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Does *MAP2* Have a Role in Predicting the Development of Anti-NMDAR Encephalitis Associated with Benign Ovarian Teratoma? A Report of Six New Pediatric Cases

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ABSTRACT

Anti-*N*-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is a potentially fatal neurologic syndrome in which patients present with a spectrum of central nervous system deficits. Sixty percent of the cases can be attributed to the presence of tumors, most often ovarian teratomas. This report examines 6 pediatric patients who presented with neurologic deficits associated with the presence of such tumors. These cases illustrate a perplexing phenomenon, where benign teratomas could have a possible association with anti-NMDAR encephalitis. The purpose of this study was to compare the histology and immunohistochemistry of tumors associated with this syndrome to ovarian teratomas found in patients presenting with no neurologic symptoms. After obtaining institutional review board approval, 57 cases of ovarian teratomas were identified at our institution over 12 years. Six patients were identified with anti-NMDAR encephalitis. A panel of immunostains, including S100, GFAP, MAP2, and NeuN was applied to patients' tumor sections as well as the 6 controls from age-matched patients. No qualitative histologic or immunohistochemical differences were seen between the study cases and control group. Because no qualitative differences were identified between the study cases and the control group, testing of paired serum and cerebrospinal fluid remains the best method for diagnosis of anti-NMDAR encephalitis. Tumor banking with molecular analysis of ovarian teratomas, including whole-genome sequencing and comparative genomic hybridization between ovarian tissue saved from patients with and without anti-NMDAR encephalitis, is necessary to fully understand the etiopathogenesis of anti-NMDAR encephalitis.

Key words: anti-NMDAR, encephalitis, MAP2, NeuN, neuronal markers, ovarian teratoma

INTRODUCTION

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is a recently recognized, antibody-mediated disease that is potentially lethal in children and young adults [1]. The anti-NMDAR antibodies are produced by the immune system in response to stimuli such as tumors, viral infection, or genetic predisposition [2–4]. The NMDA receptor is present throughout the central nervous system (CNS). Because of the important role of the NMDA receptor in the CNS for mediating synaptic transmission and plasticity, patients with this syndrome suffer from disruption in signal transmission. Typically, these patients present with a spectrum of neuropsychiatric symptoms and general bizarre behavior [2–4].

Although this disorder has been investigated with relative frequency in the adult population, very little research has been done in pediatrics. The 6 cases in this report present this newly recognized disorder in children and its association with ovarian teratomas. Such a potentially fatal disorder requires further attention to understand the relationship between ovarian teratomas and encephalitis and how to treat those affected by this syndrome. The goal of this report is to examine the histopathologic and immunohistochemical differences between ovarian teratomas associated with anti-NMDAR encephalitis and those that caused no neurologic deficits.

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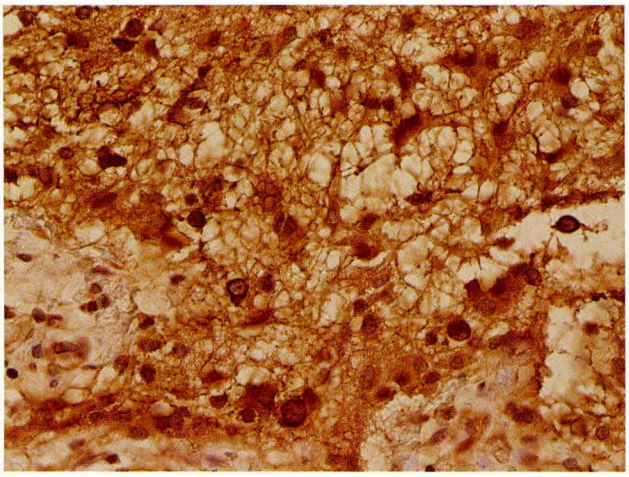

Figure 1. Section of ovarian teratoma showing strong cytoplasmic MAP2 immunostain positivity in a patient without anti-NMDAR encephalitis; ×400.

