

## PRION DISEASES

# Detection of prions in the plasma of presymptomatic and symptomatic patients with variant Creutzfeldt-Jakob disease

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Variant Creutzfeldt-Jakob disease (vCJD) is a human prion disease resulting from the consumption of meat products contaminated by the agent causing bovine spongiform encephalopathy. Evidence supporting the presence of a population of silent carriers that can potentially transmit the disease through blood transfusion is increasing. The development of a blood-screening assay for both symptomatic vCJD patients and asymptomatic carriers is urgently required. We show that a diagnostic assay combining plasminogen-bead capture and protein misfolding cyclic amplification (PMCA) technologies consistently detected minute amounts of abnormal prion protein from French and British vCJD cases in the required femtomolar range. This assay allowed the blinded identification of 18 patients with clinical vCJD among 256 plasma samples from the two most affected countries, with 100% sensitivity [95% confidence interval (CI), 81.5 to 100%], 99.2% analytical specificity (95% CI, 95.9 to 100%), and 100% diagnostic specificity (95% CI, 96.5 to 100%). This assay also allowed the detection of silent carriage of prions 1.3 and 2.6 years before the clinical onset in two blood donors who later developed vCJD. These data provide a key step toward the validation of this PMCA technology as a blood-based diagnostic test for vCJD and support its potential for detecting presymptomatic patients, a prerequisite for limiting the risk of vCJD transmission through blood transfusion.

## INTRODUCTION

Variant Creutzfeldt-Jakob disease (vCJD) was identified in 1996 in the U.K. as a zoonotic infection caused by the dietary transmission of bovine spongiform encephalopathy (1, 2). vCJD is a rare transmissible spongiform encephalopathy (TSE) with a long incubation period and with no validated test to identify affected individuals before clinical onset. Until recently, all clinical cases of vCJD evaluated for the prion protein gene (*PRNP*) were homozygous for methionine at codon 129 (59% of the Caucasian population) (3) with no cases in the alternative genotypes. The first confirmed vCJD clinical case in a patient heterozygous for methionine/valine has just been reported in the U.K. (4). Patients with vCJD display an accumulation of abnormally folded prion protein (PrP<sup>TSE</sup>) in the brain and lymphoid tissues including the spleen, lymph nodes, appendix, and tonsils (5, 6), raising concerns about the risk of blood-borne infection. The secondary transmission of prions through blood has been demonstrated in several animal models (7–10). In the U.K., four cases of infection by the vCJD

agent have been reported in recipients of nonleukodepleted red blood cell concentrate from donors who were carriers for vCJD (11–13). A fifth probable case was reported in a patient with hemophilia treated with coagulation factor VIII manufactured from plasma derived from U.K. donors (14). Infectivity in blood is thought to be split about equally between leukocytes and plasma, with negligible amounts directly associated with red blood cells or platelets (15). Whereas clinical cases of vCJD are declining worldwide, the prevalence of asymptomatic carriers in the general population remains a concern. Investigations into the prevalence of asymptomatic carriers, using immunohistochemical detection of PrP<sup>TSE</sup> in a large series of appendix specimens, indicated an unexpectedly high rate of infection (1/2000) in the general U.K. population (16, 17), which can be extrapolated to a prevalence of 1/20,000 in France where the level of exposure is considered to be 10 times lower. Detecting circulating agents in asymptomatic subjects with peripheral prion infection is thus important for public health, in particular to avoid secondary transmission through blood transfusion. A prototype enzyme-linked immunosorbent assay (ELISA) test was recently reported to identify vCJD cases by probing whole blood (18–20). Several other strategies are being explored using blood (21–23) and urine from vCJD patients (24). However, most blood tests have only reached about 70% sensitivity in blinded studies, thus falling short of the acceptance criteria of 90% required by the European Union (EU) Commission Directive 2011/100/EU for licensing for human use (25). In addition, none of these tests has proven effective for detecting presymptomatic patients. We have previously described a blood diagnostic assay based on serial prion amplification by protein misfolding cyclic amplification (PMCA) (26). To circumvent the presence of blood-associated conversion inhibitors, we first captured the prion protein using magnetic nanobeads coated with plasminogen. Plasminogen was identified as a ligand of abnormal prion proteins using specific interactions with lysine residues by

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Fischer *et al.* (27). Of particular interest is the capacity of plasminogen to bind to PrP<sup>TSE</sup> from various species including humans as well as sporadic CJD (sCJD) prions (28). Plasminogen has also demonstrated the ability to stimulate prion conversion in vitro (29).

