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Dispersion of prion protein deposits around blood vessels in variant Creutzfeldt-Jakob disease

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Abstract

In variant Creutzfeldt-Jakob disease (vCJD), a disease linked to bovine spongiform encephalopathy (BSE), florid-type prion protein (PrP^{sc}) deposits are aggregated around the larger diameter (> 10 µm) cerebral microvessels. Clustering of PrP^{sc} deposits around blood vessels may result from blood-borne prions or be a consequence of the cerebral vasculature influencing the development of the florid deposits. To clarify the factors involved, the dispersion of the florid PrP^{sc} deposits was studied around the larger diameter microvessels in the neocortex, hippocampus, and cerebellum of ten cases of vCJD. In the majority of brain regions, florid deposits were clustered around the larger diameter vessels with a mean cluster size of between 50 µm and 628 µm. With the exception of the molecular layer of the dentate gyrus, the density of the florid deposits declined as a negative exponential function of distance from a blood vessel profile suggesting that diffusion of molecules from blood vessels is a factor in the formation of the florid deposits. Diffusion of PrP^{sc} directly into the brain via the microvasculature has been demonstrated in vCJD in a small number of cases. However, the distribution of the prion deposits in vCJD is more likely to reflect molecular 'chaperones' diffusing from vessels and promoting the aggregation of pre-existing PrP^{sc} in the vicinity of the vessels to form florid deposits.

Key words: variant Creutzfeldt-Jakob disease (vCJD), disease form of prion protein (PrP^{sc}), florid deposits, clustering, diffusion, negative exponential model.

Introduction

Four subtypes of Creutzfeldt-Jakob disease (CJD) have been described to date, viz., genetic or familial CJD (fCJD) [18], iatrogenic CJD (iCJD), resulting from the direct transmission of PrP^{sc}, sporadic CJD (sCJD) [11,40], and variant CJD (vCJD) [43]. Variant CJD is the most recent subtype to be described and was first reported in the United Kingdom (UK) in 1996 [43]. The majority of vCJD patients initially develop psychiatric symptoms such as depression and/or anxiety, and these symptoms are usually followed by ataxia, involuntary movements, and cognitive impairment. Most patients affected to date are young (median age 28 years, range 14-74 years) and death occurs within 12-24 months; a significantly longer duration than observed in the more common sCJD [43].

How the majority of CJD cases acquire the disease form of prion protein (PrP^{sc}) is uncertain. Variant CJD has been linked to the consumption of beef originat-

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ing from cattle with bovine spongiform encephalopathy (BSE) [43]. BSE can be transmitted to sheep by transfusing them with whole blood from another sheep during symptom free exposure to BSE [22]. In addition, although PrP^{sc} has not been found in human serum [15], normal prion protein (PrP) is present in human blood [31]. However, there is a theoretical risk of secondary vCJD transmission from human to human via blood and blood products, organs and tissues or via contaminated surgical instruments and medical devices. This concern has resulted in the blood transfusion service in the UK implementing universal leucodepletion of cellular blood components to minimize the possible risk [16,35]. Moreover, four cases of probable vCJD transmission via the blood have now been described and one further case of secondary infection via a plasma product [12].

Two morphological types of PrP^{sc} deposit commonly occur in the brains of individuals with vCJD [9]. First, 'florid' deposits comprise a condensed 'core' of PrP^{sc} and are heavily stained with antibodies raised against PrP [29,39]. Second, diffuse deposits (also called 'fine feathery diffuse deposits' or 'fine diffuse plaques') are more lightly stained and irregular in shape and lack a condensed core [3,32]. A previous study demonstrated a positive spatial correlation between the florid deposits and the cerebral microvessels in vCJD suggesting that prions may spread into the brain via the cerebral microcirculation [10]. In sCJD, the synaptic-type PrP^{sc} deposits were also aggregated around blood vessels [7].

A number of hypotheses could account for the distribution of the florid deposits around blood vessels in vCJD. First, blood-borne prions could enter the brain via the cerebral circulation and diffuse from the vessels into the neuropil. Second, the distribution could represent an abortive attempt to clear PrP^{sc} from the brain [42]. Third, florid deposits could develop from PrP originating from the endothelia of blood vessels or associated cells [38]. Fourth, proteins acting as 'molecular chaperones', could diffuse into the brain from the cerebral circulation and influence the development of PrP^{sc} deposits adjacent to the vessels [7,8]. To clarify the factors involved, the present study tested the hypothesis that diffusion from blood vessels could be involved in the formation of the florid deposits. First, the spatial pattern of the florid deposits around the larger diameter-blood vessel profiles was studied in regions of the cerebral cortex, hippocampus, and cerebellum. Second, a mathematical model was fitted to the data which predicts the distribution of the florid deposits around a blood vessel that would result from diffusion [5].

