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Molecular Genetic Studies of Creutzfeldt-Jakob Disease

Lev G. Goldfarb,* Paul Brown, Larisa Cervenakova, and D. Carleton Gajdusek

Laboratory of Central Nervous System Studies, NINDS, National Institutes of Health, Bethesda, MD 20892

Abstract

Genetic study of over 200 cases of Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI), and kuru have brought a reliable body of evidence that the familial forms of CJD and all known cases of GSS and FFI are linked to germline mutations in the coding region of the PRNP gene on chromosome 20, either point substitutions or expansion of the number of repeat units. No pathogenic mutations have so far been found in sporadic or infectious forms of CJD, although there are features of genetic predisposition in iatrogenic CJD and kuru. In FFI and familial CJD, clinically and pathologically distinct syndromes that are both linked to the $178^{Asp \rightarrow Asn}$ substitution, phenotypic expression is dependent on a polymorphism at codon 129. Synthetic peptides homologous to several regions of PrP spontaneously form insoluble amyloid fibrils with unique morphological characteristics and polymerization tendencies. Peptides homologous to mutated regions of PrP exhibit enhanced fibrilogenic properties and, if mixed with the wild-type peptide, produce even more abundant and larger fibrous aggregates. A similar process in vivo may lead to amyloid accumulation and disease, and transmission of "baby fibrils" may induce disease in other hosts.

Index Entries: Transmissible spongiform encephalopathies; Creutzfeldt-Jakob disease; PRNP gene mutations; Prion diseases.

Introduction

Creutzfeldt-Jakob disease (CJD) is a subacute mental and neurological disorder with prominent dementia and movement abnormalities typically affecting middle-aged individuals and leading to death in 3–12 mo after onset of symptoms. Spongiform degeneration in various parts of the brain cortex is the characteristic neuropathological feature. The disease is randomly distributed around the world, with an annual mortality rate of one per million. Familial accumulation is observed in 5–10% of cases, with an autosomal dominant pattern of inheritance. Gerstmann-Sträussler-Scheinker (GSS) disease is a rare familial disorder with cerebellar ataxia as the usual presenting symptom, and mas-

*Author to whom all correspondence and reprint requests should be addressed.

Table 1
Creutzfeldt-Jakob Disease Families with the 178^{Asn} Mutation

Country of origin	Number of affected members	Neuropathologically verified	Experimentally transmitted
Finland	14	4	1
Hungary	9	5	2
Netherlands	10	4	2
Canada	6	4	0
France	14	6	1
France	3	1	0
Total	56	24	6

sive degeneration in the cerebellum with characteristic multicentric amyloid plaques. Fatal familial insomnia (FFI) is also a rare syndrome, always familial, with intractable insomnia as a leading clinical feature, associated with degeneration of thalamic nuclei. Brain suspensions from CJD and GSS patients transmit disease to experimental primates through intracerebral inoculation, proving that these disorders are at once hereditary and infectious.

Major efforts have been undertaken in the last several years to elucidate the nature of these disorders. Novel molecular biology techniques have become important tools for analysis of the genetic mechanisms controlling these disorders and in studies of the infectious agent. This review includes detailed molecular-genetic analysis of familial CJD based primarily on our own studies of this disease. In many of the CJD patients and in some of their at risk family members we performed complete sequencing of the coding region of the PRNP gene in order to identify structural defects in this gene. Studies of pathogenic mechanisms of spongiform encephalopathies based on experiments with synthetic peptides conclude this review.

Germ-Line Mutations Causing Familial Creutzfeldt-Jakob Disease

Three germ-line mutations: point substitutions at codons 178 and 200, and an expending 24-nucleotide repeat, were identified in multiple families affected with CJD.

