アンテロープにおいてはBSEの垂直感染（Aldhous, 1990）が認められ、ウシにおいてはBSEの発症が認められることから、ウシがBSEに感染する際にも垂直感染による可能性も存在するという疑いを払拭することができない（Aldhous, 1991）。

図1：仔ウシが生まれてから母ウシがBSEを発症するまでの時間的長さと、仔ウシの血液検体におけるPrP^{res}の陽性率と陰性率との関係の度数分布（* 母ウシの発症までの時間的長さが1年の際の差、$p<0.05$）

医薬品　研究報告　調査報告書

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販売名（企業名）	－		研究報告の公表状況	Neuropathology. 2009 Oct;29(5):625-31.	公表国 オーストラリア	

研究報告の概要	脳神経外科用器具、脳波計（EEG）用脳内電極、ヒト下垂体ホルモン、硬膜移植片、角膜移植、輸血を介してクロイツフェルト・ヤコブ病（CJD）に罹患した患者は400名を超えている。 医原性CJD患者の新規の罹患数は減少しているが、輸血を介して伝播された多様なCJD症例が2004年以降報告されている。 CJDの医原性感染は、依然として明らかに深刻な問題である。近年、我々はこの9年間に日本CJDサーベイランス委員会（CJD Surveillance Committee）の登録患者に実施された医療（全ての外科処置、脳神経外科処置、眼科手術、および輸血）を調査した。 孤発性CJD（sCJD）患者753名と対照被験者210名で構成した症例対照試験で、プリオン病がsCJD発症以前に調査対照の医療を介して伝播したことを示すエビデンスを見出せなかった。 これまでに報告された症例対照試験のレビューでは、輸血がCJDの有意なリスク因子であることは一度も明らかにされておらず、我々の研究でも同じ結果が得られている。 手術がsCJDの有意なリスク因子であることを報告している症例対照試験もいくつかあるが、外科処置を手術のタイプに分類すると、その結果は相互に相容れないものがあり、これは外科処置を介してのプリオン伝播の可能性がほとんどないことを示唆している。我々の試験では、sCJD患者の4.5%がsCJD発症後に手術を受けており、これには脳神経外科処置0.8%および眼科手術1.9%が含まれる。sCJD発症後ですら、脳神経外科処置を除外できないことを示唆している。 医原病リスクを低減するためには、我々はプリオン病に対して警戒を続けなければならない。
報告企業の意見	今後の対応
輸血がCJDの有意なリスク因子であることは明らかにされていないが、警戒は続ける必要があるとの報告である。 現時点まで血友病以外で血漿分画製剤からvCJD伝播が疑われた報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。 なお、当社血漿分画製剤の原料血漿は現在まで英国の血漿を使用していない。	今後ともvCJDに関する安全性情報等に留意していく。
使用上の注意記載状況・その他参考事項等	
重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はないが、しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。	

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Symposium: Prion diseases — Updated

The risk of iatrogenic Creutzfeldt-Jakob disease through medical and surgical procedures

Tsuyoshi Hamaguchi,[1] Moeko Noguchi-Shinohara,[1] Ichiro Nozaki,[1] Yosikazu Nakamura,[2,6] Takeshi Sato,[3,6] Tetsuyuki Kitamoto,[4,6] Hidehiro Mizusawa[5,6] and Masahito Yamada[1,6]

[1]Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, [2]Department of Public Health, Jichi Medical University, Shimotsuke, [3]Department of Neurology, Higashi-Yamato Hospital, Higashi-yamato, [4]Departments of Prion Protein Research, Division of CJD Science and Technology, Tohoku University Graduate School of Medicine, Sendai, [5]Department of Neurology and Neurological Science, Graduate School, Tokyo Medical and Dental University, Tokyo, and [6]Creutzfeldt-Jakob Disease Surveillance Committee, Japan

There have been more than 400 patients who contracted Creutzfeldt-Jakob disease (CJD) via a medical procedure, that is, through the use of neurosurgical instruments, intracerebral electroencephalographic electrodes (EEG), human pituitary hormone, dura mater grafts, corneal transplant, and blood transfusion. The number of new patients with iatrogenic CJD has decreased; however, cases of variant CJD that was transmitted via blood transfusion have been reported since 2004. Clearly, iatrogenic transmission of CJD remains a serious problem. Recently, we investigated medical procedures (any surgery, neurosurgery, ophthalmic surgery, and blood transfusion) performed on patients registered by the CJD Surveillance Committee in Japan during a recent 9-year period. In a case-control study comprising 753 sporadic CJD (sCJD) patients and 210 control subjects, we found no evidence that prion disease was transmitted via the investigated medical procedures before onset of sCJD. In a review of previously reported case-control studies, blood transfusion was never shown to be a significant risk factor for CJD; our study yielded the same result. Some case-control studies reported that surgery was a significant risk factor for sCJD. However, when surgical procedures were categorized by type of surgery, the results were conflicting, which suggests that there is little possibility of prion transmission via surgical procedures. In our study, 4.5% of sCJD patients underwent surgery after onset of sCJD, including neurosurgeries in 0.8% and ophthalmic surgeries in 1.9%. The fact that some patients underwent surgery, including neurosurgery, even after the onset of sCJD indicates that we cannot exclude the possibility of prion transmission via medical procedures. We must remain vigilant against prion diseases to reduce the risk of iatrogenesis.

Key words: blood transfusion, Creutzfeldt-Jakob disease, medical procedure, neurosurgery, ophthalmic surgery, prion, surgery.

INTRODUCTION

Prion diseases such as Creutzfeldt-Jakob disease (CJD) are characterized by spongiform change and abnormal prion protein deposition in the brain, and are transmissible under certain conditions. Human prion disease is divided into three categories: genetic prion diseases resulting from mutations of the prion protein (*PrP*) gene, acquired prion diseases contracted due to prion transmission via exposure to contaminated materials, and sporadic CJD (sCJD) with no *PrP* mutation or evidence of exposure to prions. Acquired prion diseases include kuru in Papua New Guinea,[1,2] variant CJD (vCJD) that may be transmitted to humans from cows with bovine spongiform encephalopathy (BSE),[3] and iatrogenic CJD transmitted via medical procedures.[4,5] To date, iatrogenic CJD has been reported in more than 400 patients, who were exposed to prion transmission via contaminated neurosurgical instruments, intracerebral electroencephalographic electrodes, human pituitary hormone, corneal transplant, or dura mater grafts.[5] The incidence of iatrogenic CJD has greatly decreased,[5] but a new type of iatrogenic CJD "vCJD transmitted via blood transfusion" was reported in 2004.[6]

Some case-control studies reported that medical procedures were possible risk factors for sporadic CJD (sCJD).[7–13] However, other studies found no significant association between medical procedures and sCJD.[14–17] Therefore, the risk posed by such procedures is unclear. Recently, we analyzed medical procedures (any surgery, neurosurgery, ophthalmic surgery, and blood transfusion) in patients registered by the CJD Surveillance Committee in Japan over a recent 9-year period to determine if there is an association between medical procedures and sCJD.[18] Here, we review reports on iatrogenic CJD, and the results of our and other studies, to determine if there is an association between medical procedures and sCJD.

Correspondence: Masahito Yamada, MD, PhD, Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, 13-1, Takara-machi, Kanazawa 920-8640, Japan. Email: m-yamada@med.kanazawa-u.ac.jp

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IATROGENIC CREUTZFELDT-JAKOB DISEASE

Dura mater graft-associated CJD

Since the first report of dura mater graft-associated CJD (dCJD) in 1987,[19,20] 196 cases have been identified worldwide,[5] and more than 50% of dCJD cases have occurred in Japan.[5] At this writing, the number of patients with dCJD in Japan has reached 132.[21] The mean age at onset of the 132 patients with dCJD was 55 years (range: 15–80 years), and the mean incubation period (duration from receipt of dura mater to onset of CJD) was 11.8 years (range: 1.2–24.8 years).[21] All the 132 patients had received dura mater grafts between 1978 and 1993.[21] Two-thirds of dCJD patients display subacute progression of neurologic manifestations that are almost identical to those of classic sCJD; however, the other one-third of dCJD patients present with atypical clinicopathologic features: relatively slow progression of neurologic manifestations, scarcity of periodic sharp-wave complexes (PSWCs) on electroencephalography (EEG), and the pathological presence of amyloid plaques immunoreactive for PrP.[22,23]

CJD transmitted via corneal transplant

In 1974, a 56-year-old woman who died of autopsy-confirmed CJD after an 8-month illness was reported in the United States. She had received a corneal graft 18 months before disease onset from a donor with autopsy-confirmed CJD.[24] This case was the first reported case of iatrogenic CJD. In 1997, a 45-year-old woman who developed CJD 30 years after corneal transplant from a donor with autopsy-confirmed CJD was reported in Germany.[25] In addition to these cases, eight CJD patients with a history of corneal transplantation have been reported; however, the CJD status of their donors was not confirmed.[26]

CJD related to treatment with human growth hormone and gonadotoropin

The occurrence of autopsy-confirmed CJD in three young adults in 1985, all of whom had been treated with cadaveric pituitary-derived human growth hormone (hGH), suggested the possibility of iatrogenic transmission of CJD.[27–29] Since these reports, more than 190 patients with hGH-related CJD have been reported worldwide.[5] France has the highest number of such patients, at more than 100. No patients have been reported in Asia.[5] A number of factors, such as the method of chromatography purification in the hormone production process, may contribute to these regional differences.[5] Interestingly, the incubation period of hGH-related CJD was shorter in patients that were homozygous for codon 129 polymorphisms (methionine [M]/valine [V]) of the PrP gene, as compared to heterozygotes, which confirms the findings of a previous report indicating that MM homozygosity at codon 129 of PrP gene is a significant risk factor for sCJD.[30] In addition, four patients with CJD who had undergone hormone therapy with cadaveric pituitary-derived gonadotropin were reported in Australia.[5]

CJD transmitted via surgical instruments and stereotactic EEG needles

In 1977, two patients who had developed CJD 15 and 18 months after stereotactic electroencephalographic exploration using silver electrodes that had been previously implanted on a patient with proven CJD were reported in Switzerland.[31] The electrodes had been sterilized with 70% alcohol and formaldehyde vapor, but one of the electrodes subsequently transmitted spongiform encephalopathy to a chimpanzee 18 months after implantation in the cerebral cortex.[32] Furthermore, in a review of a report in 1960,[33] two patients with CJD possibly transmitted via neurosurgical instruments were identified.[34,35] There has been no case of CJD transmitted via surgical instruments or stereotactic EEG needles since the 1980s.

vCJD transmitted by blood transfusion

In 2004, the first case of human-to-human secondary transmission of vCJD via blood transfusion was reported in the UK.[6] This patient had received a transfusion of non-leucodepleted red blood cells that had originated from a donor who developed clinical vCJD 3 years and 4 months after donation.[6] Two additional patients with vCJD transmitted via blood transfusion have been identified.[36] All

Table 1 Medical procedure-related risk for sporadic Creutzfeldt-Jakob disease (sCJD) divided into three categories according to age at disease onset in patients monitored by the CJD Surveillance Committee, Japan[18]

Age		*n*	Any surgery	Neurosurgery	Ophthalmic surgery	Other surgery†	Blood transfusion
All	sCJD	753	49.4%	3.3%	5.6%	44.8%	10.4%
	Control	210	49.5%	6.2%	5.2%	42.4%	9.5%
31–50	sCJD	32	50.0%	6.3%	6.3%	40.6%	3.1%
	Control	37	45.9%	10.8%	2.7%	37.8%	5.4%
	Odds ratio		1.66	0.38	2.15	0.78	0.64
	95% CI		0.04–74.09	0.02–6.64	0.05–101.51	0.02–33.39	0.05–9.09
	P		0.79	0.50	0.70	0.90	0.74
51–70	sCJD	414	43.7%	1.7%	2.2%	41.8%	9.4%
	Control	97	46.4%	5.2%	3.1%	40.2%	11.3%
	Odds ratio		0.18	0.69	2.71	5.57	0.84
	95% CI		0.02–1.73	0.13–3.62	0.24–30.38	0.62–50.05	0.40–1.77
	P		0.14	0.66	0.42	0.13	0.64
71–	sCJD	317	57.0%	5.2%	10.1%	49.2%	12.4%
	Control	60	65.0%	6.7%	10.0%	56.7%	11.7%
	Odds ratio		0.81	0.76	1.15	0.83	1.27
	95% CI		0.15–4.37	0.15–3.80	0.38–3.48	0.17–4.02	0.52–3.10
	P		0.80	0.74	0.81	0.82	0.60

† Other surgery: Surgery other than neurosurgery or ophthalmic surgery. 95% CI, 95% confidence interval.

three of these patients had MM at codon 129 of the PrP gene, as did all other previous vCJD patients. However, a fourth patient with asymptomatic infection after blood transfusion and MV at codon 129 was reported in 2004.[37] This patient died of a non-neurological disorder 5 years after receiving a blood transfusion from a donor who subsequently developed vCJD.[37] Protease-resistant PrP was detected by Western blot, paraffin-embedded tissue blot, and immunohistochemistry in tissue from the spleen, but not in brain tissue.[37] This case was the first indication that individuals with codon 129 polymorphisms other than MM could be infected by the vCJD agent.

THE RISK OF sCJD TRANSMISSION VIA MEDICAL PROCEDURES

The association between sCJD and medical procedures before disease onset

To determine if an association exists between medical procedures and sCJD, we investigated medical procedures (any surgery, neurosurgery, ophthalmic surgery, and blood transfusion) in patients registered by the CJD Surveillance Committee in Japan over a recent 9-year period.[18] We conducted an age-stratified case-control study with 753 sCJD patients and 210 control subjects. We also investigated sCJD patients who underwent neurosurgery or ophthalmic surgery at a hospital where other patients with any type of prion disease had undergone neurosurgery or ophthalmic surgery.[18] In our case-control study, the cases were patients with definite or probable sCJD, and patients with "prion diseases definitely denied" and "prion diseases probably denied" as the controls.[18] The frequencies of medical procedures before disease onset in cases and controls are shown in Table 1. Among both cases and controls, approximately 50% had a history of surgery, and approximately 10% had received a blood transfusion. There was no significant difference between cases and controls in the frequencies of any surgery, neurosurgery, ophthalmic surgery, other surgery or blood transfusion (Table 1). On logistic regression analysis, there was no significant risk associated with any investigated medical procedure (Table 1). Although the control group was relatively small, there was no evidence that prion disease was transmitted via medical procedures before onset of sCJD in this study.

The results of 11 case-control studies and a meta-analysis investigating the history of medical procedures as a risk factor for sCJD are shown in Table 2. In these studies, blood transfusion was never shown to be a significant risk for developing CJD, which conforms with our results (Table 2).[7–12,15–18] However, the association between surgical procedures and the development of CJD has been controversial (Table 2).[7–18] Our results, which indicated that surgery was not a significant risk for sCJD, were consistent with those of two previous case-control studies with large sample sizes[15,16] and a meta-analysis that included three case-control studies (Table 2).[17] In studies that claimed to reveal an association,[7–12] the results were conflicting when surgical procedures were categorized by type of surgery. With respect to neurosurgeries, one case-control study observed a significant risk for sCJD;[8] however, other studies indicated that there was no significant risk, when cadaveric dura mater grafts were excluded.[7,9–12] Ophthalmic surgery was reported to be a significant risk for sCJD in a case-control study from Australia,[9] but not in other studies.[11–14,16] In a recent study in the UK,[12] an increase in risk associated with having had surgery was observed. This association was mainly noted in the category of "other surgery," for example, stitches to skin, and the association largely disappeared when "other surgery" was excluded from the analysis. These results suggest that although the possibility of prion transmission via surgical procedures is slight, we cannot entirely exclude this because of the existence of iatrogenic CJD. A recent study reported that methodological differences might partially explain the conflicting results regarding the association between surgery and CJD.[38] In particular, it is important to consider where the control participants are recruited (hospital or community) and how information on them is obtained (from participants or proxy informants).[38] Such methodological inconsistencies are serious limitations of case-control studies.

In our study,[18] five patients with sCJD had a history of neurosurgery or ophthalmic surgery at hospitals where neurosurgery or ophthalmic surgery had been performed on patients who later developed prion diseases; however, the interval between surgeries at the same hospitals was always more than 3 years.[18] According to the Incident Panel in the UK, most instruments that have gone through 10 cycles of use and decontamination are unlikely to pose a significant risk.[39] We assume that all instruments had indeed gone through more than 10 cycles of use during the 3-year interval and that they were not infective. Therefore, it is unlikely that an infectious agent was transmitted via these surgeries. In Japan, a large number of dCJD patients have been identified, but there have been no cases of other types of iatrogenic CJD. This study confirms that there were no cases of surgical transmission among patients diagnosed with sCJD.

