Fatal Familial Insomnia and Familial Creutzfeldt-Jakob Disease: Disease Phenotype Determined by a DNA Polymorphism

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Fatal familial insomnia (FFI) and a subtype of familial Creutzfeldt-Jakob disease (CJD), two clinically and pathologically distinct diseases, are linked to the same mutation at codon 178 (Asn^{178}) of the prion protein gene. The possibility that a second genetic component modified the phenotypic expression of the Asn^{178} mutation was investigated. FFI and the familial CJD subtype segregated with different genotypes determined by the Asn^{178} mutation and the methionine-valine polymorphism at codon 129. The Met^{129}, Asn^{178} allele segregated with FFI in all 15 affected members of five kindreds whereas the Val^{129}, Asn^{178} allele segregated with the familial CJD subtype in all 15 affected members of six kindreds. Thus, two distinct disease phenotypes linked to a single pathogenic mutation can be determined by a common polymorphism.

In several Mendelian disorders, distinct phenotypes are linked to different point mutations in a single gene. Two forms of β-amyloid–related diseases, the hereditary cerebral hemorrhage with amyloidosis–Dutch type (HCHWA-D) and subtypes of familial Alzheimer's disease, are associated with different mutations in the amyloid precursor protein (APP) gene (*1*). Double point mutations in a single allele have been described in several conditions including sickle cell anemia, xeroderma pigmentosum, and GMI gangliosidosis (*2*). In these disorders, the second mutation triggers the disease or modifies its severity (*2*).

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A group of inherited and sporadic disorders known as spongiform transmissible encephalopathies, or infectious amyloidoses, or prion diseases, are characterized by the presence of an abnormal isoform of the prion protein (PrP) that is resistant to proteases (*3*). PrP is encoded by a gene (*PRNP*) located on the short arm of human chromosome 20 (*3*). On the basis of clinical and pathological characteristics, three inherited forms of spongiform encephalopathies have been recognized: Gerstmann-Sträussler-Scheinker syndrome (GSS), characterized by chronic cerebellar ataxia and dementia in association with the presence of multicentric amyloid plaques (*4*); Creutzfeldt-Jakob disease (CJD), a subacute dementing illness with widespread spongiform degeneration (*4*); and the recently described fatal familial insomnia (FFI), a subacute condition with untreatable insomnia, dysautonomia, and severe selective atrophy of thalamic nuclei (*5*). Several mutations in the coding region of *PRNP* are linked to these phenotypes. Subtypes of GSS are linked to mutations at codons 102, 117, 198, and 217 (*3*, *6*). Several subtypes of familial CJD are associated with a variable number of octapeptide coding repeats in the region of codons 51 to 91 (*3*). One subtype of familial CJD is linked to a point mutation at codon 200 (*3*). Surprisingly, the same GAC → AAC mutation in codon 178 of *PRNP*, resulting in the substitution of asparagine (Asn^{178}) for aspartic acid, is linked to one of the subtypes of familial CJD, and to FFI (*5*, *7*).

We have studied 30 affected members of 11 kindreds with the Asn^{178} mutation. The subjects affected by FFI had clinical, pathological, and transmissibility features that were different from those of the subjects with Asn^{178} CJD (Table 1) (*5*, *7–10*).

The coding region of *PRNP* has a polymorphism at codon 129 that results in two variant alleles, one coding for methionine

Table 1. Major phenotypic characteristics.

Characteristics	FFI*	CJD*
Age of onset (years)	49 ± 11†	45 ± 8†
Duration (months)	15 ± 8†	22 ± 13†
Ataxia	13/15	13/15
Myoclonus	15/15	11/15
Insomnia	13/15	0/15
Selective thalamic atrophy‡	13/13	0/5
Spongy degeneration	3/13	8/8
Transmission to primates	0/3	4/5

*References for characterization of the FFI and CJD families are (*8*) and (*9*), respectively. †Mean ± SD. ‡Refers to neuronal depopulation and reactive astrogliosis exceeding those present in other cerebral regions.

Table 2. Genotypic characteristics.

Kindred–subject*	Codon 129		Pattern†
	Mutant alleles	Normal alleles	
FFI-1–IV-37	Met	Met	1
IV-21	Met	Met	1
IV-34	Met	Met	1
IV-75	Met	Met	1
IV-16	Met	Val	2
V-58	Met	Val	2
FFI-2–IV-26	Met	Met	1
FFI-3–III-5	Met	Met	1
IV-7	Met	Met	1
V-14	Met	Met	1
IV-12	Met	Val	2
FFI-4–III-1	Met	Met	1
III-2	Met	Met	1
FFI-5–IV-1	Met	Met	1
IV-4	Met	Met	1
CJD-Str–III-6	Val	Met	3
III-9	Val	Met	3
III-11	Val	Met	3
III-14	Val	Met	3
III-15	Val	Met	3
III-17	Val	Met	3
III-19	Val	Met	3
IV-14	Val	Val	4
CJD-Wui–IV-4	Val	Met	3
CJD-LaP–III-11	Val	Met	3
CJD-Bel–II-1	Val	Val	4
CJD-Day–IV-2	Val	Val	4
IV-5	Val	Val	4
IV-6	Val	Val	4
CJD-Kui–III-8	Val	Val	4