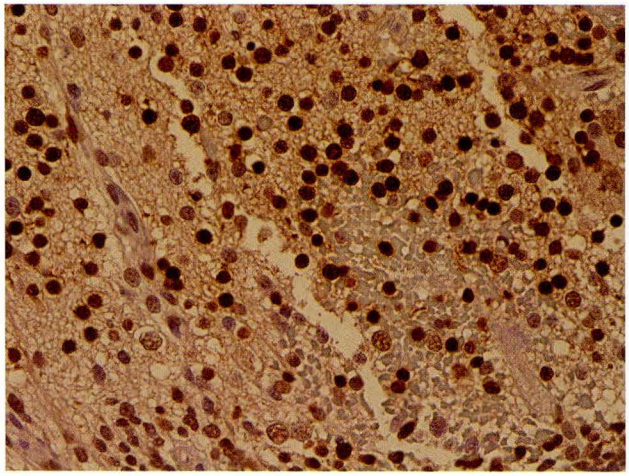

Figure 2. Section of ovarian teratoma showing strong nuclear NeuN immunostain positivity in a patient without anti-NMDAR encephalitis; ×400.

METHODS

Upon institutional review board approval, 57 cases of ovarian teratomas were identified from 2001 to 2013 at Children's Healthcare of Atlanta, GA. The medical records of those patients were examined, and 6 cases of anti-NMDAR encephalitis were identified. The study group and 6 age-matched controls were examined according to a specific protocol in which tumor samples were submitted as one cassette per centimeter of tumor size.

The excised ovarian teratomas (n = 11) and mediastinal teratoma (n = 1) were examined histopathologically with a panel of immunostains for S100, glial fibrillary acidic protein (GFAP), and neuronal markers: microtubule-associated protein 2 (MAP2) and neuronal nuclei (NeuN). The histology of the study group and the controls were examined by 2 independent pathologists (B.M.S and C.R.A) at our institution and included the presence of mature and immature neural tissue. These data allowed for a qualitative determination of histologic similarity between the study group and the matched controls. Additionally, the available results from cerebrospinal fluid (CSF) and serum anti-NMDAR titers were examined in the study group. A chi-square test was performed to determine the statistical significance of the results, and $P < 0.05$ was considered significant.

RESULTS

From our study group, 5 patients (3–18 years old) tested positive for anti-NMDAR antibody titers in CSF; additionally, 2 of those patients tested positive for anti-NMDAR antibody titers in serum (ARUP laboratories, Salt Lake City, UT, USA). One patient did not undergo this testing because that child presented with obvious neurologic symptoms before the availability of the standard anti-NMDAR encephalitis test. However, the patient was included in the study because she presented with an ovarian teratoma and symptoms consistent with anti-NMDAR encephalitis by retrospective analysis of her medical records. In this study group, one patient was diagnosed with the syndrome 1 year before the discovery of the ovarian teratoma, and another female patient tested positive for anti-NMDAR antibodies with a mediastinal teratoma.

The size of the teratomas in the study group ranged from 8 to 22 cm (mean, 15.2), and the size of the control group teratomas ranged from 10 to 19 cm (mean, 15). The control and study groups showed a spectrum of mature elements accompanied by small foci of immature neural elements in those tumors. All examined sections contained nerve tissue, and none of the teratomas in either group showed malignant components. Immature neural elements were found in both groups and represented less than 5% of the tissue examined. A chi-square test was performed. No statistically significant histopathologic differences were seen between the ovarian teratomas from the study group and the corresponding 6 controls after staining with hematoxylin-eosin and immunostains ($P = 0.414$).

In the immunohistochemical analysis of study and control groups, 12 teratomas (6 from the study group and 6 controls) were analyzed (Table 2). Antibodies generated against the neuronal marker microtubule associated protein 2 (MAP2) and neuronal nuclei (NeuN) were blindly tested in patients with ovarian teratoma with and without anti-NMDAR encephalitis. Three of the 6 patients (50%) with ovarian teratoma and anti-NMDAR encephalitis tested positive for both neuronal markers MAP2 and NeuN, and 4 of the 6 control patients (67%) with ovarian teratoma but no encephalitis were positive in those stains (Figs. 1,2). Moreover, GFAP and S100 expression was positive in all patients from our study and control groups. From our study, both the ovarian teratomas associated with anti-NMDAR encephalitis and those that followed a clinically normal course showed no significant immunohistochemical differences ($P = 0.414$).

