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Fatal familial insomnia and familial Creutzfeldt–Jakob disease: Different prion proteins determined by a DNA polymorphism

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ABSTRACT **Fatal familial insomnia and a subtype of Creutzfeldt–Jakob disease, two clinically and pathologically distinct diseases, are linked to the same mutation at codon 178 (Asp-178 → Asn) but segregate with different genotypes determined by this mutation and the methionine–valine polymorphism at codon 129 of the prion protein gene. The abnormal isoforms of the prion protein in these two diseases were found to differ both in the relative abundance of glycosylated forms and in the size of the protease-resistant fragments. The size difference was consistent with a different protease cleavage site, suggesting a different conformation of the protease-resistant prion protein present in the two diseases. These differences are likely to be responsible for the type and location of the lesions that characterize these two diseases. Therefore, the combination of the mutation at codon 178 and the polymorphism at codon 129 determines the disease phenotype by producing two altered conformations of the prion protein.**

Prion diseases, also called spongiform encephalopathies or transmissible amyloidoses, are a group of sporadic, iatrogenic, and familial diseases that affect humans and animals (1–3). The human familial forms include three major groups: (*i*) Creutzfeldt–Jakob disease (CJD), a subacute dementing illness usually associated with cerebellar signs, myoclonus, and spongiform degeneration (4, 6); (*ii*) Gertsmann–Sträussler–Scheinker syndrome (GSS), a chronic condition characterized by ataxia, dementia, and the presence of amyloid plaques (4–6); and (*iii*) fatal familial insomnia (FFI), characterized by a loss of the ability to sleep, dysautonomia, selective atrophy of the thalamus, and usually no spongiform changes (7–11). Within these three groups there are subtypes defined by specific mutations in the prion protein (PrP) gene (*PRNP*). Mutations at *PRNP* codons 102, 105, 117, 198, and 217 are associated with GSS subtypes (12, 13), while a mutation at codon 200 is linked to one CJD subtype, CJD^{200} (14). A mutation at codon 178 that results in the replacement of aspartic acid by asparagine (Asp-178 → Asn) is shared by FFI and another CJD subtype, CJD^{178} (4, 15–17). In spite of the common mutation, these two diseases are phenotypically distinct since in CJD^{178} severe sleep impairment has not been reported and there is widespread spongiosis rather than selective thalamic atrophy. Also, in contrast to FFI, CJD^{178} has been consistently transmitted to receptive animals (18). We recently showed that FFI and CJD^{178} segregate with distinct *PRNP* genotypes determined by a common methionine–valine polymorphism at codon 129 of the mutant allele that specifies methionine in FFI and valine in CJD^{178} (18).

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The hallmark of all prion diseases is the presence of an aberrant isoform of the PrP that is partially resistant to proteases (1–3). The protease resistance is thought to result from a conformational change of the normal PrP (1–3). Different protease-resistant PrP isoforms have been shown to cause distinct pathological changes in animal models (19–21). In inherited human prion diseases, it is hypothesized that each mutation results in a different PrP conformation, which in turn determines the disease phenotype (1–3). However, this proposal fails to explain FFI and CJD^{178}, which are associated with the same mutation.

In this study, we have characterized the protease-resistant PrP present in brain tissue of subjects from three FFI and three CJD^{178} kindreds and found them to differ both in size and in the relative abundance of glycosylated forms.

MATERIALS AND METHODS

Source of Tissue. The subjects and kindreds for this study have been described (18). Tissue analyzed was from five subjects from three FFI kindreds (FFI-1: IV-16, IV-37, and V-58; FFI-2: IV-27; FFI-5: IV-4) and five subjects from three CJD^{178} kindreds (CJD^{178}-Str; CJD^{178} Day IV-2; CJD^{178}-Wui: IV-4, V-5, and V-6). Two of the FFI and three of the CJD^{178} subjects were heterozygous at *PRNP* codon 129 (18).

Antibodies. The following antibodies were used: mouse monoclonal antibody 3F4 (22), which reacts with PrP residues 109–112 (23); rabbit antisera to synthetic peptides homologous to hamster PrP residues 90–104 (24) and to human PrP residues 220–231 (L.A.-G., unpublished data) and residues 23–40 (B.G., unpublished data).

