59	F	5	PCD	Gait ataxia	Normal	No	PNS-kit, Sez6l2, GAD	Yes	No	MTX-LPD	Rheumatoid arthritis	OCB	No	Chemo-therapy	Good	Probable
5	72	M	8	PCD	Gait ataxia	Mild cerebellar atrophy	No	Hu, Yo, Ri, GAD	Yes	No	Lung SCC	No	Pleocytosis; increased protein	No	Surgery	Progression	Probable
6	62	F	6	Non-PCD	Dysarthria, gait ataxia	Normal	VGCC [24]	Hu, Yo, Ri, CV2, Tr, Ma2, amphiphysin, gliadin, GAD	Yes	No	No	No	No	IVMP	No	Good	Definite
7	60	F	2	Non-PCD	Dysarthria, gait ataxia	Mild cerebellar atrophy	Sez6l2 [18]	Hu, Yo, Ri, GAD	Yes	No	No	No	No	IVMP, IVIg, PE, PSL	No	Good	Probable
8	66	F	2	Non-PCD	Gait ataxia, ULDs	Normal	No	PNS-kit, VGCC, mGluR1, GAD, CASPR2	Yes	No	No	Sjögren's syndrome	No	IVMP, PSL	No	Good	Probable
9	41	F	1	Non-PCD	Dysarthria, gait ataxia, ULDs	Normal	No	PNS-kit, GAD	Yes	No	No	No	No	IVMP, PSL	No	Good	Negative
10	70	F	36	Non-PCD	Dysarthria, gait ataxia	Moderate cerebellar atrophy	No	GAD	No	No	No	No	No	IVMP, PSL	No	Good	Negative
11	56	F	10	Non-PCD	Dysarthria, dysphagia, gait ataxia	Normal	No	GAD	Yes	No	No	No	No	IVMP, PSL	No	Good	Negative
12	63	F	2	Non-PCD	Gait ataxia	Normal	No	GAD	Yes	Yes	No	No	No	IVMP, PSL	No	Good	Probable
13	22	M	9	Non-PCD	Gait ataxia, headache, fever	Abnormal signals in brainstem	No	None of the others	Yes	No	No	No	Pleocytosis; increased protein	PSL	No	Good	Negative

Table 3 Comparison of clinical features between ACA and MSA.

	ACA	MSA	*p*-value ACA vs. MSA
Mean age of onset, years	61.2 years (range, 22–87)	58.9 years (range, 36–75)	0.171
Mean duration from onset to initial admission, months	6.2 months (range, 0–26)	39.0 months (range, 5–120)	***<0.01***
Cancer	5/13 (38.4%)	9/93 (9.7%)	***0.01353***
Brain MRI			
Cerebellar atrophy	3/13 (23.1%)	92/92 (100.0%)	***<0.01***
Hot cross bun sign	0/13 (0.0%)	71/92 (77.2%)	***<0.01***
CSF			
Pleocytosis (>5 WBCs/μL)	4/13 (30.8%)	2/55 (3.6%)	***0.01036***
Increased protein (>50 mg/dL)	4/13 (30.8%)	13/55 (23.6%)	0.723
OCBs	2/12 (16.7%)	3/36 (8.3%)	0.5872
^{123}I-IMP SPECT			
Decreased cerebellar blood flow	4/13 (30.8%)	84/88 (95.5%)	***<0.01***
SARA			
n	8	45	
Average total score	13.3	15.5	0.1766
Laterality difference ≥ 1	7/8 (87.5%)	26/45 (57.8%)	0.2336
Laterality difference ≥ 2	4/8 (50.0%)	12/45 (26.7%)	0.224

