

Familial Prion Disease Cases Without Mutation in *PRNP* Gene

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Abstract

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare and fatal neurodegenerative disorders that affect both humans and animals. The etiology of TSEs contributed to the important “protein-only” hypothesis, which postulates that proteinaceous particles known as “prions, which are devoid of nucleic acids, are the causative agents of TSEs. Human TSEs are classified as familial, acquired or sporadic. Inherited prion disease, which exhibits autosomal dominant inheritance and which accounts for approximately 15% of human TSEs, is caused by germline mutations in the prion protein gene. Here, results of mutation screening of *PRNP* in two siblings diagnosed with familial CJD are reported. We found neither a point mutation nor an insertional mutation in the patients’ DNA. The implications of this finding are discussed.

Keyword: Prion Disease, CJD, *PRNP*

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of rare and fatal neurodegenerative disorders that affect both humans and animals (Collinge 2001). The etiology of TSEs contributed to the important “protein-only” hypothesis, which postulates that proteinaceous particles known as “prions” that are devoid of nucleic acids are the sole causative agents in TSEs (Prusiner 1982). Clinical presentations of prion diseases are variable, and may include myoclonus, dysarthria, ataxia, dementia, memory and behavioral disturbances, sensory and motor deficits, cerebellar signs and occasionally visual signs (Gambetti et al., 2003). Additionally, features in magnetic resonance images (MRI) of the brain and results of laboratory tests are used as exclusion criteria for other neurological diseases with similar presentations (Collie et al., 2001). The mean age at onset is 55 years with a range of 26-84 years. Definitive diagnosis of prion disease rests on post-mortem microscopic examinations of the cortex which evidence tiny holes causing the

tissue to appear like a sponge, hence the term “spongiform” (Zlotnik and Stamp 1961).

Human TSEs are classified as familial, acquired or sporadic. Inherited prion disease, which exhibits autosomal dominant inheritance and which accounts for approximately 15% of human TSEs, is caused by germline mutations in the prion protein gene (*PRNP*; MIM 176640) (Goldfarb et al., 1994). Inherited human TSEs include familial Creutzfeldt-Jakob disease (CJD; MIM 123400), Gerstmann-Straussler-Scheinker syndrome (GSS; MIM 137440) and fatal familial insomnia (FFI; MIM 600072). Familial CJD represents the most prevalent type of human inherited TSEs. Acquired forms of the disease are caused by exposure to contaminated material such as infected animal meat or contaminated medical equipment. Acquired forms of human prion diseases include variant CJD (usually transmitted through bovine meat), iatrogenic CJD and kuru (MIM 245300) (Collinge 2001). The incidence of acquired prion diseases is very low. The majority of human cases of TSE occur sporadically and the etiology of the sporadic cases is unknown. Most sporadic cases are

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diagnosed as CJD (Prusiner 1994; Collinge 2001).

The human *PRNP* gene is located on chromosome 20 and contains two exons. The protein product of the gene contains 253 amino acids, entirely encoded within the second exon. Two types of mutations have been identified in *PRNP* (Hsiao et al., 1989; Owen et al., 1989). The first type which is uncommon affects a change in the number of octapeptide repeats within the NH₃-terminal region of the prion protein (PrP). The second type consists of point mutations that affect amino acid changes within the COOH-terminal region of the protein. The most common point mutations within *PRNP* cause Glu200Lys, p.Asp178Asn, and p.Pro102Leu. It is reported that in familial cases, the phenotypic manifestation of disease in patients harboring these mutations is affected by the sequence of nucleotides that encode codon 129 of the protein. Individuals with p.Asp178Asn mutation who are also homozygous for p.Val129 show a CJD phenotype, whereas those homozygous for p.Met129 are diagnosed with FFI (Goldfarb et al., 1992). Codon 129 apparently also affects susceptibility to the acquired and sporadic forms of human TSEs. So far all reported cases of variant CJD have been shown to be homozygous for the methionine allele, and up to 90% of sporadic CJD cases have occurred in individuals who are homozygous either for the methionine or the valine allele at codon 129. Patients were rarely heterozygous p.Val129/p.Met129 (Palmer et al., 1991). Allele frequencies of codon 129 are heterogeneous among different populations, with the frequency of the valine allele ranging from 5% in East Asia to 65% in the Americas. The genotype encoding codon p.219 also appears to affect phenotypic presentation, although to a lesser extent than p.129 (Andrews et al., 2003; Soldevila et al., 2003). The p.Lys219 codon as compared to the p.Glu219 allele is considered somewhat protective.

