Neuromyelitis Optica Brain Lesions Localized at Sites of High Aquaporin 4 Expression

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Background: Neuromyelitis optica (NMO)–IgG is a specific autoantibody marker for NMO. It binds selectively to aquaporin 4 (AQP4), which is highly concentrated in astrocytic foot processes at the blood-brain barrier and is not restricted to optic nerve and spinal cord. Although it is conventionally believed that the brain is spared, brain imaging abnormalities are not uncommon in patients with NMO.

Objective: To investigate the location of brain lesions that are distinctive for NMO with respect to the localization of AQP4 in mammalian brain.

Design: Observational, retrospective case series.

Setting: Clinical serologic cohort of patients tested for NMO-IgG for whom brain magnetic resonance images were available.

Patients: We identified 120 patients seropositive for NMO-IgG for whom brain magnetic resonance images were available.

Main Outcome Measure: Magnetic resonance imaging abnormalities.

Results: In 8 patients we observed recurring and distinctive magnetic resonance imaging abnormalities in the hypothalamic and periventricular areas that corresponded to brain regions of high AQP4 expression.

Conclusion: The distribution of NMO-characteristic brain lesions corresponds to sites of high AQP4 expression.

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Neuromyelitis optica (NMO) is a severe demyelinating disease recognized principally by its propensity to selectively affect optic nerves and the spinal cord, causing recurrent attacks of blindness and paralysis. The 1999 diagnostic criteria of Wingerchuk et al. required fulfillment of 3 absolute criteria for a diagnosis of NMO: optic neuritis, acute myelitis, and no symptoms that implicated other central nervous system structures. The criteria additionally required fulfillment of at least 1 of 3 major or 2 of 3 minor supportive criteria. Major supportive criteria were (1) brain magnetic resonance imaging (MRI) at onset of disease either normal or not fulfilling multiple sclerosis (MS) imaging criteria; (2) individual spinal cord MRI T2-weighted lesions that accompany myelitis and extend across 3 or more vertebral segments; and (3) cerebrospinal fluid leukocyte count that exceeds 50 white blood cells per microliter or 5 neutrophils per cubic millimeter, typically in the context of an acute attack. Minor supportive criteria were (1) bilateral optic neuritis, (2) severe residual visual loss, and (3) severe fixed postattack weakness.

The serum autoantibody NMO-IgG was reported as a biomarker of NMO in 2004. It is detected by indirect immunofluorescence assay on a substrate of mouse CNS tissue. Neuromyelitis optica–IgG binds selectively to the mercurial-insensitive water channel protein aquaporin 4 (AQP4), which is concentrated in astrocytic foot processes at the blood-brain barrier.

Aquaporin 4 is the predominant water channel in the brain and has an important role in brain water homeostasis. It is also expressed, to a limited extent, in the stomach, kidney, lung, skeletal muscle, and inner ear. Although abundant in optic nerve and spinal cord, AQP4 is found throughout the brain. Immunohistochemical studies show intense AQP4 immunoreactivity in the astrocytic end feet that abut capillaries and pia in the brain and spinal cord, the glial lamellae of the supraoptic nucleus in the hypothalamus, and the basolateral membranes of ependymal cells. Consistent with its localization in the CNS, AQP4 is involved in the development, function, and integrity of the interface between the brain and blood and between the brain and cerebrospinal fluid.

Despite traditional views that the lesions of NMO are restricted to optic nerves and spinal cord, recent MRI studies have revealed evidence of brain lesions in 60% of patients who, except for brain MRI findings, fulfill the 1999 criteria of Wingerchuk.

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et al\(^1\) for the diagnosis of NMO. Most imaged lesions are nonspecific. Occasional lesions resemble those regarded as typical of MS. Of pertinence to this report, some patients have distinctive lesions in the hypothalamus or brainstem that are atypical of MS.\(^9,10\) We and others have recognized a reiterative pattern of signal abnormality that appears to be characteristic of, if not specific to, NMO or its spectrum disorders.\(^9-11\) These lesions, apparent on MRI, predominantly involve the hypothalamus and occasionally extend to brain tissues that surround the third and fourth ventricles. In this observational study, we describe these lesions and report their location with respect to the reported localization of the AQP4 water channel protein in mammalian brain.

**METHODS**

During serologic evaluation for NMO-IgG, we identified 120 seropositive patients for whom brain MRIs were available for review. Of these 120 patients, 89 fulfilled the criteria of Wingerchuk et al\(^1\) for the diagnosis of NMO, except for the requirement of a normal brain MRI at onset and absence of symptoms outside optic nerves and spinal cord. We have recently described the frequency and characteristics of MRI head abnormalities in 60 patients with NMO;\(^9\) 41 of these 60 patients were NMO-IgG seropositive and are included in the 89 patients with NMO from this current study.

