Molecular Neurobiology

ISSN0893-7648/94/8(2-3): 99–103/$5.00

A Novel Mechanism of Phenotypic Heterogeneity Demonstrated by the Effect of a Polymorphism on a Pathogenic Mutation in the PRNP (Prion Protein Gene)

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Abstract

Fatal familial insomnia (FFI) is a subacute dementing illness originally described in 1986. The phenotypic characteristics of this disease include progressive untreatable insomnia, dysautonomia, endocrine and motor disorders, preferential hypometabolism in the thalamus as determined by PET scanning, and selective thalamic atrophy. These characteristics readily distinguish FFI from other previously described neurodegenerative conditions. Recently, FFI was shown to be linked to a mutation in the prion protein gene (PRNP) at codon 178, which results in the substitution of asparagine for aspartic acid. As such, FFI represents the most recent addition to the growing family of prion protein-related diseases. The mutation that results in FFI had previously been linked to a subtype of familial Creutzfeld-Jakob disease (178^{Asn} CJD). The genotypic basis for the difference between FFI and 178^{Asn}CJD lies in a polymorphism at codon 129 of the mutant prion protein gene: 129^{Met} 178^{Asn} results in FFI, 129^{Val} 178^{Asn} in CJD. The finding that the combination of a polymorphism and a single pathogenic mutation result in two distinct conditions represents a significant advance in our understanding of phenotypic variability.

Index Entries: Fatal familial insomnia; Creutzfeldt-Jakob disease; dementia; PRNP; mutation; polymorphism; phenotype; prion.

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Introduction

Fatal familial insomnia (FFI) was first described in a large Italian kindred as a neurodegenerative condition that presented with progressive insomnia, demonstrated by polysomnography and autonomic dysfunction *(1)*. Pathologic evaluation demonstrated severe neuronal loss in the anterior and dorsomedial thalamic nuclei without spongiotic, vascular, or inflammatory changes. In addition to being the first example of a genetically linked condition that affected specific thalamic nuclei, FFI underscored the importance of these nuclei in the regulation of the sleep–wake cycle, as well as in other autonomic functions.

Five additional cases of longer duration were subsequently reported *(2)*. In one of these cases, there was focal spongiosis in the cerebral cortex, but otherwise the pathology was identical to the original cases. The spongiform degeneration in this case suggested that it might be a familial prion disease. Protease-resistant prion protein (PrP) was detected, and analysis of the PRNP revealed that there was a mutation at codon 178 *(3)* that replaced the normal aspartic acid with asparagine (178^{Asn}). Following this discovery, the 178^{Asn} mutation was found in four additional FFI families *(4,5)*. The cumulative LOD score for 178 mutation in the five families is 6.5. Surprisingly, at the time that the genetic basis of FFI was being uncovered, the genetic lesion in one form of familial Creutzfeldt-Jakob was shown to be an identical mutation at codon 178 *(6)*.

Characterization of FFI and 178^{Asn} CJD

The revelation that FFI and 178^{Asn} CJD shared a common mutation necessitated a careful clinical and pathologic review of the two conditions. FFI presents clinically with progressive insomnia characterized by loss of slow wave and REM sleep, which can be rigorously established by overnight polysomnography *(1)*. This type of formal documentation is critical since approx 30% of adults over 50 yr of age report some sleep disturbance *(7)*. FFI results in an inability to generate electrophysiological sleep patterns, not merely a reduction in sleep *(8)*. In one case of 178^{Asn} CJD tested by polysomnography, no sleep disturbance was detected (*9* and M. Haltia and M. Partinen, personal communication). FFI also alters autonomic functions and circadian rhythms, while sparing mental function in the early stages of the disease. In contrast, patients affected by CJD generally present with rapidly progressive dementia *(10)*.

Pathologic examination allows further differentiation. FFI specifically affects the thalamus, and only in cases of long duration is there focal involvement of the cerebral cortex (Fig. 1). In contrast, 178^{Asn} CJD demonstrates widespread spongiosis in the cerebral cortex irrespective of disease duration, and no specific thalamic degeneration (Fig. 1). Thus, FFI and 178^{Asn} CJD can be distinguished on both clinical and pathologic grounds, leaving us with the quandary as to how a single mutation can result in these two conditions.

Codon 129 Polymorphism in PRNP

There is a well-documented polymorphism in PRNP at codon 129 that codes for either methionine or valine *(11)*. Although the valine allele is not particularly rare, around 38%, Collinge and his coworkers have shown that within the sporadic and iatrogenic cases of CJD, there is a statistically skewed distribution of alleles *(12)*. In their study, although 51% of the normal population was heterozygous at codon 129, only 11% of the sporadic cases of CJD were heterozygous. Of the homozygous cases in the normal population, 22% were valine and 78% were methionine. On the basis of these data, we reasoned that the polymorphism at codon 129 might have an effect on the expression of the 178 mutation.

