Original Article

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Sodium/Potassium-transporting ATPase Subunit Alpha-3 Antibody is not Present in Patients with Idiopathic Intracranial Hypertension

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Abstract

Aim: Increased cerebrospinal fluid (CSF) oligoclonal band prevalence, elevated cytokine levels, glial antibodies, and positive response to steroids have been described in idiopathic intracranial hypertension (IIH), suggesting autoimmune etiology in this disease. Sodium/potassium ATPases have been implicated in CSF production. Our objective was to discern unprecedented autoantibodies specific for sodium/potassium-transporting ATPase subunit alpha-3 (ATP1A3) in IIH.

Methods: Sera obtained from 31 individuals clinically diagnosed with IIH and 20 healthy control subjects were subjected to indirect immunofluorescence staining using live cultured rat brain neurons and HEK293 cells transfected with ATP1A3 cDNA.

Results: Autoantibodies targeting neuronal cell surface antigens were identified in three patients diagnosed with IIH. However, serum samples from IIH patients and healthy controls did not react with ATP1A3-transfected cells.

Conclusion: Our results indicate that ATP1A3 antibodies do not have a primary role in IIH pathogenesis. However, we provide preliminary support for the presence of anti-neuronal autoimmunity.

Keywords: Idiopathic intracranial hypertension, autoimmunity, antibody, sodium/potassium-transporting ATPase

Introduction

Idiopathic intracranial hypertension (IIH) is a disease characterized by elevated intracranial pressure (ICP) without any specified reason (1). Since this disease is a diagnosis of exclusion, it is crucial to evaluate secondary reasons that could cause an increase in ICP (2).

Clinically, headache, transient visual obscurations, pulsatile tinnitus, and neck and low back pain are the most common symptoms of the disease (3). If left untreated, optic atrophy and permanent vision loss may develop due to papilledema (4). While the disease is seen with a frequency of 0.5-2 per 100,000 in the general population, this rate rises to 12-20 per 100,000 in obese women of reproductive age, the population in which the disease is most common (5-8).

Although speculations such as cerebrospinal fluid (CSF) overexpression, deterioration in CSF drainage, and an increase in venous pressure that may cause impairment of CSF dynamics have been put forward, the pathogenesis of the disease is still not clearly identified (1). The strong link between IIH and obesity suggests that inflammation from fat cells might be involved in causing the disease; supporting this idea, levels of CCL2 and leptin were found to be higher in the CSF of IIH patients than in those without the condition (9). Increased prevalence of the disease in women of childbearing age has raised the possibility that hormonal factors may also be important. The activity of the 11β-hydroxysteroid dehydrogenase type 1 enzyme, which is an enzyme in glucocorticoid metabolism, is found to be related to CSF dynamics and may have a role in the pathogenesis of the disease (10).

Detection of oligoclonal bands (OCB) in CSF samples of IIH patients suggested that the disease could have an immunogenic background (11). Aquaporin-4 (AQP-4),

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which is a well-known water channel protein associated with water homeostasis and an antigenic target for neuromyelitis optica, has been investigated as a potential immunogenic target in IIH cases. However, different study groups have failed to establish anti-AQP-4 antibodies in serum and CSF samples of IIH patients (9,12).

Similarly, the screening of antibodies against the myelin oligodendrocyte glycoprotein antigen has also been negative. On the other hand, strong immunoreactivity with membrane antigens of hippocampal and cortical neurons was observed in sera of IIH patients, suggesting that autoantibodies directed against the neuronal surface antigens may have a role in the pathogenesis of IIH. In light of these findings, serum samples from 34 IIH patients were screened for antibodies against common neuronal surface antigens, such as NMDAR, AMPAR, CASPR2, LGI1, and GABABR, with the commercial cell-based assay (CBA) kit, and none of them were positive (13).

The perivascular astrocytic end-feet have received attention due to the close association of their area with water homeostasis in IIH pathogenesis. To establish any potential role of the anti-glial humoral response, antibodies against glial fibrillary acidic protein (GFAP) were screened in the serum samples of 58 IIH patients with live cell CBA and identified in 2 patients (14). Another study group has reported a single case of IIH with anti-GFAP antibodies in the CSF sample (15). However, because the GFAP antigen is an intracytoplasmic antigen, it was thought that anti-GFAP antibodies may not have a pathogenic role; instead, this positivity may occur as a secondary phenomenon. Nevertheless, the findings also suggest that an anti-glial immune response against an as yet unidentified target antigen may play a role in the disease pathogenesis.

