SHORT REPORT

Protease resistant prion proteins are not present in sporadic "poor outcome" schizophrenia

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Abstract

Various clinical and epidemiological data have suggested the possibility of infectious mechanisms in schizophrenia. In addition, lengthy prodromal psychiatric symptoms can presage the development of Creutzfeldt-Jakob disease, a prototypical prion disorder. Accordingly, the presence of human protease resistant prion proteins (PrP^{res}) was assessed in postmortem frontal cortical and thalamic tissues from a prospectively accrued and well characterised sample of elderly patients with chronic, sporadic, "poor outcome" schizophrenia using a sensitive immunoblot assay. No PrP^{res} was found in the brains of any of the cases, providing evidence against a role for abnormal prion proteins in the pathogenesis of schizophrenia.

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cases, may play a part in schizophrenia or schizophrenia-like psychoses.

In our prospective clinicopathological studies of schizophrenia in elderly people, we have been impressed by the frequency of profound cognitive and functional impairments suggestive of a neurodegenerative disease.⁴ However, detailed diagnostic neuropathological examinations as well as exhaustive quantitative investigations have failed to identify even subtle evidence of neurodegenerative disease pathology or neural injury.⁵ Thus the basis for the deterioration in these schizophrenic patients remains unknown.

Whereas Creutzfeldt-Jakob disease is pathologically typified by widespread spongiform changes that predominantly affect the cerebral cortex, basal ganglia, thalamus and cerebellum, sensitive assays have detected PrP^{res} in some patients with a progressive dementing disorder but no significant spongiform changes in their brains after detailed neuropathological examination.⁶⁻⁸ In this study, we applied a highly sensitive immunoblot technique for the detection of PrP^{res} in brain tissues from a sample of elderly, "poor outcome" patients with schizophrenia.

Materials and methods

Frozen frontal and thalamic tissues were obtained at postmortem from 12 patients with schizophrenia. All were participants in a prospective clinicopathological studies programme of schizophrenia⁴ and had been chronically in hospital with a primary diagnosis of schizophrenia that was confirmed by history and clinical examination using DSM-IV diagnostic criteria.⁹ The key demographic and clinical data on these patients are presented in the table. In addition to their psychiatric symptoms, eight of the patients exhibited severe and progressive cognitive and functional impairments sufficient to meet the diagnostic criteria for an additional diagnosis of dementia. Tissue blocks were dissected at postmortem and fixed in ethanol (70% EtOH, 150 mM NaCl) or 10% neutral buffered formalin and embedded in paraffin for sectioning for diagnostic studies, while the remainder of the brain was frozen and stored at -70°. Diagnostic neuropathological examinations disclosed no abnormalities in any of the subjects. In addition, one patient with neuropathologically confirmed Creutzfeldt-

Psychiatric symptoms are well recognised features of Creutzfeldt-Jakob disease, a prototypical prion disorder. However, several recent articles have indicated a possible relation between prion disease and severe psychiatric illness even in the absence of frank dementia and neurological signs. In an epidemiological meta-analysis of risk factors for Creutzfeldt-Jakob disease, Wientjens *et al*¹ found that a prior history of admission to hospital for psychotic illness was 12 times more common among patients with Creutzfeldt-Jakob disease than in the general population, with the psychiatric illness predating the first "hard" signs of Creutzfeldt-Jakob disease by decades. In addition, Samaia *et al*² described a family with a high frequency of atypical psychotic disorder associated with prominent mood disturbance and personality changes, wherein a prion protein gene mutation was found among most of the affected members. Finally, a recent analysis of new variant Creutzfeldt-Jakob disease found that most patients presented with psychiatric symptoms, including psychosis.³ Thus these reports raise the possibility that abnormal isoforms of the prion protein, such as the protease resistant form (PrP^{res}) that has been pathogenetically linked to Creutzfeldt-Jakob disease and other prion dis-

Demographic and clinical data

Patient	Age	Sex	Age of onset	Duration of illness	Dementia	Brain weight (g)	PMI
1	67	F	16	51	No	1408	8.5
2	72	F	24	48	No	1157	10.0
3	75	F	23	52	Yes	1044	16.0
4	75	M	21	54	No	1311	12.0
5	76	M	18	58	Yes	1275	8.0
6	77	F	39	38	Yes	932	8.5
7	77	F	37	40	No	1150	11.0
8	80	F	22	58	Yes	1060	11.0
9	82	M	22	60	Yes	1383	7.0
10	87	F	27	60	Yes	1270	6.5
11	89	F	26	63	Yes	1341	15
12	95	M	25	70	Yes	1080	8.5
Mean (SD)	79.3 (7.8)	8F/4M	25.0 (6.8)	54.3 (9.2)	8 Yes/4 No	1211 (152)	10.2 (3.0)

PMI=Postmortem interval.