Analysis of 15 FFI- and 15 178^{Asn} CJD-affected subjects showed a remarkably skewed distribution at codon 129 *(9)*; most notably in FFI there were no subjects homozygous for Val whereas in 178^{Asn} CJD, there were no subjects homozygous for Met (Fig. 2). However, these data did not provide a genetic explanation for the difference between FFI and 178^{Asn} CJD, since there were heterozygous individuals in both instances. Consequently, we examined codon 129 on the mutated allele, and discovered that in all cases FFI was linked to methionine and that 178^{Asn} CJD was linked to valine at codon 129. Thus, the polymorphism at codon 129 in conjunction with the mutation at codon 178 results in two different conditions.

The question remaining was whether codon 129 on the normal allele had any effect on the disease. It had been suggested previously that heterozygosity at codon 129 had a "protective" effect from iatrogenic transmission of CJD and resulted in a prolonged disease course in sporadic CJD *(12)*. The

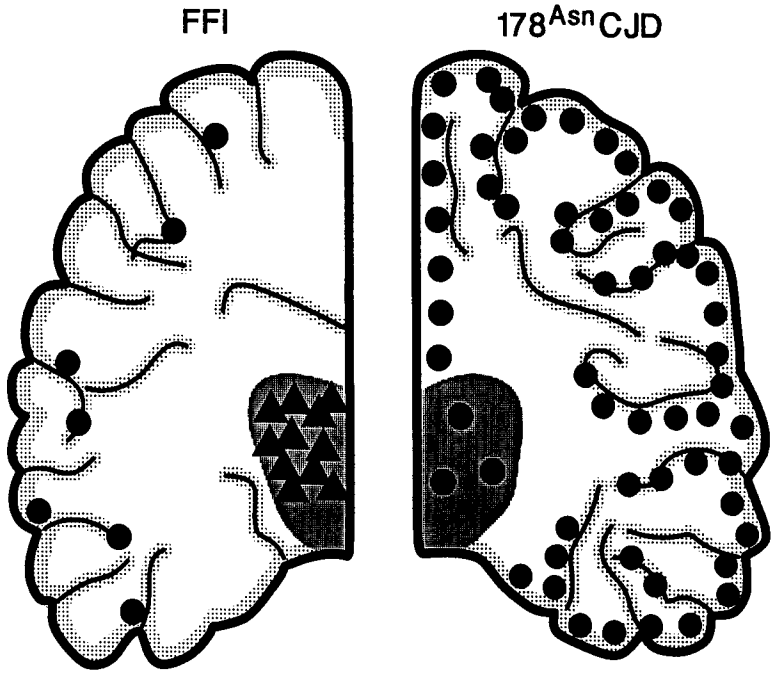

Fig. 1. Distribution of lesions in FFI and 178^{Asn} CJD. Note that in FFI, the thalamus is most severely affected, but the lesions (in the thalamus) do not include spongiform degeneration. In contrast, the lesions associated with 178^{Asn} CJD are prominent in the cerebral cortex and are characterized by spongiform degeneration. (▲ = neuronal loss and gliosis; ● = spongiform degeneration)

	Normal	FFI	CJD
Met/Met	37%	80%	0%
Met/Val	51%	20%	60%
Val/Val	12%	0%	40%
Met	62%	90%	30%
Val	38%	10%	70%

Fig. 2. Prevalence of the Met/Val polymorphism at codon 129 of PRNP in a normal Caucasian population and in patients with FFI or 178^{Asn} CJD *(9,12)*. The upper part of the figure shows the distribution of genotypes in normal, FFI, and 178^{Asn} CJD populations. The lower part of the figure shows the distribution of alleles in the three populations.

disease duration in heterozygous FFI patients was 26 ± 10 mo, whereas the homozygous FFI patients had a disease course of 12 ± 4 mo ($p < 0.002$). Similarly, in 178^{Asn} CJD patients, the disease course in the heterozygotes was 27 ± 14 mo, and in the homozygotes, 14 ± 4 mo ($p < 0.05$). In addition to the difference in disease duration, the onset of disease was markedly earlier in 178^{Asn} CJD homozygotes (39 ± 8 yr vs 49 ± 4 yr; $p < 0.01$).

