Childhood Onset in Familial Prion Disease With a Novel Mutation in the PRNP Gene

Ekaterina Rogaeva, PhD; Cindy Zadikoff, MD; Jonathan Ponesse, MD; Gerold Schmitt-Ulms, PhD; Toshitaka Kawarai, MD; Christine Sato, BSc; Shabnam Salehi-Rad, BSc; Peter St. George-Hyslop, MD, FRCPC; Anthony E. Lang, MD

Background: Up to 15% of cases of prion diseases are due to the autosomal dominant inheritance of coding PRNP mutations.

Objective: To describe the unique clinical and genetic findings in a family of East Indian origin with autosomal dominant inheritance of a novel PRNP mutation.

Design: Detailed neurological examination and sequencing analysis of the MAPT and PRNP genes.

Setting: Toronto Western Hospital, Toronto, Ontario.

Patients: Five available members of a family of East Indian origin with a rapidly progressive neurodegenerative disorder characterized by dementia, motor decline, and ataxia.

Results: We identified a novel Pro105Thr mutation in the PRNP gene in all of the 3 clinically affected family members but not in their unaffected relatives or normal controls. Although 5 of 6 affected family members had a relatively homogeneous phenotype and age at onset (range, 33–41 years), 1 of the 6 patients developed the disease at age 13 years.

Conclusions: A novel mutation in the PRNP gene was identified in all of the available, clinically affected members of this family with a rapidly progressive neurodegenerative disease. To our knowledge, the propositus represents the youngest individual with inherited prion disease described to date.

Arch Neurol. 2006;63:1016-1021

A HALLMARK OF PRION DISEASES is the structural transition of the normal cellular prion protein, PrPC, to its disease-causing isoform, PrPSc.1 The order and predominance of the clinical features of disease may vary with the prion disease subtype, and the age at onset may range from as early as the second decade of life, as seen in acquired forms of prion disease, to late in life.

Up to 15% of cases of prion diseases are due to the autosomal dominant inheritance of coding mutations of the PRNP gene encoding the prion protein.2 Some PRNP polymorphisms are able to modify the disease phenotype when they are located on the mutant allele. For example, an interaction between the Met129Val common polymorphism and the pathological Asp178Asn mutation might result in abnormal prion isoforms that differ in conformation and clinical presentation; whereas the allele of Val129 to Asn178 presents as Creutzfeldt-Jakob disease (CJD), the allele of Met129 to Asn178 segregates with fatal familial insomnia.3 Furthermore, homozygosity at codon 129 increases the risk for individuals to acquire the infectious forms of prion disease.4,5

We describe the clinical and genetic findings in an extended family with a neurodegenerative disorder characterized by a rapidly progressive cognitive and motor decline with a unique juvenile onset in the propositus. Owing to the clinical course as well as a family history consistent with autosomal dominant inheritance, we performed mutation analysis of 2 genes: the microtubule-associated protein tau gene (MAPT) associated with inherited frontotemporal dementia with parkinsonism linked to chromosome 17q and the PRNP gene. We found a nonsynonymous PRNP mutation (Pro105Thr), previously unpublished to our knowledge, segregating with the disease.

METHODS

SUBJECTS

Informed consent for research purposes, approved by the research ethics board at the University of Toronto, Toronto, Ontario, was obtained from all of the individuals involved in the study. A detailed neurological examination was performed on all of the 5 available family members recruited from the Toronto Western Hospital, Toronto. The normal control
group comprised 200 unrelated subjects of North American origin (mean ± SD age at time of examination, 72.7 ± 8.4 years).

GENETIC ANALYSIS

Genomic DNA was extracted from whole blood using a kit (Qiagen, Inc, Mississauga, Ontario). One member of the family (case 1940) was initially tested for mutations in exons 1 and 9 through 13 of the MAPT gene by direct sequencing as previously described. The entire open reading frame of the PRNP gene was sequenced in 5 family members available for the study and was captured with 2 overlapping polymerase chain reaction (PCR) fragments from genomic DNA. The primers used for the first fragment were PRP5-1F (5'-CAGACACGTCATTATGGCGGA-3') and PRP3-3R (5'-GCCTGTAGTACACTTGGTTG), and the primers for the second fragment were PRP5-3F (5'-GGTGCTGGCTGGCTAAGGAGG) and PRP3-1R (5'-TACCGCCTCCCTCAACGGTG). The PCR conditions were 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, either 58°C for 30 seconds for fragment 1 or 60°C for 30 seconds for fragment 2, and 72°C for 30 seconds, and finally 7 minutes at 72°C. Both of the PCR reactions included 1× Q-Solution (Qiagen, Inc). Variations were detected by direct inspection of the fluorescent chromatograms and by analysis using SeqScape software version 1.0 (Applied Biosystems, Foster City, Calif). The frequency of the Pro105Thr mutation in 200 controls was 0.3 U of

RESULTS

CLINICAL FEATURES

This family of East Indian origin had autosomal dominant inheritance of a rapidly progressive neurodegenerative disorder characterized by dementia and motor decline, including ataxia (Figure, A). The family record indicates that at least 6 individuals have had the disease. Five of the affected relatives had a similar age at onset (mean age at onset, 36 years; range, 33-41 years) and phenotype. This is in stark contrast to the younger age at onset (age 13 years) and unique clinical features of the proband. Three patients and 2 unaffected relatives were available for the genetic and clinical study (Table).

