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Prion Protein Gene Analysis in Three Kindreds with Fatal Familial Insomnia (FFI): Codon 178 Mutation and Codon 129 Polymorphism

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Summary

Fatal familial insomnia (FFI) is a disease linked to a GAC(Asp)→AAC(Asn) mutation in codon 178 of the prion protein (PrP) gene. FFI is characterized clinically by untreatable progressive insomnia, dysautonomia, and motor dysfunctions and is characterized pathologically by selective thalamic atrophy. We confirmed the 178^{Asn} mutation in the PrP gene of a third FFI family of French ancestry. Three family members who are under 40 years of age and who inherited the mutation showed only reduced perfusion in the basal ganglia on single photon emission computerized tomography. Some FFI features differ from the clinical and neuropathologic findings associated with 178^{Asn} reported elsewhere. However, additional intragenic mutations accounting for the phenotypic differences were not observed in two affected individuals. In other sporadic and familial forms of Creutzfeldt-Jakob disease and Gerstmann-Sträussler syndrome, Met or Val homozygosity at polymorphic codon 129 is associated with a more severe phenotype, younger age at onset, and faster progression. In FFI, young and old individuals at disease onset had $129^{Met/Val}$. Moreover, of five 178^{Asn} individuals who are above age-at-onset range and who are well, two have 129^{Met} and three have $129^{Met/Val}$, suggesting that polymorphic site 129 does not modulate FFI phenotypic expression. Genetic heterogeneity and environment may play an important role in inter- and intrafamilial variability of the 178^{Asn} mutation.

Introduction

Fatal familial insomnia (FFI) is an autosomal dominant disease characterized by untreatable inability to sleep, dysautonomia, motor dysfunctions, selective thalamic atrophy with a 7–36-mo duration, and onset between 35 and 61 years of age (Lugaresi et al. 1986; Manetto et al. 1992). We recently reported that, in two Italian kindreds, FFI is linked to a GAC→AAC mutation in codon 178 of the prion protein (PrP) gene, resulting in an Asp→Asn substitution (Medori et al. 1992*b*, 1992*c*). A proteinase-K–resistant prion protein isoform (Medori et al. 1992*c*) was found in two of the three affected brains examined (Medori et al. 1992*a*). We now report that the same mutation is present also in three unaffected members of a French family with FFI described by Julien et al. (1990). These individuals are under 40 years of age and have been examined by electroencephalograph (EEG) and single photon emission computerized tomography (SPECT) in order to identify presymptomatic functional abnormalities.

Defects of the PrP gene have been reported in several kindreds affected with progressive dementing illnesses that are variably associated with ataxia, myoclonus, EEG periodic activity, and brain spongiosis and that are mainly categorized as Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler syndrome (GSS). PrP gene sequencing in these familial disorders identified a variety of underlying mutations. Generally, mutations are associated with rather consistent phenotypes, which may lead to the assumption that different PrP gene regions account for selected features. Substitutions at codons 102, 117, and 198 (Hsiao et al. 1989, 1992; for review, see Prusiner 1991 and Brown 1992) are associated with different forms of GSS, and substitutions at codons 200 and 178 are linked to CJD (Goldgaber et al. 1989;

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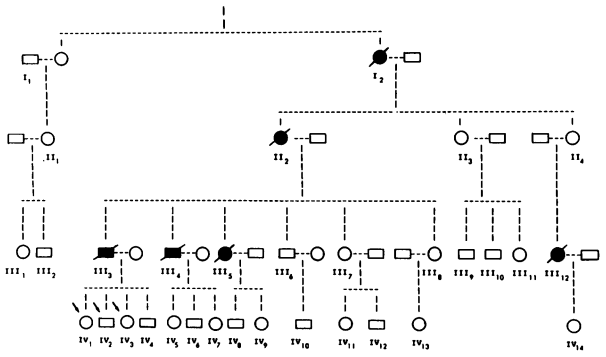

Figure 1 Pedigree of the French family (FFI3) affected with FFI. Circles denote female subjects, squares denote male subjects, and symbols with slashes through them denote deceased subjects. Blackened symbols denote affected subjects according to clinical and neuropathologic examination. Males and females are affected in each generation, as is expected in autosomal dominant diseases. The arrows indicate members who carry the 178^{Asn} mutation.