Spongiform changes within the brain and accumulation of aggregates of the protease-resistant isoform (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^C) in the brain are the definitive pathologic characteristics of TSEs (Prusiner 1998). PrP^C is a membrane glycoprotein abundantly expressed in normal neurons of the central nervous system (CNS). PrP^C under certain conditions undergoes conformational change and appears as PrP^{Sc}. The secondary structure of PrP^C is composed mostly of α -helices, whereas PrP^{Sc} contains mostly β -sheets. The structural change renders PrP^{Sc} detergent-insoluble and resistant to digestion with proteinase K. The function of PrP^C is unknown; however its presence appears to be obligatory for PrP^{Sc} propagation and development of prion pathology (Prusiner 1998). It is thought that once PrP^{Sc} is present, it impresses its conformation on host PrP^C and causes generation of more PrP^{Sc} in an autocatalytic fashion (Bueler et al., 1993). After sufficient PrP^{Sc} propagation, PrP^{Sc} aggregation in the brain ensues. In familial cases of TSEs, *PRNP* mutations increase the probability of PrP^{Sc} production. Sporadic forms of TSEs may result from spontaneous conversion of PrP^C to PrP^{Sc}, or from somatic mutations of *PRNP* gene (Cohen et al., 1994).

Here, results of mutation screening of *PRNP* in two siblings diagnosed with familial CJD are reported. We found neither a point mutation nor an insertional mutation in the patient's DNA. We discuss the implications of this finding.

Subjects and Methods

This research was performed in accordance with the Helsinki Declaration and with approval of the ethics board of the University of Tehran. All participants or their responsible guardians consented to participate after being informed of the nature of the research. Two brothers diagnosed with

familial CJD by neurologists at Hazrat Rasool Hospital were referred to us for genetic analysis (Fig. 1). Diagnosis had been based on results of clinical examinations, electroencephalographic (EEG) data, brain MRI, biochemistry, and disease progression (Masters et al., 1979). The parents of the patients were consanguineous. Blood samples were collected from all members of the family, and DNA was extracted from leukocytes by standard phenol chloroform protocols. *PRNP* exons and flanking intronic regions were amplified by polymerase chain reactions (PCR). Exon 1 which lies within a

GC-rich region, proved difficult to amplify. Ultimately, amplification was achieved by using DyNAzyme EXT DNA polymerase (F-505L, Finnzymes Oy, Finland) as recommended by the manufacturer. The PCR products were sized on 1% agarose gels so as to be able to detect potential insertion mutations. Subsequently, PCR products were sequenced by the Sanger protocol. *PRNP* reference sequences used were NG_009087.1, NM_000311.3 and NP_000302.1. The sequences of primers used for *PRNP* gene amplification and sequencing are presented in Table 1.

Table 1. *PRNP* primers.

Primer name ^a	Amplicon length(bp)	Forward primer (5'-3')	Reverse primer (5'-3')
PRNPx1	807	GTGATGGTCAGTGTCTTTCTACG	TAACCTGAAAAGCGAAGCCAAG
PRNPx2	994	CTATGCACTCATTATTATGCAG	CCAAGGGTATTGATTAGCCTATC
PRNPx2b	924	TGGATGAGTACAGCAACCAGAAC	GCCAGAGGTTTCAAGTGTGTGAC
PRNPx2c	925	GAGATTCTTAGCTCTTGGGATGC	CATTGTAAGCCTAAGGACCACG
PRNPx2d	555	TTGCATAGGACAGACTTAGGAG	CAATAGTGCCTTTGCAGTTTAC

a. PRNPx2 primers were designed to amplify the G/C rich amino acid coding region of exon 2. Other pairs amplified non-coding regions within exons 1 and 2 of *PRNP*.

Results

The age at onset of symptoms in one of the patients was 22 years. The patient reported with progressive amnesia, seizure and imbalance. At the age of 28 years, neurologic examination evidenced dementia, myoclonus, apraxia and spastic quadriparesis. At this time, the patient was mute and bedridden. His EEG was abnormal due to diffuse slowing and periodic epileptiform discharge, and his brain MRI was abnormal. His affected sibling who is four years younger had a similar history. This patient also experienced two generalized tonic clonic seizures (GTCS) during a course of three months. Wilson's syndrome was excluded because of normal levels of serum and urine

copper, and absence of Kayser-Fleischer rings. The patients were diagnosed with CJD. Familial CJD seemed the most appropriate diagnosis because of two siblings being affected and because life styles and interviews gave no indication of opportunities for contact with contaminated substances (Brown et al., 1992; Windl, et al., 1999). All other family members are healthy individuals (Fig. 1).

