医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2010 年 9 月 7 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	乾燥濃縮人アンチトロンビンⅢ		研究報告の公表状況	Annals of Neurology 2010; 68(2): 162-172	公表国 アメリカ	
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）					

研究報告の概要

目的：「プロテアーゼ感受性プリオン症(PSPr)」の 2 つの新しい遺伝子型を報告する。これは 2008 年に記載され新型のプリオン病で、被験者 11 人はプリオン蛋白質(PrP)遺伝子のコドン 129 が全員バリン同型接合(129VV)だった。2 つの新しい PSPr は、メチオニン同型接合(129MM)とメチオニン/バリン異型接合(129MV)の個人である。

方法：129MM、129MV、129VV の被験者 15 人は国立プリオン病病理監視センターで臨床、組織病理学的、免疫組織化学、遺伝子型、PrP 特性で比較評価を受けた。

結果：罹病期間(22 から 45 ヶ月の間)は、129VV と 129MV の被験者で有意に異なった。PrP 電気泳動プロフィルと共に他の殆どの表現型の機能は同様だったが、3 つの 129 遺伝子型で区別できる。主な違いは、疾患関連 PrP のプロテアーゼ消化の感受性にあり、129VV では高度であるが、129MV と 129MM では遙かに低いか、全くない。この違いは本来の命名を変え、「可変プロテアーゼ感受性プリオン症(VPSPr)」になった。被験者は誰も PrP 遺伝子コドン領域の変異はなかった。

解釈：3 つの 129 遺伝子型が全員関係し、区別でき、表現型として関係するので、VPSPr は二番目の孤発性プリオン蛋白質疾患になる。この特徴は 1920 年に報告したクロイツフェルト・ヤコブ病によく似ていた。しかし、異常プリオン蛋白質の特性は VPSPr は典型的なプリオン病とは異なることを示唆している。おそらく、ゲルストマン・ストロイスラー・シャインカー疾患の亜型と類似している。

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
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。

報告企業の意見	今後の対応
「可変プロテアーゼ感受性プリオン症(VPSPr)」という、新規の孤発性プリオン病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ないと考えるので、特段の措置はとらない。

ORIGINAL ARTICLE

Variably Protease-Sensitive Prionopathy: A New Sporadic Disease of the Prion Protein

Wen-Quan Zou, MD, PhD,[1] Gianfranco Puoti, MD, PhD,[1] Xiangzhu Xiao, PhD,[1] Jue Yuan, BA,[1] Liuting Qing, PhD,[1] Ignazio Cali, MSc,[1] Miyuki Shimoji, PhD,[1] Jan P. M. Langeveld, PhD,[2] Rudy Castellani, MD,[3] Silvio Notari, PhD,[1] Barbara Crain, MD,[4] Robert E. Schmidt, MD,[5] Michael Geschwind, MD,[6] Stephen J. DeArmond, MD, PhD,[6] Nigel J. Cairns, MD,[7] Dennis Dickson, MD,[8] Lawrence Honig, MD,[9] Juan Maria Torres, PhD,[10] James Mastrianni, MD, PhD,[11] Sabina Capellari, MD,[12] Giorgio Giaccone, MD,[13] Ermias D. Belay, MD,[14] Lawrence B. Schonberger, MD, MPH,[14] Mark Cohen, MD,[1] George Perry, PhD,[15] Qingzhong Kong, PhD,[1] Piero Parchi, MD, PhD,[12] Fabrizio Tagliavini, MD,[13] and Pierluigi Gambetti, MD[1]

Objective: The objective of the study is to report 2 new genotypic forms of protease-sensitive prionopathy (PSPr), a novel prion disease described in 2008, in 11 subjects all homozygous for valine at codon 129 of the prion protein (PrP) gene (129VV). The 2 new PSPr forms affect individuals who are either homozygous for methionine (129MM) or heterozygous for methionine/valine (129MV).
Methods: Fifteen affected subjects with 129MM, 129MV, and 129VV underwent comparative evaluation at the National Prion Disease Pathology Surveillance Center for clinical, histopathologic, immunohistochemical, genotypical, and PrP characteristics.
Results: Disease duration (between 22 and 45 months) was significantly different in the 129VV and 129MV subjects. Most other phenotypic features along with the PrP electrophoretic profile were similar but distinguishable in the 3 129 genotypes. A major difference laid in the sensitivity to protease digestion of the disease-associated PrP, which was high in 129VV but much lower, or altogether lacking, in 129MV and 129MM. This difference prompted the substitution of the original designation with "variably protease-sensitive prionopathy" (VPSPr). None of the subjects had mutations in the PrP gene coding region.
Interpretation: Because all 3 129 genotypes are involved, and are associated with distinguishable phenotypes, VPSPr becomes the second sporadic prion protein disease with this feature after Creutzfeldt-Jakob disease, originally reported in 1920. However, the characteristics of the abnormal prion protein suggest that VPSPr is different from typical prion diseases, and perhaps more akin to subtypes of Gerstmann-Sträussler-Scheinker disease.

ANN NEUROL 2010;68:162–172

Human prion diseases are prominently heterogeneous. In sporadic Creutzfeldt-Jakob disease (sCJD), the most prevalent prion disease, heterogeneity is largely predicated on the common methionine (M)/valine (V) polymorphism at codon 129 of the prion protein (PrP) gene and the disease-associated PrP (PrP^{Dis}) that are

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.22094

Received Mar 9, 2010, and in revised form May 5, 2010. Accepted for publication May 19, 2010.

Address correspondence to Dr. Gambetti or Dr. Zou, Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland, OH 44106. E-mail: pierluigi.gambetti@case.edu or wenquan.zou@case.edu

From the [1]Department of Pathology, National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH; [2]Central Veterinary Institute of Wageningen, Lelystad, the Netherlands; [3]Department of Neuropathology, University of Maryland Medical Center, Baltimore, MD; [4]Department of Neuropathology, Johns Hopkins University, Baltimore, MD; [5]Department of Neuropathology, Washington University, St. Louis, MO; [6]Department of Pathology, University of California at San Francisco, San Francisco, CA; [7]Departments of Neurology, Pathology, and Immunology, Washington University, St. Louis, MO; [8]Department of Neuropathology, Mayo Clinic-Jacksonville, Jacksonville, FL; [9]New York Presbyterian Hospital, Columbia University, New York, NY; [10]Centro de Investigación en Sanidad Animal, Madrid, Spain; [11]Department of Neurology, University of Chicago, Chicago, IL; [12]Department of Neurological Sciences, University of Bologna, Dipartimento di Scienze Neurologiche, Università di Bologna, Bologna, Italy; [13]IRCCS Foundation, National Neurological Institute, Instituto Nazionale Neurologico Carlo Besta, Milan, Italy; [14]Center of Investigation on Animal Health, Centers for Disease Control and Prevention, Atlanta, GA; and [15]College of Science, University of Texas at San Antonio, San Antonio, TX.

distinguished in types 1 and 2 based on the electrophoretic mobility of their protease-resistant regions.[1]

However, despite this remarkable heterogeneity, all well-established sporadic prion diseases (here operationally defined as nonacquired prion diseases free of mutations in the PrP gene coding region) have been shown to share the same basic pathogenetic mechanism; PrP^{Dis} interacts with the normal or cellular PrP and converts it into PrP^{Dis}, triggering an autocatalytic process that leads to the accumulation of PrP^{Dis} and ultimately to the clinical disease.[2]

In 2008, we described 11 cases affected by a new disease involving PrP; we named this disease protease-sensitive prionopathy (PSPr).[3] Subsequently, 2 additional cases of PSPr have been independently reported.[4,5] PSPr differed from known sporadic ption diseases in the clinical presentation, in the histopathologic and immunohistochemical features, and in the basic characteristics of the PrP^{Dis}. Furthermore, all 11 cases had the 129VV genotype and no mutation in the PrP gene open reading frame (ORF).

We now report 15 additional cases, all of which bear features of the PSPr as originally reported. However, the new cases also include, in addition to new 129VV subjects, individuals who are 129MV heterozygous and 129MM homozygous. Although the affected subjects belonging to the 3 genotypes share several important characteristics, they also display basic variations that allow the 3 corresponding phenotypes to be distinguished. Therefore, the new cases show that the disease originally described as PSPr, like sCJD, affects all 3 129 genotypes and to some extent mimics the 129-related phenotypic heterogeneity of sCJD, although the PSPr characteristics underline basic differences from sCJD and similarities with Gerstmann-Sträussler-Scheinker disease (GSS), a rare phenotype, which to date has been reported as exclusively associated with PrP gene mutations. In view of the increased protease-resistance of the PrP^{Dis} associated with the new 129 genotypes compared to that of the 129VV cases, we propose to revise the original PSPr label to VPSPr or "variably protease-sensitive prionopathy." Parts of these findings have been presented previously.[6–9]

Subjects and Methods

Subjects

A total of 15 affected subjects, including 3 129MM, 6 129MV, and 6 previously unreported 129VV, were examined. Thirteen affected subjects were referred to the National Prion Disease Pathology Surveillance Center (NPDPSC) (Cleveland, OH) between 2002 and 2010. All cases were symptomatic except 1 of the 129MM subjects, who died suddenly of heart problems while participating in a dementia study as a negative control, underwent autopsy, and was referred to the NPDPSC because it was noted to have spongiform degeneration (SD) on histological examination. One 129MM subject was received by Dr Fabrizio Tagliavini[10] (National Neurological Institute, Instituto Nazionale Neurologico Carlo Besta, Milan, Italy), and 1 129MV subject was received by Dr Piero Parchi (Department of Neurological Sciences, University of Bologna, Dipartimento di Scienze Neurologiche, Università di Bologna, Bologna, Italy). All the subjects including those serving as positive control as indicated were examined at autopsy following analyses of fixed and frozen tissues. Consent was obtained for using tissues for research, including genetic analyses.

Tissue Processing

Fixed and frozen brain tissues were processed as previously described; a different procedure was followed for the case received from Dr Tagliavini.[3,10–12]

Histopathology and Immunohistochemistry

Samples obtained from up to 18 brain regions were processed according to previously described procedures.[3,12] Lesion profiles were constructed using semiquantitative evaluation of SD and astrogliosis in 11 brain regions from 10 subjects, including the 3 129 genotypes. SD and astrogliosis were scored (Fig 1), and the scores from each of the brain regions were summed for each subject separately; values were averaged, their standard deviations determined, and they were plotted according to the brain region.[3,12] Vacuoles with >4μm diameter were measured individually on random photomicrographs of frontal neocortex (10/subject, ×180) using Spotsoftware version 4.6 after calibration (Diagnostic Instruments, Sterling Heights, MI).[3]

Sections from the frontal and occipital neocortices, hippocampus, basal ganglia, thalamus, cerebellar hemisphere, and midbrain were processed for PrP immunohistochemistry with the monoclonal antibody (mAb) 3F4 or 1E4 (Cell Sciences, Canton, MA).[11–17] Selected brain regions were also immunostained with the mAbs 4G8 to amyloid β or PHF1 to the tau protein.[3]

Molecular Genetics

The entire PrP ORF was amplified by polymerase chain reaction using genomic DNA (extracted from unfixed brain tissue or blood) and the primers 42F (CATAACTTAGGGTCACATTT GTCC) and 45R (CCAGATTAACCAATGGTTATTTGC); sequencing was done directly or after cloning into plasmid pSTBlue 1 (Novagen, Madison, WI) by automated sequencing.[13]

Prion Protein Characterization

REAGENTS AND ANTIBODIES. Phenylmethylsulfonyl fluoride was purchased from Sigma Chemical Co. (St. Louis, MO). Peptide N-glycosidase F (PNGase F) was purchased from New England Biolabs (Beverly, MA) and used following the

manufacturers protocol. Reagents for enhanced chemiluminescence (ECL Plus) were from Amersham Pharmacia Biotech (Piscataway, NJ). Antibodies to various sequences of human PrP included anti-C, a rabbit antiserum (220–231), 3F4, a mouse mAb (106–110), and 1E4, a mAb (97–108).[3,14–17]

BRAIN HOMOGENIZATION. The 10% (weight/volume) brain homogenates were prepared in 9 volumes of lysis buffer (100mM Tris, 150 mM NaCl, 0.5% Nonidet P40, 0.5% deoxycholate, 5mM ethylenediaminetetraacetic acid, pH 8.0) on ice using pestles with Eppendorf tubes driven by a cordless motor as previously described.[14] When required, brain homogenates were centrifuged at 1,000 × *g* for 10 minutes at 4°C to collect supernatant.

IMMUNOBLOT ANALYSIS. Samples were resolved on 15% Tris-HCl Criterion precast gels (Bio-Rad Laboratories, Hercules, CA) for gel electrophoresis, and Western blotting as described previously.[15] The proteins on the gels were transferred to Immobilon-P membrane polyvinylidene fluoride (Millipore, Billerica, MA) for 2 hours at 70V. For probing PrP, the membranes were incubated for 2 hours at room temperature with anti-PrP antibodies. Following incubation with horseradish peroxidase-conjugated sheep antimouse immunoglobulin G (IgG) or donkey antirabbit IgG at 1:3,000, the PrP bands were visualized on Kodak film (Eastman Kodak, Rochester, NY) by ECL Plus as described by the manufacturer.

Results

Clinical Features

The cases, grouped according to the 129 genotype, demographics, and clinical data and tests, along with the previous 129VV cases, are summarized in the Table.

In 129VV cases, the presentation was characterized by 1 or more components of a triad comprising psychiatric signs, in the form of behavior and mood changes, speech deficit, and cognitive impairment. Behavior and mood changes, expressed as disinhibition, euphoria, and impulsivity or loss of interest and apathy, were the most frequent (80% of the cases). Language deficits, observed in half of the cases, were characterized by anomic or semantic aphasia, or by dysarthria. Cognitive impairment, mostly of the frontal lobe type, was present at onset in 50% of the cases, alone or together with the behavioral changes and language deficits.

In the 129MV subtype, psychiatric signs were often associated with parkinsonism, followed by ataxia and myoclonus, whereas aphasia was rare; in these cases, the mean age at onset (72 years) and duration (45 months) of the disease were the most advanced and the longest, respectively, of the 3 subtypes and the duration was significantly different from those of the 129VV genotype ($p < 0.017$).

Both symptomatic 129MM subjects (1 died apparently before clinical onset of disease) presented with Parkinsonism and ataxia followed by progressive diffuse cognitive impairment and myoclonus; aphasia was reported in 1 case, but neither showed psychiatric symptoms.

As for the diagnostic tests, just 1 VV case showed signal changes consistent with CJD on magnetic resonance imaging and electroencephalography; all the other cases revealed various degrees of brain atrophy and diffuse slowing of cerebral electrical activity.

Familial occurrence of dementia was reported in about 50% (7/14 available family histories) of the 129VV cases (1 case in the present series), and in 1 129MM, but not in the 129MV genotype.

Histopathology

The hallmark common to all 129 genotypes was the presence of moderate SD comprising vacuoles in the major cerebral regions, which were relatively larger than those observed in sCJDMM1 but overall smaller than those of sCJDMM2 (see Fig 1A–E and Fig 1A-C of Gambetti et al[3]). Occasionally, the molecular layer of the cerebellum contained small homogeneous formations, with the appearance of microplaques in the 129VV and 129MV cases (see Fig 1F, G). On average, all these lesions were more severe in the 129VV and 129MV than in the 129MM cases (see Fig 1E).

The pattern of PrP immunostaining was slightly different in the 3 genotypes.

In the 129VV cases, the PrP immunostaining, as originally described, was targetlike in the cerebrum (Fig 2A) and dotlike in the cerebellar molecular layer (see Fig 2B).

In the 129MV genotype, the targetlike pattern was less recognizable (see Fig 2C). The cerebellar molecular layer showed a more plaquelike immunostaining pattern (see Fig 2D).

In the 129MM subjects, the predominant immunostaining pattern was plaquelike (see Fig 2E). The cerebellum occasionally showed small plaquelike formations (see Fig 2F).

With mAb 1E4, the patterns of PrP immunostaining were similar to those revealed by 3F4 (data not shown).

Various degrees of amyloid β immunostaining, apparently age-correlated, were also observed (data not shown).

