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Phenotypic Heterogeneity of Neurological Disorders Associated with Glutamic Acid Decarboxylase Antibodies

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Abstract—Neurological disorders associated with glutamic acid decarboxylase (GAD65) antibodies represent a relatively new field of research in the modern clinical neurology and are of great theoretical and practical interest. High titer of anti-GAD65 antibodies is a highly sensitive, though not always specific, marker of the autoimmune disorders of the central nervous system (CNS). Literature review and authors' clinical observations of the phenotypic heterogeneity of GAD65-associated disorders are presented in the paper.

Keywords: stiff person syndrome, sporadic ataxia, limbic encephalitis, GAD65 antibodies
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Glutamic acid decarboxylase (GAD), or glutamate decarboxylase, is the key enzyme in the synthesis of gamma-aminobutyric acid (GABA), which is the main inhibitory neurotransmitter in the central nervous system (CNS). GAD is mainly produced by the GABAergic neurons of the CNS and by beta cells of the pancreas. GAD exists in two isoforms: membrane-bound (GAD65) and soluble (GAD67) [4].

Production of GAD65 antibodies (which only occurs in about 1% of healthy people) leads to GABA deficiency, which, in turn, results in hyperactivity of motor units; this pathological process underlies the stiff person syndrome (SPS). SPS is one of the most common neurological disorders within a broad range of conditions associated with anti-GAD antibodies. Apart from SPS and its variants (stiff limb syndrome and progressive encephalomyelitis with rigidity and myoclonus) the latter also include sporadic ataxia, limbic encephalitis, focal epilepsy, opsoclonus-myoclonus syndrome, palatal myoclonus, and myasthenia. These conditions can be either idiopathic or manifest as paraneoplastic syndromes [20]. Other autoimmune disorders associated with anti-GAD65 antibodies include type 1 diabetes mellitus, Hashimoto's thyroiditis, polyglandular autoimmune syndrome, atrophic gastritis, vitamin B12 deficiency, and vitiligo.

SPORADIC ATAXIA

It has been shown that anti-GAD65 antibodies are found in 11% of cases of sporadic cerebellar ataxia and in 40% of cases of gluten ataxia [5]. According to one study, sporadic ataxia associated with anti-GAD antibodies is more frequent in woman; the mean age at onset was 59 years, ranging from 39 to 77 years [15]. The course of the disease is often characterized by subacute progression. Ataxia is accompanied by the other cerebellar signs (dysarthria, nystagmus) and sometimes muscle rigidity [16, 17, 23]. In the early stages, brain MRI can be unremarkable; however, in the later stages, moderate atrophy of the cerebellum can be observed [12]. Diagnostic workup in the case of sporadic cerebellar ataxia should include tests for serum anti-GAD65 antibodies, as well as for anti-gliadin antibodies along with screening for malignancies, such as breast cancer (in women) and small-cell lung cancer (in men and women), which are the most common causes of paraneoplastic cerebellar degeneration [12, 13].

The neurological symptoms of gluten sensitivity may include mild motor ataxia, dysphagia, bowel and bladder dysfunction, loss of deep sensitivity, reduction in the ankle jerk reflex, fasciculations, and amyotrophy [11]. About one-fourth of the patients have either asymptomatic, or overt gluten enteropathy with diarrhea, malabsorption syndrome, weight loss, etc. [11]. It has been shown that gluten-free diet leads to decreased levels of the anti-GAD antibodies [14].

LIMBIC ENCEPHALITIS

Clinical presentation of limbic encephalitis is characterized by personality changes, memory loss, behavioral and mood changes, productive symptoms, psychomotor agitation, disorientation, and generalized or complex partial seizures [6, 20]. Encephalitis can be a part of paraneoplastic syndrome. It can occur both as manifestation of a tumor and during antineoplastic therapy [12]. Along with antibody tests MRI is another important diagnostic tool in workup for limbic

encephalitis. Increased T2/FLAIR signal intensity is often observed in mesial temporal lobes and sometimes in hypothalamus and basal parts of frontal lobes [6, 20]. Electroencephalography (EEG) shows in these patients reduced bioelectrical activity, either diffuse or focal (in frontal and (or) temporal lobes only), and spike-and-wave discharges.