Table 2 Review of case-control studies and a meta-analysis of medical procedure-related risk for sporadic Creutzfeldt-Jakob disease (sCJD)

	Year	Case	Control	Country	Medical procedures associated with sCJD
Kondo & Kuroiwa[7]	1982	60	56	Japan	Surgery within 5 years before onset of disease
Davanipour, *et al.*[8]	1985	26	40	USA	Injury to, or surgery for the head, face, or neck
					Suture
					Ocular tonometry
Collins, *et al.*[9]	1999	241	784	Australia	Surgery
					Surgeries for heart, hemorrhoids, gallbladder, hernia, cataract/eye, varicose veins, carpal tunnel and hysterectomy
Nakamura, *et al.*[10]	2000	52	102	Japan	Surgery with cadaveric dura mater
Ward, *et al.*[11]	2002	326	326	France, Germany, Netherlands, UK	Surgery
					Gynecologic surgery and other surgery (any surgery other than neurologic, eye, ear, gallbladder, gastrointestinal, gynecologic operations, tonsilectomy and appendectomy)
Ward, *et al.*[12]	2008	431	454	UK	Surgery
					Other surgery
Mahillo-Fernandez, *et al.*[13]	2008	167	3059	Sweden, Denmark	Major surgical procedures conducted 20 or more years before onset of sCJD
Harries-Jones, *et al.*[14]	1988	92	184	UK	None
van Duijn, *et al.*[15]	1998	405	405	Belgium, France, Germany, Italy, Netherlands, UK	None
Zerr, *et al.*[16]	2000	405	405	Belgium, France, Germany, Italy, Netherlands, UK	None
Wientjens, *et al.*[17] (meta-analysis)	1996	178	332	Japan, USA, UK	None
Ours[18]	2009	753	210	Japan	None

Surgical procedures after onset of sCJD

Surgical procedures after onset of sCJD might result in secondary transmission of the disease through the use of contaminated instruments. In particular, neurosurgery is categorized as a high-risk procedure, and ophthalmic and olfactory surgery as medium-risk procedures, for transmission of the infectious agent for sCJD, according to the guidelines of the CJD Incident Panel in the UK.[39] We found that 34 (4.5%) sCJD patients had undergone some type of surgery before receiving a diagnosis of prion disease, and that six (0.8%) had undergone neurosurgery and 14 (1.8%) had undergone ophthalmic surgery (Table 3).[18] The six cases that underwent neurosurgery did so within 3 months after sCJD onset: the procedures were performed for subdural hematoma ($n = 3$), aneurysm ($n = 2$), and meningioma ($n = 1$) (Table 3).[18]

Our findings suggest that a delayed diagnosis of sCJD may be linked to an increase in the risk of secondary transmission of prion diseases via surgical instruments. Among the neurosurgery cases, the symptoms of sCJD were misdiagnosed as those of other neurological diseases, and the surgeries were performed near disease onset. In ophthalmic surgery, all the patients underwent surgery for

Table 3 Sporadic Creutzfeldt-Jakob disease (sCJD) patients who underwent neurosurgery or ophthalmic surgery after onset of sCJD in a study of patients investigated by the CJD Surveillance Committee, Japan[18]

Patient no.	Reason for surgery	Interval between surgery and onset of sCJD symptoms	Age (years) at onset of sCJD	Symptom at onset of sCJD
1	Subdural hematoma	0 M	71	Dementia
2	Subdural hematoma	0 M	77	Psychiatric symptoms
3	Subdural hematoma	1 M	57	Dementia
4	Meningioma	1 M	74	Vertigo
5	Aneurysm	2 M	46	Dementia
6	Aneurysm	3 M	67	Vertigo
7	Cataract	0 M	60	Gait disturbance
8	Cataract	0 M	61	Dementia
9	Cataract	0 M	63	Visual impairment
10	Cataract	0 M	71	Visual impairment
11	Cataract	0 M	74	Visual impairment
12	Cataract	0 M	74	Visual impairment
13	Cataract	1 M	66	Dementia
14	Cataract	1 M	74	Psychiatric symptoms
15	Cataract	1 M	85	Visual impairment
16	Cataract	2 M	79	Tremor
17	Cataract	4 M	81	Visual impairment
18	Cataract	8 M	77	Psychiatric symptoms
19	Cataract	10 M	57	Dementia
20	Cataract	14 M	64	Visual impairment

M, months.

cataract, and 50% of the patients (7/14) presented with visual disturbance as the initial symptom of sCJD (Table 3).[18] These findings are similar to those of a report from the UK,[40] and those of our previous study.[41] Visual disturbance might prompt ophthalmic surgery. Of greater concern is the fact that three patients underwent surgery eight or more months after sCJD onset. In our studies,[18,41] all surgeons who provided us with information regarding instrument cleaning and sterilization procedures reused some surgical instruments. However, the sterilization methods were inadequate to sterilize against infectious PrP, according to WHO guidelines.[42] These inadequate methods included the use of ethylene oxide gas and incomplete autoclaving. Neurosurgeons and ophthalmologists must become better informed about prion diseases and the necessity of using disposable instruments whenever possible. Furthermore, a more sensitive method for the early diagnosis of sCJD is required, because clinical diagnosis is sometimes difficult, particularly in atypical sCJD cases, which include the MM2, MV2, VV1, and VV2 phenotypes[43–46] according to six phenotypes of sCJD based on codon 129 *PrP* polymorphisms and type of protease-resistant PrP as determined by Western blotting.[47] Even neurologists sometimes misdiagnose atypical sCJD in patients with other neurodegenerative disease, such as Alzheimer's disease or progressive supranuclear palsy.[43]

CONCLUSIONS

According to the conflicting results of case-control studies, including ours, we cannot assert that medical procedures are risk factors for the development of sCJD. However, the fact that some sCJD patients had surgeries, including neurosurgery, even after the onset of sCJD, indicates that we cannot completely exclude the possibility of transmission of prion diseases via medical procedures. Neurosurgeons, ophthalmologists, other surgeons and physicians must pay more attention to the possibility of prion diseases in order to reduce the risk of transmission. In addition, careful long-term surveillance of prion diseases is necessary.

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医薬品
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研究報告　調査報告書

識別番号・報告回数				報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
一般的名称	乾燥濃縮人アンチトロンビンⅢ				2010年3月15日	該当なし	
販売名（企業名）	①ノイアート静注用500単位（ベネシス） ②ノイアート静注用1500単位（ベネシス） ③ノイアート（ベネシス）		研究報告の公表状況	AABB Weekly Report/2010/03/12		公表国 アメリカ	

研究報告概要

とのようにプリオン病が脳を破壊するのかを研究しているアメリカ国立保健研究所の科学者は、プリオン関連の障害に特有のスポンジ様の脳の損傷を引き起こさないマウスでプリオン病の新しい形状を観察した。
NIH プレス・リリースによると、新しいプリオン病は、脳アミロイド血管障害（脳動脈を損傷するアルツハイマー病に関連した状態）に似ている。
プリオン病（感染性海綿状脳症として知られている）は主に脳に損傷を与え、牛における狂牛病又はウシ海綿状脳症と散発性 CJD、vCJD を含む。
本研究は、スコットランドで National Institute of Allergy and Infections Diseases と Veterinary Laboratories Agency によって行われ、プリオン病の通常の徴候の多くが発現した。しかしながら、プリオン病を代表するニューロン内外のスポンジ様の穴は観察されなかった。
その代わりに、マウスの脳は血管の外でトラップされたプリオン蛋白質プラークの大きな蓄積（これは脳の動脈、静脈そして毛細血管を損傷させる）を含んでいた。
この研究から得られた知見は、プリオン病の治療の開発において、アルツハイマー病と同様に科学者の役に立つであろう。

報告企業の意見

マウスを用いた動物実験で、プリオン病に特有のスポンジ様の脳損傷を起こさない新しい形状が観察されたことについての報告である。
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてノイアート静注用500単位の記載を示す。
2. 重要な基本的注意
(1)略
2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

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IN THIS ISSUE

Cord Blood Workshop Answers Industry Questions

Approximately 225 people from across North and South America, Europe, and Asia attended the Cord Blood Licensure Workshop held this week in Rockville, Md. The March 8-10 event provided information on the elements and steps involved in the biologics license application process as outlined in a Food and Drug Administration guidance. The guidance pertains to the manufacturing of minimally manipulated, unrelated, allogeneic placental/umbilical cord blood and applicable regulatory requirements compliance. A draft guidance on investigational new drug applications for HPC-Cs was released at the same time as the licensure guidance and provides recommendations for use of HPC-Cs that are not licensed but are needed for treatment of a patient, with a serious or life-threatening disease or condition. Participants raised concerns about the ability to meet the October 2011 deadline to obtain a license for these products, inquired about how the guidance would affect their patients, and sought clarification on the submission process for BLAs and INDs. FDA speakers noted that some items are facility-specific and should be addressed in pre-BLA meetings, encouraging those with additional questions to submit them to the docket, which is referenced in an Oct. 20, 2009, Federal Register notice. Presenters also emphasized the unique opportunity that licensure presents for collaboration among those in the cellular therapy community, as these could be the first licensed allogeneic cellular therapy products.

Handouts from the workshop will be posted in the coming weeks on AABB's Live Learning Center for attendees to access.

Event Calendar

March 18-21 – 2010 South Central Association of Blood Banks Annual Meeting and Exhibit Show read more »

March 21-22 – SCABB Immunohematology Reference Lab Workshop read more »

March 24 – AABB Audioconference: Distance Education: Is It an Answer to the Personnel Shortage? read more »

March 24-25 – National Cancer Institute's 3rd Annual Biospecimen Research Network Symposium read more »

March 31 – AABB Audioconference: Molecular Approaches to Rh Problems read more »

Full Calendar read more »

Poster Created to Help Meet New Standard 1.5 Requiring Process for Reporting Quality Concerns to AABB

AABB has developed a poster for facilities to use in the workplace to help them comply with a new standard that requires a process for personnel to be able to anonymously communicate concerns about quality or safety to AABB. Standard 1.5 is included in the 26th edition of Standards for Blood Banks and Transfusion Services, which took effect Nov. 1, 2009. Facilities are not required to use the poster; it is an aid to compliance with the intent of this new standard. Any questions can be directed to AABB's Department of Accreditation and Quality.

Latest Issue of AABB News Explores New Government Health Care Leadership

The March issue of AABB News focuses on the changes in health care leadership since President Obama took office — and how this affects the transfusion and cellular therapy communities. In one article, Howard Koh, MD, MPH, assistant secretary for health at the U.S. Department of Health and Human Services, speaks to AABB News about HHS' priorities — including an increased focus on preventive medicine and forging new relationships with industry. Another article examines the changes in store at the National Institutes of Health under Director Francis Collins, MD, PhD. This issue — to be mailed next week — also includes an update on the Donor Hemovigilance System and a column about how to record references in standard operating procedures.

Enrollment Under Way for National Hemovigilance System

One month following the launch of the Hemovigilance Module of the Centers for Disease Control and Prevention's National Healthcare Safety Network, approximately 60 facilities have agreed to enroll. The module is a surveillance system that allows for the real-time tracking of adverse events associated with blood transfusions as well as the quick identification of trends within a facility. All hospitals with transfusion medicine services are encouraged to join the module as well as AABB's special data analysis group within the system. The goal of AABB's Hemovigilance Module group is to provide individual institutions with in-depth analyses and recommendations for specific enhancements to patient safety and reductions in health care costs. To join, facilities should express their interest to AABB and complete the Intent to Participate form. Assistance with the NHSN enrollment process also is being offered by AABB. Interested facilities should visit the AABB Web site or contact Barbee I. Whitaker, PhD, director of data and special programs at AABB, for further guidance.

FDA, Makers of WinRho SDF Warn of Potentially Fatal Complications in ITP Patients

The Food and Drug Administration issued a MedWatch announcement on Wednesday alerting the medical community to potentially fatal risks of intravascular hemolysis in patients being treated for immune thrombocytopenic purpura with WinRho SDF. In the announcement, FDA indicated that the manufacturer and distributor of WinRho SDF, Cangene Corporation and Baxter Healthcare Corporation, have sent a letter informing health care professionals that a new boxed warning has been added to the product labeling, which specifies what complications can

result and provides guidelines for proper administration and follow-up to help ensure WinRho SDF is used safely and effectively. The letter also advises of specific changes to the warnings, contraindications, precautions, and dosage and administration. Other resources included in the announcement include prescribing information and patient information. This notification does not apply to patients receiving WinRho SDF for the suppression of Rh isoimmunization.

Global Cellular Therapy Organizations Gather New Data

The Alliance for Harmonisation of Cellular Therapy Accreditation — of which AABB is a member — has partnered with the World Marrow Donor Association to gather data on cellular therapy collection facilities. Collection facilities are asked to complete a form seeking certain license and accreditation data to include in this registry, which will serve as a resource for WMDA members and regulatory organizations.

WMDA also is in the process of updating country-specific import and export requirements. This data — which was originally collected in 2003 — is located in the regulatory section of the WMDA Web site.

＊「NIH Scientists Find New Form of Prion Disease That Damages Brain Arteries」

National Institutes of Health scientists studying how prion diseases damage the brain have observed a new form of prion disease in mice that does not cause the sponge-like brain deterioration characteristic of prion-related disorders. According to an NIH press release, the new prion disease resembles cerebral amyloid angiopathy, a condition related to Alzheimer's disease that damages brain arteries. Prion diseases — known as transmissible spongiform encephalopathies — primarily damage the brain and include mad cow disease or bovine spongiform encephalopathy in cattle, sporadic Creutzfeldt-Jakob disease and variant CJD. The study, conducted by scientists at NIH's National Institute of Allergy and Infectious Diseases and the Veterinary Laboratories Agency in Scotland, revealed many of the usual signs of prion disease. However, the sponge-like holes in and around nerve cells typical of prion disease were not observed. Instead, the mouse brains contained large accumulations of prion protein plaques trapped outside blood vessels, which damages arteries, veins and capillaries in the brain. The knowledge gained from this study may help scientists in developing therapies for prion diseases as well as Alzheimer's disease.

Region Watch

Demand for most frozen products is increasing; the need for cryoprecipitate and fresh frozen plasma is especially steady. A robust supply of frozen products easily serves these needs, according to the National Blood Exchange. Platelet products are readily available throughout the country, and the surplus of red blood cells continues to climb.

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Managing Editor: Sherkiya Wedgeworth

医薬品
医薬部外品　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2010年2月3日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②③乾燥抗HBs人免疫グロブリン ④⑤ポリエチレングリコール処理抗HBs人免疫グロブリン		研究報告の公表状況	Haemophilia 2010; 1-9	公表国 イギリス	
販売名 (企業名)	①ヘブスブリン筋注用200単位　(ベネシス) ②ヘブスブリン筋注用1000単位　(ベネシス) ③ヘブスブリン　(ベネシス) ④ヘブスブリンIH静注1000単位　(ベネシス) ⑤静注用ヘブスブリン-IH　(ベネシス)					

研究報告の概要

英国プール血漿由来濃縮因子製剤によって1980～2001年の間に出血障害の治療を行った全ての英国の患者はvCJD感染リスクが増加した可能性があることを告知されている。我々は、vCJDのリスクが高いと考えられる17人の神経学的にasymptomaticな血友病患者に関連したプロテアーゼ耐性プリオン（PrP^{res}）の検出について報告する。
11の剖検および7の生検材料からPrP^{res}を分析した。
脾臓サンプルのひとつはウエスタンブロット分析でPrP^{res}強陽性であった。73歳の患者からのこの組織は、神経症状はなかったが、プリオン蛋白遺伝子のコドン129はメチオニン/バリンのヘテロ型であった。彼はvCJD感染ドナーからのドネーションも含むプール血漿から製造された第Ⅷ濃縮因子9000単位以上およびvCJD感染ドナーからのドネーションを含むかわからない400,000単位の投与を受けた。彼は14ユニットの赤血球製剤も投与された、そしていくつかの外科的処置及び侵襲的な内視鏡的処置を受けていた。食事、手術、内視鏡、輸血そして英国製のプラスマ由来製剤を投与されたことを通しての曝露の相対的なリスクの評価において、この患者にもっともありそうな感染経路は英国製のプラスマ製剤を投与されたことであると示唆された。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
2. 重要な基本的注意
(1)略
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2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国プール血漿由来凝固因子製剤を投与された血友病患者のvCJD感染リスクが増加している可能性があることより、血友病患者の生体サンプルからプロテアーゼ耐性プリオン（PrP^{res}）の検出を試みたところ、73歳の患者脾臓サンプルからPrP^{res}が検出された。この患者のプリオン蛋白遺伝子のコドン129はメチオニン/バリンのヘテロ型であり、最もありそうな感染経路は英国製のプラスマ製剤であったとする報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Haemophilia

ORIGINAL ARTICLE

Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia

A. PEDEN,* L. MCCARDLE,* M. W. HEAD,* S. LOVE,† H. J. T. WARD,* S. N. COUSENS,‡ D. M. KEELING,§ C. M. MILLAR,¶ F. G. H. HILL** and J. W. IRONSIDE*

*National Creutzfeldt–Jakob Disease Surveillance Unit, University of Edinburgh, Edinburgh; †Department of Neuropathology, Frenchay Hospital, Bristol; ‡London School of Hygiene and Tropical Medicine, London; §Oxford Haemophilia and Thrombosis Centre, Churchill Hospital, Oxford; ¶Department of Haematology, Imperial College London, London; and **Department of Haematology, Birmingham Children's Hospital, Birmingham, UK

Summary. All UK patients with bleeding disorders treated with any UK-sourced pooled factor concentrates between 1980 and 2001 have been informed that they may be at an increased risk of infection with variant Creutzfeldt–Jakob disease (vCJD). We describe a study to detect disease-associated, protease-resistant prion protein (PrP^{res}) in 17 *neurologically* asymptomatic patients with haemophilia considered to be at increased risk of vCJD. Materials from 11 autopsy and seven biopsy cases were analysed for PrP^{res}. The tissues available from each case were variable, ranging from a single biopsy sample to a wide range of autopsy tissues. A single specimen from the spleen of one autopsy case gave a strong positive result on repeated testing for PrP^{res} by Western blot analysis. This tissue came from a 73-year-old male patient with no history of neurological disease, who was heterozygous (methionine/valine) at codon 129 in the prion protein gene. He had received over 9000 units of factor VIII concentrate prepared from plasma pools known to include donations from a vCJD-infected donor, and some 400 000 units not known to include donations from vCJD-infected donors. He had also received 14 units of red blood cells and had undergone several surgical and invasive endoscopic procedures. Estimates of the relative risks of exposure through diet, surgery, endoscopy, blood transfusion and receipt of UK-sourced plasma products suggest that by far the most likely route of infection in this patient was receipt of UK plasma products.

Keywords: haemophilia, plasma, prion protein, spleen, vCJD

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) was identified in the UK in 1996 [1] and subsequently shown to be caused by a transmissible agent with identical properties to the bovine spongiform encephalopathy (BSE) agent [2,3], most likely as a consequence of consumption of BSE-contaminated meat products [4]. Variant CJD represents the only known example of a human prion disease caused by exposure to an infectious prion agent from a non-human source. It is also unique in that the transmissible agent is detectable in a much wider tissue distribution than is the case for other forms of human prion disease. Both infectivity and the protease-resistant form of disease-associated prion protein (PrP^{res}) are readily detectable in a range of tissues apart from that of the central nervous system in vCJD, particularly in lymphoid tissues and the peripheral nervous system, albeit at lower levels than in the central nervous system [5,6].

