The diagnosis of prion diseases

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SUMMARY

Prion diseases of humans and other animals have clinical, economic and political significance. Pre-mortem diagnosis is therefore of great importance. Clinical diagnostic criteria and the current status of the available diagnostic tests are reviewed and possible future developments discussed. Presently, most diagnostic tests are indirect, relying on findings that may not be confined to prion diseases rather than on their particular intrinsic nature. They are therefore not absolutely specific nor are they 100% sensitive. The electroencephalogram (EEG), cerebral imaging and cerebrospinal fluid (CSF) analysis are the main techniques employed. However, there is hope that useful blood tests could be developed which would be simpler and less invasive. Also, there is hope that more specific tests will become available.

Key words: CJD, Prion, EEG, CSF, 14-3-3.

INTRODUCTION

The prion diseases or transmissible spongiform encephalopathies (TSEs) comprise a group of animal and human illnesses that share similar neuropathological features, a common molecular biological underpinning and, under certain circumstances, a transmissible nature (Table 1). Scrapie, a naturally occurring disease of sheep and goats, has been recognized for over 250 years. The first cases of bovine spongiform encephalopathy (BSE) were seen in 1985–86 and were probably infected as calves in 1981–82 (Collee & Bradley, 1997*a*, *b*). Kuru had a specific geographical distribution and a unique mode of infection, being confined to the Eastern highlands of Papua New Guinea and related to ritual mourning cannibalism.

The most important human TSE, Creutzfeldt–Jakob disease (CJD), was described by Jakob in 1921 and its transmissibility was demonstrated in 1968 by the intracerebral inoculation of a non-human primate with cerebral material from a patient who had died of the illness. Until recently, CJD was recognized in three main or classical forms; sporadic, genetic and iatrogenic. In 1996, a fourth type was reported, named new variant CJD (nvCJD), and it has been shown that this represents the infection of humans with BSE (Tables 2 and 3) (Will *et al*. 1996). The unusual nature of these illnesses along with the strange characteristics of their causative agents has stimulated much scientific interest. This has been intensified by the economic and political implications of BSE and the concern that there may be a resultant epidemic of nvCJD.

THE NATURE OF PRIONS

The methods of modern molecular biology and genetics have increased our understanding of TSEs. The hallmark of these diseases is the accumulation in the CNS tissues of an abnormal protein designated PrP^{RES}. Normal cells contain the PrP gene (*PRNP*) which encodes for a normal cellular protein designated PrP^{c}. The organization and structure of *PRNP* is similar in all mammals that have been studied with the entire open reading frame (ORF) of the gene contained in a single exon. Sequence homology among mammalian PrP molecules ranges from 85% to 95%. All mammalian PrP molecules contain 2 consensus sites for N-linked glycosylation and a C-terminal glycosylinositol phospholipid (GPI) anchor. PrP^{c} is expressed in a variety of tissues with the highest levels of mRNA being found in brain. It is localized to cell membranes and appears to have an important role in CNS cells but its precise function is unknown. In TSEs, the normal PrP^{c} is produced but then undergoes a post-translational modification to the abnormal PrP^{RES}. The primary structure of these 2 protein forms is identical being encoded for by the same gene but PrP^{RES} has a different conformation which confers upon it certain physicochemical properties (Table 4). Its resistance to protease activity gave rise to the terminology currently used; PrP^{c} standing for 'cellular' PrP and PrP^{RES} standing for 'resistant' PrP.

In cases of infection (such as with human growth hormone (hGH) treatment or nvCD), it is the introduction of exogenous PrP^{RES} which causes the normal host protein to undergo change to the abnormal form. In genetic disease, the PrP formed has an abnormal primary structure reflecting the gene mutation and this somehow predisposes to disease. The cause in sporadic cases is unknown. The accumulation of PrP^{RES} in the tissues is associated with disease and with infectivity. Disease results either from the aggregation of the abnormal PrP^{RES} or the depletion of the normal PrP^{c} (as it is converted into PrP^{RES}) or both. There may be other, as yet undetermined, pathological mechanisms. The