STIFF PERSON SYNDROME

Progressive muscle rigidity and painful spasms, mainly in axial muscles, are the hallmark of PSP. Spasms can be triggered by various sensory stimuli (exaggerated startle response and hyperekplexia). Freezing episodes, postural instability, and falls can also be observed [16, 20]. Long-term muscle hypertonia often leads to skeletal deformations (lumbar hyperlordosis or ankylosis). Manifestation of brainstem (eye movement disorders, dysphagia, dysarthria), pyramidal, and autonomic (profuse perspiration, tachycardia, mydriasis, hypertension, neurogenic bladder dysfunction) disturbances is indicative of progressive encephalomyelitis with rigidity and myoclonus (PERM), which can be considered both as a form of SPS and as a distinct condition [18, 20].

Currently, the prevalence of SPS is estimated to be 1 : 1000000. The mean age at onset is 46 years, ranging from 13 to 81 years. However, rare cases with manifestation in early childhood and even infancy have also been described [3]. This disorder is more frequent in women (the prevalence ratio in women and men is 2 : 3). In 70% of cases, there is a comorbid autoimmune endocrine disorder. Patients with primary SPS usually have good prognosis; carefully selected therapy provides compensation of the disorder and enables high quality of life and social activity [9].

Diagnosis

The main diagnostic tool for recognizing SPS is electromyography (EMG). Typical EMG pattern is characterized by continuous firing of motor units at rest; conduction velocity in peripheral neurons and parameters of motor unit potential remain normal; no signs of denervation are observed. Anti-GAD antibodies are found in 80–90% of patients; in the rest of the cases, antibodies against amphiphysin, glycine receptors GlyR1 and DPPX, or others can be found [20].

The differential diagnosis of SPS can be challenging, since the clinical signs are polymorphic, and many other neurological conditions may manifest with generalized dystonia. A range of disorders should be considered in the differential diagnosis, such as:

—torsion dystonia;

—psychogenic movement disorders;

—atypical myelopathy;

—intoxication caused by tetanospasmin or strychnine;

—Satoyoshi syndrome;

—neuromyotonia;

—McArdle disease (glycogen storage disease type V);

—fibromyalgia;

—scleroderma (Buschke disease); and

—ankylosing spondylitis (Bekhterev's disease).

Bizarre hyperkinetic movements and their dependence on emotional and sensory stimuli, as well as frequent cases of agoraphobia and basophobia caused by the fear of sudden and painful dystonic paroxysm can lead to misdiagnosis of psychogenic movement disorder. Variability and fluctuations in movements' pattern along with tests for autoantibodies and EMG are needed for verification of diagnosis.

Generalized dystonia, rare forms of paroxysmal kinesigenic and non-kinesigenic dystonias, and myoclonus dystonia can be misinterpreted as SPS [2]. Physician should carefully examine patient for dystonia features, dyskinesia, and corrective gestures.

Satoyoshi syndrome is an orphan disease which is characterized by progressive painful muscle spasms, diarrhea, malabsorption, multiple endocrine disorders, alopecia, dysmenorrhea, and signs of status dysraphicus, such as shortness, epiphysis abnormalities, bone cysts, acroosteolysis, fractures, and early development of osteoarthritis [10]. Satoyoshi syndrome is thought to be an autoimmune disorder caused by antinuclear antibodies. However, cases with positive anti-GAD65 antibodies were also described [11].

Neuromyotonia has pronounced and specific clinical signs, which help to differentiate it from other neurological syndromes. The most prominent feature of myotonia is involvement of the distal limb muscles; the neurological signs include myokymia, fasciculations, and myotonic phenomena associated with muscle movements and percussion [24]. Myotonia never leads to persistent skeletal deformations and continuous severe muscle stiffness, whereas SPS do [24].

Glycogen storage disease type V (commonly referred to as McArdle disease) is a hereditary autosomal recessive muscle phosphorylase deficiency caused by point mutations of *PYGM* gene [8, 25]. Painful muscle cramps, the core clinical sign of this disorder, always result in rhabdomyolysis, which leads to myoglobinuria. The severity of myoglobinuria varies, and in some cases it can cause acute renal failure [8].