Material and methods

Cases

Ten cases of vCJD (details in Table I), all fulfilling the criteria for a pathological diagnosis of vCJD [24], were obtained from the National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK. The principles embodied in the 1975 Helsinki declaration (as modified Edinburgh, 2000) were followed with respect to experiments involving material of human origin. None of the vCJD cases had any of the known mutations of the *PrP* gene or family history of prion disease, and there was no evidence of the known types of iatrogenic aetiology. The pattern of PrP^{sc} deposition typical of vCJD was observed in all cases with florid-type deposits in the cerebral cortex, cerebellum, basal ganglia, thalamus, and brain stem [23].

Preparation of material

Blocks of the frontal cortex (FC) (B8) at the level of the genus of the corpus callosum, parietal cortex (PC) (B7) at the level of the splenium of the corpus callosum, occipital cortex (OC) including the calcarine sulcus (B17), and temporal cortex at the level of the lateral geniculate body were taken from each brain. Within the temporal lobe, the inferior temporal gyrus (ITG) (B22) and parahippocampal gyrus (PHG) (B28) were studied. In addition, a block of the right cerebellar cortex was taken from each case at the level of the superior cerebellar peduncle. Tissue was fixed in 10% phosphate buffered formal-saline and embedded in paraffin wax. For quantitative analysis, coronal 7 µm sections were immunolabelled using the monoclonal antibody 12F10 (dilution 1 : 250) which binds to a region of human PrP downstream of the neurotoxic domain adjacent to helix region 2: residues 142-160 [28] (kindly provided by Prof. G. Hunsmann, The German Primate Centre, Gottingen, Germany). Immunoreactivity was enhanced by formic acid (98% for 50 minutes) and autoclaving (121°C for 10 minutes) pretreatment [20]. Sections were treated with Dako Biotinylated Rabbit anti-Mouse (RAM) (dilution 1 : 100) and Dako ABComplex HRP kit for 45 minutes (Amersham, UK). Diaminobenzidine tetrahydrochloride was used as the chromogen. Immunolabelled sections

Table I. Demographic data and gross brain details of the variant Creutzfeldt-Jakob disease (vCJD) cases.

Case	Gender	Age at onset (yrs)	Duration (yrs)	Brain weight (gm)	Atrophy
A	F	39	2	586L	None
B	F	28	1	1375	None
C	F	28	1	NA	NA
D	M	19	1	NA	NA
E	M	30	1	699R	None
F	M	48	2	1470	None
G	F	34	1	810L	None
H	M	18	1	1434	None
I	M	24	1	NA	NA
J	F	21	2	1394	None

M – male, F – female, R – right hemisphere only, L – left hemisphere only, NA – data not available

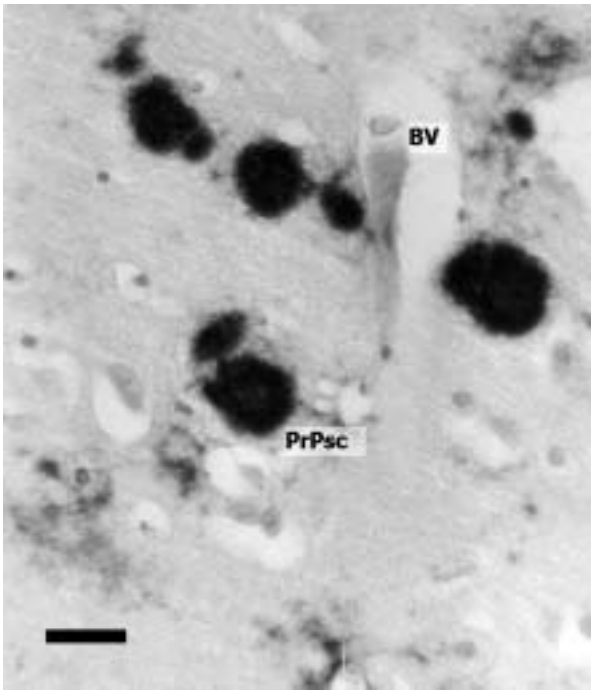

Fig. 1. Cluster of florid PrP^{sc} deposits surrounding a larger diameter blood vessel (BV) in the superior frontal cortex of a patient with variant Creutzfeldt-Jakob disease (vCJD) (magnification bar = 30 µm).

were also stained with haematoxylin for 1 minute to reveal the blood vessel profiles.

