**SUPPLEMENTAL INFORMATION (CASE REPORTS)**

Patient #1

This 30 year-old woman was admitted to the hospital on April 5, 2005 because of headache, hyperthermia and disorientation for 3 days. She had no past medical history of interest. At admission her temperature was 100o F. The neurological examination only revealed short-term memory deficits. Extensive diagnostic testing of CSF and serum were negative or non-specifically abnormal (i.e., lymphocytic pleocytosis). EEG demonstrated 8-9 Hz slow activity. The MRI of the brain revealed T2 and FLAIR hyperintensities involving the medial aspect of the temporal lobes (Figure 1 A). CT scan of chest, abdomen and pelvis revealed a 5 cm ovarian mass that was considered a benign ovarian cyst (Figure 2 A). The patient was diagnosed with limbic encephalitis and treated with acyclovir and methylprednisolone (1 g/day for 3 days). This resulted in a decrease of the hyperthermia but the patient became more restless and anxious. She subsequently developed generalized tonic seizures and decreased level of consciousness. The EEG showed diffuse wave activity. For the next 2 weeks the patient required sedation and mechanical ventilation. During this time involuntary movements and episodes of epilepsy partialis continua were noted, and she was treated with plasma exchange and IVIg. The hyperthermia and seizures subsided and the level of consciousness started to improve. The CSF pleocytosis and protein concentration progressively improved and by the end of May were normal. In June, a follow-up MRI demonstrated significant improvement of the medial temporal lobe abnormalities (Figure 1 B). The patient then developed constipation and a bulging mass was noted in the lower abdomen. A follow-up CT revealed a significant interval increase of the size of the ovarian mass that now measured approximately 10 cm (Figure 2 B). A few days later, the patient underwent removal of the tumor with pathological diagnosis of immature teratoma. The tumor contained hair follicles, cartilage tissue, areas with a glandular appearance, and cerebral cortex-like tissue containing neurons. In July, the patient was discharged from the hospital. By the time of her discharge the WAIS-R was 84, and she received neurocognitive rehabilitation. In April 2006, the neurological examination and EEG were normal, and she was back to work as a physician. The NR2 antibodies were identified in a retrospective evaluation of archived frozen serum and CSF obtained at the time of symptom presentation.

Patient #2

This 35 year-old woman was admitted to the hospital in August 2005 for headache and a pure amnestic syndrome predominantly involving short-term memory. An EEG obtained on the day of admission, revealed generalized slow and disorganized activity without epileptic discharges. A second EEG obtained 24 hours later showed similar findings with episodes of bilateral frontal large amplitude slow waves. Within the next 4 days, the patient developed mild personality changes and 3 tonic-clonic seizures, without recovery of the level of consciousness after the second seizure. She was then sedated and transferred to the ICU with mechanical ventilation. CT scan of the brain was normal. The MRI showed FLAIR and T2 hyperintensities in the medial temporal lobes (Figure 1 C). Extensive diagnostic testing of CSF and serum were negative or non-specifically abnormal (Table 2). A CT of the chest, abdomen and pelvis revealed a small left ovarian cyst; the presence of the cyst was confirmed with an abdominal ultrasound but it was felt to be unrelated to the neurologic disorder. Initial treatments included acyclovir and valproic acid. After the seizures were controlled, a decrease of sedation revealed persistent short-term memory loss without focal neurological deficits. She subsequently developed partial motor seizures in the left lower extremity and dystonic movements in the left upper extremity, followed by partial complex status requiring sedation, multiple antiepileptic medications, and mechanical ventilation. Treatments with corticosteroids, plasma exchange and IVIg did not result in improvement. By mid-October, the patient was treated with intravenous cyclophosphamide; this resulted in normalization of the CSF pleocytosis, but no neurological improvement. Follow-up MRIs revealed progressive atrophy, predominantly involving the temporal lobes (Figure 1 D). Multiple attempts to decrease the sedation revealed persistent coma, with preserved brainstem reflexes, but emerging status epilepticus. The patient died 4 months after symptom presentation. NR2 antibodies were detected a few weeks before the patient’s death.

The autopsy revealed that the left ovary was largely replaced by a cyst (3.5 x 2.5 x 1 cm) that contained fat and hair. The microscopic examination was consistent with a teratoma containing diverse mature tissues derived from the 3 germinal layers: skin, respiratory epithelium, fat, cartilage, bone, choroidal epithelium, and brain tissue composed of glial cells and neurons.