Figure1

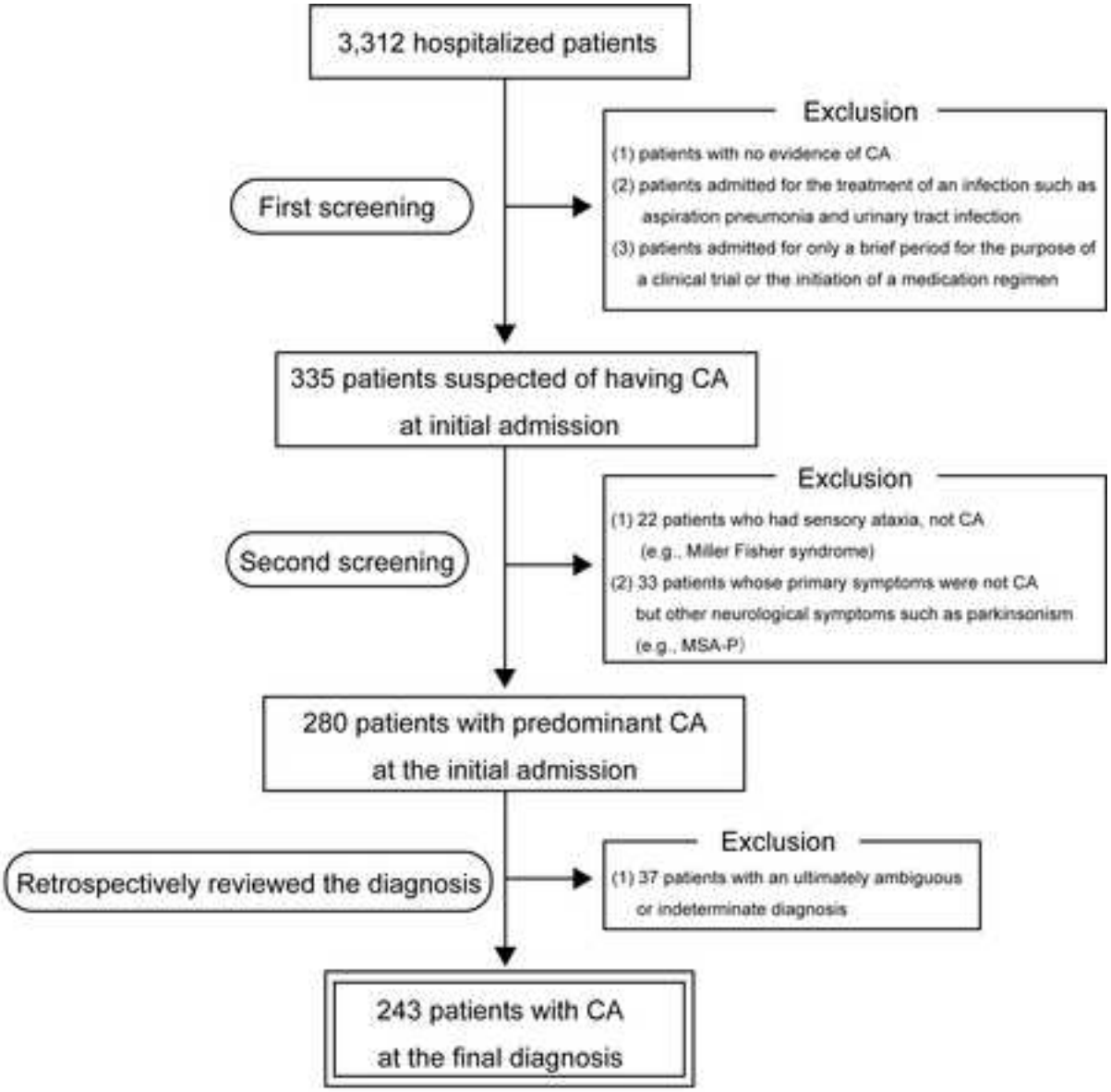

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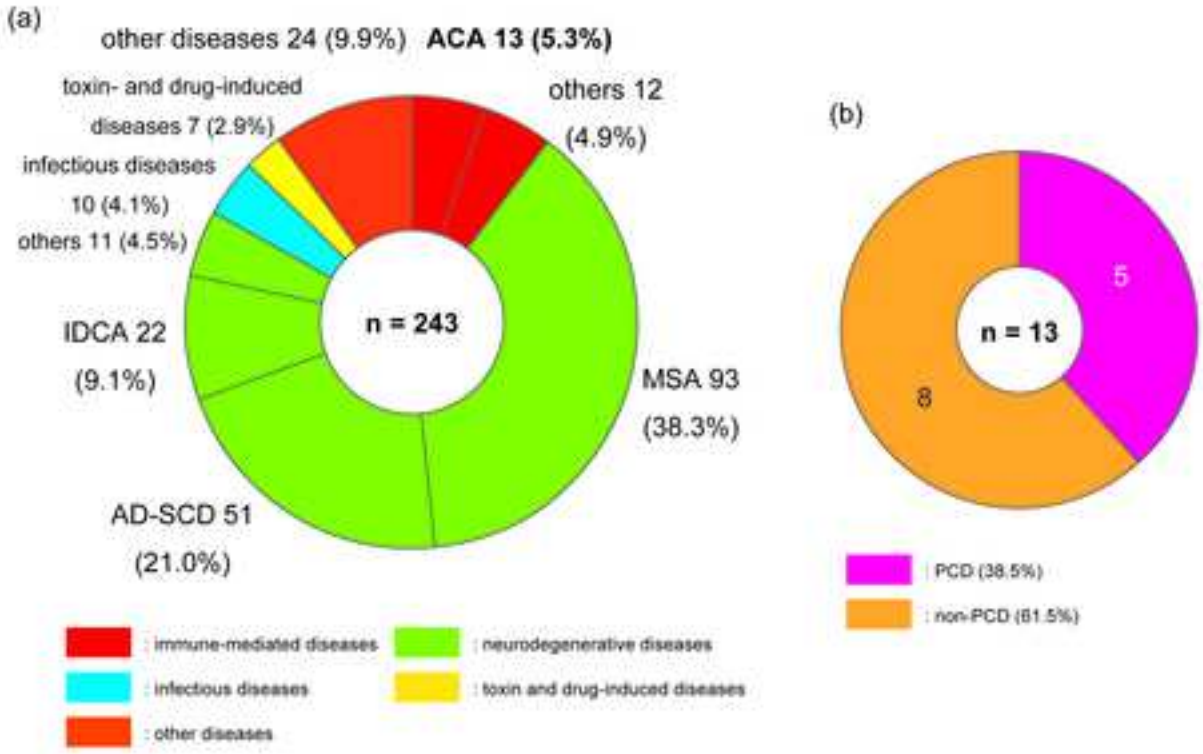
Figure2


(a)
other diseases 24 (9.9%)
ACA 13 (5.3%)
toxin- and drug-induced diseases 7 (2.9%)
others 12 (4.9%)
infectious diseases 10 (4.1%)
others 11 (4.5%)
IDCA 22 (9.1%)
n = 243
MSA 93 (38.3%)
AD-SCD 51 (21.0%)
: immune-mediated diseases
: neurodegenerative diseases
: infectious diseases
: toxin and drug-induced diseases
: other diseases
(b)
5
n = 13
8
: PCD (38.5%)
: non-PCD (61.5%)