Brief Communications

Repetitive transcranial magnetic stimulation does not replicate the Wada test

Article abstract—The authors compared inferior frontal speech arrest from repetitive transcranial magnetic stimulation (rTMS) with bilateral Wada tests in 17 epilepsy surgery candidates. Although rTMS lateralization correlated with the Wada test in most subjects, rTMS also favored the right hemisphere at a rate significantly greater than the Wada test. Postoperative language deficits were more consistent with Wada results. Available methods for inducing speech arrest with rTMS do not replicate the results of Wada tests.

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Repetitive transcranial magnetic stimulation (rTMS) has well-documented effects on several aspects of speech and language. The most striking is the arrest of meaningful speech output during rapid stimulation over the inferior frontal region. Speech arrest (SA) is consistently easier to obtain over the left hemisphere, but prior studies of rTMS have drawn different conclusions concerning its sensitivity and accuracy for lateralization of language function. The largest published series that compared rTMS with unilateral Wada tests reported that complete SA could be obtained in only 14 of 21 epilepsy patients. One patient in this series had discordant lateralization to the right hemisphere by rTMS criteria, which was interpreted as a correlation between rTMS and the Wada test of 95%.

We reported previously that rTMS at the unexpectedly slow rate of 4 Hz produces reliable SA with safety and relative comfort. Here we compare the results of this technique with bilateral Wada tests in a series of patients undergoing evaluation for epilepsy surgery. This group includes the largest number of epilepsy patients to undergo complete bilateral testing and to experience complete SA.

Methods. Patients included 17 of 18 consecutive epilepsy surgery candidates undergoing presurgical Wada tests at Emory University Hospital. One patient was dropped from consideration because of severe baseline dysarthria, which was considered likely to compromise the validity of both procedures. The others gave informed consent to a protocol approved by the Human Investigations Committee, Emory University School of Medicine. All patients were taking their customary doses of anticonvulsants at the time of testing.

Motor threshold was determined in the right first dorsal interosseous muscle according to the NIH technique. rTMS methods have been described previously, and include a stimulation rate of 4 Hz using a high-efficiency iron-core magnetic stimulation coil with maximum induced electrical field beneath its center. While the patient counted briskly upward, the stimulator was activated and the coil was moved carefully toward the lateral frontal region of each hemisphere to determine the scalp position of lowest threshold SA and the output level at which complete interference occurred. Using the lowest level of complete SA determined for either side, patients were retested over both hemispheres while counting and reading aloud. Complete SA was defined as either total mutism or the production of only simple sounds with no language quality. Testing parameters for rTMS did not exceed recent safety recommendations.

Wada tests were performed bilaterally using a variant of the Montreal technique. Lateralization of rTMS and Wada test results was ranked according to the criteria in the table. Parametric and nonparametric comparisons were made using SPSS 6.1.1 (SPSS Inc., Chicago, IL).

Results. Sixteen of 17 patients had complete arrest of counting during rTMS over one or both hemispheres. Three were completely mute; 13 were able to produce only simple, unintelligible sounds without language quality. The remaining patient had severe, asymmetric dysarthria only on left-side stimulation, and not on the right at equal intensity. He declined testing at a higher intensity because of pain. In all patients the two hemispheres could be compared at a stimulation intensity that produced strong effects on at least one side. Apparent rTMS lateralization was always the same during reading as during counting. Aside from discomfort, no adverse effects of rTMS were observed.

By Wada testing, all patients were left-hemisphere dominant. Using rTMS, 12 of 17 patients appeared left dominant and had rank scores equivalent to their Wada results. Five of 17 patients showed a greater tendency toward right-side speech by rTMS. SA occurred at the...
same intensity bilaterally in three patients, whereas one patient with entirely left-side language by Wada testing appeared entirely right dominant using rTMS criteria. Lateralization by Wada test and rTMS was correlated \((r = 0.57, p < 0.05, \text{Spearman’s rank correlation coefficient})\), and the overall tendency for left-hemisphere lateralization by rTMS was different from chance \((p < 0.01, \text{binomial distribution})\). However, the proportion of studies with some indication of right-side speech output was greater for rTMS than for the Wada test \((p < 0.05, \text{Wilcoxon’s signed rank test})\).

The patient with totally discordant results scored 14 of 14 for right-hand dominance on a standardized handedness inventory \(^7\); she reported a left-handed son and an ambidextrous daughter. Her MRI results demonstrated right mesial temporal sclerosis, and seizures originated in the right temporal lobe.

Fifteen of 17 patients had epilepsy \((7 \text{ left temporal, 7 right temporal, and 1 right frontal})\). Five patients, all of whom underwent left temporal resections, experienced mild postoperative language difficulty in the form of anomia or paraphasic errors. Three of these five appeared completely left dominant for language by both Wada test and rTMS. Two of the five appeared primarily left dominant by Wada test, but bilaterally dominant by rTMS. The patient who appeared entirely right dominant by rTMS underwent right temporal lobe resection, and experienced no postoperative language difficulty.

**Discussion.** Speech lateralization by rTMS clearly correlates with left-hemisphere language dominance in most subjects. But compared with the Wada test, rTMS gives an excessive yield of apparent bilateral or right-hemisphere lateralization. When postoperative language deficits were present in this series, they were always compatible with Wada test results. Furthermore, in the three patients in whom rTMS predicted the possibility of a different outcome, postoperative language function was more consistent with the Wada test. Two patients with bilateral speech by rTMS, but left-hemisphere dominance by Wada test, had postoperative language deficits after left temporal lobectomy. One patient with right-hemisphere speech by rTMS but left dominance by Wada test had no deficit after right temporal lobectomy. Compared with previous series, the use of a less painful rTMS technique allowed us to obtain complete SA more consistently, without anticonvulsant withdrawal; to rank both rTMS and Wada results; and to perform more detailed comparisons between them. Although safe and tolerable enough for widespread use, lateralization of SA by rTMS does not adequately reproduce the Wada test.

This failure may relate to the specific features of magnetic SA, which appears to occur at the motor cortex rather than over classic language areas.\(^8\) The most prominent effects of rTMS are consistent with a motor speech disorder, rather than with a general impairment of language. Thus the side of lowest rTMS threshold may reflect simply a preference for skilled motor performance, similar to but not completely congruous with handedness. The fact that most patients with minor infarcts to the dominant frontal operculum ultimately recover nearly normal speech suggests that the ability for speech output is represented to some degree bilaterally in most people, and may adapt to injury more easily than other aspects of language. Other evidence suggests that motor speech may be an insufficient index of language lateralization, independent of how it is estimated. Of the several measures made during the course of the Wada test, the duration of SA after injection of anesthetic is, by itself, a poor predictor of cerebral dominance.\(^9\)

Other approaches to TMS, such as stimulation over Wernicke’s area or the use of an auditory short-term memory task, might seem to be better means of addressing the critical goal of lateralizing language comprehension and verbal memory. Unfortunately, despite many attempts, TMS effects during such tasks have not been sufficiently robust to give straightforward results in individual subjects.\(^10\) Clinically useful lateralization of language by rTMS may require further development of these methods.

**Table Ranks for Wada and rTMS lateralization**

<table>
<thead>
<tr>
<th>Ranks</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wada lateralization</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Left-hemisphere language only</td>
</tr>
<tr>
<td>2</td>
<td>Left dominant, but some evidence of right-hemisphere language</td>
</tr>
<tr>
<td>3</td>
<td>Bilateral language function</td>
</tr>
<tr>
<td>4</td>
<td>Right dominant, but some evidence of left-hemisphere language</td>
</tr>
<tr>
<td>5</td>
<td>Right-hemisphere language only</td>
</tr>
<tr>
<td>rTMS lateralization</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Complete left-hemisphere SA at a level that produced no effect on the right</td>
</tr>
<tr>
<td>2</td>
<td>Complete left-hemisphere SA at a level that produced mild to moderate speech interference on the right</td>
</tr>
<tr>
<td>3</td>
<td>Complete SA bilaterally at a level that was not distinguishable ((\pm 2% \text{ difference, expressed as full-scale stimulator output}))</td>
</tr>
<tr>
<td>4</td>
<td>Complete right-hemisphere SA at a level that produced mild to moderate speech interference on the left</td>
</tr>
<tr>
<td>5</td>
<td>Complete right-hemisphere SA at a level that produced no effect on the left</td>
</tr>
</tbody>
</table>

rTMS = repetitive transcranial magnetic stimulation; SA = speech arrest.

**References**

TLE patients with postictal psychosis: Mesial dysplasia and anterior hippocampal preservation

**Article abstract**—The authors studied six patients with refractory temporal lobe epilepsy and postictal psychosis using quantitative MRI and histopathology, and compared the results with 45 patients with temporal lobe epilepsy without postictal psychosis. Total hippocampal volumes were not different between the two groups. However, patients with postictal psychosis had a relatively preserved anterior hippocampus, and temporal lobe dysplasia was more frequent \( (p = 0.006, \text{chi-square test}) \). These findings may be associated with the clinical symptoms.

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It has long been recognized that some patients with refractory temporal lobe epilepsy (TLE) develop postictal psychosis (PIP).1 Patients have delusions or hallucinations, starting shortly after an epileptic seizure. Psychotic symptoms may last for several days or even weeks, and they never occur without a preceding seizure.2 The onset of episodes of PIP is typically much later than the onset of TLE.

The reasons for PIP are unknown. Based on previous clinical observations, two risk factors have been suggested. First, because patients with PIP often lack a history of prolonged febrile convulsions, they may have less damaged hippocampi than patients without PIP.3 Second, dysplastic malformations may represent a risk factor for developing PIP.3,4

In this study, we performed a systematic investigation of hippocampal morphometry using quantitative MRI, and examined microdysplastic features histopathologically. We hypothesized that patients with PIP would demonstrate less atrophic hippocampi and more dysplastic abnormalities.

**Methods.** **Subjects.** All patients with refractory TLE with PIP who were identified during intensive presurgical evaluation5 between 1993 and 1997, and who had MR images of sufficient quality for quantitative analysis, were included. There were three men and three women, with a mean age of 39 years, who participated in the study. PIP was diagnosed by a psychiatrist, based on established criteria.6 Patients were not included if they had a history of nonictal psychotic disorder, relevant recent substance abuse, or when other organic factors were suspected. In four of the six patients with PIP, psychotic episodes were observed directly while they were inpatients.

The control group consisted of a consecutive series of 45 patients with refractory TLE who were investigated in a similar way, and in whom psychiatric assessment excluded PIP (23 men, 22 women; mean age, 35 years).

The study was approved by the hospital’s committee on human ethics.

**MRI methods.** In all patients, MRI was performed using a similar protocol on a 1.5-T Siemens SP Magnetom scanner (Siemens, Munich/Erlangen, Germany). Quantitative assessment included bilateral hippocampal and hemi- cranial volumes, and T2 relaxometry.7 Hippocampal volumes were corrected for intracranial volumes.8 Quantitative MRI findings of 45 patients have already been reported.9

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The posterior-to-anterior distribution of the hippocampal volume was presented graphically. The slice order number was determined for each slice separately. Side-to-side comparison of the cross-sectional area was performed between the slices with the same order number, allowing correction for asymmetry. First, the posterior-to-anterior distribution was assessed visually in each individual for prominent patterns in the two groups. Second, the volume deficit at each slice position, calculated by subtracting the ipsilateral side from the contralateral side, was averaged for the two groups. Differences between the two groups were assessed at each slice position using the Mann–Whitney U test.

Histologic evaluation. Temporal lobe surgery was undertaken in 50 patients. Histologic assessment was performed by a neuropathologist, who was blinded to all other results, to determine the presence of hippocampal sclerosis (HS) and dysplasia.

In hippocampal or dentate gyri, dysplasia was diagnosed when there were definite architectural abnormalities, such as nodularity of the stratum radiatum margin of the CA1 segment of the pyramidal neuronal layer, excessive numbers of single neurons in the stratum radiatum, or nodular collections of aberrant dentate granule cell neurons. In the stratum lacunosum moleculare, the presence of single large or grouped small neurons was regarded as dysplastic, as was the presence of nodular collections (two or more together) of mature-appearing neurons in the white matter.

In the neocortical specimens, dysplasia was diagnosed when there were groups of medium or large neurons in cortical layer 1, when aberrant neuronal clustering with “bare” areas occurred in layers 2 through 6, or when nodular collections of neurons were present in the white matter. Features known to occur in control subjects and requiring quantification for assessment of significance were not regarded as dysplastic.

Statistical analysis. Demographic variables and quantitative MRI results were analyzed with separate Mann–Whitney U tests and chi-square tests for differences between patients with and without PIP. To correct for multiple comparisons, significance was set at 1%.

Results. Onset of PIP was, on average, 20 years after the onset of TLE. Clinical variables were not different between patients with and without PIP (table). Visual analysis of the MR image showed HS in four of the six patients with PIP. Another patient had widespread temporal dysplasia, and the sixth patient had a small dysembryoplastic neuroepithelial tumor. Of the 45 patients with TLE without PIP, 34 had MRI features of HS. Four had widespread temporal dysplasia, and the remaining seven patients had other findings.

The total hippocampal volume on the focus side was not different in patients with PIP (2,395 ± 986 cm³) compared with patients without PIP (2,406 ± 639 cm³). There was also no difference between patients with and without PIP in the contralateral hippocampal volume, the hemispheric volumes, and hippocampal T2 relaxometry (Mann–Whitney U test).

However, the distribution of the hippocampal volume showed a difference. Visual analysis of the distribution graphs demonstrated in all six patients with PIP a relative preservation of the anterior hippocampus. The ipsilateral
The hippocampus was as long as the contralateral. In contrast, in patients without PIP, volume loss was distributed diffusely, and the ipsilateral hippocampus was shorter (figure 1). The average ipsilateral hippocampal volume deficit in PIP and non-PIP patients is shown in figure 2. There was no difference between the two groups in the posterior hippocampus, but there was less volume deficit in PIP patients in the anterior hippocampus (12th slice; \( p = 0.003 \), Mann–Whitney U test).