Characterization of PrP^{Dis} in the 3 129 Genotypes

PRP^{DIS} ELECTROPHORETIC PROFILE AND PROTEINASE K RESISTANCE. The ladderlike electrophoretic profile of the proteinase K (PK)-resistant PrP^{Dis} fragments, the

TABLE: Summary of Cases

Codon 129	Distribution, % (No.)	Onset, yr ± SD (range)	Duration, mo ± SD (range)	Main Neurological Signs: Psychiatric,[a] %	Aphasia, %	Parkinsonism, %	Ataxia, %	Myoclonus,[b] %	Frontal Type Dementia	Diagnostic Tests: 14-3-3, Positive/ Total or %	EEG, Typical/ Total or %	MRI,[c] Typical/ Total or %	Family History of Dementia
VPSPr													
MM	12 (3)[d]	64 & 78[e]	41 & 50	0	50	100	50	100	0	1/2	1/2	0/2	1/2
MV	23 (6)	72 ± 7[f] (65–81)	45 ± 24[g] (7–72)	83	17	67	67	40	50	0/2	0/2	0/6	0/5
VV	65 (17)[h]	65 ± 8[e,f] (48–77)	23 ± 17[g] (10–60)	71	47	30	71	5	60	1/7	0/14	1/17	7/13
Total % distribution	100	67 ± 10 (48–81)	30 ± 21 (7–72)	68	48	48	64	20	52	2/12	1/18	1/25	8/20
sCJD[i]													
MM1	68	65 (42–91)	4(1–18)	28	23	7	33	97	NA	95%	80%	75%	—
MV1	2	62 (51–72)		12	25	0	75	100	NA		71%		
VV1	1	37 (19–55)	21(10–49)	0	33	33	0	67	NA	100%	0%	100%	—
MM2	2	64 (49–77)	16 (9–36)	0	33	33	17	67	NA	75%	0%	43%	—
MV2	9	60 (41–81)	17 (5–72)	34	11	22	81	77	NA	80%	7%	86%	—
VV2	16		6 (3–18)	19	0	6	100	66	NA		7%	70%	—
Totals	98[j]	63	6	26	18	8	49	87	NA	89%	56%	72%	

[a]Psychiatric symptoms include depression, psychosis, and personality/behavioral changes.
[b]Generally appeared late in the disease.
[c]Twenty-one of 25 VPSPr patients showed significant cerebral atrophy.
[d]One of the 3 129MM subjects who died of accidental causes before onset of clinical disease has been excluded.
[e]$p < 0.025$;
[f]$p < 0.045$;
[g]$p < 0.03$ (statistical analysis by GrapliPad [La Jolla, CA] Prisma 5 software).
[h]Including the 11 129VV cases previously published.[3]
[i]Data adapted from Gambetti et al,[1] Parchi et al,[12] and Zou et al[17]; cases with co-occurrence of disease-associated prion protein type 1 and 2 have been omitted.[14,18,19]
[j]Does not include sporadic fatal insomnia (2%).[20]
SD = standard deviation; EEG = electroencephalogram; MRI = magnetic resonance imaging; VPSPr = variably protease-sensitive prionopathy; M = methionine; V = valine; sCJD = sporadic Creutzfeldt-Jakob disease.

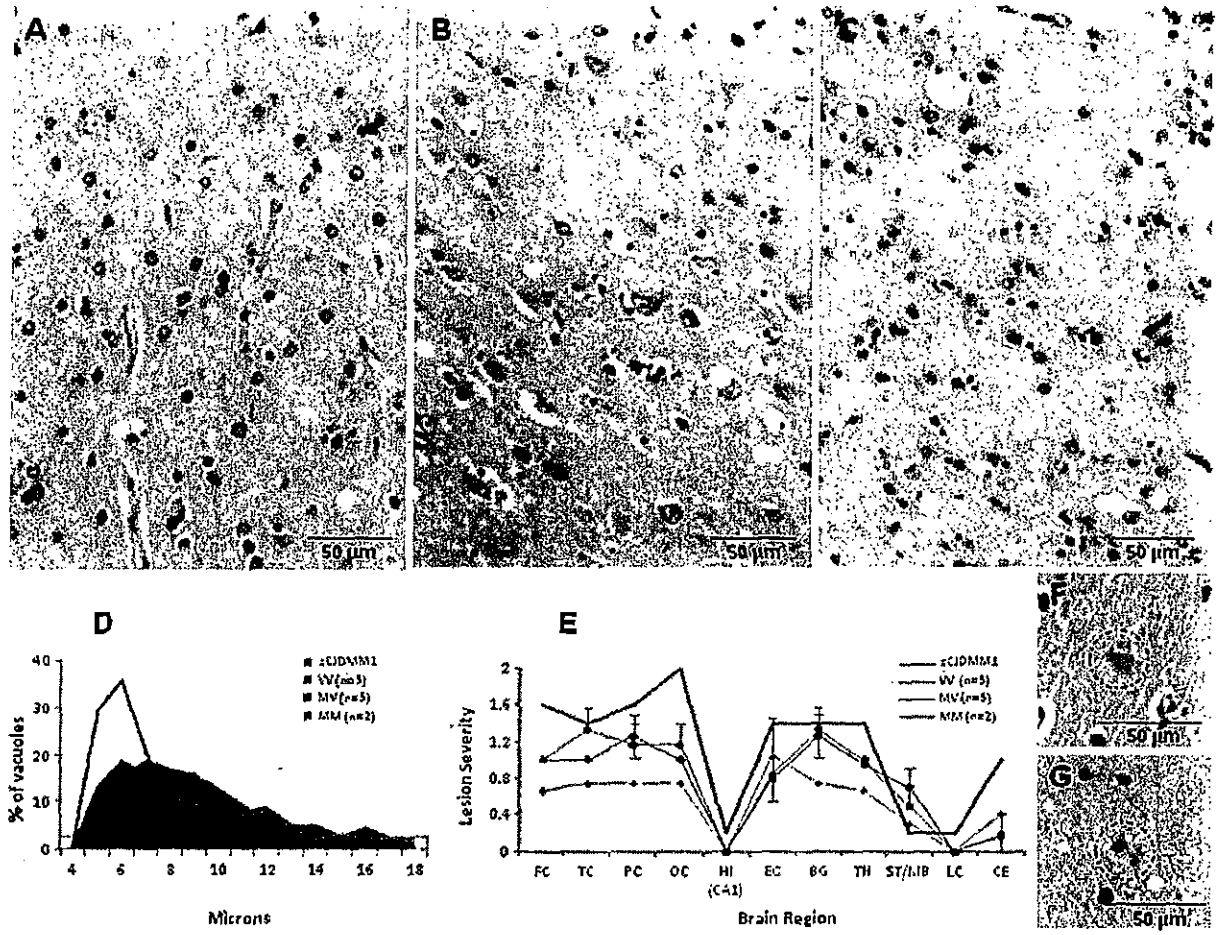


FIGURE 1: Histopathology with vacuole size and lesion profiles in the cases belonging to the 3 129 genotypes of variably protease-sensitive prionopathy (VPSPr). The spongiform degeneration is qualitatively similar in all 3 129 genotypes (A, 129VV; B, 129MV; C, 129MM). (D) As originally shown in the 129VV cases,[3] the spongiform degeneration is made of a significant percentage of relatively large and midsize vacuoles on average significantly larger than those of common sporadic Creutzfeldt-Jakob disease (sCJD) subtypes (diameters: VPSPr [combined] 9.3 ± 3.4μ vs sCJDMM1 5.8 ±1.2μ; $p < 0.0001$ [Student t test]), resulting in an elongated vacuole size distribution in the vacuole size histogram. (E) The lesion profiles are very similar in the 3 129 genotypes, but show less severe lesions in the 129MM genotype than in the 129VV and 129MV genotypes. FC, TC, PC, and OC= frontal, temporal, parietal, and occipital cortices; HI = CA1 of hippocampus; EC = entorhinal cortex; BG = basal ganglia; TH = thalamus (medial-dorsal nucleus); ST/MB = striatum/midbrain; LC = pons (locus coeruleus); CE = cerebellar cortex. The vertical bars refer to standard deviations. Spongiform degeneration was scored on a 0 to 4 scale (0, not detectable; 1, mild; 2, moderate; 3, severe; and 4, confluent), and astrogliosis on a 0 to 3 scale (0, not detectable; 1, mild; 2, moderate, and 3, severe). (F, G) Homogeneous micro deposits with the appearance of plaques were observed in the molecular layer of the cerebellum in some cases associated with the 129VV (F) and 129MV (G) genotypes, but not in the 3 129MM cases. (A–C, F, G) Hematoxylin & eosin. M = methionine; V = valine.

distinctive feature of the PSPr 129VV cases, was shared by all the affected subjects belonging to the 129MM and 129MV genotypes, although, due to the higher PK resistance, the representation of most PrP^{Dis} fragments was greater in 129MM and 129MV than in 129VV (Fig 3A).[3] The ladderlike profile demonstrated with 1E4 consisted of 5 major bands migrating at approximately 26kDa, 23kDa, 20kDa, 17kDa, and 7kDa (hereafter identified as VPSPr26, VPSPr23, VPSPr20, VPSPr17, and VPSPr7) (see Fig 3A). In contrast, PrP^{Dis} types 1 and 2 from sCJD formed the classical pattern of 3 bands migrating at 32/30kDa, 28/26kDa, and 21/19kDa (see Fig 3A).

PrP^{Dis} preparations from the 3 genotypes were probed with mAb 1E4 or 3F4 after treatment with various amounts of PK. When PrP^{Dis} fragments were considered all together, both 1E4 and 3F4 confirmed the relatively high PK resistance of PrP^{Dis} in the 129MM cases, intermediate in the 129MV cases, and low or entirely lacking in the 129VV cases (see Fig 3). However, both mAbs also showed the heterogeneous resistance of the individual fragments to PK, which was confirmed with

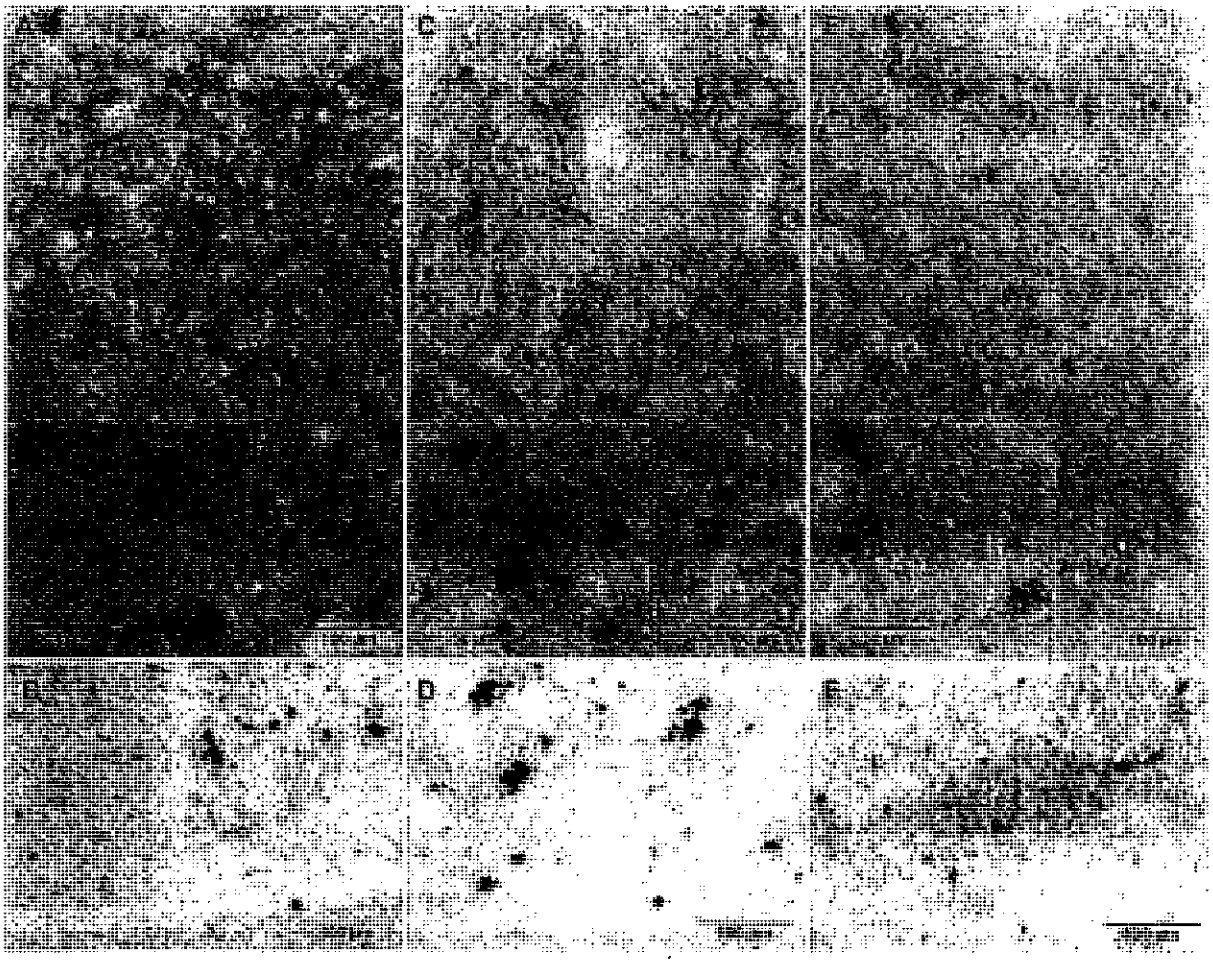


FIGURE 2: Prion protein immunohistochemistry in the 3 variably protease-sensitive prionopathy 129 genotypes. The cerebral cortex (A, C, E) and cerebellar molecular layer (B, D, F) best exemplify the predominant immunostaining patterns. (A) The pattern in the 129VV genotype is often targetlike, with a larger stained granule or clusters of granules surrounded by smaller granules in a focal or more diffuse background of punctate or synaptic staining (*inset:* higher magnification of the same cortical region). (B) The molecular layer of the cerebellum shows relatively large granules that are often compact and intensely stained. (C) In the 129MV genotype, the targetlike pattern is generally less obvious, as large granules are more often isolated; focal or larger areas of synaptic staining are also present (*inset:* as above). (D) In the cerebellum, the granules are fewer, are more loose, and have a plaquelike appearance. (E) The 129MM genotype often shows a plaquelike immunostaining pattern (*inset:* as above). (F) The cerebellum shows small formations. Immunostaining was done with monoclonal antibody 3F4. M = methionine; V = valine.

