occurred 75 km south of the port of Skikda (*10*). The hypothesis of recent importation of the plague bacillus in Kehailia is therefore tempting but is tempered by the fact that 1) the grain is primarily imported from Europe, which is not affected by plague, and from North America where natural foci exist but have very limited areas of overlap with those regions where cereal grains are grown, 2) no higher mortality rate in the murine population of the port was noted, 3) no human cases occurred in this sector of the city, and 4) a 3IS–restriction fragment length polymorphism (*11*) analysis grouped these strains in a cluster clearly distinct from the strains isolated from Africa and America (V. Chenal-Francisque et al., unpub. data).

The geographic concentration of the cases in 2 foci, both contiguous in the mountainous area of Tessala, suggested the existence of a natural focus in this area. Moreover, *Meriones* are present in Tessala, and these rodents are a well-known potential reservoir of *Y. pestis* (*12*). The outbreak occurred at harvest time, and it is possible that the abrupt reduction in the source of food pushed the wild rodents to approach houses in which grain was stored.

The current challenge in terms of public health is to determine if this animal reservoir has disappeared or if it is well established in the ecosystem. The capture of 3 seropositive small mammals (2 *Mus musculus* and 1 *Aleterix algerius*) in July 2004 (J.L. Soares et al., unpub. data) and the identification of several *Y. pestis* infected fleas in the same area (*13*) favor the second option.

Beyond the local problem, the proximity of a possible natural reservoir of plague to Oran, a large international commercial port, raises the possibility of the risk for an urban outbreak. At the time of the investigation, the sanitation in the city and port were poor and rodents proliferated. These urban rodents could come in contact with infected rodents from rural areas in the uncontrolled dumps at the periphery or through a dry riverbed that penetrates as far as the city center. Because of Oran's population density and the commercial activities of its seaport, a plague outbreak would have international implications.

This outbreak is a textbook illustration of the unexpected and sudden reemergence of an infectious disease epidemic that is potentially highly lethal. It also demonstrates that the danger of a plague outbreak is not limited to the currently indexed natural foci.

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"So many scientists think that once they figure it out, that's all they have to do, and writing it up is just a chore. I never saw it that way; part of the art of any kind of total scholarship is to say it well."

—Stephen Jay Gould

別紙様式第2-1

No. 21

医薬品 研究報告 調査報告書

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○英国の若年女性におけるクロイツフェルト・ヤコブ病、プリオンタンパク質遺伝子コドン129VV、および新規PrP^{Sc}型

背景:変異型クロイツフェルト・ヤコブ病(vCJD)は、ウシ海綿状脳症と因果関係のある後天性プリオン疾患であり、若い成人に多く発現する。調査した臨床例は全て、プリオンタンパク遺伝子(PRNP)のコドン129がメチオニンホモ接合体であり、典型的な神経病理所見を伴い、分子学的系統は典型的なPrP^{Sc}タイプ4であった。トランスジェニックマウスのモデル試験では、他のPRNP遺伝子型もウシ海綿状脳症に感染しやすいことが示されているが、特徴的な表現型を発現すると考えられる。

目的:PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の組織病理学的、分子学的検討。

デザイン:症例報告、剖検、分子学的解析。

設定・施設:neurology referral centerおよびMRC(医学研究審議会)プリオン部門の研究所。

結果:剖検所見は非定型孤発型CJDであり、灰白質と白質の変性が顕著で、プリオンタンパク質(PrP)の広域な沈着があった。解析用のリンパ網内系組織は得られなかった。小脳組織由来のPrP^{Sc}(PrPのスクレイピーアイソフォーム)の分子解析は、vCJDで見られるものと同等の新規PrP^{Sc}型を示した(PrP^{Sc}タイプ4)。しかし、金属イオンキレート剤EDTA存在下においてプロテアーゼ切断部位が変化したことにより、典型的なvCJD パターンと区別することができた。