The patient’s brain weighted 1,200 g, and revealed atrophy of the temporal lobes and hippocampi; the white matter, brainstem, and cerebellum were macroscopically normal. The microscopic studies revealed severe abnormalities in the hippocampi including, a striking loss of pyramidal neurons, predominantly in Sommer’s sector, with extensive gliosis and microglial proliferation (Figure 7 A). A similar degree of neuronal degeneration and intense gliosis was present in the amygdala. In the other brain regions, the pathological findings were mild; these included a few areas of neuronal degeneration and gliosis in the neocortex, and rare loss of Purkinje cells of the cerebellum. Lymphocytic infiltrates were mild and scattered in the leptomeninges overlying the parahippocampal areas, cingulate region, insula and mesencephalon. Moderate perivascular lymphocytic infiltrates were also present in parahippocampi, and a few in the thalami, insula and medulla (Figure 7 B-D). Significant deposits of IgG were identified in the hippocampus, and at much lesser degree in other brain regions, resembling the reactivity of the patient’s NR2 antibodies with rat brain (Figure 6).

Patient #3

In July 2005 this 25 year-old woman started having memory problems. She was forgetting names of people and recent conversations. In addition, she began having panic attacks, confusion, abnormal sleep patterns and hallucinations. She went to a local hospital where it was felt that she had a psychiatric disorder, and she was transferred to a psychiatric center. A brain CT and general blood tests were normal. The CSF revealed 15 WBC/l (100% lymphocytes) and normal protein concentration. A brain MRI and MR angiography were normal. She developed hyperthermia and occasional jerky movements of the extremities. An EEG revealed epileptic activity and the patient was transferred to the hospital of one of the authors. At admission, she was staring off into space, was minimally reactive, and uncooperative with the examination. Her neck was supple and she had mild right facial weakness, marked increase of muscle tone and hyperreflexia in all extremities. Her blood pressure was elevated and she was tachycardic (160 beats per minute). A transthoracic echocardiogram was normal, and an EEG did not reveal epileptic discharges. Extensive diagnostic tests were normal except for CSF pleocytosis. She had episodes of diaphoresis and hyperthermia (101.5o F). During repeat examinations she continued staring off into space, occasionally moaning but unable to communicate, with frequent abnormal movements including, cycling and fist clenching. At times she was catatonic, but withdrew to painful stimuli. A brain MRI showed mild FLAIR hyperintensities in the medial temporal lobes and the right frontal cortical region. A CT scan of chest, abdomen and pelvis revealed a left ovarian cyst. Treatment with methylprednisolone 1 g/ day for 5 days resulted in mild improvement of symptoms. NR2 antibodies were mildly positive in serum and clearly positive in CSF obtained a few days later. She then developed adynamic ileus and became acutely worse with fever, hypotension, and signs of peritonitis. Urgent exploratory laparotomy revealed a perforation in the transverse colon, and she underwent a reversible colonic ostomy and removal of the ovarian cyst. The pathological evaluation was consistent with mature teratoma. Her neurological status started to improve within 3 days of surgery. Two weeks later, she was significantly improved and received 5 days of plasma exchange prior to discharge to a rehabilitation facility. A follow-up 7 months later during her admission for reversal of the colonic ostomy, showed a normal neurological examination, and she was back to her normal activities. A repeat brain MRI was normal.