Pathologic examination diagnosed dysplasia in all five operated patients with PIP (100%; see the table). The patient with temporal dysplasia on MRI had the most extensively distributed dysplasia on histopathology. In contrast, dysplasia was found in only 16 of the 45 patients without PIP (36%; \( p = 0.006 \), chi-square test).

**Discussion.** Two findings characterized refractory TLE patients with PIP. Patients with PIP had an unusual distribution of the hippocampal volume and a higher frequency of mesial dysplasia. We did not confirm earlier speculations that patients with PIP would have a reduced total volume of the ipsilateral hippocampus. Total hippocampal volumes and relaxation times were not different between patients with and without PIP. However, patients with PIP showed a relative preservation of the anterior hippocampus. The ipsilateral hippocampus was at least as long as the contralateral, and it demonstrated less volume loss in the anterior portion. This is unusual for refractory TLE. Typically, hippocampal volume loss is distributed diffusely, and the ipsilateral hippocampus is shorter than the contralateral.

It is known that patients with presumably epileptogenic dysplastic abnormalities are at risk for PIP. Our results document that when HS is the epileptogenic lesion, the presence of dysplasia is also a risk factor for PIP. Defining dysplasia in temporal lobectomy specimens is difficult and has excited considerable controversy in the past. Based on this controversy, only established criteria have been used in this study.

The appearance of PIP after only 10 to 20 years of seizures is puzzling, but suggests it may be related to slow plastic changes in the epileptogenic temporal lobe, such as axonal remodeling. Episodes of PIP, which occur only after certain seizures, are short-
lived. They presumably reflect a transient metabolic or electrophysiologic disturbance on a substrate of a temporal lobe epileptogenic abnormality. Our data suggest that changes in this substrate may be more likely to lead to PIP episodes in the presence of dysplasia or when there is relative preservation of the anterior hippocampal structures.

Acknowledgment
The authors acknowledge the help of their radiology team: Dr. Greg Fitt, Dr. Anne Mitchell, and Ari Syngeniotis.

References

Adult myoclonic epilepsy: A distinct syndrome of idiopathic generalized epilepsy

Article abstract—The authors present 11 cases of idiopathic generalized epilepsy that began in adulthood at a mean age of 39 years. All patients had myoclonic jerks, five had absence seizures, and nine had infrequent generalized tonic-clonic seizures. A majority had a family history of seizures. EEG in all patients showed generalized epileptiform abnormalities, whereas neuroimaging and neurologic examination results were normal. This series appears to represent a previously undescribed idiopathic generalized epilepsy syndrome of adult myoclonic epilepsy.

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Classification of the idiopathic generalized epilepsies (IGE) is based principally on seizure types and age at onset. Seizure types, however, overlap in the IGE syndromes. For example, typical absence seizures are included in the description of juvenile myoclonic epilepsy (JME) and myoclonic jerks sometimes occur in patients with generalized tonic-clonic seizures on awakening (GTCA). As Janz emphasized in a recent review, “the age at onset is the strongest single indicator of a biologic difference between the four major syndromes of IGE.” The large majority of initial seizures in the IGE occur during childhood or adolescence, although exceptional cases are reported in early adulthood. Family and twin studies suggest that childhood absence epilepsy and JME are genetically distinct syndromes, and gene abnormalities associated with JME have been localized to chromosomes 6p, 9, and 15q.

We present 11 cases of IGE characterized by myoclonic seizures beginning in adulthood with accompanying absence or GTC seizures. These patients were initially misdiagnosed with localization-related epilepsy due to the difficulty in classifying them by the current international system and absence of previously reported similar cases. We propose that this series of patients with IGE may represent a distinct syndrome of adult myoclonic epilepsy (AME).

Methods. Patients and clinical evaluation. Four patients were identified at the University of Alabama–Birmingham (UAB) Epilepsy Center, two were evaluated at the Georg–August Universitat Gottingen, and five came from the Epilepsy Clinic of Vanderbilt University, between 1993 and 1996. Clinical history, neurologic examination, and family history were obtained by a board-certified neurologist in our outpatient clinics. The seizures were classified by the guidelines of the International League Against
All patients received EEG obtained with 21 channel machines in accordance with the American Society of Clinical Neurophysiology Guidelines. EEG were interpreted independently by three board-certified electroencephalographers. Brain MRI was obtained in 10 patients using a 1.5 tesla unit, including T1-weighted and T2-weighted images in axial, coronal, and sagittal planes; the other patient received a CT scan of the brain.

**Results. Patients.** The seizure types; age at onset; EEG, neurologic examination, and neuroimaging results; and family history of the 11 patients are presented in the table. The age at seizure onset ranged from 28 to 53 years, with a mean age of 39 years. Retrospective assessment at UAB indicated that the patients were 0.5% of all newly referred epilepsy patients and 10% of all newly referred IGE cases at an adult tertiary care center. Detailed histories identified no indication that myoclonic, absence, or tonic-clonic seizures began before the ages listed in the table. Nine of the patients and all but one affected family member were women. The neurologic and mental status examinations did not identify abnormalities in any patient. All patients completed secondary education without apparent academic difficulties and were employed full-time. No prenatal or perinatal problems, CNS infections, febrile seizures, or head trauma with loss of consciousness were reported.

**Seizure classifications.** Myoclonic jerks were the predominant seizure type in all patients, consisting of brief, synchronous jerks of the upper or upper and lower extremities. These were usually isolated jerks, but sometimes occurred in flurries of repetitive jerks. In two patients the repetitive jerks had caused falls. No consistent time of occurrence was reported, but three patients described a tendency for seizures to occur with fatigue. Absence seizures in patients were described by the patients’ family members as brief episodes of staring with subtle eye blinking and jaw movement lasting 5 seconds without postictal confusion or behavior changes. All patients with GTC seizures reported rates of fewer than 1 per year. Several patients reported that the GTC seizures occurred in the morning soon after waking from sleep. No aura or focal features were described, and all terminated within 3 minutes. No signs or symptoms indicative of partial seizures were reported.

**EEG and MRI.** The background rhythms appeared normal in all patients in the waking, drowsy, and sleeping states. Patients 1 through 4 and 7 through 11 had recurrent 1- to 4-second runs of generalized, anteriorly predominant, 3 to 4 Hz, 50 to 125 μV spike or polyspike and slow wave complexes. The epileptiform complexes were more frequent during drowsiness, but occurred during wakefulness and stage I and II sleep. An example of a spike and slow wave burst from Patient 3 is shown in figure 1. Patients 5 and 6 had similar activity induced by photic strobe stimulation at 10 to 16 Hz consistent with a photoparoxysmal response. The epileptiform activity appeared to be activated by hyperventilation in Patient 2. Detailed visual inspection with attention to the gray–white junction throughout all regions of each lobe in all MRI and CT scans revealed no abnormalities. Although quantitative evaluations of MRI have described widespread structural changes in another series of IGE, these analyses were not performed on our patients.

**Treatment response.** Valproate controlled all seizures for a sustained period of more than 2 years before the most recent clinical assessment in Patients 5 through 7 and 9 through 11. Absence and myoclonic seizures continued at a reduced rate in Patient 1 while treated with maximum tolerated doses of valproate. All seizures were subsequently controlled in Patient 1 with 100 mg lamotrigine twice a day. Patients 2 and 4 experienced only infrequent myoclonic jerks on valproate, but requested to convert to lamotrigine due to weight gain. They have remained without myoclonic or absence seizures for over 2 years. Patient 3 had frequent absence and myoclonic seizures on phenytoin, carbamazepine, and gabapentin, but is seizure-free for more than 1 year on lamotrigine. Patient follow-up was at least 6 months for all patients.

**Table Summary of age at seizure onset, clinical evaluation, and family history of 11 patients with idiopathic generalized myoclonic epilepsy beginning in adulthood**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at onset of myoclonic seizures, y</th>
<th>Age at onset of absence seizures, y</th>
<th>Age at onset of GTC seizures, y</th>
<th>EEG</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>37</td>
<td>—</td>
<td>poly S/W</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>34</td>
<td>—</td>
<td>poly S/W</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>S/W</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>S/W</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>—</td>
<td>28</td>
<td>PPR</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>—</td>
<td>41</td>
<td>PPR</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>—</td>
<td>32</td>
<td>S/W</td>
<td>Sister with febrile seizures</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>—</td>
<td>51</td>
<td>S/W</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>27</td>
<td>27</td>
<td>S/W</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>—</td>
<td>29</td>
<td>S/W</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>41</td>
<td>—</td>
<td>38</td>
<td>poly S/W</td>
<td>Sister with febrile seizures</td>
</tr>
</tbody>
</table>

All patients had normal results on neurologic, MRI, and CT examination.

GTC = generalized tonic-clonic; S/W = generalized spike and slow wave complexes occurring in 1–4 second bursts at 3–5 Hz; PPR = photoparoxysmal response.
Family history. No seizures were reported in the family members of Patients 2, 3, 5, 9, and 10. Patient 1 described GTC seizures in her first cousin beginning at 18 years of age. She was uncertain about the presence of myoclonic seizures. Patient 6 reported that her mother and three maternal aunts experienced absence and GTC seizures on awakening beginning after the age of 30 years; her sister had an isolated GTC seizure during pregnancy. Her mother continues to show 3 to 5 Hz polyspike and slow wave bursts in her EEG at 74 years of age, but has been seizure-free on phenobarbital for several years. The family pedigree of Patient 6 is shown in figure 2. Patients 7 and 11 each had a sister with simple febrile seizures in early childhood. Patient 8 had a son who developed GTC seizures following encephalitis in childhood.

Discussion. The nosologic classification of IGE is often difficult due to the overlap of seizure types of the four described non-neonatal syndromes of childhood absence, juvenile absence, JME, and GTCA. Substantial evidence, however, supports that these are clinically distinct syndromes with genetic differences. In a recent review of the IGE, Janz emphasized that concordance of IGE syndromes within families and syndromic concordance between monozygotic twins supports a genetic difference. He suggests that these observations should encourage clinicians “to play a more active role in defining the phenotypes, identifying informative families, and helping select possible linkage or gene target.”

We present 11 cases of IGE beginning in adulthood with myoclonic jerks as the predominant seizure type. Except for age at onset, the clinical and EEG features are typical for an IGE. Although these cases could represent phenotypic variants of JME or GTCA, two patients did not experience GTC seizures and the age at onset was beyond the upper range reported in prior systematic descriptions of the syndromes. One patient, furthermore, had strong concordance of age at onset among multiple family members in different generations, supporting a ge-
Corpus callosum size in autism

Article abstract—The size of the seven subregions of the corpus callosum was measured on MRI scans from 22 non–mentally retarded autistic subjects and 22 individually matched controls. Areas of the anterior subregions were smaller in the autistic group. In a subsample, measurements were adjusted for intracranial, total brain, and white matter volumes and the differences between groups remained significant. No differences were found in the other subregions. This observation is consistent with the frontal lobe dysfunction reported in autism.

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Structural and functional studies have detected a variety of brain abnormalities in autism suggesting the underdevelopment of the neocortical neural networks, including the circuitry of the frontal systems. Reduced metabolic correlations have been reported in a group of mostly high-functioning individuals with autism (HFA) involving the frontal and parietal cortex and subcortical structures. A PET study of blood flow in preschool autistic children revealed evidence of delayed maturation of the frontal lobes. Recently, a study of saccadic eye movements in HFA reported significant abnormalities in voluntary saccades subserved by the circuitry of frontal systems.

Recent neuroanatomic studies of the corpus callosum (CC), an index of neural connectivity between brain regions, provide impetus for investigating its role in autism. The CC is topographically organized

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Corpus callosum size in autism

One non-Japanese report of myoclonic seizures beginning after early adulthood described two patients, one with absence seizures in adolescence and another with EEG features atypical for IGE. Plaster et al. recently reported gene localization of familial AME (FAME) to chromosome 8q24. The 21 patients from four Japanese families differed from our series due to the presence of persistent tremulous finger movements, progressively worsening myoclonus, and lack of absence seizures. None of our patients displayed these findings. Based on the prominent phenotypic differences, we believe that FAME is a different clinical syndrome from AME.

If age at onset signifies a biologic difference between the IGE, our series may represent a distinct syndrome of AME. The family pedigree in Patient 6 suggests an autosomal dominant inheritance with high penetrance, but that of the entire sample indicates more complex genetics as seen in most other IGE. Recognition of this syndrome is critical for further phenotypic and genotypic definition. Accurate characterization of the IGE is necessary for appropriate clinical diagnosis and management of patients with these syndromes, as well as development of new treatments based on more complete understanding of their genetic and biochemical causes.

References


Figure. Subregions of the corpus callosum (CC) based on the organization according to Witelson.\textsuperscript{5} Regions denote anatomic label (cortical region[s]). Region 1 = rostrum (caudal-orbital prefrontal, inferior premotor); region 2 = genu (prefrontal); region 3 = rostral body (premotor, supplementary motor); region 4 = anterior midbody (motor); region 5 = isthmus (superior temporal, posterior parietal); region 7 = splenium (occipital, inferior temporal). ACC = most anterior point of the CC; PCC = most posterior point of the CC; M = most superior point of the CC at its midpoint; M1 = most inferior point of the CC at its midpoint; S = superior point on the splenium; S1 = inferior point on the splenium; G = most anterior point on the inner convexity of the CC.

