FFI Cases from the United States, Australia, and Japan

Paul Brown[1], Larisa Cervenáková[1], James M. Powers[2]

[1] Laboratory of CNS Studies, National Institute of Neurological Disorders and Stroke, National Institute of Health, Bethesda MD

[2] Department of Pathology, University of Rochester Medical Center, Rochester NY.

In this section we describe a previously unreported United States family with the signature D1178N (cis-129M) PRNP genotype of fatal familial insomnia (FFI), and provide references and short commentaries for already published FFI families in the United States, Australia, and Japan.

Unpublished US family

This family (JCW) has an exceptionally well documented geneology, with English origins traced back to the 13th century. The first family member to arrive in the United States landed 15 years after the sailing of the Mayflower and settled in New England from which various family branches have since spread through other parts of the country. With respect to FFI, information from the generation of family members born during the 1940's implicates several individuals in three previous generations who died from a neurological illness with "muscle twitching": the first died at age 40 in 1894, and three of his four children were similary affected, dying at ages 40, 50, and 52 with death certificate diagnoses of cerebellar degeneration, cerebral edema and degenerative encephalopathy. In these three at-risk pedigrees, five of six children (including the proband's mother, maternal aunt, and second cousin) and one grandchild (the proband) have died of the disease. Their medical records are summarized here.

Case 1: The proband, a 33 year-old male executive, had the onset of severe, persistent insomnia in January 1974, followed one month later by auditory and visual hallucinations and myoclonic jerking. These symptoms continued and over the next several months he also noted a 40 Ib weight loss. On admission to the hospital, he appeared acutely ill, with a rapid pulse, elevated blood pressure and fever for which no explanation was evident. He was disoriented and confabulatory, and had generalized tremulousness with myoclonus. All admission laboratory studies, including cerebrospinal fluid (CSF) analysis, were normal. During his extended hospital stay he had innumerable additional laboratory studies including an electroencephalogram (EEG), computed tomography (CT) scan, carotid arteriogram, and brain biopsy, all of which were interpreted as being normal except for EEG which showed constant diffuse slow wave activity. His clinical course was one of unremitting deterioration, and despite the absence of characteristic changes in the biopsy and EEG, a consensus was reached that the most likely diagnosis was CJD. He had intermittent febrile episodes attributed to central nervous system pathology, continued insomnia, weight loss, hallucinations, and myoclonus. In his final weeks, he became globally demented, with horizontal nystagmus, bilateral ptosis, bilateral ankle clonus, primative reflexes, and difficulties with swallowing and respiratory secretions. He died nine months after the onset of illness.

At postmortem, the brain weighed 1540 g and was grossly normal. Microscopic examination revealed only a mild diffuse gliosis. A review of the slides at the NIH by Dr. Colin Masters confirmed this observation and also noted Hirano bodies in the hippocampi.

Experimental transmission studies were performed in 1972. A 10 % saline homogenate of frozen brain was inoculated intracerebrally into four guinea pigs and two capuchin monkeys. The guinea pigs died 2-3 years later without evidence of neurological disease and the monkeys also died without neurological disease after observation periods of 17 and 19 years. In addition, supernatant fluid from brain tissue cultures were inoculated intracerebrally into two spider monkeys they died 26 months and 5 1/2 years later without neurological disease.

Molecular genetic studies were performed in 1997. DNA was extracted from stored frozen brain tissue and the PRNP gene amplified by PCR. Full sequencing of the open reading frame revealed a heterozygous D178N mutation, and heterozygous M129V polymorphism, with methionine specified on the mutant allele. Restriction endonuclease analysis confirmed the codon 129 and 178 genotypes.

Case 2: Five years later in June 1977, the proband's 57-year old maternal aunt was briefy hospitalized for evaluation of the subacute onset of gait ataxia and slurred speech, associated with a 12 Ib weight loss over the preceding two months. She was noted to be anxious

Corresponding Author:
Dr Paul Brown, Building 36, Room 5B20, NIH, Bethesda, MD 20892; Fax: (301) 496-8275

and mentally slow, with gait and truncal ataxia; CT scan was normal, and CSF showed slight elevation of the protein (57mg%). During the next several months her gait worsened, her weight decreased by a further 50 Ibs, and she developed ocular dysmetria, intention tremor of both arms, and ankle clonus. She was re-hospitalized in the spring of 1978, with failing memory, progressive gait and truncal ataxia, dysarthria, ocular dysmetria, extensor plantar responses, startle myoclonus, and continued weight loss. CT scan showed mild cortical atrophy, and EEG showed mild slowing. She was hospitalized a third time in September 1978 with dehydration and fever. She was now severely demented, with visual hallucinations, marked intention tremor, continuous myoclonic and choreiform movements of the arms, hyper-reflexia, and bilateral grasp reflexes. EEG was unchanged. She improved on rehydration and antibiotics and was discharged to a nursing home where she died 20 months after the onset of illness. At no time had she ever been noted to have clinically evident insomnia or other disruption of normal sleep patterns.