Various transporters expressed in the choroid plexus (CP) can participate in CSF production. It is known that in the CP epithelium, sodium potassium (Na/K) ATPase modulates the osmotic gradient and water transport (16). The functionality of the Na/K ATPase is important for CSF production because ouabain, the Na+/K+-ATPase inhibitor, reduces CSF production by 50% (17). Furthermore, autoantibodies against sodium/potassium-transporting ATPase subunit alpha-3 (ATP1A3) have been detected in two clinical cases of paraneoplastic syndrome (18,19).

The aim of the study was to screen autoantibodies specific to ATP1A3 in IIH.

Materials and Methods

Compliance with Ethical Standards

Ethical approval of the study was obtained from the Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (date: 12.12.2023, and approval number: 24). The study followed the guidelines set forth in the Helsinki Declaration II. The informed consent forms of all the subjects who participated in the study were signed by patients.

Participants

All assays employed a control group comprising twenty healthy individuals matched for age and gender. We incorporated thirty-one adult patients who were diagnosed with IIH using the updated diagnostic criteria (20). These individuals were monitored at our headache outpatient clinic for a period exceeding one year. The demographic and clinical characteristics of the patients are detailed in Table 1.

Investigation of Anti-neuronal Antibodies

All participants' sera were stored at -80 °C until analysis. All patients were treatment-naive and symptomatic at the time of sera collection. Antibodies for NMDAR, AMPAR, LGI1, CASPR2, GABABR, GAD, and glycine receptor were investigated by CBAs utilizing human embryonal kidney 293 (HEK293) cells, as described previously (Euroimmun, Luebeck, Germany). ATPase subunit alpha-3 antibodies were investigated in HEK293 cells transfected with ATP1A3 cDNA by immunofluorescence staining, as per the manufacturer's instructions (Euroimmun) (18,19). As a positive control, a murine monoclonal antibody against the cytoplasmic N-terminus of ATP1A3 (Dianova, Hamburg, Germany) followed by anti-mouse IgG-Cy2 (Jackson Research, Suffolk, UK) was used as reported previously (19).

Immunofluorescence on Live Neurons

The immunoreactivity of IgG against neuronal surface antigens was evaluated using a CBA using rat cortical neurons isolated from newborn rat embryos (P1), as

Table 1. Demographic and clinical features of the patients

Clinical characteristics	**n=31**
Age (Mean ± SD)	32.61±9.53
Gender (Female/Male)	28 (90.3%)/3 (9.7%)
BMI (Mean ± SD)	33.46±6.96
Onset of the disease (age) (Mean ± SD)	29.78±8.12
Disease duration (month) (Mean ± SD)	82.15±56.27
First symptom (%) Headache Vision disturbances	 90.48 9.52
Symptoms (%) Blurred vision Tinnitus Diplopia Papilledema Vision lost	 90.48 63.66 9.1 82.61 27.27
CSF opening pressure (mmH_2O) (Mean ± SD)	402.27±160.48

SD: Standard deviation, BMI: Body mass index, CSF: Cerebrospinal fluid

described (21). Cultured neurons were incubated with sera (1:250) in a neurobasal medium for one hour at room temperature. Following this incubation, neurons were fixed using 3% paraformaldehyde and subsequently exposed to a 1:100 dilution of Alexa Fluor 488-conjugated anti-human IgG (Abcam, Cambridge, UK) for 45 minutes. The evaluation of immunostaining was conducted using an inverted fluorescence microscope (Leica Microsystems Ltd., Heerbrugg, Switzerland). Positive outcomes were identified by the presence of a green color, which was generated through the binding of a secondary antibody and displayed a range from moderate to strong intensity.

Statistical Analysis

No statistical analysis was conducted due to the descriptive nature of our study.

The power analysis was conducted based on the antibody positivity rate of 3% detected in our previous GFAP study. In the power analysis, the required number of patients to be included in the study was determined by setting the type 1 error (alpha) at 0.05 and the type 2 error (beta) at 0.20 (14).

Results

Identification of IIH Patients with Neuronal Surface Antibodies

To analyze serum antibodies targeting neuronal surface antigens, we employed a specialized immunocytochemistry method. To prevent antibodies directed against intracellular antigens, sera were added to cell culture before fixation and permeabilization steps. This approach revealed serum IgG specifically reacting with cell surface antigens and axonal-dendritic projections in cultured neurons in 3 out of 31 IIH patients, while none of the 20 healthy controls exhibited such reactivity (Figure 1). None of the IIH, or healthy control, sera tested positive for well-characterized anti-neuronal antibodies investigated through CBAs. This observation indicates that the binding of neuronal surface IgG in IIH sera was not attributable to well-characterized anti-neuronal antibodies associated with autoimmune encephalitis. Likewise, neither the IIH serum nor the healthy control serum reacted with ATP1A3 transfected HEK293 cells. In contrast, the commercial monoclonal antibody for ATP1A3, which served as a positive control, reacted strongly with ATP1A3 transfected cells, but did not react with cells that had other neuronal antigen cDNAs (Figure 2).