(figure) and matures throughout childhood into young adulthood. Gender differences, testosterone levels, and handedness have been reported to affect CC anatomy.\textsuperscript{5,6} Studies of autistic subjects with a wide range of functioning have documented quantitative abnormalities of the CC and reported the presence of an overall size reduction.\textsuperscript{2,7,8} A smaller body and posterior subregions of the CC were also observed in two different studies examining individuals with autism with and without mental retardation.\textsuperscript{7,8}

We investigated the size of the CC as an index of interhemispheric connectivity. The total cross-sectional area of the CC as well as its seven subregions were measured in 22 HFA and 22 individually matched controls. We hypothesized that the total size of CC would be smaller in HFA compared with the control group, and that the anterior subregions would be most affected, as frontal lobes are the most complex and mature last.

Table 1 Demographic characteristics of the 22 individually matched pairs

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Autistic patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22.4 (10.1)</td>
<td>22.4 (10.0)</td>
</tr>
<tr>
<td>Full-scale IQ</td>
<td>100.4 (14.7)</td>
<td>100.5 (14.2)</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>103.0 (16.4)</td>
<td>100.5 (14.7)</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>97.5 (12.6)</td>
<td>99.6 (12.5)</td>
</tr>
<tr>
<td>Educational level</td>
<td>10.5 (3.2)</td>
<td>10.9 (2.8)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>3.8 (1.7)</td>
<td>3.6 (1.4)</td>
</tr>
</tbody>
</table>

No significant differences were found. Data are presented as mean (SD).

Methods. Autistic subjects met the following inclusion criteria: 1) diagnosis through expert clinical evaluation in accordance with accepted clinical descriptions of HFA and two structured research diagnostic instruments (the Autism Diagnostic Interview\textsuperscript{2} and Autism Diagnostic Observation Scale\textsuperscript{3}); 2) full-scale and verbal IQ >70; 3) absence of known neurologic conditions as determined by neurologic history, examination, and laboratory testing; and 4) ability to provide (or a guardian providing) informed consent. The study was confined to men because sample size was too small to accommodate structural variability associated with gender.

Healthy control subjects met the following criteria: 1) they were neurologically and psychiatrically normal, without developmental disorders; and 2) had a negative family history of autism and neuropsychiatric disorders. Control subjects were individually matched with autistic subjects with regard to race, full-scale IQ, and age, and group matched on handedness and socioeconomic status of the family of origin, as measured by the Hollinghead method (table 1).

MRI scans were conducted using a 1.5-tesla Signa system (GE Medical Systems, Milwaukee, WI). The imaging protocol consisted of two T1-weighted series of images (repetition time [TR], 500 msec; echo time [TE], 20 msec): a coronal scouting series of 5-mm thick images (1-mm gap) to identify the midline plane and a sagittal series of 3-mm thick images (interleaved) parallel to the midline plane. An acceptable sagittal series was one that produced a true midline image as defined by clear visualization of the full extent of the fornix and aqueduct of Sylvius, the anterior commissure, and the primary and the prepyramidal cerebellar fissures.

Images were digitally transferred to optical disks. CC area measurements were made on the midsagittal scans using the approach described by Witelson\textsuperscript{4} (see figure). Scans for 16 participants with autism and 19 control subjects were of adequate quality for measurement of total brain (TBV), intracranial (ICV), and white matter volumes (WMV). There were no differences in the demographics of the resulting participant subject groups. ICV was calculated by summing up areas of successive coronal slices, including CSF, and multiplying by the slice thickness. Images were traced along the edge of the brain in all slices, without excluding dura mater. TBV was calculated by summing up areas of successive coronal slices of the ICV tracings after excluding CSF, dura mater, the cerebellum, and brainstem, and multiplying by slice thickness.

Data were analyzed using the IMAGE software (version 1.45) developed by the National Institutes of Health.\textsuperscript{9} Measurements of regions of interest were performed by an individual (A.H.) who was blinded to the subjects’ diagnostic status, after adequate interrater (A.H. and E.D.) and intrarater reliability (all intraclass correlation coefficients were >0.91; n = 10). Between-group differences were analyzed with paired, two-tailed Student’s t-tests. Analysis of covariance was used to examine differences in the structures of interest after adjustment for TBV, ICV, and WMV. Adjustment for TBV and ICV is essential to account for differences in CC that may be related to brain and body size.

Results. The genu of the CC and the genu plus rostrum were significantly smaller in the autistic group when compared with the normal control subjects. No significant dif-
Table 2 Corpus callosum area measurements (cm²)*

<table>
<thead>
<tr>
<th>Structure</th>
<th>Autistic patients†</th>
<th>Control subjects</th>
<th>p Value</th>
<th>ICV‡</th>
<th>p Value</th>
<th>TBV</th>
<th>p Value</th>
<th>WMV</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>F</td>
<td>p Value</td>
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<td>p Value</td>
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<td>Area</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>0.24 (0.10)</td>
<td>0.27 (0.13)</td>
<td>0.27</td>
<td>3.20</td>
<td>0.08</td>
<td>2.77</td>
<td>0.17</td>
<td>2.93</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>1.30 (0.31)</td>
<td>1.47 (0.25)</td>
<td>0.05</td>
<td>7.20</td>
<td>0.01</td>
<td>7.15</td>
<td>0.01</td>
<td>9.35</td>
<td>0.00</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>1.55 (0.31)</td>
<td>1.74 (0.32)</td>
<td>0.03</td>
<td>5.66</td>
<td>0.02</td>
<td>5.18</td>
<td>0.03</td>
<td>7.02</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.88 (0.19)</td>
<td>0.95 (0.17)</td>
<td>0.13</td>
<td>2.76</td>
<td>0.11</td>
<td>2.18</td>
<td>0.15</td>
<td>2.17</td>
<td>0.15</td>
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<tr>
<td>4</td>
<td>0.74 (0.14)</td>
<td>0.79 (0.13)</td>
<td>0.11</td>
<td>2.64</td>
<td>0.11</td>
<td>2.25</td>
<td>0.14</td>
<td>2.44</td>
<td>0.12</td>
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<tr>
<td>5</td>
<td>0.72 (0.14)</td>
<td>0.70 (0.12)</td>
<td>0.37</td>
<td>0.00</td>
<td>0.99</td>
<td>0.01</td>
<td>0.92</td>
<td>0.01</td>
<td>0.91</td>
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<tr>
<td>6</td>
<td>0.61 (0.14)</td>
<td>0.65 (0.13)</td>
<td>0.16</td>
<td>3.78</td>
<td>0.06</td>
<td>3.22</td>
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<tr>
<td>7</td>
<td>1.79 (0.36)</td>
<td>1.87 (0.30)</td>
<td>0.15</td>
<td>0.61</td>
<td>0.44</td>
<td>0.40</td>
<td>0.53</td>
<td>0.49</td>
<td>0.48</td>
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<tr>
<td>Total</td>
<td>6.29 (0.97)</td>
<td>6.71 (1.00)</td>
<td>0.08</td>
<td>3.82</td>
<td>0.06</td>
<td>3.24</td>
<td>0.08</td>
<td>4.31</td>
<td>0.05</td>
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</table>

Data comparing autistic and control subjects expressed as mean (SD).

* Areas segmented according to Witelson.
† Corpus callosum area measurements for the 22 matched pairs, df (1,43).
‡ Comparison of the corpus callosus regions for the 16 autistic subjects and 19 matched controls when adjusting for intracranial volume (ICV), total brain volume (TBV), and white matter volume (WMV); df (1,32).

Differences in the sizes of the other segments of the CC were found, even after combining the different subregions. There was also a trend in reduction in the total cross-sectional area (table 2). These findings were more pronounced after adjusting for ICV, TBV, and WMV (see table 2). Interestingly, when subjects were divided according to age (21 years), the genu and the genu plus rostrum in the younger group remained smaller in autistic (n = 12) compared with normal control subjects (n = 13) (genu: Student’s t-test = −2.29; df = 23; p = 0.03; genu plus rostrum: Student’s t-test = −2.29; df = 23; p = 0.03). This was not the case in the older group of autistic patients (n = 10) compared with control subjects (n = 9) (genu: Student’s t-test = −0.966; df = 17; p = 0.35; genu plus rostrum: Student’s t-test = −0.55; df = 17; p = 0.6). No significant differences were observed in the total area or other subregions of the CC in either age group.

Discussion. This study revealed a decrease in the size of the anterior regions of the CC, and a strong trend toward a decrease in the overall size that may have reached a significant level with a sample size of 45. The greatest reduction in CC area in the autistic participants was found in the genu (region 2), involving the projections from prefrontal cortex. This is consistent with the cognitive, neurophysiologic, and behavioral evidence of frontal lobe dysfunction reported recently in the literature.²⁴ There have been reports of deficits in executive function,² spatial working memory,² and the capacity for suppressing context-inappropriate responses in autism.¹ Interestingly, the orbital frontal cortex, which projects through the rostrum (region 1), has not been investigated in autism but is thought to play a significant role in the ritualistic behavior of obsessive-compulsive disorder and may make a similar contribution to such behavior in autism.⁹ Furthermore, our finding of decreased size of the anterior regions of the CC may reflect the regional enlargement of parietal, temporal, and occipital but not the frontal regions.¹⁰ This discrepancy may also reflect an increase in intrahemispheric connectivity and a decrease in the interhemispheric one.

The reduction in the total cross-sectional area of the CC observed here may indicate a decrease in interhemispheric connectivity, whereas the increase in TBV may indicate an increase in local connectivity. This dissociation between the size of the CC and TBV may indicate a developmental delay in the emergence of more widespread connectivity of the neural systems for the higher order cognitive abilities impaired in autism. Our findings of the reduction in size of the anterior subregions of the CC differ from those reported previously⁷,⁸ and may reflect the differences in the maturational stage of the brain between the autism groups, in that the subjects included in the previous studies were more severely affected than those in the current one. Alternatively, and perhaps less likely, the differences in results between studies may reflect the disproportionate number of women in prior control groups, the different segmentation strategies implemented, and the lack of control for handedness. Therefore, further imaging studies of this structure need to examine larger sample sizes with varying levels of autism severity, and should carefully consider potential confounding variables such as TBV, handedness, gender, and age. In the absence of such studies, a meta-analysis of the available data from different investigators would be desirable to resolve these contradictions.

Acknowledgment
The authors thank Dr. John Holtuurn and Elizabeth Dick for assistance in the measurement procedures. The efforts and com-
mitment of the participants and their families in this study are gratefully acknowledged.

References

We report a 22-year-old man with metachromatic leukodystrophy (MLD) due to a novel mutation in the arylsulfatase A (ASA) gene, who presented with an isolated polyneuropathy.

Case report. A 22-year-old man developed acute left-hand weakness 2 weeks before our evaluation. There was no known antecedent injury and the his medical history was otherwise unremarkable. His parents were second cousins of Asian Indian background. Family history was remarkable for Erb’s palsy in a younger brother. The patient was a bright, athletic, and energetic young man who was employed as a financial analyst for a large company. The patient’s examination was remarkable for moderate weakness of left hypothenar and interosseous hand muscles, and sensory loss involving the left hand (digits 4 and 5). Results of the neurologic examination were otherwise normal. About 6 months after his first evaluation, he awoke one evening with similar numbness involving the right hand. One year following his initial evaluation, all symptoms had completely resolved.

Nerve conduction studies, performed during the initial evaluation (table), showed evidence of a left ulnar mononeuropathy superimposed upon a diffuse, uniformly demyelinating, sensorimotor polyneuropathy. Electromyography (EMG) was remarkable for fibrillations and positive waves, and reduced recruitment of larger-amplitude motor unit action potentials limited to the left first dorsal intersosseous, abductor digiti minimi, and flexor carpi ulnaris. Results of the following studies were normal or unremarkable: cranial MRI scan, CSF studies, serum chemistries, visual and brainstem evoked potentials, EEG, and mutation analyses for the Charcot–Marie–Tooth neuropathies 1A, 1B, and 1X. The Wechsler Adult Intelligence Scale III testing revealed a full scale IQ score of 124, verbal score of 128, and performance score of 114.

Epon-embedded sections of sural nerve, 1-mm thick, revealed a mild reduction in myelinated fibers, many thinly myelinated fibers, and Schwann cell inclusions (figure 1). Onion bulb formations were rare and regenerating axon clusters were absent. Ultrastructural studies revealed prismatic Schwann cell inclusions with periodicity of 5.6 to 5.8 nm (see figure 1). Morphometric data revealed the following values: myelinated fiber density, 3.966/mm² (normal, 6,906 ± 329/mm²); mean fiber area, 26.7 μm² (based

Adult-onset MLD: A gene mutation with isolated polyneuropathy

Article abstract—A 22-year-old man presented with recurrent ulnar mononeuropathies and diffusely slow nerve conduction velocities. Arylsulfatase A (ASA) activity from leukocytes and fibroblasts was reduced, and urinary sulfatides were increased. Sural nerve biopsy revealed a reduction in myelinated fibers and Schwann cell inclusions. Results of studies of CNS integrity, including cranial MRI, evoked potentials, and neuropsychologic tests, were normal. Molecular genetic analyses revealed a novel homozygous missense mutation (Thr286Pro) in the ASA gene.

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Table Nerve conduction study results

<table>
<thead>
<tr>
<th>Nerve stimulation record sites</th>
<th>Amplitude, uV or mV</th>
<th>Distal latency, ms</th>
<th>Conduction velocity, m/sec</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>L</td>
<td>N</td>
</tr>
<tr>
<td>Sural sensory</td>
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<td></td>
<td></td>
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<tr>
<td>Foreleg–ankle</td>
<td>5.6</td>
<td>5.3</td>
<td>&gt;6</td>
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<td>Median sensory</td>
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<tr>
<td>Wrist–digit 2</td>
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<td>Wrist–digit 5</td>
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<td>Forearm–wrist</td>
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<tr>
<td>Ankle–EDB</td>
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<tr>
<td>Wrist–hypothenar</td>
<td>7.9</td>
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<tr>
<td>Below elbow–hypothenar</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*F-wave latencies: tibial = 74.0 msec (N < 55); median = 34.5 msec (N < 31).
†The proximal tibial compound muscle action potential waveforms are markedly desynchronized.