Preparation and Analysis of Protease-Resistant PrP. Brain tissue was homogenized in 9 volumes of lysis buffer (100 mM NaCl/10 mM EDTA/0.5% Nonidet P-40/0.5% sodium deoxycholate/10 mM Tris, pH 7.4) and digested with proteinase K at 100 μg/ml for 1 hr at 37°C. Digestion was terminated by the addition of 2 mM phenylmethylsulfonyl fluoride and boiling in electrophoresis sample buffer (3% SDS in 62.5 mM Tris, pH 6.8). For deglycosylation, these proteinase K-treated samples were digested for 2 hr with recombinant glycopeptide N-glycosidase (PNGase F; New England Biolabs) as specified by the supplier, precipitated with 4 volumes of methanol at −20°C, and resuspended in electrophoresis sample buffer. Presence of the glycosyl phosphatidylinositol anchor was determined by phase-partitioning with Triton X-114 (25).

Abbreviations: CJD, Creutzfeldt–Jakob disease; FFI, fatal familial insomnia; GSS, Gertsmann–Sträussler–Scheinker syndrome; *PRNP*, prion protein gene; PrP, prion protein; PNGase, glycopeptide N-glycosidase.

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Gel Electrophoresis and Immunoblots. Samples were resolved on 12% polyacrylamide gels and transferred to Immobilon P (Millipore) for 2 hr at 60 V. Immunoblots were developed by the enhanced chemiluminescence system (ECL, Amersham) as described by the manufacturer. Quantitative analysis of the immunoblots was carried out with a computer-assisted laser scanner (LKB Ultroscan XL).

Analysis of Amino- and Carboxyl-Terminal Fragments. Brain homogenates (1 g in 10 volumes of 320 mM sucrose/1 mM EDTA/10 mM Tris, pH 7.5) were centrifuged at 5000 × *g* for 10 min, and the supernatants were centrifuged at 100,000 × *g* for 60 min. The final pellets were homogenized in lysis buffer, digested with proteinase K, and deglycosylated as described above and then digested with endoproteinase Asp-N (sequencing grade, Boehringer) as described (26). Briefly, the samples were denatured with 6 M guanidine hydrochloride in 50 mM Tris·HCl (pH 8.0), reduced with 2 mM dithiothreitol, carboxymethylated with 6 mM sodium iodoacetate, and precipitated with 10 volumes of ethanol at −20°C. The pellet was resuspended in 0.1% SDS/50 mM sodium phosphate, pH 8.0, and digested overnight at 37°C with endoproteinase Asp-N. Peptides were electrophoretically resolved in 10–18% (wt/vol) Tris–tricine gels (27) and detected on immunoblots as above. For analysis of the intact PrP amino-terminal fragment in subjects homozygous for either valine or methionine at codon 129, the same procedure was followed except that digestion with proteinase K was omitted.

RESULTS

Proteinase K treatment of brain tissue from FFI subjects generated two major fragments of 28 and 26 kDa (Fig. 1). A minor fragment migrating at 19 kDa was seen at longer film exposures. Similar preparations from CJD[178] subjects demonstrated three fragments of 29, 27, and 21 kDa (Fig. 1). The stoichiometry among these fragments in CJD[178] (1:1.53:0.45) was significantly different from that in FFI (1:0.69:0.09). All fragments from both diseases included the PrP region between residue 90 and the site of attachment of the glycosylphosphatidylinositol anchor at residue 231, as determined by epitope-mapping with antisera to PrP residues 90–104 and 220–231 (data not shown).