Goldfarb et al. 1991; Brown et al. 1992), whereas insertions cause atypical mixed forms (Collinge et al. 1990; Owen et al. 1991). However, the 178^{Asn} mutation was reported by Nieto et al. (1991) and Goldfarb et al. (1991) and later was reported by Goldfarb et al. (1992*a*) and Brown et al. (1992) in seven families with atypical CJD. Several factors, such as intragenic and genetic heterogeneity and environment, may account for the different phenotype and also for intrafamilial phenotypic variability. PrP gene variants such as polymorphic codon 129 are known to affect genetic susceptibility to prion diseases. Val or Met homozygosity at codon 129 is excessively frequent in sporadic and iatrogenic CJD, showing that this polymorphism is important in the phenotype expression (Goldfarb et al. 1989; Collinge et al. 1990; Palmer et al. 1991; Hsiao et al. 1992). We searched for intragenic abnormalities in the affected and asymptomatic members from the three FFI kindreds who carry the 178 mutation and looked for the role of the polymorphic site at codon 129 in this disease. A recent paper by Goldfarb et al. (1992*b*) showed segregation of the $129^{Met}/178^{Asn}$ allele in 15 FFI patients from different kindreds.

Patients and Methods

The family tree of the French family is shown in figure 1. All affected members underwent thorough medical investigation and have been described in detail by Julien et al. (1990). They had behavioral changes, insomnia, and hallucinations. Tests performed on affected subjects confirmed the presence of dysautonomia and the lack of EEG sleep patterns, as in FFI kindred 1 (FFI1) and FFI kindred 2 (FFI2). Patients III-3 and III-4, who underwent histopathologic examination, showed severe atrophy of anterior and dorsomedial thalamic nuclei (Julien et al. 1990). Cerebral and cerebellar cortices had mild to minimal gliosis but no spongiosis. The three asymptomatic members who carry the mutation at codon 178 underwent EEG and polygraphic recordings and SPECT. They had normal EEG and sleep patterns and had only mildly reduced perfusion in the basal ganglia on SPECT. FFI1 and FFI2 pedigrees are published elsewhere (Medori et al. 1992*a*, 1992*c*).

Peripheral blood DNA was obtained from 90 affected and asymptomatic members of the three FFI kindreds and from 20 unrelated individuals (Medori et al. 1992*c*). PCR amplification and sequencing of the prion coding region was carried out as described elsewhere (Medori et al. 1992*b*) from affected members IV-16 (59 years old) and V-58 (35 years old) of FFI1 and from IV-3 of FFI3. *Tth*111I (Boehringer Mannheim) digestion of the PrP gene coding region was carried out as described elsewhere (Medori et al. 1992*c*). The amplified coding region underwent restriction analysis also by using *Bsa*AI (pyAC/GTpu; New England Biolabs) enzyme according to the manufacturer's recommendations. Allele-specific–genotyped 129^{Met}, 129^{Val}, and $129^{Met/Val}$ DNA samples (provided by Dr. J. Collinge) were used as internal controls. We analyzed 13 members of the French family, 41 members of Italian FFI1, 31 members of Italian FFI2 (Medori et al. 1992*a*), and 20 unrelated controls. Restriction products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under a UV lamp.

Results

Members IV-1 and IV-2 of the French family showed heterozygous 178A(GAC)Asp→(AAC)Asn mutation (fig. 2). Since this mutation abolishes a normal *Tth*111I restriction site, the amplified coding region of the PrP gene digested with this enzyme shows three bands of 800, 556, and 244 bp in these individuals and only two 556- and 244-bp bands in normal subjects. This $178^{Asn(AAC)}$ mutation was confirmed by direct sequencing of the amplified open reading frame from member IV-3 of the same family (fig. 3). *Tth*111I restriction analysis searching for 178^{Asn} mutation carriers showed this substitution in 41%, 31%, and 30% of the FFI1, FFI2, and FFI3 members, respectively, at risk in each generation. All controls showed normal $178^{ASP(GAC)}$.