PK titration experiments. These analyses showed that the VPSPr7 fragment was highly PK-resistant in all 3 genotypes. In contrast, the other 4 fragments appeated to follow 2 distinct patterns, which were similar and involved pairs of the same fragments in both 129MV and 129MM; VPSPr26 and VPSPr20 increased and decteased rapidly in amount peaking at 10μg/ml of PK and generated fairly narrow bell-shaped curves. In contrast, borh PSPr23 and PSPr17 increased at a lower rate, peaked between 25 and 50μg/ml of PK, and remained relatively well represented even at 100μg/ml of PK. The representations of the 2 pairs of fragments were significantly different at 100μ/ml PK concentration in both 129 genotypes (129MM, $p < 0.02$; 129MV, $p < 0.005$). As expected, the PK tesistance of the 129VV fragments was much lowet, except for VPSPr7. Combined, the immunoblots and quantitative analyses argue that VPSPr23 and VPSPr17 have the strongest resistance to PK and likely form secondarily from VPSPr26 and VPSPr20 following treatment with high PK concentrations. It has to be noted, however, that the PK sensitivity of the 129VV preparations was in part related to the mAb used. When probing with 1E4 instead of 3F4, all fragments present in the VPSPr-129MM and -129MV preparations were also detectable in the preparations from the 129VV genotype, even if they displayed different ratios. Therefore,

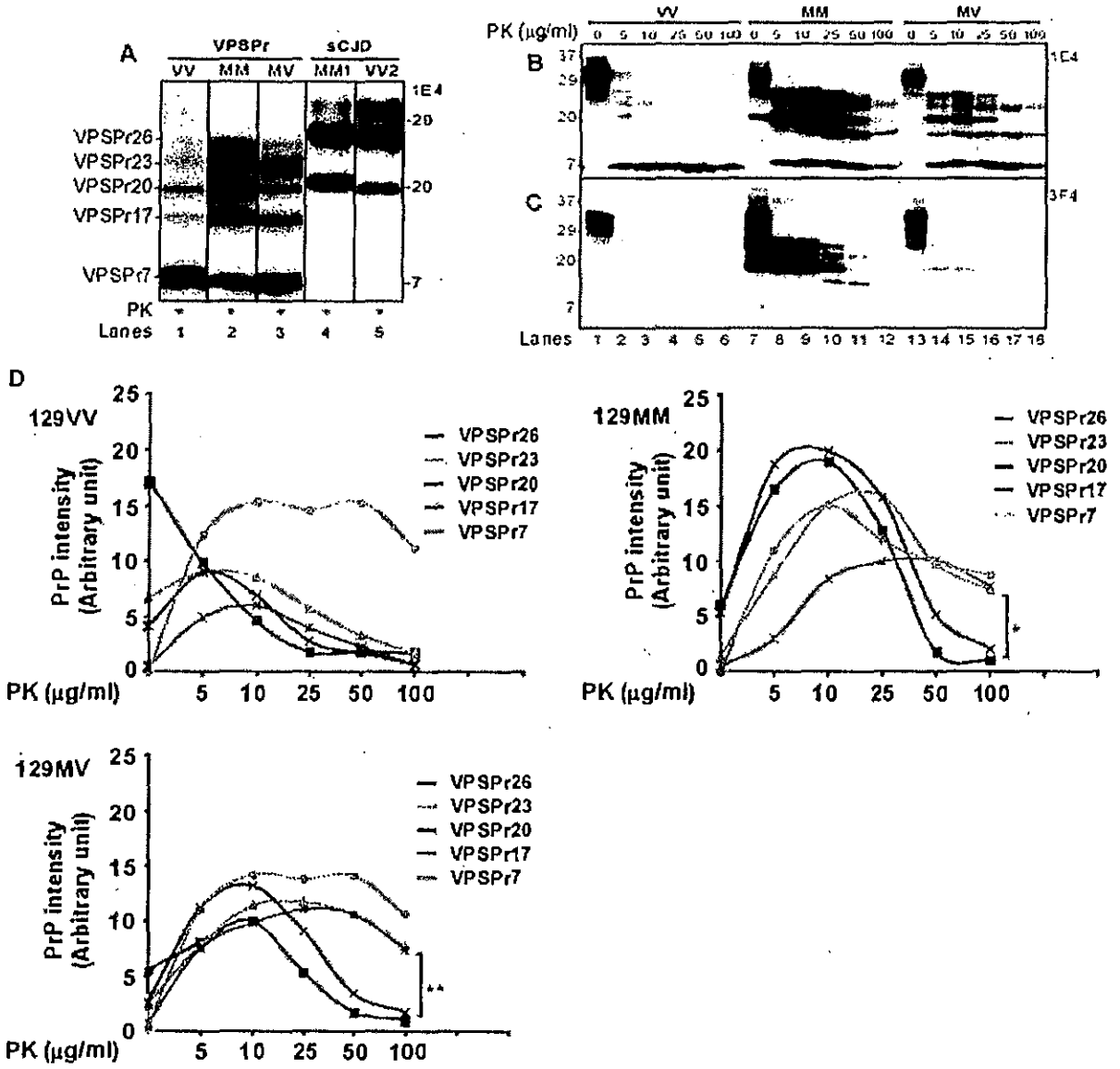


FIGURE 3: Electrophoretic profiles and proteinase K (PK) titration of PK-resistant disease-associated prion protein (PrP^{Dis}) from variably protease-sensitive prionopathy (VPSPr) associated with the 129VV, 129MM, or 129MV genotype. (A) The Western blots of the total brain homogenates (BHs) treated with 25μg/ml of PK and probed with the monoclonal antibody 1E4 reveal 5 PrP bands migrating approximately to 26kDa, 23kDa, 20kDa, 17kDa, and 7kDa, forming a ladderlike pattern in all 3 (129VV, 129MM, and 129MV) genotypes of VPSPr (VPSPr26, VPSPr23, VPSPr20, VPSPr17, and VPSPr7) *(lanes 1–3)*. The faint band that migrates at approximately 30kDa in VPSPr-129VV *(lane 1)* likely represents the incomplete PK digestion of the normal diglycosylated, N-terminus truncated PrP fragment or associated monoglycosylated full-length PrP. In contrast, BHs from sporadic Creutzfeldt-Jakob disease (sCJD) associated with the 129MM genotype and the PrP^{Dis} type 1 (sCJDMM1) or sCJDVV2 (sCJD with the 129VV genotype and PrP^{Dis} type 2) show the typical 3 PK-resistant PrP fragments of type 1 and 2 migrating between 31kDa and 19kDa (lanes 4 and 5). (B, C) PK titration of PrP^{Dis}. Brain homogenates from 129VV, 129MM, and 129MV genotypes were treated with PK at various concentrations between 0 and 100μg/ml. (B) Probed with 1E4. (C) Probed with 3F4. (D) PK titration with quantitative analysis of the individual VPSPr fragments. The curves represent the relative amounts of the individual VPSPr fragments at increasing PK concentrations (0–100μg/ml) after probing the immunoblots with 1E4 in each of the 3 129 genotypes. The relative representations of the bands corresponding to the VPSPr fragments were determined by densitometry and expressed as averages of 129VV (n = 3), 129MM (n = 2), and 129MV cases (n = 3). Comparative analysis of the curves from each of the 3 129 genotypes confirms the PK sensitivity of all the fragments in 129VV cases, with the exception of VPSPr7, which is resistant to PK in all 3 genotypes. The remaining 4 fragments follow similar patterns in both the 129MM and 129MV genotypes; VPSPr26 and VPSPr20 form rapidly but are digested at PK concentrations >10μg/ml; VPSPr23 and VPSPr17 are resistant up to 100μg/ml of PK (*$p < 0.02$; **$p < 0.005$). M = methionine; V = valine.

the PK treatment might not only break down the PrP^{Dis} associated with the 129VV genotypic form, but also generate fragments relatively undetectable by the mAb 3F4. Alternatively, PrP^{Dis} associated with VPSPr-129VV might have a low immunoreactivity with 3F4, even without PK treatment.

IDENTIFICATION OF THE PRP^{DIS} CORE FRAGMENTS AND THEIR COMPARISON WITH THOSE OF THE GSS VARIANT LINKED TO THE A117V MUTATION. Various amounts of VPSPr20, VPSPr17, and VPSPr7 were demonstrated by 1E4 in all 3 129 genotypes after deglycosylation and up to 50μg/ml of PK treatment (Fig 4A). Because the deglycosylation eliminated VPSPr26 and VPSPr23, these 2 fragments likely are the glycosylated isoforms of VPSPr20 and VPSPr17, respectively. This would explain the shared level of PK resistance of these 2 fragments (see Fig 3D). Of notice, the same PK-resistant fragments were well represented also without deglycosylation, suggesting that VPSPr20 and VPSPr17 are present as both glycosylated and unglycosylated isoforms (see Fig 3A, B). With 3F4, only VPSPr20 and VPSPr17 were detectable in the 129MM and 129MV cases, whereas again the 129VV genotype showed no PK-resistant PrP (see Fig 4B). The combined and individual resistance of the deglycosylated fragments to PK was comparable to that of the glycosylated isoforms.

Further characterization of the core fragments with the antibody anti-C (C-terminal residues 220–231) demonstrated 4 PrP bands migrating at approximately 20kDa, 18kDa, 12–13kDa, and 8kDa, of which only the 20kDa band matched VPSPr20 detected with 1E4 and 3F4 (see Fig 4C). The 3 fragments undetected by 1E4 and 3F4 must comprise the C-terminal region (reactive with anti-C) and must lack the 97–112 sequence

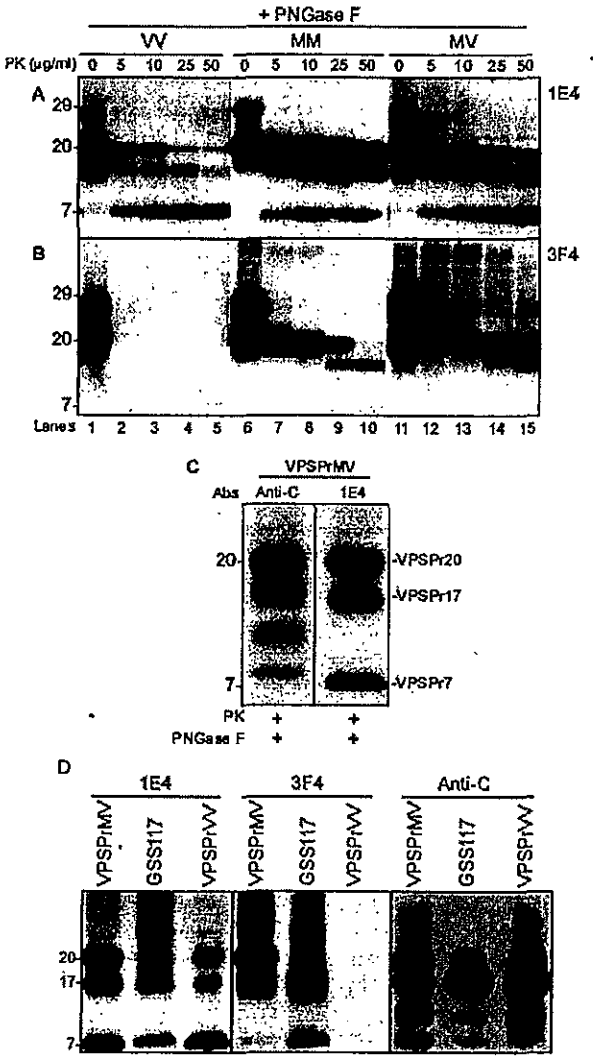


FIGURE 4: Proteinase K (PK)-resistant core fragments of variably protease-sensitive prionopathy (VPSPr) and their comparison with the disease-associated prion protein (PrP^{Dis}) fragments associated with Gerstmann-Sträussler-Scheinker disease (GSS)-A117V. All brain homogenates were treated with increasing concentrations of PK and with peptide N-glycosidase F (PNGase F). (A) With 1E4, all immunoblots from cases with the 129VV,129MM, and 129MV genotypes essentially show variably protease-sensitive prionopathy (VPSPr)-20, VPSPr17, and VPSPr7. However, the PK resistance of these bands varies according to the 129 genotype and the individual bands within the same genotype in a way roughly similar to that shown in Figure 3. (B) The immunoblots probed with 3F4 reveal 2 major bands in the 129MM and 129MV genotypes, which, however, exhibit a quite different pattern of resistance to PK in the 2 genotypes. As expected, no PK-resistant PrP bands are detected in the 129VV genotype. (C) Additional analysis of the core fragments following treatment with 25μg/ml of PK and PNGase, using the antibody anti-C to the PrP C-terminal residues 220–231. Compared to 1E4, anti-C demonstrates 4 bands of 20kDa, 18kDa, 12–13kDa, and 8kDa, respectively, of which only the 20kDa band has the same gel mobility as VPSPr20 detected with 1E4. The other 3 bands, including 18kDa, 12–13kDa, and 8kDa, do not match the bands detected with 1E4. (See text for explanation). (D) Brain homogenates from VPSPr-129MV, GSS117, and VPSPr-129 were treated with PK and PNGase F prior to Western blotting and probed with 1E4 *(left panel)*, 3F4 *(middle panel)*, and anti-C *(right panel)* antibodies. With monoclonal antibody 1E4, bands matching VPSPr20, VPSPr17, and VPSPr7 are detected in the GSS-A117V preparations, but the GSS-V117V bands immunoreact much less with 1E4 than the bands of VPSPr-129MV and VPSPr-129VV. The 23kDa band is seen more prominently in GSS-A117V. With 3F4, the VPSPr17 and VPSPr7 bands are shared by GSS-A117V and VPSPr-129MV, but the 20kDa band is missing in GSS-A117V. The VPSPr7 band is much less reactive in 129MV. As previously, the 129VV is not reactive with 3F4. Anti-C reveals apparently the same bands in all 3 preparations, but with significantly different ratios. M = methionine; V = valine.

containing the 1E4 and 3F4 epitopes. Therefore, 6 core fragments of relative molecular weights between 20kDa and 7kDa were identified by the combined use of 1E4 and anti-C. Several PrP C-terminal fragments of similar relative molecular weight have been previously reported.[18–20] Considerable similarities were observed in the electrophoretic mobilities of the PK-resistant core fragments from the 129MV, 129VV, and 129MM genotypes and GSS-A117V (see Fig 4D). Also in GSS-A117V, the mAb 1E4 demonstrated the presence of 3 bands of 20kDa, 17kDa, and 7kDa described in VPSPr, which, however, displayed different immunoreactivities. Comparable variations in antibody immunoreactivity and band representation were seen with 3F4 and anti-C (see Fig 4D). Therefore, most of the PK-resistant PrP^{Dis} fragments appeared to have similar sizes in VPSPr and GSS-A117V, but different ratios and antibody reactivities.

Discussion

At variance with the series of 11 cases of PSPr described in 2008 and 2 cases subsequently published by others—all 13 of which were 129VV homozygous at the PrP gene—the 15 cases reported here also include affected subjects who are 129MV and 129MM, in addition to new 129VV subjects.[3–5] Comparative analyses indicated that all these cases are affected by the same disease process, and that most of the heterogeneity that we observed results from distinct 129 genotypes.

These cases are likely to be affected by the same disease because of the overall similarity in major phenotypical characteristics, including the clinical features, which prominently exhibit aphasia, ataxia, and parkinsonian signs; SD, displaying vacuoles comparable in size in the 3 genotypes but otherwise different from the vacuoles of other common prion diseases; and finally PrP immunostaining patterns, which also display comparable general features in all these cases. However, the 2 most striking similarities reside in the ladderlike electrophoretic pattern of the PK-resistant fragments and in the unique immunoreactivity of PrP^{Dis} with mAb 1E4. Cumulatively, these findings suggest that all these cases share a similar molecular mechanism of PrP^{Dis} formation.

Significant clinical differences among the 129VV and 129MV groups (only 2 129MM symptomatic subjects were available) occurred in the mean age at onset and in disease duration (see Table). PrP immunostaining patterns were also distinguishable in the 3 groups. An additional difference might lay in disease prevalence, which appeared to be highest in 129VV subjects (65% of the cases), followed by the 129MV (23%) and 129MM subjects (12%) (see Table). However, 2 distinctive features were evident among the 3 groups; these were: (1) the apparent resistance to PK digestion, which was generally much lower in the 129VV cases than in the 129MM and 129MV cases; and (2) the immunoreactivity of the PK-resistant PrP^{Dis} with mAb 3F4, which was strong in the 129MM cases, weak in the 129MV cases, and lacking in the 129VV cases. Cumulatively, these findings argue that, although PrP^{Dis} may be formed by a similar mechanism in the 3 genotypes, the conformation or aggregation is likely different, and this difference results in variable resistance to PK, variable accessibility by 3F4, or both.

These findings also indicate that, in the present series of cases, it is the 129 genotype that modifies the phenotypic characteristics, including PK resistance and antibody immunoreactivity of PrP^{Dis}. However, the possibility that phenotypic heterogeneity is caused by other variations in PrP^{Dis} among the 3 groups or by a combination of different 129 genotypes and PrP^{Dis} characteristics, as is the case with sCJD, cannot be excluded.

The variations in prevalence are likely to be associated with the 129 genotype as well. This also is a feature of sCJD, in which 129MM cases account for about 70% of the total, 129MV for 11%, and 129VV for 17%.[1,12] It is remarkable that the effect of the 129 genotype on disease prevalence in our series of cases appears to be the opposite of that in sCJD. The high percentage of 129VV subjects described to date (20 of 28 known subjects, including the 2 cases reported elsewhere) and the apparent rarity of 129MM subjects (only 3 of 28 subjects) suggest that the prevalence of VPSPr is directly related to the presence of the 129V allele.[3–5] Indeed, at least 1 129V allele is present in 25 of the 28 known cases of VPSPr. The prevalence of the 3 129 genotypes in VPSPr is quite different from that in normal Caucasian populations, in which the 129MM genotype accounts for 43% of subjects, the 129MV for 49%, and the 129VV for 8%.[21]

The present findings raise a number of questions concerning the nature of VPSPr and its place within the group of known prion diseases.