AH = abductor hallucis; EDB = extensor digitorum brevis; N = normal value; NR = no response.

on 333 fibers) (normal, 37.8 ± 1.9 μm²); and mean g-ratio, 0.78 (based on 200 fibers) (normal, 0.65).

Methods. Enzyme activity for ASA was determined in leukocytes and cultured skin fibroblasts. The $K_m$ and $V_{max}$ for fibroblast ASA activity were determined using nitrocatchol sulfate as substrate, using assay conditions as previously described, with the substrate concentration ranging from 0.2 to 8 mM.

DNA was extracted from peripheral blood leukocytes using the Puregene extraction kit (GENTRA Systems, Minneapolis, MN). Briefly, erythrocytes were first lysed and removed by centrifugation. The remaining cell pellet was then resuspended in cell lysis solution. Subsequent to protein precipitation, the supernatant was removed to a clean microcentrifuge tube containing 300 μL 100% isopropanol to precipitate DNA. DNA hydration solution was then added and the sample allowed to rehydrate overnight at room temperature or at 65 °C for 1 hour. DNA concentrations were determined by absorbance spectrophotometry. Screening of mutations 459 + 1 G—A (allele I) and C2381T (allele A) of the ASA gene was performed essentially as described, in individual amplification reactions with endonucleases Xba I and Pst. Screening of mutation T799G was performed as described by Gomez Lira et al. Genomic DNA was amplified by restriction site generating PCR (RG-PCR), using a modified F primer 5’TGAC-CAGGGCCTGGTCCCGA3’ in exon 3, which creates a restriction site for Sau IIIA1. The amplified product was digested for 3 hours and then analyzed by 12% acrylamide electrophoresis. The eight exons and exon–intron boundaries of the ASA gene were amplified as previously described. We performed heteroduplex analysis on the amplified products by standard procedures, mixing amplified fragments of the patient with amplified control fragments to create heteroduplexes. Direct sequencing of all eight exons and exon–intron boundaries was performed using an automatic ABI 377 PRISM DNA Sequencer (Applied Biosystems, Foster City, CA). Genomic DNA was amplified and then digested with Bsa I for 3 hours at 50 °C. Digested products were analyzed by 15% acrylamide gel electrophoresis.

Results. Arylsulfatase A activity was 6.8 nmol/mg protein/h from leukocytes (normal, 44 ± 11) and 172 nmol/mg protein/h from cultured fibroblasts (normal, 673 ± 408). Kinetic analyses for fibroblast ASA revealed a $K_m$ value of 2.2 mM for normal control fibroblasts and 2.3 mM for the patient. The $V_{max}$ value was 985 nmol/mg protein/h for the normal control pooled fibroblasts and 44 nmol/mg pro-

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tein/h for the patient. The urinary sulfatide level was 0.58 nmol/mg creatinine (normal, 0.10 ± 0.05).

Direct sequencing of all amplified fragments demonstrated a homozygous transition, A1505C, in exon 5 (figure 2). The transition determines a substitution of threonine by proline at codon 286 (Thr286Pro) in the amino acid chain. The mutation lies near the primer F used to amplify exon 5. Because the mutation abolishes a Bsa I restriction site, it was possible to confirm the transition by restriction and electrophoresis of the digested products. Sequencing of exon 7 revealed that the patient was homozygous for a common polymorphism (Thr391Ser). The three most common MLD mutations (459 + 1 G→A, C2381T, and T799G) and the pseudodeficiency allele (Asn350Ser) in exon 6 were not detected. Sequencing of the entire coding sequence and exon–intron boundaries revealed no other sequence alteration.

**Discussion.** Metachromatic leukodystrophy is an autosomal recessive disorder due to a deficiency of ASA, the lysosomal enzyme that converts cerebroside sulfatide, a major component of myelin, to cerebrosides. The ASA gene, localized at 22q13.31-qter, contains eight exons along a 1.5-kb coding sequence and, thus far, over 60 mutations have been identified. The reduced activity of the ASA enzyme leads to accumulation of sulfatides in Schwann cells and oligodendrocytes. It is speculated that the accumulating sulfatides cause neurologic dysfunction by rendering the molecular structure of myelin unstable, interfering with the function of Schwann cells and oligodendrocytes, or reducing the critical temperature needed for lipid bilayer stability.

Based on age at onset and disease severity, three forms of MLD are recognized: late-infantile, juvenile, and adult. Adult-onset MLD is the least common form; the initial symptoms usually include progressive intellectual decline, behavioral changes, and frank psychosis. The diagnosis of MLD is confirmed by demonstrating a deficiency of ASA activity in leukocytes or cultured skin fibroblasts, and increased urinary sulfatide levels. Supportive data include EMG evidence of a demyelinating sensorimotor polyneuropathy, cranial MRI evidence of a diffuse white matter disease, neuropsychologic testing evidence in support of a progressive dementia, and sural nerve

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**Figure 1. Sural nerve histopathology.** (A and B) Semithin transverse (×400 before reduction) and longitudinal (×1000 before reduction) sections stained with toluidine blue show a mild reduction in myelinated fibers, many thinly myelinated fibers, and Schwann cell inclusions (arrows). (C) Electron microscopy demonstrates prismatic inclusions within a Schwann cell (×39,000 before reduction). (D) At higher magnification, these inclusions show a periodicity of 5.6 to 5.8 nm (×73,000 before reduction).
histopathologic evidence of a demyelinating polyneuropathy with metachromatically stained granules and characteristic Schwann cell inclusions.6,9

To our knowledge, this is the second reported case of MLD with isolated peripheral nervous system involvement.10 In contrast to the previous report, our patient’s polyneuropathy was subclinical and only discovered during the EMG workup for his left hand weakness. Recurrent mononeuropathies, as in our patient, have not been previously reported in MLD. It is possible that these were unrelated to MLD; however, given the absence of recognized ulnar nerve compression or trauma, a relationship seems plausible. Akin to the clinical findings in other neuropathies (e.g., hereditary neuropathy with liability to pressure palsies), we suspect that the MLD may have increased our patient’s liability to compressive ulnar mononeuropathies. The novel homozygous Thr286Pro missense mutation adds to the growing list of previously reported ASA gene mutations. The substitution of threonine by proline may have a major effect upon polypeptide structure and orientation because of the restrictions imposed by the proline ring and, therefore, is likely responsible for producing the mutant ASA enzyme.

Acknowledgment
The authors thank Dr. Keshav Rao for referring the patient, Dr. Richard Simon for performing the sural nerve biopsy, and Conceitina Gillies for performing the electron microscopy.

References
CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy

Article abstract—Familial hemiplegic migraine is caused by CACNA1A missense mutations in 50% of families, including all families with cerebellar ataxia. A patient with healthy parents, who experienced prolonged attacks of migraine with hemiplegia, coma, and seizures, is reported. The patient also had mental retardation, permanent cerebellar ataxia with cerebellar atrophy, and right-sided brain atrophy. This patient carried a de novo Tyr 1385 Cys mutation in the CACNA1A gene and illustrates a novel phenotype associated with CACNA1A mutations.

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Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura. The first FHM gene, CACNA1A, encodes the pore-forming α1A subunit of neuronal P/Q-type Ca2+ channels. Whereas the pure form of FHM is genetically heterogeneous, all cases with cerebellar symptoms studied to date are due to mutations in the CACNA1A gene.

Some unusual clinical aspects have been described in FHM. First, patients may undergo severe attacks with confusion, coma, fever, and hemiplegia lasting for days or even weeks. Diagnosis of FHM may be very difficult to establish in such cases. Second, FHM patients may develop a disabling ataxia with marked cerebellar atrophy. Finally, some sporadic cases of hemiplegic migraine have been reported, which differ from familial cases only by the absence of any affected relative.

We report a patient who had recurrent, severe, and prolonged attacks of hemiplegic migraine. In addition, she had mental retardation and early-onset disabling ataxia with cerebellar atrophy. Genetic screening led to the identification of a novel de novo missense mutation in the CACNA1A gene in this hemiplegic migraine patient.

Patient and methods. The proband, aged 33 years, and her two parents, aged 57 and 58 years, of French Caucasian ancestry, were examined. The patient had a normal birth; she was unable to sit until 2.5 years of age and did not walk until age 7. On examination, she had a severe cerebellar gait ataxia, bilateral limb incoordination, a slight dysarthria, and gaze-evoked nystagmus. She had also mental retardation with an estimated IQ of 40.

At age 7, the patient developed fever (39°C) over 1 hour and a dense left hemiplegia with altered consciousness. EEG on day 7 recorded delta activities over the entire right hemisphere extending to the left frontal region and focal spike-and-wave complexes in the right retrolenticular region. Blood examinations excluded a metabolic or infectious encephalopathy. She remained stuporous for 7 days and hemiplegic for 10 days and then gradually recovered over 4 weeks without sequelae.

The second attack, at age 8 years, consisted in a right hemiplegia with fever, headache, and vomiting that gradually subsided over 3 weeks. The third attack occurred at age 25. The patient had a rapid onset of mental obtundation, vomiting, photophobia, right hemiplegia, and fever. CSF was normal on day 1, but showed an aseptic meningitis with 114 cells/mm³ (95% polynuclear) on day 2. Brain MRI on day 7 showed diffuse left cortical abnormalities suggestive of edema, a right cerebral hemisphere atrophy, and a cerebellar atrophy (figure 1). Contrast angiography on day 15 disclosed multiple segmental narrowing of the intracranial branches of the left carotid artery and of the basilar artery and a vasodilatation of the distal branches of the left hemisphere arteries. Temperature and consciousness returned to normal on day 2. CSF was normal on day 5. The motor deficit fully recovered over 4 weeks. On a follow-up MRI with MR angiogram, the left cerebral hemisphere and the vessels returned to normal.

At age 31, shortly after the patient’s exposure to the sun, a left hemiparesis progressed to hemiplegia within a few hours, with a fever up to 40°C, photophobia, severe headache, nuchal rigidity, and mental obtundation. Several left-sided tonic contractions occurred. The motor deficit fully recovered in 10 days. The patient’s most recent attack occurred at age 33, again during the summer. She presented with a rapidly progressive obtundation, vomiting, mutism, and a right hemiparesis that worsened over a few hours. Brief recurrent episodes of tonic eye deviation suggestive of partial seizures were noticed. CSF was normal. MRI showed diffuse left cortical abnormalities suggestive of edema in addition to the unchanged cerebellar and right cerebral atrophy. On day 3, she was afebrile with normal consciousness. After 3 months, examination showed a persistent slight aphasia and normal MRI results, except for the atrophy (data previously reported).

The parents never had migraine attacks, and their neurologic examination was normal.

DNA from the proband and her two parents was extracted from peripheral blood using standard procedures. In addition, DNA from 100 unrelated healthy subjects...
(French Caucasian individuals) was also available for the study. All 47 exons of CACNA1A were screened using a combination of single-strand conformation polymorphism and sequencing analysis, as previously described. To exclude false paternity and confirm the de novo nature of the mutation, the proband and her parents were genotyped with three intragenic markers, two CA repeats flanking the 6-cM interval containing CACNA1A (D19S221 and D19S226), and eight polymorphic CA repeats located on different chromosomes, using procedures previously described. The three CACNA1A intragenic markers were the GCG repeat in exon 1, D19S1150 (CA repeat located in intron 7), and the CAG repeat contained in exon 47. The eight other markers used to establish paternity and maternity were D1S233, D3S1192, D5S407, D6S282, D7S479, D9S152, D10S537, and D14S80.

Results. A novel missense mutation located in exon 26 was identified. This A/G substitution at codon 1385 (codon TAC/TGC) leads to the replacement of a preexisting tyrosine for a cysteine. This mutation was absent in the panel of 200 normal chromosomes. Sequence analysis of exon 26 in both parents showed homozygosity for the normal sequence (TAC/TAC at codon 1385) (figure 2).

Polymorphic marker study allowed us to exclude false paternity as well as false maternity (data not shown) and to establish the de novo nature of this mutation. The number of CAG repeats at the 3’ coding end of the gene was in the normal range (13/13).

Discussion. We have characterized the first de novo CACNA1A mutation occurring in a hemiplegic migraine patient. Several arguments strongly suggest that this Tyr 1385 Cys mutation causes the hemiplegic migraine syndrome of our patient, is indeed deleterious, and is not a polymorphism. First, the mutation was not detected in 200 control chromosomes. Second, it was absent in both biologic healthy parents. Last, it affects segment 5 of the third domain of the channel, an important functional and highly conserved domain involved in calcium selectivity and shown to be affected in previously reported FHM families.

One of the reasons why the phenotype of this patient is so severe and peculiar may be owing to the nature of the mutation itself, which has never so far been reported in FHM families. The clinical phenotype of this patient is indeed remarkable by the association of particularly severe hemiplegic migraine...
attacks with unusual permanent neurologic signs. Hemiplegic migraine attacks lasted up to several weeks with a massive but fully reversible hemiplegia associated with coma, hyperthermia, meningeal signs, and partial seizures. To firmly conclude the role of this novel mutation, one would need to genetically study previously reported cases similar to ours. Electrophysiologic studies would also be helpful to better understand the very strong genotype/phenotype variations that exist between different hemiplegic migraine mutation carriers, despite the fact that these mutations are located in the same regions.

The severe cerebellar atrophy in our patient is also probably related to the Tyr 1385 Cys mutation as, first, 20% of FHM families have associated permanent cerebellar signs, second, the length of the CAG repeat was within the normal range excluding SCA6, and third, P/Q-type voltage-dependent calcium channels are the major calcium channels in Purkinje cells.