In our PMCA-based assay (30), the capture step enables the concentration of prion proteins present in 500  $\mu$ l of plasma and their separation from blood-associated conversion inhibitors. Our assay has already achieved the level of performance required to detect PrP<sup>TSE</sup> at the femtomolar range in plasma, buffy coat, or whole-blood samples using either citrate or EDTA as anticoagulant. Here, by testing rare specimens from U.K. and French CJD surveillance centers, we further assess the performance of our assay against the strict requirements outlined in the amended Decision 2002/364/EC of the European Directive 98/79/EC (25) regarding the common technical specifications for vCJD blood-screening assays. Our assay allowed us to diagnose in a blinded fashion the presence of PrP<sup>TSE</sup> in samples from 18 of 18 symptomatic vCJD cases among a large sample collection, including samples from patients with sCJD, with 100% sensitivity. It also permitted PrP<sup>TSE</sup> detection in plasma samples collected 14 and 31 months before clinical onset in two French blood donors who later developed vCJD.

## RESULTS

### Analytical sensitivity of the vCJD diagnostic assay

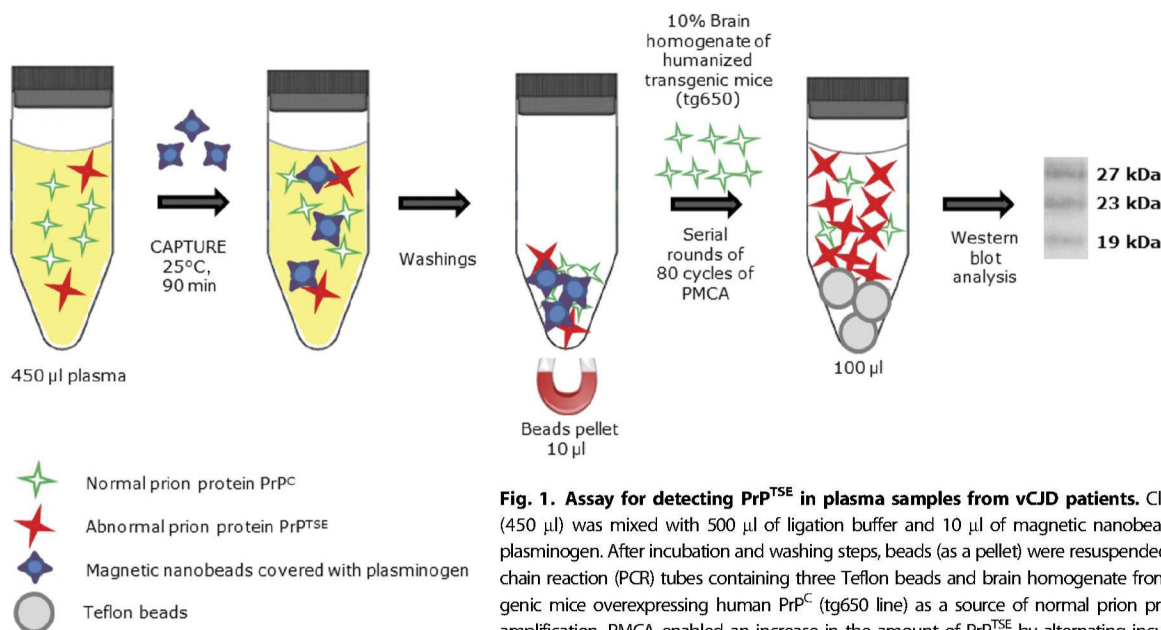
Our vCJD diagnostic assay consisted of a plasminogen-bead capture step followed by serial PMCA (Fig. 1). We validated our vCJD assay for analytical sensitivity (test detection threshold) using a blinded reference panel consisting of plasma (in citrate anticoagulant) spiked with World Health Organization (WHO) reference materials from the National Institute for Biological Standards and Control (NIBSC) (NHBY0/0003 for brain and NHSY0/0009 for spleen from vCJD patients) (30). To refine these sensitivity data, we prepared an additional panel of human plasma samples spiked with serial dilutions of the same vCJD WHO reference

brain tissue ranging from  $10^{-5}$  to  $10^{-12}$ . After three PMCA rounds of 80 cycles each, PrP<sup>TSE</sup> could be detected at a dilution of  $10^{-9}$  (Fig. 2). Additional rounds of PMCA did not increase this level of analytical sensitivity. The number of misfolded PrP<sup>TSE</sup> molecules in the  $10^{-10}$  dilution of the brain (and lower dilutions) and recovered by the plasminogen beads as the PMCA seed input was likely insufficient to initiate the amplification process. However, this  $10^{-9}$  dilution of analytical sensitivity is 4 to 5 log higher than the acceptance criteria of  $10^{-4}$  dilution required by the Common Technical Specifications defined in the European Commission Directive regarding the performances of vCJD screening assays (25).