In our series of 26 VPSPr cases collected to date, 8 subjects apparently had familial dementia; they were all 129VV except for 1 129MM. One of the 2 VPSPr-129VV cases reported by others also had a definitive family history of neurodegenerative disease.[4] This raises the possibility that VPSPr is a familial disease with a locus other than the ORF of the PrP gene (which is free of mutations), a condition analogous to that of familial Alzheimer disease.[3,22] Whether the VPSPr subjects reported to date also include inherited cases belonging solely to the 129VV and 129MM genotype remains to be determined.

Well-recognized prion diseases, which are associated with the classic PrP^{Dis} commonly identified as PrP27-30, such as all sCJD subtypes and several subtypes of familial CJD and sporadic and familial fatal insomnia, are transmissible with relative ease to receptive animals.

Inoculated animals develop a full-blown disease with clinical signs, SD, and presence of a PrP27-30 that generally reproduces the characteristics of the PrP27-30 present in the inoculum.[1,23,24] In contrast, other prion diseases, especially GSS, a rare phenotype that to date has been reported as exclusively associated with PrP gene mutations, have been more difficult to transmit or have been reported not to be transmissible at all.[25–28] For example, inoculation of brain homogenate from a subtype of GSS linked to the P102L mutation and characterized by the immunoblot presence of only a PK-resistant fragment of 7kDa similar to that present in VPSPr did not cause a symptomatic disease in recipient transgenic mice, but elicited the formation of PrP amyloid deposits in the absence of abnormal PrP.[26] Similarly, inoculation of PK-sensitive recombinant PrP polymerized into amyloid fibers generated a prion disease in PrP overexpressing transgenic mice that was apparently asymptomatic but caused SD and deposition of PK-sensitive abnormal PrP, 2 features shared by the 129VV genotype of VPSPr, only late in the life of the inoculated animals, consistent with very long incubation times.[29] Furthermore, similar transmission patterns on inoculation of brain homogenates from affected animals or humans have been observed in other neurodegenerative diseases, such as Alzheimer disease and diseases of the tau protein or tauopathies.[30,31] Experiments on the transmissibility of VPSPr are ongoing. Preliminary data indicate that VPSPr transmissibility, if it occurs at all, is not efficient, and it could be more like that of GSS-P102L associated with PrP^{Dis} 7kDa or of PK-sensitive PrP amyloid fibers, which require long incubation times and do not shorten the life span of the affected animals.[26,29]

It is intriguing that GSS also shows characteristics of the phenotype and of the PrP^{Dis} associated with some of the mutations that resemble those of VPSPr.[25] They include long disease duration, multiple PK-resistant fragments, and variable PK resistance of PrP^{Dis}. Our comparative analysis of the electrophoretic profiles in VPSPr and GSS-A117V reveals provocative similarities. This finding raise the issue of whether VPSPr might be viewed as the sporadic form of GSS.

Regardless of its relationship with GSS, the finding that VPSPr affects all 3 129 genotypes, resulting in distinct disease phenotypes and PrP^{Dis} characteristics, establishes VPSPr as the second "sporadic" prion protein disease, after sCJD.

Acknowledgment

This study was supported by the NIH (grants NIA AG14359 and AG08702, P.G.; NINDS R01NS062787, W.-Q.Z.), Centers for Disease Control and Prevention (grant CCU 515004, P.G.), Britton Fund (P.G.), CJD Foundation (W.-Q.Z.), Alliance BioSecure(W.-Q.Z.), University Center on Aging and Health with the support of the McGregor Foundation and President's Discretionary Fund (Case Western Reserve University) (W.-Q.Z.), and National Institute on Aging (grant AG05681, J.M.).

We thank the families of the patients for their generous cooperation; the CJD Foundation for its invaluable help; Drs N. Rance, P. Kirby, B. Zabel, M. DeVries, and J. Powers for help in obtaining the cases; D. Kofsky and P. Scalzo for skillful histologic and immunohistochemical preparations; Y. Cohen and W. Chen for insightful diagnostic Western blot preparations; Dr M. Zheng for contributing to sequencing of the PrP gene; and S. Berri, J. Blevins, and K. Glisic for ably assisting in managing the cases and preparing the manuscript.

Potential Conflicts of Interest

Nothing to report.

References

1. Gambetti P, Kong Q, Zou WQ, et al. Sporadic and familial CJD: classification and characterization. Br Med Bull 2003;66:213–239.
2. Prusiner S. Prions. Proc Natl Acad Sci U S A 1998;95:13363–13383.
3. Gambetti P, Dong Z, Yuan J, et al. A novel human disease with abnormal prion protein sensitive to protease. Ann Neurol 2008; 63:677–708.
4. Jansen C, Head MW, van Gool WA, et al. The first case of protease-sensitive prionopathy (PSPr) in The Netherlands: a patient with an unusual GSS-like clinical phenotype. J Neurol Neurosurg Psychiatry. 2010 Jun 14. [Epub ahead of print].
5. Head MW, Knight R, Zeidler M, et al. A case of protease sensitive prionopathy in a patient in the UK. Neuropathol Appl Neurobiol 2009;35:628–632.
6. Gambetti P, Puoti GF, Kong Q, et al. Novel human prion disease affecting 3 prion codon 129 genotypes: the sporadic form of Gerstmann-Sträussler-Scheinker disease? J Neuropathol Exp Neurol 2009:68;554.
7. Gambetti P, Kong Q, Zou WQ. A novel human prion disease affecting subjects with three prion protein codon 129 genotypes: could it be the sporadic form of Gerstmann-Sträussler-Scheinker disease? Prion 2008;124.
8. Gambetti P. A novel human prion disease affecting subjects with three prion protein codon 129 genotypes: could it be the sporadic form of Gerstmann-Sträussler-Scheinker disease? Prion 2009:45.
9. Capellari S, Powers JM, Petersen RB, et al. A novel molecular and clinico-pathologic phenotype of prion disease in an American. Neurology 1998;50:A235.
10. Giaccone G, Di Fede G, Mangieri M, et al. A novel phenotype of Creutzfeldt-Jakob Disease. J Neurol Neurosurg Psychiatry 2007; 78:1379–1382.
11. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1996;39:767–778.
12. Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999;46:224–233.

13. Kong Q, Huang S, Zou WQ, et al. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J Neurosci 2005;25:7944–7949.

14. Cali I, Castellani R, Alshekhlee A, et al. Co-existence of scrapie prion protein types 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effects on the phenotype and prion type characteristics. Brain 2009;132:2643–2658.

15. Yuan J, Xiao X, McGeehan J, et al. Insoluble aggregates and protease-resistant conformers of prion protein in uninfected human brains. J Biol Chem 2006;281:34848–34858.

16. Yuan J, Dong Z, Guo JP, et al. Accessibility of a critical prion protein region involved in strain recognition and its implications for the early detection of prions. Cell Mol Life Sci 2008;65:631–643.

17. Zou WQ, Langeveld J, Xiao X, et al. PrP conformational transitions alter species preference of a PrP-specific antibody. J Biol Chem 2010;285:13874–13884.

18. Zou WQ, Capellari S, Parchi P, et al. Identification of novel proteinase K-resistant C-terminal fragments of PrP in Creutzfeldt-Jakob disease. J Bio Chem 2003;278:40429–40436.

19. Colucci M, Moleres F, Xie Z, et al. Gerstmann-Straussler-Scheinker: a new phenotype with "curly" PrP deposits. J Neuropathol Exp Neurol 2006;65:642–651.

20. Taguchi Y, Shi ZD, Ruddy B, et al. Specific biarsenical labeling of cell surface proteins allows fluorescent- and biotin-tagging of amyloid precursor protein and prion proteins. Mol Biol Cell 2009; 20:233–244.

21. Zimmermann K, Turecek PL, Schwarz HP. Genotyping of the prion protein gene at codon 129. Acta Neuropathol 1999;97:355–358.

22. Selkoe DJ, Podlinsky MB. Deciphering the genetic basis of Alzheimer's disease. Ann Rev Genom Hum Genet 2002;3:67–99.

23. Korth C, Kaneko K, Groth D, et al. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci USA 2003;100: 4784–4789.

24. Asante EA, Linehan J, Desbruslais M, et al. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J 2002; 21:6358–6366.

25. Kong Q, Surewicz WK, Peterson RB, et al. Inherited prion diseases. In: Prusiner SB, ed. Prion biology and disease. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2004:673–775.

26. Piccardo P, Manson JC, King D, et al. Accumulation of prion protein in the brain that is not associated with transmissible disease. Proc Nat Acad Sci USA 2007;104:4712–4717.

27. Tateishi J, Kitamoto T, Hoque MZ, et al. Experimental transmission of Creutzfeldt-Jakob disease and related diseases to rodents. Neurology 1996;46:S32–537.

28. Hegde RS, Tremblay P, Groth D, et al. Transmissible and genetic prion diseases share a common pathway of neurodegeneration. Nature 1999;402:822–826.

29. Colby DW, Wain R, Baskakov IV, et al. Protease-sensitive synthetic prions. PLoS Pathog 2010;6:e1000736.

30. Meyer-Luehmann M, Coomaraswamy J, Bolmont T. Exogenous induction of cerebral β-amyloidogenesis is governed by agent and host. Science 2006;313:1781–1784.

31. Clavaguera F, Bolmont T, Crowther RA, Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 2009;11:909–913.

32. Krasnianski A, Kallenberg K, Collie DA, et al. MRI in the classical MM1 and the atypical MV2 subtypes of sporadic CJD: an inter-observer agreement study. Eur J Neurol 2008;15:762–771.

33. Heinemann U, Krasnianski A, Meissner B, et al. Creutzfeldt-Jakob disease in Germany: a prospective 12-year surveillance. Brain 2007;130: 1350–1359.

34. Castellani RJ, Colucci M, Xie Z, et al. Sensitivity of 14-3-3 protein test varies in subtypes of sporadic Creutzfeldt-Jakob disease. Neurology 2004;63:436–442.

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2010 年 7 月 29 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	乾燥濃縮人血液凝固第Ⅷ因子		研究報告の公表状況	European Medicines Agency/2010/07/24	公表国 欧州	
販売名（企業名）	コンコエイト-HT（ベネシス）					

研究報告の概要

この文書は、2003 年 2 月に公表され、2004 年 6 月と 2010 年 XXX に改訂された「クロイツフェルト-ヤコブ病と、血漿由来医薬品および尿由来医薬品」についての CPMP(脚注 2)の見解表明書(position statement) (EMEA/CPMP/BWP/2879/-02)の第 2 改訂版であり、1998 年 2 月に公表された「新しい変異型 CJD と血漿由来医薬品」についての CPMP の見解表明書に置き換わるものである。

要約

疫学的なエビデンスが集積してきたが、それらのエビデンスは血漿由来医薬品によってクロイツフェルト-ヤコブ病(CJD)の伝播が起こるとの考え方を裏付けるものではない。あるドナーがドネーション後に孤発性、家族性、もしくは医原性 CJD であることが確認された場合に、血漿由来医薬品のリコールを求めることは妥当ではない、とのこれまでの CHMP の立場には変化はない。

変異型 CJD(vCJD)は新たに出現してきた疾患であり、症例数が最終的にどの程度となるのかは不確かである。vCJD は孤発性 CJD と比較すると末梢組織中への分布がより広く感染性/異常プリオンタンパク質のレベルがより高い。英国で輸血によるヒトへの医原性 vCJD 感染が明確な 4 例から vCJD が輸血を介して伝播しうるものである、ということの強力なエビデンスが得られた。2009 年に、中程度の純度の第 VIII 因子の投与を受けた血友病 A 患者で感染性物質が検出されたが。この製剤は英国で 1998 年より前に採取されたプール血漿から調製されたものであった。

英国に居住していることは vCJD の危険因子とされており、そのことから英国は英国起原の血漿から分画を今後行わないことを決定するに至った。危険期間中に英国に長期間滞在したドナーから血液/分画用血漿のドネーションを受けないようにすることはこの英国の決定と一貫した措置である。1980 年初頭から 1996 年末までの間に英国に累積で 1 年間以上滞在したドナーを、血液/分画用血漿のドネーションから排除することを勧告する。

あるドナーについて英国での滞在に基づけば排除すべきであったことがドネーション後に判明した場合に、当該バッチを回収することは勧告されていないが、それは、このような排除が非常に慎重を期した予防措置だからである。

現在得られているデータでは、ヒト血漿中に vCJD の感染性が仮に存在していたとしても、血漿由来医薬品の製造工程で vCJD の感染性は低減されることが示されている。製造者にはその製造者の用いている製造工程で感染性を低減させる能力がどの程度あるか、ステップごとに調べて推定することが求められている。製造者がこの推定を行う際には、マイルストーンの各々でこの分野の専門家と相談することを勧告する。CHMP とそのバイオテクノロジー作業部会(BWP:Biotechnology Working Party)は、これらの勧告事項と取るべきアクションについて検討を続けるつもりである。

この勧告事項のサポートとして、CHMP と BWP は、外部の専門家の関与も依頼して、vCJD の危険性に関して製造工程の調査方法に関するガイダンスを作成したが、CHMP と BWP は生ずる可能性のある問題点について討議する用意がある。

このような立場を取る理由は、仮に将来、血液および血漿由来医薬品製造のための血漿を採取し集めている国々においてさらに vCJD の症例が発生したならば、以前に TSE 感染性を低減しうることが示されていた製造工程は、過去の製剤の安全性の再保証を与えることとなり、分画を継続することを妥当とすることの助けとなろう。

スクレイピーに感染したげっ歯類の尿中、および慢性消耗性疾患のシカの尿中に低レベルの TSE 感染性物質が検出されている。しかし、尿由来の医薬品による CJD もしくは vCJD の伝播の疫学的証拠はない。尿由来医薬品の製造工程の総合的レビューでは、ある製剤が比較

使用上の注意記載状況・
その他参考事項等

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

	的少数で特徴のよく分かっているドナー集団由来のものである場合には、ドナー選択基準を適用することが可能であることを指摘している。さらに、そのレビューでは、TSE 感染性が出発材料中に存在していたとした場合でも、理論的にはその感染性を低減することができると考えられるステップを、少なくとも 1 つ製造工程中に有していることを指摘している。尿由来医薬品は英国で採取された尿を原料とはしないこととなっている。 　このレビューおよびその他の考慮事項に基づいて、可能であれば、尿ドナーパネルを選択するための除外基準の使用が、予防的措置として勧められる。血漿由来医薬品の製造のための出発材料を提供する血液/血漿ドナーに用いるものとして CJD および vCJD に関しても同じ除外基準が適用されるべきであるが、血液/血漿ドナーとは異なり、それらの判定基準は各ドネーションごとにはチェックされない。尿由来医薬品の製造者が、血漿由来医薬品のためのアプローチと類似のものに従って、製造工程の TSE 感染性物質の低減/排除能を評価することを勧告する。	2. 重要な基本的注意 (1)略 1)略 2)略 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
これは血漿由来と尿由来製品（EMEA/CPMP/BWP/2879/02)のクロイツフェルトーヤニブ病に関する CPMP の見解表明書の第２改訂(案）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研究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。

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

London, 24 June 2010
EMEA/CPMP/BWP/2879/02/rev 2
Committee for Medicinal Products for Human Use (CHMP)

CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

Draft[1]

Draft Agreed by Biologics Working Party	May 2010
Adoption by CHMP for release for consultation	24th June 2010
End of consultation (deadline for comments)	30th September 2010
Agreed by Biologics Working Party	<Month YYYY>
Adoption by CHMP	<DD Month YYYY>

Comments should be provided using this template. The completed comments form should be sent to Alberto.Ganan@ema.europa.eu

Keywords	Creutzfeldt-Jacob disease, human Transmissible Spongiform Encephalopathies, plasma-derived medicinal products, urine-derived medicinal products, sporadic CJD, genetic CJD, iatrogenic CJD, variant CJD, blood infectivity, transmissibility

[1] Delete once the reflection paper is adopted.