Morphological methods

In the cerebral cortex, the density of the florid deposits, and the incidence of blood vessels was measured parallel to the pia mater along strips of tissue (3200 to 6400 µm in length) using 50 × 250 µm contiguous sample fields. A micrometer eyepiece with 100 horizontal divisions was used as the sample field. The sample fields were located within cortical laminae II/III and V/VI, the short edge of the field being aligned with guidelines marked on the slide and located parallel to the pia mater. In the hippocampus, measurements commenced at the beginning of CA1 and extended as far as the CA2/CA3 boundary; the short dimension of the sample field being aligned with the alveus. In the dentate gyrus, the sample fields were aligned with the top of the granule cell layer. In the cerebellum, the short edge of the sample field was aligned with the edge of the section to sample the outer region of the molecular layer. Florid deposits (Fig. 1) are easily distinguishable from diffuse deposits and consist of a condensed 'core' of PrP^{sc} surrounded by a peripheral ring of small vacuoles. All blood vessel profiles visible in the section were sampled. The vessel profiles were quantified by 'lattice sampling', i.e., by

counting the number of times the intersections of the grid lines encountered a vessel [3].

Correlations between the densities of the florid deposits and the frequency of contacts with blood vessels were tested for each brain region using Pearson's correlation coefficient *r*. The degree of correlation between the abundance of two histological features depends on field size [4]. Hence, to determine the scale at which the correlation was evident, counts of histological features in adjacent sample fields were added together successively to provide data for increasing field sizes, e.g., 50 × 250 µm (field size 1), 100 × 250 µm (field size 2), 200 × 250 µm (field size 3) etc., up to a size limited by the length of the strip sampled. Pearson's *r* was calculated for each field size and the change in *r* with field size examined for each brain region. Hence, significant correlations at the smallest field sizes (50 µm, 100 µm) suggest a close spatial relationship between PrP^{sc} deposits and blood vessels. By contrast, a significant correlation present at a larger field size only is likely to reflect the general abundance of florid deposits and blood vessels in the section [27,30]. The dimension of the florid plaque clusters in each tissue was estimated using spatial pattern analysis [2,6].

Dispersion of florid plaques around blood vessels

Transient or local leakage of proteins from blood vessels is likely to have a diminishing effect with distance from a blood vessel. Mathematically, randomly diffusing particles from a point source will result in a spatial distribution of particles which declines as a negative exponential function of distance from the source [37]. Hence, the distribution of the florid deposits was studied at different distances from a sample of prominent blood vessel profiles [5]. A sample of the larger vessel profiles was selected from each region from each vCJD case. Each blood vessel profile fulfilled the following criteria: 1) florid deposits were present around the selected vessel, 2) the vessel diameter was at least 10 µm measured parallel to the pia mater, and 3) the vessel profile was separated, at least on one side, by a distance of at least 2200 µm from the nearest large blood vessel. The number of florid deposits was then counted at different distances from each vessel in seven, 50 × 250 µm vertically oriented, contiguous sample fields with the short edge parallel with the pia mater. At least 10 blood vessels were studied from each brain region and a negative exponential model fitted to the combined data. The simplest type of exponential model was fitted to the data in which the number of florid deposits (Y) was assumed to decline as a negative exponential function of distance (X) from the blood vessel, i.e., $Y = a(exp^{-bx})$ [5,37]. To fit this model: 1) an exponential function was fitted to the original data and 2) a linear function was fitted to the logarithm (*Log*) of the density of the florid deposits plotted against distance from the source [40].

Results

Figure 1 shows a vertically penetrating arteriole in the upper laminae of the frontal cortex surrounded by florid PrP^{sc} deposits. The florid deposits are clearly aggregated around the vessel profile; while the diffuse PrP^{sc} deposits are less frequent close to the vessel.

The estimated dimensions of the clusters of the florid PrP^{sc} deposits and the spatial correlations between the densities of the florid deposits and the larger diameter blood vessels are summarized in Table II. The mean cluster size of the florid deposits varied from 50 µm to 628 µm; the largest clusters of deposits being present in the occipital cortex and the smallest in sectors CA1/2 of the hippocampus, and in the molecular layer of the dentate gyrus. In neocortical regions, the percentage of cases in which a significant positive correlation between the florid deposits and blood vessels was present varied from 70% to 90%. Correlations between the florid deposits and the blood vessels were observed less consistently in the hippocampus and cerebellum.