PROBAND AND RELATIVES

The proband (case 6548) was a 15-year-old boy who first became uncharacteristically anxious and paranoid at age 13 years. Within a month, he developed dysarthria and gait difficulties; soon after, he had trouble with self-care. His initial examination results were significant for saccadic pursuit, dysarthria, tongue fasciculations, generalized spasticity, truncal and appendicular ataxia with a wide-based gait, and hyperreflexia with clonus at both ankles and extensor planter responses. When first seen in our unit (15 months after symptom onset), he was anarthric, dysphagic, made few purposeful movements, and required total care. However, he remained seemingly cognitively intact, used blinking to communicate, and attempted to follow commands as best he could. Twenty-one months after disease onset, he had become cachectic and unable to communicate. He cried continuously, no longer followed commands, made no purposeful movements, and developed generalized contractures. He had an unrevealing extensive workup (5 months after symptom onset) for neurometabolic disorders prior to presentation at the Toronto Western Hospital. A magnetic resonance image showed increased signal of the white matter in the centrum semiovale and cortical spinal tracts on T2-weighted and fluid-attenuated inversion recovery sequences. Electroencephalographic results were normal. Cerebrospinal fluid study results were normal, although the 14-3-3 protein test was not performed.

The proband’s mother (case 6549), who presented to the Toronto Western Hospital at the same time as her son, was a 40-year-old woman who became symptomatic 9 months after her son first showed signs of anxiety. She initially had fatigue and gait instability. She started falling early in the disease course, requiring a walker only 6 months after symptom onset and becoming wheelchair dependent a few months later. Her initial examination results were significant for dysarthria, slow saccades, paratonia, bilateral postural tremor of the upper extremities with distal polymyoclonus, mild appendicular apraxia, upper and lower limb ataxia, dystonic posturing of the right foot, brisk reflexes, and a wide-based gait. Within 4 months of her initial examination, she developed a pseudobulbar affect with emotional lability and performed poorly on a Frontal Assessment Battery (score, 7 of 18) and a Mini-Mental State examination (score, 15 of 24). Her gait had become extremely apraxic as well as ataxic. A workup done prior to presentation at the Toronto Western Hospital included normal (or negative) brain magnetic resonance imaging, electroencephalography, and cerebrospinal fluid study results, including normal 14-3-3 protein test results.

The proband’s maternal uncle (case 1940) was examined in 1996 in Toronto (8 years before the presentation of the propositus to our unit) at age 35 years for a progressive motor and cognitive disorder. He initially had right-sided numbness and weakness. He was examined 4 months after symptom onset. His score was 20 of 28 on a Mini-Mental State examination (demonstrating difficulties in all of the domains), and he was noted to have a pseudobulbar affect. The physical examination results were significant for dysarthria, tongue fasciculations, paratonia, decreased strength in his distal extremities, bilateral appendicular ataxia, diminished temperature sensation on the right, hyperreflexia, and an ataxic gait. Multiple diagnostic considerations were explored, and investigations included normal brain magnetic resonance imaging and routine cerebrospinal fluid studies (it is not known whether the presence of 14-3-3 protein was tested). An electroencephalogram showed diffuse slow waves, especially involving the temporal lobes, and intermittent spikes in the left frontal lobe. However, no diagnosis was made.
Figure. Family with a Pro105Thr mutation in the PRNP gene. A, The pedigree structure of the Toronto family shows the inheritance of prion disease (with age at onset and PRNP mutation status indicated as appropriate). Filled symbols indicate individuals with prion disease; quarter-filled symbol, possible prion disease based on the information obtained from the family members; question mark, unknown disease status; diagonal lines, deceased individuals; and arrow, proband for whom the sequencing analysis was performed. The sex of each individual has been masked to protect family confidentiality. B, The DNA sequence fluorescent chromatograms of the PRNP mutation as well as codon 129 are shown for each patient.
At the time of his assessment, blood was sent to our laboratory for MAPT gene testing in view of his family history (as described later). He returned to India and died there 3 years after the disease onset.