7 Westferry Circus • Canary Wharf • London E14 4HB • United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu

CHMP position statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products

Table of contents

This is the second revision of the CPMP[2] Position Statement on "Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products" (EMEA/CPMP/BWP/2879/02) published in February 2003 and revised in June 2004 and XXX 2010, which replaced the CPMP Position Statement on "New variant CJD and plasma-derived medicinal products" (CPMP/201/98) issued in February 1998.

Summary

Cumulative epidemiological evidence does not support transmission of sporadic, familial and iatrogenic Creutzfeldt-Jakob disease (CJD) by plasma-derived medicinal products. There is no change to the previous CHMP position that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, familial or iatrogenic CJD.

Variant CJD (vCJD) is an emerging disease and the eventual number of cases of the disease is uncertain. There is a wider distribution and higher level of infectivity/abnormal prion protein in peripheral tissues than is seen with sporadic CJD. Four instances of apparent iatrogenic vCJD infection by blood transfusion in man in the UK provide strong evidence that vCJD is transmissible through blood transfusion. In 2009, the agent was detected in a haemophilia A patient who received intermediate purity FVIII prepared from pooled plasma sourced in the UK before 1998.

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding to no longer fractionate from UK plasma. It is consistent with this decision to exclude donors who have spent long periods in the UK during the risk period from donating blood/plasma for fractionation. It is recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation.

There is no recommendation to recall batches if information that would have excluded a donor based on his/her stay in the UK becomes available post-donation, since this is a very conservative precautionary measure.

Available data indicate that the manufacturing processes for plasma-derived medicinal products would reduce vCJD infectivity if it were present in human plasma. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity using a step-wise approach. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. CHMP and its Biotechnology Working Party (BWP) will keep progress with these recommendations and the actions to be taken under review.

In support of this recommendation, CHMP and BWP, with the involvement of external experts, have developed guidance on how to investigate manufacturing processes with regard to vCJD risk and CHMP and BWP are available to discuss issues that might arise.

The rationale for this position is that if, in the future, further cases of vCJD occur in countries collecting blood and plasma for the manufacture of plasma-derived medicinal products, a process previously shown to be able to reduce TSE infectivity will provide reassurance on the safety of past products, and could help to justify continuing fractionation.

Low levels of infectious TSE agents have been detected in the urine of scrapie-infected rodents and in the urine of deer with Chronic Wasting Disease. However, there is no epidemiological evidence of CJD or vCJD transmission by urine derived medicinal products. A general review of manufacturing

[2] In May 2004 there was a change in the name of the EMEA's scientific committee for human medicines from CPMP to CHMP.

processes for urine-derived medicinal products indicates that it is feasible to apply donor selection criteria when a product is derived from a relatively small and well-defined donor population. In addition, it indicates that manufacturing processes have at least one step that might be theoretically capable of reducing TSE infectivity if it were present in the starting material. It is noted that urine-derived medicinal products are not sourced from urine collected in the UK.

On the basis of this review and other considerations, the use of exclusion criteria for selection for a urine donor panel is encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the manufacture of plasma-derived medicinal products but, unlike blood/plasma donors, these criteria would not be checked at each donation. Manufacturers of urine-derived medicinal products are recommended to evaluate the capacity of the manufacturing process to reduce/eliminate TSE agents by following a similar approach to that for plasma-derived medicinal products.

1. Introduction

Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative disease belonging to the group of human Transmissible Spongiform Encephalopathies (TSEs) or prion diseases. Mortality rate of TSEs ranges approximately from 1.5 to 2 persons per million population per year. TSEs can occur sporadically (sporadic CJD (sCJD) and sporadic fatal insomnia), be associated with mutations of the prion protein gene (genetic TSEs (gTSE)), or result from medical exposure to infectious material (iatrogenic CJD (iCJD)). In 1996, a variant form of CJD (vCJD) was identified.[1] There is strong evidence that vCJD is caused by the agent responsible for bovine spongiform encephalopathy (BSE) in cattle.[2,3,4] The most likely hypothesis is that vCJD has occurred through exposure to BSE contaminated food.

Human TSEs, including in particular vCJD, were addressed in expert meetings/workshops at the EMEA in January 1998, January 1999, December 1999, May 2000, and December 2000. A CPMP Position Statement on variant CJD and plasma-derived medicinal products was issued in February 1998[5f] and the outcome of the subsequent meetings was published on the EMEA website.[5] An EMEA Expert Workshop on Human TSEs and Medicinal Products was held on 19-21 June 2002. This provided the scientific basis for a new CPMP Position Statement issued in 2003.[5b] A further EMEA Expert Workshop was held in January 2004 to review the current state of knowledge of vCJD, in the light of the recent report of a possible human transmission by blood transfusion.[6] In addition, the Workshop discussed the CPMP Discussion document on the investigation of manufacturing processes with respect to vCJD.[5a] In October 2005, a follow-up workshop was held to discuss the number of vCJD cases reported in France and other European countries and the potential effect of additional donor exclusion measures. Urine-derived medicinal products were specifically discussed at an EMEA expert workshop in July 2007[5g] after publication of experiments indicating transmission of prions via urine using a hamster model.

Blood and blood components for transfusion are outside the scope of this Position Statement. Recommendations on the suitability of blood and plasma donors and the screening of donated blood in the European Community were described in Council Recommendation 98/463/EC.[7c] European legislation on human blood and blood components entered into force on 8 February 2003[7a] Under this legislation, a Commission Directive on certain technical requirements for blood and blood components, including eligibility criteria for donors, entered into force in April 2004.[7b] In addition, Council of Europe Recommendation No. R (95) 15 contains a technical appendix on the use, preparation and quality assurance of blood components and details the current requirements for donors.[79]

In December 2003, following the announcement of a possible case of vCJD transmission by blood transfusion, Commissioner Byrne made a statement highlighting EU activities in the area of vCJD and announcing a meeting of the Working Group of the Blood Regulatory Committee to consider the latest information available from the UK.[7d] The meeting took place in January 2004 and a summary statement was produced.[7e]

The Scientific Steering Committee (SSC) and the Scientific Committee on Medicinal Products and Medical Devices (SCMPMD) of the European Commission have published a number of opinions relating to TSEs, which are of relevance to blood and blood components for transfusion, as well as to plasma-derived medicinal products.[8] WHO Guidelines on TSEs are also of relevance to both blood components for transfusion and plasma-derived medicinal products.[9] The Council of Europe has made recommendations for blood and blood components for transfusion.[10]

2. Human TSEs current status

2.1. Sporadic, genetic and iatrogenic forms of human TSEs

There is no evidence that sporadic, genetic or iatrogenic forms of human TSEs have been transmitted from person to person through exposure to plasma products or urinary derived medicinal products. Systematic surveillance for CJD of all types has been undertaken in a number of countries, including a collaborative study in the EU since 1993,[11,12] and no case of sporadic, genetic or iatrogenic CJD has been causally linked to prior treatment with plasma products. Cases of sporadic CJD with a history of drug treatment for infertility have not been identified but there is uncertainty about the validity of this observation. (See the report of the 2007 EMA expert meeting for further details.[5g]) Although there is evidence that plasma products have not been implicated in transmission of sporadic, genetic or iatrogenic CJD, the strength of the evidence excluding transmission by urinary derived medicinal products is less secure.

2.2. Variant CJD

The official UK figures for vCJD at the beginning of April 2010 were a total of 172 definite or probable vCJD cases.[13] (One case diagnosed in Hong Kong was classified as a UK case and is included in the UK figures.) Outside of the UK, there have been 25 cases in France[15], 5 in Spain, 4 in the Republic of Ireland, 3 in the Netherlands, 3 in the USA, 2 in Portugal and Italy and single cases in Canada, Saudi Arabia and Japan. 2 of the Irish cases, 2 of the US cases, 1 French case and the Canadian case had spent more than 6 months in the UK during the period 1980-1996 and were probably infected while in the UK. [14] The third US case has been reported as most likely infected when living in Saudi Arabia. The possibility of cases occurring in other countries cannot be excluded.

Two cases of vCJD identified in Spain occurred in the same family. No family links have been reported in any other vCJD cases to date.

All definite and probable cases, which have been genotyped so far, are Met-Met homozygotes at codon 129 of the prion protein (PrP) gene.[16] In 2009 a possible case of variant CJD was reported in the UK with a heterozygous codon 129 genotype. [17]

Analysis of the UK figures for the quarterly incidence of deaths indicates that vCJD incidence in the UK is currently in decline. However, interpretation requires caution as there may be a long tail or more than one peak to the epidemic.[18]

A UK study screening specimens from surgically removed appendices and tonsils for accumulation of prion protein in the lymphoreticular system has been carried out in order to try and obtain some estimation of the number of people that might be incubating vCJD in the UK.[19] Three positive appendix specimens have been found as a result of the screening of 12,674 appendix and tonsil specimens. However, the pattern of lymphoreticular accumulation in two of these samples was dissimilar from that seen in known cases of vCJD, raising the possibility that they may be false positives. With respect to this possibility, the authors comment that although it is uncertain whether immunohistochemical accumulation of prion protein in the lymphoreticular system is specific for vCJD, it has not been described in any other disease, including other forms of human prion disease or a range of inflammatory and infective conditions. Subsequent genetic analysis of residual tissue samples from these 2 cases found that both were valine homozygotes at codon 129 in the prion protein gene[20] This finding might account for the immunohistochemical features in these cases; all patients who have developed vCJD and have undergone a comparable genetic analysis have been methionine homozygotes at codon 129 in the prion protein gene.

Statistical analysis on this finding of 3 positive specimens gives the following estimations of numbers who may be incubating vCJD:

237 infections per million population (95% confidence interval (CI): 49-692 per million)

Assuming that this estimate relates to those aged 10-30 years[3], 3,808 individuals (CI 785-11 128) aged 10-30 years may be incubating vCJD in the UK.

These estimations are higher than predictions from modelling of the clinical data (upper 95% confidence interval of 540 future cases).[21] It is not known whether those incubating vCJD will eventually develop clinical disease. However, estimates of numbers possibly incubating are important with respect to any potential for secondary transmission (e.g. by blood donation, surgical instruments) while individuals are in the incubation phase. It should be noted that plasma-derived medicinal products have not been manufactured from donations collected in the UK since 1998.

A larger study of an archive of tonsil tissue from 63,007 people of all ages removed during routine tonsillectomies has been published.[22] 2,753 samples were from the 1961- 1985 birth cohort in which most cases of vCJD have arisen and 19,808 were from the 1986-1995 birth cohort that may also have been orally exposed to bovine spongiform encephalopathy. None of the samples were unequivocally reactive to two enzyme immunoassays and none of the initial reactives were positive for PrP^{TSE} by immunohistochemistry or immunoblotting. The estimated 95% confidence interval for the prevalence of PrP^{TSE} in the 1961-1995 birth cohort was 0-113 per million and in the 1961-1985 birth cohort 0-289 per million. These estimates are lower than the previous study of appendix tissue, but are still consistent with this study. Archiving of tonsil tissues continues and further studies are planned.

3. Human tissue distribution of infectivity/abnormal prion protein.

Tissue distribution has been investigated by detection of the abnormal prion protein PrP^{TSE} or by infectivity assays. Detection of PrP^{TSE} in tissues has often been associated with infectivity, however it should be noted that, in some circumstances, infectivity can be present without detection of PrP^{TSE} or PrP^{TSE} be present in absence of infectivity.[23] This may be related to limitations of assay methods for PrP^{TSE}, however, in some cases the reason for this finding is not known. It is thus recommended that

[3] The reason the age range of 10-30 years is specified is because 83% of the samples were from individuals in this age range.

any study on tissue or fluid distribution of the abnormal prion protein be confirmed with an infectivity assay.

A wider distribution and higher level of PrP^{TSE} in human peripheral tissues, including the lymphoreticular system, has been found in vCJD compared with sporadic CJD.[24;25,26] Limited data from infectivity assays of vCJD tissues are consistent with the PrP^{TSE} findings.[27] In clinical vCJD cases high titres of infectivity are found in the brain and spinal cord and lower levels in spleen and tonsil[27]. While PrP^{TSE} and infectivity are occasionally found in the spleen of sporadic CJD, the levels of PrP^{TSE} are lower than in vCJD.[8i] It is also suspected that lymphoid tissue involvement in sCJD is associated with a relatively long duration of clinical illness whereas it occurs preclinically in vCJD. PrP^{TSE} accumulations have been observed in muscles of some patients with both sporadic and variant CJD.[28]

It is likely that the distribution of PrP^{TSE} and infectivity in iCJD is more similar to sCJD than vCJD.[29] Data are lacking for gCJD.

4. Infectivity in blood and transmissibility via blood

4.1. Animal blood

Low levels of infectivity have been found in the blood of rodents experimentally infected with animal and human TSE agents.[30,31,32,33] Experiments indicate that approximately half the infectivity is in the cellular components, mainly the buffy coat, and the remainder in the plasma. Experimental studies indicate that the vCJD agent behaves in a similar way (qualitatively and quantitatively) to a genetic TSE agent[4] when adapted to RIII/Fa/Dk mice.[33] Infectivity has also been detected in buffy coat of a prosimian microcebe experimentally infected with a macaque-adapted BSE strain.[34]

The infectivity in rodent blood was transmitted by intravenous inoculation, but 5-7 fold less efficiently than by the intracerebral route.[31] In one study with mouse-adapted vCJD agent, the intravenous and intracerebral routes were found to be equally efficient for the buffy coat fraction but not for the plasma fraction.[33] However, studies in primates show that survival times were similar after intravenous or intracerebral inoculation of infected brain material.[35,36] Unpublished studies presented at scientific meetings[37,38] indicate that blood of primates experimentally infected with human TSE agent is infectious from about half way through the incubation period.

Furthermore, information from intra-species transfusion experiments indicates that experimental BSE in orally infected sheep or natural scrapie infection in sheep can be transmitted to sheep by blood transfusion.[39,40] Transmission efficiency was high for both BSE and natural scrapie, and the majority of transmissions resulted from blood collected more than half way through the incubation period[41]. The level of infectivity in sheep blood cannot be established from these experiments.

The European Union has provided funding for animal transmission projects.

4.2. Human blood

The tracing of recipients of blood transfusion from UK donors who have subsequently developed vCJD (the TMER study) has revealed four instances of secondary transmission.[42] These individuals had received transfusion of non-leucodepleted red cells from donors who were clinically healthy at the time of donation but subsequently (17–40 months later) developed variant CJD. Three of the four patients developed disease after incubation periods ranging from 6.5 to 8.5 years; the fourth died 5 years after

[4] Mouse-adapted GSS strain of human TSE (brain tissue obtained from a case of Gerstmann-Sträussler-Scheinker syndrome).

transfusion of an illness unrelated to prion disease but tested positive for PrP^{TSE} in the spleen and lymph nodes. This asymptomatic prion-infected patient was heterozygous (methionine/valine) at codon 129 of the *PRNP* gene. Taken together, these instances are strong evidence that vCJD is transmissible through blood transfusion.

Recently, another presumed case of prion infection was identified in an elderly haemophilic patient who was heterozygous at codon 129 in the prion protein gene.[43] The patient, who died of unrelated pathology, had received large quantities of UK-sourced fractionated plasma products, including some units derived from plasma pools which contained plasma from a donor who later developed variant CJD. This patient was identified through an intensive search for PrP^{TSE} positivity in all post-mortem tissues, although only 1 of 24 samples taken from the spleen tested positive. Whether someone with this limited distribution of PrP^{TSE} would be infectious is unknown, but from a public health perspective, this patient represents a warning that some plasma-derived products might contain residual prion infectivity.

The surveillance described above emphasises the importance of the TMER study for identifying the risk of blood transfusion in transmitting vCJD. Moreover, national databases of blood donors and the maintenance of traceability from donor to recipient and vice versa are essential to establish whether a vCJD case has been a blood donor (UK experience has shown that questioning of family members is unreliable for establishing whether a patient has been a blood donor). Traceability is a specific requirement in Article 14 of Directive 2002/98/EC.[7a]

Infectivity or PrP^{TSE} were not detected in blood of vCJD cases using methods capable of detecting infectivity/PrP^{TSE} in peripheral tissues such as tonsil or spleen, indicating that if infectivity is present it is at levels below the sensitivity of these methods. [27,24]

There is no epidemiological evidence that blood of sporadic CJD may transmit disease.[44,45] Prospective studies, similar to the TMER study, are in progress in the UK and USA and have not yet revealed any possible case of sporadic CJD linked to blood transfusion. However, current data are scanty to unequivocally exclude the possibility that such an event could occur in a small number of cases with a long (10 or more years) incubation period.[46]

A review of transmission studies to detect infectivity in the blood of humans with CJD (sporadic, iatrogenic and variant) shows that although experimental transmissions to animal models have occasionally been reported[47-50], other studies failed to detect infectivity.[51,27] It remains possible that PrP^{TSE} is present at low levels in the blood of clinically affected cases of sCJD. Data are lacking for gCJD but the assumption is that the tissue distribution of infectivity will be more similar to sCJD than vCJD.