Table 1. Summary of patients with anti-NMDAR encephalitis

	Patient					
	1	**2**	**3**	**4**	**5**	**6**
Age (years)	3	3	12	15	18	8
Symptoms	• Seizures • Feeding intolerance with abdominal distention • Choreiform movement • Unable to follow commands • Loss of speech with ataxia	• Increased movement • Severe jerkiness • Stiffness in legs	• Seizures • Feeding intolerance/ constipation • Dysphagia • Altered mental status • Insomnia • Inability to verbalize • Drooling • Agitation	• Movement disorder • Aphasia • Autonomic dysregulation • Cognitive impairment • Insomnia • Dysphagia	• Seizures • Insomnia • Cognitive impairment • Agitation	• Altered mental status • Choreiform movement • Verbal and auditory hallucination
Tumor location/size (cm)	Ovarian/22	Ovarian/17	Ovarian/12	Mediastina/8	Ovarian/20	Ovarian/12
CSF anti-NMDAR Ab	Test unavailable	Positive	Positive	Positive	Positive	Positive
Serum anti-NMDAR Ab	Test unavailable	Positive	Negative	Positive	Test unavailable	Test unavailable
NeuN	Negative	Test unavailable	Positive	Negative	Negative	Positive
MAP2	Negative	Test unavailable	Positive	Negative	Negative	Positive
Treatment	• Teratoma resection • Acyclovir • NG tube placement • Antiepileptics	• Teratoma resection • IViG • NG tube placement • Rituximab • Antiepileptics	• Teratoma resection • IviG • Plasma exchange • Rituximab • Antiepileptics	• Teratoma resection • IVIG • Plasma exchange • Rituximab • Cytoxan	• Teratoma resection	• Teratoma resection
Recovery	Only short-term known (first 6 months after onset): • Aphasia/dysphasia • Nonambulatory • Seizure disorder	• Aphasia/dysphasia • Nonambulatory • Seizure disorder • Upper extremity choreathetoid movement	• Aphasia • Limited speech • Limited ambulation • Seizure disorder	• Expressive aphasia • Memory and other cognitive deficits	Unknown	Unknown

anti-NMDAR indicates anti-*N*-methyl-D-aspartate receptor; IViG, intravenous immunoglobulin G; Ab, antibody; NeuN, neuronal nuclei; MAP2, microtubule-associated protein 2; NG, nasogastric.

Table 2. Summary of neurologic markers for ovarian teratomas with and without NMDAR encephalitis

	Patient type				
	Total patients	**Positive MAP2**	**Negative MAP2**	**Positive NeuN**	**Negative NeuN**
Ovarian teratoma without anti-NMDAR encephalitis (control)	6	4	2	4	2
Ovarian teratoma with anti-NMDAR encephalitis	6	3	3	3	3

NMDAR indicates *N*-methyl-D-aspartate receptor; MAP2, microtubule-associated protein 2; NeuN, neuronal nuclei.

All patients subsequently underwent magnetic resonance imaging and tumor resection. Postsurgical follow up and treatment varied among the patients but included some or all of the following: plasmapheresis, intravenous immunoglobulin G (IViG), and rituximab. At the conclusion of this study, there was a 100% survival rate. However, there was not a 100% recovery rate from the neurologic deficits for all patients. All 6 patients displayed some level of persistent neuropsychologic symptoms, including aphasia, seizures, and cognitive defects. A full summary of the clinical symptoms, treatment, and recovery of each patient can be found in Table 1.

DISCUSSION

Anti-NMDAR encephalitis is a newly recognized neurologic syndrome [1] associated with the presence of several tumors, and it presents a diagnostic challenge to attending physicians [5]. This condition is characterized by a spectrum of neuropsychiatric symptoms, which, in most cases, develop before the diagnosis of a tumor. Irani and Vincent [6] divided anti-NMDAR encephalitis development into 2 stages. The first stage is exemplified by psychiatric symptoms, confusion, amnesia, and seizures [6]. The second stage, which follows 10 to 20 days later, is characterized by movement disorders, autonomic disability, and reduction of consciousness [6]. Some patients develop cardiac dysrhythmias, thus, stressing the importance of early diagnosis and treatment. About 75% of patients with NMDAR antibodies recover or have mild sequelae; all other patients (25%) remain severely disabled or die [1].

Epidemiologically, published data show a female predominance in the adult and pediatric population (8.5:1.5) [7]. Approximately 60% of anti-NMDAR encephalitis symptoms are triggered by a tumor, mainly ovarian teratomas (mature, 70%; immature, 30%), but may be associated with sex cord stromal tumors or neuroendocrine tumors [5,7,8]. This condition has also been linked with testicular and mediastinal teratomas on rare occasions (5%) [5]. In 40% of cases, no tumor is identified, but encephalitis symptoms persist [9]. In cases in which no tumor was identified, alternative etiologies such as a prodromal viral infection or genetic predisposition were proposed [9,10].