178^{Asn} Mutation

This mutation was first identified by direct sequencing of the coding region of the PRNP gene in two members of a Finnish CJD kindred *(1)* and a member of the US-Dutch family *(2)*. The GAC-to-AAC substitution at codon 178 results in an amino acid change from aspartic acid to asparagine. Using the *Tth III* 1 restriction test for screening, we initially identified 7 CJD families as carrying the 178^{Asn} mutation, but a subsequent analysis showed that one of the families had fatal familial insomnia caused by the same 178 mutation. Data describing the six families with dementing illness and spongiform encephalopathy characteristic for CJD and no insomnia or thalamic degeneration are shown in Table 1. Twenty-four patients were neuropathologically verified and six experimentally transmitted to primates, mostly to squirrel monkeys, by intracerebral inoculation of 10% brain suspensions. All 15 patients available for genetic screening showed the mutation, as well as 10 of 26 first degree relatives, but none of 83 unrelated controls. Tight linkage (LOD score of 5.3) was established between the mutation and disease in the Finnish and US-Hungarian families. In a pedigree of the original Finnish family (Fig. 1), of 13 individuals in generation III, seven tested positive for the 178^{Asn} mutation and all have died of CJD. The six others who tested negative (including the III-23 twin of a positive-testing CJD victim) all survived beyond the age of risk. This observation suggests that the disease penetrance is close to 100% and that intrafamilial transmission of disease to mutation-negative family members do not occur.

200^{Lys} Mutation

This mutation was first found in siblings from a Polish family *(3)*, then in seemingly sporadic patients from Slovakia and Chile, and a Sephardic Jewish family from Greece *(4)*. It was later established that all patients from a CJD cluster in Slovakia *(5)*, as well as all patients from a similar cluster in Libyan Jews living in Israel *(6,7)*, other