Jakob disease was included as a positive control.

Immunoblots were performed as previously described.⁷ Briefly, frozen samples of frontal cortex and thalamus were homogenised in nine volumes of lysis buffer and aliquots were digested with proteinase K (100 or 50 µg/ml) for 1 hour at 37°C, resolved on 12 or 14% polyacrylamide gels, transferred to Immobilon P, and incubated overnight at 4°C with monoclonal antibody 3F4, which recognises the human PrP residues 109–112,¹⁰ found in all human prion disease variants.¹¹ Samples equivalent to 2 mg wet tissue were loaded in lanes of the gels. The immunoblots were developed using the enhanced chemoluminescence system (ECL, Amersham, UK).

Results

Western blot analysis found a typical 19 kDa banding pattern (PrP^{res} type 2) in the positive control case, confirming the neuropathological diagnosis of Creutzfeldt-Jakob disease (figure). By contrast, no PrP^{res} was found in any of the schizophrenia cases.

Discussion

Aside from the associations of psychiatric illness with the later development of frank Creutzfeldt-Jakob disease noted above, there are clinical, genetic, and epidemiological features of schizophrenia that would be compatible with a latent viral or cryptic prion disease model.^{12–13} These include a high degree of heritability, regional prevalence differences, a history of urban birth and household crowding, and a history of poliomyelitis which also

may be a risk factor for Creutzfeldt-Jakob disease.¹ Furthermore, there has been increasing recognition of the cognitive impairments that are found in schizophrenia^{14–16} as well as the dementia that can accompany the disease in late life.^{4–17} This raises the spectre of neurodegenerative processes contributing to the disorder. Despite intensive neuropathological investigation, the defining neurobiological signature(s) of schizophrenia and its dementia remain an enigma.^{18–19}

Our sample of elderly schizophrenic patients with poor outcome may be especially useful in the study of possible neurodegeneration or post-maturation neural injury mechanisms in schizophrenia. These patients have required continuous stay in hospital, despite numerous efforts towards deinstitutionalisation. Furthermore, most exhibited coexistent severe dementia in their final years. Thus if neurodegenerative processes are present in schizophrenia, it would be more likely in an older, severely affected sample than in a younger or better functioning group. We have expended considerable efforts to discover excessive neuropathological lesions suggesting such processes, including neuron loss, neurofibrillary and amyloid lesions, astrogliosis, ubiquitination, and microglial proliferation, but have found none.⁵

In this study, we used a sensitive and specific method for the detection of PrP^{res} in a small, but very well characterised cohort of elderly schizophrenic patients. Whereas none of our cases had spongiform or other abnormal findings on neuropathological examination, this technique has been demonstrated to detect PrP^{res} even in the absence of such lesions. To

Western blot of proteinase K treated brain homogenates from a patient with sporadic Creutzfeldt-Jakob disease (lane 1) and from six subjects with schizophrenia (lanes 2–6), probed with the antibody 3F4. (A) Whereas the Creutzfeldt-Jakob disease sample shows protease resistant prion protein (PrP^{res}) with a typical type 2 pattern,⁸ no PrP^{res} is seen in any of the patients with schizophrenia. Prolonged exposure of the film (B) did not disclose specific PrP^{res} peptides in the samples from patients with schizophrenia but only aspecific bands (also stained omitting the primary antibody). All samples from patients with schizophrenia were loaded 20 times more than the Creutzfeldt-Jakob disease sample.

maximise its sensitivity, we used tissue from more than one brain region, including the thalamus, a region which is consistently affected in all variants of human prion diseases. Furthermore, the 2 mg equivalent of wet tissue that was loaded into each lane of the immunoblot gels is 20 times more than what is typically used for PrP^{res} detection in suspected cases of prion diseases. Our failure to detect PrP^{res} by immunoblot provides further evidence against the presence of abnormal prion proteins as an infectious aetiology in schizophrenia.

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