Scleroderma-like disorders include a group of systemic connective tissue diseases, such as Buschke disease, systemic scleroderma, limited scleroderma, eosinophilic fasciitis, secondary induced (i.e., paraneoplastic) scleroderma, and pseudo-sclerodermic syndromes. These disorders are characterized by skin lesions in the form of diffuse or limited thickening, which leads to fibrosis and atrophy [1]. Patients may complain of constant stiffness and feeling of skin constricting that don't depend on any external triggers

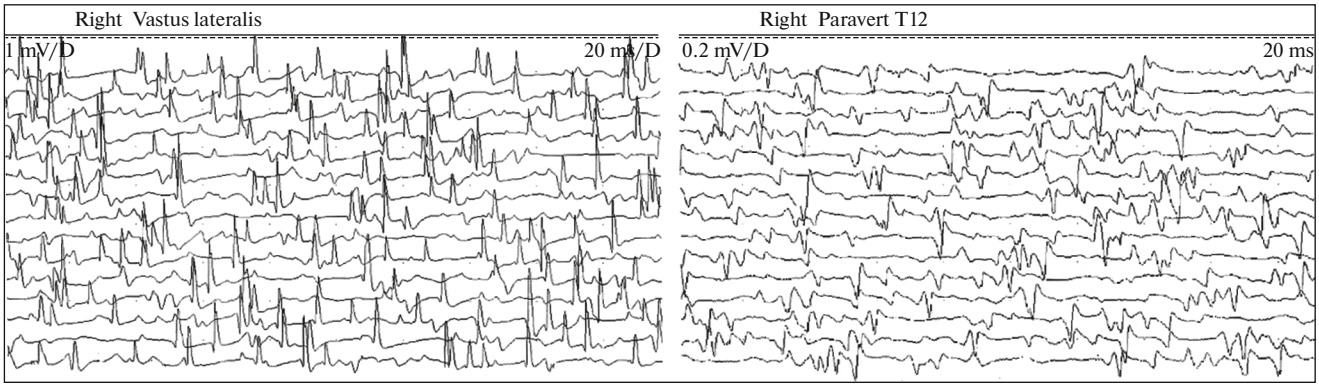

Fig. 1. Electromyography: continuous firing of motor units without any periods of "dense" peaks; parameters of motor unit potentials are within the normal range for this age.

(except for Raynaud's phenomenon, in some cases). Affected skin is strained and pale or cyanotic; skin folds are formed hardly.

Ankylosing spondylitis (Bekhterev's disease) is a chronic systemic inflammatory disease of joints and spine. It belongs to the group of seronegative polyarthritis. The prominent features of this disease are lumbar and sacral pain and stiffness, which usually start at rest, especially in the second half of the night and in the morning, and are reduced by movements and exercises [7]. Pain usually worsens at rest and during sleep. Other typical signs include irreversible rigidity of the spine and muscle hypertonia following by their atrophy. Neuroimaging allows to detect intervertebral disc ankylosis at early stages; it is one of the main methods for diagnosis of all abovementioned disorders.

Treatment

The most common medicines used for symptomatic treatment of SPS are GABAergic drugs which block the increased activity of spinal motoneurons, primarily benzodiazepines (such as diazepam and clonazepam) and baclofen [20]. The first-line drug is diazepam, which can be used either as monotherapy or in combination with clonazepam and baclofen. The range of effective doses is broad due to high variability of individual sensitivity in patients. Levetiracetam, which facilitates GABAergic signal transmission, has been found to be effective for the symptomatic treatment not only of SPS, but also of PERM [19]. Antiadrenergic drugs (such as tizanidine and clonidine) do not usually provide sufficient therapeutic effect. In severe cases, if a patient is refractory to standard therapy, repeated administrations of botulinum toxin type A to paraspinal muscles can be considered. Treatment of comorbid endocrine disorders is also important and can reduce rigidity and severity of the spasms.

Other methods for the treatment of SPS include use of corticosteroids [21], plasmapheresis, and intravenous administration of immunoglobulin [22]. In severe cases, if all the abovementioned approaches do not provide therapeutic effect, cytostatic drugs (azathioprine, cyclophosphamide, mycophenolate, or rituximab) can be considered as alternative [20].

Clinical Cases

Three patients with idiopathic SPS were admitted to the Department of Neurogenetics of the Research Center of Neurology over the past year (Table 1). In all three cases, manifestation of SPS was subacute. The physical status before the onset was unremarkable. Clinical features included progressive muscle rigidity and painful tonic spasms of the axial muscles, which worsened after harsh light, auditory or other stimuli.