*Kindreds FFI-1 and FFI-2 are Italian; FFI-3 and FFI-5 are American of British and German extractions; FFI-4 is French (*5*). CJD-Str is Finnish; CJD-Wui and CJD-Bel are French; CJD-Day, CJD-Kui, and CJD-LaP are American of Dutch, Hungarian, and French-Canadian origins, respectively (*7*). Subject identification as described (*5*, *7*). †See Fig. 1 for examples.

(Met^{129}) and the other for valine (Val^{129}) (*11*). The 129 polymorphism is common in the normal Caucasian population; the reported frequencies of the methionine and valine alleles are 0.62 and 0.38, respectively (*11*). Homozygosity for either allele predisposes individuals to infectious forms of spongiform encephalopathies such as kuru and iatrogenic CJD as well as to sporadic CJD (*11, 12*) or affects the age of onset in inherited forms such as GSS and a subtype of spongiform encephalopathy associated with a 144-bp insertion (6, *13*). We have tested the hypothesis that the polymorphism at codon 129 is involved in determining the disease phenotype, FFI or CJD, expressed by the pathogenic mutation.

The *PRNP* coding region was examined by sequencing and restriction analyses in the two groups of 15 subjects each with established FFI or Asn^{178} CJD phenotype (*14, 15*) (Table 2). We confirmed the presence of the Asn^{178} mutation in all 30 subjects (*14*). Codon 129 of the Asn^{178} allele was found to encode Met in all FFI subjects and Val in all those with the Asn^{178} CJD phenotype (*14*) (Fig. 1 and Table 2). Sequencing of the coding region of both alleles from 11 subjects representing three FFI and three Asn^{178} CJD kindreds revealed no genotypic variations, other than those related to the polymorphism at codon 129 (*15*). Therefore, the FFI and Asn^{178} CJD phenotypes are linked to two distinct haplotypes: Met^{129}, Asn^{178} for FFI and Val^{129}, Asn^{178} for CJD ($P < 0.005$, two-tailed Fisher's exact test).

Analysis of codon 129 in both PRNP alleles (Fig. 1 and Table 2) showed that 12 FFI-affected subjects were homozygous for methionine (Pattern 1), and three were heterozygous with Met^{129} on the Asn^{178} allele (Pattern 2). Nine Asn^{178} CJD-affected individuals, most of which were contributed by the "Str" kindred, were heterozygous with Val^{129} on the Asn^{178} allele (Pattern 3), and the remaining six were homozygous for valine (Pattern 4).

The three FFI subjects heterozygous at codon 129 had a significantly longer mean duration of the disease than the 12 homozygous subjects for methionine whereas the age of onset was not significantly different between the two groups (Table 3). Disease onset was significantly later and duration significantly longer in heterozygous than homozygous Asn^{178} CJD subjects. Thus, by prolonging the duration or delaying the onset of the disease, heterozygosity at codon 129 apparently slows the disease process in both FFI and Asn^{178} CJD providing partial protection against the disease as has been suggested for the sporadic and inherited forms of prion diseases (6, *11–13*). Spongiosis of cerebral cortex was observed only in heterozygous FFI subjects, raising the possibility that valine at residue 129 is needed to express spongiosis in FFI.

These findings indicate that in the kindreds examined the presence of methionine at position 129 of the PrP molecule encoded by the Asn^{178} mutant allele results in a disease process primarily localized to the thalamus (FFI phenotype), whereas valine at the same position is associated with a more widespread disease process (CJD phenotype). In contrast, the amino acid specified at codon 129 of the normal allele modifies the severity rather than the topography of the disease process, affecting duration and age of onset in both FFI and Asn^{178} CJD. Thus, a common polymorphism can modify both qualitatively and quantitatively the phenotypic expression of a pathogenic mutation linked to a Mendelian disorder.

The primary event in the pathogenesis of sporadic prion diseases is thought to be an acquired conformational change of the normal PrP isoform that acts as a template, engendering the formation of insoluble PrP aggregates (*3*). In inherited prion diseases, mutant PrP isoforms would spontaneously assume different conformations depending on the mutation (*3, 4*). An interaction between methionine or valine at position 129 and asparagine at position 178 might result in two abnormal PrP isoforms that differ in conformation and cause distinct diseases.