### Diagnostic sensitivity and analytical specificity of the vCJD assay

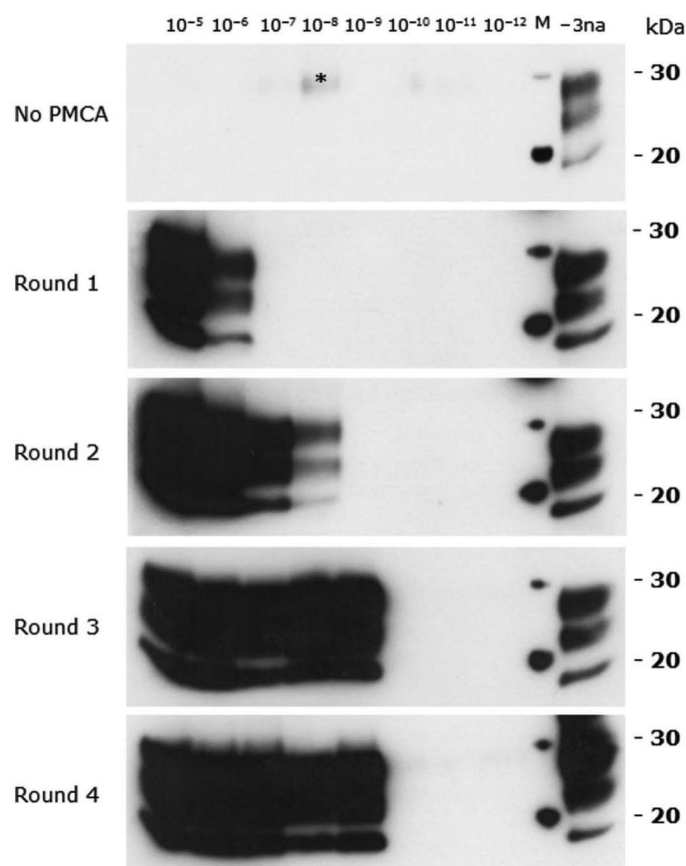
To evaluate the diagnostic sensitivity and the analytical specificity of our assay, we analyzed in a blinded fashion a total of 152 plasma samples from French and British patients, including 18 patients with vCJD, 67 with sCJD, and 67 with non-CJD neurological diseases. In addition, plasma samples from 104 blood donors were tested as healthy controls to evaluate diagnostic specificity (Table 1).

Among the 152 plasma samples from patients, our assay blindly identified all 18 cases of clinical vCJD, thus achieving a diagnostic sensitivity of 100% [95% confidence interval (CI), 81.5 to 100]. As shown in Table 2, of the 18 vCJD samples, 1 was positive after two rounds of PMCA, 8 were positive after three rounds of PMCA, and 9 were positive after four rounds. Whereas the detection limit was reached after three rounds of PMCA for brain material spiked into the plasma (exogenous prions), one additional round of PMCA increased the sensitivity of the assay when plasma samples from vCJD patients (endogenous prions) were tested. Blood samples were obtained for all patients shortly before death, except for five that were collected at earlier clinical phases of the disease. The number of rounds required to obtain a positive signal showed no correlation with the time at which the tested sample was collected before the patient's death.



**Fig. 1. Assay for detecting PrP<sup>TSE</sup> in plasma samples from vCJD patients.** Clarified plasma (450  $\mu$ l) was mixed with 500  $\mu$ l of ligation buffer and 10  $\mu$ l of magnetic nanobeads coated with plasminogen. After incubation and washing steps, beads (as a pellet) were resuspended in polymerase chain reaction (PCR) tubes containing three Teflon beads and brain homogenate from healthy transgenic mice overexpressing human PrP<sup>C</sup> (tg650 line) as a source of normal prion protein for PrP<sup>TSE</sup> amplification. PMCA enabled an increase in the amount of PrP<sup>TSE</sup> by alternating incubation (29 min 40 s at 37°C) and sonication steps (20 s at 240 W). After 80 cycles of PMCA, samples were analyzed by Western blot and/or subjected to a subsequent round of PMCA by refreshing the substrate.