All three patients underwent comprehensive examination, including neuroimaging (MRI of the brain and cervical, thoracic, lumbar, and sacral spine), abdominal and pelvic ultrasound, chest X-ray, EEG, and EMG. In addition to complete blood cell count and urinalysis, following serum markers were assessed: tumor markers (carcinoembryonic antigen, alpha-fetoprotein, human chorionic gonadotropin, CA-125, CA-15-3), antineuronal antibodies (Hu, Yo-1, CV2, PNMa2, Ri, AMPH), muscle markers (creatine phosphokinase, myoglobin), rheumatic markers (rheumatoid factor, C-reactive protein, fibrinogen, antistreptolysin O), and thyroid and parathyroid hormones. In all cases, only EMG and serum anti-GAD65 test were abnormal (Fig. 1), which became the basis for the final diagnosis. Levels of antibodies did not correlate with the phenotype and severity of disturbances.

All patients received treatment widely used in SPS: benzodiazepines and adjunctive therapy including centrally acting muscle relaxant baclofen and anticonvulsant levetiracetam. Patient K. (32 y.o.; Fig. 2) and patient M. (33 y.o.) had classic variants of SPS: both of them responded well to the treatment and rapidly achieved full recovery. The third patient (patient S., 54 y.o.) had an intermediate form of disorder between SPS and PERM. Before the first visit to Research

Clinical characteristics of patients

	K., 32 years	M., 33 years	S., 54 years
Disease duration	2 months	3 months	4 years
Clinical features stiffness in paravertebral muscles of lower thoracic and upper lumbar spine	+	+	+
Stiffness in rectus abdominis muscles	+	+	+
Stiffness in arm muscles	+ (proximal)	–	–
Stiffness in leg muscles	_	+ (adductor muscles of the thigh)	–
Lumbar hyperlordosis	+	+	+
Limited range of motions in lower thoracic and lumbar spine	+/–	+	++
Gait disturbances	–	–	++
Titer of anti-GAD65 antibodies (normal: <5.0 U/mL)	>1000 U/mL	268.3 U/mL	787.4 U/mL
Comorbid condition	Hyperthyroidism; Graves' disease, grade 1	Hashimoto's thyroiditis; cryptogenic epilepsy with complex partial seizures	Hashimoto's thyroiditis
Other neurological signs	–	–	Internuclear ophthalmoplegia, right Babinski sign
Treatment	Diazepam 30 mg/day baclofen 50 mg/day	Diazepam 20 mg/day baclofen 30 mg/day clonazepam 1 mg/day levetiracetam 1750 mg/day	Clonazepam 7 mg/day levetiracetam 750 mg/day clozapine 6.25 mg/day

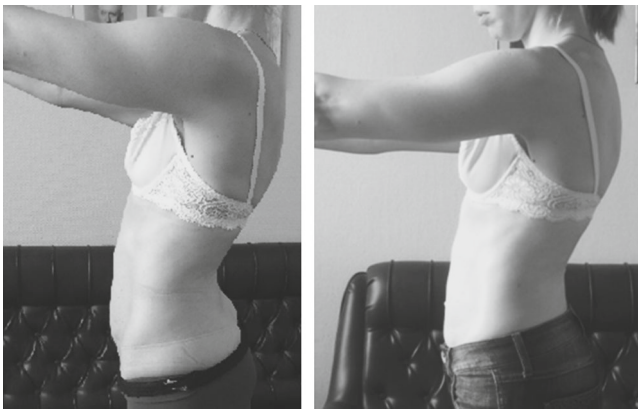

Fig. 2. Lumbar hyperlordosis and stiffness of abdominal muscles before and during the treatment.

Center of Neurology, patient S. received pulse therapy with prednisolone and plasmapheresis; however, only clonazepam provided minimal therapeutic effect. By the time of the first visit, this patient could barely take care of herself and needed assistance in walking. Significant improvement was achieved with levetiracetam and small doses of clozapine, an atypical antipsychotic, which also has an antidepressant, hypnotic, sedative, mood stabilizing, and anxiolytic action.

CONCLUSIONS

The diversity of antigenic targets and production of anti-GAD65 antibodies both in idiopathic and paraneoplastic syndromes lead to phenotypic heterogene-

ity of disorders associated with anti-GAD65 antibodies and difficulties in their diagnosis.

Tests for serum anti-GAD65 antibodies with radioimmunoassay or enzyme-linked immunosorbent assay are available to establish the diagnosis. However, occurrence of seronegative forms should also be considered. Immunoassay, adequate interpretation of test results along with careful analysis of neuroimaging and EMG data provide accurate differential diagnosis and allow to reveal associations between clinical signs and the antibody profile.

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