Parasitology (1998), **117**, S3–S11. Printed in the United Kingdom
https://doi.org/10.1017/S0031182099004138 Published online by Cambridge University Press

Table 1. The transmissible spongiform encephalopathies

Disease	Host	Notes
Animal diseases		
Scrapie	Sheep, goats	Naturally occurring animal disease
Chronic wasting disease	Mule deer, elk	Naturally occurring animal disease
Transmissible mink encephalopathy	Mink on mink farms	Cause uncertain
Feline spongiform encephalopathy	Domestic cats, zoo species	Due to BSE contamination of feed
BSE	Cattle	Contamination of feed
Human diseases		
Kuru	Man	Confined to Papua New Guinea. Now rare
CJD	Man	Worldwide distribution
Gerstmann Sträussler Schencker	Man	Rare familial disease
Fatal familial insomnia	Man	Rare familial disease

Table 2. Recognized forms of CJD

CJD type	Notes
Classical sporadic	Random world-wide distribution. Cause uncertain
Genetic	Genetic/familial due to *PRNP* mutations. Autosomal dominance
Iatrogenic	Accidental transmission: surgical, hormone treatments, etc.
New variant	First reported 1986. Essentially in UK. Shown to be due to BSE

Table 3. Types of CJD frequency in UK

Clinical type	Frequency pre-1996*	Frequency 1996*
Classical sporadic	83 %	66 %
Genetic	10 %	10 %
Iatrogenic	7 %	7 %
New variant	—	17 %

* Note: Figures are approximate and for UK only; based on deaths due to definite or probable CJD.

precise nature of the infectivity is not yet clear. However, the most widely accepted hypothesis is that the PrP^{RES} is itself the infectious agent or the major component of it. This novel view that the agent is a protein that replicates without nucleic acid is the prion hypothesis. The alternative view is that there is some nucleic acid-containing agent associated with the PrP^{RES}. The idea of an infectious, replicating protein without nucleic acid is an interesting one that explains the resistance of the infective agent to a number of physicochemical procedures that would be expected to destroy the integrity of nucleic acids. However, it is not so successful in explaining the phenomenon of agent strain variation. TSE agents do demonstrate certain properties which are reminiscent of the strain variation that characterizes agents such as viruses which is usually explained on the basis of their nucleic acid content. For example, one can demonstrate that scrapie from a certain source behaves in a particular and consistent manner in laboratory animals (in terms of incubation period and distribution of neuropathological abnormalities), whereas scrapie from a separate source behaves differently.

Analysis of PrP^{RES} has revealed that it exists in different glycoforms and that there is a characteristic glycoform pattern associated with different forms of disease (Collinge *et al*. 1996). It is therefore of note that the glycoform pattern found in new variant disease differs from that seen in classical sporadic cases but is like that of BSE.

Table 4. PrP^{RES} and PrP^{c}: physico-chemical properties

PrP^{c}	PrP^{RES}
Mostly α helix structure	Significant β sheet structure
Soluble in detergents	Insoluble in detergents – tends to aggregate
Relatively protease sensitive	Relatively protease resistant
Normal cellular turnover	Forms amyloid structures

https://doi.org/10.1017/S0031182099004138 Published online by Cambridge University Press

DIAGNOSIS

General points

The discussion will centre on the human disease of CJD but some comment will be made on BSE and a number of the points made about CJD can potentially be generalized to other prion diseases. The diagnostic methods that have been employed in CJD include purely clinical evaluation, routine investigations, such as the EEG, CSF examination and magnetic resonance imaging (MRI), and neuropathological analysis.

It should be emphasized that a vital role of investigatory procedures in suspect cases of CJD is the exclusion of other possible diagnoses. At present, the absolute diagnosis of CJD depends on neuropathological findings.

Clinical criteria

There are well-established clinical criteria for the diagnosis of classical forms of CJD i.e. sporadic, genetic and iatrogenic. The standard criteria are set out in Table 5. The criteria for sporadic disease include an investigation result, namely the EEG and this is discussed below. Iatrogenic disease is suggested by a history of exposure to a known risk factor (such as receiving a human dura mater graft or treatment with cadaveric hGH). Genetic disease is suggested by an appropriate family history. However, in some cases of apparently sporadic disease, a *PRNP* mutation may be found. This is usually because of undisclosed family history or premature death of antecedents from other causes before they had time to develop symptoms of CJD. This indicates the importance of confirming genetic CJD by *PRNP* gene sequencing, which is discussed below.