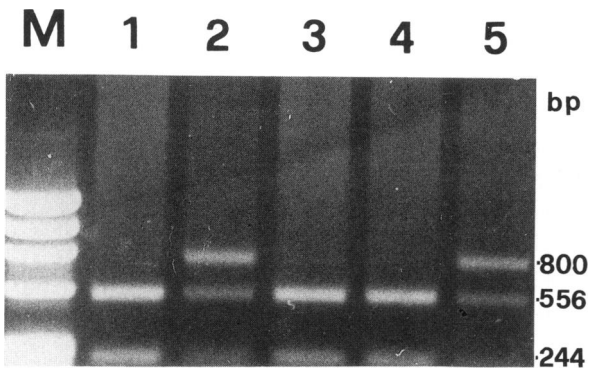

Figure 2 *Tth*111I digestion of the 800-bp–amplified PrP coding region from five FFI3 family members. Three bands of 800 bp, 556 bp, and 244 bp are present in members carrying the GAC→AAC mutation which abolishes the *Tth*111I restriction site in one of the two alleles (IV-1 [lane 2] and IV-2 [lane 5]). Only two fragments are present in family members without the mutation (III-6 [lane 1], IV-4 [lane 3], and IV-5 [lane 4]), as both alleles carry the restriction site. Lane M, DNA marker (*Bgl*I- and *Hin*fI-digested pBR328 DNA).

Analysis of polymorphic codon 129 by using *Bsa*AI enzyme showed one 800-bp band in 129^{Met}, two 800-bp and 413+387-bp bands in $129^{Met/Val}$, and one 413+387-bp band in 129^{Val} (fig. 4). Homozygous 129^{Met} was present in 60%, 44%, and 60% of the members from FFI1, FFI2, and FFI3, respectively (table 1). Analyzing affected individuals, we found that FFI1 members IV-21 and IV-37 and FFI2 member IV-48 carried homozygous 129^{Met}, whereas FFI1 members IV-16 and V-58 had heterozygous $129^{Met/Val}$ (fig. 5). By direct sequencing, we confirmed both the 178 GAC→AAC mutation and the heterozygous $129^{Met/Val}$ in IV-16 and V-58, and we ruled out additional abnormalities.

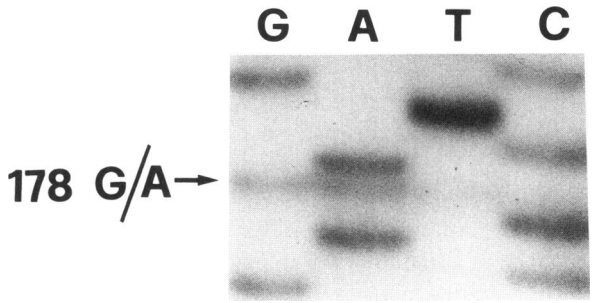

Figure 3 Autoradiograph of sequencing gel from part of the PrP coding region from FFI3 member IV-3. The gel shows a heterozygous G-to-A transition (arrow) generating the substitution Asp→Asn at amino acid 178.

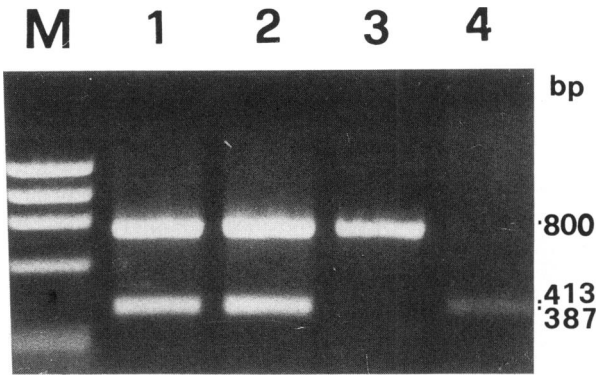

Figure 4 *Bsa*AI restriction analysis of normal unrelated control individuals. The two bands of 800 bp and 413+387 bp in lanes 2 and 3 indicate the presence of a restriction site for the enzyme in one allele and therefore the presence of $129^{Met/Val}$. The single 800-bp band seen in lane 4 shows the lack of restriction site for the *Bsa*AI enzyme and therefore the presence of $Met^{(ATG)}$ in both alleles. The single 413+387-bp band shown in lane 4 indicates the presence of *Bsa*AI restriction site $Val^{(GTG)}$ in both alleles. Lane M, Size marker (*Hae*III-digested OX-174RF DNA). Lanes 1 and 2, Heterozygous $129^{Met/Val}$. Lane 3, Homozygous 129^{Met}. Lane 4, Homozygous 129^{Val}.