The relationship between the density of florid deposits and distance from the larger diameter blood vessels is shown for a single brain region (Frontal cortex, laminae V/VI) in Fig. 2. The untransformed densities show that the florid deposits declined rapidly with distance from a vessel. In addition, the density of the florid deposits, expressed on a logarithmic scale, declined linearly with distance from the blood vessels ($r = 0.97$, $P < 0.001$) consistent with a negative exponential model. The data for all brain regions is summarised in Table III. The relationship between the logarithm of density of the florid deposits and distance from the blood vessels was fitted successfully by a negative exponential model in all regions examined with the exception of the molecular layer of the dentate gyrus.

Table II. Mean cluster sizes of the florid prion protein (PrP^{sc}) deposits and the spatial correlation (Pearson's *r*) with larger diameter (> 10 μm) blood vessel profiles in various brain regions.

			Brain region							
Case		Laminae	SFC	PC	OC	ITG	PHG	HC	DG	CB
A	Size	II/III	400	100	50	50	200	800	R	R
		V/VI	50	100	100	50	≥ 1600			
	r	II/III	0.42**	0.34**	0.03	0.40**	0.48**	–	–	–
		V/VI	0.31*	–0.01	0.25*	0.09	0.07			
B	Size	II/III	≥ 1600	800	200	800	50	50	R	50
		V/VI	200	Reg	800					
	r	II/III	–0.01,	0.38**	0.19	0.27*	0.34**	–	–	–
		V/VI	0.15	0.29*	–0.01					
C	Size	II/III	200	≥ 1600	100	50	≥ 1600	–	–	100
		V/VI	100	200	800	≥ 1600	50			
	r	II/III	0.36**	0.40**	0.42**	0.25*	0.30*	0.42*	–	0.10
		V/VI	0.29*	0.31*	0.12	0.25*	0.27*			
D	Size	II/III	50	400	100	100	200	50	–	800
		V/VI	50	R	≥ 1600	50	R			
	r	II/III	0.45**	0.39**	0.41**	0.44**	0.42**	0.65**	–	0.62**
		V/VI	0.33**	0.21	–	0.61**	0.48**			
E	Size	II/III	100	100	100	800	50	–	50	100
		V/VI	50	200	200	50				
	r	II/III	0.47**	0.11	0.45**	0.30*	0.13	–	0.82**	0.12
		V/VI	0.33**	0.50**	0.16	0.45**				
F	Size	II/III	R	R	400	200	R	–	50	400
		V/VI	100	100	800	400	100			
	r	II/III	0.52**	0.45**	0.40**	0.55**	0.32**	–	0.14	0.38**
		V/VI	0.44**	0.16	0.46**	0.35**	0.53**			
G	Size	II/III	400	≥ 1600	≥ 1600	50	R	50	–	50
		V/VI	800	50	800	100				
	r	II/III	0.46**	0.24	0.61**	0.51**	0.35**	0.46**	0.24	0.65**
		V/VI	0.50**	0.40**	0.29*	0.54**	0.28*			
H	Size	II/III	Reg	R	400	200	400	50	R	–
		V/VI	400	200	≥ 1600	200	100			
	r	II/III	0.49**	0.36**	0.36**	0.49**	0.51**	0.30	0.44*	–
		V/VI	0.36*	0.10	0.58**	0.46**	0.21*			
I	Size	II/III	400	100	≥ 1600	200	50,	–	–	> 1600
		V/VI	400	50	R	100				
	r	II/III	0.46**	0.33**	0.34**	0.41**	0.29*	0.21	–	0.11
		V/VI	0.36**	0.45**	0.43**	0.18	0.42**			
J	Size	II/III	R	Reg	Reg	50	50	–	50	50
		V/VI	Reg	R	50	Reg	200			
	r	II/III	0.50**	0.40**	0.25*	0.70**	0.50**	–	0.52**	0.24
		V/VI	0.27*	0.31*	0.44**	0.66**	0.40**			