The differential diagnosis of sporadic CJD is arguably narrower than that of new variant. It is also somewhat easier to exclude other possibilities with fairly simple investigation. This is partly a reflection of the intrinsic natures of the diseases: a rapidly progressive dementia occurring in middle or late life is an easier clinical matter than a more slowly progressive neuropsychiatric condition in youth. However, it is also a reflection of numbers; there is now a considerable worldwide experience of sporadic disease while, at the time of writing, what we know of new variant is based on a total experience of 24 patients. There are, at present, no agreed validated clinical diagnostic criteria for new variant disease.

Routine investigations

A number of investigations are necessary to exclude other possible diagnoses. Cerebral imaging, such as CT (computed tomography) or MRI, may exclude structural disease or other conditions such as cerebrovascular disease. Other imaging procedures such as a chest X-ray may be necessary to look for evidence of neoplasia. Blood tests may be directed at a variety of disorders including toxic or metabolic conditions, evidence of vasculitis and paraneoplastic disease (using appropriate antibody tests). The range of differential diagnosis is potentially wide and the assessment of possible CJD cases calls for a clinician with neurological experience Three investigations require detailed comment: the EEG, cerebral imaging and CSF examination.

EEG. Periodic sharp-wave complexes are a very characteristic finding in the EEG in sporadic CJD (Fig. 1). Indeed, this EEG finding is the single criterion that differentiates 'probable' from 'possible' cases in the agreed diagnostic criteria. However, only about two-thirds of all cases show this pattern and there are other conditions in which periodic complexes are seen, Unfortunately, there have been few attempts to devise and evaluate the validity of objective criteria for a 'typical' CJD EEG and less specific patterns may be misleadingly reported as 'CJD-like'. Formal EEG criteria were used in one phase of surveillance in England and Wales. In a period of nearly 2 years, 52 cases were notified who turned out not to have CJD. Nearly half of these were referred because of an EEG report stating that there were CJD-like features but when the EEG records were reviewed using the formal criteria, only 2 of them showed the typical abnormality. In these 2 instances there were metabolic disturbances which explained both the clinical features and the EEG changes (England and Wales Surveillance Study 1980–4, unpublished data). In this same period, about 60% of definite sporadic cases had a characteristic EEG at some point in their illness. A report from the German CJD surveillance system examined the EEGs of 29 cases according to different strictly-defined criteria and found a specificity of 86% and a sensitivity of 67% for sporadic CJD (Steinhoff *et al*. 1996).

Some of the other possible causes of this EEG pattern (such as metabolic disorders) should be readily distinguishable from CJD on simple clinical grounds. Other diseases (such as Alzheimer's disease) may give rise to greater difficulties but do not commonly cause the characteristic pattern. In the UK surveillance system since 1990, 249 definite CJD cases have been identified and there have been 2 instances of a CJD-like EEG in cases of pathologically proven Alzheimer's disease. The characteristic pattern is not so commonly seen in other forms of classical CJD and, to date, it has not been seen in new variant disease. The EEG is a fairly accessible and non-invasive test with an important role in the diagnosis of sporadic CJD. The German and UK surveillance systems have recently agreed new formal EEG criteria which are to be prospectively evaluated.

Cerebral imaging. Given the devastating nature of these illnesses, one might expect that cerebral

https://doi.org/10.1017/S0031182099004138 Published online by Cambridge University Press

Table 5. Diagnostic criteria for CJD

Sporadic CJD	
Definite	Neuropathological confirmation (and/or immunocytochemistry)
Probable	I Progressive dementia
	II At least two of these clinical features:
	1. Myoclonus
	2. Visual or cerebellar
	3. Pyramidal/extrapyramidal
	4. Akinetic mutism
	III Typical EEG
Possible	I Progressive dementia
	II Duration < 2 years
	III At least 2 of the above clinical features
	IV No EEG or atypical EEG
Familial/genetic CJD	I Definite or probable CJD
	Definite or probable CJD in 1st degree relative
	II Neuropsychiatric disorder
	Disease-specific *PRNP* mutation
Iatrogenic CJD	Progressive cerebellar syndrome in a pituitary hormone recipient
	Sporadic CJD with a recognized exposure risk