This patient has a hemispheric brain atrophy that has not so far been reported in migraine. This atrophy could be the consequence of repeated and severe hemiplegic migraine attacks leading to permanent neuronal injury. However, in our patient, the atrophy was unilateral, whereas attacks occurred on either side. Another hypothesis would be that of a cerebral hemiatrophy secondary to the focal seizures, as reported in childhood focal status epilepticus. However, our patient had only rare focal seizures, always occurring during the severe hemiplegic migraine attacks in the absence of focal status epilepticus.

It is also possible that there is a link between the early-onset profound mental retardation and the Tyr 1385 Cys mutation, particularly as no other metabolic or congenital etiology could be identified. In addition, a mild mental retardation has been noted in some FHM-affected family members. Mental retardation has also been reported in episodic ataxia type 2 families with CACNA1A mutations leading to protein truncation.

References

Indomethacin reduces CSF pressure in intracranial hypertension

Intracranial hypertension (IH) is a syndrome of unknown cause that predominantly occurs in obese women. IH is characterized by increased intracerebral pressure (ICP), normal CT or MRI findings, and a normal CSF composition.1 Symptomatic cases due to sinus venous thrombosis, drug side effects, or hormonal disequilibrium must be excluded. Patients complain of headaches, transient visual obscurations, pulsatile tinnitus, diplopia, and visual loss.1

IH is treated by lowering ICP, preventing visual loss secondary to papilledema, and reducing headache intensity. Conservative therapy includes normalization of weight, repeated lumbar puncture, and drug therapy. Only acetazolamide has been shown to decrease CSF pressure. Other diuretics are frequently used in IH, but their effects are often difficult to interpret because symptoms frequently resolve following lumbar puncture.1 When conservative therapy fails, surgery (optic nerve sheath fenestration, lumbo-peritoneal or ventriculoperitoneal shunting) is the only alternative to prevent visual loss.

Because normalization of body weight, although having a favorable effect on IH, is time consuming, additional conservative therapies are desirable to spare patients the prospect of surgery. Recent reports indicate that indomethacin may lower ICP.2,6 Indomethacin is not an established modality for treating increased ICP, and to the best of our knowledge, its use for treatment of IH has not been reported previously. We report on the effects of IV indomethacin administration on CSF pressure in eight patients with IH.

Patients and methods. Eight patients with documented elevation of CSF opening pressure were included. All patients underwent at least one MRI examination, including contrast-enhanced imaging, and a diagnostic lumbar puncture. Routine blood tests included measurement of thyrotropin, thyroxine, and tri-iodothyronine as a screening for endocrinopathy. Vitamin A or drug-induced IH were excluded by the patient’s drug history. Patients 1 through 7 were diagnosed with idiopathic IH according to the classification criteria of the International Headache Society.2 Patient 8 was diagnosed to have symptomatic IH due to a non–space-occupying meningioma en plaque. Patients 1 through 7 either did not tolerate acetazolamide therapy or repeated lumbar puncture, or refused surgery after progressive visual loss during treatment with acetazolamide. All patients gave their consent to participate in the study after being informed in detail about potential risks and side effects of indomethacin.

All patients were placed in a comfortable lateral position. A 20-gauge needle was introduced between the third and fourth lumbar vertebrae, and a manometer was immediately attached. The CSF opening pressure was determined when the reading remained stable for over 5 minutes (maximal accepted variation ±10 mm H2O); then 50 mg indomethacin was injected intravenously over 1 minute and CSF pressure was documented for the next 10 minutes. All patients were asked about side effects. Thereafter, 20 to 30 mL of CSF were drawn to further lower CSF pressure.

Results. The clinical data from the patients are shown in the table. CSF opening pressure was significantly elevated in all patients (mean, 400 mm H2O; range, 350 to 500 mm H2O; normal, ≤200 mm H2O). During indomethacin administration, all patients showed a marked reduction (mean, 139 mm H2O; range, 80 to 200 mm H2O) of CSF pressure within 1 minute. CSF opening pressure was stable in all patients for the following 10 minutes. Transient side effects were observed in four patients (dizziness lasting 1 to 4 minutes), but they were well tolerated. The table presents CSF pressure data for all patients. After lumbar puncture all patients reported headache relief. CSF composition was normal in all patients. Long-term indomethacin treatment (75 mg orally) was initiated in five patients in addition to standard therapy with acetazolamide or repeated lumbar puncture. All patients reported improvement of headache and tinnitus on follow-up investigations. Ophthalmologic follow-up showed improvement of papilledema grade and visual field findings (enlargement of blind spot, peripheral visual loss) in all patients. No patient required surgery.

Discussion. Eight patients with markedly elevated CSF opening pressure documented by direct measurement during a therapeutic lumbar puncture...
were treated with indomethacin, 50 mg IV. In all patients indomethacin induced a prompt reduction of CSF pressure of 80 to 200 mm H₂O lasting at least 10 minutes. There were no serious side effects. It is difficult to determine whether headache relief was due to the analgesic effect of indomethacin, the CSF-pressure-lowering effect of indomethacin, or the lumbar puncture itself. Although end-expiratory pCO₂ levels were not measured, it is unlikely that hyperventilation caused the reduction in CSF pressure, as all patients exhibited stable CSF pressure over 5 minutes and prompt pressure reduction during the injection.

The literature reveals two patients with Bartter's syndrome who developed IH while receiving indomethacin treatment, probably due to salt and water retention. The follow-up results in our five patients on long-term indomethacin comedication were favorable, and there were no clinical signs of water retention. Water retention might be a contraindication for treatment of IH with indomethacin. Our experience with long-term indomethacin use suggests no adverse effects in IH. As none of our patients had indomethacin monotherapy we can draw no conclusions as to its real long-term effect on lowering ICP in IH.

Elevated ICP can be explained only by a disturbance of the volume equilibrium of the brain tissue, CSF, or blood. The pathophysiology of IH is unknown. Postulated mechanisms include: 1) an increase in brain interstitial fluid; 2) elevated intracranial blood volume; 3) an increased rate of CSF formation; and 4) an increase in venous outflow pressure with either reduced CSF absorption or a compensatory increase in CSF pressure to sustain bulk flow.

According to the pathophysiologic models of IH, treatment should influence the vascular compartment. Several observations reveal that indomethacin influences cerebral hemodynamics. The effects of indomethacin on ICP have been studied in animals, normal volunteers, premature infants, and patients with traumatic brain edema. Indomethacin effectively lowers ICP, improves cerebral perfusion pressure, and causes a substantial dose-related reduction of the cerebral blood flow (CBF). Three mechanisms are believed to cause CBF reduction: 1) the formation of vasodilating prostaglandins is prevented via cyclo-oxygenase inhibition; 2) hyperventilation is induced with mild decreases in pCO₂; and 3) cerebral blood vessels are constricted.

Other cyclo-oxygenase inhibitors have no effect on CBF, either during normocapnia or hypercapnia. Even low doses of indomethacin, which may not affect in vitro platelet aggregation, have been shown to reduce CBF. Thus, the inhibition of vasodilatation via cyclo-oxygenase inhibition cannot be the sole mechanism of CBF reduction, and the changes of

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**Table Clinical data**

<table>
<thead>
<tr>
<th>Patient no./age, y/sex</th>
<th>Height, cm/body weight, kg</th>
<th>Duration of clinical signs</th>
<th>Signs and symptoms</th>
<th>MRI findings</th>
<th>CSF opening pressure, mm H₂O</th>
<th>CSF pressure after IV indomethacin, mm H₂O</th>
<th>Medication (day of puncture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/51/M</td>
<td>180/111</td>
<td>6 y</td>
<td>Papilledema, headache</td>
<td>Normal</td>
<td>360</td>
<td>240</td>
<td>Cilazapril 5 mg/d</td>
</tr>
<tr>
<td>2/28/F</td>
<td>168/73</td>
<td>3 mo</td>
<td>Visual obscurations, tinnitus, papilledema, headache</td>
<td>Tomograms and angiography normal</td>
<td>370</td>
<td>190</td>
<td>Acetazolamide 0.75 g/d</td>
</tr>
<tr>
<td>3/24/F</td>
<td>170/111</td>
<td>3 mo</td>
<td>Mild headache, papilledema congenital nystagmus</td>
<td>Tomograms and angiography normal</td>
<td>400</td>
<td>250</td>
<td>None</td>
</tr>
<tr>
<td>4/41/F</td>
<td>158/90</td>
<td>4 y</td>
<td>Visual loss, optic atrophy</td>
<td>Empty sella sign</td>
<td>475</td>
<td>275</td>
<td>Acetazolamide 1 g/d, furosemide 40 mg/d, bisoprolol 2.5 mg/d, ramipril 5 mg/d, prednisolone 12 mg/d</td>
</tr>
<tr>
<td>5/50/M</td>
<td>180/125</td>
<td>2 mo</td>
<td>Papilledema, tinnitus, mild headache</td>
<td>Normal</td>
<td>500</td>
<td>350</td>
<td>None</td>
</tr>
<tr>
<td>6/21/F</td>
<td>167/87</td>
<td>6 wk</td>
<td>Papilledema, tinnitus, headache</td>
<td>Tomogram and angiography normal</td>
<td>420</td>
<td>340</td>
<td>Acetazolamide 1 g/d, furosemide 40 mg/d, furosemide 40 mg/d, fortocortin 4 mg/d, ranitidin 300 mg/d</td>
</tr>
<tr>
<td>7/48/F</td>
<td>158/72</td>
<td>6 mo</td>
<td>Papilledema, headache</td>
<td>Empty sella sign</td>
<td>350</td>
<td>200</td>
<td>Acetazolamide 0.5 g/d, ramipril 5 mg, xipamid 40 mg/d, doxazosin 4 mg/d, atorvastatin 5 mg/d</td>
</tr>
<tr>
<td>8/24/M</td>
<td>171/76</td>
<td>About 1 y</td>
<td>Mild headache</td>
<td>Right frontal non–space–occupying meningioma en plaque</td>
<td>350</td>
<td>250</td>
<td>None</td>
</tr>
</tbody>
</table>

CSF composition was normal in all patients.
CBF produced by indomethacin cannot account solely for the change in the pCO2 levels.\textsuperscript{3}

The ICP-lowering effects of indomethacin can be explained best by vasoconstriction and a consecutive reduction of the CBF.\textsuperscript{5} After IV administration of indomethacin there is a significant increase in the arteriovenous oxygen difference. Several studies have shown that indomethacin does not cause cerebral ischemia, because the cerebral metabolic rate of oxygen or the net production of lactate does not significantly change after IV administration.\textsuperscript{4,6} In healthy volunteers autoregulation is preserved, because the vasoconstrictive effects of indomethacin are still influenced by hypoxemia and hypercapnia.\textsuperscript{3} Indomethacin does not influence the effects of other standard therapies for ICP control, but it does cause a rebound increase in ICP after drug discontinuation.\textsuperscript{6}

References

Complications during apnea testing in the determination of brain death: Predisposing factors

Apnea testing is a mandatory part of the determination of brain death, but several reports question its safety.\textsuperscript{1–3} Potential complications of apnea testing include severe hypotension, pneumothorax, and cardiac arrhythmia. These complications could compromise procurement of the patient’s vital organs when transplantation is anticipated. More importantly, concerns about these complications may translate into deferral of the apnea test or replacement by a confirmatory test. Patients with preexisting systemic factors (e.g., poor oxygenation, previous cardiac arrhythmia, hypotension, electrolyte abnormalities) may be at increased risk of complications during apnea testing.

American Academy of Neurology (AAN) practice guidelines for performing the apnea test were developed to standardize the procedure and potentially minimize complications during the test, but supportive data have not been recently published.\textsuperscript{4} We therefore investigated the complications of the apnea test in brain death to determine 1) how many tests were performed according to the AAN guidelines and 2) whether inappropriately performed tests had increased risk of complications.

Methods. Serial data were available for 145 apnea procedures performed in 121 patients with a clinical diagnosis...
of brain death at Mayo Medical Center from 1990 to 1999.
Brain death was declared when an irreversible cata-
strophic structural brain lesion resulted in unresponsiv-
earthiness to noxious pain stimuli, absence of brainstem reflexes
(pupillary light responses, corneal reflexes, vestibulo-
ocular tests, tracheobronchial stimulation), and apnea.
Testing for apnea was ideally performed using previ-
ously described guidelines.4 In brief, patients with a clin-
ical diagnosis of brain death were preoxygenated with
100% oxygen for at least 10 minutes. During the apnea test,
patients were disconnected from the ventilator and observed for effective respiratory movements, typically for
5 to 10 minutes. Blood pressure, oxygen saturation, and
heart rate were monitored during the procedure. Arterial
blood gas samples were taken for analysis before discon-
nection from the ventilator and immediately before resum-
ing ventilatory support. An apnea test was considered
positive (i.e., supporting a diagnosis of brain death) if the
post-test arterial partial pressure of carbon dioxide
(PaCO₂) was >60 mm Hg or increased >20 mm Hg from a
normal pretest level and there were no observable effective
respiratory movements. Pre- and post-test EKG and
rhythm strips were also reviewed for dysrhythmias.

The following factors that could potentially increase the
risk of complications during the apnea test were identified in
each patient: failure to preoxygenate with 100% oxygen for
at least 10 minutes before testing; pretest hypotension
(systolic blood pressure <90 mm Hg); administration of
vasopressor drugs; pretest lipo-threatening cardiac ar-
rhythmia; acid–base abnormalities (pH < 7.3 or > 7.5);
and electrolyte abnormalities (plasma sodium >170 or
<120 mEq/L, serum potassium >6.0 or <3.0 mEq/L, se-
um calcium >10.5 or <8.0 mEq/L). The complications
were categorized as a decrease in systolic blood pressure
by 15% or the development of ventricular arrhythmia.

Continuous data were analyzed with linear regression,
and categorical data were analyzed with logistic regression
or Fisher’s exact test where appropriate. Factors were con-
idered significant if the p value was <0.05.