Discussion

In our previous study, GFAP and CACNA1H antibodies were detected in two different headache syndromes (14,22). In this study, we exclusively examined neuronal cell-surface antibodies, focusing on their pathogenic effects in autoimmune encephalopathies (23,24). In 9.7% of patients with IIH, we detected rare neuronal surface antibodies, potentially contributing to the pathogenesis of IIH by reacting with neuronal membrane antigens. However, a newly discovered putative neuronal surface antibody targeting ATP1A3 was not found in IIH patients. Previously, we had established that individuals with IIH display CSF OCB, elevated levels of serum/CSF cytokines and pro-inflammatory adipokines, and increased levels of neuron-specific enolase, serving as an indicator of neuronal damage (11,25,26). In addition, we also identified anti-GFAP antibodies in some IIH patients, indicating anti-astrocyte autoimmunity in this disorder (14). Our current findings substantiate the proposition that individuals with IIH may indeed host antibodies targeting neuronal surface proteins. In aggregate, these outcomes strongly imply that inflammation and potentially antigen-specific autoimmunity play a role in the pathogenesis of IIH.

The sodium/potassium transporting ATPase, a pivotal cellular component, consists of a catalytic alpha subunit, an ancillary non-catalytic beta subunit, and an additional regulatory subunit. *ATP1A* genes, encompassing ATP1A3, encode the alpha subunit, serving as the catalytic component behind the active enzyme. This enzyme orchestrates the hydrolysis of ATP, concurrently facilitating the exchange of sodium and potassium ions across the plasma membrane. The ensuing electrochemical gradient of sodium and potassium ions not only fuels the energetic

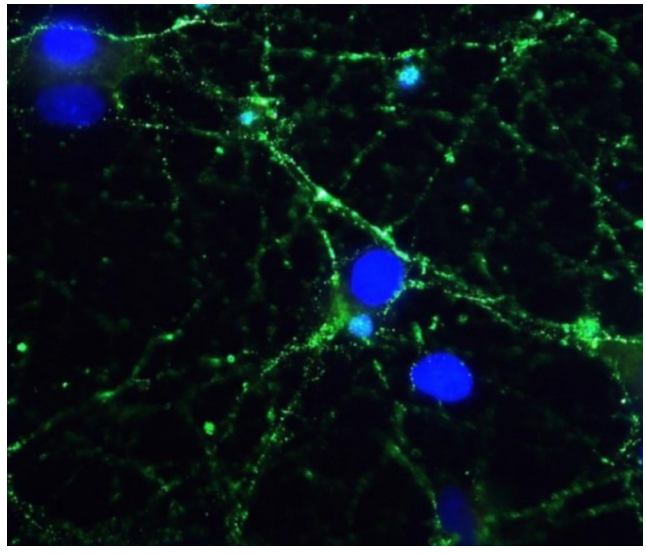

Figure 1. Immunolabeling of cultured live rat neurons with serum IgG of an IIH patient using indirect immunofluorescence. The serum IgGs of the patient show reactivity with the membrane of neuronal cell body and axonal/dendritic protrusions (green). Original magnification (800x, oil lens), counterstained with DAPI (blue)

IIH: Idiopathic intracranial hypertension

dynamics of cellular processes but also underpins the active transport of diverse nutrients (27).

It has been well demonstrated that ATP1A3 is expressed by the neuronal membrane and is thus exposed to potentially hazardous effects of circulating antibodies (28). It was therefore tempting to conclude that ATP1A3 antibodies may disrupt the function of the sodium/potassium-transporting ATPase, which plays a role in osmoregulation, the transport of various nutrient molecules, and the electrical excitability of neurons (29). Osmotic regulation is potentially impaired in IIH, as evidenced by amelioration of symptoms through the use of diuretics acting on Na-K transport, such as acetazolamide and furosemide (30). Given the substantial amino acid identity shared among various ATP1 proteins, there is a likelihood that individuals with IIH may possess antibodies targeting additional ATP1 proteins. This hypothesis warrants further comprehensive investigation.