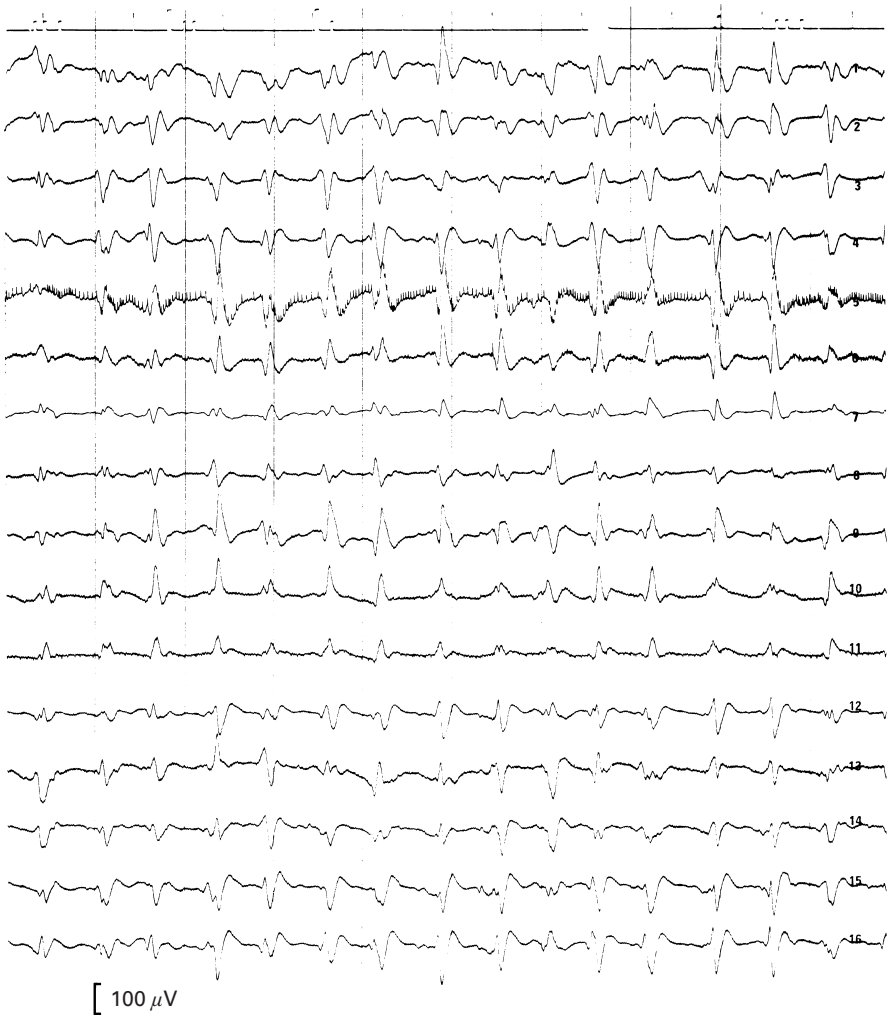

Fig. 1. The typical EEG pattern in classical sporadic CJD, showing generalized periodic complexes.

imaging would be positively helpful in diagnosis as well as excluding other possibilities. It is, therefore, a little surprising and disappointing to find that the cerebral CT is usually normal in CJD patients even with severe dementia, cerebellar ataxia, cortical blindness and myoclonus. However, one could argue

https://doi.org/10.1017/S0031182099004138 Published online by Cambridge University Press