The remaining 31 of the 120 seropositive study patients had relapsing, recurrent, longitudinally extensive transverse my-
elitis (LETM) without optic neuritis. This type of myelitis is the most sensitive and specific clinical characteristic of NMO-related disorders. Furthermore, patients with LETM who are seropositive for NMO-IgG are at high risk of relapse or the development of NMO. Brain MRI in 8 of these 120 patients revealed the reiterative and distinctive signal pattern abnormality that is the subject of this communication.

**RESULTS**

Clinical and demographic findings for the 8 patients are listed in Table 1. These 8 patients represented 6 of the 89 patients with NMO (patients 2-5, 7, and 8) and 2 of 31 patients with relapsing LETM (patients 1 and 6). Patient 6, who had no clinical symptoms or signs of optic neuritis, had a delayed visual evoked response consistent with subclinical optic neuropathy. The MRIs (Figure 1) from patients 1 through 6 (Table 2) illustrate the distribution of the NMO brain lesions we consider characteristic of NMO. Patient 7 had extensive signal abnormality on both T2 and fluid-attenuated inversion recovery with prominent periventricular signal abnormality in serial axial images from lower pons to lateral ventricles (Figure 2). White dots indicate the location of AQP4 protein in high concentration (based on published immunohistochemical studies of rodent and human brain). Patient 8, ascertained serologically but not evaluated clinically at Mayo Clinic, had MRIs of the head reported on by our neuroradiology department as follows: “There was periventricular enhancement around the left occipital horn and along the left anterior callosal body”; these images were not available for inclusion in this report.

**Figure 2.** Serial axial sections of brain from patient 7 at the level of lateral ventricles (image 1) through the third ventricle, diencephalon (images 2 and 3), midbrain (image 4), and pons (images 5 and 6). The fluid-attenuated inversion recovery signal abnormality is contiguous throughout the periventricular and periependymal tissues and involves the hypothalamus. White dots indicate areas of high aquaporin 4 expression in the periependymal regions, which correspond to regions of magnetic resonance imaging abnormalities (arrows).
The beneficial effects of plasmapheresis\textsuperscript{14} and anti–B-cell therapy (rituximab)\textsuperscript{15} in patients with acute NMO are consistent with NMO being an autoantibody-mediated disease. Although its pathogenicity is not yet proved, NMO-IgG has proved to be a sensitive and specific marker for a spectrum of NMO-related disorders, including relapsing myelitis\textsuperscript{2,13} and relapsing optic neuritis.\textsuperscript{2} Neuromyelitis optica–IgG has been shown to interact specifically with the AQP4 water channel protein \textsuperscript{3} in vitro. The distribution of AQP4 at glial-fluid interfaces in the mouse spinal cord\textsuperscript{16} coincides with sites of NMO-IgG\textsuperscript{2} binding and is similar to the pattern of immunoglobulin and complement deposition in lesions of autopsy and biopsy spinal cord specimens of patients who have active acute-stage NMO.\textsuperscript{17} These observations support our hypothesis that AQP4-IgG plays a pathogenic role in NMO.

The anatomical and cellular distribution of AQP4 in mammalian tissues, including brain and spinal cord, has been investigated extensively.\textsuperscript{4-7} Venero et al\textsuperscript{18} reported high AQP4 messenger RNA expression in periventricular organs of rodent brain. An immunolocalization study\textsuperscript{3} performed with normal human brain tissue demonstrated restriction of AQP4 to astroglial cell membranes, particularly in subpial and subependymal zones around the ventricles, as observed in other mammals.\textsuperscript{4-7}

Although most brain lesions encountered in patients with NMO are nonspecific, lesions in the brainstem and hypothalamus appear to be relatively characteristic for NMO.\textsuperscript{9-11} Vernant et al\textsuperscript{20} described 8 Antillean women with an NMO-like illness of whom 3 had endocrinopathies with MRI lesions in the hypophysis and inferior hypothalamus. In addition to our 3 cases with NMO and MRI evidence of hypothalamic involvement,\textsuperscript{9} Poppe et al\textsuperscript{10} described 2 patients who presented with otherwise classic NMO and developed clinical manifestations of hypothalamic dysfunction with lesions that involved the hypothalamus as the sole parenchymal lesions in the brain.

In consideration of these reports and the recently discovered serologic marker NMO-IgG, Wingerchuk and colleagues\textsuperscript{21} have proposed revised diagnostic criteria for definite NMO. These criteria require optic neuritis, myelitis, and at least 2 of 3 supportive criteria: (1) MRI evidence of a contiguous spinal cord lesion 3 or more vertebral