The brain weighed 970 g and was grossly atrophic but otherwise unremarkable. Microscopic examination revealed mild to moderate neuronal loss and gliosis in the temporal cortex, insula, and pulvinar with occasional areas of spongiform change in the parietal and temporal cortex and insula. Neuronal loss and gliosis were mild to moderate in the cerebellar vermis and dentate nuclei, and marked in the olivary nuclei. A section cut through the posterior thalamus showed patchy neuronal loss and gliosis. These changes were confirmed on review at the NIH by Dr. Colin Masters.

Experimental transmission studies were performed in 1979. A 10 % saline homogenate of frozen brain was inoculated intracerebrally into a spider monkey. The animal died without neurological disease after an observation period of 10 1/2 years.

For molecular genetic studies (1997), DNA was extracted from stored frozen brain tissue and the PRNP gene amplified by PCR. Full sequencing of the open reading frame revealed a heterozygous D178N mutation, and heterozygous M129V polymorphism, with methionine specified on the mutant allele. Restriction endonuclease analysis confirmed the codon 129 and 178 genetypes.

Case 3: Two years after her sister's death, in the autumn of 1980, the proband's mother experienced the insidious onset of gait imbalance, followed several months later by intermittent episodes of double vision. Hospital evaluation in April 1981 revealed mild decrease in cognition and upper extremity ataxia, and a CT scan showed severe midline cerebellar degeneration. Over the next several months, her symptoms progressed and she also complained of hearing loss. By April 1982, she was confined to a wheelchair and had lost effective speech; examination showed hypokinetic dysarthria, paratonia with extra-pyramidal rigidity and resting tremor, myoclonus, dysmetria, intention tremor and ataxia of all extremities. Her condition further deteriorated during the next month, and she died 20 months after the onset of neurological symptoms. Insomnia or other sleep disorders were not observed to have occurred at any time during the patient's illness.

The brain weighed 1030g and appeared markedly atrophic. Microscopic examination revealed a mild degree of neuronal loss, gliosis, and spongiform change in the temporal cortex; neuronal loss and gliosis was marked in the inferomedial portion of the thalamus, and slight to moderate in the inferior olives. There was also marked Purkinje cell loss in the anterior cerebellum.

No tissue was available for experimental transmission and molecular genetic studies.

Case 4: A 68 year old cousin of the proband's mother began to have bifrontal headaches in November 1991 which were extensively but unfruitfully evaluated with ENT, ophthalmology and neurological consultations (including CT and MRI scans). Various therapeutic efforts were ineffective, and over a period of several months she lost her appetite and about 65 lbs of weight. During a hospitalization in May 1992, she was noted to be confused and disoriented with supranuclear gaze abnormalities, twitching of the hands and head, and generalized muscle wasting. CSF was normal. She was confined to a nursing home and readmitted to the hospital in November, when she appeared severely demented, poorly responsive, and cachectic. She showed diffuse spasticity and myoclonus, hypertonus, and primative reflexes. An EEG showed semi-periodic multifocal high voltage sharp waves on a background of generalized slowing. She was transferred to a nursing home, where she died in March 1993, 15 months after the onset of illness. Like the proband's aunt and mother, the cousin was never observed to have clinically evident insomnia or other sleep abnomality.

The brain was removed under biohazard conditions and gross observations were not recorded. Microscopic examination of biopsy tissue specimens from the frontal cortex, parietal cortex, and cerebellum revealed a fine vacuolation of the neocortical neuropil, either pancorti-

cal, concentrated in the deepers layers, or focally prominent in layer II, associated with mild astrocytosis. Occasional vacuoles were seen in the molecular layer of the cerebellum.

No frozen tissue was available for inoculation. Analysis of DNA extracted form formalin-fixed brain tissue blocks failed to detect a mutation in codon 178 by restriction endonuclease testing, and sequencing was unsuccessful. These results were felt to be due to degraded DNA in the fixed specimen.

Other US families

One well-documented family (McK) with English ancestry was originally included as Family 23 in a 1981 review of familial CJD by Masters *et al*. (9), then reported in detail in 1986 as "familial myoclonic dementia masquerading as CJD" by Little *et al*. (7), before finally finding its rightful place as FFI in 1992 in an article by Petersen *et al*. (12). Several members had either insomnia or somnolence (which can be a clue to unrecognized insomnia); several others had no evident sleep disturbances. Brain tissue from two members was tested for the presence of PrP and infectivity: both had small amounts of PrP in the cerebral cortex (3), and neither transmitted disease to primates (2).