結論:本患者に見られたプリオン系統の特徴を明らかにし、ウシ海綿状脳症との因果関係を検討するには、さらに試験が必要である。本症例は、PrP^{Sc}のプロテアーゼ切断パターンの金属イオン依存性を検討するため、EDTAによるプリオン疾患の分子解析の重要性を明らかにしている。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
PRNP コドン129がバリンホモ接合である非定型孤発型CJDの若年英国人女性の症例報告である。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

3-1

OBSERVATION

Creutzfeldt-Jakob Disease, Prion Protein Gene Codon 129VV, and a Novel PrP^{Sc} Type in a Young British Woman

Simon Mead, PhD, MRCP; Susan Joiner, MSc; Melanie Desbruslais, BSc; Jonathan A. Beck, BSc; Michael O'Donoghue, PhD; Peter Lantos, FRCP; Jonathan D. F. Wadsworth, PhD; John Collinge, FRS

Background: Variant Creutzfeldt-Jakob disease (vCJD) is an acquired prion disease causally related to bovine spongiform encephalopathy that has occurred predominantly in young adults. All clinical cases studied have been methionine homozygotes at codon 129 of the prion protein gene (*PRNP*) with distinctive neuropathological findings and molecular strain type (PrP^{Sc} type 4). Modeling studies in transgenic mice suggest that other *PRNP* genotypes will also be susceptible to infection with bovine spongiform encephalopathy prions but may develop distinctive phenotypes.

Objective: To describe the histopathologic and molecular investigation in a young British woman with atypical sporadic CJD and valine homozygosity at *PRNP* codon 129.

Design: Case report, autopsy, and molecular analysis.

Setting: Specialist neurology referral center, together with the laboratory services of the MRC [Medical Research Council] Prion Unit.

Subject: Single hospitalized patient.

Main Outcome Measures: Autopsy findings and molecular investigation results.

Results: Autopsy findings were atypical of sporadic CJD, with marked gray and white matter degeneration and widespread prion protein (PrP) deposition. Lymphoreticular tissue was not available for analysis. Molecular analysis of PrP^{Sc} (the scrapie isoform of PrP) from cerebellar tissue demonstrated a novel PrP^{Sc} type similar to that seen in vCJD (PrP^{Sc} type 4). However, this could be distinguished from the typical vCJD pattern by an altered protease cleavage site in the presence of the metal ion chelator EDTA.

Conclusions: Further studies will be required to characterize the prion strain seen in this patient and to investigate its etiologic relationship with bovine spongiform encephalopathy. This case illustrates the importance of molecular analysis of prion disease, including the use of EDTA to investigate the metal dependence of protease cleavage patterns of PrP^{Sc}.

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THE ORIGINAL RECOGNITION of variant Creutzfeldt-Jakob disease (vCJD) was based on a case series of young patients with rapidly progressive dementia, a geographic and temporal association with bovine spongiform encephalopathy (BSE), and novel neuropathological findings consisting of abundant florid prion protein (PrP) plaques.[1] Molecular strain typing allowed identification of a unique type of PrP^{Sc} (the scrapie isoform of PrP) (type 4) in the brain that was distinct from those seen in classic (sporadic or iatrogenic) CJD and similar to that seen in BSE prion infection of cattle and other species.[2] Subsequent biological strain typing in both conventional and transgenic mice confirmed that vCJD and BSE were caused by the same prion strain.[3,4]

Variant CJD also differs markedly from classic CJD in having prominent and consistent involvement of lymphoreticular tissue, allowing its diagnosis by tonsil biopsy findings.[5-7] To date, more than 160 individuals have died of vCJD in the United Kingdom; the number infected by BSE prions and who may develop prion disease in the years ahead is unknown because human prion incubation periods may exceed 50 years.[8]