The proband’s mother had 7 siblings, including case 1940. Two other sisters died in their 30s, 2 to 3 years after the onset of a rapidly progressive dementing disorder (although no further history is available for these relatives). The proband’s maternal grandfather died in his 60s (cause unknown), and his maternal grandmother was alive and well. The proband’s maternal great aunt died in her 40s of a subacute dementing illness (Figure, A).

The proband’s 43-year-old father (case 6551) and 12-year-old brother (case 6550) were alive and well. Based on the family history and the rapidly progressive clinical course, a familial prion disease was suspected. Although the maternal uncle (case 1940) had died 6 years earlier, blood was still available for testing.

**GENETIC ANALYSIS**

Mutation analysis performed on the maternal uncle (case 1940) did not reveal any sequence variations in the MAPT gene. However, in the PRNP gene, we identified a novel nonsynonymous mutation: a heterozygous C-to-A substitution at messenger RNA nucleotide position 413 (GenBank accession number NM_000311) resulting in the Pro105Thr mutation. This mutation was not found in 200 normal controls.

Segregation analysis was performed for all of the 5 family members available for study (Figure, A). The 3 clinically affected individuals (cases 1940, 6548, and 6549) inherited the Pro105Thr mutation, which was absent in 2 unaffected relatives (cases 6550 and 6551) (Figure, B). As the maternal uncle was homozygous for methionine at codon 129, the Pro105Thr substitution must map to a Met129 allele of the PRNP gene. The proband (case 6548), who was affected at the youngest age and exhibited a different, more severe phenotype than the rest of the family, was heterozygous for the Met129Val variation whereas the patients experiencing a later age at onset (but with equally rapidly progressive disease) (cases 1940 and 6549) were homozygous for methionine (Figure, B). No other PRNP polymorphisms were detected in the family, and the octapeptide repeats were of normal size between codons 51 and 91.

---

### Table. Clinical Details

<table>
<thead>
<tr>
<th>Individual</th>
<th>Age at Onset, y</th>
<th>Presenting Symptom</th>
<th>Clinical Features and Disease Course</th>
<th>Disease Duration</th>
<th>Examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband (case 6548)</td>
<td>13</td>
<td>Anxiety</td>
<td>Saccadic pursuit, dysarthria, gait difficulties, tongue fasciculations, spasticity, dystonia, ataxia, hyperreflexia, preserved cognition until late; required total care within 15 mo of disease onset</td>
<td>Alive 2 y after symptom onset but doing very poorly</td>
<td>Increased signal in white matter of centrum semiovale and corticospinal tracts on T2-weighted and FLAIR sequences on head MRI; normal or negative results on routine CSF studies (including lactic acid level and oligoclonal bands), EEG, evoked potentials, metabolic screen, genetic testing for Huntington disease, spinocerebellar ataxias, and skin biopsy</td>
</tr>
<tr>
<td>Proband’s mother (case 6549)</td>
<td>39</td>
<td>Fatigue and gait instability</td>
<td>Dysarthria, slow saccades, paraparesis, postural tremor of upper extremities with polymyalgia, ataxia, apraxia, dystonia, hyperreflexia, pseudobulbar affect, dementia; wheelchair bound within 9 mo owing to a combination of ataxia and apraxia of gait</td>
<td>Alive but requiring full care 1 y after symptom onset</td>
<td>Normal brain and cervical spine MRI, EEG, and lumbar puncture results (including 14-3-3 protein)</td>
</tr>
<tr>
<td>Proband’s maternal uncle (case 1940)</td>
<td>35</td>
<td>Right-sided numbness and weakness</td>
<td>Rapidly progressive dementia, ataxia, and pseudobulbar affect, dysarthria, tongue fasciculations, paraparesis, weakness</td>
<td>Died 3 y after symptom onset</td>
<td>Diffuse slow waves (bilateral temporal lobe predominant) and intermittent left frontal spikes on EEG; normal brain MRI, evoked potential, and routine CSF study results</td>
</tr>
<tr>
<td>Proband’s maternal aunt</td>
<td>33</td>
<td>NA</td>
<td>Rapidly progressive dementia</td>
<td>Died 2-3 y after disease onset</td>
<td>NA</td>
</tr>
<tr>
<td>Proband’s maternal aunt</td>
<td>33</td>
<td>NA</td>
<td>Rapidly progressive dementia</td>
<td>Died 2-3 y after disease onset</td>
<td>NA</td>
</tr>
<tr>
<td>Proband’s maternal great aunt</td>
<td>Early 40s</td>
<td>NA</td>
<td>Rapidly progressive dementia</td>
<td>Died a few years after disease onset</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; EEG, electroencephalography; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; NA, not available.
COMMENT