Size data preceded by ≥ indicate large scale clustering of PrP^{sc} deposits of at least 1600 μm. Data not preceded by ≥ indicate clusters that are regularly distributed parallel to the pia mater. (–) density of florid PrP^{sc} deposits too low to determine spatial patterns, R – random distribution, Reg – regular distribution SFC – superior frontal cortex, PC – parietal cortex, OC – occipital cortex, ITG – inferior temporal gyrus, PHG – parahippocampal gyrus, HC – hippocampus sectors CA1/2, DG – dentate gyrus, CB – molecular layer of the cerebellum in 10 cases of variant Creutzfeldt-Jakob disease (vCJD)
**P < 0.05, **P < 0.01*

Discussion

The data suggest: 1) florid PrP^{sc} deposits were clustered around the larger diameter blood vessels, the dimension of the clusters varying with brain region and 2) the density of florid deposits declined as a negative exponential function with distance from a larger diameter blood vessel. Hence, the data are consistent with the hypothesis that diffusion from blood vessels is involved in the pathogenesis of the florid deposits.

There are several factors that could influence the deposition of PrP^{sc} around blood vessels. First, the distribution of PrP^{sc} could represent an abortive attempt to clear PrP^{sc} from the brain via the cerebral circulation as has been suggested for the removal of β-amyloid (Aβ) in Alzheimer's disease (AD) [42]. Extracellular fluid is drained from the brain to the cervical lymph nodes via the perivascular channels and therefore, PrP^{sc} deposits around vessels could be attributable to overloading of this system, i.e., to failed or inhibited clearance. This process, however, should affect both the florid and the diffuse PrP^{sc} deposits

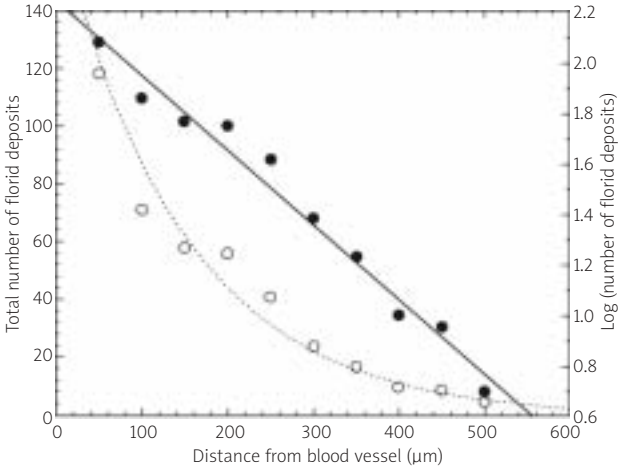

Fig. 2. The density of florid prion protein (PrP^{sc}) deposits at different distances from large diameter blood vessel profiles in laminae V/VI of the frontal cortex in a case of variant Creutzfeldt-Jakob disease (vCJD) (Fit to negative exponential model $r = -0.97$, $P < 0.001$).

Table III. Results of fitting a negative exponential model to the numbers of florid prion protein (PrP^{sc}) deposits at different distances from larger diameter (> 10 μm) blood vessels in various brain regions.

Region	Laminae	Pearson's 'r'	Regression equation	Probability
SFC	II/III	–0.92	Y = 2.24 – 0.0029X	$P < 0.001$
SFC	V/VI	–0.97	Y = 4.65 – 0.0061X	$P < 0.001$
PC	II/III	–0.94	Y = 5.57 – 0.0065X	$P < 0.001$
PC	V/VI	–0.92	Y = 5.20 – 0.0088X	$P < 0.001$
OC	II/III	–0.87	Y = 5.85 – 0.0097X	$P < 0.001$
OC	V/VI	–0.91	Y = 4.28 – 0.0042X	$P < 0.001$
ITG	II/III	–0.95	Y = 5.07 – 0.009X	$P < 0.001$
ITG	V/VI	–0.95	Y = 4.98 – 0.009X	$P < 0.001$
PHG	II/III	–0.92	Y = 5.65 – 0.011X	$P < 0.001$
PHG	V/VI	–0.93	Y = 4.53 – 0.0061X	$P < 0.001$
HC	CA1/2	–0.96	Y = 3.11 – 0.014X	$P < 0.001$
DG	ML	–0.64	Y = 2.41 – 0.0054X	$P > 0.05$
CB	ML	–0.79	Y= 3.02 – 0.005X	$P < 0.01$

SFC – superior frontal cortex, PC – parietal cortex, OC – occipital cortex, ITG – inferior temporal gyrus, PHG – parahippocampal gyrus, HC – hippocampus sectors CA1/2, DG – dentate gyrus, CB – molecular layer of the cerebellum in 10 cases of variant Creutzfeldt-Jakob disease (vCJD)

but the latter do not exhibit any spatial relationship with the microvessels [4]. In addition, as in AD, there should be more resistance to drainage of Aβ in older patients resulting in increased accumulation of amyloid close to the vessel. This is less likely to be a factor in vCJD as the majority of cases were young (mean age 29 years). In addition, there was no correlation between the cluster size of PrP^{sc} deposits and age of the patient at death.