**Fig. 2. Analytical sensitivity of the vCJD detection assay.** The analytical sensitivity of the assay was assessed by analyzing plasma samples supplemented with log dilutions of vCJD reference brain tissue ranging from  $10^{-5}$  to  $10^{-12}$ . The PrP<sup>TSE</sup> signal was assessed by means of Western blot analysis using the 3F4 antibody after proteinase K digestion. For each sample, 20  $\mu$ l of the product was loaded onto the gel. As a Western blot control, a  $10^{-3}$  dilution of the vCJD reference brain sample without any amplification (no PMCA) was loaded on each gel (-3na). The results obtained after each serial round of PMCA are shown. The asterisk indicates a faint signal from incomplete PrP<sup>C</sup> digestion. M, molecular weight markers.

As shown in Table 1, our assay also showed high analytical specificity, with only 1 of 134 potentially cross-reacting blood specimens from patients with sCJD, Alzheimer's disease, Parkinson's disease, and other neurological diseases giving a positive result (99.2% specificity; 95% CI, 95.9 to 100). The positive specimen was from a 65-year-old patient in the U.K. with sCJD (homozygous for methionine at codon 129 with a 2a molecular typing; type MM2a). Two other plasma samples from type MM2a sCJD patients were tested in this study and gave negative results for PrP<sup>TSE</sup>. Results obtained on the whole U.K. panel (20 plasma samples) are shown in Fig. 3. Representative results obtained on French plasma samples are shown in Fig. 4A. No PrP<sup>TSE</sup> signal was detected in the 104 plasma samples from blood donors (Table 1) even after five rounds of PMCA, thus indicating a diagnostic specificity of 100% (95% CI, 96.5 to 100).

We then compared by Western blotting the PrP<sup>TSE</sup> molecular signature obtained for the clinical vCJD amplified samples with that of the reference brain samples from patients with sCJD or vCJD (Figs. 3 and 4B). Samples from all the vCJD patients displayed the characteristic type 2 mobility (19 kDa) and clear predominance of the diglycosylated isoform (27 kDa), whether amplified from plasma (Fig. 4B;

vCJD plasma) or from crude brain tissue (Fig. 4B; vCJD brain homogenate). A similar type 2 mobility was also seen on the amplified products obtained from the U.K. patient samples (Fig. 3), including the 10 vCJD samples and strikingly with the sCJD patient classified as MM2a [UK-14; Fig. 3].

### Preclinical detection of abnormal prions in plasma

In France, since 1999, as an additional control for blood safety, two 500- $\mu$ l nonleukodepleted aliquots of the plasma of all blood donations are archived in liquid nitrogen for 5 years by the Etablissement Français du Sang (French blood service). Three of the 27 patients who developed clinical signs of vCJD had been regular blood donors (cases 8, 9, and 13). Eleven sequential archived plasma vials from two of these three donors were traced and specifically archived for long-term conservation. These vials correspond to the seven last blood donations from donor A (case 8) and the four last donations from donor B (case 13). No archived samples could be traced for case 9. Donors A and B donated once or twice a year before they developed clinical signs of vCJD 7 and 2 months, respectively, after their last blood donation. Thus, we analyzed 11 nonleukodepleted plasma samples from donors A and B starting from 55 to 30 months, respectively, before clinical symptom onset. For donor A (Fig. 5A), although the first four plasma samples (A1 to A4) were negative after six successive rounds of PMCA, we detected PrP<sup>TSE</sup> 31 months before clinical onset (A5) and in subsequent blood donations (A6 and A7) after three or four PMCA rounds. In donor B, after six PMCA rounds, PrP<sup>TSE</sup> detection was negative 30 months before clinical onset (B1) and became positive 14 months later (B2), which was 16 months before the appearance of clinical signs of the disease (Fig. 5B). Subsequent blood donations (B3 and B4) were also positive for vCJD prions after three or four PMCA rounds. These results demonstrate the presence of PrP<sup>TSE</sup> in the plasma of individuals during the presymptomatic phase of vCJD.