We describe unique clinical and genetic findings in a family with a prion-related neurodegenerative disease. We detected a novel heterozygous Pro105Thr mutation on a Met129 allele of the PRNP gene in all of the 3 examined patients but not in their unaffected relatives (or in a cohort of 200 normal controls), thereby leaving little doubt regarding the pathogenic nature of the mutation. Significantly, the Pro105Thr mutation alters an evolutionarily conserved codon positioned within a functionally important domain (near a high-affinity copper binding site).8

According to a short note in the mutation database (http://www.mad-cow.org/prion_point_mutations.html), there has been 1 other, poorly characterized family with the Pro105Thr mutation. Unfortunately, the records of this family are lost from the Brain Bank of the National Institutes of Health, Bethesda, Md (written communication, Lev G. Goldfarb, PhD, March 24, 2005). Apparently, one of the family members developed symptoms similar to sporadic CJD at age 30 years and tested positively for the Pro105Thr mutation. It is not clear whether the Pro105Thr mutation was located on the Met129 allele since this individual was heterozygous for the Met129Val polymorphism. The disease was transmitted from the father, who died from it at age 42 years.

The same PRNP codon is also affected by a different substitution (Pro105Leu) in several Japanese families with Gerstmann-Straussler-Scheinker syndrome.9,10 Although some of the clinical features between the Japanese families and our Toronto (East Indian) family do overlap, there are important differences. Spastic paraparesis, although present in the proband and a prominent feature in the Japanese families, is not a major manifestation of the other members of the Toronto family. Moreover, the time from symptom onset to death was longer in the Japanese families.9,10 Such phenotypic diversity is likely attributed to the different nature of the substitutions; in addition, the Pro105Leu mutation in the Japanese families was detected on a Val129 allele as opposed to the Met129 allele affected in the Toronto cases.

Although the classical magnetic resonance imaging abnormalities associated with sporadic CJD have been found in familial forms of the disease,11,12 none of the members of the Japanese families or the Toronto family who were studied demonstrated the classical high signal in the basal ganglia. Although this has been reported in T2-weighted and fluid-attenuated inversion recovery sequences, diffusion-weighted imaging, a much more sensitive test,13,14 was not performed.

Although 5 of 6 affected family members described here have a relatively homogeneous phenotype (rapidly progressive dementia and motor dysfunction, including ataxia) and age at onset (range, 33–41 years), the proband is unique in a number of respects (Table). He first developed symptoms of the disease at age 13 years. To our knowledge, this case represents the youngest individual with inherited prion disease described to date and certainly the youngest to date with motor features. Moreover, his phenotype differed from the other family members in that he displayed marked psychiatric disturbances at disease onset with cognition preserved until relatively late. Recently, an Uruguayan family with a novel PRNP mutation (Gly114Val) was described, some of whose members also developed psychiatric symptoms at onset as young as 18 years. However, in that family, there was a longer delay from the onset of psychiatric symptoms to the development of motor symptoms than in our proband.15

Boessenberg et al6 found that those who developed sporadic CJD at a younger age (defined as age <50 years; range, 19–49 years) had psychiatric features at onset more often than those who developed sporadic CJD at an older age (age >50 years). They also found that in young-onset sporadic CJD, motor symptoms followed psychiatric symptoms more quickly than in variant CJD. The presentation of our proband is clearly similar to that in young-onset sporadic CJD described by Boessenberg and colleagues. The phenotypic differences observed between our proband and his family members with the disease therefore support this observation that perhaps the age at onset may play an important role in phenotypic manifestations of prion diseases.

Given that particular PRNP polymorphisms can substantially modify the clinical presentation of prion diseases,2 we evaluated whether the phenotype in affected members of our family was influenced by PRNP variations. The Met129Val polymorphism was the only PRNP variation revealed by sequencing analysis of the entire reading frame in all of the 5 individuals available for this study. The genotypes for the Met129Val polymorphism did not explain the observed high variability in the age at onset in the Toronto family, as Met129Val heterozygosity (observed in case 6548 with juvenile onset) is considered to be a protective genotype.17 Notably, the proband was the only member in the Toronto family for whom genetic transmission occurred from mother to child. It is possible that the unusually early onset of the disorder observed in this child may have been caused by an exposure to the infectious prion conformer PrPSc in the womb during the disease latency period of the mother (at age 25 years).

Accepted for Publication: November 21, 2005.

Correspondence: Anthony E. Lang, MD, Toronto Western Hospital, Morton and Gloria Shulman Movement Disorders Center, Mcl-7, 399 Bathurst St, Toronto, Ontario M5T 2SB, Canada (lang@uhnres.utoronto.ca).


Funding/Support: This work was supported by grants from the Connaught Grant, Japan-Canada and Cana-
Acknowledgment: We thank the patients for taking part in this research.

REFERENCES