### Table 1. Clinical Characteristics of 8 Patients With NMO and Brain Lesions

<table>
<thead>
<tr>
<th>Patient No./Sex/ Age at Onset, y/Race</th>
<th>No. of ON Attacks</th>
<th>No. of LETM Attacks</th>
<th>Initial Head MRI Results</th>
<th>Time From Onset to Abnormal Imaging Result, mo</th>
<th>Symptoms Other Than ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/48/As</td>
<td>0</td>
<td>3</td>
<td>Abnormal</td>
<td>Unknown</td>
<td>Transient encephalopathy–confusional amnestic syndrome</td>
</tr>
<tr>
<td>2/F/18/W</td>
<td>2</td>
<td>&gt;6</td>
<td>Normal</td>
<td>29‡</td>
<td>Complex partial seizures</td>
</tr>
<tr>
<td>3/M/5/W*</td>
<td>1</td>
<td>3</td>
<td>Abnormal</td>
<td>4</td>
<td>Transient diplopia, upbeat and gaze-evoked nystagmus</td>
</tr>
<tr>
<td>4/F/34/W</td>
<td>3</td>
<td>2</td>
<td>Normal</td>
<td>96</td>
<td>Diplopia without objective findings</td>
</tr>
<tr>
<td>5/F/13/H*</td>
<td>3</td>
<td>6</td>
<td>Normal</td>
<td>6</td>
<td>Nausea</td>
</tr>
<tr>
<td>6/F/34/W</td>
<td>0†</td>
<td>2</td>
<td>Normal</td>
<td>12</td>
<td>Dysarthria, diplopia, left facial numbness</td>
</tr>
<tr>
<td>7/F/38/AA*</td>
<td>2</td>
<td>2</td>
<td>Normal</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>8/M/36/W</td>
<td>3</td>
<td>5</td>
<td>Normal</td>
<td>54</td>
<td>Vertigo</td>
</tr>
</tbody>
</table>

**Abbreviations:** AA, African American; As, Asian; H, Hispanic; LETM, longitudinally extensive transverse myelitis (>3 vertebral segments); MRI, magnetic resonance imaging; NMO, neuromyelitis optica; ON, optic neuritis; W, white.

*Described previously.\textsuperscript{9}

†Prolonged visual evoked potentials bilaterally.

‡The MRI performed at 29 months showed nonspecific foci of signal abnormality in the deep white matter; images shown in the figure were taken 88 months after disease onset.

### Table 2. MRI Characteristics of 8 Patients With NMO and Brain Lesions

<table>
<thead>
<tr>
<th>Patient No./Sex/ Age at Onset, y/Race</th>
<th>Region of Head MRI Signal Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>1/F/48/As</td>
<td>+</td>
</tr>
<tr>
<td>2/F/18/W</td>
<td>−</td>
</tr>
<tr>
<td>3/M/5/W*</td>
<td>−</td>
</tr>
<tr>
<td>4/F/34/W</td>
<td>−</td>
</tr>
<tr>
<td>5/F/13/H*</td>
<td>+</td>
</tr>
<tr>
<td>6/F/34/W</td>
<td>−</td>
</tr>
<tr>
<td>7/F/38/AA*</td>
<td>+</td>
</tr>
<tr>
<td>8/M/35/W</td>
<td>−</td>
</tr>
</tbody>
</table>

**Abbreviations:** AA, African American; As, Asian; E, enhancing; H, Hispanic; MRI, magnetic resonance imaging; NMO, neuromyelitis optica; ON, optic neuritis; W, white; +, present; −, absent.

*Described previously.\textsuperscript{9}

†Extending into the cerebellar peduncles.
segments in length, (2) brain MRI nondiagnostic for MS at the onset of disease, and (3) detection of NMO-IgG in serum. These revised criteria acknowledge that both clinical and subclinical evidence of brain involvement are compatible with a diagnosis of NMO. In support of a broader definition of an “NMO-spectrum disorder,” Weinshenker and colleagues have documented that 40% of patients who present with a single episode of LETM are seropositive for NMO-IgG and that seropositivity predicts high risk of a relapse of transverse myelitis or subsequent development of optic neuritis (fulfilling criteria for a definite diagnosis of NMO).13 We now recognize NMO-IgG–seropositive patients with recurrent LETM as having a limited form of NMO. This was our rationale for including patients with either NMO or “NMO spectrum disorders” as subjects of this report.

The MRI brain lesions that are characteristic of NMO occur adjacent to the ventricular system at any level but are more commonly found around the third and fourth ventricle and the aqueduct of Sylvius than around the lateral ventricles. The corpus callosum is sometimes involved. The distribution of these characteristic NMO brain lesions mirrors the periventricular and hypothalamic localization of AQP4. It is not yet known and remains to be demonstrated experimentally whether inflammatory sequelae follow the binding of NMO-IgG to AQP4. We anticipate that detailed immunohistochemical studies of autopsy or biopsy brain tissue specimens from patients with NMO, as well as imaging and immunopathologic studies of CNS tissues in animals immunized with AQP4 (or injected with NMO-IgG), will establish the extent of brain tissue involvement beyond the optic nerves.

In contrast to the severe clinical manifestations of lesions that involve optic nerves and spinal cord in patients with NMO, the brain lesions described in this report were minimally or not symptomatic and were observed to resolve in some patients. It is conceivable that focal accumulations of water may account for the MRI abnormalities we report, consistent with the critical role of AQP4 in sustaining brain water homeostasis.8 To our knowledge, this is the first report to correlate a distinctive radiologic pattern with a putative autoantigen.

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REFERENCES