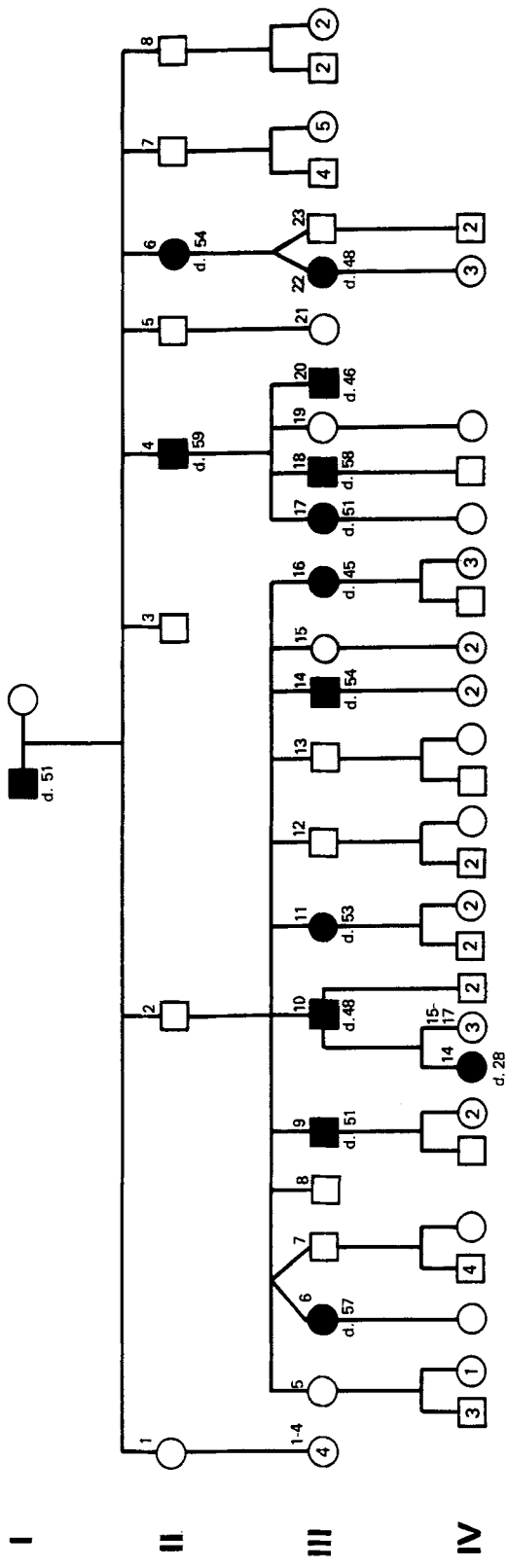

Fig. 1. Abridged pedigree of Finnish family "Str." Affected members are shown as neuropathologically and/or clinically verified cases (solid) of Creutzfeldt-Jakob disease. Number of unaffected individuals at risk is shown inside symbols. Cases III-6 and III-9 have been transmitted to experimental primates.

Table 2
Creutzfeldt-Jakob Disease Families with the 200^{Lys} Mutation

Country of origin	Number of families	Number of affected members	Neuropathologically confirmed	Experimentally transmitted
Slovakia	25	41	29	4
Poland, Germany	3	10	4	2
Libya	13	16	6	1
Tunisia	2	9	3	2
Greece	2	7	3	2
Chile	6	11	4	5
US	3	4	2	0
Total	54	98	51	16

Sephardic families originating in Greece and Tunisia *(8)*, and all tested familial CJD patients in Chile *(9)*, carried the same 200^{Lys} mutation.

The codon 200 GAG to AAG change results in a substitution of glutamic acid to lysine in the encoded protein. Restriction endonuclease analysis with *Bsm* A1 and single nucleotide extension reaction *(10)* were used for screening.

The current annual mortality rate of CJD in the Slovakian clusters is approx 200/million population/yr (in some Northern villages it approaches 2000/million); the Libyan Jewish population in Israel is characterized by rates close to 100/million/yr; and the frequency of CJD in some populations in Chile is 18/million/yr. The codon 200^{Lys} mutation was detected in 54 families with 98 known CJD cases (Table 2) by genetic screening of the CJD patients and/or their first-degree relatives. Fifty-one patients were neuropathologically verified and 16 experimentally transmitted to primates. All tested CJD patients and 24 of 71 first-degree relatives, but none of 103 unrelated healthy control individuals coming from the same populations had the mutation. A very strong association was demonstrated between the mutation and disease by comparing frequencies of the mutation in patients and control individuals ($X^2 = 90$ for Slovakia and $X^2 = 34$ for Libya).

Eleven of the mutation-carrying families have emigrated from the cluster areas to Western countries. Thirty cases of CJD were reported in the second, third, or fourth generations after emigration, among people who have never revisited the country of origin. For example, individuals in the left and the right parts of the pedigree shown in Fig. 2 still live in the Northern Slovakian CJD cluster region; the part of the family shown in the middle emigrated from Slovakia in the beginning of the current century and moved to the Chicago area. The last three generations of this American branch were born in the US and have never revisited Slovakia. Still, six family members developed typical CJD; all six were neuropathologically verified, and one experimentally transmitted. The fact that branches of some families migrating from cluster areas to other countries continue to have CJD over several generations, argues against a role of local environmental factors, and supports the view that familial CJD is a primarily genetic disorder, in which a germ-line mutation is responsible for disease.

The codon 200^{Lys} mutation probably originated in Spain and was dispersed in the Middle Ages by the mass migration of expelled Sephardic Jews to North African and European countries, and the migration of Spanish people to Latin American countries, including Chile. Historical data suggest that Sephardic Jewish communities were established in the areas of Krakow, Vienna, and Prague, surrounding Slovakia. Accumulation of the 200^{Lys} allele in some other populations does not yet have an adequate explanation.

Unlike 178^{Asn}, the codon 200^{Lys} mutation often occurs in families with "skipped" generations, with cases limited to a single generation, or even in apparently sporadic cases. The disease penetrance was found to be 0.56, i.e., presence of the 200^{Lys} mutation results in disease in approx half of the carriers.