All clinical cases of vCJD studied have had a methionine-homozygous (MM) genotype at polymorphic codon 129 of the prion protein gene (*PRNP*).[9] The extension of BSE prion-related disease to individuals with valine-homozygous (VV) or heterozygous (MV) genotypes at *PRNP* codon 129 has been predicted by comparison with other acquired human prion diseases[10,11] and by transgenic mouse

models.[12-14] These models also predict that infection of VV and MV genotypes with BSE or vCJD prions may result in propagation of distinct prion strain types and that patients with VV or MV genotypes might present with clinical, pathological, and molecular phenotypes distinct from that of vCJD.[12-14]

To date, we know of no reported cases of clinical vCJD occurring in the VV or MV genotypes. However, PrP^{Sc} has been reported in lymphoid tissues, but not in the brain, of a patient with *PRNP* 129 MV who had received blood from a person with preclinical vCJD and who died of an unrelated cause.[15] In addition, abnormal PrP immunoreactivity has been reported in anonymous archived lymphoid tissue from 2 individuals with *PRNP* 129 VV.[16] It is unknown whether the individual with the MV genotype would have gone on (or if those with VV will go on) to develop clinical disease and, if so, whether the phenotype will fit the case definition of vCJD.

METHODS

Brain homogenates (10% w/v) were prepared in Dulbecco phosphate buffered saline lacking Ca^{2+} or Mg^{2+} ions. Aliquots were analyzed with or without proteinase K digestion (50 µg/mL final protease concentration, 1 hour, 37°C) by immunoblotting with anti–PrP monoclonal antibody 3F4[17] as described previously.[7,18] Metal ion–dependent conformations of PrP were determined as previously described.[19] Genomic DNA was extracted from peripheral blood, and the entire *PRNP* open reading frame was amplified by polymerase chain reaction and sequenced as described previously.[20]

REPORT OF A CASE

A 39-year-old woman presented to an optician in January 1999 with episodes of blurred vision and photophobia, but no abnormality was found. Two months later, she noted memory impairment, diplopia, dysarthria, and an unsteady gait of fluctuating severity. Five months after onset, the gait and limb ataxia had progressed, although walking was still possible, and the memory loss became more profound. The patient then developed paranoid ideation, aggression, restless nocturnal behavior, anorexia, and mood disturbance. By 5½ months after onset, she could not walk and was unsteady sitting, and limb movements were clumsy.

Examination showed dysarthria, broken pursuit eye movements without nystagmus, impaired upgaze, and stereotyped involuntary movements of the legs. However, limb power, vibration, proprioception, tendon reflexes, and plantar responses were normal. During the ensuing 4 weeks, speech ceased and incontinence and jerky involuntary limb movements became evident. Eight months after onset, the patient was mute but could follow some commands. She was able to visually fixate and follow moving objects but also had abnormal, spontaneous horizontal roving eye movements with a supranuclear vertical gaze palsy. Her face was impassive with occasional twitching movements, brisk facial reflexes, and trismus. There were prominent jerking movements of all limbs brought out by use; power was relatively preserved and the plantar responses were extensor.

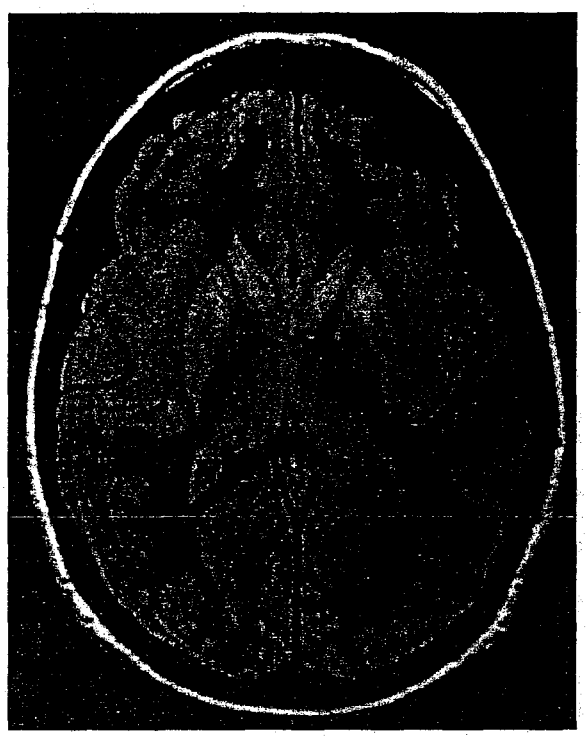

Figure 1. T2-weighted axial magnetic resonance image showing diffuse increased signal within both caudate nuclei and each putamen.