Coincidentally, an unrelated family (WG) having both a codon 178 mutation and a 24-bp deletion was reported as CJD by Bosque *et al*. (1) and an apparently isolated case of FFI with the same genotypic combination was described in 1995 by Reder *et al*. (13). The probability of a blood relationship between the two families led to an intensive geneological search that eventually yielded a previously unrecognized connection that had occurred three generations earlier! The family is of special interest also in being the first in which brain tissue from an affected member transmitted disease to experimental animals, establishing FFI as a full-fledged member of the group of transmissible spongiform encephalopathies (TSE) (16).

Australian families

Two apparently unrelated families living on the eastern seabord of Australia have recently been reported - one of mixed Irish-Danish descent, in the state of Queensland (15), and the other, of Irish descent, in the state of Victoria (10). The Queensland family (CE) includes the proband, with typical FFI, and her son, the youngest known patient with this disease, whose illness began at age 20 and did not include clinically apparent insomnia. The Victoria family (DO) is remarkable, first, for the fact that the proband was an adopted child whose blood connection to an FFI family would not have been discovered had not she and an affected member of her real family both been seen by the same neurologist, and second, for its clinical heterogeneity; of six patients with well documented clinical histories, one had a typical FFI phenotype, two had some FFI features, two had a typical CJD phenotype, and one had an illness resembling "autosomal dominant cerebellar ataxia." Geneological investigation has failed to identify any relationship between the two families as far back as the middle-19th century, but their common Irish descent leaves open the distinct possibility of a connection in the more distant past.

Japanese family

A case of genomically proven, clinically typical FFI was reported in a family with no other known affected members including the patient's parents, who had died of non-neurological illnesses at the ages of 62 and 80 years, and four siblings, who remain alive and well at ages over 60 years (11). None of these unaffected family members were tested for the presence of the codon 178 mutation, leaving unanswered the question of a "first-time" mutation in this family, or of unrevealed illegitimacy or adoption (like the proband of the Victorian Australian family).

General Comments

In our newly reported family, only one of the four affected members who had adequate medical records was observed to have had insomnia, an observation in keeping with the inconsistent occurrence of insomnia in most other families that have been described. In point of fact, there is as much phenotypic heterogeneity in FFI as there is in CJD and the Gerstmann- Sträussler-Scheinker syndrome (GSS); and so it should neither surprise nor disappoint that FFI can exist without insomnia, just as TSE can exist without spongiform change. It has been proposed that only EEG sleep studies can provide proof of insomnia, but without the symptom there is no motive to obtain a sleep study, and with the symptom, EEG proof is unnecessary. The same may be said about other symptoms referable to the autonomic system and the performance of detailed autonomic and endocrine testing.

The clinical diagnosis of FFI is further complicated by the fact that 10-15% of patients with CJD report disturbances of sleep, including insomnia, among their early symptoms (2). Although usually not a prominent symptom, it can in rare patients mimic the intensity of FFI. Chapman *et al*. (4) reported a case of familial CJD

in a patient with a E200K mutation whose intractable insomnia was a major feature of her illness, and who on autopsy had prominent neuronal loss and gliosis of the mediodorsal thalamic nuclei, in addition to widespread cortical spongiform change. Another patient with no family history of neurological disease and a normal PRNP genotype (i.e. a case of sporadic disease) developed intractable insomnia and agitation 18 months into an illness that terminated at 24 months; at autopsy he had severe neuronal loss and gliosis of the dorsomedial and anterior ventral thalamic nuclei together with spongiform foci in the cerebral cortex (5).

Biologically, FFI is remarkable not only for its dependence on the genotypic combination of a pathogenic mutation and a polymorphism (6), and for the prominence of thalamic pathology that generates the insomnia and other autonomic signs, but also for the comparatively small amount of "prion" protein in the brain and the difficulty with which experimental transmission can be accomplished. We look forward to the time when all of these distinctive features can be explained by a single molecular biological mechanism, perhaps involving the topographical distribution of PrP receptors and subtle but important differences in binding affinities between differently folded mutant PrP isoforms and one (ore more) receptors. Two proteins that bind with PrP have recently been reported to occur in brain cells: one is a 37-kD laminin receptor precursor (14), and the other is a 66-kDa otherwise uncharacterized membrane protein (8). If one or both prove to be functioning PrP receptors, the next step will be to investigate their distribution and affinity characteristics.

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