Since 2004, four instances of vCJD infection (three clinical cases, one asymptomatic) in the UK have been associated with the transfusion of non-leucodepleted packed red cells from asymptomatic donors who subsequently died from vCJD [7–10]. The National Blood Authorities in the UK have taken a

Correspondence: Prof. James W. Ironside, National Creutzfeldt-Jakob Disease Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK.
Tel.: +44 131 537 3109; fax: +44 131 343 1404;
e-mail: james.ironside@ed.ac.uk

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number of steps to reduce the likelihood of secondary transmission of vCJD by blood components [11]. It is known that plasma donations from asymptomatic individuals infected with vCJD have also contributed to some batches of pooled clotting factor concentrate (termed 'vCJD-implicated batches'). The potential vCJD infectivity of these batches has been estimated by the UK CJD Incidents Panel (CJDIP) based on findings from a risk assessment commissioned by the Department of Health (DH) [12], together with batch-specific manufacturing data. Variant CJD-implicated batches of clotting factor concentrates factor VIII (FVIII) and IX were assessed to be likely to carry sufficient levels of vCJD infectivity to warrant the implementation of public health measures in recipients to minimize the possible risk of onward transmission [12]. A public health notification exercise of patients with bleeding disorders was conducted in 2004 by the Health Protection Agency and Scottish Centre for Infection and Environmental Health on behalf of the UK Departments of Health, at which time it was considered likely that further batches of UK-sourced plasma products would become implicated as future cases of vCJD arose [13]. Therefore, on the advice of the UK Haemophilia Centre Doctors' Organisation (UKHCDO), all patients with bleeding disorders who had been treated with any UK-sourced pooled factor concentrates between 1980 and 2001 were informed that they may be at an increased risk of infection with vCJD and were required to take measures to prevent the possibility of secondary spread of infection. This inclusive 'population' approach was endorsed by the CJDIP, DH and the Haemophilia Society.

To date, 170 cases of vCJD have been identified in the UK, including the three clinical cases in which infection is likely to have been transmitted by non-leucodepleted packed red cells transfused from asymptomatic donors who subsequently died from vCJD [7,9,10]. The annual incidence and death rate for vCJD have both declined in UK over the past few years, but the prevalence of vCJD infection in the UK remains uncertain. A retrospective study to detect disease-associated prion protein in paraffin-embedded sections of tonsil and appendix tissue indicated that the prevalence of vCJD infection might be higher than the current number of clinical cases recorded would suggest, with three positive cases being found in 12 674 tissue samples studied, giving an estimated prevalence rate of 237 vCJD infections per million in the UK population (although with wide confidence intervals) [14,15]. Further investigations on a large series of tonsil samples found a prevalence of disease-associated prion protein in tonsils from a 1961–1995 combined birth cohort of 0/32 661 with a 95% confidence interval of 0–113 per million [16]. In the 1961–1985 cohort, the prevalence of zero with a 95% confidence interval of 0–289 per million was lower than, but still consistent with, the results of the previous survey of tonsil and appendix tissues by Hilton *et al.* [14]. The prevalence of vCJD infection in the general UK population could therefore be around 1 in 10 000, based on an approximate average value between the results of these studies [14,16,17].

To date, no case of vCJD has been identified in any recipient of UK-sourced plasma products. In 2001 DH commissioned and funded a project to undertake active surveillance of UK patients with haemophilia for the possibility of vCJD infection. This study included the prospective and retrospective analysis of lymphoid tissues and brain tissue in biopsy material and/or autopsy material for the presence of the PrP^{res} isoform characteristic of vCJD.

We report the laboratory findings in this study, demonstrating for the first time the presence of PrP^{res} in the spleen of a UK adult haemophilic patient who at the time of death had no neurological signs or symptoms attributable to vCJD.

Materials and methods

Collection of tissue samples

Ethical approval was obtained for the project entitled 'Surveillance of new variant CJD-UKHCDO' (MREC/01/2/11) and the study was administered through the UKHCDO. All haemophilic patients undergoing surgical procedures involving the central nervous system and lymphoid tissue (including tonsil, lymph nodes and spleen) were encouraged to participate in the study. This applied only to patients who were to undergo surgical biopsy or resection of relevant tissues for medical reasons and was therefore opportunistic. Consent was obtained from patients for the analysis of biopsy samples and from relatives of the patient for autopsy tissues following the death of a patient undergoing either a hospital or Coroner's autopsy.

Cases and tissue specimens

Material from 11 autopsy cases and seven biopsy cases from 17 patients had tissue samples submitted to the National CJD Surveillance Unit for investigation. One patient had biopsy samples submitted on two occasions, and another patient had both biopsy and autopsy materials examined. The number of tissues available from each case was variable, ranging from single lymphoid tissue samples from living patients to a wide range of autopsy tissues (brain, tonsil, spleen, lymph node, appendix) in others. The samples were analysed in this study by a combination of Western blotting, paraffin-embedded tissue (PET) blotting and immunohistochemistry for disease-associated, protease-resistant prion protein (PrP^{res}). Cases of clinically suspected CJD that were given an alternative final pathological diagnosis were used as negative controls, as they lack PrP^{res} in the brain and peripheral tissues. Ethical approval for the acquisition and use of this autopsy material for research on transmissible spongiform encephalopathies in the National CJD Surveillance Unit brain bank is covered by LREC 2000/4/157 (JWI). The polymorphic status of codon 129 of the prion protein gene (*PRNP*) of each case was determined by restriction fragment length polymorphism as described previously [18].

NaPTA precipitation/Western blot analysis for PrP^{res}

Frozen central nervous system (cerebral frontal cortex, cerebellum, spinal cord) and lymphoreticular (spleen, tonsil, appendix) tissues (when available) from cases in this study and from vCJD and non-CJD control patients were homogenized to 10% (w/v) in 2% sarkosyl/PBS using the FastPrep™ instrument (Anachem, Cambridge, UK) and 500 μL samples of this homogenate were analysed by sodium phosphotungstic acid precipitation followed by high-sensitivity Western blotting (NaPTA/WB), as described previously [8,19,20]. At least four samples of spleen and other lymphoid tissues (when available) were studied.

Criteria for assigning positives

Samples of frozen brain (frontal cortex) and spleen from non-CJD neurological control patients were available for use as negative controls in the Western blots in this study. As a positive control in the NaPTA/WB analyses of either central nervous system tissue or lymphoreticular tissue, 10% (w/v) vCJD brain homogenate (3 μL) was diluted into 500 μL of a 10% (w/v) homogenate of either brain or spleen tissue from a non-CJD control patient. These spiked homogenates were then diluted with a further 500 μL of 2% sarkosyl/PBS as described in the standard protocol used for all the test samples [8]. Samples of tissue from haemophilic patients in this study were assessed by comparison with positive and negative control samples run on the same gel. The following criteria were established before interpreting the results: a positive result was assigned if at least two bands were observed to co-migrate with the corresponding PrP^{res} bands in the positive control and no bands were seen in the lane containing non-CJD control sample, after maximum exposure to HyperFilm ECL (GE Healthcare Life Sciences, Buckinghamshire, UK).

Centrifugal concentration/Western blotting

A number of samples of tissue homogenate prepared in 2% sarkosyl/PBS as described above were re-analysed using the centrifugal concentration/Western blot method described by us previously [6].

Densitometric analysis of PrP^{res} levels and glycoform ratios

For densitometric analysis, immunoblot images were scanned using a Bio-Rad GS-800 Densitometer and images were analysed and processed with QUANTITY ONE™ software (Bio-Rad, Hertfordshire, UK). Immunoblot images were included in the densitometric analysis if all three bands (di-, mono- and unglycosylated) were in the linear range.

Immunohistochemistry and PET blotting

Paraffin-embedded tissue blot analysis was carried out as described by us previously [21], using a modified version of the method of Schulz-Shaeffer *et al.* [22]. Immunohistochemistry for disease-associated prion protein was performed using a panel of four different anti-prion protein antibodies as previously described [21].

Results

Biochemical analysis

The high-sensitivity Western blot (NaPTA/WB) analyses were conducted on receipt of tissue and were subject to the availability of frozen tissue specimens, which varied between patients (Table 1). One sample of spleen out of the initial four tested from one of these patients gave a very strongly positive signal for PrP^{res} producing a poorly resolved smear, but with the highest densities in the region of the immunoblot typical for authentic PrP^{res}. A smaller volume of the positive homogenate (50 μL rather than 500 μL) was re-analysed by NaPTA/WB in order to obtain better resolution of the immunoreactive bands, and a positive signal was confirmed according to our criteria (Fig. 1). The glycoform ratio of this positive sample was consistent with vCJD, showing a predominance of the diglycosylated form of PrP^{res}.

Table 1. Summary of frozen tissue samples analysed by NaPTA/WB for PrP^{res}

Case number (*PRNP* codon 129)	Tonsil	Spleen	Lymph node	Appendix	Brain	Bone marrow	Gut
1 (MV)	–	–	–	–	0/9	–	–
2 (MV)	–	0/4	0/3	0/4	0/8	–	–
3 (MV)	0/4	0/12	0/4	–	0/12	–	–
4 (MM)	–	–	0/4	–	0/8	–	–
5 (MM)	–	–	–	–	0/8	–	–
6 (VV)	–	0/3	–	0/4	0/14	0/4	0/8
7 (MM)	0/3	0/3	–	0/3	0/3	–	–
8 (MM)	–	–	–	–	0/16	–	–
9 (MV)	–	1/26	0/2	–	0/11	–	–
10 (MM)	–	0/4	0/4	–	0/8	–	–

Depending on availability a minimum of four samples were tested from the tissue listed above. The results are given as the number of PrP^{res} positive samples as a proportion of the total number of independent samples tested for each tissue specimen.
A dash (–) indicates that no samples were available for analysis; M, methionine; V, valine; PrP^{res}, protease resistant prion protein; NaPTA/WB, sodium phosphotungstic acid precipitation/Western blotting.

The remaining 100 μL aliquot of this homogenate was analysed by the centrifugal concentration/Western blotting protocol and was again strongly positive (data not shown). Densitometry was used to compare the total signal (of all three PrP^{res} bands) with a dilution series of PrP^{res} samples from vCJD brain run in parallel with this and the previous sample. This analysis indicated that the level of PrP^{res} in this spleen sample was 3–5% of that found in vCJD brain.

NaPTA/WB analysis of a further 22 samples taken from the available spleen tissue from this case failed to show any evidence of PrP^{res} (Table 1). Exhaustive immunohistochemical and PET blot analysis of this tissue was similarly negative and NaPTA/WB, immunohistochemistry and PET blotting all failed to detect the presence of PrP^{res} in lymph node, frontal cortex or cerebellum in this case (Table 1). All other tissues from the remaining cases were negative by each of the methods used.

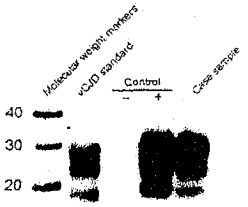

Fig. 1. Sodium phosphotungstic acid (NaPTA) precipitation/Western blotting analysis of spleen tissue samples for the presence of protease-resistant prion protein (PrP^{res}). A sample of spleen homogenate from case 9 (case sample) corresponding to 5 mg of tissue was analysed alongside spleen samples from a control case with non-CJD neurological disease (control) corresponding to 50 mg of tissue. One of the latter control samples (+) had been spiked with an amount of variant Creutzfeldt–Jakob disease (vCJD) brain homogenate, corresponding to 300 μg of tissue, prior to NaPTA precipitation. Standard vCJD brain PrP^{res}, corresponding to 100 μg of brain tissue, analysed without prior NaPTA precipitation, was run in the lane marked 'vCJD standard'. The molecular weight markers (in kDa) are shown in the leftmost lane.

To make a more quantitative assessment of the glycoform ratio in the positive specimen, we again used densitometry. The glycoform ratio of the specimen positive by NaPTA/WB mapped close to, but at the extreme diglycosylated side of the area defined by Western blot analysis of vCJD brain tissue, including vCJD brain tissue 'spiked' into negative control spleen and analysed by NaPTA/WB (Fig. 2). The glycoform ratio of this positive specimen was also more predominantly diglycosylated than the samples of vCJD spleen PrP^{res} used as positive controls in this study.

Genetic analysis

The results of the *PRNP* codon 129 analysis on each of the eight cases studied are included in Table 1. The case containing PrP^{res} in the spleen was heterozygous (methionine/valine) at this codon.

Immunohistochemistry and PET blotting

Immunohistochemistry and PET blot analysis on all the PET blocks in this study were negative in all cases, including the case in which PrP^{res} was detected biochemically in the spleen.

Case history

The clinical history of the haemophilic patient in whom PrP^{res} was detected in the spleen was reviewed in detail as follows:

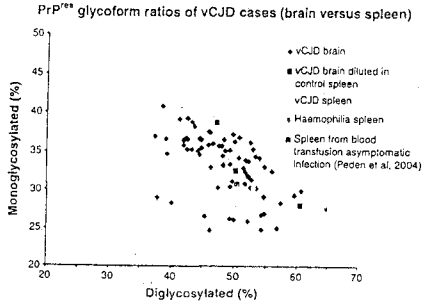

Fig. 2. Scattergram analysis of percentage diglycosylated and percentage monoglycosylated isoforms found in individual cases of variant Creutzfeldt–Jakob disease (vCJD). The glycoform ratio of protease-resistant prion protein (PrP^{res}) following sodium phosphotungstic acid (NaPTA) precipitation in the positive spleen sample from the Haem UK study case 9 (light blue diamond) is compared with vCJD brain PrP^{res} without NaPTA precipitation (dark blue diamonds), vCJD brain PrP^{res} diluted in non-CJD neurological control spleen homogenate with NaPTA precipitation (red squares), endogenous vCJD spleen PrP^{res} with NaPTA precipitation (yellow triangles). The glycoform ratio of spleen from a single case of preclinical vCJD infection following blood transfusion in an asymptomatic *PRNP* codon 129 MV individual (green square) is also shown.

The patient had severe haemophilia A (FVIII <1%). He was one of 10 children and all six of his affected brothers had died at an early age. He never developed antibodies to FVIII. He suffered from severe haemophilic arthropathy and despite multiple orthopaedic surgical procedures was wheelchair-bound by the age of 36 years. He also suffered from recurrent gastrointestinal (GI) bleeding and at the age of 39 sustained an intracerebral haemorrhage. He had multiple exposures to UK-sourced plasma-derived FVIII receiving 754 '500-unit' vials between 1980 and 2001 (approximately 400 000 units as the vials were overfilled). When tests became available, he was found have both antibodies to hepatitis C and to have the virus detectable in his blood. However, his liver function tests remained normal and he did not develop any clinical signs of liver disease. He was treated with two vCJD-implicated FVIII 8Y batches in 1994 (Batch FHC 4237, 1000 units) and 1996 (Batch FHB 4547, 8025 units), the latter given over a 3-day period for a bleed into the right hip joint. Both batches included a donation from a single donor who subsequently died from vCJD in 1997.

It is also recorded that the patient had been transfused with red blood cells in 1998 (3 units), in 1999 (5 units), in 2003 (3 units) and in 2007 (3 units). The red cell transfusion in 1998 was unlikely to have been leucodepleted, but the remaining transfusions were likely to have been leucodepleted. Apart from the earlier orthopaedic procedures, the patient had undergone multiple lower GI endoscopic procedures from 1980 to 2007, with polyp resections on five occasions between 2003 and 2007; an upper GI endoscopy without biopsy was performed in 1999.

At the age of 73, he was admitted to hospital in 2008 with chest pain, having fallen out of bed 2 days previously. On examination, he was noted to be in pain with a blood pressure of 135/80 mmHg with a heart rate of 95/min. He was fully conscious (Glasgow coma scale score 15/15) and showed no evidence of cognitive impairment or any other neurological abnormalities. A 5- to 6-cm haematoma was noted over the posterior aspect of the left side of his chest. Two days later, he deteriorated suddenly with a loss of consciousness and development of hypotension. He was suspected to have sustained an intracranial haemorrhage and died the following morning. An autopsy was performed under HM Coroner's instructions, which found a thrombosed fusiform aneurysm of the left iliac artery with extension of thrombus into the lower aorta. The elbow and knee joints were swollen with evidence of previous surgery to the left knee and multiple cutaneous bruises were present over the left upper limb and left side of the trunk.

Examination of the brain revealed a cavitated old haemorrhagic infarct in the right frontal lobe, but no evidence of recent haemorrhage was noted and no histological evidence of a spongiform encephalopathy was identified. The heart, spleen, lymph nodes and appendix all appeared normal and showed no evidence of accumulation of abnormal prion protein on immunohistochemistry. The liver showed evidence of a prominent mononuclear inflammatory cell infiltrate in the portal tracts with centrilobular microvacuolation and steatosis, in keeping with the history of hepatitis C infection. Sections of the iliac artery aneurysm showed the features of a longstanding aneurysm with a patchy infiltrate of chronic inflammatory cells in part of the wall. There was also evidence of both previous and fresh haemorrhage into the thrombus within the aneurysm, with foci of acute haemorrhage that were contiguous with foci of haemorrhage into the adjacent vessel wall. The most likely interpretation of these findings is that the patient's fall caused bleeding into the wall of the large left iliac artery aneurysm and accumulation of this haemorrhage resulted in occlusion of the vessel

with rapid propagation of blood clot upwards into the aorta resulting in hypotension, loss of consciousness and death.

Following the autopsy and with appropriate consent, frozen tissue samples from the brain, spleen and lymph node were submitted to the National CJD Surveillance Unit, along with fixed samples from the heart, liver, spleen, lymph node and appendix and iliac artery aneurysm.