There was a strong family history of late-onset cerebellar ataxia consistent with autosomal dominant inheritance. A polyglutamine expansion in ataxin 3 associated with spinocerebellar ataxia type 3 was found in a symptomatic family member, but our patient did not share this expansion.

Normal results of the following investigations were found: complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, electrolytes, liver function, thyroid function, enzyme-linked immunosorbent assay for syphilis, vitamin B_{12}, folate, ferritin, vitamin E, and serum ceruloplasmin. Tests for antinuclear, antineuronal, anti–Purkinje cell, and antiganglioside antibodies were negative. Nerve conduction studies showed no evidence of a peripheral neuropathy. The electroencephalogram 6 months after onset was reported as normal, but at 7 and 8 months electroencephalograms showed diffuse slow-wave activity, without epileptiform changes or periodic discharges typical of CJD. Cerebrospinal fluid examination showed a normal cell count, protein level, and glucose level, and oligoclonal immunoglobulin bands were absent. The protein S100b level of 4.39 ng/mL (reference cutoff, <0.38 ng/mL), neuron-specific enolase level of 98 ng/mL (reference cutoff, <20 ng/mL), and 14-3-3 protein were all abnormal values.

A magnetic resonance image of the brain (**Figure 1**) showed diffuse cerebellar atrophy and diffuse increased signal within both caudate nuclei and each putamen. Tonsil biopsy was not possible because of a previous tonsillectomy from which little tissue remained. Genetic testing for mutations associated with spinocerebellar ataxia 1, 2, 3, 6, and 7 and Friedreich ataxia gave negative re-