Patients who presented with a tumor displayed a stronger immune response to the condition than did those with no detectable tumor, and had better outcomes because of tumor excision [4,8,9]. Lymphocytic pleocytosis of the CSF is the most consistent finding in these patients [4,9], which is most likely attributed to the antibodies that are formed against the 2 subunits of the NMDA receptor, NR1 and NR2 [3,4,9,11]. These heteromers bind glycine and glutamate, respectively, and are both required to form a functional receptor [2,4,7]. The NR1 subunit is present throughout the CNS, which explains the wide variety of symptoms seen in patients with this syndrome [9,11]. The NR2 NMDA receptors have a major role in synaptic transmission, remodeling, dendritic sprouting, hippocampal long-term potentiation, as well as memory formation and learning [2]. The NR2 subunit is itself composed of 4 individual components, which are coded by specific genes and vary in their location and presence at different developmental stages [2,11].

The pathogenesis of anti-NMDAR encephalitis is due to the development of antibodies that specifically target the extracellular *N*-terminal of the NR1 subunit of the NMDA receptor heteromer [3,4,7,9]. The direct antagonism of the NMDA receptor by the antibody, similar to the action of typical pharmacologic blockers of the receptor, such as phencyclidine and ketamine, may lead to neural defects [7]. Importantly, however, a reduction in the density of NMDAR functional heteromers on the postsynaptic knob, because of receptor internalization once the antibody has been bound, is most likely the cause of neuronal damage and the typical signs of anti-NMDAR encephalitis [6,7].

These newly discovered anti-NMDAR antibodies are enigmatic in their mechanism of entrance to the CNS, leading to encephalitis. In most cases, the serum NMDAR antibody levels are higher than CSF levels are, implying that antibody production starts in the periphery and is transferred to the CNS. In anti-NMDAR encephalitis, the antibodies seem to be able to cross the blood brain barrier (BBB) without disruption of the membranes [6], and the severity of this syndrome correlates with the concentration of antibodies in the serum and CSF of patients [4,5]. Martinez-Hernandez and colleagues [10] were able to detect the presence of plasma cells in the CSF using CD138 immunostain, and the hypothesized that B cells

cross the BBB in the CNS where they differentiate into antibody-secreting cells. Although CSF pleocytosis has been observed in most patients, no complement deposits were detected in the CNS [2,5]. The lack of NMDA receptor destruction by the complement system may explain the reversibility of this condition [9].

In addition to using the presence of NMDAR antibodies in CSF as a marker for NMDAR encephalitis, studies performed by Dalmau and colleagues [9] and Martinez-Hernandez and colleagues [10] found that all teratomas associated with anti-NMDAR encephalitis contained nerve tissue and tested positive for NMDA receptors and MAP2 (a neuron and dendrite cell marker) [2,7]. Although each patient in our study, where the test was available, were positive for anti-NMDAR encephalitis serum and CSF antibody titers, the neuronal markers MAP2 and NeuN did not show any significant difference between the study group and the control group. Testing CSF for anti-NMDAR antibodies is readily available and relatively inexpensive. Therefore, testing for anti-NMDAR encephalitis with CSF remains the best diagnostic tool to date for the diagnosis of this syndrome. Interestingly, normal ovaries have been found to express NMDAR subunits, which may explain the female predominance and disease onset in the absence of a tumor [8,12]. Therefore, we postulate that other genetic factors or predispositions allow only a few cases of ovarian teratomas to be paired with this encephalitis.

A recent study identified an association of a maternally inherited microdeletion at chromosome band 6p21.32 in a case of a 3-year-old boy with anti-NMDAR encephalitis [13]. The authors suggest that the copy number of *HLA* genes on chromosome 6 is a possible predisposing factor for autoimmune diseases and the development of anti-NMDAR encephalitis [4]. However, no other reports have reproduced these results.

As one can conclude from these case studies, anti-NMDAR encephalitis is a poorly understood phenomenon that needs more-focused attention from pathologists. Moreover, ovarian teratomas associated with the syndrome have been found to be histologically identical to teratomas from patients without neurologic symptoms. Tumor banking with molecular analysis of ovarian teratomas, including whole-genome sequencing and comparative genomic hybridization between ovarian tissue saved from patients with and without anti-NMDAR encephalitis, is necessary to fully understand the etiopathogenesis of this rare, debilitating, and potentially fatal case of encephalitis. Additionally, genetic counseling and HLA typing may further explain the possible genetic predisposition of this disease.

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