Discussion

We describe the pathological analysis of tissues from a group of 17 UK patients with haemophilia considered to be at increased risk of vCJD through exposure to UK-sourced plasma products during the period between 1980 and 2001. Eleven out of 17 patients had died, of whom six patients had previously recorded treatment with vCJD-implicated batches, including one patient who had received treatment with an implicated batch made from the same plasma pool as batch FHB 4547 (received by the index case). Another patient (not included in this study), who is still alive, has received treatment with two vCJD-implicated batches, one of which contained plasma from the donor of implicated batches FHC 4237 and FHB 4547. None of the patients in this study showed any evidence of a neurological disease consistent with vCJD. Immunohistochemistry and PET blot analysis for the abnormal form of the prion protein was consistently negative in all central and peripheral tissues examined. A single specimen from the spleen of one of these patients did, however, give a strong positive result on repeated testing for PrP^{res} by Western blot analysis. The positive result had all of the expected characteristics of a true positive result in terms of the electrophoretic mobility, abundance and glycoform ratio of vCJD PrP^{res}, and more specifically that of vCJD lymphoreticular tissue [6,8]; however, exhaustive re-sampling of other regions of the residual spleen tissue failed to identify any similar findings. Immunohistochemistry and PET blotting of the spleen from this case were also negative for abnormal PrP.

We therefore investigated the possibility that the positive results derived from an unexplained misidentification or contamination of samples in the laboratory. Meticulous review of the audit trail for specimen receipt, storage, sampling and analysis of this case found no opportunity for specimen misidentification, substitution or cross-contamination. Additionally, the glycoform ratio in the positive spleen sample clearly rules out sample contamination with vCJD brain, as both the abundance and glycoform ratio are consistent with those expected from a vCJD lymphoreticular tissue [6,8]. We therefore conclude that the spleen of this case had a highly discrete positive region with readily detectable levels of PrP^{res}, having a glycoform pattern typical of vCJD. In a previous report, we described the detection of PrP^{res} in the spleen of another asymptomatic UK patient (who did not have haemophilia), who 5 years prior to death had received a transfusion of packed red cells from a donor who subsequently died from vCJD [8]. In this case, the levels of PrP^{res} in the spleen were highly variable, with only one of the eight regions tested giving a similar result to the index case described above. Another five regions sampled gave a weak PrP^{res} signal, while the remaining two regions sampled were negative. The earlier patient was also a heterozygote (methionine/valine) at codon 129 in the *PRNP* gene. However, immunohistochemistry in that case showed positive staining for abnormal prion protein in occasional follicles in the spleen, unlike the current case.

These observations together suggest that the distribution of PrP^{res} in the spleen of asymptomatic patients is highly variable and that multiple samples need to be analysed to ensure (as in our current case) that false negative results are avoided. Immunoblotting for PrP^{res} is more sensitive than immunohistochemistry and PET blot analysis, so it is not surprising that the immunohistochemical and PET blot findings in the current case were negative, although it should be noted that the amount of fixed tissue available for immunohistochemistry and PET blot analysis was smaller in quantity than the frozen spleen tissue for biochemical analysis. It is also conceivable that the distribution of PrP^{res} in the spleen may be influenced by the *PRNP* codon 129 polymorphism, as the distribution of PrP^{res} in lymphoid tissue of scrapie-affected sheep is variable between different *PRNP* genotypes [23].

The detection of PrP^{res} in the spleen of this patient with haemophilia, who had no evidence of any neurological disease (including vCJD) in life, requires careful interpretation. There are four known possible routes of exposure to vCJD infection that may have resulted in this finding, namely via the food chain, transfusion with donor red cells, surgical and invasive endoscopic procedures, and finally via treatment with UK-produced FVIII, including two vCJD-implicated batches.

Dietary acquisition of vCJD infection is considered unlikely in this individual, who was aged 73 when he died, based on the observed incidence of vCJD in this age group. None of the 14 blood donors to this patient has developed vCJD, but there remains the

possibility that this group of donors could include asymptomatic carriers of vCJD infection. The investigation of patients who have also undergone endoscopy with the endoscope used on this patient has found no clinical cases of vCJD to date.

This patient's only proven link to a vCJD source is the receipt of two separate batches of FVIII, which contained plasma from a donor who subsequently developed vCJD 4.5 years after the first donation. As part of the 2004 notification exercise, the recipients of 98% of these batches have been identified and to date there have been no reports of neurological diseases (including vCJD) in this haemophilia cohort. FVIII made from another vCJD-implicated batch from the same donor was received by one of the other haemophilic patients included as an autopsy case in this study, in whom no evidence of PrP^{res} was identified in the brain, spleen, tonsil or lymph node. The interval between treatment and death in that patient was 3 years shorter than that in the patient with PrP^{res} detected in the spleen in this study. The vCJD donor had also made earlier blood donations; the Transfusion Medicine Epidemiology Review records one surviving recipient of non-leucodepleted red cells who is well [24]. Furthermore, there have been no reports of neurological events in patients with bleeding disorders who have received other batches of clotting factor concentrates linked to this donor.

Estimates of the relative levels of risk to which this individual was exposed, through diet, surgery/endoscopy, blood transfusion and receipt of plasma products, suggest that by far the most likely route through which this individual was infected is through receipt of UK-sourced plasma products [25] (Table 2). It is known that the individual concerned was exposed to some 9000 units of FVIII prepared from plasma pools that included donations from a donor who went on to develop vCJD and was presumed to have been infected at the time of donation. There is no chromatographic step in the production of FVIII 8Y, which may reduce the clearance of any potential prion contamination. However, as the plasma products concerned were produced from very large pools of donors (c. 20 000), and because this individual received many units from batches not known to be implicated (c. 400 000), it is highly likely that this individual was also exposed to infectivity in presently unimplicated batches.

While there is clear evidence of the transmission of vCJD infectivity by non-leucodepleted packed red cell transfusion in humans [7–10], and transmission of scrapie and BSE by whole blood and buffy coat transfusion in sheep [26], uncertainty remains about the risk of transmission of vCJD by UK-sourced plasma. Because of this uncertainty, precautionary public health measures to prevent onward transmission of vCJD were introduced in 2004 for patients with bleeding disorders who had been treated with UK plasma-sourced products between 1980 and 2001. The current situation, with its accompanying uncertainties for the future, causes ongoing concern for these patients and their families.

Conclusion

We believe that the findings in this case indicate vCJD infection in the spleen of this UK haemophilic patient, albeit in a very restricted distribution that may relate to the small dose of infectivity likely to

Table 2. Summary of haemophilia risk calculations assuming a population prevalence of one in 10 000.

Route	Estimated risk	Assumptions
Diet	1 in 10 000*	Background risk
Blood [12,28]	7–14 in 10 000	Assuming transmission probability is between 0.5 and 1
Endoscopy with biopsy [25,29]	1–6 in 10 000	Reduced risk based on a general surgical model (set of 20 instruments) by a factor of 10 (1 small biopsy head)
Implicated plasma products [12,25]	0.2–0.6 ID50s implies risk of 1000–3000 in 10 000	Linear dose response; the possibility of unidentified vCJD-infected donors to the plasma pools is also taken into account c. 400 000 units of factor VIII, to which unidentified vCJD-infected donors may have contributed
Non-implicated plasma products [12,25]	>2 ID50s implies infection very likely	

vCJD, variant Creutzfeldt–Jakob disease.
*The assumed prevalence of vCJD infection in the general UK population is one in 10 000, based on an approximate average value between the results of the study by Hilton *et al.* [14] and the more recent National Tonsil Archive Study [16,17].

have been present in the UK plasma products used in treatment, and perhaps also to the heterozygous *PRNP* codon 129 genotype in this patient. Continuing surveillance for vCJD infection, both symptomatic (passive) and asymptomatic (active), is required to help clarify the degree of overall risk in this group of patients from treatment with UK-sourced plasma products. The findings also have implications for laboratory methodology in the proposed autopsy-based prevalence study of vCJD infection in the UK [27].

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Disclosures

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医薬品
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研究報告の概要

最近、血漿製剤を介したプリオン伝播の可能性に関する論文がいくつか報告されており、感染性プリオンの混入の可能性のある血漿製剤のリスク評価として製造工程の除去効果の評価は重要である。
我々は孔径15nmのウイルス除去膜の評価を行った。
ナノろ過直前のアンチトロンビンサンプルに2つの異なる方法で調製したプリオン物質をスパイクした。動物への感染実験による感染性プリオン除去能は≧4.72及び4.00（2回の独立したスパイク実験）であった。しかしながら、感染性は15nmろ過サンプルの超遠心後の上清および沈殿物の両方に検出され、完全な除去の困難さを示していた。
このデータは、より小さなand/or可溶性の状態（直径15nm未満）で一定量の感染性プリオンタンパク質が存在するとの結論を支持している。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)略
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3)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤の製造工程におけるウイルス除去膜（平均孔径15nm）による感染性プリオンタンパク質の除去能力を評価したところ、感染性プリオンタンパク質は15nmのウイルス除去膜を通過しうることが確認されたことについての報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁（HPA）はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えるものではないと考えるので、特段の措置はとらない。

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Infectious prion protein in the filtrate even after 15nm filtration

Mitchiro Yunoki [a,c,*], Hiroyuki Tanaka [a], Takeru Urayama [a,b], Yuta Kanai [b,d], Aya Nishida [a], Maki Yoshikawa [a], Yuji Ohkubo [a], Yoshiyasu Kawabata [a], Katsuro Hagiwara [b], Kazuyoshi Ikuta [c]

[a] Research & Development Division, Benesis Corporation, Japan
[b] School of Veterinary Medicine, Rakuno Gakuen University, Japan
[c] Department of Virology, Research Institute for Microbial Diseases, Osaka University, Japan
[d] Current address: Thailand-Japan Research Collaboration Centre on Emerging and Re-emerging Infectious (RCC-ERI), Thailand

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ABSTRACT

The evaluation of the removal efficacy during manufacturing is important for the risk assessment of plasma products with respect to possible contamination by infectious prions, as recently reported in several papers on the potential for prion transmission through plasma products. Here, we evaluated a virus removal filter which has 15 nm pores. An antithrombin sample immediately prior to nanofiltration was spiked with prion material prepared in two different ways. The removal (log reduction factor) of prion infectivity using animal bioassays was ≥4.72 and 4.00 in two independent filtrations. However, infectivity was detected in both the pellet and supernatant following ultracentrifugation of the 15 nm filtered samples, indicating difficulty in complete removal. The data supports the conclusion that a certain amount of infectious prion protein is present as a smaller and/or soluble form (less than ~15 nm in diameter).

1. Introduction

Although the risk of transmission of classical or sporadic Creutzfeldt-Jakob disease (sCJD) through blood transfusion is theoretically possible, no verifiable case of transmission has been reported. However, the risk of contracting variant CJD (vCJD) through blood transfusion has been of increasing concern, particularly since the report of a fourth possible transmission case [1,2]. In addition, two investigations of cases involving recipients of plasma products manufactured from pooled source plasma containing a vCJD-infected donor were recently reported. In the first of these reports, abnormal prion protein was detected in a patient without symptoms of vCJD, revealed vCJD abnormal prion protein at post mortem in the patient (a haemophiliac) who had been treated with a Factor VIII product derived from a source material containing plasma that included a donor who developed vCJD after the donation. The UK Health Protection Agency retained their position of 'at risk' for UK derived plasma products [3]. The FDA considers 'the estimated risk' is highly uncertain but is most likely to be extremely small in the case of US-licensed plasma products [4]. A follow-up review of the case reported that the patient was more likely to have been infected by potential subclinical vCJD donors present in normal donor plasma, than by smaller quantities of plasma derived from the donor who had developed vCJD [5,6]. In another report, vCJD abnormal prion protein was not found in a post mortem examination of a patient with common variable immunodeficiency (CVID) who had been treated with an intravenous immunoglobulin (IVIG) product derived from a source material containing plasma from a donor who later developed vCJD post mortem without symptoms of vCJD [7].

Experimental studies in animal models have demonstrated the transmission of bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD), scrapie, CJD and vCJD through transfusion [2]. Furthermore, infectivity was detected in plasma derived from vCJD-infected mice [8]. To reduce the risk of transmission through biologics derived from raw materials potentially contaminated with infectious prion protein, such as plasma, safety measures against pathogen contamination should be employed. Such measures include decreasing the potential prion load, evaluating the risk of the product and employing prion removal step(s) in the manufacturing process wherever possible [9–11].

Nano-filtration has been reported as a very effective tool for the removal of prions [12–14]. These reports suggested that the biological properties of infectious prions in the spiking material could affect

* Corresponding author. Osaka Research Laboratory, Research & Development Division, Benesis Corporation, 3-16-89, Kashima, Yodogawa-ku, Osaka 532-8505, Japan. Tel.: +81 6 6300 2458; fax: +81 6 6300 2404.
E-mail address: YunokiMitchiro@m.kmt-pharma.co.jp (M. Yunoki).

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evaluations of clearance. Foster [15] also reviewed the significance of the method of preparing the spiking material for clearance studies but further research is required due to a lack of consensus. Although infectious activity peaks markedly at 17–27 nm [16], our recent study reveals that even a 15 nm filter could not remove all infectious prion [14]. Our objective in this study is to clarify the infectivity of prion protein that penetrated the 15 nm filter.

2. Materials and methods

2.1. Quantitative removal capacity of 15 nm filter

To evaluate the quantitative removal capacity of a 15 ± 2 nm virus removal filter virus removal filter (Pnanova 15 N, 0.001 m^2, (P-15 N), Asahi Kasei Medical Co., Ltd, Tokyo, Japan) we used a sample of the antithrombin preparation (Neuart®, Benesis Corp., Osaka, Japan) taken immediately before the P-15 N step. Briefly, the microsomal fraction as a spiking material was prepared as follows. Brain homogenates from hamster adopted scrapie 263 K strain infected hamsters in PBS (10% w/v) were centrifuged at low speed (1,000 g for 20 min, at 4 °C) and the supernatant was treated with the 0.1% detergent lysolecithin (37 °C for 30 min). Then the homogenate was centrifuged at high speed (9,100 g for 10 min at 4 °C) and the supernatant was extensively sonicated on full power (20 kHz, 550 W, Misonix XL2020, Qsonica LLC., USA) for 5 min with 2 min intervals every 1 min sonication (5 ml/tube without cymbal rod). The homogenate obtained was sequentially filtered using 0.45, 0.22 and 0.1 μm filters was used as a spiking material. The starting material was spiked (1:50 v/v) and then filtered using P-15 N. The samples, before and after the filtration of two independent runs were titrated to determine the reduction of the PrP^{res} by Western blotting (WB2 method in reference 14). An animal bioassay (BA) was also performed to determine the reduction in infectivity. For the BA, four to five-week old specific pathogen free and viral antibody-free male Syrian hamsters were inoculated i.c. with 0.05 ml/animal of the ten-fold serially diluted sample. Six animals were used for each diluted sample. The animals were monitored for general health and clinical signs, and euthanized once advanced clinical signs were evident or at the end of the assay period (383 days). A histopathological analysis was performed on all brains from animals sacrificed in the study and log reduction factors were calculated following titre determinations by the method of Kärber. This investigational TSE clearance study was performed in accordance with GLP and guidances at BioReliance, Glasgow UK and Rockville US facilities [10,17,18].

2.2. Property of P-15 N-filtered samples

To determine the characteristics of prion infectivity in the filtrate, an analysis of filtrates from additional spiked runs was performed by ultracentrifugation and qualitative (200 days) infectivity assay. Microsomal fraction as spiking material was prepared as described in 2.1 (without detergent treatment) following ultracentrifugation to purify the microsomal fraction. The microsomal fraction was then extensively sonicated at 20 kHz, 200 W (Bioruptor UCD-200 T, Cosmobio Co., Ltd., Japan), 10 min with 1 min intervals every 1 min sonication (2 ml/tube with cymbal rod) and subsequently filtered using 0.22 μm filters. This filtrate was used to spike samples. The spiked (1:20 v/v) antithrombin samples were passed through a 15 nm filter. The resultant log reduction factor by Western blotting was ≥2.8 and infectivity was detected in the filtered sample [14]. The filtered sample was ultracentrifuged at 15 0000 g for 60 min at 4 °C and the pellet was resuspended with PBS. The resuspended pellet and supernatant were inoculated i.c. to three female-specific pathogen-free Syrian Hamsters with 0.02 ml/animal of these undiluted samples. As a control, a non-ultracentrifuged filtrate sample was also inoculated. The animals were euthanized once advanced clinical signs were evident or at the end of the assay period (200 days). A histopathological analysis of the brain from all sacrificed animals was also performed described as previous study [14].

3. Results

3.1. Capacity of the 15 nm filter to remove prion

The capacity to remove prions from the antithrombin preparations during Planova 15 N filtration using either extensively sonicated lysolecithin treated prions or extensively sonicated microsomal fractions are summarized in Table 1. The log reduction factors (LRFs) using the lysolecithin spike in the animal experiments were ≥4.72 and 4.00, respectively for the duplicate runs. These results revealed that the Planova 15 N filtration is "effective but not complete" for the removal of infectious prion contamination. One of the experiments showed that a small amount of infectious prion was still detectable in the filtrate. These results demonstrate that even 15 nm filtration may not be able to completely remove infectious prion (Table 1).

3.2. Qualitative removal capacity of 15 nm filter and subsequent analysis of the filtered sample

To clarify the properties of the infectious prion, the pellet and supernatant derived from the 15 nm filtrate (using a sonicated microsomal spike material) after ultracentrifugation were investigated. PrP^{res} was not detected by Western blot assay either in the filtrate, or in the supernatant and pellet by ultracentrifugation of the filtrate. In contrast, infectivity was detected in all samples by animal bioassay, a more sensitive assay method (Table 1). This result showed that a certain amount of infectious prion was able to penetrate the 15 nm virus removal filter and was not pelleted by ultracentrifugation. Of note, one of two animals which were inoculated with the supernatant showed slightly faster disease progression than other animals after the appearance of clinical signs in the study. However, histopathological observations did not show any clear differences between the supernatant and pellet fractions after ultracentrifugation.