that the finding of a normal cerebral CT in such a striking clinical setting should in itself suggest that CJD be considered as a diagnosis. When MRI became a widely available technique, it was hoped that the more detailed images it provides would prove more diagnostically useful. A number of publications have discussed the MRI findings in CJD, in particular suggesting that abnormalities in the basal ganglia may be diagnostically helpful. However, in 1996, a letter reported the MRI experience of the UK surveillance system: 96 out of 256 definite or probable cases were identified as having had a cerebral MR during their illness. Eight had iatrogenic CJD, 4 had familial disease and the remainder had sporadic CJD. Only 4 (3 sporadic and 1 hGH related) were reported as showing high signal abnormalities of the basal ganglia on MRI. The authors agreed that the findings might be relatively specific for CJD and therefore useful in particular cases, but felt that MR imaging was not a sensitive test (Zeidler *et al*. 1996). However, this analysis was based on the original hospital radiology reports and there was no organized attempt to independently review the individual images. It is therefore possible that subtle but definite changes may have been unrecognized and under-reported. There were also many patients in whom there were no available MR data. A separate report from the German surveillance system in the same year suggested that MR imaging was in fact much more sensitive in the diagnosis of CJD. The authors reported on the MR findings in 29 patients who had died of CJD (14 definite and 15 probable CJD). They found moderate to marked bilateral, symmetrical, increased signal intensity in the putamen and caudate nucleus on T2- and proton density-weighted MR images in 79% of cases. T1-weighted images did not show signal intensity abnormalities. In 4 cases, high signal intensity was seen in the occipital cortex and similar changes were seen in the cerebellar cortex in a single case. Patients with a disease duration of less than 4 months showed no substantial atrophy but significant and progressive atrophic changes were seen in longer duration cases. The MR images were reviewed retrospectively but by radiologists blinded to the diagnosis (Finkenstaedt *et al*. 1996). Further work is being undertaken by the German surveillance system and unpublished data support the conclusions of this limited published study. If further analysis supports these findings, then the MRI may become a very useful diagnostic tool in sporadic CJD. Bilateral hyperintense abnormalities in the basal ganglia on T2-weighted MR images may be seen in conditions other than CJD such as cerebral hypoxia, carbon monoxide poisoning, encephalitis, mitochondrial disorders and Wilson's disease. However, in the clinical context, other investigation results and other MR features should differentiate most, if not all, of these possibilities from CJD. Of even greater interest is the possibility that nvCJD may give rise to a rather different and characteristic MRI signature. Unpublished data from current UK surveillance indicates that signal change in the posterior and medial thalamus may prove to be a relatively sensitive and specific indication of the diagnosis. Further evaluation of the MRI in classical and new variant forms of disease is required.

A recent publication (de Silva *et al*. 1998) described the SPECT findings in 2 cases of nvCJD. However, the abnormalities were non-specific and simply supported the idea that the patients had an organic encephalopathy rather than a primarily psychiatric condition.

CSF. The CSF is examined for 2 separate reasons. First, to exclude other conditions and, secondly, to undertake specific protein analysis which may be positively helpful in diagnosing CJD. Routine microscopy of the CSF in CJD is normal. Therefore, the finding of significant CSF pleocytosis immediately indicates another condition. The total protein level may be mildly or moderately elevated in CJD but this is a very non-specific finding in CNS disease. However, the identification of particular brain-specific proteins may be diagnostically helpful. Interest has centred on S100, NSE (neuron-specific enolase), tau protein and 14-3-3. It should be stressed that all of these proteins are normal brain proteins which may be released into the CSF in disease states; they are not specifically related to the underlying mechanisms of CJD. In particular, they are not specifically related to PrP. Nonetheless, they are often found in significant amounts in the CSF in CJD in a manner which may be helpful in differential diagnosis. The published data largely concern classical sporadic CJD.

CSF NSE levels are usually high in CJD but may be elevated in a number of other conditions including brain trauma, brain tumours, subarachnoid haemorrhage and cerebral infarction. A German study found that values above 35 ng ml^{-1} are indicative of the disease, with a sensitivity of 80% and a specificity of 92% (Zerr *et al*. 1995*b*, 1996).