24-Nucleotide Repeat Expansion

The wild-type sequence between codons 51 and 91 of the PRNP coding region consists of a 27-nucleotide sequence followed by four very similar but not identical 24-nucleotide repeats. Owen et al. *(11,12)* first reported a six extra 24-nucleotide repeat sequence in the PRNP gene in a British family with atypical CJD described in greater detail by Poulter

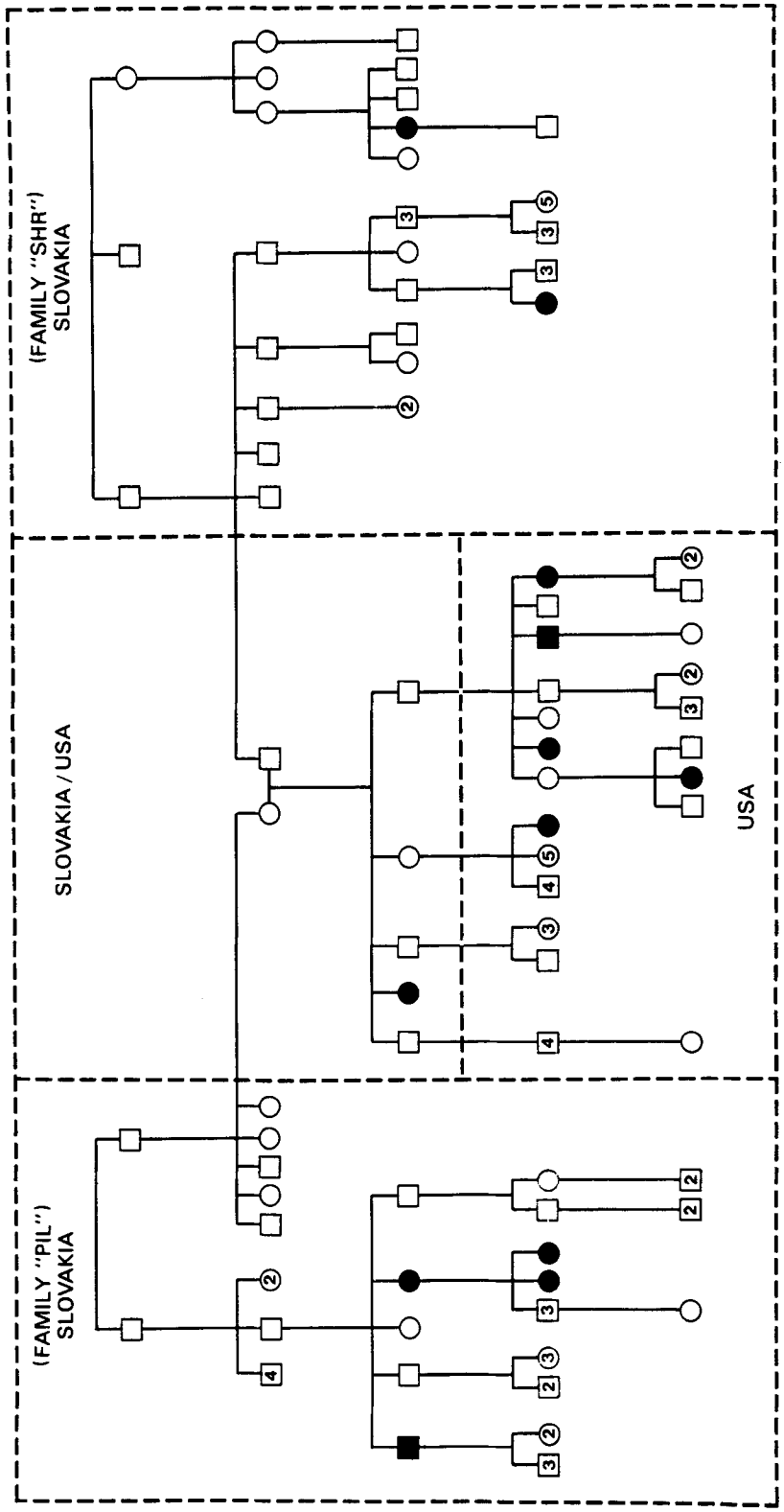

Fig. 2. Pedigree of a Slovakian family, of which the branch in the middle has left. The Slovakian CJD cluster area came to the US in 1902. Members of the last two generations of this American branch were born in the US. Six individuals from these two generations developed CJD and died of this disease. All six were pathologically verified and one experimentally transmitted.