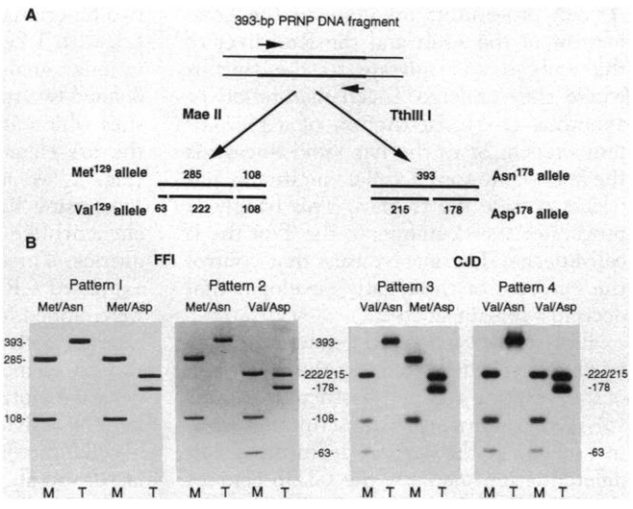

Fig. 1. Analysis of FFI and CJD genotypes. (**A**) A 393-bp fragment between nucleotides 320 and 713 of the *PRNP* coding region was amplified and digested with Mae II or TthIII 1 (*14*). Digestion products are shown. (**B**) Gel analysis of restriction digests. M, Mae II; T, TthIII 1. Pattern 1, FFI subject homozygous for Met^{129}; pattern 2, FFI subject heterozygous at codon 129; pattern 3, CJD subject heterozygous at codon 129; pattern 4, CJD subject homozygous for Val^{129}. In the heterozygous cases the Asn^{178} segregates with Met^{129} in FFI and Val^{129} in CJD. Sizes are in base pairs.

Table 3. Age of onset and duration of the disease.

Phenotype	Codon 129	Number of subjects	Age of onset (years) (mean ± SD)	Disease duration (months) (mean ± SD)
FFI	Met/Val	3	44 ± 11 (NS)*	26 ± 10 ($P < 0.002$)*
FFI	Met/Met	12	50 ± 5	12 ± 4
CJD	Val/Met	9	49 ± 4 ($P < 0.01$)*	27 ± 14 ($P < 0.05$)*
CJD	Val/Val	6	39 ± 8	14 ± 4

*Two-tailed Student's *t* test. NS, not significant. Comparisons were between heterozygous and homozygous individuals.

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8. In seven subjects from four kindreds (FFI 1, IV-37, IV-21, V-58, IV-16; FFI 2, IV-26; FFI 4, III-2; FFI 5, IV-4) sleep was investigated with polysomnography or overnight recording; severe insomnia with marked reduction or loss of slow waves and REM sleep stages was observed (*5*). In subject FFI 3, IV-7 sleep study revealed an atypical REM sleep pattern (W. Pendlebury, unpublished data). Insomnia was not reported in FFI 3 subjects IV-12 and V-14; the latter, however, was reported to have somnolence, (*10*). Dysautonomia, was recorded in all but four cases (FFI 3, IV-7, V-14; FFI 4, III-1; and FFI 5, IV-1) (*5*). Thalamic atrophy affected anterior ventral and mediodorsal nuclei in all 13 cases examined at autopsy. Other thalamic nuclei were variably involved. Cerebral cortex was unremarkable or had a moderate degree of astrogliosis. Widespread spongiosis was present only in two subjects (FFI 1, V-58 and IV-16) who had a clinical course of 25 and 34 months (*5*). These two subjects also had high-voltage synchronized spike and wave activity in electroencephalogram recording while the others had diffuse slowing (*5*). A single focus of initial spongiosis was seen in the entorhinal cortex of FFI 5 subject IV-4 who had a course of 13 months (*5*). Detailed histopathological examination was not carried out in FFI 3 subjects III-5 and IV-12 (*10*). Transmission of the disease to primates was unsuccessful with brain homogenates from FFI 3 subjects III-5, IV-5, IV-12 (*7*). Linkage analysis between disease and the Asn^{178} mutation carried out in kindred FFI 1 gave a cumulative lod score of 6.16 (*5*).
9. The Asn^{178} CJD subtype is characterized by early and severe memory loss (*7*). EEG shows diffuse slowing, but no periodic activity (*7*). Sleep physiological studies were performed in at least one patient with nonspecific findings (P. Brown, unpublished data). Neuropathological examination was carried out at autopsy in subjects CJD-Str III-9, III-14, CJD-Wui IV-4, CJD-Day IV-2, and IV-5, and by biopsy of cerebral cortex in subjects CJD-Str III-6, III-11, and CJD-Bel II-1 (*7, 16*). There was moderate or severe spongiosis with moderate or mild astrogliosis of the cerebral cortex in all cases. The thalamus was affected by spongiosis in several cases but less severely than the cerebral cortex. Autopsy examination of nine members of the CJD-Wui (IV-2), CJD-Day (III-1; III-7; III-8), CJD-Kui (II-9; II-10; III-4), and CJD-LaP (II-3; II-4) kindreds not included in this study revealed changes similar to those observed in the tested subjects (*16, 17*). Experimental transmission was accomplished with tissue from subjects CJD-Str III-6; CJD-Wui IV-4; CJD-Day IV-5; CJD-Kui III-8; it was unsuccessful with subject CJD-Bel II-1 (*7*). Linkage analysis between disease and the Asn^{178} mutation carried out in kindreds CJD-Str and CJD-Day gave a cumulative lod score of 5.30 (*8*).
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14. DNA was extracted from blood, frozen brain tissue, or fixed and paraffin-embedded brain tissue by standard techniques. Either a portion or the entire *PRNP* coding region was amplified by the polymerase chain reaction (PCR) under standard conditions (primer sequences available on request). The number of amplification cycles ranged from 25 to 35, depending on the source of the DNA, with each cycle comprised of denaturation at 94°C, annealing at 60°C, and extension at 72°C. Analysis of the genotype was based on digestion of the amplified DNA with TthIII 1 to screen for the Asn^{178} mutation and Nsp I or Mae II for analysis of codon 129.
15. Both PRNP alleles from an individual were analyzed by restriction enzyme digestion after cloning the amplified DNA (*14*) into a plasmid vector and identifying the Asn^{178} and Asp^{178} alleles by digestion with TthIII 1. Complete sequencing of the *PRNP* coding region was performed for 11 patients. Both alleles were sequenced using Sequenase (version 2.0; U.S. Biochemicals) or Taq DNA polymerase (Applied Biosystems). The resulting sequences were compared to that previously published (*18*).
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Ikaros, an Early Lymphoid-Specific Transcription Factor and a Putative Mediator for T Cell Commitment