S100 is an acidic calcium-binding protein consisting of a heterodimer of two isomeric subunits, alpha and beta. The beta isomer (S-100b) is present in glial cells. Otto *et al*. (1977) described the S100 levels in CSF in a group of 135 patients referred to the German CJD surveillance unit. They found that a cut-off level of 8 ng ml^{-1}, provided a sensitivity of 84·2% and a specificity of 90·6% for the diagnosis of CJD. CSF S-100b levels are being measured in the continuing UK surveillance study but there are no published data at present. A further publication from the German surveillance group has reported the results of analysing serum concentrations of S100. With a cut-off point of 213 pg ml^{-1}, a sen-

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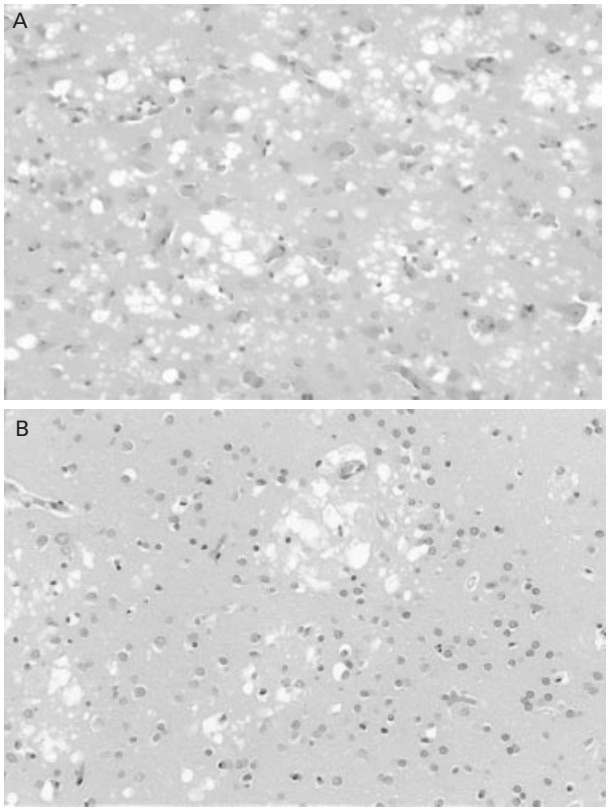

Fig. 2. The neuropathological appearances in classical sporadic and new variant CJD. (A) The cerebral cortex in sporadic CJD shows vacuolation of the grey matter (spongiform change), which in several areas becomes confluent to produce cyst-like spaces. Haematoxylin and eosin, ×200. (B) The cerebral cortex in new variant CJD shows patchy spongiform change around fibrillary plaques of prion protein, the 'florid' plaque (centre). Haematoxylin and eosin, ×200. Courtesy of Dr J. W. Ironside, CJD Surveillance Unit, Edinburgh.

sitivity of 77·8% and a specificity of 81·1% were found for a diagnosis of CJD (Otto *et al*. 1988). The authors suggest that further studies with serial tests are required and also comment that it may prove to be useful in diagnosing BSE in cattle since there is a high degree of homology between bovine and human S100.