Table 3
24-Nucleotide Repeat in the PRNP Gene
in Creutzfeldt-Jakob Disease and Nonneurological Control Patients

Number of repeats	Medical condition	Order of repeats
5	Normal	R1,R2,R2,R3,R4
7	CJD	R1,R2,R2,R2a,R2a,R3,R4
9	Cirrhosis	R1,R2,R2,R3,R2,R3,R2,R3,R4
10	CJD	R1,R2,R2,R3,R2,R3g,R2,R2,R3,R4
11	CJD	R1,R2,R2,R2,R3,R2,R3g,R2,R2,R3,R4
12	CJD	R1,R2,R2c,R3,R2,R3,R2,R3,R2,R3g,R3,R4
13	GSS	R1,R2,R2,R3,R2,R2,R2,R2,R2,R2,R2,R2a,R4
14	Dementing illness	R1,R2,R2,R3,R2,R3g,R2a,R2,R2,R2,R3g,R2,R3,R4

Table 4
Phenotypic Expression of Mutations Associated with Familial Creutzfeldt-Jakob Disease

		Familial CJD		
	Sporadic CJD	200^{Lys}	178^{Asn}	24-bp repeat expansion
Age at onset, yr; mean ± SD	62 ± 9	55 ± 8	46 ± 7	34 ±10
Duration of illness, mo; mean ± SD	6 ± 8	8 ± 18	22 ± 13	84 ± 5

et al. *(13)* and Collinge et al. *(14)*. We *(15)* confirmed that the area of tandem 24-bp repeats between codons 51 and 91 is variable. Of a total of 532 individuals screened for the number of repeat units, members of five CJD families and one nonfamilial nonneurological control patient were identified as having an expanded number of 24-bp repeat units in this region (Table 3). The nonneurological patient who died at age 63 of advanced micronodular cirrhosis had no family history of neurological disease, no clinical or pathological signs of spongiform encephalopathy, and brain tissue did not transmit disease to experimental primates. The PRNP coding region was completely sequenced, and a total of nine repeats were detected. No irregular nucleotide substitutions were seen. In contrast, the CJD patients from American families "Kel," "Ken," "Lar," and "Ald" with 7, 10, 10, and 12 repeats, and a French family "Che" with 13 repeats, all had irregular nucleotide substitutions, which may be the cause of instability. The reported CJD affected families with 11 *(12)* and 14 *(15)* repeats also had irregular substitutions. All members of a single family (affected or unaffected) always had the same number of repeats with the same irregular substitutions. The occurrence of extra coding repeats in the PRNP gene is associated with neuropathologically verified and experimentally transmitted familial CJD with an unusually early age of onset and prolonged duration of disease.

Other Mutations

Kitamoto et al. *(17)* have recently identified two new mutations in four cases of apparently sporadic CJD with clinically and pathologically typical disease. One case had a valine to isoleucine change at codon 180, two cases had a methionine to arginine substitution at codon 232, and the fourth case had both mutations.

Phenotypic Expression of Different Mutations

The age of onset and the disease duration in familial CJD varies with the type of mutation. At the time of disease onset, the patients carrying the 200^{Lys} mutation were in their fifties, patients from the 178^{Asn} families were in their mid-forties, and most of CJD patients with expanding number of 24-nucleotide tandem repeats were in their thirties (Table 4). The disease duration had the opposite distribution: It was the longest in the repeat expansion patients and increasingly shorter in the 178^{Asn} and 200^{Lys} cases.