For the purpose of risk assessments, it is recommended that, as a worst case assumption, a relative efficiency of the intravenous and intracerebral routes of 1:1 should be used.[52] This is because the accumulated information now available from animal studies indicates that the intravenous route can be an efficient route of transmission and in certain cases can give a transmission rate and/or an incubation period similar to the intracerebral route (see also 4.1).

5. Detection techniques

Several techniques are under development for the detection of PrP^{TSE} in blood including methods based on epitope protection[53] and PrP^{TSE} specific antibodies[54]. Approaches based on surrogate markers are also under investigation. Development and validation of all methods is on-going but there is no screening test yet. Confirmatory tests that have been proposed include Protein Mis-folding Cyclic Amplification (PMCA) [55] which is extremely sensitive, but has not yet been validated.

Several WHO reference preparations are available and further materials are under development[9b]. These reference preparations will allow calibration of assays versus infectivity bioassays, and can be used for collaborative studies to compare the performance of different assays to see whether they are sufficiently sensitive and specific to justify further evaluation for screening blood.

PrP^{TSE} detection methods for screening human blood for evidence of infection are being considered for inclusion as Annex II List A devices under the IVD Directive. There are very few samples of blood or plasma from clinically affected patients or from individuals known to have been infected at a particular time. This contrasts with other blood borne agents such as viruses. Alternative development and evaluation strategies have been proposed to assess whether a candidate assay is sufficiently promising to be given access to the available samples.[56]

6. Leucoreduction and specific prion affinity filters

Leucoreduction is used in transfusion medicine to reduce the level of white blood cells in blood and blood components. It was implemented in the UK in 1999.

The rationale for considering leucoreduction as a precautionary measure is:

- The lymphoreticular involvement in vCJD
- The detection of low levels of infectivity, in studies with rodents, in the buffy coat (associated with white blood cells).

The SCMPMD opinion on leucoreduction [8a, 8b] for blood and blood components for transfusion states that it might be a precautionary step to remove white cells as completely as possible. For plasma for fractionation the opinion states the following:

'Taken together, there is no compelling scientific evidence to date for the introduction of leucoreduction of plasma for fractionation, or other methods aiming at removal of cells and debris, as a precaution against vCJD transmission. The question should be further explored by suitable experiments.'

Results reported at the 2002 EMEA Workshop, suggested that leucoreduction does not provoke fragmentation of cells and lysis. Results of a comprehensive study involving a number of different filters and procedures indicate that leucodepletion is not detrimental in terms of the generation of microvesicles or the release of prion proteins[57].

Infectivity data from hamster studies indicate that leucoreduction alone is not totally protective against prion transmission, with between 42 to 72 percent reduction in infectivity of whole blood[58,59].

Specific affinity ligands that bind prion proteins are being evaluated for their ability to reduce TSE infectivity present in blood and plasma.

A study in hamsters showed that a leucocyte-reduction filter based on modified polyester fibres exhibited a prion clearance capability between 99.0 to 99.9 percent on the endogenous and exogenous infectivity of red cell concentrates[60].

Initial studies using leucoreduced human red blood cell concentrates spiked with hamster brain-derived scrapie infectivity indicate that some ligands immobilised on a chromatographic resin matrix are capable to removing 3 to 4 log ID_{50} per ml[59]. A further study using scrapie-infected hamster whole blood demonstrated an overall reduction of infectivity of more than 1.22 log ID[61].

The prion binding capacity of an affinity ligand chromatography step has been investigated in the processing of a plasma medicinal product using hamster brain derived spiking material[62]. This preliminary data requires further evaluation before conclusions can be drawn on possible efficacy.

7. Manufacturing processes for plasma-derived medicinal products

Taking account of the available data concerning blood infectivity, it is of utmost importance to investigate the capacities of the manufacturing process (fractionation) to eliminate/inactivate the infectious material potentially present in the plasma pool used as the starting material for preparation of plasma-derived products. Initial results from animal studies, using blood from rodents infected by intracerebral inoculation, indicated that the fractionation process contributes to the removal of endogenous plasma infectivity.[30,31] Information reported at the EMEA Workshops in 2002 and 2004 suggested that endogenous infectivity might persist through the fractionation process to a greater extent than would be expected from spiking studies,

Many investigational studies have now been carried out with different strains of agent and spiking materials of different nature and purity, and using different assays to follow the partition of PrP^{TSE} and/or infectivity. In most cases, the correlation between the capacity to partition PrP^{TSE} and infectivity has been demonstrated for the spiking preparations used until now (mainly brain homogenates of various strains). It is now confirmed that biochemical assays can be useful for spiking experiments to investigate manufacturing processes in a reasonable timeframe and less costly protocols than the *in vivo* bioassay. However it is still necessary to correlate such results with those from infectivity assays in animals. Cell-based assays may also be useful if properly validated for this purpose.

Studies aimed at investigating the contribution of the various manufacturing steps to reduction of infectivity (including precipitation followed by centrifugation or depth filtration, chromatography and nanofiltration) have accumulated convergent data supporting the removal of infectivity by steps that are commonly used in the manufacture of plasma-derived medicinal products.[62-68] For coagulation factors derived from cryoprecipitate, downstream fractionation using various precipitating agents or conditions allow to discard PrP^{TSE} in the precipitates. Reduction level achieved may vary according to the specific manufacturing process and probably depends on the concentration of the precipitating agent and salts, and the pH. Chromatographic steps, classically used in the separation of coagulation factors but also in the purification of other plasma derivatives have been described to remove TSE infectivity or PrP^{TSE}. Again, the reduction factors may be variable according to the fraction eluted. However, caution is still needed in the interpretation of those data since the effectiveness of a given step is dependent on a number of variables including the process conditions and the state/nature of the agent in the spiking preparation sample and in the spiked product intermediate. Consequently, effectiveness of removal may vary from one manufacturer to another. In addition, recent studies have highlighted the fact that removal capacity may be variable according to the state of dispersion of the agent in the spiking preparation particularly for steps based on retention mechanisms.

Overall, there is a need i) to investigate the partitioning or removal capacities of the various fractionation steps used in the preparation of the plasma-derived medicinal products, ii) to investigate the partition and removal of endogenous infectivity and the extent to which this is comparable with data from spiking studies, iii) to gain better knowledge of the form of infectivity present in blood in order to confirm the relevance of the spiking material used in the validation studies.

8. Infectivity in urine

Low levels of infectivity have been detected in urine of scrapie-infected rodents by several research groups and in the urine of deer with Chronic Wasting Disease.[5g, 9c]

Gregory *et al.*[69] demonstrated that the disease could be transmitted by intracerebral inoculation of pooled urine from scrapie-sick hamsters. The infectivity titre of the urine was calculated to be around 3.8 infectious doses/ml. Titration of kidney and urinary bladders from the same animals gave 20,000-fold greater concentrations. Histologic and immunhistochemical examination of these tissues showed no indication of inflammation or other pathologic changes, except for occasional deposits of disease-associated prion protein in kidneys.

Kariv-Inbal *et al.*[70] have observed transmission of the disease after intraperitoneal (i.p.) administration of enriched urine fractions from scrapie sick hamsters. Transmission via the oral route was also investigated. The recipient hamsters remained without symptoms but secondary transmission was observed after inoculation of brain extract from an asymptomatic hamster.

Seeger *et al.*[71] have studied transmission via urine using mouse models of chronic inflammation. They have detected prionuria in scrapie infected mice with coincident chronic lymphocytic nephritis. Transmission has been shown upon intracerebral inoculation of purified proteins from pooled urine collected from scrapie sick or presymptomatic mice. In contrast, prionuria was not observed in scrapie infected mice displaying isolated glomerulonephritis without interstitial lymphofollicular foci or in scrapie infected wild type mice lacking inflammatory conditions.

Prionuria was also detected in chronic wasting disease (CWD) of deer. Experiments by Haley *et al.*[72] provided evidence that concentrated urine from deer at the terminal stage of the disease, that also showed mild to moderate nephritis histopathologically, was infectious when inoculated into transgenic mice expressing the cervid PrP gene. In addition, the urine collected from the CWD sick deer that was used for mouse inoculation, showed positive results when assayed for PrP^{TSE} by serial rounds of protein misfolding cyclic amplification (PMCA) assay. The concentration of abnormal prion protein was very low as indicated by undetectable PrP^{TSE} by traditional assays and prolonged incubation periods and incomplete TSE attack rates in the transgenic mice.

Using the highly sensitive PMCA technology Gonzalez-Romero *et al.*[73] and Murayama *et al.*[74] have detected PrP^{TSE} in urine of scrapie sick hamsters. The results by Gonzalez-Romero *et al.* suggest that the concentration of PrP^{TSE} in urine is in average 10-fold lower than in blood. Animal experiments have demonstrated that *in vitro* generated PrP^{TSE} by PMCA starting from urine produced a disease indistinguishable from the one induced by infected brain material.[73]

Epidemiological evidence in the last 25 years, during which urinary-derived medicinal products and particularly gonadotrophins have been widely used, does not suggest a risk from sporadic CJD. Since epidemiological evidence has identified the few cases of iatrogenic transmission of CJD through the use of pituitary-derived gonadotrophins, it is possible that transmission from urinary-derived gonadotrophins would have been detected if it had occurred.

9. Recommendations and proposals

9.1. Sporadic, genetic and iatrogenic CJD and plasma-derived medicinal products

Cumulative epidemiological evidence does not support transmission of sporadic, genetic and iatrogenic CJD by blood, blood components or plasma-derived medicinal products.[75, 76, 12] Nevertheless, rigorous epidemiological studies for tracing blood-related sCJD cases have not yet reached sufficient statistical power to formally exclude the possibility of blood transmission in a small number of cases. Moreover, the experimental evidence of peripheral tissue infectivity in various subtypes of sCJD is very limited but available data show presence of infectivity in spleen and lymph nodes in human TSEs other than vCJD.

The implementation of appropriate actions in relation to CJD depends on accurate diagnosis in suspected cases. There is a potential for diagnostic confusion between sporadic and variant CJD, particularly in younger age groups.

Donor selection criteria include criteria to exclude donors who might be at higher risk of developing CJD. The following permanent deferral criteria are specified in Commission Directive 2004/33/EC: Persons who have a family history which places them at risk of developing a TSE, or persons who have received a corneal or dura mater graft, or who have been treated in the past with medicines made from human pituitary glands.[7b] Precautionary recalls of batches of plasma-derived medicinal products after post-donation reports of CJD or CJD risk factors in a donor contributed to severe shortages of certain products.[9a]

On the basis of the current epidemiological evidence, the CHMP recommendation that recall of plasma-derived medicinal products is not justified where a donor is later confirmed as having sporadic, genetic or iatrogenic CJD or CJD risk factors is maintained.

9.2. Variant CJD and plasma-derived medicinal products

Uncertainties still exist concerning the number of cases of vCJD that will occur although the number of cases is in decline in the UK and France. Variant CJD has a different distribution of infectivity in tissue outside the central nervous system to sporadic CJD.

There is now strong epidemiological evidence of human to human transmission of vCJD by blood transfusion (see Section 4.2). In addition, one vCJD infection was detected in a patient with haemophilia treated with high doses of intermediate purity factor VIII. Estimates of the relative risks of exposure through diet, surgery, endoscopy, blood transfusion and receipt of UK-sourced plasma products suggest that the most likely route of infection in the patient with haemophilia was receipt of UK plasma products. At least one batch came from a pool containing a donation from a donor who later developed vCJD.[43,77,]

The following measures are aimed at minimising the risk of transmission of the agent by plasma-derived medicinal products.

9.2.1. Exclusion Criteria

a) Consideration of Country-based exclusions

There is currently no screening test to detect donors who may be incubating the disease or in the early clinical stages. Therefore, other approaches are considered in order to try and identify donors who may present a higher risk.

UK plasma

Residence in the UK is a recognised risk factor for vCJD and has led to the UK deciding no longer to fractionate from UK plasma.

Exclusion of donors based on cumulative period of time spent in the UK

Since UK donors are excluded from donating plasma for the manufacture of plasma-derived medicinal products in the UK, it is consistent to exclude donors who have spent long periods in the UK. This is supported by the finding of vCJD cases, which have a risk factor of long periods spent in the UK, in other countries[5].

It is, therefore, recommended that donors who have spent a cumulative period of 1 year or more in the UK between the beginning of 1980 and the end of 1996 are excluded from donating blood/plasma for fractionation. Countries are highly encouraged to choose their national cumulative period limit for plasma-derived medicinal products according to a nationally calculated benefit/risk balance, which will take into account the endogenous risk of BSE exposure (and introduction in the food chain) and the risk of shortages of blood and plasma for the manufacture of medicinal products. The national limit is recommended to be of cumulative periods in the UK below or equal to 1 year.

Countries may still apply a stricter limit than 1 year for exclusion of donors for blood/plasma collected for fractionation within the country (e.g. 6 months) but will accept plasma-derived medicinal products from other countries provided that at least the one-year time limit is applied.

The rationale for this recommendation is to exclude donors who have the highest individual risk from stays in the UK and to be consistent with the UK decision to no longer fractionate from UK plasma. This is further explained in the first version of this Position Statement published in February 2003.[5b]

French plasma and plasma from other BSE-exposed European countries

France published an analysis of the risk of transmission of vCJD by blood and its derivatives sourced from French plasma in December 2000.[78g] This concluded that plasma collected in France could continue to be used for fractionation. The safety margin for plasma-derived medicinal products was considered to be sufficient. However, introduction of additional steps to further increase the safety margin of some products was recommended (e.g. nanofiltration of Factor VIII introduced in January 2001). Leucodepletion for plasma for fractionation, as for plasma for transfusion products, was also recommended in 2001 as a precautionary measure. The subsequent risk-analyses published in 2002, 2003, 2004, 2005, 2007 and 2009 re-confirmed these conclusions and acknowledged that the size of epidemic was revised to a lower estimate by more recent modeling, and the risk to collect blood from vCJD-incubating donors lower than previously estimated. [78]

Based on the limited data on human exposure to BSE-risk materials in other European countries it is still difficult to estimate the epidemiological risk in those countries which have small number of vCJD cases or have not yet reported any vCJD cases.

Donors who have spent a cumulative period of time in France and other BSE-exposed countries

Exclusion of donors who have spent a cumulative period of time in France is not recommended because of the lower risk associated with time spent in France compared with time spent in the UK (the risk in France is estimated to be 1/10 of that in UK). Since the previous version of the Position Statement, endogenous vCJD cases occurred in some other countries (see Section 2. Human TSEs current status) placing them close to or lower than France in terms of incidence and ratio of risk in

[5] Two cases in Ireland, two cases in US, one case in France and the Canadian case associated with long periods spent in the UK.

comparison to UK. Exclusion of donors who have spent time in other European countries having a risk ratio in the same order of magnitude as France is not recommended.

Concluding remarks

Country-based exclusions may appear unjustified in the sense that the vast majority of donors who will be excluded will not develop the disease. There is a lack of spare plasma capacity to make up for shortfalls if countries that are major producers of plasma-derived medicinal products discontinue the use of nationally collected plasma for fractionation.

b) Other possible exclusion criteria

Commission Directive 2004/33/EC indicates that further deferral criteria for vCJD may be recommended as a precautionary measure.[7b]

Other possible exclusion criteria that could be considered include permanent exclusion of recipients of blood transfusion (general exclusion or exclusion of recipients of transfusion in UK[6]), transplant recipients, and donors who have undergone neurosurgery.