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In a screen for transcriptional regulators that control differentiation into the T cell lineage, a complementary DNA was isolated encoding a zinc finger protein (Ikaros) related to the *Drosophila* gap protein Hunchback. The Ikaros protein binds to and activates the enhancer of a gene encoding an early T cell differentiation antigen, CD3δ. During development, Ikaros messenger RNA was first detected in the mouse fetal liver and the embryonic thymus when hematopoietic and lymphoid progenitors initially colonize these organs; no expression was observed in the spleen or the bone marrow. The pattern of Ikaros gene expression and its ability to stimulate CD3δ transcription support the model that Ikaros functions in the specification and maturation of the T lymphocyte.

T cell progenitors originate in the bone marrow of the adult and the fetal liver of the embryo and migrate to the thymus where they undergo T cell maturation or apoptosis (*1–5*). Restriction of a pluripotent progenitor to the lymphoid lineage is the first step toward T cell commitment and occurs outside the thymus. This lymphoid progenitor then commits to the T or the B cell lineage. The mechanisms that control the outcome of these early developmental decisions remain unclear.

Early events in T cell differentiation may be characterized by studying the regulation of transcription of T cell–restricted antigens (*6*). We examined the transcriptional control of one of the earliest definitive T cell differentiation markers, the CD3δ gene of the CD3–T cell receptor (TCR) complex (*5, 7*). In order to identify a transcription factor expressed at, or earlier than, T cell commitment, which could function as a genetic switch regulating entry into the T cell lineage, we characterized the T cell–specific enhancer mediating CD3δ gene expression (*8, 9*). This enhancer contains two functionally distinct elements, δA and δB, with T cell–restricted activity (*9*). Mutational analysis of the δA element has defined two transcriptionally active binding sites which are required for full activity of the δA element and the CD3δ enhancer (Fig. 1, A and B), a CRE [cyclic AMP (adenosine 3′,5′-monophosphate) response element]-like region and a G-rich sequence. Three isoforms of the ubiquitously expressed CRE-binding protein (CRE-BP) were cloned from T cells for their ability to interact with the CRE-like binding site of the δA element (*10*). Although dominant negative mutations in CRE-BP down-regulate the activity of this enhancer element in T cells, the expression of this transcription factor in all hematopoietic and non-hematopoietic cells suggests that it is unlikely to be the switch that activates the CD3δ enhancer in the early prothymocyte progenitor (*10*).

A variant of the δA element with a mutated CRE binding site (δAmu1-CRE) was used to screen a T cell expression library as described (*10*). A lymphoid-restricted cDNA was cloned that encodes a previously uncharacterized zinc finger protein (Ikaros). The Ikaros protein contains 431 amino acids and five $CX_2CX_{12}HX_3H$ zinc finger motifs organized in two separate clusters (Fig. 2A). The first cluster of three fingers is located 59 amino acids from the

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