Second, the distribution of the florid deposits around blood vessels could reflect PrP^{sc} deposition in association with the endothelia of blood vessels or adjacent cells. In experimental amylotrophic leukospongiosus, for example, in which the disease agent is $PrP_{27\text{-}30}$, there were pathological changes in astrocytes adjacent to capillaries, their foot processes, and in pericytes accompanied by PrP^{sc} deposition in the basement membrane and endothelium of altered blood vessels coincident with disturbance of the blood brain barrier [38]. Hence, if these cellular components were involved exclusively in the formation of the florid deposits it would result in a single ring of deposits around affected vessels rather than a negative exponential decline in density. Nevertheless, if smaller capillaries were also involved, these vessels could decrease as a negative exponential function with distance from a larger blood vessel profile and result in a similar distribution of the florid deposits. There is no evidence, however, that the density of smaller capillaries decreases as a negative exponential function with distance from the larger vessels [4] and moreover, it is frequently the diffuse rather than the florid deposits which show the closest association with components of the smaller capillary vessel walls [4].

Third, a negative exponential distribution of florid deposits could represent diffusion from blood vessels. If diffusion is involved, then one explanation is haematogenous spread of PrP^{sc} into the brain [13-15,21,33] followed by diffusion from the brain microvessels. Scrapie prion is one of the proteins able to negotiate the blood-brain barrier early in the course of the disease and even before overt damage is present [34]. In addition, a dysfunctional blood-cerebral spinal fluid barrier has been observed in 6 out of 25 CJD patients [25] and could also encourage the leaking of PrP^{sc} into the brain. Nevertheless, neither the more abundant diffuse PrP^{sc} deposits nor the vacuoles show a spatial relationship with the blood vessels in vCJD [4] which argues against the pathology of vCJD originating by diffusion of prions from blood vessels. In addition, in patients with CJD in which there is evidence of amyloid in association with vessel walls (capillary amyloid angiopathy), no PrP^{sc} has been observed in association with the vessels [19]. Furthermore, PrP^{sc} has not been detected in blood plasma in human patients with CJD [15] although it is possible that disease causing prions may be present in blood at levels below those currently detectable. More recently, the presence of PrP^{sc} associated with white blood cells was investigated in cases of sCJD [17]. No evidence of PrP^{sc} was found in purified granulocytes, monocytes, B cells, CD4(+) T cells, CD8(+) T cells, natural killer cells, nonclassical gamma delta T cells, or in platelets. Hence, if white blood cells do harbor prion infectivity in vCJD, levels are likely to be low and not sufficient to explain the density of PrP^{sc} around the vessels.

An alternative hypothesis is that as in sCJD and AD [7,8], diffusion of proteins other than PrP^{sc} may influence the formation of the florid deposits. Degeneration of neurons and glial cells close to blood vessels could compromise the integrity of the brain microvasculature and increase the leakage of proteins. Blood proteins, such as apolipoprotein E (apo E), and amyloid-P have been recorded in association with PrP^{sc} deposits [1,26,35]. The most likely protein to originate from blood vessels is amyloid-P, since the liver is the only tissue of the body which exhibits its mRNA [26]. Amyloid-P is a feature of all types of systemic amyloid deposit and is evident in lesions in various diseases most notably in the Aβ deposits in AD [26]. Plasma proteins such as amyloid-P could therefore diffuse from vessels and act as 'molecular chaperones' encouraging the aggregation of PrP^{sc} molecules to form florid deposits around blood vessels.

In conclusion, there are spatial correlations between the florid-type PrP^{sc} deposits and the cerebral microvessels in vCJD. The florid deposits are aggregated around the larger diameter blood vessels and their density declines exponentially with distance from a prominent blood vessel consistent with diffusion from blood vessels. Although there are small numbers of vCJD cases in which blood transfusion may have been the origin of the disease, it is less likely that the spatial relationship observed in the present study reflects the direct leakage of blood-borne prions into the brain and, as in sCJD, more likely that it reflects the leakage of proteins from the vessels enhancing the aggregation of PrP^{sc} in the vicinity of the blood vessels.

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