Patient #4

This 17 year-old woman suddenly developed mood lability, bizarre behavior, hallucinations, disorganized thinking and catatonic-like episodes. She was initially seen in the emergency room of a Children’s Hospital and from there was sent to a Psychiatric Institute. However, she was discharged shortly thereafter, because it was felt that the patient was not cooperating with therapy. Subsequently, the patient was seen in the emergency room of 2 institutions, and eventually admitted to the psychiatric unit of an outside facility. She was noted to be emotionally detached, restless, and wandering aimlessly, with episodes of yelling, and was unable to carry a meaningful conversation. The diagnosis of acute psychosis was initially considered, but she developed hyperthermia (101.1o F) and was transferred to a medical service to rule out meningitis. At admission, she was noted to have episodes of catatonia alternating with periods of normality. A CT of the brain was normal; CSF analysis revealed 1 RBC and 10 WBC/l (95% lymphocytes, 5% monocytes), and normal protein and glucose concentrations. Treatment with acyclovir, antibiotics, and lorazepam had been started on her arrival. A few hours later she was found to be unresponsive due to possible status epilepticus and was transferred to the ICU. EEG monitoring revealed diffuse slowing, but no evidence of seizure activity. An MRI showed a punctuate FLAIR hyperintensity in the right frontal lobe that was not detected in 2 MRIs obtained over the next few days. During this time the patient was treated with fosphenytoin and clonazepam and kept sedated with fentanyl, midazolam and lorazepam. She developed episodes of hyperthermia, pupillary dilatation, agitation and hypertension that were attributed to possible malignant hyperthermia, and the medication was changed to ketamine and midazolam. Extensive diagnostic tests were unrevealing. On considering the possible causes of the patient’s symptoms, a past history of removal of an ovarian teratoma suggested a paraneoplastic etiology; tumors markers CEA, -HCG, and -fetoprotein were within normal values. A pelvic MRI revealed a left 7 cm heterogeneous mass that was resected 3 days later. The pathology was consistent with immature teratoma. A few days after surgery (while on plasma exchange and IVIg) the patient’s mental status mildly improved; she was able to make eye contact and occasionally follow simple commands, but she was unable to clear secretions and had choreoathetoid movements and intermittent oculogyric crises. The CSF revealed NR2 antibodies. Upon consultation with one of the authors she was started on intravenous corticosteroids and cyclophosphamide. Three days later (12 days post-surgery), significant improvement was noted; she was appropriately responsive to questions and was able to be extubated. She was discharged to a rehabilitation facility, with significant improvement of the dyskinesias, but with generalized tremulousness and occasional refusal to talk. Follow-up several months later, revealed a normal neurological examination, and the patient was back to high school achieving good grades.

Patient #5

This 32 year-old woman was in her usual state of good health until December 2005 when she developed headaches, behavioral changes and seizure-like episodes. She was taken to a local hospital, where blood tests, EEG and brain MRI were reported as normal. In a few days she became more confused and was found wandering in her neighborhood. She was taken to a psychiatric center where a generalized tonic-clonic seizure was witnessed and she was then transferred to a general hospital. At admission she was extremely confused and with frequent facial twitches; no focal sensorimotor deficits were identified. An EEG revealed bifrontal periodic lateralized epileptiform dischargers (PLEDS), but multiple subsequent EEGs revealed only generalized slow activity. The patient was initially treated with phenytoin and subsequently with levetiracetam. The CSF showed 40 WBC/l (84% lymphocytes) with normal glucose and protein concentration. For 2 months she remained profoundly encephalopathic, with episodes of agitation, unable to follow commands or communicate, and occasional episodes of facial twitching. She required a PEG, but was not intubated and did not require ventilatory support. Two brain MRIs were normal, and 2 additional CSF studies showed decreasing pleocytosis with mild transient elevation of protein concentration (53 mg/dL). Other diagnostic tests of CSF and serum were unrevealing. A CT of the chest, abdomen and pelvis was reported as showing an incidental ovarian cyst. Upon consultation with one of the authors, the possibility of an ovarian teratoma was suggested, and radiological re-evaluation confirmed the presence of calcium. NR2 antibodies were identified in patient’s serum and CSF, and resection of the ovarian cyst was recommended. While awaiting surgery she received plasma exchange for 2 days resulting in significant improvement; she was able to speak and follow commands. The next day, the tumor was removed (mature cystic teratoma of the ovary) and she then received three additional daily plasma exchanges, after which she was oriented to person, place and time, without agitation, and was able to participate in physical therapy. The PEG was removed and she was discharged to a rehabilitation center. At the last follow-up 7 months later, her MMSE and neurological examination were normal; she had married and was back at work.