The most specific of the protein tests is the detection of 14-3-3. A report in 1986 described the finding of two 30 kDa proteins (designated proteins 130 and 131 or p130/p131) by two-dimensional electrophoresis in the CSF from cases of CJD (Harrington *et al*. 1986). Later it was determined that these were identical to the brain protein 14-3-3 and a simple immunoassay for this protein was developed (Hsich *et al*. 1996). The German Surveillance study has reported a sensitivity of 84% and a specificity of 100% in sporadic CJD, using two-dimensional electrophoresis detection of 14-3-3 (Zerr *et al*. 1996). Another report concerning a 14-3-3 antibody-based assay in CJD has claimed a sensitivity of 98% and a specificity of 99% (Lee & Harrington, 1996). The test does appear to have a high sensitivity and a very high specificity for classical sporadic CJD. It is not understood why this protein should be found so particularly in CJD as opposed to other dementing illnesses and it is clear that some false positives and negatives can occur. It is also the case that sporadic CJD is often diagnosable on other grounds (such as the combination of clinical features and EEG findings) and a positive test may not add a great deal to a case already meeting the criteria for 'probable' sporadic CJD. The main use of the test is likely to be in those cases which meet only the 'possible' sporadic criteria. Indeed, it has been proposed that surveillance systems might expand the definition of 'probable' sporadic CJD to include those cases who do not have the characteristic EEG finding but who have a positive CSF 14-3-3 test. Unfortunately, preliminary results from the UK suggest that the sensitivity of the test is much lower in the new variant form than in classical forms (Unpublished data from UK CJD Surveillance). In other words, it might not prove to be very useful in the type of CJD for which most diagnostic help is needed. Further evaluation of this test (in all forms of CJD) is needed and is indeed being undertaken in the UK and elsewhere. Another area of interest has concerned the possible role of apolipoprotein E (ApoE) in CJD. This is produced by astrocytes in the CNS and it is thought to be involved in the mobilization and redistribution of lipids in repair and growth. It is upregulated in glial cells in response to neural damage and deafferentation. It exists in three common isoforms coded for by specific alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) of the *APOE* gene. ApoE has been detected in some prion protein deposits. In 1994, Amouyel *et al*. claimed that the $\epsilon 4$ allele was a risk factor for CJD and that the $\epsilon 2$ allele modified the clinical course. However, this has not been universally accepted (Nakagawa *et al*. 1995; Zerr *et al*. 1995*a*). Interestingly, a recent report (Hochstaser *et al*. 1997) described the finding of elevated levels of bovine ApoE in the CSF of BSE cattle and suggested that this may be useful as a diagnostic test in suspect BSE cases. This is an area which probably deserves more research.

Neuropathology

The neuropathological abnormalities seen in CJD are characteristic ones that allow definitive diagnosis of the disease (Fig. 2). However, these are essentially to be found in the brain and one therefore requires cerebral tissue for diagnostic confirmation. This is obviously available at autopsy but this is not helpful for *in vivo* diagnosis and permission for autopsy may not be granted. Cortical biopsy may be undertaken in life but this is not an entirely straightforward affair with some risk to the patient and a potential for contamination. Disposable instruments must be used and the tissue handled by those experienced in such

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matters. Finally, there is always the possibility that an unrepresentative area of cortex is biopsied and no firm diagnosis made.

The most specific diagnosis of a transmissible disease entails the direct or indirect identification of the transmitting organism. The closest candidate for this in CJD is PrP^{RES}. Certainly, PrP^{RES} has not been found in tissues from normal individuals or those with diseases other than TSEs. Unfortunately, the principal area of PrP deposition is the central nervous system and thus the process requires brain tissue with the disadvantages discussed above. However, there is a single case report describing the identification of PrP^{RES} in tonsillar tissue taken from an individual with nvCJD (Hill *et al*. 1997). This might prove to be the basis of a non-CNS tissue-based diagnosis for the nv form and, while tonsillar biopsy is not an entirely simple procedure, it is arguably more straightforward than cerebral biopsy. Before it can become established, it needs further careful evaluation. It is also possible that other, perhaps more accessible tissue, such as lymph node may be useful in the detection of PrP^{RES} and thus nvCJD. Such reticuloendothelial involvement has not been reported in sporadic CJD and these considerations presently remain confined to the new variant form. The identification of PrP^{RES} depends on an antibody directed against PrP but the standard antibody employed does not distinguish between the normal cellular PrP^{c} and the disease-related PrP^{RES}. Therefore, all tissue to be tested has to undergo pretreatment with an agent that will destroy PrP^{c} but not PrP^{RES} (such as proteinase K). In experienced hands, this is a reliable technical method but does make for additional complexity and needs to be undertaken by suitably equipped laboratories and appropriately trained staff. There are three general methods with employ the antibody detection of PrP^{RES}. They all depend on the binding of antibody to PrP^{RES} (after suitable treatment to remove PrP^{c}) and then processing to show the sites of bound antibody. They share the basic ability to detect the presence of the abnormal protein but individually have different properties which can provide additional useful information. First, there is immunocytochemistry in which paraffin-embedded tissue sections are treated with the antibody and then processed in a manner to show the deposition. This method allows identification of the abnormal protein, allows analysis of the form of its deposition at a microscopic level (such as the detection of plaque formation) and also permits the examination of the general cellular architecture. It is essentially a histological method and one cannot analyse the structure of the detected protein. Secondly, there is the histoblot. This method uses slices of tissue which are treated with the antibody and then processed in order to show the areas of antibody binding. Aside from the simple detection of protein, this technique allows some analysis of the general distribution of the protein in different areas of the CNS. Finally, one can use homogenized tissue with Western blotting which allows the analysis of the protein, including assessment of the glycosylation pattern. This is potentially very helpful in indicating the type of CJD since, for example, different patterns have been reported in sporadic and new variant CJD. Recently, a report has claimed the development of an antibody specifically directed to PrP^{RES} (Korth *et al*. 1997). If this method is validated, then it will make the laboratory identification of TSEs somewhat easier.