4. Discussion

Clarification as to the real form of infectious prion protein in infectious human and animal plasma is very important in order to

Table 1
Scrapie PrP^{Res} and infectivity in samples generated with 15 nm filtration and subsequent ultracentrifugation

Spiking material	Quantitative		Qualitative	
	WB	BA	WB	BA
	lysolecithin treated and Extensively sonicated		Extensively sonicated	
Before filtration	6.1 / 6.1	7.97 / 8.30	3.6	+ve[c]
After filtration	<2.6 / <2.6	<3.25 / 4.30	<0.8	+ve[d]
Log reduction	≥3.5 / ≥3.5	≥4.72 / 4.00	≥2.8	NA
Pellet[a]	NA	NA	<1.0	+ve[e]
Supernatant[b]	NA	NA	<1.0	+ve[f]

+ve, scrapie positive. NA, not applicable.
[a] :Pellet fraction of ultracentrifuged filtrate.
[b] Supernatant fraction of ultracentrifuged filtrate.
[c] Clinical sign was observed from 90 ~ 118 days post infection.
[d] Clinical sign was observed from 111 ~ 175 days post infection.
[e] Clinical sign was observed from 111 ~ 113 days post infection.
[f] Clinical sign was observed from 125 ~ 175 days post infection.

evaluate the risks of prion contamination in plasma products and biopharmaceutical medicines. Some results suggesting the form of infectious prion protein in human and animal plasma have been reported. A genetically-modified animal plasma containing GPI-anchor less prion protein had some infectivity [19,20]. On the other hand, a high titer of prion remained in the supernatant of an ultracentrifuged microsomal fraction derived from scrapie-infected brain, although PrP^{res} was not detected by Western blot assay [21]. Although these results were obtained under experimental conditions, it suggests that the infectious prion protein may exist in animal plasma as a soluble or soluble-like form. Ultracentrifugation has been commonly used for the concentration of the prion protein. The ultracentrifugation and subsequent preparation of the spiking material should be done carefully in order to ensure that such preparations do not exclude such soluble-like prion protein. To avoid over estimating removal, pelleting of the spike by ultracentrifugation should not be used. However, preparation methods or employing treatment which generate small size of infectious prion such as sonication and/or detergent treatment following the ultracentrifugation (as performed in this study) should be used. Many studies to evaluate prion removal during manufacturing have been performed, however studies of the appropriateness of the spiking materials derived from prion-infected brain are limited. We reported that extensively-sonication and/or treatment with a detergent such as sarkosyl and lysolecithin were useful for the preparation of spiking material for analyzing particle size [14]. Hence, preparation methods without pelleting the prion by ultracentrifugation or with the treatment which generates soluble-like prion in the supernatant following ultracentrifugation will lead to more acceptable results for the evaluation of TSE removal, especially when an animal study is included.

In this study, we evaluated the prion removal performance of nano-filtration on a lab scale using a 15 nm Planova filter and a sample of antithrombin which was spiked with infectious prion protein. Two types of spiking material were used. Both spiking materials used in this study seemed to contain soluble-like infectious prion protein because of the preparation methods employed sonication treatment which seems to generate the soluble-like form infectious prion. Hence, the results of the filtrate sample and LRF in the studies can be considered realistic for evaluation of the filtering process with respect to prion removal.

Residual infectivity was detected in the filtered process sample of antithrombin preparations which was spiked with extensively sonicated or detergent/sonication-treated spiking material. Furthermore, the filtered sample was ultracentrifuged and subsequently the infectivity was detected in pellet and supernatant fractions after ultracentrifugation. These results showed that 15 nm filtration which is the filter of smallest pore size for virus removal removes infectious prion protein effectively but not completely under the filtration condition of antithrombin preparation. Other prion removal options such as other filter devices, column chromatography and fractionations during processing steps have also been reported [13]. One should choose a suitable spiking material for a process evaluation study, before starting the study. The combination of several different process steps for prion removal is likely to improve the removal of all forms of potential prion contamination and thus safeguard against contamination.

The results of this study also revealed that some infectious prion protein was less than 15 nm in diameter, apparently as a low molecular weight and/or soluble form. Unfortunately, the properties or presence of such a soluble-like infectious prion protein in blood have not been clarified. The properties of this form could be very important to evaluate the risk of prion contamination in biological products. Hence, further investigations are required, especially of the properties of soluble-like prion protein in blood and plasma.

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医薬品 研究報告 調査報告書

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研究報告の概要

○コドン129ヘテロ接合性の変異型CJD患者
30歳男性が、13ヶ月前から人格変化、進行性不穏、知能低下を呈し、2008年6月に入院した。患者は重度下肢痛および記憶低下を訴えた。2ヵ月後、幻視を発現し、腹部に腫瘍があるという妄想を持った。その後3ヶ月間に症状は悪化し、2008年10月の精神状態検査のスコアは26/30であった。追跡眼球運動は衝動性であり、口とがらし反射があった。腕に軽度運動失調があり、下肢には腱反射亢進と左足底伸展反応を伴う重度失調があった。歩行に2本の杖を必要とした。既往歴には、頸部リンパ節除去および扁桃摘出術(15年前)があったが、輸血歴やヒト組織の移植歴はなかった。EEGは徐波活性を示した。CSFのタンパク、ブドウ糖、血球数は正常であったが、14-3-3タンパク質が陽性であった。脳MRI所見は、視床枕徴候と一致した。評価したすべての神経放射線医が視床枕徴候を陽性と見なしたわけではないが、定量評価で尾状核と比べ視床枕核の高い対称性信号が示された。遺伝性、代謝性、自己免疫性疾患(腫瘍誘発性疾患を含む)の広範なスクリーニング検査結果は陰性であった。PRNP解析は、既知疾患に関連する突然変異を示さなかった。コドン129はヘテロ接合性だった。特徴的臨床症状、疾患の進行、他の診断の除外、ならびにMRI所見に基づき、変異型クロイツフェルトヤコブ病(vCJD)の臨床診断が下された。患者の年齢が若く、臨床症状、MRI所見、ならびにEEGでpseudoperiodic complexesが見られないことを複合的に考慮し、孤発性CJDの可能性は低いと判断した。患者の保護者はそれ以上の検査は望まなかった。患者の容態は悪化し、2009年1月に死亡した。剖検は実施されなかった。

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使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

プリオンタンパク遺伝子コドン129はヘテロ接合性で、臨床症状、疾患の進行、他の診断の除外、MRI所見から変異型クロイツフェルトヤコブ病と診断された患者の症例報告である。
プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、アルブミンを介して伝播するという証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考える。

今後の対応

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人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を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

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MedDRA/J Ver.12.0J

JRC2010T-003

Case Report

Variant CJD in an individual heterozygous for *PRNP* codon 129

Diego Kaski, Simon Mead, Harpreet Hyare, Sarah Cooper, Ravi Jampana, James Overell, Richard Knight, John Collinge, Peter Rudge

Lancet 2009; 374: 2128

MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK (D Kaski MRCP, S Mead PhD, H Hyare FRCR, Prof J Collinge FRS, P Rudge FRCP); Institute of Neurological Sciences, Glasgow University, Glasgow, UK (S Cooper MRCP, R Jampana FRCR, J Overell FRCP); and National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK (Prof R Knight FRCP)

Correspondence to: Prof John Collinge, MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK
j.collinge@prion.ucl.ac.uk

A 30-year-old man was admitted to hospital in June, 2008, with a 13-month history of personality change, progressive unsteadiness, and intellectual decline. He complained of severe leg pain and poor memory. 2 months later he developed visual hallucinations and falsely believed he had an abdominal tumour. Symptoms worsened over the next 3 months. In October, 2008, his score on the mini mental state examination was 26/30. Pursuit eye movements were saccadic. He had a pout reflex. There was mild ataxia in the arms. His legs were severely ataxic with brisk tendon reflexes and a left extensor plantar response. He needed two crutches to walk. Medical history included tonsillectomy and removal of a cervical lymph node 15 years previously but he had never had a blood transfusion or received implantation of other human tissues.

EEG showed slow wave activity. CSF protein, glucose, and cell count were normal but the 14-3-3 protein was positive. MRI of the brain was consistent with the pulvinar sign (figure A). Although not all neuroradiologists consulted considered the pulvinar sign positive, quantitative assessment showed symmetrical higher signal in the pulvinar nuclei than the caudate nuclei (figure B). Extensive screens for genetic, metabolic, and autoimmune diseases, including those induced by neoplasia, were negative. *PRNP* analysis did not show any known disease-associated mutations; codon 129 was heterozygous. A clinical diagnosis of variant Creutzfeldt-Jakob disease (vCJD) was made on the basis of a characteristic clinical onset and progression, exclusion of other diagnoses, and MRI findings. Sporadic CJD was judged unlikely given the combination of young age, clinical features, MRI findings, and absence of pseudoperiodic complexes on EEG. His carers did not want further investigation. His condition deteriorated and he died in January, 2009. Autopsy was not done.

Human prion diseases have acquired, sporadic, and inherited aetiologies, show wide phenotypic heterogeneity, and are associated with propagation of infectious prions of many distinct strain types.[1] Since 1994, about 200 cases of vCJD, causally related to bovine spongiform encephalopathy (BSE) prions, have been identified worldwide. vCJD is generally seen in young adults, has characteristic neuropathological features and tissue distribution of infectivity, and a distinctive type 4 (London classification) molecular strain type.[2] A polymorphism at codon 129 (encoding methionine or valine) of the human prion protein gene (*PRNP*), constitutes a powerful susceptibility factor in all types of prion disease. In vCJD, every case genotyped to date has been methionine homozygous. In the other acquired prion diseases, cases have occurred in all genotypes but with different mean incubation periods,[3] which can span decades;[4] *PRNP* codon 129 heterozygotes generally have the longest incubation periods. There is a report of a recipient of a blood transfusion from a donor incubating vCJD who died of unrelated causes but showed signs of prion infection at autopsy and was *PRNP* codon 129 heterozygous.[5] Animal studies have suggested that different clinicopathological phenotypes could occur in people with various *PRNP* codon 129 genotypes.[4,5] The majority of the UK population have potentially been exposed to BSE prions but the extent of clinically silent infection remains unclear. About a third of the UK population are *PRNP* codon 129 methionine homozygous. If individuals with other genotypes are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases, which may or may not meet diagnostic criteria for vCJD, would be expected in these *PRNP* codon 129 genotypes. However, prion disease susceptibility and incubation periods are also affected by other genetic loci, and the possibility remains that cases of vCJD to date may have unusual combinations of genotypes at these loci, yet to be fully characterised.

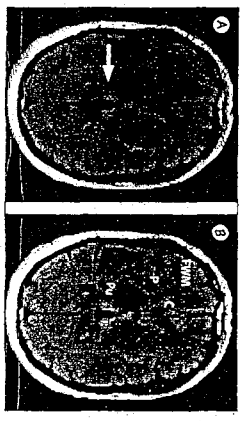

Figure: MRI
(A) Increased signal intensity in the pulvinar nucleus bilaterally (arrow). (B) ADR signal intensity in the pulvinar (Pu) is higher than in the head of the caudate nuclei (C), putamen (Pt), and right frontal white matter (FWM).

Contributors
All authors were involved in discussion about diagnosis, care of the patient, and preparation of the report. Written consent to publish was obtained.

Conflicts of interest
JC is a director and shareholder of D-Gen Ltd, an academic spin-out company in the field of prion disease diagnosis, decontamination, and therapy. The other authors declare that they have no conflicts of interest.

References

1 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; **24**: 519–50.
2 Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. *Lancet* 2006; **367**: 2068–74.
3 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004; **364**: 527–29.
4 Asante E, Linehan J, Gowland I, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc Natl Acad Sci USA* 2006; **103**: 10759–64.
5 Wadsworth JD, Asante E, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004; **306**: 1793–96.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
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販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要

○プリオン病最新情報 英国:国立CJDサーベイランスユニット、月次vCJD・CJD統計、2010年1月5日時点
英国のCJDサーベイランスユニットから公表されたvCJDを始めとするプリオン病の患者数に関する最新情報である。
vCJD確定例または可能性例総数は前月から変化なく166名のままである。生存患者は4名であるため、2009年までのvCJD症例数は合計170例である。
2009年中に新たに2症例が記録されたが、全体としては英国におけるvCJD流行は減少しつつあるとする見解に一致している。
vCJDによる死亡患者は1995年に初めて確認され、死亡患者数のピークは2000年の28名であった。その後2001年に20名、2002年に17名、2003年に18名、2004年に9名、2005年に5名、2006年に5名、2007年に5名、2008年に1名、2009年に2名となっている。
プリオン病患者全体としては、2009年の12ヶ月間に143名の照会があった。このうち、孤発性CJD:59名、家族性CJD:1名、医原性CJD:1名、GSS:3名、vCJD:2名だった。

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使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
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血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
英国CJDサーベイランスユニットの統計によると、2010年1月5日の時点でvCJD死亡患者総数は170名であり、英国におけるvCJD流行は収まりつつあるとする見解に一致するとの報告である。 プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、アルブミンを介して伝播するという証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考える。	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人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を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

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Back

Archive Number 20100107.0076
Published Date 07-JAN-2010
Subject PRO/AH/EDR> Prion disease update 2010

PRION DISEASE UPDATE 2010
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[With the continuing decline in the number of cases in the human population of variant Creutzfeldt-Jakob disease -- abbreviated previously as vCJD or CJD (new var.) in ProMED-mail -- it has been decided to broaden the scope of the occasional ProMED-mail updates to include some other prion-related diseases. In addition to vCJD, data on other forms of CJD: sporadic, iatrogenic, familial, and GSS (Gerstmann-Straussler-Scheinker disease), are included also since they may have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:
[1] UK: National CJD Surveillance Unit - monthly statistics as of 5 Jan 2010
[2] France: Institut de Veille Sanitaire- monthly statistics as of 4 Jan 2010
[3] US National Prion Disease Center - not updated since 7 Nov 2009
[4] Portuguese vCJD case - pathology
[5] vCJD codon 129 heterozygote
[6] vCJD codon 129 heterozygote - Lancet paper
[7] Prion evolution & a new reagent

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[1] UK: National CJD Surveillance Unit - monthly statistics as of 5 Jan 2010
Date: Tue 5 Jan 2010
Source: UK National CJD Surveillance Unit, monthly statistics [edited]
<http://www.cjd.ed.ac.uk/figures.htm>

The number of deaths due to definite or probable vCJD cases remains 166. A total of 4 definite/probable patients are still alive, so that the total number of definite or probable vCJD cases remains 170 for the year 2009.

Although 2 new cases vCJD were recorded in 2009, the overall picture is still consistent with the view that the vCJD outbreak in the UK is in decline, albeit now with a pronounced tail. The 1st cases were observed in 1995, and the peak number of deaths was 28 in the year 2000, followed by 20 in 2001, 17 in 2002, 18 in 2003, 9 in 2004, 5 in 2005, 5 in 2006, 5 in 2007, one in 2008, and 2 in 2009.

Totals for all types of CJD cases in the UK in the year 2009

During the 12 months of 2009, there have been 143 referrals, 59 cases of sporadic CJD, one case of familial CJD, one case of iatrogenic CJD, 3 cases of GSS, and 2 cases of vCJD.

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Communicated by:
ProMED-mail <promed@promedmail.org>

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[2] France: Institut de Veille Sanitaire - monthly statistics as of 4 Jan 2010
Date: Mon 4 Jan 2010
171
Source: IVS - Maladie de Creutzfeldt-Jakob et maladies apparentees

[in French, trans. & summ. Mod.CP]
<http://www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html>

During the 12 months of 2009, there were 1486 referrals, 85 cases of sporadic CJD, 10 cases of familial CJD, 3 cases of iatrogenic CJD, and 2 confirmed cases of vCJD.

A total of 25 cases of confirmed or probable vCJD has now been recorded in France since 1997. The 25 confirmed cases comprise 13 females and 12 males. All 25 are now deceased. Their median age is 37 (between 19 and 58). Seven were resident in the Ile-de-France and 18 in the provinces. All the identified cases have been Met-Met homozygotes. No risk factor has been identified. One of the 25 had made frequent visits to the United Kingdom.

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Communicated by:
ProMED-mail <promed@promedmail.org>

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[3] US National Prion Disease Center - not updated since 7 Nov 2009
Date: Sat 7 Nov 2009
Source: US National Prion Disease Pathology Surveillance Center [edited]
<http://www.cjdsurveillance.com/pdf/case-table.pdf>

(Report not updated since 7 Dec 2009): During the period 1 Jan 2009 to 7 Nov 2009, there were 341 referrals, of which 198 were classified as Prion disease, comprising 133 cases of sporadic CJD, 33 of familial CJD, and no cases of iatrogenic CJD or vCJD.

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Communicated by:
ProMED-mail <promed@promedmail.org>

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[4] Portuguese vCJD case - pathology
Date: Fri 1 Jan 2010
Source: J Neurol Neurosurg Psychiatry 2010 Jan;81(1):112-4. [edited]
<http://jnnp.bmj.com/content/81/1/112.abstract>

Title: Variant Creutzfeldt-Jakob disease: the first confirmed case from Portugal shows early onset, long duration and unusual pathology.

Authors: Barbot C, Castro L, Oliveira C, Carpenter S.
At: Department of Neuropaediatrics, Hospital Maria Pia, Porto, Portugal.

Summary:
We present clinical and autopsy findings in the 1st case of variant Creutzfeldt-Jakob disease diagnosed and confirmed in Portugal. Onset was at 11 years, the earliest onset reported, and the course (32 months) relatively long. Western blot showed protease resistant prion protein, mainly of type 4 (2B) isoform. The cerebral cortex revealed severe spongiform change with numerous amyloid plaques, which did not fit the definition of florid plaques. In the striatum, spongiform change was limited, but the extracellular space was dilated. Other reports have found marked spongiform change in the striatum and little in the cortex. Massive neuronal loss, in excess of what has been described, was found in the thalamus and pontine grey. The cerebellum showed, as expected, severe loss of granule cells, moderate loss of Purkinje cells and marked immunopositivity for the prion protein. Differences between our findings and previous ones probably result from the patient's long survival.