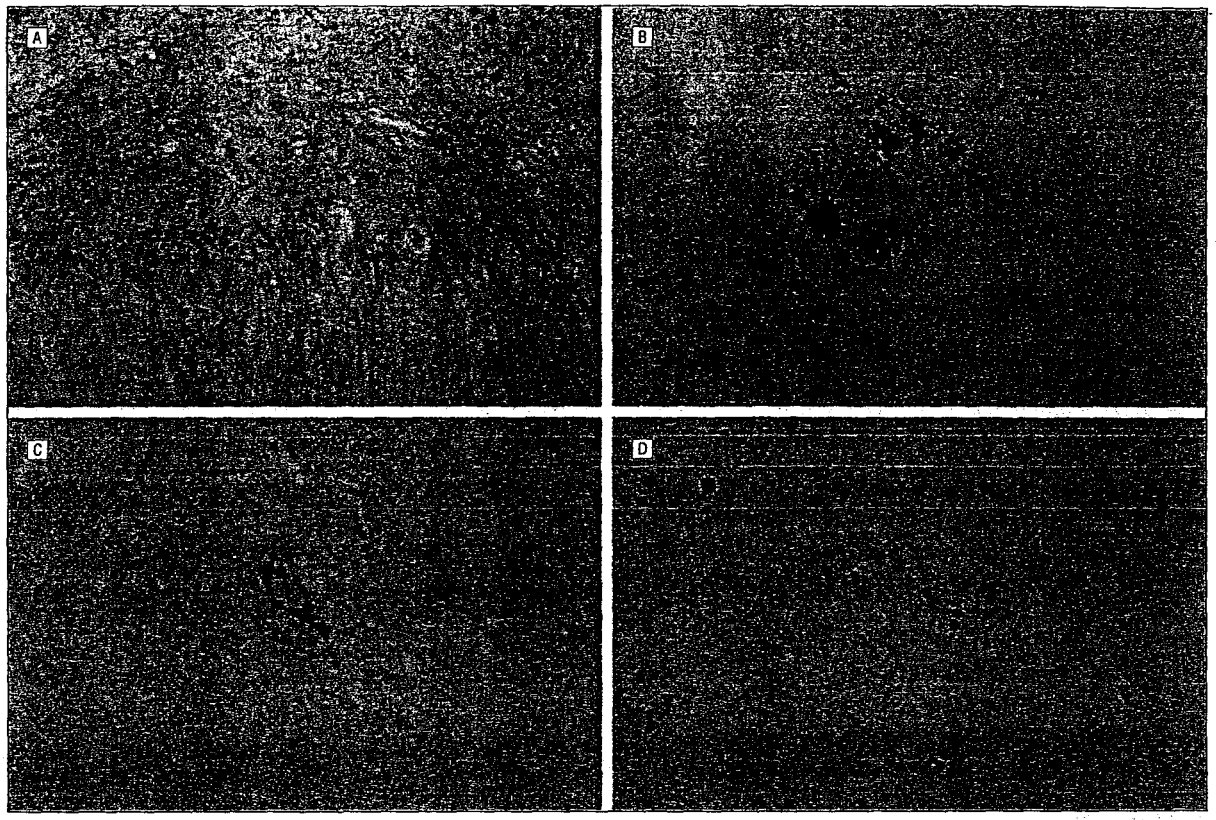

Figure 2. Immunohistochemical analysis of brain sections from the patient. A, Glial fibrillary acidic protein immunohistochemistry of the molecular and granule cell layers of the cerebellum showing neuronal loss and Bergmann astrocytosis (original magnification ×20). B, Granular prion protein staining in the cerebellum (original magnification ×40). C, Perineuronal prion protein staining in the temporal lobe (original magnification ×20). D, Prion protein plaques in the temporal lobe (original magnification ×20).

sults. Sequencing of the *PRNP* open reading frame was normal on 2 separate occasions. A polymerase chain reaction performed with primers designed to amplify the octapeptide repeat region of *PRNP* did not demonstrate an insertion mutation. The codon 129 polymorphism was homozygous for valine.

Fourteen months after onset, the patient died and an autopsy was performed.

AUTOPSY FINDINGS

Histopathologic examination was limited to the brain and spinal cord (**Figure 2**). The findings were atypical of sporadic CJD in the severity of white matter degeneration and the extensive nature of PrP deposition in the cortex and white matter. The frontal cortex showed extremely severe neuronal loss with striking astrocytosis and prominent spongiform vacuolation. There was severe overall loss of white matter, in places reminiscent of infarction. Deposition of PrP was extensive throughout the cortex and white matter. In places this was a diffuse punctate deposition similar to the recognized synaptic pattern. Occasionally, individual cells, mainly pyramidal neurons, were outlined by PrP deposition and had a fine granular intracellular deposition. More dense deposits, similar to plaques, were seen in the cortex. Also in the white matter, PrP deposits were seen ranging from a couple of micrometers to much larger plaquelike deposits, although these were not florid.

Temporal, parietal, and occipital lobes showed histologic features similar to those described in the frontal lobe, the occipital lobe being most severe. The hippocampus was relatively well preserved. In the caudate, putamen, and amygdala there was neuronal loss, astrocytosis, and microglial activation. The thalamus, midbrain, and substantia nigra showed mild to moderate spongiform change, neuronal loss, and astrocytosis with intraneuronal and extracellular punctate deposits. The pons and medulla were less severely affected than the midbrain with punctate PrP deposits. The cerebral peduncles were severely affected, with nearly complete loss of myelin. The cerebellum was very severely affected, with a dramatic loss of Purkinje and granule cells accompanied by vacuolation and astrocytosis. The cerebellar white matter showed severe white matter loss similar to incipient infarcts. Deposition of PrP in the cerebellum was marked with accumulation of punctate deposits resembling plaques, most commonly in the granule cell layer. In the white matter the deposits were denser still, occasionally plaquelike or forming irregular linear deposits.