Electrophoresis of the exon 2 amplicon of *PRNP* gave no indication of change in the number of octapeptide repeats encoded within the exon (not shown). Furthermore, sequencing results of *PRNP* exons in the affected individuals showed no sequence variation as compared to the reference

sequence of the gene, except heterozygosity at c.385A>G in both patients (Fig. 2). This heterozygosity results in corresponding heterozygosity at position p.129 (p.Val129/p.Met129) of the encoded protein. Both patients were homozygous for the p.Lys219 allele, which is putatively protective.

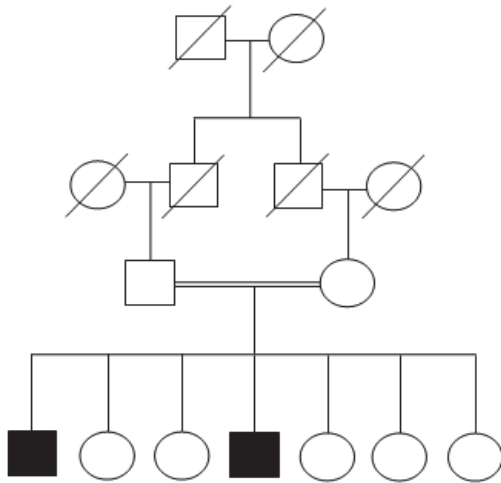


Figure 1. Pedigree of the family affected with CJD. Dark shaded: diagnosed as CJD; unshaded: unaffected.

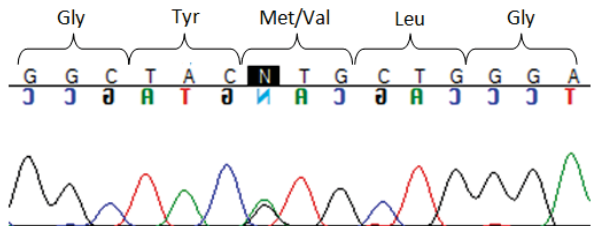


Figure 2. PRNP p.Val129/p.Met129 polymorphism. Chromatogram showing PRNP c.385A>G heterozygous variation in a CJD affected individual.

Discussion

To date coding variations of *PRNP* gene are reported as the only cause of disease in all cases of human inherited prion diseases including familial CJD (Mastrianni et al., 1996). Furthermore, mutations in *PRNP* have been observed in all familial cases of prion disease that have been genetically screened

(Windle et al., 1996). Here we report two siblings who are affected with apparently familial CJD but without any mutation in the amino acid coding exon, the non-coding exon, and flanking intronic regions of *PRNP*. The only variation in the DNA of both patients was a codon 129 allele polymorphism in the heterozygous state. Earlier studies showed that homozygosity of either allele at this position decreases the age at onset of familial CJD (Baker et al., 1991). However, onset of disease in our patients who were heterozygous was notably early and far below the average age at onset. Previously, the youngest reported age at onset was 26 years (Gambetti et al., 2003). In addition to there being multiple affected individuals in the same family, heterozygosity of codon 129 also makes the sporadic status for our patients very unlikely. We have no definitive explanation for absence of mutations in the DNA of our patients. One possibility is that an intronic variation that affects mRNA processing and ultimately the protein sequence exists, but was not detected because the long intron between the two exons of the gene was not sequenced. Alternatively, it is worth considering that genes other than *PRNP* may cause prion diseases. According to several studies there is a lack of correlation between PrP^{Sc} deposition and disease severity, and this may indicate that in addition to accumulation of PrP^{Sc}, the PrP^C to PrP^{Sc} conversion process may be important in the etiology of the disease (Hsiao et al., 1990; Bueler et al., 1994; Mallucci and Collinge 2005). It can be considered that genes that affect the conversion process or that render PrP^C unstable and more prone to convert to PrP^{Sc} may exist. It is expected that such genes would be able to contribute to the etiology of prion diseases. Consanguinity in the parents suggests that an unknown gene that affects disease status in a recessive fashion may exist in the family. It is necessary to genetically

examine larger numbers of familial prion cases in order to discriminate between these possibilities and address the possible existence of other prion causing genes. Clearly, this will be difficult as prion diseases are rare and familial cases constitute only a small percent of all the cases.

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