Of all asymptomatic members with 178^{Asn} mutation, five individuals were above the upper limit of age onset. Two subjects, 62 years old and 68 years old, had homozygous 129^{Met}, and three members, 61 years old, 62 years old, and 63 years old, had $129^{Met/Val}$ (table 1). In agreement with published data (Owen et al. 1990), 43% of unrelated control cases showed 129^{Met}, and 15% showed 129^{Val}.

Discussion

These results confirm the association between FFI and the PrP gene 178^{Asn} mutation in a third unrelated FFI kindred from southern France. Affected members from the three families present with insomnia due to the inability to produce sleep patterns, dysautonomia, and motor dysfunctions. Selective thalamic atrophy is consistently present, while only the two FFI1 patients with the longest disease duration have mild spongy degeneration and EEG periodic activity. These clinical and pathological features differ from the phenotype linked to the same codon 178 mutation found in the Finnish family "Str" (Haltia et al. 1979) and the French families "Wui" and "Bel" (Buge et al. 1978; Cathala et al. 1986), as well as in the American-Hungarian "Day," the American-Dutch "Kui," the American-English "McK," and the American–French-Canadian "LaP" families (Friede et al. 1964; May et al. 1968; Masters et

Table 1

Presence of Polymorphic Codon 129 in All Three FFI Kindreds, Affected Members, and Asymptomatic Members

	Proportion of Polymorphic Codon 129 in FFI Kindreds (%)		
	129^{Met}	$129^{Met/Val}$	129^{Val}
FFI1	60	40	0
FFI2	44	36	0
FFI3	60	40	0
Control	43	42	15
	Member (age [in years] at onset)		
	129^{Met}	$129^{Met/Val}$	
Personally observed affected:			
FFI1	IV-21 (52) and IV-37 (45)	IV-16 (59) and V-58 (35)	
FFI2	IV-48 (50)		
	Member (present age [in years])		
Asymptomatic: FFI1	IV-43 (62) and IV-13 (68)	IV-18 (63), IV-21 (61), and IV-55 (62)	

al. 1981) by Nieto et al. (1991), Goldfarb et al. (1991, 1992*a*), and Brown et al. (1992). In these seven families, the disease shows a slower progression, with a 9–51-mo range, and generally does not present with total inability to sleep and dysautonomia. In contrast to FFI, on postmortem examination, moderate to severe spongiosis is a consistent finding, while the marked selective thalamic atrophy is not observed. Phenotypic heterogeneity may be explained on the basis of genetic diversity. However, additional intragenic abnormalities which might have explained the phenotypic difference have not been found by sequencing the PRP coding region from FFI affected members. Most frequently, allelic heterogeneity is due to mutations within the gene coding regions; however, it cannot be excluded that mutations of other nontranslated regulatory elements account for the diverse phenotypic expression. Therefore, genetic diversity has to be considered.

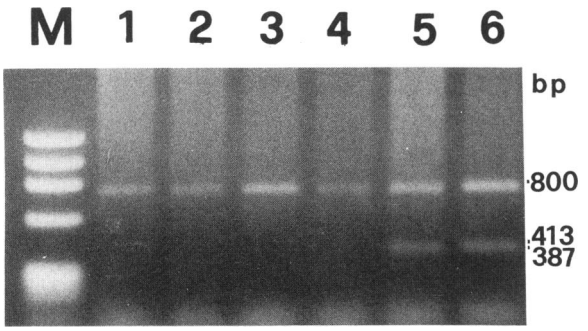

Figure 5 *Bsa*AI digestion of the 800-bp amplified PrP coding region in samples from six members of FFI1, FFI2, and FFI3 carrying the 178^{Asn} mutation. The presence of one 800-bp band in lanes 2–5 indicates homozygous 129^{Met}. In lanes 5 and 6, the two bands of 800 bp and 413+387 bp indicate heterozygous $129^{Met/Val}$. Lane M, Size marker (*Hae*III-digested OX-174RF DNA). Lane 1, FFI3 member IV-1. Lane 2, Affected FFI2 member IV-48 (age at onset = 50 years). Lanes 3–6, FFI1 members IV-21 (age at onset = 51 years), IV-37 (age at onset = 61 years), IV-16 (age at onset = 59 years), and V-58 (age at onset = 35 years).