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Communicated by:
Terry S. Singeltary Sr. <flounder9@verizon.net>

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[5] vCJD codon 129 heterozygote
Date: Fri 19 Dec 2009
Source: BBC News, Health [edited]
<http://news.bbc.co.uk/1/hi/health/8419459.stm>

A 30-year-old man thought to have died in January [2009] from vCJD belonged to a genetic group that had not shown any signs of the disease, scientists say. In the UK, 166 people have died of vCJD, linked to eating BSE [bovine spongiform encephalopathy] infected beef, and all were thought to have shared a certain gene.

Writing in the Lancet, scientists say that the victim, a resident of, Lanarkshire [Scotland], had a different version of the gene. They estimate that up to 350 people in this group could get vCJD. Scientists have always thought that a 2nd wave of vCJD cases would emerge some time after the 1st. This is the 1st indication that this theory is being born out, with the identification of the 1st probable vCJD patient outside of the initial genetic group, BBC science correspondent Pallab Ghosh reports.

The father believes his son was incubating the disease for much of his life. It is probable because the diagnosis is based on observations of the progression of the disease rather than post-mortem tests which would have provided absolute confirmation of the disease, he adds.

The case report written by Professor John Collinge of the National Prion Clinic and colleagues is a reminder that the disease has not gone away. Many thousands of people may be carrying the infection, and although they will never show any symptoms, they have the potential to infect others.

vCJD is caused by infectious agents called prions. Prion diseases affect the structure of the brain or other neural tissue and are currently untreatable. Disease-causing prions are thought to consist of abnormally folded proteins, which spread by encouraging the normal healthy prion protein found on the surface of most cells in the body to change shape. Tests showed that the patient had a heterozygous version of the gene which codes for the human prion amino acids valine (V) or methionine (M). People can be V V (homozygous), M M (homozygous) or M V (heterozygous). Since 1994, around 200 cases of vCJD have been identified worldwide, and all those tested have been M M homozygous. [However, genetic analysis of 2 out of 3 prion-positive appendix samples in the tissue-based prevalence study in 2001-2004 showed that both were valine homozygous (VV) at codon 129 in the prion protein gene (Ironside et al, Brit Med J 2006). - Mod.CP]. However, this most recent victim was M/V heterozygous. It is thought that 47 percent of the population have this version of the gene. Professor Collinge said: "The majority of the UK population have potentially been exposed to BSE prions, but the extent of clinically silent infection remains unclear. About 1/3rd of the UK population are M/M homozygous. If individuals with other genotypes [M/V and V/V] are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases would be expected."

The scientists have previously looked at another prion disease in New Guinea called "kuru" [which was induced by eating infected human brain tissue. - Mod.CP]. The original cases were all M/M, but more recently, M/V cases have appeared. They say this indicates that M/V people can get prion diseases like kuru but have a much longer incubation period.

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Communicated by:
ProMED-mail <promed@promedmail.org>

[The abstract of the Lancet paper upon which the above report is based is reproduced below. - Mod.CP]

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[6] vCJD codon 129 heterozygote - Lancet paper
Date: Thu 18 Dec 2009
Source: Lancet 2009; 374: 2128 [edited]
<http://press.thelancet.com/vcjd.pdf>

[A Case Report published in the 18 Dec 2009 issue of the Lancet by Professor John Collinge, MRC Prion Unit and National Prion Clinic,

UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London]

A 30-year-old man was admitted to hospital in June 2008 with a 13-month history of personality change, progressive unsteadiness, and intellectual decline. He complained of severe leg pain and poor memory. Two months later, he developed visual hallucinations and falsely believed he had an abdominal tumour. Symptoms worsened over the next 3 months. In October 2008, his score on the mini mental state examination was 26/30. Pursuit eye movements were saccadic [a rapid movement of the eye between fixation points]. He had a pout reflex. There was mild ataxia in the arms. His legs were severely ataxic with brisk tendon reflexes and a left extensor plantar response. He needed 2 crutches to walk. Medical history included tonsillectomy and removal of a cervical lymph node 15 years previously, but he had never had a blood transfusion or received implantation of other human tissues.

EEG showed slow wave activity. CSF protein, glucose, and cell count were normal, but the 14-3-3 protein was positive. MRI [magnetic resonance imaging] of the brain was consistent with the pulvinar sign (illustrated in the original text). Although not all neuroradiologists consulted considered the pulvinar sign positive, quantitative assessment showed symmetrical higher signal in the pulvinar nuclei than the caudate nuclei (illustrated in the original text). Extensive screens for genetic, metabolic, and autoimmune diseases, including those induced by neoplasia, were negative. PRNP analysis did not show any known disease-associated mutations; codon 129 was heterozygous. A clinical diagnosis of variant Creutzfeldt-Jakob disease (vCJD) was made on the basis of a characteristic clinical onset and progression, exclusion of other diagnoses, and MRI findings. Sporadic CJD was judged unlikely given the combination of young age, clinical features, MRI findings, and absence of pseudoperiodic complexes on EEG. His care givers did not want further investigation. His condition deteriorated, and he died in January 2009. Autopsy was not done.

Human prion diseases have acquired, sporadic, and inherited aetiologies, show wide phenotypic heterogeneity, and are associated with propagation of infectious prions of many distinct strain types (1). Since 1994, about 200 cases of vCJD, causally related to exposure to bovine spongiform encephalopathy (BSE) prions, have been identified world-wide. vCJD is generally seen in young adults, has characteristic neuropathological features and tissue distribution of infectivity, and a distinctive type 4 (London classification) molecular strain type (1). A polymorphism at codon 129 (encoding methionine or valine) of the human prion protein gene (PRNP) constitutes a powerful susceptibility factor in all types of prion disease. In vCJD, every case genotyped to date has been methionine homozygous. In the other acquired prion diseases, cases have occurred in all genotypes but with different mean incubation periods (1), which can span decades (2); PRNP codon 129 heterozygotes generally have the longest incubation periods. There is a report of a recipient of a blood transfusion from a donor incubating vCJD who died of unrelated causes but showed signs of prion infection at autopsy and was PRNP codon 129 heterozygous (3). Animal studies have suggested that different clinicopathological phenotypes could occur in people with various PRNP codon 129 genotypes (4,5). The majority of the UK population have potentially been exposed to BSE prions but the extent of clinically silent infection remains unclear. About 1/3rd of the UK population are PRNP codon 129 methionine homozygous. If individuals with other genotypes [V/V or V/M] are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases, which may or may not meet diagnostic criteria for vCJD, would be expected in these PRNP codon 129 genotypes. However, prion disease susceptibility and incubation periods are also affected by other genetic loci, and the possibility remains that cases of vCJD to date may have unusual combinations of genotypes at these loci, yet to be fully characterised.

References:

(1) Collinge J. Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 2001; 24: 519-50.

(2) Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century - an acquired human prion disease with very long incubation periods. Lancet 2006; 367: 2068-74.

(3) Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004; 364: 527-29.

(4) Asante E, Linehan J, Gowland I, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. Proc Natl Acad Sci USA 2006; 103: 10759-64.

(5) Wadsworth JD, Asante E, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 2004; 306: 1793-96.

[Acknowledgment: MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK (D Kaski MRCP, S Mead PhD, H Hyare FRCR, Prof J Collinge FRS, P Rudge FRCP); Institute of Neurological Sciences, Glasgow University, Glasgow, UK (S Cooper MRCP, R Jampana FRCR, J Overell FRCP); and National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK (Prof R Knight FRCP)]

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[To put this work in perspective, parts of a British Medical Journal editorial by Maurizio Pocchiari are reproduced below. - Mod.CP.

Date: 21 May 2009
Source: BMJ 2009;338:b435 [edited]
<http://www.bmj.com/cgi/content/full/338/may21_2/b435>

"Prevalence of variant CJD in the UK

The number of cases of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom has decreased since 2000, but controversy remains about how many people carry the infectious agent and will eventually develop disease. Clewley and colleagues in a limited study add to the debate by assessing 63 007 pairs of tonsils for the only available marker of prion disease, the pathological, partially protease resistant, prion protein. Although more than half of the samples came from people born between 1961 and 1995, when the risk of exposure to bovine spongiform encephalopathy (BSE) infection was high, no convincingly positive tonsil specimens were detected. This study estimated that the prevalence of vCJD in the British population is zero, but with a large confidence interval of 0 to 113 per million.

This result agrees with one UK survey of 2000 tonsil specimens, but it differs from another survey of 1427 tonsils and 11 247 appendices, which found that more than 10 000 people might be incubating the disease. However, despite the discrepancy, the 95 percent confidence intervals of the 2 studies overlap, indicating that the results do not differ significantly and that many people in the UK may be carriers.

The chance that no one in the UK is incubating the disease, as suggested by the lower confidence limit of Clewley and colleagues' study, is unlikely because backup calculations predict up to 100 new cases of vCJD in the next 50 years. This prediction seems reasonable unless most cases of vCJD were missed by surveillance in the past years.

Until December 2008, all 210 people reported to have vCJD (164 in the UK, 46 in other countries) were homozygous for methionine at the polymorphic codon 129 of the prion protein gene (PRNP), suggesting that genetic factors strongly influence the development of disease. Whether people who are heterozygous for methionine and valine or homozygous for valine at this codon (about 60 percent of the population) will develop vCJD in the future is still unknown. However, data from gene targeted transgenic mice indicate that these people are also susceptible to BSE and vCJD, although incubation periods are longer than in those who are homozygous for methionine."

Interested readers should consult the original article for further information and references. - Mod.CP]

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[7] Prion evolution & a new reagent
Date: 1 Jan 2010
Source: BBC Health News [edited]
<http://news.bbc.co.uk/1/hi/health/8425320.stm>

Abnormal prion proteins cause at least 20 fatal diseases. Scientists have shown for the 1st time that "lifeless" prion proteins, devoid of all genetic material, can evolve just like higher forms of life. The Scripps Research Institute in the US says the prions can change to suit their environment and go on to develop drug resistance.

Prions are associated with 20 different brain diseases in humans and animals. The scientists say their work suggests new approaches might be necessary to develop therapies for these diseases. In the study, published in the journal Science [see below], the scientists transferred prion populations from brain cells to other cells in culture and observed the prions that adapted to the new cellular environment out-competed their brain-adapted counterparts. When returned to the brain cells, the brain-adapted prions again took over the population.

Charles Weissmann, head of Scripps Florida's department of infectology who led the study, said: "On the face of it, you have exactly the same process of mutation and adaptive change in prions as you see in viruses. This is a timely reminder that prion concerns are not going away and that controls to stop abnormal prions being transmitted to humans through the food system or through blood transfusions must be vigorously maintained."

Professor John Collinge, Medical Research Council Prion Unit stated that: "This means that this pattern of Darwinian evolution appears to be universally active. In viruses, mutation is linked to changes in nucleic acid sequence that leads to resistance. Now, this adaptability has moved one level down -- to prions and protein folding -- and it's clear that you do not need nucleic acid (DNA or RNA) for the process of evolution."

Mammalian cells normally produce cellular prion protein or PrPC. During infections, such as the human form of mad cow disease, known as vCJD, abnormal or mis-folded proteins convert the normal host prion protein into its toxic form by changing its conformation or shape. "It was generally thought that once cellular prion protein was converted into the abnormal form, there was no further change," Prof. Weissmann said. "But there have been hints that something was happening. When you transmit prions from sheep to mice, they become more virulent over time. Now we know that the abnormal prions replicate and create variants, perhaps at a low level initially. But once they are transferred to a new host, natural selection will eventually choose the more virulent and aggressive variants."

Professor John Collinge, of the Medical Research Council's (MRC) Prion Unit, described the research as exciting confirmation of a hypothesis that he had proposed 2 years ago, that there could be a "cloud" or whole array of prion proteins in the body. He called it the cloud hypothesis: "The prion protein is not a clone, it is a quasi-species that can create different protein strains even in the same animal. The abnormal prion proteins multiply by converting normal prion proteins. The implication of Charles Weissmann's work is that it would be better to cut off that supply of normal prion proteins rather than risk the abnormal prion adapting to a drug and evolving into a new more virulent form. You would do this by trying to block the sites on the normal prion protein that the abnormal form locks on to to do its conversion. We know there is an antibody that can do this in mice, and the Medical Research Council's Prion Unit have managed to engineer a human antibody to do this. It is currently undergoing safety tests, and we hope to move to clinical trials by the end of 2011."

Professor Collinge said the MRC was also trying to find more conventional chemical compounds to do this and has been collaborating

with the chemical company GlaxoSmithKline (GSK). He said: "They have given us access to their chemical libraries, which contain millions of compounds, and we have already identified some that may work well. This is a timely reminder that prion concerns are not going away and that controls to stop abnormal prions being transmitted to humans through the food system or through blood transfusions must be vigorously maintained."

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[The abstract and the reference for the Science paper descried above are the following: Science DOI: 10.1126/science.1183218, Published Online 31 Dec 2009.
<http://www.sciencemag.org/cgi/content/abstract/science.1183218>.
Darwinian Evolution of Prions in Cell Culture. By Jiali Li, Shawn Browning, Sukhvir P. Mahal, Anja M. Oelschlegel, Charles Weissmann
At: Department of Infectology, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458, USA.

Abstract: "Prions are infectious proteins consisting mainly of PrPSc, a sheet-rich conformer of the normal host protein PrPC, and occur in different strains. Strain identity is thought to be encoded by PrPSc conformation. We found that biologically cloned prion populations gradually became heterogeneous by accumulating "mutants," and selective pressures resulted in the emergence of different mutants as major constituents of the evolving population. Thus, when transferred from brain to cultured cells, "cell-adapted" prions out competed their "brain-adapted" counterparts, and the opposite occurred when prions were returned from cells to brain. Similarly, the inhibitor swainsonine selected for a resistant substrain, whereas in its absence, the susceptible substrain outgrew its resistant counterpart. Prions, albeit devoid of a nucleic acid genome, are thus subject to mutation and selective amplification."

From a theoretical standpoint, this work has great significance. Nonetheless, the immediate interest of the BBC News report is the information that Professor John Collinge's MRC group has succeeded in engineering a humanised monoclonal antibody that interacts with the sites on the normal prion protein that the abnormal form locks onto to achieve its conversion and that it is hoped eventually to move to clinical trials of this reagent. - Mod.CP]

[see also:
2009

Prion disease update 2009 (10) 20091103.3784
vCJD - Italy: susp. 20091024.3671
Prion disease update 2009 (09) 20091005.3461
Prion disease update 2009 (08) 20090908.3170
Prion disease update 2009 (07) 20090806.2783
Prion disease update 2009 (06) 20090706.2433
Prion disease update 2009 (05) 20090602.2054
Prion disease update 2009 (04) 20090406.1337
vCJD, 5th death - Spain (Cantabria) 20090307.0953
Prion disease update 2009 (03) 20090305.0918
Prion disease update 2009 (02) 20090202.0463
Prion disease update 2009 (01) 20090108.0076
2008

Prion disease update 2008 (14): new vCJD wave imminent? 20081218.3980
Prion disease update 2008 (13) 20081201.3780
Prion disease update 2008 (12) 20081103.345
Prion disease update 2008 (11) 20081006.3159
vCJD, mother & son - Spain: (Leon) 20080926.3051
Prion disease update 2008 (10) 20080902.2742
vCJD - Spain: susp. 20080410.1311
Prion disease update 2008 (05) 20080408.1205
Prion disease update 2008 (01): correction 20080104.0046
Prion disease update 2008 (01) 20080102.0014
2007

Prion disease update 2007 (08) 20071205.3923
Prion disease update 2007 (07) 20071105.3602

Prion disease update 2007 (06) 20071003.3269
Prion disease update 2007 (05) 20070901.2879
Prion disease update 2007 (04) 20070806.2560
Prion disease update 2007 (03) 20070702.2112
Prion disease update 2007 (02) 20070604.1812
Prion disease update 2007 20070514.1542
CJD (new var.) update 2007 (05) 20070403.1130
CJD (new var.) update 2007 (04) 20070305.0780
CJD (new var.) update 2007 (03) 20070205.0455
CJD (new var.) update 2007 (02): South Korea, susp 20070115.0199
2006

CJD (new var.), blood transfusion risk 20061208.3468
CJD, transmission risk - Canada (ON) 20061207.3457
CJD (new var.) update 2006 (12) 20061205.3431
CJD (new var.) update 2006 (11) 20061106.3190
CJD (new var.) update 2006 (10) 20061002.2820
CJD (new var.) - Netherlands: 2nd case 20060623.1741
CJD (new var.) - UK: 3rd transfusion-related case 20060209.0432
CJD (new var.) update 2006 (02) 20060206.0386
CJD (new var.) update 2006 20060111.0101
2005

CJD (new var.) update 2005 (12) 20051209.3547
CJD (new var.) update 2005 (11) 20051108.3270
CJD (new var.) update 2005 (10) 20051006.2916
CJD (new var.) update 2005 (02) 20050211.0467
CJD (new var.) - UK: update 2005 (01) 20050111.0095
2004

CJD, genetic susceptibility 20041112.3064
CJD (new var.) - UK: update 2004 (14) 20041206.3242
CJD (new var.) - UK: update 2004 (10) 20040909.2518
CJD (new var.) - UK: update 2004 (02) 20040202.0400
CJD (new var.) - UK: update 2004 (01) 20040106.0064
CJD (new var.) - France: 8th case 20041022.2864
CJD (new var.) - France: 9th case 20041123.3138
CJD (new var.), blood supply - UK 20040318.0758
CJD (new var.), carrier frequency study - UK 20040521.1365
2003

CJD (new var.) - UK: update 2003 (13) 20031216.3072
CJD (new var.) - UK: update 2003 (01) 20030108.0057
2002