The 178^{Asn} mutation patients were distinctive in that the initial sign of disease was invariably an

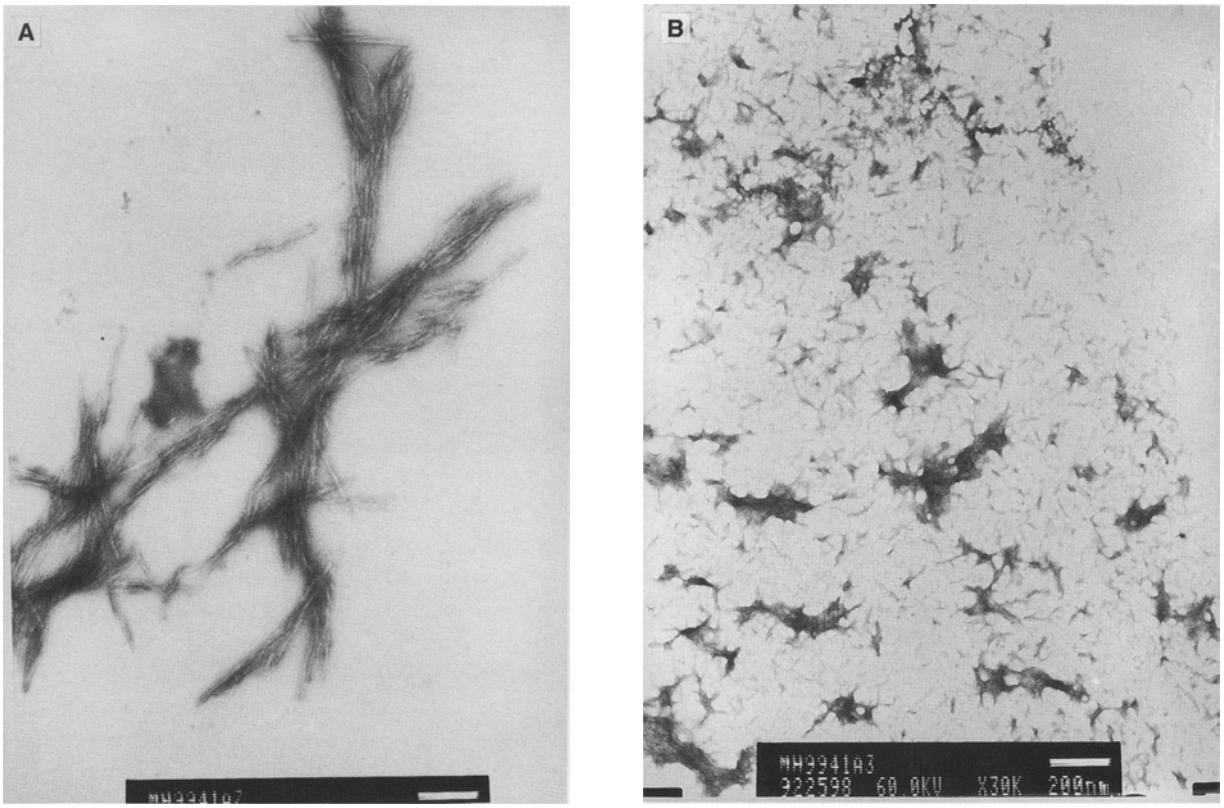

Fig. 3. Electron photomicrographs of aggregates of distinct rod-like fibrils 8–12 nm in width and several hundred nm in length, formed by **(A)** mutant 200^{Lys} peptide and **(B)** scattered short, ill-defined fibrils 6–8 nm in thickness, and <100 nm in length with clumps of fibrilar material, formed by mutant 178^{Asn} peptide (bar = 200 nm; 2% uranyl acetate stain).

insidious memory loss, and during the course of illness the triphasic periodic slow waves characteristic for all other familial and sporadic CJD patients were never seen.