PrP^{Sc} TYPING STUDIES

Western blot analysis was performed on fresh frozen cerebellar tissue from the patient. Identical results were ob-

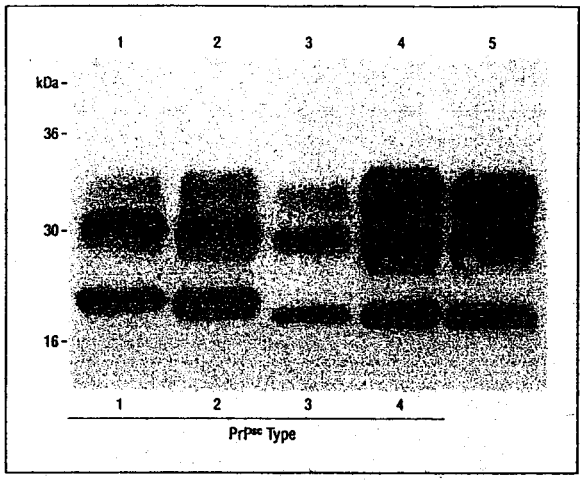

Figure 3. Immunoblotting of 10% brain homogenate after limited proteinase K digestion using anti–prion protein (PrP) monoclonal antibody 3F4. Lanes 1, 2, and 3 show 3 types of PrP^{Sc} (the scrapie isoform of PrP) seen in sporadic and iatrogenic cases of Creutzfeldt-Jakob disease; lane 4 shows PrP^{Sc} type 4, which is uniquely seen in brain tissue from patients with variant Creutzfeldt-Jakob disease.[21] Lane 5 shows PrP^{Sc} from the cerebellum of our patient demonstrating the same predominance of the high-molecular-mass diglycosylated PrP glycoform and a molecular mass of all PrP fragments similar to those of PrP^{Sc} type 4.

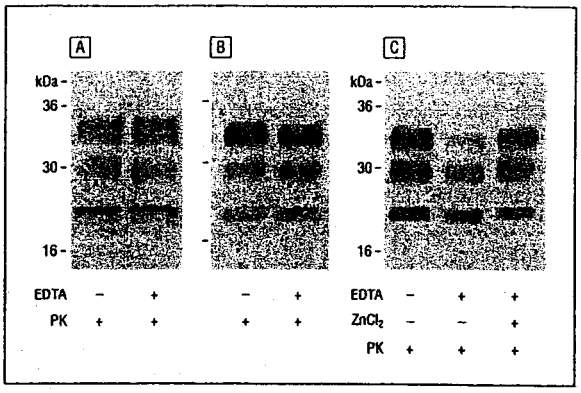

Figure 4. Immunoblotting of 10% brain homogenate after limited proteinase K (PK) digestion using anti–prion protein (PrP) monoclonal antibody 3F4. A, Cerebellum from a patient with variant Creutzfeldt-Jakob disease demonstrating a doublet of low-molecular-mass nonglycosylated bands of PrP^{Sc} (the scrapie isoform of PrP) with an identical pattern of PrP fragments observed after proteolysis in the presence of 25mM EDTA. B, Cerebellum from our patient demonstrating a doublet of low-molecular-mass nonglycosylated PrP^{Sc} bands. All bands migrate with lower apparent molecular mass following proteolysis in the presence of 25mM EDTA. C, Aliquots of cerebellum homogenate from our patient digested directly with proteinase K or after treatment with 25mM EDTA and sequential washing of insoluble pellets with *N*-ethyl morpholine buffer either lacking (–) or containing (+) 20μM zinc chloride ($ZnCl_2$).[19]