CJD (new var.) - UK: update Dec 2002 20021207.5997
CJD (new var.) - UK: update Jan 2002 20020111.3223
2001

CJD (new var.), incidence & trends - UK (02) 20011124.2875
CJD (new var.), incidence & trends - UK 20011115.2816
CJD (new var.) - UK: reassessment 20011029.2671
CJD (new var.) - UK: update Oct 2001 20011005.2419
CJD (new var.) - UK: regional variation (02) 20010907.2145
CJD (new var.) - UK: update Sep 2001 20010906.2134
CJD (new var.) - UK: update Aug 2001 20010806.1872
CJD (new var.) - UK: 9th Annual Report 20010628.1231
CJD (new var.) - UK: update June 2001 20010622.1198
CJD (new var.) - UK: update 3 Jan 2001 20010104.0025]
.....cp/msp/dk

別紙様式第2-1

No. 23

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 12. 25	該当なし	
一般的名称	新鮮凍結人血漿		研究報告の公表状況	UK Department of Health, Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO). Available from: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@ab/documents/digitalasset/dh_108860.pdf	公表国 英国	
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)					

研究報告の概要 180

○英国血液・組織・臓器の安全性にかかる諮問委員会 2009年10月27日第8回会議議事要旨 プリオンフィルターについて
メンバーはこれまでの会議でプリオンフィルターについて議論を重ね、有効性と安全性双方の分析について最新の情報を入手してきた。この情報は、プリオンフィルター処理赤血球の安全性を分析する臨床試験(the PRISM trial)及び製剤についての個別の有効性分析から得られ、ワーキンググループメンバーに報告された。臨床試験の初期結果は有望だったが、完了までまだ時間がかかることを指摘した。メンバーはメーカーと他の研究から得られた情報に加えて、保健省の有効性評価のデータを評価した。動物を使用した内部の有効性試験からデータが得られるのは2014年になる。
以上の情報と分析から委員会は以下の通り結論する。
・プリオンの感染性を低減させるフィルターに今では十分なエビデンスがあることを確信している。
・1996年1月1日以降に生まれた人には、PRISM臨床試験の完了を条件としてフィルター処理赤血球を使用すること。
委員会は、プリオンフィルター処理が実施された場合、フィルターの普及率や有効性についてさらにデータが得られた場合は見直しを行うとした。
委員会はこれまでに、vCJDリスク対策として、16歳未満の患者とヘモグロビン上昇患者には2倍量赤血球(DDRC)を使用するよう推奨していた。プリオンフィルター処理赤血球を使用する患者については、DDRCの推奨は撤回される。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
英国の血液・組織・臓器の安全性にかかる諮問委員会で、1996年1月1日以降に生まれた人の輸血にプリオンフィルター処理赤血球を使用することが推奨されたとの報告である。	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、1980～96年の英国1ヶ月をはじめ、欧州等38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。今後もCJD等プリオン病に関する新たな知見及び情報を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

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SaBTO

Advisory Committee on the Safety of Blood, Tissues and Organs

Summary of the Eighth Meeting, 27 October 2009

1. Consent for blood transfusion

Members were reminded that questionnaires regarding informed consent for blood transfusion had been finalised by a working group consisting of SaBTO members and other experts. Two questionnaires have been developed which are specifically for either Healthcare Professionals or Patient groups. The working group had agreed the management of the consultation process with the Department of Health. The consultation process will be UK wide. Participants will be given 12 weeks to respond, after which time the consultation will close and the responses will be analysed.

2. MSBTO Guidance update

Members noted the urgent need for this update, which was expected to be forthcoming shortly.

3. Prion Filtration

Members had discussed prion filtration at previous meetings, and had asked to be kept updated on progress of both efficacy and safety assessments. This was provided via a presentation from the vCJD working group, with new data from both the ongoing clinical trial to assess safety of prion filtered red blood cells (the PRISM trial) and independent efficacy assessments of the performance of the same product. Early results from the clinical trial are encouraging, but members noted that the trial is still some way from completion. Members were appraised of data from the Health Protection Agency's independent evaluation of efficacy, in addition to information from the manufacturer and another independent study. The committee noted that independent data from animal based, endogenous studies of efficacy will not be available until 2014.

Having considered the information and analysis provided, the committee:

- is satisfied that there is now sufficient evidence that this particular filter reduces infectivity;
- recommends that filtered red cells be provided to those born since 1 January 1996, subject to satisfactory completion of the PRISM clinical trial.

The committee also noted that, if implemented, the continuing requirement for prion filtration should be reviewed in the event that either further data on prevalence or efficacy of the filters becomes available.

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JRC2009T-066

The committee had previously recommended the introduction of double dose red cells (DDRC) as a vCJD risk-reduction measure for under 16s and patients with haemoglobinopathies. SaBTO recommended that DDRC be rescinded for those groups receiving prion filtered blood.

B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料3において集積報告を行っているため、添付していない）。

感染症定期報告の報告状況(2010/3/1～2010/5/31)

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用調査
100113	2010/3/29	91089	CSLベーリング	フィブリノゲン加第XIII因子	アンチトロンビン	ヒト血液	米国、ドイツ、オーストリア	製造工程	有	有	無
100114	2010/3/29	91090	CSLベーリング	人血清アルブミン 人血液凝固第XIII因子 フィブリノゲン加第XIII因子	人血清アルブミン	ヒト血液	米国、ドイツ、オーストリア	有効成分添加物	有	有	無
100115	2010/3/29	91091	CSLベーリング	フィブリノゲン加第XIII因子	アプロチニン液	ウシ肺	ウルグアイ、ニュージーランド	有効成分	無	有	無
100116	2010/3/29	91092	CSLベーリング	フィブリノゲン加第XIII因子	トロンビン末	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
100117	2010/3/29	91093	CSLベーリング	フィブリノゲン加第XIII因子	フィブリノゲン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
100119	2010/3/30	91096	バクスター	乾燥濃縮人血液凝固第VIII因子	乾燥人血液凝固第VIII因子	人血漿	米国	有効成分	無	有	無
100120	2010/3/30	91097	バクスター	乾燥濃縮人血液凝固第VIII因子	人血清アルブミン	人血漿	米国	添加物	無	有	無
100127	2010/4/15	100065	CSLベーリング	フィブリノゲン加第XIII因子 人血液凝固第XIII因子	人血液凝固第XIII因子	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
100128	2010/4/15	100066	CSLベーリング	抗破傷風人免役グロブリン	破傷風抗毒素	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
100144	2010/4/23	100124	バクスター	人血清アルブミン	人血清アルブミン	人血漿	米国	有効成分	無	有	無

感染症発生症例一覧

	番号	感染症の種類 器官別大分類	感染症の種類 基本語	発生国	性別	年齢	発現時期	転帰	出典	区分	備考
第14回	1	感染症および寄生虫症	A型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年2月8日
第14回	1	感染症および寄生虫症	B型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年2月8日
第14回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年2月8日
第13回	報告なし										
第12回	1	感染症および寄生虫症	HIV感染	ドイツ	男	35	不明	不明	症例報告	外国製品	識別番号3-08000029 報告日:2009年02月17日
第12回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	35	不明	不明	症例報告	外国製品	識別番号3-08000029 報告日:2009年02月17日
第12回	2	感染症および寄生虫症	C型肝炎	ドイツ	女	77	2009/1/5	不明	症例報告	外国製品	識別番号3-08000039 報告日:2009年02月17日
第11回	1	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 報告日:2008年4月30日
第11回	2	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 報告日:2008年5月29日
第10回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 報告日:2008年4月1日
第10回	2	感染症および寄生虫症	B型肝炎	日本	女	33	2007/8/7	回復	症例報告	当該製品	識別番号1-07000093 報告日:2007年10月11日
第9回	報告なし										
第8回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日
第8回	1	臨床検査	C型肝炎抗体陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日
第8回	1	臨床検査	C型肝炎RNA陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日
第7回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	63	2005年11月	不明	症例報告	外国製品	識別番号:3-06000004 報告日:2006年5月18日
第6回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号:3-05000494 報告日:2005年12月27日
第6回	1	感染症および寄生虫症	輸血後肝炎	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号:3-05000494 報告日:2005年12月27日
第6回	1	臨床検査	抗HBs抗体陽性	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号:3-05000494 報告日:2005年12月27日
第6回	2	感染症および寄生虫症	B型肝炎	ドイツ	女	77	2005/9/28	未回復	症例報告	外国製品	識別番号:3-05000493 報告日:2005年12月27日
第5回	1	感染症および寄生虫症	ウイルス性肝炎	ドイツ	女	55	1995年	不明	症例報告	外国製品	識別番号:3-04000122 報告日:2005年6月8日
第4回	1	臨床検査	C型肝炎陽性	フランス	男	68	2004/08	不明	症例報告	外国製品	識別番号:3-04000088 報告日:2004年11月22日
第3回	報告なし										
第2回	報告なし										

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 1. 19	該当なし	
一般的名称	人血清アルブミン				公表国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)		研究報告の公表状況	Kaski D, Mead S, Hyare H, Cooper S, Jampana R, Overell J, Knight R, Collinge J, Rudge P. Lancet. 2009 Dec 19;374(9707):2128.	英国	

研究報告の概要 168

○コドン129ヘテロ接合性の変異型CJD患者

30歳男性が、13ヶ月前から人格変化、進行性不穏、知能低下を呈し、2008年6月に入院した。患者は重度下肢痛および記憶低下を訴えた。2ヵ月後、幻視を発現し、腹部に腫瘍があるという妄想を持った。その後3ヶ月間に症状は悪化し、2008年10月の精神状態検査のスコアは26/30であった。追跡眼球運動は衝動性であり、口とがらし反射があった。腕に軽度運動失調があり、下肢には腱反射亢進と左足底伸展反応を伴う重度失調があった。歩行に2本の杖を必要とした。既往歴には、頸部リンパ節除去および扁桃摘出術(15年前)があったが、輸血歴やヒト組織の移植歴はなかった。EEGは徐波活性を示した。CSFのタンパク、ブドウ糖、血球数は正常であったが、14-3-3タンパク質が陽性であった。脳MRI所見は、視床枕徴候と一致した。評価したすべての神経放射線医が視床枕徴候を陽性と見なしたわけではないが、定量評価で尾状核と比べ視床枕核の高い対称性信号が示された。遺伝性、代謝性、自己免疫性疾患(腫瘍誘発性疾患を含む)の広範なスクリーニング検査結果は陰性であった。PRNP解析は、既知疾患に関連する突然変異を示さなかった。コドン129はヘテロ接合性だった。特徴的臨床症状、疾患の進行、他の診断の除外、ならびにMRI所見に基づき、変異型クロイツフェルトヤコブ病(vCJD)の臨床診断が下された。患者の年齢が若く、臨床症状、MRI所見、ならびにEEGでpseudoperiodic complexesが見られないことを複合的に考慮し、孤発性CJDの可能性は低いと判断した。患者の保護者はそれ以上の検査は望まなかった。患者の容態は悪化し、2009年1月に死亡した。剖検は実施されなかった。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
赤十字アルブミン20%静注10g/50mL
赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見	今後の対応
プリオンタンパク遺伝子コドン129はヘテロ接合性で、臨床症状、疾患の進行、他の診断の除外、MRI所見から変異型クロイツフェルトヤコブ病と診断された患者の症例報告である。 プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、アルブミンを介して伝播するという証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考える。	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人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を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

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JRC2010T-003

Case Report

Variant CJD in an individual heterozygous for PRNP codon 129

Diego Kaski, Simon Mead, Harpreet Hyare, Sarah Cooper, Ravi Jampana, James Overell, Richard Knight, John Collinge, Peter Rudge

Lancet 2009; 374: 2128

MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK (D Kaski MRCP, S Mead PhD, H Hyare FRCR, Prof J Collinge FRS, P Rudge FRCP); Institute of Neurological Sciences, Glasgow University, Glasgow, UK (S Cooper MRCP, R Jampana FRCR, J Overell FRCP); and National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK (Prof R Knight FRCP)

Correspondence to: Prof John Collinge, MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK
j.collinge@prion.ucl.ac.uk

A 30-year-old man was admitted to hospital in June, 2008, with a 13-month history of personality change, progressive unsteadiness, and intellectual decline. He complained of severe leg pain and poor memory. 2 months later he developed visual hallucinations and falsely believed he had an abdominal tumour. Symptoms worsened over the next 3 months. In October, 2008, his score on the mini mental state examination was 26/30. Pursuit eye movements were saccadic. He had a pout reflex. There was mild ataxia in the arms. His legs were severely ataxic with brisk tendon reflexes and a left extensor plantar response. He needed two crutches to walk. Medical history included tonsillectomy and removal of a cervical lymph node 15 years previously but he had never had a blood transfusion or received implantation of other human tissues.

EEG showed slow wave activity. CSF protein, glucose, and cell count were normal but the 14-3-3 protein was positive. MRI of the brain was consistent with the pulvinar sign (figure A). Although not all neuroradiologists consulted considered the pulvinar sign positive, quantitative assessment showed symmetrical higher signal in the pulvinar nuclei than the caudate nuclei (figure B). Extensive screens for genetic, metabolic, and autoimmune diseases, including those induced by neoplasia, were negative. *PRNP* analysis did not show any known disease-associated mutations; codon 129 was heterozygous. A clinical diagnosis of variant Creutzfeldt-Jakob disease (vCJD) was made on the basis of a characteristic clinical onset and progression, exclusion of other diagnoses, and MRI findings. Sporadic CJD was judged unlikely given the combination of young age, clinical features, MRI findings, and absence of pseudoperiodic complexes on EEG. His carers did not want further investigation. His condition deteriorated and he died in January, 2009. Autopsy was not done.

Human prion diseases have acquired, sporadic, and inherited aetiologies, show wide phenotypic heterogeneity, and are associated with propagation of infectious prions of many distinct strain types.[1] Since 1994, about 200 cases of vCJD, causally related to exposure to bovine spongiform encephalopathy (BSE) prions, have been identified worldwide. vCJD is generally seen in young adults, has characteristic neuropathological features and tissue distribution of infectivity, and a distinctive type 4 (London classification) molecular strain type.[1] A polymorphism at codon 129 (encoding methionine or valine) of the human prion protein gene (*PRNP*), constitutes a powerful susceptibility factor in all types of prion disease. In vCJD, every case genotyped to date has been methionine homozygous,[2] the other acquired prion diseases, cases have occurred in all genotypes but with different mean incubation periods,[1] which can span decades.[3] *PRNP* codon 129 heterozygotes generally have the longest incubation periods. There is a report of a recipient of a blood transfusion from a donor incubating vCJD who died of unrelated causes but showed signs of prion infection at autopsy and was *PRNP* codon 129 heterozygous.[4] Animal studies have suggested that different clinicopathological phenotypes could occur in people with various *PRNP* codon 129 genotypes.[4,5] The majority of the UK population have potentially been exposed to BSE prions but the extent of clinically silent infection remains unclear. About a third of the UK population are *PRNP* codon 129 methionine homozygous. If individuals with other genotypes are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases, which may or may not meet diagnostic criteria for vCJD, would be expected in these *PRNP* codon 129 genotypes. However, prion disease susceptibility and incubation periods are also affected by other genetic loci, and the possibility remains that cases of vCJD to date may have unusual combinations of genotypes at these loci, yet to be fully characterised.

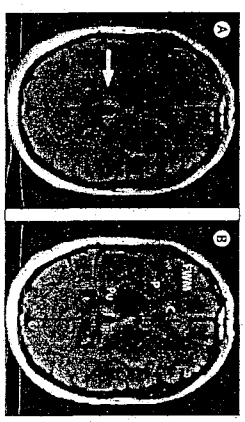

Figure: MRI

(A) Increased signal intensity in the pulvinar nucleus bilaterally (arrow). (B) MR signal intensity in the pulvinar (Pu) is higher than in the head of the caudate nuclei (C), putamen (P), and right frontal white matter (FWM).

Contributors

All authors were involved in discussion about diagnosis, care of the patient, and preparation of the report. Written consent to publish was obtained.

Conflicts of interest

JC is a director and shareholder of D-Gen Ltd, an academic spin-out company in the field of prion disease diagnosis, decontamination, and therapy. The other authors declare that they have no conflicts of interest.

References

1 Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Annu Rev Neurosci* 2001; 24: 519–50.

2 Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. *Lancet* 2006; 367: 2068–74.

3 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004; 364: 527–29.

4 Asante E, Linehan J, Gowland I, et al. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc Natl Acad Sci USA* 2006; 103: 10759–64.

5 Wadsworth JD, Asante E, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004; 306: 1793–96.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2010. 1. 15	該当なし	
一般的名称	人血清アルブミン		研究報告の公表状況	ProMED. 20100107.0076, 2010 Jan 07. 情報源:UK: National CJD Surveillance Unit - monthly statistics as of 5 Jan 2010.	公表国 英国	
販売名(企業名)	赤十字アルブミン20(日本赤十字社) 赤十字アルブミン25(日本赤十字社) 赤十字アルブミン20%静注4g/20mL(日本赤十字社) 赤十字アルブミン20%静注10g/50mL(日本赤十字社) 赤十字アルブミン25%静注12.5g/50mL(日本赤十字社)					

研究報告の概要 170

○プリオン病最新情報 英国:国立CJDサーベイランスユニット、月次vCJD・CJD統計、2010年1月5日時点
英国のCJDサーベイランスユニットから公表されたvCJDを始めとするプリオン病の患者数に関する最新情報である。
vCJD確定例または可能性例総数は前月から変化なく166名のままである。生存患者は4名であるため、2009年までのvCJD症例数は合計170例である。
2009年中に新たに2症例が記録されたが、全体としては英国におけるvCJD流行は減少しつつあるとする見解に一致している。
vCJDによる死亡患者は1995年に初めて確認され、死亡患者数のピークは2000年の28名であった。その後2001年に20名、2002年に17名、2003年に18名、2004年に9名、2005年に5名、2006年に5名、2007年に5名、2008年に1名、2009年に2名となっている。
プリオン病患者全体としては、2009年の12ヶ月間に143名の照会があった。このうち、孤発性CJD:59名、家族性CJD:1名、医原性CJD:1名、GSS:3名、vCJD:2名だった。

使用上の注意記載状況・その他参考事項等

赤十字アルブミン20
赤十字アルブミン25
赤十字アルブミン20%静注4g/20mL
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赤十字アルブミン25%静注12.5g/50mL

血液を原料とすることに由来する感染症伝播等

報告企業の意見

英国CJDサーベイランスユニットの統計によると、2010年1月5日の時点でvCJD死亡患者総数は170名であり、英国におけるvCJD流行は収まりつつあるとする見解に一致するとの報告である。
プリオン病の原因とされる異常プリオンがコーン分画工程で効果的に除去されるとの成績と併せて、これまでの疫学研究では如何なるプリオン病も、アルブミンを介して伝播するという証拠は無い。また本製剤の使用は一時的かつ限定的であることから伝播のリスクは非常に低いものと考える。

今後の対応

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人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を収集するとともに、血漿分画製剤の製造工程における病原因子の除去・不活化技術の向上に努める。

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[in French, trans. & summ. Mod.CP]
<http://www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html>

During the 12 months of 2009, there were 1486 referrals, 85 cases of sporadic CJD, 10 cases of familial CJD, 3 cases of iatrogenic CJD, and 2 confirmed cases of vCJD.