Moreover, in Brown's and Goldfarb's families, the disease is fully penetrant, while FFI seems to have a low penetrance. Five individuals from the FFI1 who carry the 178^{Asn} mutation are above the upper limit of age onset and yet are asymptomatic. Intragenic variants such as polymorphic codon 129 (Goldfarb et al. 1989) may modulate disease expression. $129^{Met(GTG)}$ and $129^{Val(ATG)}$ homozygosity influences not only occurrence but also early onset and faster progression in familial CJD and GSS (Doh-Ura et al. 1989; Baker et al. 1991; Prusiner 1991; Goldfarb et al. 1992*b*). 129^{Met}

and, more often, 129^{Val} are excessively frequent in sporadic and iatrogenic CJD (Collinge et al. 1991; Palmer et al. 1991), indicating that these genotypes confer increased susceptibility to prion diseases. In FFI, of the five 178^{Asn} asymptomatic individuals, two, one 62 years old and the other 68 years old, show homozygous 129^{Met}, while the remaining three have $129^{Met/Val}$. These findings suggest that the low penetrance of FFI is not affected by homozygosity in this polymorphic site.

Moreover, in other prion diseases, 129^{Met} and 129^{Val} homozygosity is associated with earlier onset and shorter course. The analysis of codon 129 in the FFI affected members showed that FFI1 member V-58, who was 35 years of age at disease onset, and FFI member IV-16, who was 59 years of age at disease onset, carry $129^{Met/Val}$. Since only these last two patients were kept under constant hospital care, it is difficult to compare the disease course in all five patients. However, heterozygosity associated with early and late onset suggests that this polymorphic site does not influence the age at FFI presentation. In other human diseases with intrafamilial phenotypic variability, such as fucosidosis (Willems et al. 1988) and Waardenburg syndrome (Asher and Friedman 1990), modifier loci have been postulated. The FFI mutant phenotype is not influenced by additional intragenic mutations and polymorphic site 129 and may reflect the action of modifier loci or environmental influences.

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References

Asher JH, Friedman TB (1990) Mouse and hamster mutants as models for Waardenburg syndrome in humans. J Med Genet 27:618–626

Baker HF, Poulter M, Crow TJ, Frith CD, Lofthouse R, Ridley RM (1991) Aminoacid polymorphism in human prion protein and age at death in inherited prion disease. Lancet 337:1286

Brown P (1992) The phenotypic expression of different mutations in transmissible human spongiform encephalopathy. Rev Neurol 148:317–327

Brown P, Goldfarb L, Kovanen J, Haltia M, Cathala F, Sulima M, Gibbs CJ, et al (1992) Phenotypic characteristics of familial Creutzfeldt-Jakob disease associated with the codon 178^{Asn} PRNP mutation. Ann Neurol 31:282–285

Buge A, Escourolle R, Brion S, Rancurel G, Hauw JJ, Mehaut F, Gray F, et al (1978) Maladie de Creutzfeldt Jakob familiale: étude clinique et anatomique de trois cas sur huit répartis sur trois générations. Transmission au single écureuil. Rev Neurol 134:165–181

Cathala F, Brown P, Chatelain J, Castaigne P, Gajdusek C (1986) Maladie de Creutzfeldt-Jakob en France: intérêt des formes familiales. Presse Med 15:379–382

Collinge J, Owen F, Poulter M, Leach M, Crow TJ, Rossor MN, Hardy J, et al (1990) Prion dementia without characteristic pathology. Lancet 336:7–9

Collinge J, Palmer MS, Dryden AJ (1991) Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. Lancet 337:1441–1442

Doh-Ura K, Tateishi J, Sasaki H, Kitamoto T, Sakaki Y (1989) Pro-leu change at position 102 of prion protein is the most common but not the sole mutation related to Gerstmann-Straussler syndrome. Biochem Biophys Res Commun 163:974–979

Friede RL, DeJong RN (1964) Neuronal enzymatic failure in Creutzfeldt-Jakob disease. Arch Neurol 10:181–195

Goldfarb LG, Brown P, Goldgaber D, Asher DM, Strass N, Graupera G, Piccardo P, et al (1989) Patients with Creutzfeldt-Jakob disease and kuru lack the mutation in the PRIP gene found in Gerstmann Sträussler syndrome, but they show a different double-allele mutation in the same gene. Am J Hum Genet Suppl 45:A189