Discussion

Taken in sum, our data show that the codon 129 polymorphism operates to modify the disease process both qualitatively and quantitatively in association with the codon 178 mutation (Fig. 3). Codon 129 acts as a binary switch on the mutant allele resulting in two clinically and pathologically distinct entities. $129^{Met}\backslash 178^{Asn}$ causes FFI, which is characterized by insomnia and a pathology largely confined to the thalamus, whereas $129^{Val}\backslash 178^{Asn}$ causes 178^{Asn} CJD, which lacks the FFI sleep disturbance and possesses widespread cortical pathology. Phrased in another way, the 129 polymorphism on the mutant allele appears to dictate the disease topography, which in turn is reflected in the clinical presentation. On the normal allele, codon 129 acts as a modulator of the disease severity. Individuals heterozygous at codon 129 are partially "protected," with a prolonged disease course compared to homozygotes in both FFI and 178^{Asn} CJD and a delayed onset of disease in 178^{Asn} CJD. Thus, the 129 polymorphism on the normal allele dictates the disease severity.

One of the inherent difficulties in genetic analysis of rare diseases is that the sample sizes are small.

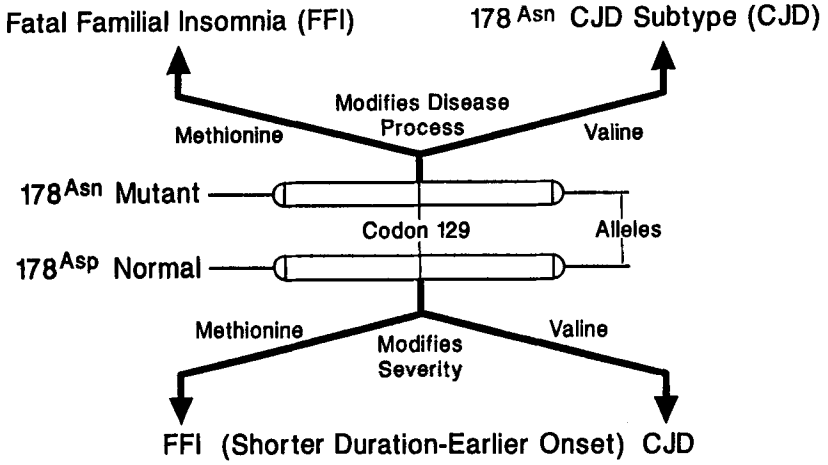

Fig. 3. Effect of the codon 129 polymorphism on expression of the 178^{Asn} mutation.

As a result, it is possible that a mutation or polymorphism linked to a disease may actually be just a marker for the disease-causing locus rather than the cause or modifier of the disease. We have considered this possibility of the association of the codon 129 polymorphism with FFI and 178^{Asn} CJD. Two recent cases have clarified this issue.

In the first case, described by Bosque et al. *(13)*, the proband presented with low-grade fever, night sweats, and a rash. These symptoms were followed by claustrophobia and insomnia. Eighteen months after the onset of symptoms, PET scanning showed reduction of activity in the thalamus and medial frontal lobes. The subject died 25 mo after onset of symptoms. A collaborative review of the neuropathology in this case (C. L. Vnencak-Jones, M. Johnson, and P. Gambetti, personal communication) revealed that the lesions were similar to those in FFI of long duration. In accord with this diagnosis, most of the other affected relatives are reported to have suffered from insomnia *(13)*. Analysis of the PRNP in this family demonstrated the codon 178^{Asn} mutation on a codon 129 methionine allele, as one would expect, but in addition there was a 24-base deletion resulting in the juxtaposition of codons 82 and 91. As predicted from the relatively long disease course, the proband was found to be heterozygous at codon 129 (C. L. Vnencak-Jones, personal communication). The 24-bp deletion present in this case is a rare polymorphism, estimated at 1–3% *(14,15)*, that is, in and of itself, apparently not pathogenic *(15)*.

A second case with a genotype apparently identical to that described by Bosque has recently been identified (A. Reder, R. Roos, and J. P. Spire, personal communication). In this case, the diagnosis of FFI was made during the life of the patient based on polysomnography. The histopathology was also consistent with the diagnosis of FFI. Thus, the two families with a 129^{Met}, 178^{Asn}, and 24-bp deletion on the same allele manifest a condition that is more similar to FFI than to 178^{Asn} CJD. Since these families are not related to the previous FFI kindred, we conclude that the 129 polymorphism provides the switch resulting in FFI or 178^{Asn} CJD, rather than acting as marker for another locus.

This represents the first observation that a common polymorphism can modify the expression of a pathogenic mutation. This novel mechanism may help explain phenotypic heterogeneity that has been observed in other familial diseases.

Acknowledgments

We thank all the physicians and families whose cooperation was, and continues to be, vital to these studies. In addition, Mark Cohen for critical reading of the manuscript and Sandra Richardson for expert technical assistance. This work was supported by NIA AG-08992-02, NIH NIA 1R01 AGNS08155-02, NATO 910672, and the Britton Fund.

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