A total of 25 cases of confirmed or probable vCJD has now been recorded in France since 1997. The 25 confirmed cases comprise 13 females and 12 males. All 25 are now deceased. Their median age is 37 (between 19 and 58). Seven were resident in the Ile-de-France and 18 in the provinces. All the identified cases have been Met-Met homozygotes. No risk factor has been identified. One of the 25 had made frequent visits to the United Kingdom.

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Communicated by:
ProMED-mail <promed@promedmail.org>

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[3] US National Prion Disease Center - not updated since 7 Nov 2009
Date: Sat 7 Nov 2009
Source: US National Prion Disease Pathology Surveillance Center [edited]
<http://www.cjdsurveillance.com/pdf/case-table.pdf>

(Report not updated since 7 Dec 2009): During the period 1 Jan 2009 to 7 Nov 2009, there were 341 referrals, of which 198 were classified as Prion disease, comprising 133 cases of sporadic CJD, 33 of familial CJD, and no cases of iatrogenic CJD or vCJD.

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Communicated by:
ProMED-mail <promed@promedmail.org>

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[4] Portuguese vCJD case - pathology
Date: Fri 1 Jan 2010
Source: J Neurol Neurosurg Psychiatry 2010 Jan;81(1):112-4. [edited]
<http://jnnp.bmj.com/content/81/1/112.abstract>

Title: Variant Creutzfeldt-Jakob disease: the first confirmed case from Portugal shows early onset, long duration and unusual pathology.

Authors: Barbot C, Castro L, Oliveira C, Carpenter S.
At: Department of Neuropaediatrics, Hospital Maria Pia, Porto, Portugal.

Summary:
We present clinical and autopsy findings in the 1st case of variant Creutzfeldt-Jakob disease diagnosed and confirmed in Portugal. Onset was at 11 years, the earliest onset reported, and the course (32 months) relatively long. Western blot showed protease resistant prion protein, mainly of type 4 (2B) isoform. The cerebral cortex revealed severe spongiform change with numerous amyloid plaques, which did not fit the definition of florid plaques. In the striatum, spongiform change was limited, but the extracellular space was dilated. Other reports have found marked spongiform change in the striatum and little in the cortex. Massive neuronal loss, in excess of what has been described, was found in the thalamus and pontine grey. The cerebellum showed, as expected, severe loss of granule cells, moderate loss of Purkinje cells and marked immunopositivity for the prion protein. Differences between our findings and previous ones probably result from the patient's long survival.

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Communicated by:
Terry S. Singeltary Sr. <flounder9@verizon.net>

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[5] vCJD codon 129 heterozygote
Date: Fri 19 Dec 2009
Source: BBC News, Health [edited]
<http://news.bbc.co.uk/1/hi/health/8419459.stm>

A 30-year-old man thought to have died in January [2009] from vCJD belonged to a genetic group that had not shown any signs of the disease, scientists say. In the UK, 166 people have died of vCJD, linked to eating BSE [bovine spongiform encephalopathy] infected beef, and all were thought to have shared a certain gene.

Writing in the Lancet, scientists say that the victim, a resident of, Lanarkshire [Scotland], had a different version of the gene. They estimate that up to 350 people in this group could get vCJD. Scientists have always thought that a 2nd wave of vCJD cases would emerge some time after the 1st. This is the 1st indication that this theory is being born out, with the identification of the 1st probable vCJD patient outside of the initial genetic group, BBC science correspondent Pallab Ghosh reports.

The father believes his son was incubating the disease for much of his life. It is probable because the diagnosis is based on observations of the progression of the disease rather than post-mortem tests which would have provided absolute confirmation of the disease, he adds.

The case report written by Professor John Collinge of the National Prion Clinic and colleagues is a reminder that the disease has not gone away. Many thousands of people may be carrying the infection, and although they will never show any symptoms, they have the potential to infect others.

vCJD is caused by infectious agents called prions. Prion diseases affect the structure of the brain or other neural tissue and are currently untreatable. Disease-causing prions are thought to consist of abnormally folded proteins, which spread by encouraging the normal healthy prion protein found on the surface of most cells in the body to change shape. Tests showed that the patient had a heterozygous version of the gene which codes for the human prion amino acids valine (V) or methionine (M). People can be V V (homozygous), M M (homozygous) or M V (heterozygous). Since 1994, around 200 cases of vCJD have been identified worldwide, and all those tested have been M M homozygous. [However, genetic analysis of 2 out of 3 prion-positive appendix samples in the tissue-based prevalence study in 2001-2004 showed that both were valine homozygous (VV) at codon 129 in the prion protein gene (Ironside et al, Brit Med J 2006). - Mod.CP]. However, this most recent victim was M/V heterozygous. It is thought that 47 percent of the population have this version of the gene. Professor Collinge said: "The majority of the UK population have potentially been exposed to BSE prions, but the extent of clinically silent infection remains unclear. About 1/3rd of the UK population are M/M homozygous. If individuals with other genotypes [M/V and V/V] are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases would be expected."

The scientists have previously looked at another prion disease in New Guinea called "kuru" [which was induced by eating infected human brain tissue. - Mod.CP]. The original cases were all M/M, but more recently, M/V cases have appeared. They say this indicates that M/V people can get prion diseases like kuru but have a much longer incubation period.

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Communicated by:
ProMED-mail <promed@promedmail.org>

[The abstract of the Lancet paper upon which the above report is based is reproduced below. - Mod.CP]

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[6] vCJD codon 129 heterozygote - Lancet paper
Date: Thu 18 Dec 2009
Source: Lancet 2009; 374: 2128 [edited]
<http://press.thelancet.com/vcjd.pdf>

[A Case Report published in the 18 Dec 2009 issue of the Lancet by Professor John Collinge, MRC Prion Unit and National Prion Clinic,

UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London]

A 30-year-old man was admitted to hospital in June 2008 with a 13-month history of personality change, progressive unsteadiness, and intellectual decline. He complained of severe leg pain and poor memory. Two months later, he developed visual hallucinations and falsely believed he had an abdominal tumour. Symptoms worsened over the next 3 months. In October 2008, his score on the mini mental state examination was 26/30. Pursuit eye movements were saccadic [a rapid movement of the eye between fixation points]. He had a pout reflex. There was mild ataxia in the arms. His legs were severely ataxic with brisk tendon reflexes and a left extensor plantar response. He needed 2 crutches to walk. Medical history included tonsillectomy and removal of a cervical lymph node 15 years previously, but he had never had a blood transfusion or received implantation of other human tissues.

EEG showed slow wave activity. CSF protein, glucose, and cell count were normal, but the 14-3-3 protein was positive. MRI [magnetic resonance imaging] of the brain was consistent with the pulvinar sign (illustrated in the original text). Although not all neuroradiologists consulted considered the pulvinar sign positive, quantitative assessment showed symmetrical higher signal in the pulvinar nuclei than the caudate nuclei (illustrated in the original text). Extensive screens for genetic, metabolic, and autoimmune diseases, including those induced by neoplasia, were negative. PRNP analysis did not show any known disease-associated mutations; codon 129 was heterozygous. A clinical diagnosis of variant Creutzfeldt-Jakob disease (vCJD) was made on the basis of a characteristic clinical onset and progression, exclusion of other diagnoses, and MRI findings. Sporadic CJD was judged unlikely given the combination of young age, clinical features, MRI findings, and absence of pseudoperiodic complexes on EEG. His care givers did not want further investigation. His condition deteriorated, and he died in January 2009. Autopsy was not done.

Human prion diseases have acquired, sporadic, and inherited aetiologies, show wide phenotypic heterogeneity, and are associated with propagation of infectious prions of many distinct strain types (1). Since 1994, about 200 cases of vCJD, causally related to exposure to bovine spongiform encephalopathy (BSE) prions, have been identified world-wide. vCJD is generally seen in young adults, has characteristic neuropathological features and tissue distribution of infectivity, and a distinctive type 4 (London classification) molecular strain type (1). A polymorphism at codon 129 (encoding methionine or valine) of the human prion protein gene (PRNP) constitutes a powerful susceptibility factor in all types of prion disease. In vCJD, every case genotyped to date has been methionine homozygous. In the other acquired prion diseases, cases have occurred in all genotypes but with different mean incubation periods (1), which can span decades (2); PRNP codon 129 heterozygotes generally have the longest incubation periods. There is a report of a recipient of a blood transfusion from a donor incubating vCJD who died of unrelated causes but showed signs of prion infection at autopsy and was PRNP codon 129 heterozygous (3). Animal studies have suggested that different clinicopathological phenotypes could occur in people with various PRNP codon 129 genotypes (4,5). The majority of the UK population have potentially been exposed to BSE prions but the extent of clinically silent infection remains unclear. About 1/3rd of the UK population are PRNP codon 129 methionine homozygous. If individuals with other genotypes [V/V or V/M] are similarly susceptible to developing prion disease after BSE prion exposure, but with longer incubation periods, further cases, which may or may not meet diagnostic criteria for vCJD, would be expected in these PRNP codon 129 genotypes. However, prion disease susceptibility and incubation periods are also affected by other genetic loci, and the possibility remains that cases of vCJD to date may have unusual combinations of genotypes at these loci, yet to be fully characterised.

References:

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(2) Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century - an acquired human prion disease with very long incubation periods. Lancet 2006; 367: 2068-74.

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[Acknowledgment: MRC Prion Unit and National Prion Clinic, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK (D Kaski MRCP, S Mead PhD, H Hyare FRCR, Prof J Collinge FRS, P Rudge FRCP); Institute of Neurological Sciences, Glasgow University, Glasgow, UK (S Cooper MRCP, R Jampana FRCR, J Overell FRCP); and National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK (Prof R Knight FRCP)]

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[To put this work in perspective, parts of a British Medical Journal editorial by Maurizio Pocchiari are reproduced below. - Mod.CP.

Date: 21 May 2009
Source: BMJ 2009;338:b435 [edited]
<http://www.bmj.com/cgi/content/full/338/may21_2/b435>

"Prevalence of variant CJD in the UK

The number of cases of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom has decreased since 2000, but controversy remains about how many people carry the infectious agent and will eventually develop disease. Clewley and colleagues in a limited study add to the debate by assessing 63 007 pairs of tonsils for the only available marker of prion disease, the pathological, partially protease resistant, prion protein. Although more than half of the samples came from people born between 1961 and 1995, when the risk of exposure to bovine spongiform encephalopathy (BSE) infection was high, no convincingly positive tonsil specimens were detected. This study estimated that the prevalence of vCJD in the British population is zero, but with a large confidence interval of 0 to 113 per million.

This result agrees with one UK survey of 2000 tonsil specimens, but it differs from another survey of 1427 tonsils and 11 247 appendices, which found that more than 10 000 people might be incubating the disease. However, despite the discrepancy, the 95 percent confidence intervals of the 2 studies overlap, indicating that the results do not differ significantly and that many people in the UK may be carriers.

The chance that no one in the UK is incubating the disease, as suggested by the lower confidence limit of Clewley and colleagues' study, is unlikely because backup calculations predict up to 100 new cases of vCJD in the next 50 years. This prediction seems reasonable unless most cases of vCJD were missed by surveillance in the past years.

Until December 2008, all 210 people reported to have vCJD (164 in the UK, 46 in other countries) were homozygous for methionine at the polymorphic codon 129 of the prion protein gene (PRNP), suggesting that genetic factors strongly influence the development of disease. Whether people who are heterozygous for methionine and valine or homozygous for valine at this codon (about 60 percent of the population) will develop vCJD in the future is still unknown. However, data *from gene* targeted transgenic mice indicate that these people are also susceptible to BSE and vCJD, although incubation periods are longer than in those who are homozygous for methionine."

Interested readers should consult the original article for further information and references. - Mod.CP]

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[7] Prion evolution & a new reagent
Date: 1 Jan 2010
Source: BBC Health News [edited]
<http://news.bbc.co.uk/1/hi/health/8435320.stm>

Abnormal prion proteins cause at least 20 fatal diseases. Scientists have shown for the 1st time that "lifeless" prion proteins, devoid of all genetic material, can evolve just like higher forms of life. The Scripps Research Institute in the US says the prions can change to suit their environment and go on to develop drug resistance.

Prions are associated with 20 different brain diseases in humans and animals. The scientists say their work suggests new approaches might be necessary to develop therapies for these diseases. In the study, published in the journal Science [see below], the scientists transferred prion populations from brain cells to other cells in culture and observed the prions that adapted to the new cellular environment out-competed their brain-adapted counterparts. When returned to the brain cells, the brain-adapted prions again took over the population.

Charles Weissmann, head of Scripps Florida's department of infectology who led the study, said: "On the face of it, you have exactly the same process of mutation and adaptive change in prions as you see in viruses. This is a timely reminder that prion concerns are not going away and that controls to stop abnormal prions being transmitted to humans through the food system or through blood transfusions must be vigorously maintained."

Professor John Collinge, Medical Research Council Prion Unit stated that: "This means that this pattern of Darwinian evolution appears to be universally active. In viruses, mutation is linked to changes in nucleic acid sequence that leads to resistance. Now, this adaptability has moved one level down -- to prions and protein folding -- and it's clear that you do not need nucleic acid (DNA or RNA) for the process of evolution."

Mammalian cells normally produce cellular prion protein or PrPC. During infections, such as the human form of mad cow disease, known as vCJD, abnormal or mis-folded proteins convert the normal host prion protein into its toxic form by changing its conformation or shape. "It was generally thought that once cellular prion protein was converted into the abnormal form, there was no further change," Prof. Weissmann said. "But there have been hints that something was happening. When you transmit prions from sheep to mice, they become more virulent over time. Now we know that the abnormal prions replicate and create variants, perhaps at a low level initially. But once they are transferred to a new host, natural selection will eventually choose the more virulent and aggressive variants."

Professor John Collinge, of the Medical Research Council's (MRC) Prion Unit, described the research as exciting confirmation of a hypothesis that he had proposed 2 years ago, that there could be a "cloud" or whole array of prion proteins in the body. He called it the cloud hypothesis: "The prion protein is not a clone, it is a quasi-species that can create different protein strains even in the same animal. The abnormal prion proteins multiply by converting normal prion proteins. The implication of Charles Weissmann's work is that it would be better to cut off that supply of normal prion proteins rather than risk the abnormal prion adapting to a drug and evolving into a new more virulent form. You would do this by trying to block the sites on the normal prion protein that the abnormal form locks on to to do its conversion. We know there is an antibody that can do this in mice, and the Medical Research Council's Prion Unit have managed to engineer a human antibody to do this. It is currently undergoing safety tests, and we hope to move to clinical trials by the end of 2011."

Professor Collinge said the MRC was also trying to find more conventional chemical compounds to do this and has been collaborating

with the chemical company GlaxoSmithKline (GSK). He said: "They have given us access to their chemical libraries, which contain millions of compounds, and we have already identified some that may work well. This is a timely reminder that prion concerns are not going away and that controls to stop abnormal prions being transmitted to humans through the food system or through blood transfusions must be vigorously maintained."

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[The abstract and the reference for the Science paper descried above are the following: Science DOI: 10.1126/science.1183218, Published Online 31 Dec 2009.
<http://www.sciencemag.org/cgi/content/abstract/science.1183218>.
Darwinian Evolution of Prions in Cell Culture. By Jiali Li, Shawn Browning, Sukhvir P. Mahal, Anja M. Oelschlegel, Charles Weissmann
At: Department of Infectology, Scripps Florida, 130 Scripps Way, Jupiter, FL 33458, USA.

Abstract: "Prions are infectious proteins consisting mainly of PrPSc, a sheet-rich conformer of the normal host protein PrPC, and occur in different strains. Strain identity is thought to be encoded by PrPSc conformation. We found that biologically cloned prion populations gradually became heterogeneous by accumulating "mutants," and selective pressures resulted in the emergence of different mutants as major constituents of the evolving population. Thus, when transferred from brain to cultured cells, "cell-adapted" prions out competed their "brain-adapted" counterparts, and the opposite occurred when prions were returned from cells to brain. Similarly, the inhibitor swainsonine selected for a resistant substrain, whereas in its absence, the susceptible substrain outgrew its resistant counterpart. Prions, albeit devoid of a nucleic acid genome, are thus subject to mutation and selective amplification."

From a theoretical standpoint, this work has great significance. Nonetheless, the immediate interest of the BBC News report is the information that Professor John Collinge's MRC group has succeeded in engineering a humanised monoclonal antibody that interacts with the sites on the normal prion protein that the abnormal form locks onto to achieve its conversion and that it is hoped eventually to move to clinical trials of this reagent. - Mod.CP]

[see also:
2009

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2006

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2005

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2004

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2003

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2002

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