Goldfarb LG, Brown P, Haltia M, Cathala F, McCombie WR, Kovanen J, Cervenakova L, et al (1992*a*) Creutzfeldt-Jakob disease cosegregates with the codon 178Asn PRNP mutation in families of European origin. Ann Neurol 31:274–281

Goldfarb LG, Haltia M, Brown P, Nieto A, Kovanen J, McCombie WR, Trapp S, et al (1991) New mutation in scrapie amyloid precursor gene (at codon 178) in Finnish Creutzfeldt-Jakob kindred. Lancet 337:425

Goldfarb LG, Petersen RB, Tabaton M, Brown P, LeBlanc A, Montagna P, Cortelli P, et al (1992*b*) Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. Science 258:806–808

Goldgaber D, Goldfarb LG, Brown P, Asher DM, Brown WT, Lin WS, Teener JW, et al (1989) Mutations in familial Creutzfeldt-Jakob disease and Gerstmann-Straussler-Scheinker's syndrome. Exp Neurol 106:204–206

Haltia M, Kovanen J, Van Crevel H, Th G, Bots AM, Stefanko S (1979) Familial Creutzfeldt-Jakob disease. J Neurol Sci 42:381–389

Hsiao K, Baker HF, Crow TJ, Poulter M, Owen F, Terwilliger JD, Westaway D, et al (1989) Linkage of a prion protein missense variant to Gerstmann-Straussler syndrome. Nature 338:342–345

Hsiao K, Dlouhy SR, Farlow MR, Cass C, DaCosta M, Conneally PM, Hodes ME, et al (1992) Mutant prion proteins

in Gerstmann-Straussler-Scheinker disease with neurofibrillary tangles. Nature Genet 1:68–71

Julien J, Vital C, Deleplanque B, Lagueny A, Ferrer X (1990) Atrophie thalamique subaigue familiale: troubles mnesiques et insomnie totale. Rev Neurol 3:173–178

Lugaresi E, Medori R, Montagna P, Baruzzi A, Cortelli P, Lugaresi A, Tinuper P, et al (1986) Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. N Engl J Med 315:997–1003

Manetto V, Medori R, Cortelli P, Montagna P, Tinuper P, Baruzzi A, Rancurel G, et al (1992) Fatal familial insomnia: clinical and pathological study of five new cases. Neurology 42:312–319

Masters CL, Gajdusek DC, Gibbs CJ Jr (1981) The familial occurrence of Creutzfeldt-Jakob disease in Alzheimer's disease. Brain 104:535–558

May WW, Itabashi HH, DeJong RN (1968) Creutzfeldt-Jakob disease II: clinical, pathological and genetic study of a family. Arch Neurol 19:137–149

Medori R, Montagna P, Tritschler HJ, LeBlanc A, Cortelli P, Tinuper P, Lugaresi E, et al (1992*a*) Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. Neurology 42:669–670

Medori R, Tritschler HJ, Gambetti P (1992*b*) Production of single stranded DNA for sequencing: an alternative approach. Biotechniques 12:346–348

Medori R, Tritschler HJ, LeBlanc A, Villare F, Manetto V, Chen HY, Xue R, et al (1992*c*) Fatal familial insomnia is a prion disease with a mutation at codon 178 of the prion gene. N Engl J Med 326:444–449

Nieto A, Goldfarb LG, Chodosh HL, McCombie WR, Trapp S, Asher DM, Gajdusek DC (1991) Codon 178 mutation in ethnically diverse Creutzfeldt-Jakob disease families. Lancet 337:622–623

Owen F, Poulter M, Collinge J, Crow TJ (1990) Codon 129 changes in the prion protein gene in Caucasians. Am J Hum Genet 46:1215–1216

Owen F, Poulter M, Collinge J, Leach M, Tarulata S, Lofthouse R, Chen Y, et al (1991) Insertion in the prion protein gene in atypical dementia. Exp Neurol 112:240–242

Palmer MS, Dryden AJ, Hughes JT, Collinge J (1991) Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature 352:340–342

Prusiner SB (1991) Molecular biology of prion diseases. Science 252:1515–1522

Willems PJ, Garcia CA, de Smedt MCH, Martin-Jimenez R, Darby JK, Duenas DA, Granado-Villar D, et al (1988) Intrafamilial variability in fucosidosis. Clin Genet 34:7–14