Results. Patient demographic data (table 1) demonstrate
an average age of patients studied of 39 ± 20 years and
nearly equal numbers of men and women. The various
causes of death were similar in patients with and without
complications.

Patients had a wide range of oxygenation (PaO₂, alveo-
lar–arterial oxygen gradient [PₐA – aO₂] before and after
testing; table 2). Pretest arterial PaCO₂ and pH varied less
and were close to the desired range (PaCO₂ = 40 mm Hg
and pH = 7.40). There was a significant increase in arte-
rival PaCO₂ and decrease in arterial pH following comple-
tion of the apnea test. The average rate of rise in arterial
PaCO₂ was 3.7 ± 1.8 mm Hg/min. The above respiratory
parameters were similar in apnea tests with and without
complications.

Systolic and diastolic blood pressures were significantly
higher before apnea tests with complications, but the dif-
fferences were not clinically relevant (see table 2). Systolic
and diastolic blood pressures did not significantly change
following apnea tests without complications, and systolic
and diastolic blood pressures were lower following apnea
tests with complications.

Complications occurred in 38 of 145 (26%) apnea tests.
Unfavorable conditions before the apnea test were present
in 70 of 145 (48%) procedures (table 3). Complications
developed in 27 of these 70 patients (39%) but in only 11 of
74 patients (15%) without these unfavorable factors (p <
0.05). Complications occurred most frequently in patients
with inadequate preoxygenation (14/26) and acid–base or
electrolyte abnormalities (6/29), but were less frequent in
patients with prior cardiac arrhythmia (6/26), with hypot-
ension (3/16), or requiring inotropic agents (25/106). Hy-
potension occurred in 35 of 145 cases (24%). Cardiac
arrhythmia was less common, occurring in 4 of 145 cases
(<1%).

Only one patient began to breathe (at a PaCO₂ of 34
mm Hg) following withdrawal of ventilatory support, but
repeat testing eventually revealed apnea. One patient had
a cardiac arrest during apnea testing.

Discussion. Approximately one in four apnea tests
was associated with cardiovascular complications,
and the rate of complications nearly doubled in tests
without adequate precautions. The majority (85%) of
apnea tests, however, were performed without comp-
lications when adequate precautions were taken.
This suggests that attention to pretest conditions
can minimize potentially adverse circulatory effects
during apnea testing.

Inadequate preoxygenation was clearly associated
with increased complications. Preoxygenation re-
moves alveolar nitrogen stores and facilitates oxy-
gen transport. Thus, performance of apnea testing
in poorly preoxygenated patients can lead to signif-
ificant hypoxemia, even during short periods of no
effective ventilation despite delivery of 100% oxy-
gen to the patient. Hypoxemia is thought to con-
tribute to cardiac arrhythmia or hypotension
during apnea testing.5 Cardiac arrhythmia, hypo-
tension, and increased PₐA – aO₂ gradient from
pulmonary edema occurring before apnea testing
were not associated with an increased risk of
complications.

Hypotension was the most frequent complication
(24%), whereas cardiac arrhythmia with the poten-
tial for ventricular fibrillation or arrest was much
less common (<1%). Hypotension results from the peripher-
al vasodilating and possibly the cardiac de-
pressant effects of carbon dioxide and acidosis
coupled with impaired central autonomic reflexes in
brain-dead patients.6 A small prospective study
indicated no problems with hypotension during well
controlled apnea testing.7 Jeret and Benjamin,1
however, suggested that hypotension occurs in a
significant number of patients (39%). The rela-
tively high average PaCO₂ following the apnea
tests in which hypotension occurred (89 mm Hg)
could have contributed to the increased incidence of
hypotension in their study.

Guidelines for the apneic oxygenation procedure
have been well established, but because of the poten-
tial complications, alternative methods have been
proposed. Lang8 has suggested a possible method of


CO₂ augmentation. This method can produce excessive hypercarbia and lead to severe pulmonary arterial hypertension due to persistent spinal sympathetic vasosconstriction. Another suggested alternative mode of assessing apnea uses hypoventilation to increase PaCO₂, but this approach requires continuous end-

Table 1 Patient demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients with complications, n = 29</th>
<th>Patients without complications, n = 92</th>
<th>Total, n = 121</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>12/17</td>
<td>46/46</td>
<td>58/63</td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>43 ± 18</td>
<td>37 ± 21</td>
<td>39 ± 20</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed head injury</td>
<td>10</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>11</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>Intraparenchymal hemorrhage</td>
<td>5</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Hypoxic–ischemic encephalopathy</td>
<td>2</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>3</td>
<td>4</td>
<td>7</td>
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<tr>
<td>Gunshot wound</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2 Pre- and post-test hemodynamic and respiratory parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Complicated apnea tests, n = 38</th>
<th>Uncomplicated apnea tests, n = 107</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>204 ± 125</td>
<td>248 ± 147</td>
<td>0.09</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td>37 ± 7</td>
<td>38 ± 8</td>
<td>0.5</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.39 ± 0.10</td>
<td>7.39 ± 0.10</td>
<td>0.9</td>
</tr>
<tr>
<td>FiO₂, mm Hg</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>P(A – a)O₂, mm Hg</td>
<td>279 ± 176</td>
<td>269 ± 149</td>
<td>0.8</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>126 ± 30</td>
<td>108 ± 22</td>
<td>0.0007</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>71 ± 23</td>
<td>58 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>Posttest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>212 ± 139</td>
<td>206 ± 141</td>
<td>0.7</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td>66 ± 12</td>
<td>69 ± 10</td>
<td>0.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.17 ± 0.08</td>
<td>7.18 ± 0.09</td>
<td>0.6</td>
</tr>
<tr>
<td>FiO₂, mm Hg</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>P(A – a)O₂, mm Hg</td>
<td>490 ± 233</td>
<td>478 ± 200</td>
<td>0.6</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>93 ± 29</td>
<td>109 ± 25</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic pressure, mm Hg</td>
<td>54 ± 19</td>
<td>57 ± 17</td>
<td>0.5</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.4 ± 1.1</td>
<td>36.6 ± 1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Apnea time, min</td>
<td>12 ± 14</td>
<td>9 ± 5</td>
<td>0.1</td>
</tr>
<tr>
<td>ΔPaCO₂/time, mm Hg/min</td>
<td>3.2 ± 1.9</td>
<td>3.8 ± 1.6</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.

PaCO₂ = arterial partial pressure of carbon dioxide; FiO₂ = fraction of inspired oxygen; P(A – a)O₂ = alveolar–arterial oxygen gradient; ΔPaCO₂ = PaCO₂ post-test – PaCO₂ pretest.

tidal PaCO₂ monitoring or serial arterial blood gas sampling and can result in unpredictable rates of PaCO₂ accumulation. Gradual increases in PaCO₂ may not effectively stimulate respiratory centers. A rapid increase in arterial PaCO₂ to levels of ~60 mm Hg (or 20 mm Hg above normal baseline) is thought to maximally stimulate medullary respiratory centers because CSF is unable to buffer acidosis with blood bicarbonate owing to its slower diffusion than CO₂.

Table 3 Predisposing factors and apnea test complications

<table>
<thead>
<tr>
<th>Variable</th>
<th>Complications, n</th>
<th>Y</th>
<th>N</th>
<th>Complications, %</th>
<th>p Value</th>
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<td>Adequate precautions</td>
<td>11</td>
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<tr>
<td>Inadequate precautions</td>
<td>27</td>
<td>43</td>
<td>39</td>
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<tr>
<td>Predisposing</td>
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<tr>
<td>No preoxygenation</td>
<td>14</td>
<td>12</td>
<td>54</td>
<td>0.0009</td>
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<td>Electrolyte abnormality</td>
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<td>6</td>
<td>50</td>
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<td>7</td>
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<tr>
<td>Inotropic drugs</td>
<td>25</td>
<td>81</td>
<td>23</td>
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<td>Hypotension</td>
<td>3</td>
<td>13</td>
<td>18</td>
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</tbody>
</table>
This study demonstrates that a significant number of apnea tests are performed without adequate precautions or adherence to established AAN practice guidelines. The reason is unclear but likely represents unfamiliarity with the complexity of the apnea test. This is probably not isolated to our institution, as a survey of clinicians in California and Colorado found that <15% of apnea tests were performed adequately. An effort needs to be made to improve the proper execution of the apnea test because this may reduce the risk of cardiovascular complications.

References

Cortical MRI findings associated with rapid correction of hyponatremia

Since Victor and Adams first described central pontine myelinolysis, extrapontine abnormalities have been recognized, and the term osmotic demyelination syndrome (ODS) has been coined. ODS classically follows a marked osmolarity shift, usually by rapid correction of hyponatremia. Pathogenesis remains uncertain. Indeed, debate continues regarding the duration of hyponatremia, rate of correction, and other factors that cause ODS. Diagnosis is often clinical. Neuroimaging may support the diagnosis by showing characteristic lesions, but radiologic findings often lag clinical disease by days to weeks. We present two cases of ODS with extensive gadolinium-enhancing cortical MRI signal abnormalities—findings rarely reported in the literature.1,2

Methods. The two patients presented were cared for or consulted with the authors at San Francisco General Hospital, San Francisco, CA, and Hospital Angeles del Pedregal, Mexico City, Mexico.

Article abstract—The authors describe two patients with clinical manifestations of the osmotic demyelination syndrome (ODS) and unusual MRI findings of gadolinium-enhancing peripheral cortical abnormalities. They propose that these represent extrapontine manifestations of ODS because neither patient had a notable hypoxic–ischemic insult. Recognizing this imaging appearance is important because prognosis in ODS may be less uniformly grim than for hypoxia–ischemia.

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Nicole Calakos, MD, PhD; Nancy Fischbein, MD; J. Richard Baringer, MD; and Cheryl Jay, MD

Case reports. Patient 1. Patient 1 is a 43-year-old, nonalcoholic woman who was found obtunded and dysarthric, without evident cardiorespiratory arrest, head trauma, or seizure. On evaluation she was stable hemodynamically, stuporous, nonverbal, tremulous, rigid, and hypertensive, with dilated reactive pupils. Her serum sodium level was 114 mmol/L. After IV saline was administered her mental status improved and she reported recent diarrhea and thirst. Two days earlier her serum sodium level was 137 mmol/L. The patient’s medical history was notable for schizoaffective disorder, hepatitis C infection, and remote suicide attempts. Medications included haloperidol, benzpropine, fluoxetine, trazodone, lithium, and clonazepam. The results of her liver function tests and abdominal CT were normal.

IV saline was administered at 100 mL/hour. The next morning she was agitated, nonverbal, and moved her extremities spontaneously. Her sodium level 14.5 hours after her initial value was 164 mmol/L. She excreted 4 to 8 L of dilute urine per day and was diagnosed with diabetes insipidus (DI). Desmopressin transiently increased urine osmolality. Her serum antidiuretic hormone level was low, consistent with peripheral DI resulting from lithium.

During the next 3 days her sodium level ranged from 160 to 170 mmol/L despite aggressive free water re-
placement. MRI results on hospital day (HD) 3 were normal. On HDs 4 to 6, her sodium levels normalized to 135 to 145 mmol/L after treatment with indomethacin for DI. On HD 6, the patient developed complex partial seizures, followed the next day by generalized status epilepticus, managed with IV fosphenytoin and mechanical ventilation. Her blood chemistry values were normal and there was no hypoxic event. Head CT revealed multifocal punctate hemorrhages (figure 1).

Examination the next day revealed spontaneous eye opening without cognitive interaction, preserved brainstem reflexes, spontaneous movement of the arms, spasticity, hyperreflexia, and extensor plantar responses. MRI on HD 10 demonstrated a high signal on T2-weighted images bilaterally in the deep gray nuclei and cortical areas (figure 2A); the central pons was normal (figure 2B). After gadolinium administration, the deep gray nuclei and linear cortical areas enhanced (figure 2, C and D). Imaging alone could not distinguish whether these peripheral abnormalities were confined to subcortical white matter, gray matter, or the gray–white junction. By HD 19, MRI showed

Figure 1. Noncontrast head CT of Patient 1 on hospital day 9. (A) Hypodensity of the basal ganglia is noted. (B) Multifocal linear peripheral hemorrhages at the cortical–subcortical junction and hypodensity of the bilateral basal ganglia are noted.

Figure 2. Cranial MRI of Patient 1 on hospital days 10 (A through D) and 38 (E, F). (A) T2-weighted imaging demonstrates abnormal signal of bilateral caudate nuclei, putamina, thalami, and cortical/subcortical frontal and peri-Sylvian regions. (B) T2-weighted imaging demonstrates normal central pons. (C) T1-weighted post–gadolinium imaging demonstrates enhancement of the bilateral deep gray nuclei and linear cortical/subcortical regions. Pre–gadolinium T1-weighted images were unremarkable except for the presence of a small right frontal hemorrhage that was detected on initial CT. (D) T1-weighted post–gadolinium sagittal image demonstrates extensive linear peripheral enhancement. (E) T1-weighted image demonstrates intrinsic signal shortening in deep gray nuclei and cortical/subcortical regions. (F) T1-weighted post–gadolinium image demonstrates resolution of gadolinium enhancement.
extensive T1 shortening peripherally and persistent gadolinium enhancement.

The patient remained clinically stable, with preserved wakefulness, impaired awareness, and spastic quadripareisis. Three weeks after admission, she began to open her eyes, track objects visually, and regain purposeful leg movements. MRI on HD 38 showed progressive T1 shortening and substantial resolution of enhancement (figure 2, E and F). By 6 months, the patient could speak intelligibly, read, and developed symptoms consistent with her previously diagnosed schizoaffective disorder. She regained use of her hands, but required bilateral assistance to walk. MRI 1 year later showed diffuse volume loss with nearly complete resolution of T1 shortening and T2 prolongation, and focal tissue loss within the globus pallidi bilaterally (not shown).