### DISCUSSION

Here, we report a detection method in plasma allowing the diagnosis of clinical vCJD with 100% sensitivity, 99.2% analytical specificity, and 100% diagnostic specificity. Despite the rarity of the samples, we have tested 18 blood samples from vCJD patients from France and the U.K. Eighteen of 18 clinical vCJD cases were identified in a blinded fashion. Such results satisfy the requirements for both sensitivity and specificity mandated by the European Commission Directive (25). In a related study in this issue, Concha-Marambio *et al.* provided further support for the validity of the PMCA technology for detecting vCJD in human blood samples with 100% sensitivity and specificity (31).

The finding that 1 of 67 sCJD patients tested positive for PrP<sup>TSE</sup> in plasma may suggest that our assay could sporadically amplify type MM2a PrP<sup>TSE</sup> under conditions optimized for vCJD detection. The presence of infectivity in the blood of patients with sCJD has recently been reported (32) but requires further investigation. Jackson *et al.* (20), when determining the diagnostic performance of their prototype vCJD ELISA blood test, also found two patients with sCJD who tested positive, indicating some reactivity of their assay for sCJD in whole blood. Lastly, and as suggested by the results obtained in prevalence studies on appendix tissue (16), we cannot exclude the possibility that the MM2a U.K. sCJD patient testing positive in our assay was also a carrier of vCJD infection in lymphoid tissue. No peripheral tissues are available from this case to investigate this hypothesis.

Table 1. Analytical performance of the plasminogen-based capture and amplification assay.

Diagnosis		Patients with positive detection of Pr <sup>TSE</sup> in plasma samples. No./total no.	Analytical performance, % (95% CI)
Clinical CJD	vCJD	18/18	Diagnostic sensitivity, 100% (81.5 to 100)
	Probable	4/4	
	Definite	14/14	
	sCJD	1/67	Analytical specificity, 99.2% (95.9 to 100)
	Probable	0/27	
	Definite	1*/40	
Non-CJD	Other neurodegenerative diseases	0/15	
	Alzheimer's disease	0/9	
	Lewy body dementia	0/3	
	Parkinson's disease	0/2	
	Frontotemporal dementia	0/1	
	Other nonneurodegenerative diseases	0/52	
	Metabolic and toxic encephalopathies	0/14	
	Paraneoplastic encephalitis and cancer	0/12	
	Neurovascular disease	0/7	
	Infectious diseases	0/6	
	Autoimmune encephalopathies	0/3	
	Other disorders**	0/10	
Blood donors	Healthy controls	0/104	Diagnostic specificity, 100% (96.5 to 100)
	Presymptomatic vCJD***	2/2	

\*U.K. patient aged 65 years, sCJD type MM2a. \*\*Epilepsy (n = 2), brain anoxia (n = 2), psychiatric diseases (n = 2), celiac disease (n = 1), cerebellar ataxia (n = 1), confusion (n = 1), and polyradiculoneuropathy (n = 1). \*\*\*Both patients are distinct from the eight French patients with clinical vCJD sampled during the clinical course of the disease.

Using recombinant PrP as a substrate instead of brain homogenate and intensive agitation instead of sonication, an alternative method called real-time quaking-induced conversion (RT-QuIC) has emerged as a powerful tool for prion detection (33, 34). Independent studies have shown that the detection in the cerebrospinal fluid or in the olfactory epithelium of PrP<sup>TSE</sup> for the diagnosis of sCJD by RT-QuIC may be 99 to 100% specific (35–37). However, as yet, RT-QuIC appears to be less efficient in detecting PrP<sup>TSE</sup> from vCJD patients using various full-length recombinant PrP (38), although the use of hamster-sheep chimeric PrP may improve its sensitivity (22). From a clinical point of view, to date, RT-QuIC–derived methods have not been used to diagnose vCJD. Two detection assays have been described for the detection of vCJD prions in blood with sensitivities close to 70%. The first one combines a solid-state binding matrix to capture and concentrate PrP<sup>TSE</sup> from whole-blood samples with direct immunodetection (18). Having demonstrated 98.1% analytical specificity (95% CI, 93.3 to 99.8%) with two sCJD positives detected and 100% diagnostic specificity (95% CI, 99.9 to 100%) in 5000 U.S. blood donor samples (20), this assay appears promising as a screening assay. The

second assay is a PMCA-based assay that has allowed the detection of PrP<sup>TSE</sup> in white blood cells from three of four patients with clinical vCJD (21). The diagnosis of vCJD may also be achieved using urine samples according to a third PMCA-based assay (24), which has also shown promising results: 92.9% sensitivity on 14 symptomatic vCJD patient samples (95% CI, 66.1 to 99.8%) and 100% diagnostic specificity on 52 healthy control samples (95% CI, 93.2 to 100%). There is no evidence that these assays can detect PrP<sup>TSE</sup> in carriers incubating the disease, who have not yet shown clinical symptoms.