Genetic testing

The *PRNP* ORF has been sequenced and mutations may be detected on genetic analysis of material obtained from a blood sample. There are a number of recognized mutations which are associated with disease. Familial CJD results with an autosomal dominant pattern of inheritance. Gerstmann Sträussler Syndrome and Fatal Familial Insomnia are very rare prion diseases with phenotypes and neuropathologies that differ somewhat from CJD. At present, we are moving from an essentially clinical phenomenological approach (backed by neuropathology) with its eponymous nomenclature to a view based more on underlying mechanism and cause. It is therefore perhaps logical to regard these two unusual entities and familial CJD as being different expressions of a single problem rather than as quite different diseases that happen to share some common features. Point missense mutations are the commonest finding but there are also repeat insertions in one region of the ORF which contains octapeptide repeats. Deletions in this octapeptide repeat region have also been reported but their precise significance is uncertain (Cervenáková *et al*. 1996). It is a generally held view that the mutations cause the disease but it is possible that they affect susceptibility to some undetermined external cause. Even in non-familial forms of disease, *PRNP* has some influence. At codon 129 of the gene, there is a common polymorphism whereby either methionine (M) or valine (V) may be coded. Whereas only 37% of the normal UK population are MM homozygotes, 79% of classical sporadic cases have this genetic make-up. Therefore, this common polymorphism must in some way predispose to CJD.

CONCLUSION

At present, conclusive proof of the diagnosis of a prion disease depends on neuropathological examination of CNS tissue. In the classical forms of CJD, pre-mortem diagnosis is relatively reliable in a good proportion of cases. Clinical diagnostic criteria supported by negative tests for other possible

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diseases are reasonably well established. In sporadic disease, the EEG is of additional help and in familial disease *PRNP* gene sequencing can be undertaken on a blood sample. Iatrogenic disease is generally clear from the past medical history. However, clinical diagnosis is not absolutely reliable and calls for particular neurological expertise. There are also atypical forms of classical disease. In addition, the situation for new variant CJD is much less satisfactory, there being no well established clinical criteria and a potentially wider differential diagnosis. Also, the EEG does not show the characteristic periodic pattern. MR imaging may prove to be very helpful in both classical and new variant forms. CSF protein analysis, especially for 14-3-3 may support the clinical diagnosis especially in classical sporadic disease. However, all non-neuropathological tests to date are non-specific, many are 'high tech', and some are relatively invasive. The pre-mortem detection of a specific abnormality, namely the presence of PrP^{RES}, depends on cerebral biopsy. Biopsy of reticulo-endothelial tissue such as tonsil or even lymph node may prove to be helpful in nvCJD but the first of these is not necessarily an easy technique. Blood detection of brain-related proteins such as S100 may provide a simpler test but this is unlikely to be very specific. A major development would be the ability to detect PrP^{RES} in a blood sample. The discovery of an antibody specific to PrP^{RES} (rather than to either form of PrP) should make all PrP^{RES} detection methods easier.

It is vital, whenever possible, that all suggested diagnostic tests are prospectively evaluated in established surveillance systems.

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ADDENDUM

Since the original submission of this article, the number of confirmed cases of nvCJD in the UK has risen to 38 (36 definite and 2 probable). In addition, European collaborative CJD surveillance has adopted a revision of the diagnostic criteria for sporadic CJD, so that a positive 14-3-3 csf test can elevate an otherwise ''possible'' case to that of ''probable''. Provisional clinical diagnostic criteria for nvCJD have been agreed and currently are being evaluated.