N-deglycosylation of proteinase K-treated samples resulted in a single immunoreactive PrP band that corresponded in mobility to the smallest protease-resistant fragment, the 19-kDa fragment in FFI and the 21-kDa fragment in CJD[178] (Fig. 1). Therefore, the number of protease-resistant fragments are accounted for by the degree of glycosylation, with the smallest fragment being unglycosylated (Fig. 1). The underrepresentation and the faster electrophoretic mobility of the unglycosylated fragment in FFI, when compared with the corresponding CJD[178] fragment, shows that in these two diseases the fragments generated by proteinase K differ both in the degree of glycosylation and in size. We also compared the protease-resistant PrP fragments of FFI and CJD[178] to those obtained from another familial CJD subtype, CJD[200], to determine whether they differed in prion diseases with *PRNP* mutations other than Asp-178 → Asn. The fragments generated from the CJD[200] case by treatment with proteinase K and the single fragment obtained after subsequent deglycosylation differed from those of FFI and CJD[178]. Thus, each of the pathogenic mutations examined generates distinct PrP isoforms (Fig. 1).

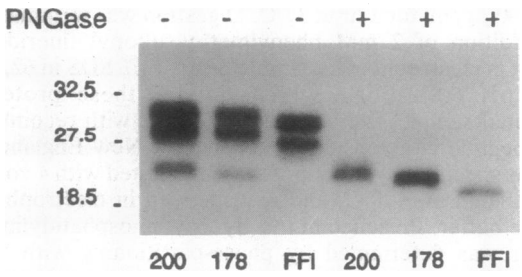

FIG. 1. Protease-resistant PrP fragments from three types of familial prion diseases. Samples of brain tissue from cases of CJD[200] (lane 200), CJD[178] (lane 178), and FFI were digested with proteinase K (PNGase, lanes −), and these digested samples were also deglycosylated with PNGase F (PNGase, lanes +). Immunoblots were probed with the monoclonal antibody 3F4.

No variation in either pattern or mobility of the PrP fragments was seen in the FFI kindreds, while minimal variations were occasionally seen in the CJD[178] kindreds (Fig. 2). PrP fragments from subjects homozygous or heterozygous at *PRNP* codon 129, in either FFI or CJD[178] kindreds were not qualitatively different. Thus, the type of protease-resistant PrP is highly consistent and apparently is determined only by the mutant allele.

To investigate further the difference in the sizes of the protease-resistant PrP fragments in FFI and CJD[178], we analyzed the amino- and carboxyl-terminal peptides obtained after digestion with endoproteinase Asp-N. Carboxyl-terminal peptides, inclusive of the glycosyl-phosphatidylinositol anchor as determined by phase partition in Triton X-114, had identical electrophoretic mobility (Fig. 3*A*). In contrast, the amino-terminal peptides showed differences in mobility that corresponded to those of the deglycosylated fragments in FFI and CJD[178] (Fig. 3*B*). To exclude the possibility that the difference in electrophoretic mobilities is merely due to the different amino acid at position 129, we analyzed amino-terminal peptides (PrP residues 23–143) from individuals homozygous for either methionine or valine at that position and from one heterozygous for a deletion of one of the octapeptide repeats within *PRNP* codons 76 and 91. These fragments had identical mobilities, and the peptide with the deletion, differing by only 0.78 kDa, was well resolved (Fig. 3 *C*). Thus, the different amino acid at residue 129 is not by itself responsible for the difference in migration of the protease-resistant PrP fragments in FFI and CJD[178]. Also, the PrP amino-terminal fragment we analyzed is not known to be posttranslationally modified (28), and variations in glycosylation are unlikely to have affected proteinase K cleavage because the different glycosylated PrP isoforms within each disease were apparently cleaved at the same site (see Fig. 2). Therefore, differences in electrophoretic mobility of the protease-resistant fragments are most likely due to different conformations of PrP, which expose different sites to proteinase K cleavage in the two diseases. Proteolytic cleavage is considered to be a useful probe of conformational change (29–31).