Patient 2. Patient 2 is a 47-year-old hypertensive man with prior alcoholic hepatitis who was admitted after falling, hitting his head, and briefly losing consciousness. Four days previously, he felt unwell and increased his lisinopril and diltiazem. He received IV saline with glucose for 3 days. After discharge, he drank copious fluids, developed peripheral edema, and became dysarthric, agitated, and confused.

On readmission he was in an agitated delirium, without abnormal eye movements or other focal neurologic signs. On HD 2, his serum sodium level was 100 mmol/L. When given IV saline, his sodium level rose to 120 mmol/L.
hours later. He deteriorated, with obtundation progressing to coma, by HD 4. Examination revealed normal oculocephalic reflexes, quadriaparesis, areflexia, and absent plantar responses. On HD 6, he developed seizures that were treated successfully with clonazepam. At this time his sodium level was 131 mmol/L—its highest level during hospitalization. Noncontrast MRI results on HDs 5 and 7 were normal. MRI on HD 13 showed a high signal on proton density-weighted images in the central pons (not shown), bilateral putamina and external capsules, and linear parietal cortical areas with gadolinium enhancement (figure 3, A and B).

One month later, he began to open his eyes, blink, yawn, and withdraw from pain. Later, he regained sleep–wake cycles, but not cognition. MRI at 2 months showed a cavitated mid-pontine lesion, intrinsic T1 shortening of the basal ganglia and cortex, persistently enhancing putaminal lesions, and some resolution of linear peripheral enhancement (figure 3, C through E). Six months later he withdrew to minor stimuli. MRI at 6 months showed resolution of intrinsic T1 signal abnormality, severe generalized volume loss of the brain parenchyma, focal cavitation within the thalami, and the pontine lesion seen earlier (figure 3F). Contrast enhancement resolved entirely (not shown). Minimal clinical improvement occurred over another year.

Discussion. These patients illustrate many well-described characteristics of ODS. Additionally, they highlight variability among ODS patients, including differences in risk factors and outcome. Both demonstrate unusual findings of extensive linear cortical abnormalities detected by MRI that enhanced initially with gadolinium. Although cortical lesions have been described previously in ODS, pathologic findings have been variable.2–7 They include laminar necrosis, laminar demyelination with gliosis, or both.2,4,6,7 These results make it difficult to conclude from radiologic imaging the pathologic basis of the cortical abnormalities.

It has been hypothesized that brain injury in ODS results primarily from hypoxia–ischemia (H-I), but typical laminar necrosis resulting solely from H-I seems unlikely in these two patients. No marked hypoxemia or hypotension was identified in either patient, both of whom were cared for in step-down and intensive care units with close hemodynamic monitoring. Furthermore, both patients sank into coma after correction of their hyponatremia, which is the usual clinical course for ODS and is not consistent with H-I. One explanation for neuroimaging findings resembling laminar necrosis would be that hyponatremia increases vulnerability to mild H-I. In animal studies, H-I impairs compensatory mechanisms in hyponatremia to prevent cerebral edema.8 In both animal studies and postmenopausal women, mortality approaches 100% with concomitant respiratory insufficiency,8,9 suggesting that the combination of hyponatremia and H-I is particularly deleterious. Thus we propose that regardless of whether the pathology underlying the MRI findings is demyelinating or necrotic, its presence is the result of ODS alone or because of ODS increasing susceptibility to minor H-I.

In our two patients we also documented the evolution of MRI changes in ODS. The earliest and most extensive abnormalities were T2 prolongation and gadolinium enhancement. Over time, corresponding T1 signal abnormalities developed and gadolinium enhancement resolved. T1 signal abnormalities resolved by 6 to 12 months. Evolution of these imaging features likely reflects the pathogenesis of ODS. T2 prolongation and gadolinium enhancement may represent the early phase of ODS, which has been proposed to involve blood–brain barrier breakdown as a result of osmotic stress and consequent vasogenic edema.10 Linear T1 shortening, observed later as gadolinium enhancement resolved, may represent accumulation of myelinolytic cellular breakdown products, consistent with this proposed mechanism. Of note, the acute-phase MRI pattern was atypical of H-I in that the cortex is usually more diffusely involved with prominent cortical edema and sulcal effacement, and delayed MRI white matter changes reflecting axonal degeneration were absent.

Acknowledgment
The authors acknowledge the input and advice of Drs. Robert Laureno and Alisa Gean, and the courtesy of Dr. Bruno Estanol in allowing consultation on his patient.

References
Thalamic microglial activation in ischemic stroke detected in vivo by PET and \[^{11}C\]PK11195

**Article abstract**—Using quantitative PET, the authors studied the binding of \[^{11}C\]PK11195, a marker of activated microglia, in the thalamus of patients with chronic middle cerebral artery infarcts. All patients showed increased \[^{11}C\]PK11195 binding in the ipsilateral thalamus, indicating the activation of microglia in degenerating projection areas remote from the primary lesion. A persistent increase in \[^{11}C\]PK11195 binding suggests active, long-term thalamic microstructural changes after corticothalamic connection damage.

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After cerebral ischemia in rodents, increased binding of \[^{3}H\]PK11195—a specific ligand for the peripheral benzodiazepine binding site (PBBS)—is observed in and around the infarcted tissue, colocalizing with invading cells of mononuclear–phagocytic lineage. However, in distant macrophage-free areas with intact blood–brain barrier, the increased binding of PK11195 is a result of the presence of activated microglia, the brain’s intrinsic macrophage, rather than astrocytes that under cell culture conditions may express high levels of PBBS. Increased \[^{3}H\]PK11195 binding can be observed in areas remote from a primary ischemic lesion, particularly in the thalamus, indicating pathologic changes secondary to retrograde or anterograde degeneration of connecting fibers.

We investigated the potential of PET and \[^{11}C\]PK11195 to assess the microglial reaction in secondary thalamic lesions in patients with infarcts in the territory of the middle cerebral artery (MCA).

**Methods.** **Patients.** Seven patients (six men and one woman; mean age, 65 ± 16 years; age range, 39 to 88 years) with unilateral infarct in the territory of MCA were studied between 2 and 24 months after insult. Contiguous T1-weighted MRI, 3-mm-thick axial slices were obtained on the same day as the PET scan using an MRMAX 0.5-T device (General Electric, Milwaukee, WI). In five patients the infarct was large and involved the MCA territory, extending to deep regions in two subjects. In the other two patients, the infarct was smaller, limited to deep structures in one and extending to both cortical and deep MCA territories in the other (table). In Patient 1, the infarct also involved the anterior cerebral artery territory. In Patient 7, MRI revealed old ipsilateral centrum semiovale infarcts.

This study was approved by the ethical committee of the Commissariat à l’Energie Atomique.

**Scans.** All patients were scanned with a CTI 953B PET camera (CTI/Siemens, Knoxville, TN) operated in two-dimensional acquisition mode. A measured attenuation was performed. After a bolus injection of \[^{11}C\]PK11195 (16.7 ± 8 mCi; specific radioactivity, 472 ± 94 mCi/µM) was administered, data were acquired over 60 minutes in 11 progressively increasing time frames from 1 to 10 minutes. Each slice was reconstructed using a Hanning filter. No axial filtering was performed. The final image spatial resolution was 8.4 × 8.4 × 4.8 mm full width at half maximum.

Parametric images of regional \[^{11}C\]PK11195 binding were generated by a three-parameter (simplified) reference region model. Instead of the previously used cerebellar reference region, which in stroke patients may potentially show remote metabolic changes resulting from cerebellar diaschisis, an area (20 cm² in the contralateral hemisphere) previously shown to be unaffected by the primary pathology was defined. The following ratios and rate constants were calculated: the ratio of delivery of radioligand between a target region and the reference region; and an efflux rate constant from the target tissue and a binding potential (BP) defined as \(BP = k_3/k_4\), where \(k_3\) and \(k_4\) are the exchange rates between the free and specifically bound radioligand. The parametric BP maps were generated using a basis function approach on a voxel-by-voxel basis.

Volumes of interest corresponding to right and left thalamus were defined anatomically on the T1-weighted MR image. Mean BP values for the thalamus were determined from the parametric BP maps after coregistration with the individuals’ own MR image.

**Results.** In all patients, regionally increased \[^{11}C\]PK11195 binding was seen in the thalamus ipsilateral to the stroke side (figure). Additionally, in some patients we observed areas of intense binding around subcortical lesions and along subcortical white matter tracts (i.e., the internal capsule, the corticospinal tract). Some increased binding was seen around but not within the cortical and most likely necrotic infarct tissue.

Quantitative analysis of thalamic \[^{11}C\]PK11195 binding revealed BP values ranging from 0.2 to 0.99 in the ipsilateral thalamus (mean ± SD, 0.61 ± 0.30) and from 0
In a previous study that used a more sensitive scanning protocol (i.e., an ECAT HR⁺⁺ PET scanner (CTI/Siemens, Knoxville, TN) operated in a three-dimensional acquisition mode) but the same method of quantification, normal control values in healthy tissue were found to be close to zero. Compared with the data from this study, the group of stroke patients had increased binding (Student’s t-test, unequal variance, \( p = 0.0019 \)) in the ipsilateral but not in the contralateral thalamus (thalamic BP control values, 0.10 ± 0.06).

**Discussion.** The current study shows that a long-lasting significant increase in \[^{11}C\]PK11195 binding can be detected in vivo in the thalamus ipsilateral to the affected hemisphere in stroke patients. In experimental models of stroke, increased \[^{11}C\]PK11195 binding...
binding is seen in the posterior thalamic complex and lateral ventral nuclei of the thalamus ipsilateral to the cortical infarcts involving sensorimotor and anterior auditory cortices\(^1\) whereas more restricted cortical infarcts increased binding in only the latter region.\(^6\) This increase is observed 7 days after infarct\(^1\) and persists beyond a period of 4 weeks.\(^8\) Immunocytochemical data strongly suggest that the increase in \(^{[11]}\text{C}\)PK11195 binding in areas remote from the focal infarct lesion is the result of the increased expression of peripheral benzodiazepine binding sites by activated microglia.\(^8\) This view is supported by the observation that after transient global forebrain ischemia in rats, the time course of increased hippocampal \(^{[3]}\text{H}\)PK11195 binding correlates with microglial activation but not with the changes in glial fibrillary acid protein messenger RNA expression in reactive astrocytes.\(^8\) Similarly, no significant \(^{[11]}\text{C}\)PK11195 PET signal was found in the astrocyte-rich scar tissue of epilepsy patients with hippocampal sclerosis. In these patients the disease was stable and thus showed no disease progression, which itself might cause an activation of microglia.\(^9\)

An important role for microglia in other models of acute, subacute, and chronic brain injury has been reported previously.\(^9\) During the early stages of neuronal injury when the affected brain tissue still appears histologically normal, microglia express numerous molecules with important immune functions and may carry out subtle microstructural changes, such as the removal of synapses (or “synaptic stripping”\(^10\)) from injured neurons. This may lead to a neuronal deafferentation that is potentially reversible if the neuron survives. During later stages, when neuronal cell death has occurred, microglia undertake neuronophagia, removing degenerating synaptic terminals and neuronal debris.\(^10\) Thus, activated thalamic microglia after cortical stroke could be an early mark of subtle thalamic damage and later of definitive neuronal loss resulting from the damage to the thalamocortical/corticothalamic projection fibers. The persistent increase in \(^{[11]}\text{C}\) PK11195 binding suggests that an active tissue pathology leading to microglia activation is present in the ipsilateral thalamus many months after the stroke event. Whether the observation of late pathologic sequelae implies a rationale for late therapeutic intervention remains open. Currently, no experimental study has followed thalamic PK11195 binding beyond 28 days after cortical lesion formation.\(^9\)

References
A new mutation in the prion protein gene: A patient with dementia and white matter changes

The prion gene is localized on the short arm of chromosome 20 and normally contains five octarepeats. The prion protein (PrP) is a sialoglycoprotein that is expressed predominantly in neurons, and its function is unknown. Numerous point mutations and octarepeat inserts in the PrP gene are associated with the accumulation of abnormal, partially protease-resistant protein, and are responsible for the inherited forms of spongiform encephalopathies or prion diseases. We describe the clinical characteristics, MRI abnormalities, and molecular findings in an individual from a Dutch family with a new mutation in the prion gene.

Individual II-1 (index patient). In 1991, a 64-year-old woman with progressive memory impairment for 3 years was seen at the outpatient clinic of another hospital. She took antihypertensive medication. On neurologic examination she had a Mini-Mental State Examination (MMSE) score of 22 points (maximum score, 30 points) and a Cambridge Cognitive Examination (CAMCOG) score of 74 points (maximum score, 107 points). On further neurologic examination, positive snout and palmonental reflexes were noted. Her gait was unsteady.

CT showed severe cerebral atrophy and bilateral frontal leukoencephalopathy. Routine laboratory examination results were normal except for an elevated cholesterol level of 8.9 mmol/L (normal, 3.7 to 7.9 mmol/L) and an elevated triglyceride level of 3.7 mmol/L (normal, 0.6 to 2.2 mmol/L). A diagnosis of probable AD was made.

We saw the patient 3 years later when she was admitted to a psychiatric clinic for observation of behavioral problems. At that time her MMSE score was 5 points, her attention and concentration were impaired, she was dystrophic and used semantic paraphasias, her orientation to time and place was disturbed, the fluency of her speech was reduced, and she had agraphia, apraxia, and acalculia. Her long-term as well as her short-term memory were impaired. Positive pseudobulbar reflexes were noted, and forced laughing and crying were present. Her gait was ataxic. Further neurologic examination was normal.