Caution is needed because of the risk of loss of donors and consequent supply problems. Since such criteria could apply to both blood and blood components, and plasma-derived medicinal products, it was appropriate to consider this further within the scope of Directive 2002/98/EC.[7a] The technical meeting of blood experts, convened by the European Commission in January 2004, considered exclusion criteria, as well as blood component preparation and processing, recipient tracing and surveillance, and optimal use of blood.[7e]

9.2.2. Leucoreduction and specific prion affinity filters

The benefit of inclusion of leucoreduction to improve the safety of plasma has not been demonstrated.

At present it is not appropriate to recommend the introduction of leucoreduction for the safety of plasma-derived products.

Efficacy of introducing recently developed affinity media / filters is still under investigation.

9.2.3. Manufacturing processes for plasma-derived medicinal products

The available data support the reduction of infectivity by steps in the manufacturing process. Manufacturers are required to estimate the potential of their specific manufacturing processes to reduce infectivity. This should follow a step-wise approach as described below and illustrated in the accompanying flow diagram. It is recommended that manufacturers consult the relevant competent authorities at each of the milestones in this estimation. A decision to undertake an infectivity assay and/or to add a further manufacturing step(s) to increase reduction capacity should only be made after a careful consideration of all benefit-risk factors for a certain product.

Firstly, manufacturers should compare their own processes to those with published data on reduction of infectivity in order to estimate the theoretical potential of their specific manufacturing processes to reduce infectivity. *(Flow diagram, step 1)*

Whereas the general information available on manufacturing processes provides useful background information, the actual effectiveness of a manufacturing process might be dependent on the specific

[6] In April 2004, the UK implemented exclusion of persons who have previously received transfusions of whole blood components since January 1980, as a precautionary approach.

process conditions. Manufacturers should consider the relevance of the published data to their specific manufacturing processes and whether the removal capacity can be expected to be comparable.

If it cannot be concluded that the removal capacity would be expected to be comparable, it is recommended that manufacturers undertake product-specific investigational studies on key steps in their manufacturing processes using biochemical assays. Priority should be given to studies on products with the lowest potential removal capacity. *(Flow diagram, step 2)*

Investigations using biochemical assays may be sufficient if a clear correlation with infectivity data has already been established for similar processes (e.g. ethanol fractionation). If such a correlation is not established (e.g. a novel step) and the step is considered critical for removal of infectivity for the specific product (e.g. it is the only step for removal), the investigations should be confirmed using an infectivity assay for the critical step(s). *(Flow diagram, step 3)*

The above steps will allow manufacturers to estimate the reduction capacity of their manufacturing processes. *(Flow diagram, step 4)*

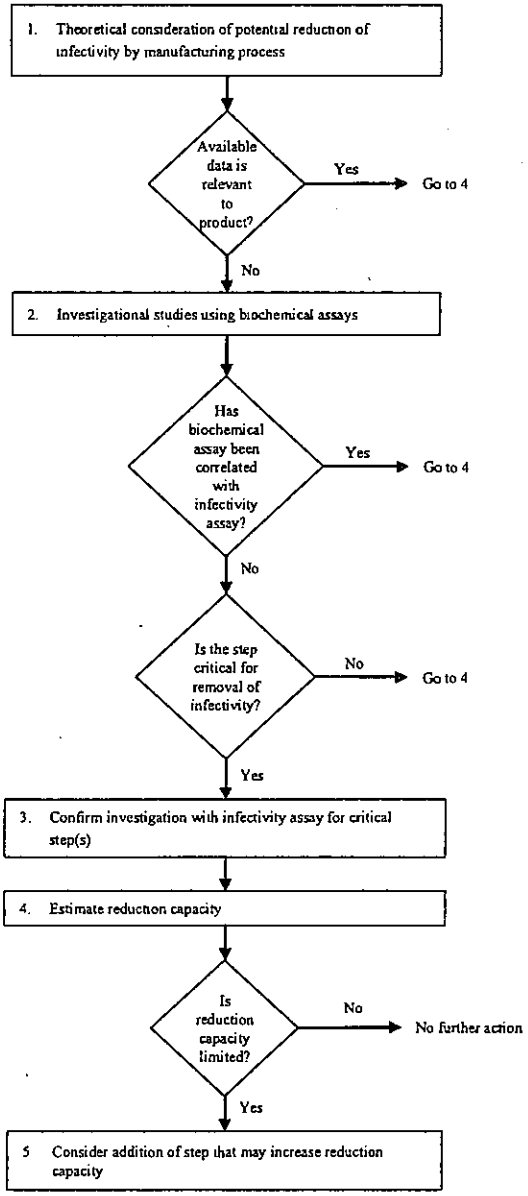
In cases where the overall reduction capacity is limited, manufacturers should consider the addition of steps that may increase the removal capacity where this is feasible without compromising the safety, quality and availability of the existing products. Discussion with the relevant competent authorities is recommended. *(Flow diagram, step 5)*

The outcome of the estimates of the theoretical potential of manufacturing processes to reduce infectivity and the results of product-specific investigational studies should be reported to the relevant competent authorities for the medicinal products concerned, as information becomes available. Applicants submitting new marketing authorisation applications for plasma-derived medicinal products will be expected to include such information in the application dossier. The outcome of the estimation of the theoretical potential to reduce infectivity should always be included in the application.

In support of these recommendations, CHMP's Biotechnology Working Party, with the involvement of external experts, has developed guidance on how to investigate manufacturing processes with regard to vCJD risk.[5a]

Figure 1: Plasma-Derived Medicinal Products: estimation of potential reduction capacity of specific manufacturing processes

Important Note: this flow diagram should be read in conjunction with the preceding text in 9.2.3. It is recommended to consult the relevant competent authorities at the milestones in this estimation. Give priority to studies on products with the lowest potential removal capacity.



9.2.4. Recall of batches where information becomes available post-donation

In view of the lack of adequate information on vCJD, it is prudent to recall batches of plasma-derived medicinal products where a donor to a plasma pool subsequently develops vCJD. Recall should also include medicinal products containing plasma-derived products as excipients. However, in both cases, consequences for essential medicinal products where alternatives are not available will need careful consideration by the competent authorities.

A case-by-case consideration would be appropriate where plasma-derived products have been used in the manufacture of other medicinal products. This consideration would include the nature of the product, the amount used, where it is used in the manufacturing process and the downstream processing.

Look-back to identify the fate of donations should be taken as far as possible. Regulatory authorities, Official Medicines Control Laboratories, surveillance centres and the supply chain should be informed of all batches of product and intermediate implicated whether or not supplies of the batch are exhausted.

There is no recommendation to recall batches if information becomes available post-donation, which would have excluded a donor based on his/her stay in the UK since this donation exclusion criteria is a very conservative precautionary measure (see 9.2.1).

9.2.5. Albumin used as an excipient or in manufacturing processes

The available data on the removal of infectivity during the fractionation process used in the manufacture of albumin indicates that the risk of transmission of infectivity by albumin would be particularly low. Nevertheless, in the case of albumin used as an excipient, recall is still recommended as a precautionary measure where a donor to a plasma pool subsequently develops vCJD. A single batch of albumin may be used to produce a number of batches of a medicinal product because of the small amounts that are typically used as an excipient. As a consequence, a recall could affect complete stocks of a product and create severe shortages. Therefore, to avoid a negative impact on supply, companies should consider the origin of plasma and select countries where the probability of having to recall batches is as limited as possible.

Development of substitutes for plasma-derived albumin used as an excipient or in manufacturing processes is encouraged although it is recognised that this can be difficult (requiring development and validation and usually non-clinical and clinical investigations) and should thus be considered as a long-term approach.

9.2.6. Substitution with alternative products

Use of alternative products to plasma-derived medicinal products could be considered, where these are available. It is felt that this choice should remain with users, taking into account the needs of the individual patient. It should be noted that plasma-derived products such as albumin may be used in the manufacture of recombinant products.

9.2.7. Optimal Use

Optimal use of plasma-derived medicinal products is encouraged, as this will maximise the benefits of the products compared with any potential risk.

9.3. Urine-derived medicinal products

The recommendations for urine-derived medicinal products are based on the following considerations:

There is no epidemiological evidence of CJD and vCJD transmission by urine-derived medicinal products.

TSE infectivity in urine has been reported in some animal models.

The review of manufacturing processes described below.

614 Investigational studies of infectivity reduction by the manufacturing processes should be done following
615 the same general, stepwise approach as recommended for plasma derived medicinal products (see
616 Section 9.2.3).[5a]

617 Results from different assay systems are not necessarily directly comparable (Western blot, cell based
618 assays, bioassay). The approach recommended for plasma-derived medicinal products would be
619 applicable (i.e. confirm reduction capacity using infectivity assays for steps critical for reduction of
620 infectivity if a clear correlation between data from biochemical assays and infectivity assays has not
621 been established for similar process steps). For inactivation studies, investigation of different TSE
622 strains should be considered as they may vary in resistance.

623 Potential accumulation of prions on chromatographic columns or a potential batch to batch
624 contamination due to carry-over of prions should be addressed in the studies.

625 Bibliographic data could be acceptable as additional supportive data to the investigational studies
626 provided. Similarity of the compared process and materials should be established. Extrapolation of
627 results for plasma-derived medicinal products is not justified particularly for chromatographic steps at
628 the beginning of the manufacturing process because of the high protein content in plasma.

629 General review of the manufacturing processes indicates that, in each manufacturing process, there is
630 at least one step that might be theoretically capable of reducing infectivity if it were present in the
631 starting material. In cases where the reduction capacity is limited, manufacturers should consider the
632 addition of steps that may increase the overall removal capacity.

633 For particular products, such as hormones from a relatively small well-defined donor population, some
634 manufacturers have put in place limited exclusion criteria for the selection of a donor for inclusion in a
635 donor panel. For other products manufactured from very large donor pools (e.g. urokinase), such
636 measures are more difficult to apply.

637 Urine should be collected from countries where there is a surveillance system for both human and
638 animal TSEs. It is noted that urine-derived medicinal products are not sourced from urine collected in
639 the UK.

640 On the basis of these considerations, the use of exclusion criteria for selection for a donor panel are
641 encouraged, as a precautionary measure, where feasible. The same exclusion criteria should be applied
642 with respect to CJD and vCJD as used for blood/plasma donors providing starting material for the
643 manufacture of plasma-derived medicinal products. Although these criteria would not be checked at
644 each donation unlike blood/plasma donors, manufacturers should follow up the donor criteria at
645 defined intervals. The exclusion of donors with known inflammation of kidney and/or chronic renal
646 inflammatory diseases is encouraged.

647 Record keeping for traceability is recommended for products where it is possible to trace back to donor
648 level.

References

1. Will RG, Ironside JW, Zeidler M, Cousens SN, Estbeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A. Smith PG. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996; 47:921-25.

2. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttle A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. Nature 1997;389:498-501.

3. Hill AF, Desbruslais M, Joiner S, Sidle KCL, Gowland I, Collinge J. The same prion strain causes vCJD and BSE. Nature 1997;389:448-450.

4. Scott MR, Will R, Ironside J, Nguyen HO, Tremblay P, DeArmond SJ, Prusiner SB. Compelling transgenic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc. Natl. Acad. Sci 1999;96:15137-15142.

5. **EMEA**

5a Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to vCJD risk. October 2004, EMEA/CPMP/BWP/5136/03.
http://www.ema.europa.eu/pdfs/human/bwp/513603en.pdf

5b CPMP Position Statement on Creutzfeldt-Jakob disease and plasma-derived and urine-derived medicinal products. February 2003, EMEA/CPMP/BWP/2879/02.
http://www.ema.europa.eu/pdfs/human/press/pos/287902en.pdf

5c Summary Report of EMEA Expert Meeting on Human TSEs and medicinal products derived from human blood and plasma, 1 December 2000, EMEA/CPMP/BWP/450/01, 28 March 2001.
http://www.emea.europa.eu/pdfs/human/regaffair/045001en.pdf

5d Report of EMEA Expert Workshop on Human TSEs and plasma-derived medicinal products, 15-16 May 2000, EMEA/CPMP/BWP/1244/00, 26 July 2000.
http://www.ema.europa.eu/pdfs/human/regaffair/045001en.pdf

5e EMEA Workshop on application to pharmaceuticals of assays for markers of TSE, February 1999, CPMP/257/99.
http://www.ema.europa.eu/pdfs/human/bwp/025799en.pdf

5f CPMP Position Statement on New variant CJD and plasma-derived medicinal products, February 1998, CPMP/201/98.
http://www.ema.europa.eu/pdfs/human/press/pos/020198en.pdf

5g Report on Expert Workshop on CJD Risk and Urine-derived medicinal products EMEA, London - 12-13 July 2007.
http://www.ema.europa.eu/pdfs/human/bwp/47271709en.pdf

6. Llewelyn CA, Hewitt PE, Knight RSG, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363:417-421.

7. **European Commission**

7a Directive 2002/98/EC of the European Parliament and Council setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83 EC, OJ L 33, 8.2.2003, pp. 30-40.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:033:0030:0040:EN:PDF

7b Commission Directive 2004/33/EC implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components, OJ L 91,30.3.2004, pp.25-39.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:091:0025:0039:EN:PDF

7c Council Recommendation of 29 June 1998 on the suitability of blood and plasma donors and the screening of donated blood in the European Community (98/463/EC), OJ L 203 pp.14-26. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:203:0014:0026:EN:PDF

7d Statement of Commissioner Byrne on possible vCJD transmission by blood. Brussels, 18 December 2003, IP/03/1781. http://europa.eu/rapid/pressReleasesAction.do?reference=IP/03/1781&format=HTML&aged=0&language=EN&guiLanguage=en

7e Summary Statement of Technical Meeting of Blood Experts related to vCJD transmission. Luxembourg 20 January 2004, D(2004) FMD/360007.

8. European Commission: SSC, SCMPMD, SCENIHR

SCMPMD

8a Opinion on the Safety of Human-Derived Products with Regard to TSE's, adopted on 18 January 2002. http://ec.europa.eu/health/ph_risk/committees/scmp/documents/out40_en.pdf

8b Opinion on update of the opinion on the Risk Quantification for CJD Transmission via Substances of Human Origin, adopted on 16 February 2000. http://ec.europa.eu/health/ph_risk/committees/scmp/documents/out28_en.pdf

8c Opinion on the Policy Regarding the Use of Blood and Blood Products adopted by Written Procedure on 24 March 1999. http://ec.europa.eu/health/scientific_committees/emerging/opinions/scmpmd/scmp_out20_en.htm

8d Opinion on the risk quantification for CJD transmission via substances of human origin, adopted on 21/10/98. http://ec.europa.eu/health/ph_risk/committees/scmp/documents/out12_en.pdf

SSC

8e Opinion on the Implications of the Recent Papers on Transmission of BSE by Blood Transfusion in Sheep (Houston et al, 2000; Hunter et al, 2002), adopted September 2002. http://ec.europa.eu/food/fs/sc/ssc/out280_en.pdf

8f Opinion on the Implications of the Houston et al paper in The Lancet of 16 September 2000 on the Transmission of BSE by blood transfusion in sheep. (The Lancet, Vol.356, pp999-1000;955-956;1013). http://europa.eu.int/comm/food/fs/sc/ssc/out143_en.pdf

8g Oral exposure of Humans to the BSE agent: infective dose and species barrier adopted by the SSC at its meeting of 13-14 April 2000 following a public consultation via Internet between 6 and 27 March 2000. http://ec.europa.eu/food/fs/sc/ssc/out79_en.pdf

8h Opinion on the Human Exposure Risk (HER) via food with respect to BSE - Adopted on 10 December 1999. http://ec.europa.eu/food/fs/sc/ssc/out67_en.pdf

SCENIHR

8i Opinion on the Safety of Human-derived Products with regard to Variant Creutzfeldt-Jakob Disease adopted on 28-29 September 2005 http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_004.pdf

9. **WHO**

9a WHO Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products. 2003. http://www.who.int/biologicals

9b WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies. 2006

http://www.who.int/biologicals

9c WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies. Updated 2010.
http://www.who.int/biologicals

10. Council of Europe Recommendation Rec(2001)4 on the prevention of the possible transmission of variant Creutzfeldt-Jakob Disease (vCJD) by blood transfusion.
http://www.coe.int/t/dg3/health/recommendations_en.asp

11. Ladogana A et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology. 2005; 64(9):1586-91.

12. Dorsey K et al. Lack of evidence of transfusion transmission of Creutzfeldt-Jakob disease in a US surveillance study. Transfusion (2009) 49: 977-984.