tained from separately analyzed tissue samples from opposite poles of the cerebellum. The glycoform ratio and fragment sizes resembled PrP^{Sc} type 4 seen in vCJD (**Figure 3**). The nonglycosylated band was seen as a doublet, as is seen for PrP^{Sc} in the cerebellum in vCJD (**Figure 4**). The effect of adding the metal ion chelator EDTA to the cerebellum homogenate before proteinase K cleavage was to reduce the apparent molecular weight of PrP^{Sc} fragments. This reflects the involvement of metal ions (most likely copper and zinc) in the conformation of PrP and determination of accessible protease cleavage sites.[19] This deduction was verified by showing that application of zinc ions to EDTA-treated samples before proteolysis resulted in preservation of the original PrP^{Sc} fragment size (Figure 4C). Although similar dependence on metal ions is observed for some PrP^{Sc} conformers associated with sporadic CJD,[19,21] this is not observed with PrP^{Sc} type 4 propagated in vCJD[19,21] (Figure 4). Therefore, these findings reflect a novel PrP^{Sc} type when compared with the diversity we and others have so far documented.[21,22]

COMMENT

Does the PrP^{Sc} typing suggest a BSE-related cause, or can our findings be accommodated by the spectrum seen in sporadic CJD cases worldwide? The molecular strain typing of the patient's brain material demonstrated a novel PrP^{Sc} type when compared with our archived cases.[21] There is as yet no internationally agreed-on classification of PrP^{Sc} type. Parchi and colleagues[23] identified 2 PrP^{Sc} types in sporadic CJD. However, Hill et al[21] described 3 PrP^{Sc} types associated with sporadic and iatrogenic CJD (types 1-3) and PrP^{Sc} type 4 associated with vCJD. The PrP^{Sc} type 5 has, to our knowledge, been observed only in mice expressing human PrP 129V inoculated with vCJD.[3,12] Hill et al[21] recently described a novel PrP^{Sc} type 6 in sporadic CJD.

The PrP^{Sc} type from our case has features similar to PrP^{Sc} type 4 (vCJD) in the predominance of the diglycosylated band; however, it is distinct from PrP^{Sc} type 4 in the dependence of the protease cleavage pattern of PrP^{Sc} on metal ions, suggesting a distinct PrP^{Sc} conformation. Unfortunately, only cerebellum was available for Western blotting in this case, although in vCJD cases from which whole brain was available we have not found evidence of any regional variation in PrP^{Sc} type. Others have reported coexistence of Gambetti PrP^{Sc} type 1 in the brain from patients with vCJD as a minority component.[24] It would also have been interesting to look for peripheral lymphoreticular PrP deposition because this is prominent in vCJD, but that tissue was not available for analysis. Transmission of BSE isolates to transgenic mice expressing human PrP 129 valine results in clinical prion disease with undetectable PrP^{Sc}; however, transmission of vCJD isolates to the same mice produces PrP^{Sc} type 5 that shares the same predominance of diglycosylated PrP^{Sc} to that of PrP^{Sc} type 4, and these data suggest that the molecular signature of BSE may be preserved after BSE transmission to *PRNP* codon 129 VV humans.[3,12] Transmission studies of the current case in transgenic mice are now being undertaken to investigate transmission characteristics.

We have described a novel PrP^{Sc} type that would be designated type 7 by our classification. A firm connection between novel PrP^{Sc} types and BSE cannot be made on the basis of a single case, and it will be important to see whether other similar cases occur in the United Kingdom and other BSE-exposed countries but not elsewhere and to perform detailed transmission studies of prions from this patient into transgenic and conventional mice to compare with BSE-derived isolates from

cattle and other species. Two other cases of prion disease with valine homozygosity and atypical features have been reported in the United Kingdom and the Netherlands. One of these cases was atypical because of very young onset and a protracted psychiatric history[25]; the other was notable because certain clinical and molecular features of the case overlapped with those of vCJD, including Western blot analysis of autopsied brain showing a predominance of a diglycosylated PrP^{Sc} isoform.[26]

We recommend keeping an open mind about the etiology of such cases during the ensuing years. These cases emphasize the importance both of continued surveillance of prion disease and the further development and refinement of molecular classification of prion diseases of humans and animals. It will also be important to assess lymphoreticular involvement in subsequent cases either at diagnostic tonsil biopsy or at autopsy.

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