Brain MRI revealed extensive leukoencephalopathy and severe atrophy of the neocortex without cerebellar atrophy or abnormal intensities in the basal ganglia (figure 1).

Vascular dementia, including cerebral autosomal dominan arteriopathy with subcortical infarcts and leukoencephalopathy, was considered in the differential diagnosis. However, the gradual course rather than a stepwise deterioration, the cortical symptoms, and the absence of cortical or lacunar infarcts on neuroimaging argued against this diagnosis. The presence of a possible positive family history for dementia and the gait disorder did raise a suspicion of human prion disease.

The patient died in 1995, almost 7 years after the onset of her illness. An autopsy was not performed.

Methods and results. Clinical and genealogic assessment. A pedigree was made based on the information of family members and the original hospital records. The clinical characteristics are described next and shown in the table.

Case reports. Individual I-1. The father of our index patient was referred to a psychiatric clinic with memory problems and agitation when he was 60 years old. Physical examination revealed dysarthria, anemia, disorientation, and short-term memory problems. Additional neurologic examination results were normal. Serum investigation revealed a disturbed renal function. Syphilis reactions were negative. EEG showed slow activity, which was attributed to renal insufficiency. No further examinations were performed. The patient died 7 months after the onset of his illness. No information about his family history could be obtained.

Individual I-2. The patient’s mother was referred to a psychiatric clinic at 37 years of age with neurotic depression. At the age of 52 she was readmitted with a severe dementia syndrome. She had been diagnosed with hypertension and diabetes mellitus. On neurologic examination she was hallucinating, had a dementia syndrome with cortical features, and had an ataxic gait. A diagnosis of hypertensive encephalopathy was made. CSF examination revealed a slight pleocytosis (36/3 leukocytes/μL) with 173 leukocytes/μL polymuclear cells. Further investigations were not recorded, and the patient died 14 months later.

Two of her siblings died during childhood, and five other siblings died without signs of a neurodegenerative disease at the ages of 63, 83, 83, 85, and 99 years. It is unknown at what age another of her siblings died.
Individual II-3. At the age of 49 years, the patient’s brother complained of memory disturbances. Neuropsychological investigation revealed slowness of visual and psychomotor functions, and verbal memory dysfunction for new and unstructured material. These deficits were attributed to a depression, which was considered in the differential diagnosis. MRI was not performed. The patient died at age 54 as a result of mesothelioma.

Molecular studies. Blood samples of the proband (Individual II-1) and her brother (Individual II-3) were obtained for DNA analysis. The open reading frame of the PrP gene was amplified by PCR using the primers PrPF72-R530, which generate in the normal PrP gene fragments of 459 base pairs (bp). The oligonucleotide sequence of the primers were TGCTGGTTCTTTGTGGC (forward primer F72) and GTGGCAATGGGGTTGGTTC (reverse primer R530). Restriction endonuclease assay was performed using NcoI restriction endonuclease according to standard conditions. After amplification of the region PrPF72-R530, the proband showed a normal allele with a fragment of 459 bp and an allele with an increased fragment of 507 bp. The PCR products revealed an identical twice-repeated 24-nucleotide sequence inserted between the fourth (normally R3) and fifth (R4) octarepeat (figure 2). The nucleotide sequence of this repeat has been described previously by Goldfarb et al. and is designated as R2a. The inserted octapeptides consisted of a variant of the R2 sequence with a point substitution from GGG to GGA in the seventh triplet—both coding for glycine. The order of the octarepeat sequences in Individual II-1 is different from the family of American ancestry (figure 3). Moreover, in the third octarepeat, the seventh triplet in the normally appearing R2 octarepeat is also changed from GGG to GGA. This repeat is equal to the R2a region of the insertion mutation. In the fourth octarepeat, the first triplet in the normally appearing R3 octarepeat is changed from CCC to CCT, which is equal to R2. Thus, R3 is missing in the PrP gene of our

Table Patient clinical characteristics

<table>
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<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Clinical features</th>
<th>Comorbidity</th>
<th>EEG</th>
<th>CSF</th>
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<td>M</td>
<td>54</td>
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<td>—</td>
<td>—</td>
<td>Died of mesothelioma</td>
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proband. There was homozygosity for valine at codon 129. Individual II-3 had no mutation in the PrP gene.

Discussion. The patient described has a novel variant of a mutation in the PrP gene: a two-octapeptide insert and two nucleotide substitutions in the other octapeptides. In a population of approximately 15 million people in the Netherlands, the mutation described here represents the second prion gene abnormality in a subject of Dutch ancestry. With a moderately progressive dementia of presenile onset, the clinical characteristics of this patient are consistent with those of patients with inherited prion disease reported previously. In the family described by Goldfarb et al., the proband had a history of a rapidly progressive cortical dementia developing from a mutistic to a comatose state, and died 3 months after the onset of her illness. However, the mother of this patient had a two-repeat octapeptide mutation and demonstrated a very gradually progressive global dementia in 13 years.

Neuropathologic examination was not performed in our patient, therefore we cannot define the influence of cerebrovascular changes associated with hypertension and hypercholesterolemia. The nature of the white matter abnormalities remains obscure, but can possibly be attributed to Wallerian degeneration. Most CT or MRI abnormalities in human prion diseases in previous reports mention either no abnormalities, cortical atrophy, or high signal intensities in the basal ganglia. White matter abnormalities, as we report, appear to be rare in spongiform encephalopathy, which is referred to as the panencephalopathic type of Creutzfeldt–Jakob disease (CJD). There are only a few case reports of sporadic CJD with white matter degeneration and cortical atrophy. Including our patient, at least four patients with a hereditary prion disease have had white matter changes.

References

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<td>R2 R2</td>
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**Figure 2. A comparison of the octarepeat region in the normal allele and in the affected subject.**

**Figure 3. A comparison of the order of octarepeats in the wild type, in the American family described by Goldfarb et al. and in our patient. Shaded cells indicate changes from the wild type.**

**Table 1.**
MRI findings in Möbius syndrome: Correlation with clinical features

Article abstract—The authors studied the MRI findings of three patients with Möbius syndrome. Möbius syndrome is a rare congenital disorder characterized by complete or partial facial diplegia accompanied by other cranial nerve palsies. MRI demonstrated brainstem hypoplasia with straightening of the fourth ventricle floor, indicating an absence of the facial colliculus. These MRI features suggest the diagnosis of Möbius syndrome and correlate with the clinical and neurophysiologic findings.

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Möbius (or Moebius) syndrome is a rare disease characterized by facial diplegia associated with paralysis of the lateral gaze movements.1 It is usually associated with numerous cranial and musculoskeletal anomalies.

Necropsy study has shown defects ranging from hypoplasia to necrosis of the cranial nerve nuclei.2 The radiologic findings in Möbius syndrome have been described.3,4 However, there are only three published reports to date containing MRI features of this disorder.5,6,7 We report the MRI findings of three patients with Möbius syndrome.

Case Report. Patient 1. An MRI study was performed a 28-year-old woman presenting with congenital bilateral facial palsy. She was unable to blink her eyes, smile, or frown. She had convergent strabismus with a bilateral deficit of horizontal gaze eye movement. Vertical gaze was intact. Other features of this patient included a small mandible, hearing loss, microstomia, speech and swallowing difficulties, tongue hemiatrophy, and metacarpi–phalangeal deformities. Pupil reflexes were normal. Motor tone, strength, and deep tendon reflexes were normal.

MRI revealed alterations in the morphology of the brainstem, including straightening of the floor of the fourth ventricle, absence of the medial colliculus in the pons (VIth and VIIth nuclei), and an absence of the hypoglossal eminence in the medulla oblongata (hypoglossal nuclei) (figure 1, C, D, and E). The sagittal T1 sequence also showed tongue atrophy.

Facial compound muscle action potential (CMAP) responses were reduced in amplitude. Brainstem auditory evoked potentials (BAEP) were increased in the IVth central conduction, indicating brainstem dysplasia.

Patient 2. An 8-year-old girl presented with facial diplegia and convergent strabismus secondary to bilateral palsy of the facial and abducens nerves. She had no family or gestational antecedents. Physical examination showed bilateral blindness with chororetinal coloboma, right deafness, ear malformation, bilateral epicanthus, micrognatia, and severe mental retardation with a history of self-aggression. The patient also presented with bilateral equinovarus foot.

The BAEP showed low-voltage answer and late latencies. An MRI study demonstrated alterations in brainstem shape and in the floor of the fourth ventricle (figure 2, A and B).

Patient 3. A 28-year-old woman was referred to us for chronic headache. She had mild convergent strabismus since childhood. She had no family or gestational antecedents. Physical examination showed facial diplegia. Eye movements were limited in abduction bilaterally.

An MRI study showed an alteration of brainstem morphology, consisting of a V-shaped depression in the floor of the fourth ventricle (figure 3, A and B).

Discussion. Möbius syndrome consists of complete or partial facial diplegia, often accompanied by other cranial nerve palsies.1 This disorder is usually associated with numerous cranial malformations that can affect any of the cranial nerves, though the IIIrd, IVth, VIth, VIIth, VIIIth, IXth, Xth, and XIIth cranial nerves are most often involved. The cerebellum, hypothalamus, and pituitary gland can also be affected, and oculofacial and limb malformations have been noted.2,6,7,8 Associations with a number of other syndromes have been described, including Poland syndrome,3 arthrogryposis, Robin syndrome, and Corey–Fineman–Ziter syndrome.

The age of our patients at presentation (8 and 28 years of age) is unusual. Möbius syndrome, a congenital disorder, is typically diagnosed in neonates or infants, but many of these patients live beyond infancy,7 and some have been described in the third and fourth decade of life.8

The etiology of Möbius syndrome is multifactorial, and several determinants have been suggested, including genetic, ischemic, and infectious factors.7 The use or ingestion of thalidomide, alcohol, benzodiazepines, cocaine, or misoprostol8 during pregnancy has been implicated in rare instances.

An etiopathologic classification of Möbius syndrome into four types has been proposed9: central lesion in the brainstem nuclei due to congenital origin (type I); primary peripheral nerve involvement (type II); central lesion in the brainstem nuclei due

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to an anoxic–infectious cause (type III); and myopathic cause (type IV). However, electrophysiologic studies indicate a brainstem process predominantly affecting the facial nuclei and their internuclear connections, rather than a supranuclear or muscular site of involvement.

Most patients with Möbius syndrome have normal CT head scan results except for medial deviation of the eyes. The small number of reported CT findings include hypoplastic or dysplastic brainstem, hypoplastic cerebellum consistent with Dandy–Walker variant malformation, and bilateral symmetric calcifications adjacent to the fourth ventricle floor at the level of the VIth cranial nerve nuclei.

Autopsy has demonstrated specific findings in patients with normal CT studies—focal necrosis and calcifications, and hypoplasia with agenesis of the respective cranial nerve nuclei.

To our knowledge, only three reports containing MRI features of Möbius syndrome have been pub-

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**Figure 1.** Patient 1. (A) T1-weighted sagittal sequence of a normal subject showing the normal shape of the floor of the fourth ventricle (arrowhead). (B) Axial T1-weighted sequence at the inferior pons level of a normal subject showing the facial colliculus, also known as medial eminence (arrow). (C) T1-weighted sagittal sequence: tongue atrophy (arrow) and straightening in the floor of the fourth ventricle (arrowhead). (D) Axial T1-weighted sequence at the inferior pons level: the facial colliculus or medial eminence (arrowhead) is not visualized, suggesting absence of the VIth nuclei and genu of the facial nerve. (E) Axial T1-weighted sequence at the level of the upper medulla: the hypoglossal eminence (arrowhead) is not observed due to absence of the XIIth nuclei.

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**Figure 2.** Patient 2. (A) Sagittal T1-weighted sequence demonstrates straightening of the floor of the fourth ventricle (arrowhead). (B) Axial T1-weighted sequence demonstrates a morphologic alteration of the fourth ventricle floor at the level of the inferior pons due to absence of the medial eminence.

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**Figure 3.** Patient 3. (A) Sagittal T1-weighted sequence demonstrates straightening of the floor of the fourth ventricle (arrowhead). (B) Axial T1-weighted sequence demonstrates V-shaped depression in the fourth ventricle floor (arrowhead) at the level of the inferior pons. The morphologic alteration of the fourth ventricle floor is due to an absence of the facial colliculus.
One of these, which also used a CT study, described focal calcification in the pons within the abducens nuclei, associated with hypoplasia of the brainstem and cerebellum. The authors suggested that the calcifications indicated a secondary origin (type III). The other two reports included one case with pons hypoplasia and one case with pontomedullary hypoplasia.

MRI findings in all of our patients revealed alterations in brainstem morphology without abnormal signal intensity. The sagittal sequence demonstrated straightening in the floor of the fourth ventricle (see figures 1C, 2A, and 3A). In a patient with a consistent clinical description of Möbius syndrome, this subtle MRI finding, reflecting hypoplasia of the pons and medulla, support the diagnosis. The cause of this morphologic alteration can be visualized in the axial slices. The medial colliculus is absent at the level of the pons, suggesting hypoplasia of the VIth and VIIth nuclei (see figures 1E, 2B, and 3B). Additionally, the hypoglossal eminence is absent at the level of medulla oblongata, suggesting hypoplasia of the hypoglossal nuclei (see figure 1E).

The nuclei of the VIth and VIIth cranial nerves are located in the lower part of the pons at the level of the fourth ventricle floor. The axons of the motor nuclei of the VIIth nerve have a posterior medial course, curving around the VIth par nuclei and forming the internal genu of the facial nerve. Subsequently they have an anterior lateral course, emerging from the pons to the pontocerebellum cistern. The abducens nuclei and the internal genu of the facial nerve form the medial prominence in the ventricle floor (see figure 1, A and B) known as the colliculus facialis or medial eminence.

Caudally, the XIIth nerve nuclei form the hypoglossal eminence, at the level of the upper medulla.

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