13. UK Monthly Creutzfeldt Jakob disease statistics.
http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/CJDGeneralInformation/CJDGeneralArticle/fs/en?CONTENT_ID=4032396&chk=5shT1Z

14. Worldwide vCJD statistics.
www.eurocjd.ed.ac.uk/vCJD.htm

15. French Monthly Creutzfeldt Jakob disease statistics.
http://www.invs.sante.fr/publications/mcj/donnees_mcj.html

16. Ward HJ, MacKenzie JM, Llewelyn CA, Knight RS, Hewitt PE, Connor N, Molesworth A, Will RG,(2009) Variant Creutzfeldt-Jakob disease and exposure to fractionated plasma products.Vox Sang. 2009 Oct;97(3):207-10. Epub 2009 Jun 16.

17. Kaski D, Mead S, Hyare H, Cooper S, Jampana R, Overell J, Knight R, Collinge J, Rudge P. Variant CJD in an individual heterozygous for PRNP codon 129. Lancet (2009) 374: 2128,

18. Andrews N. Further evidence that vCJD incidence in the UK is currently in decline. Eurosurveillance Weekly 2004;8(20):13/05/2004.

19. Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. Journal of Pathology, 2004;203:733-739.

20. Ironside JW, Bishop MT, Connolly K, Hegazy D, Lowrie S, Le Grice M, Ritchie DL, McCardle LM, Hilton DA. Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study. BMJ. 2006 May 20;332(7551):1186-8.

21. Ghani AC, Donnelly CA, Ferguson NM, Anderson RM. Updated projections of future vCJD deaths in the UK. BMC Infect Dis 2003;3:4.

22. Kelly C. Tonsil study provides estimate of the number of people in the UK who could be incubating vCJD. Eurosurveillance Weekly 2004;8(24):10/06/2004.

23. Piccardo P, Manson J, King D, Ghetti B, Barron RM: Accumulation of prion protein in the brain that is not associated with transmissible disease. PNAS 104: 4712-4717, 2007.

24. Wadsworth JD, Joiner S, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet 2001;358:171-180.

25. Head MW, Ritchie D, Smith N, McLoughlin V, Nailon W, Samad S, Masson S, Bishop M, McCardle L, Ironside JW. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease. American Journal of Pathology 2004;164:143-153.

834 26. Glatzel M, Abela E, Maissen M, Aguzzi A. Extraneural pathologic prion protein in sporadic
835 Creutzfeldt-Jakob disease. New England Journal of Medicine 2003;349:1812-1820.
836
837 27. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease
838 infectivity in extraneural tissues. Lancet 2001;358:208-209.
839
840 28. Peden AH, Ritchie DL, Uddin HP, Dean AF, Schiller KA, Head MW, Ironside JW Abnormal prion
841 protein in the pituitary in sporadic and variant Creutzfeldt-Jakob disease. J Gen Virol. 2007
842 Mar;88 (Pt 3):1068-72.
843
844 29. Head MW, Ritchie D, Smith N, McLoughlin V, Nailon W, Samad S, Masson S, Bishop M, McCardle
845 L, Ironside JW. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-
846 Jakob disease: an immunohistochemical, quantitative, and biochemical study. Am J Pathol. 2004
847 Jan;164(1):143-53.
848
849 30. Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN. The distribution of
850 infectivity in blood components and plasma derivatives in experimental models of transmissible
851 spongiform encephalopathy. Transfusion 1998;38:810-816.
852
853 31. Brown P, Cervenáková L, McShane LM, Barber P, Rubenstein R, Drohan WN. Further studies of
854 blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an
855 explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans.
856 Transfusion 1999;39:1169-1178.
857
858 32. Taylor DM, Fernie K, Reichl HE, Somerville RA. Infectivity in the blood of mice with a BSE-
859 derived agent. Journal of Hospital Infection 2000;46:78.
860
861 33. Cervenakova L, Yakovleva O, McKenzie C, Kolchinsky S, McShane L, Drohan WN, Brown P.
862 Similar levels of infectivity in the blood of mice infected with human-derived vCJD and GSS
863 strains of transmissible spongiform encephalopathy. Transfusion 2003;43:1687-1694.
864
865 34. Bons N, Lehmann S, Mestre-Francès N, Dormont D, Brown P. Brain and buffy coat transmission
866 of bovine spongiform encephalopathy to the primate *Microcebus murinus*. Transfusion
867 2002;42:513-516.
868
869 35. Herzog C, Salès N, Etchegaray N, Charbonnier A, Freire S, Dormont D, Deslys J-P, Lasmézas CI.
870 Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or
871 oral infection. Lancet 2004;363:422-28.
872
873 36. Lasmézas CI, Fournier J-G, Nouvel V, Boe H, Marcé D, Lamoury F, Kopp N, Hauw J-J, Ironside J,
874 Bruce M, Dormont D, Deslys J-P. Adaptation of the bovine spongiform encephalopathy agent to
875 primates and comparison with Creutzfeldt-Jakob disease: Implications for human health. Proc.
876 Natl. Acad. Sci. 2001;98:4142-4147.
877
878 37. Transmissible Spongiform Encephalopathy Advisory Committee June 12th 2009.
879 www.fda.gov/TransmissibleSpongiformEncephalopathyAdvisory Committee.
880
881 38. Comoy, E., Durand,V., Lescoutra,N., Freire,S., Correia,E., Ruchoux,M.M., Brown,P.,
882 Lasmezas,C.,Deslys,J-P. 2008 Abstract 0C3.12 PRION 2008, Madrid,8-10th October 2008.
883
884 39. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion
885 in sheep. Lancet 2000;356:999-1000.
886
887 40. Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F.
888 Transmission of prion diseases by blood transfusion. Journal of General Virology 2002;83:2897-
889 2905.
890
891 41. Houston,F., McCutcheon,S., Goldmann,W., Chong,A., Foster, J., Siso,S., Gonzalez,L., Jeffrey,M.,
892 Hunter,N., (2008) Prion diseases are efficiently transmitted by blood transfusion in sheep. Blood
893 vol 112; 4739-4745
894
895 42. Gillies M, Chohan G, Llewelyn CA, MacKenzie J, Ward HJ, Hewitt PE, Will RG. A retrospective case
896 note review of deceased recipients of vCJD-implicated blood transfusions. Vox Sang 97: 211-218,

2009.

43. Bennett P, Ball J: vCJD risk assessment calculations for a patient with multiple routes of exposure. Department of Health, 9 June 2009. Available at http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_100357.

44. Dorsey K, Zou S, Schonberger LB, et al.: Lack of evidence of transfusion transmission of Creutzfeldt-Jakob disease in a US surveillance study. Transfusion 49: 977–984, 2009.

45. Hamaguchi T, Noguchi-Shinohara M, Nozaki I, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Yamada M. The risk of iatrogenic Creutzfeldt-Jakob disease through medical and surgical procedures. Neuropathology 29: 625-631, 2009.

46. Mahillo-Fernandez I, de Pedro-Cuesta J, Bleda MJ, et al.: Surgery and risk of sporadic Creutzfeldt-Jakob disease in Denmark and Sweden: registry-based case-control studies. Neuroepidemiology 31:229–240, 2008.

47. Manuelidis EE, Kim JH, Mericangas JR, Manuelidis L. Transmission to animals of Creutzfeldt-Jakob disease from human blood. Lancet 2: 896-7, 1985.

48. Tateishi J. Transmission of Creutzfeldt-Jakob disease from human blood and urine into mice. Lancet 2: 1074, 1985.

49. Tamai Y, Kojima H, Kitajima R, Taguchi F, Ohtani Y, Kawaguchi T, Miura S, Sato M, Ishihara Y. Demonstration of the transmissible agent in tissue from a pregnant woman with Creutzfeldt-Jakob disease. N Engl J Med 327: 649, 1992.

50. Deslys JP, Lasmézas C, Dormont D. Selection of specific strains in iatrogenic Creutzfeldt-Jakob disease. Lancet 343: 848-849, 1994.

51. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 35: 513-529, 1994.

52. Lasmézas CI, Fournier JG, Nouvel V, Boe H, Marcé D, Lamoury F, Kopp N, Hauw JJ, Ironside J, Bruce M, Dormont D, Deslys JP. Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-- Jakob disease: implications for human health. Proc Natl Acad Sci U S A 98: 4142-7, 2001.

53. http://www.amorfix.com/technology.html

54. Korth,C., Stierli,B., Streit,P., Moser,M., Schaller,O., Fischer,R., Scuhlz-Schaeffer,W., Ktrezschmar,H., Raeber,A., Braun,U., Ehrensperger,F., Hornemann,S., Glockshuber,R., Riek,R., Billeter,M., Wuthrich,K., Oesch,B. (1997) Prion -(PrPSc)- specific epitope defined by a monoclonal antibody. Nature vol 390 74-77.

55. Castillo, J., Saa,P., Morales,R., Abid, K., Maundrell, K., Sotto, C., (2006) Protein misfolding cyclic amplification for diagnosis and prion propagation studies. Methods, Enzymol vol 412, 3-21

56. www.nibsc.ac.uk/spotlight/cjd_resource_centre/cjd_tests.aspx

57. Krailadsiri P, Seghatchian J, Macgregor I, Drummond O, Perrin R, Spring F, Prescott R, Williamson L, Prowse C, Anstee D, Turner M. The effects of leukodepletion on the generation and removal of microvesicles and prion protein in blood components. Transfusion 2006; 46: 407-17

58. Gregori L, McCombie N, Palmer D, et al Effectiveness of leucoreduction for the removal of infectivity of transmissible spongiform encephalopathies from blood. Lancet 2004; 364: 529-31.

59. Gregori L, Lambert B C, Gurgel P V, Gheorghiu L, Edwardson P, Lathrop J T, MacAuley C, Carbonell R G, Burton S J, Hammond D, Rohwer R G.
Reduction of transmissible spongiform encephalopathy infectivity from human red blood cells with prion affinity ligands. Tranfusion 2006; 46: 1152-1161

60. Sowemimo-Coker SO, Pesci S, Andrade F, Kim A, Kascsak RB, Kascsak RJ, Meeker C, Carp R. Pall leukotrap affinity prion-reduction filter removes exogenous infectious prions and endogenous infectivity from red cell concentrates. Vox Sanguinis 2006; 90: 265-275

61. Gregori L, Gurgel P V, Lathrop J T, Edwardson P, Lambert B C, Carbonell R G, Burton S J, Hammond D J, Rohwer R G. Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins. Lancet 2006; 368: 2226-2230

62. Neisser-Svae A, Bailey A, Gregori L, Heger A, Jordan S, Behizad M, Reichl H, Romisch J, Svae T-E. Prion removal effect of a specific affinity ligand introduced into the manufacturing process of a pharmaceutical quality solvent/detergent (S/D)-treated plasma OctaplasLG®. Vox Sanguinis 2009; DOI: 10. 1111/j 1423-0410.2009.01206x

63. Foster PR, Removal of TSE agents from blood products, Vox Sang. 2004 Jul;87 Suppl 2:7-10

64. Flan B, Arrabal S, Manufacture of plasma-derived products in France and measures to prevent the risk of vCJD transmission: precautionary measures and efficacy of manufacturing processes in prion removal. Transfus Clin Biol. 2007 May;14(1):51-62.

65. Poelsler G, Berting A, Kindermann J, Spruth M, Hämmerle T, Teschner W, Schwarz HP, Kreil TR. A new liquid intravenous immunoglobulin with three dedicated virus reduction steps: virus and prion reduction capacity.Vox Sang. 2008 94, 184-192

66. Stucki M, Boschetti N, Schäfer W, Hostettler T, Käsermann F, Nowak T, Gröner A, Kempf Investigations of prion and virus safety of a new liquid IVIG product. Biologicals. 2008 Jul;36(4):239-47

67. Yunoki M, Tanaka H, Urayama T, Hattori S, Ohtani M, Ohkubo Y, Kawabata Y, Miyatake Y, Nanjo A, Iwao E, Morita M, Wilson E, MacLean C, Ikuta K. Prion removal by nanofiltration under different experimental conditions. Biologicals. 2008 Jan;36(1):27-36.

68. Diez JM, Caballero S, Belda FJ, Otegui M, Gajardo R, Jorquera JI. Elimination capacity of a TSE-model agent in the manufacturing process of Alphanate/Fanhdi, a human factor VIII/VWF complex concentrate. Haemophilia. 2009 Nov;15(6):1249-57.

69. Gregori L, Kovacs GG, Alexeeva I, et al (2008) Excretion of transmissible spongiform encephalopathy infectivity in urine. Emerg Infect Dis. Sep;14(9):1406-12.

70. Kariv-Inbal Z, Ben-Hur T, Grigoriadis NC, Engelstein R, Gabizon R (2006) Urine from scrapie-infected hamsters comprises low levels of prion infectivity. Neurodegener Dis 3: 123–128.

71. Seeger H, Heikenwalder M, Zeller N, Kranich J, Schwarz P, et al. (2005) Coincident scrapie infection and nephritis lead to urinary prion excretion. Science 310: 324–326.

72. Haley NJ, Seelig DM, Zabel MD, Telling GC, Hoover EA (2009) Detection of CWD Prions in Urine and Saliva of Deer by Transgenic Mouse Bioassay. PLoS ONE 4(3): e4848. doi:10.1371/journal.pone.0004848

73. Gonzalez-Romero D, Barria MA, Leon P, Morales R, Soto C (2008) Detection of infectious prions in urine. FEBS Lett 582: 3161–3166.

74. Murayama Y, Yoshioka M, Okada H, Takata M, Yokoyama T, et al. (2007) Urinary excretion and blood level of prions in scrapie-infected hamsters. J Gen Virol 88: 2890–2898.

75. Cervenakova L, Brown P, Hammond DJ, Lee CA, Saenko EL. Review article: Factor VIII and transmissible spongiform encephalopathy: the case for safety. Haemophilia 2002;8:63-75.

76. Ricketts MN, Brown P. Transmissible spongiform encephalopathy update and implications for blood safety. Clin Lab Med 2003;23:129-37.

77. Peden A. et al. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. Haemophilia 2010;16: 296-304.

78. **AFSSaPS.**

78a Analyse du risque de transmission de la variante de la maladie de Creutzfeldt-Jakob (vMCJ) et de la forme sporadique de la maladie de Creutzfeldt-Jakob par les produits de santé d'origine humaine– Septième actualisation des travaux du groupe d'experts pluridisciplinaire. Rapport de juillet 2009.
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78b. Analyse du risque de transmission de la variante de la maladie de Creutzfeldt-Jakob (vMCJ) et de la forme sporadique de la maladie de Creutzfeldt-Jakob par les produits de santé d'origine humaine– Sixième actualisation des données du rapport du groupe d'experts adhoc de décembre 2000 – Rapport de novembre 2007.
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78c. Evaluation of the risk of transmission of Creutzfeldt-Jakob disease agent by blood and its constituents – Experts' group meeting of 16 november 2004.
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78d. Analysis of the risk of transmission of variant of Creutzfeldt-Jakob disease by health products and by tissues and fluids of human origin – Update of findings of ad hoc group report of December 2000 – Report of February 2004.
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78e. Analysis of the risk of transmission of the variant Creutzfeldt-Jakob disease by medicinal products products of human origin and labile blood products – Data update of the ad hoc group report dated December 2000 –March 2003 report.
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78f. Analysis of the risk of transmission of the variant Creutzfeldt-Jakob disease by medicinal products products of human origin and labile blood products – Data update of the ad hoc group report dated December 2000 –February 2002 report
http://www.afssaps.fr/Dossiers-thematiques/Creutzfeldt-Jakob-et-produits-de-sante

78g. Analysis of the risk of transmission of new variant Creutzfeldt-Jakob disease by blood and its derivatives", Recommendations of expert group convened at the initiative of AFSSaPS, 11 December 2000. http://afssaps.sante.fr

78h. Revision of measures to minimise the risk of TSE transmission via blood products", Report of expert group convened under the aegis of the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSaPS) and the Etablissement Français du Sang (EFS), February 2000. http://afssaps.sante.fr

EDQM

79. "Guide to the preparation, use and quality assurance of blood components", Recommendation No. R (95) 15, 15th ed. 2009. Council of Europe.