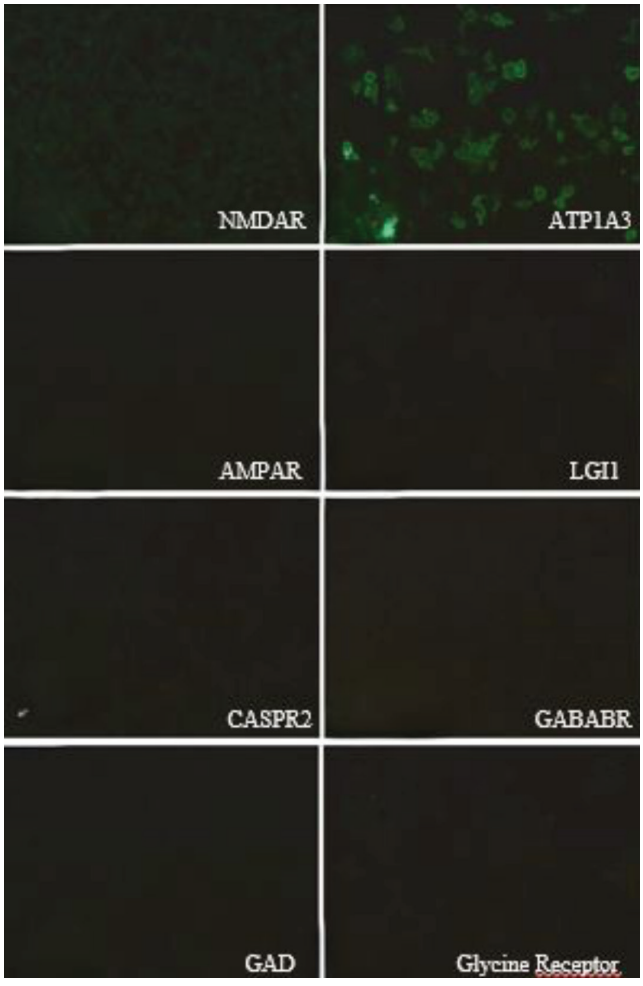

Figure 2. Immunofluorescence assays conducted with HEK293 cells transfected with different neuronal cDNA. The commercial monoclonal antibody against ATP1A3, used as a positive control, strongly immunoreacted with ATP1A3 transfected cells (green) but not with cells transfected with cDNA of other neuronal antigens (NMDAR, AMPAR, LGI1, CASPR2, GAD, Glycine receptor). Original magnification (20x)

ATP1A3: ATPase subunit alpha-3

Headache is the most typical symptom of IIH, and mutations in the *ATP1A* genes are associated with a plethora of neurological disorders, including hemiplegic migraine (31). As a matter of fact, ATP1A3 mutations are associated with epileptic encephalopathy, alternating hemiplegia of childhood, cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss-*CAPOS* syndrome, rapid-onset dystonia-parkinsonism, and recurrent episodes of cerebellar ataxia (32), indicating the remarkable importance of this subunit in neuronal functions. Notably, anti-neuronal antibodies were previously identified in a different headache syndrome, HaNDL, providing evidence for the participation of humoral autoimmunity in specific headache syndromes (22). Intriguingly, ATP1A3 antibodies have already been identified in paraneoplastic neurological syndrome patients presenting with ataxia and gaze palsy, emphasizing the immunogenic action of this particular subunit and potential involvement in the pathogenesis of neurological disorders (18,19).

Study Limitations

We only used sera in this study. Further exploration of CSF samples from IIH patients might yield more clinically relevant anti-neuronal antibodies. Lastly, similar antibody identification assays targeting different Na-K channels need to be conducted with a larger cohort of IIH patients to reliably establish the presence of such antibodies.

Conclusion

We have provided proof-of-concept evidence for the presence of novel neuronal surface antibodies in IIH. The exact pathophysiological significance of these antibodies in IIH needs to be further scrutinized.

Ethics

Ethics Committee Approval: This study was approved by the Istanbul University, Istanbul Faculty of Medicine, Clinical Research Ethics Committee (date: 01.12.2023, approval no.: 24).

Informed Consent: The informed consent forms of all the cases who participated in the study were signed by patients.

Footnotes

Authorship Contributions

Concept: C.I.K., Design: C.I.K., Data Collection or Processing: S.S., C.U., Analysis or Interpretation: C.I.K., E.T., Writing: C.I.K., E.T.

Conflict of Interest: No conflicts of interest were declared by the authors.

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