Patient #6

In mid December 2005, this 24 year-old woman experienced fever, nausea, vomiting and diarrhea. She was given antibiotics for a suspected urinary tract infection and gastroenteritis. She complained of bitemporal headaches and neck stiffness and her balance became poor. Family members noticed that she was acting paranoid, talking to herself, and appeared to have auditory hallucinations. She became progressively agitated and was brought to a local emergency room from where she was admitted to the psychiatry unit. A brain MRI demonstrated increased T2 signal in a sulcal pattern involving the parietal hemispheres, with mild contrast enhancement of the overlying meninges. Her temperature was 102o F and erythrocyte sedimentation rate was 112. She was incoherent, tremulous, and was witnessed to have a generalized seizure. An endotracheal tube was placed and she was transferred to the ICU. The CSF revealed 5 RBC/l, 219 WBC (mostly lymphocytes with a few monocytes), protein concentration 129 mg/dL and normal glucose concentration. Antibiotics (ampicillin, vancomycin, ceftriaxone) and acyclovir were started, but discontinued 48 hours later when all cultures and CSF PCR for HSV1/2 were negative. Her seizures were treated with phenytoin, benzodiazepines and propofol. When the EEG demonstrated a lack of seizure activity, propofol and all sedatives were slowly withdrawn but her consciousness remained severely impaired and she remained ventilator dependent. A tracheostomy and PEG tube were placed, and she was transferred to the hospital of one of the authors. At the time of the transfer, she had a generalized convulsion for which she received lorazepam, phenytoin and phenobarbital. Upon arrival, she was heavily sedated, without spontaneous movements and without overbreathing the ventilator. When the sedation was tapered down, myoclonic jerks and dyskinetic movements mostly involving the right side of the face and right arm were observed. EEG monitoring only showed generalized slowing; there was no electrical correlate with the rhythmic motor activity.

Over the course of 2 months, the patient seemed to develop sleep/wake cycles. During the “awake” periods, the myoclonic-dyskinetic movements became severe involving both arms and facial musculature. There were signs of autonomic instability; her temperature fluctuated between 93o F and 106o F. At times, she was hypotensive requiring pressor medications, and at other times, she was severely hypertensive (systolic pressure 240 mm Hg). During her “awake” periods, she was tachypneic (up to 50 respirations per minute) requiring sedation; during the “sleep” phases, she did not trigger the ventilator.

During the patient’s hospitalization multiple diagnostic tests of CSF and serum were negative or unrevealing. Three additional brain MRIs during the first month of the disease did not show significant changes compared with the initial study. MR with arterial and venous angiography was normal. A ventriculo-peritoneal shunt was placed for persistently elevated intracranial pressure. A right parieto-occipital biopsy revealed diffuse proliferation of reactive microglial cells and occasional microglial nodules in cerebral cortex and white matter. Some of these nodules showed a centrally located, degenerating neuron surrounded by microglial cells (“neuronophagia”). A few vessels revealed hemosiderin-laden macrophages in the perivascular space; a few lymphocytes were also observed in the perivascular space. Immunostaining for GFAP and CD68 showed abundant reactive astrocytes and numerous microglial cells. No evidence of vasculitis or white matter change was noted. Studies for bacterial, fungal, HSV infection were negative. No viral inclusions were detected using electron microscopy.

A trial of intravenous methylprednisolone failed to improve her neurological condition. An MRI in March showed no significant interval change. The patient’s course over her final two months in the hospital was characterized by a variety of nosocomial infections, for which she was treated with antibiotics. Three months after symptom presentation, the family requested comfort care only; the ventilator was disconnected and the patient died within hours. Archived CSF and serum of this patient were examined after the patient’s death and demonstrated NR2 antibodies.

The autopsy revealed a mature cystic teratoma of the right ovary (1.5 x 1 cm) that among other tissues, also contained MAP2-positive neurons and dendritic processes. The patient’s brain weighted 1,590 g. Sections of the superior temporal gyrus displayed severe reactive gliosis and axons showing Wallerian degeneration. The hippocampal formation showed rare neurons undergoing neuronophagia (with minimal neuronal drop out) and severe gliosis; there was an increased number of microglial cells and microglial nodules. The gliosis was most severe in CA4. Minimal changes (microglial proliferation, gliosis or rare swollen axons) were noted in frontal cortex, basal ganglia, and occipital cortex. No significant drop out of Purkinje cells or Bergmann’s gliosis was detected in the cerebellum. There was significant microglial proliferation with neuronophagia in the arcuate nuclei of the medulla. Microglial proliferation was also noted in sections of the spinal cord, involving the motor neurons of the ventral horns, along with reactive gliosis and swollen axons, indicative of Wallerian degeneration; some nerve roots had endoneural edema. No inflammatory infiltrates were noted in the spinal cord.