Mutations Increase the Amyloidogenicity of PrP

Synthetic polypeptides corresponding to several sequences of PrP were shown to produce insoluble fibrils *(18,19)*. Our experiments *(20)* confirmed that peptides analogous to regions of PrP encoded by normal and mutant alleles at the regions of codons 178 and 200 spontaneously formed amyloid fibrils with unique morphological characteristics and aggregation tendencies. Two peptides from the area of codons 195–213 were 200^{Glu}: GENFTETDVKMMERVVEQM and 200^{Lys}: GENFTKTDVKMMERVVEQM and two peptides from the area of codons 169–185 were 178^{Asp}: YSNQNNFVHDCVNITIK and 178^{Asn}: YSNQNNFVHNCVNITIK.

Electron microscopic study revealed the presence of fibrils in 10 mg/mL solutions of 200^{Glu}, 200^{Lys}, 178^{Asp}, and 178^{Asn} peptides. Peptide 200^{Glu} produced long rod-like fibrils showing moderate aggregation. The number of fibrils was approx between 2 and 7/grid square. The mutant 200^{Lys} peptide produced similar fibrils, but these had a stronger tendency to aggregate and covered noticeably more grid area. A mixture of both peptides produced very abundant and large fibrillar aggregates. The normal 178^{Asp} peptide produced shorter and narrower fibrils than did the codon-200 peptides. The fibrils in the mutant (178^{Asn}) peptide solution were morphologically similar to 178^{Asp}, except that the number of aggregates and the overall amount of material was noticeably greater. These aggregates were structurally different from those seen in the peptides of the codon 200 mutation area: The fibrils were much thinner and the smaller and thinner aggregates could be seen on the background of many needle-like separate fibrils (Fig. 3). The mixed

Table 5
Fiber Formation by Synthetic Peptides
Homologous to Different Region of PrP

Peptide	Fiber formation		Birefrigence
	Disperse	Aggregates	
200^{Glu}	+	+	++
200^{Lys}	+	+++	+++
$200^{Glu} + 200^{Lys}$	+	++++	+++
178^{Asp}	+	+	+
178^{Asn}	+	++	+
$178^{Asp} + 178^{Asn}$	+	+++	++

solution of 178^{Asp} and 178^{Asn} peptides showed a pattern of fibrils and aggregates similar to 178^{Asn}, with larger aggregates and the amount of fibrilar material greater than in either 178^{Asn} or 178^{Asp} peptide solutions. Semiquantitative estimation of fibril production by various peptides is summarized in Table 5. Congo red staining confirmed the presence of amyloid-like fibrils in each of the preparations.

The results of this study suggest that synthetic peptides homologous to several regions of PrP spontaneously form insoluble amyloid fibrils with unique morphological characteristics and polymerization tendencies. Peptides homologous to mutated regions of PrP exhibit enhanced fibrilogenic properties and, if mixed with the wild-type peptide, produce even more abundant and larger fibrous aggregates. Fibril production (an intrinsic feature of PrP, apparently independent of the function of its normal precursor protein) enhanced by mutations is viewed as the primary event leading to amyloid accumulation and disease. Inoculation of "baby fibrils" to an intact animal may initiate the same process.

Conclusion

Mutations in the PRNP gene—point mutations in codons 178 and 200 and 24-nucleotide repeat expansion—are all associated with familial CJD. The presence of a single heterozygous mutation in each studied case of familial CJD and in approx half of the first-degree relatives, the absence of these mutations in unrelated control individuals, and the evidence for linkage between the individual mutations and the disease, indicate that familial CJD is a primarily genetic disorder with an autosomal-dominant pattern of inheritance. Occurrence of the disease in several generations in branches of families emigrating from cluster areas to different countries and continents argues against a role for local environmental factors and strongly favors the contention that mutations are the cause of familial CJD. There is an overall correlation between individual mutations and clinical patterns in each of the genetically different subsets of spongiform encephalopathy. Synthetic peptides homologous to various regions of PrP form distinctive amyloid fibrils and mixtures of mutant and normal peptides are more fibrilogenic than their normal or mutant counterparts alone.

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