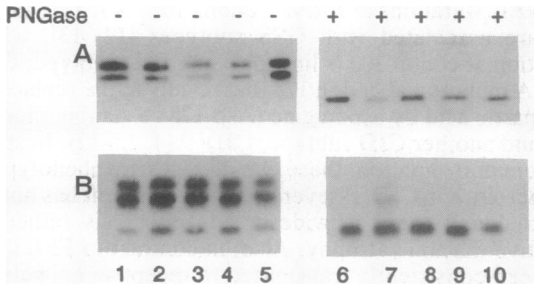

FIG. 2. Analysis of five subjects from three FFI kindreds and five subjects from three CJD[178] kindreds as described in Fig. 1. (*A*) Samples were from FFI-2 IV-26 (lanes 1 and 6), FFI-5 IV-4 (lanes 2 and 7), FFI-2 IV-27 (lanes 3 and 8), FFI-1 IV-16 (lanes 4 and 9), and FFI-1 V-58 (lanes 5 and 10). (*B*) Samples were from CJD[178] Wui IV (lanes 1 and 6), CJD[178] Wui V (lanes 2 and 7), CJD[178] Wui VI (lanes 3 and 8), CJD[178] Str (lanes 4 and 9), and CJD[178] Day IV-2 (lanes 5 and 10).

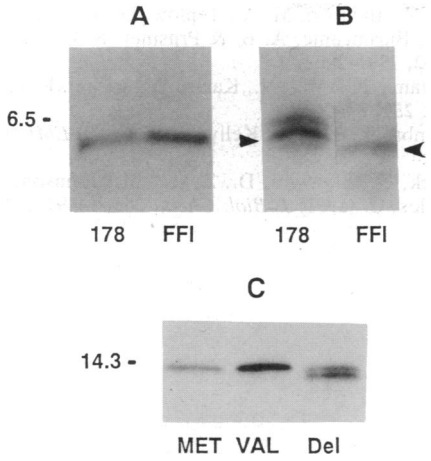

FIG. 3. Comparison of carboxyl- and amino-terminal fragments from CJD[178] (lane 178) and FFI. (*A* and *B*) After digestion with proteinase K and deglycosylation, samples were treated with endoproteinase Asp-N. Carboxyl-terminal fragments were detected with antiserum to PrP residues 220–231 (*A*). Amino-terminal fragments (arrowheads) were detected with monoclonal antibody 3F4, which recognizes PrP residues 109–112 (*B*). The slower moving band in CJD[178] is due to incomplete cleavage at Asp-143. (*C*) Samples from subjects homozygous for methionine (lane MET) or valine (lane VAL) at *PRNP* codon 129 and from a subject with a deletion of an octapeptide repeat (lane Del) were deglycosylated and digested with endoproteinase Asp-N. The amino-terminal fragments, spanning from the predicted signal peptide cleavage site at residue 23 to Asp-143, were identified by reactivity with both monoclonal antibody 3F4 (shown in *C*) and an antiserum to PrP residues 23–40 (not shown).

DISCUSSION

The present findings show that FFI and CJD[178] are associated with distinct PrP isoforms that differ in the degree of glycosylation and in the size of the fragments generated by proteinase K treatment. Because the sequence of the mutated PrP in FFI and CJD differs only at position 129 (18), the amino acid (methionine or valine) encoded at this position is likely to be responsible for the observed differences and may function as a conformational modifier. Thus, the mutant asparagine at position 178 might destabilize the PrP molecule while the methionine or valine at 129 could determine two distinct conformations. The interaction between the pathogenic mutation and the polymorphism at codon 129 may also play a role in modifying the disease phenotype in other familial prion diseases.

Our findings point to a change in conformation in the amino-terminal region of the protease-resistant PrP and differences in the relative abundance of glycosylated forms and possibly other factors as determinants of the topography and the type of lesion in prion diseases. A correlation between pathological phenotype and "type" of protease-resistant PrP has been repeatedly observed in experimental models of transmitted prion diseases, prompting the suggestion that differences in PrP conformation and glycosylation may target the exogenous protease-resistant PrP to selected cell populations, which then generate endogenous abnormal PrP and undergo pathologic changes (19–21). The present data add further complexity to this pathologic mechanism because in the genetic forms, in contrast to the transmitted models, the mutated PrP could be expressed constitutively in virtually all cells. Since the topographic distribution of protease-resistant PrP in FFI brains is highly selective (unpublished data), some cell populations appear to protect themselves either by preventing conversion of the mutated PrP into the pathogenic PrP or by "neutralizing" the pathogenic PrP before irreversible cytological damage occurs.

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