Here, we report the identification of asymptomatic patients with detectable PrP<sup>TSE</sup> in the blood before clinical onset. The observed kinetics of the appearance of detectable PrP<sup>TSE</sup> in plasma indicates that a presymptomatic diagnosis is possible up to 31 months before the first clinical symptoms appear. This is consistent with the intervals of 17 to 40 months between the blood donations most likely responsible for secondary vCJD transmission in the U.K. and the onset of vCJD symptoms observed in the corresponding donors (11, 13, 39). The number of PMCA rounds required to obtain a positive signal varied between three and four during the incubation period in each



asymptomatic case and between two and four after clinical onset. The kinetics of circulating PrP<sup>TSE</sup> during the incubation period and during the clinical phase of the natural disease is unknown in humans. It remains unclear whether circulating PrP<sup>TSE</sup> continuously increases

during the incubation period and whether it reaches a steady state during the clinical phase. It is worth noting that the presence of PrP<sup>TSE</sup> can be scarce in the lymphoid tissues of some symptomatic vCJD patients, leading to false negatives on tonsil biopsy (3, 40). Our results suggest that circulating PrP<sup>TSE</sup> during incubation and disease usually fluctuates within the limits of detection corresponding to three or four rounds of our PMCA assay, which is equivalent to the PrP<sup>TSE</sup> contained in 2 to 20 pg of vCJD brain tissue. A quantitative PMCA assay as described by Chen *et al.* (41) may be a useful approach to precisely assess PrP<sup>TSE</sup> concentrations in the blood of vCJD patients.

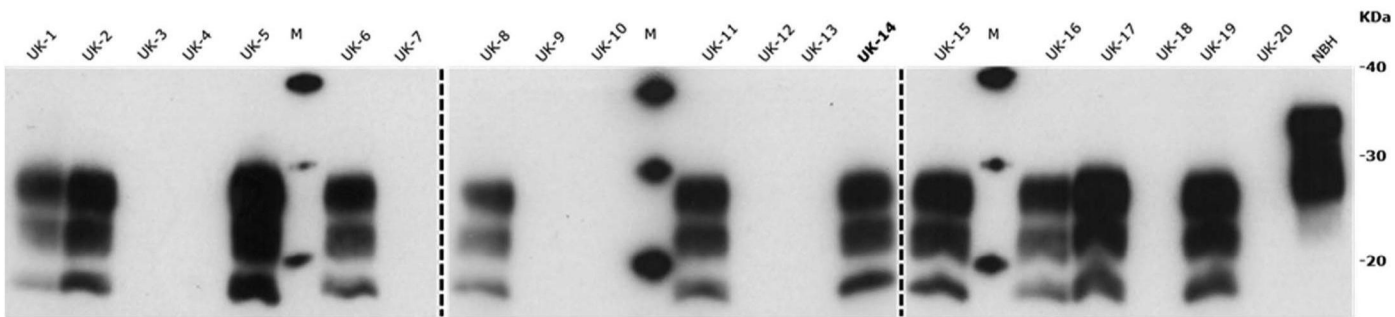
Our findings demonstrate that measurable amounts of PrP<sup>TSE</sup> with seeding activity can be found in the blood of humans at a presymptomatic stage. This has profound implications for public health and risk management, particularly in transfusion medicine. Our results provide a first estimation of the level of circulating abnormal prions in the plasma of patients in the final years of the incubation period for vCJD, which is not dissimilar from that observed in symptomatic patients. In France, no case of transfusion-transmitted vCJD has been reported so far in patients treated with blood products from the three vCJD patients who donated before symptom onset. This observation suggests that the precautionary measures introduced in France to safeguard blood transfusion, in particular leukodepletion, may have reduced the risk of transmission. With a limit of detection in the femtomolar range, our assay should allow a more precise evaluation of the residual risk of vCJD prion distribution in the different blood components. It may also be well suited for use as a complementary confirmatory assay, ensuring that any positive results from high-throughput blood-screening assays are true positives.

From a clinical point of view, a diagnostic method with 100% sensitivity is of special interest for diagnosis in young patients showing clinical signs that do not fulfill the criteria of probable vCJD or in older patients with atypical forms of rapidly progressive dementia (42).

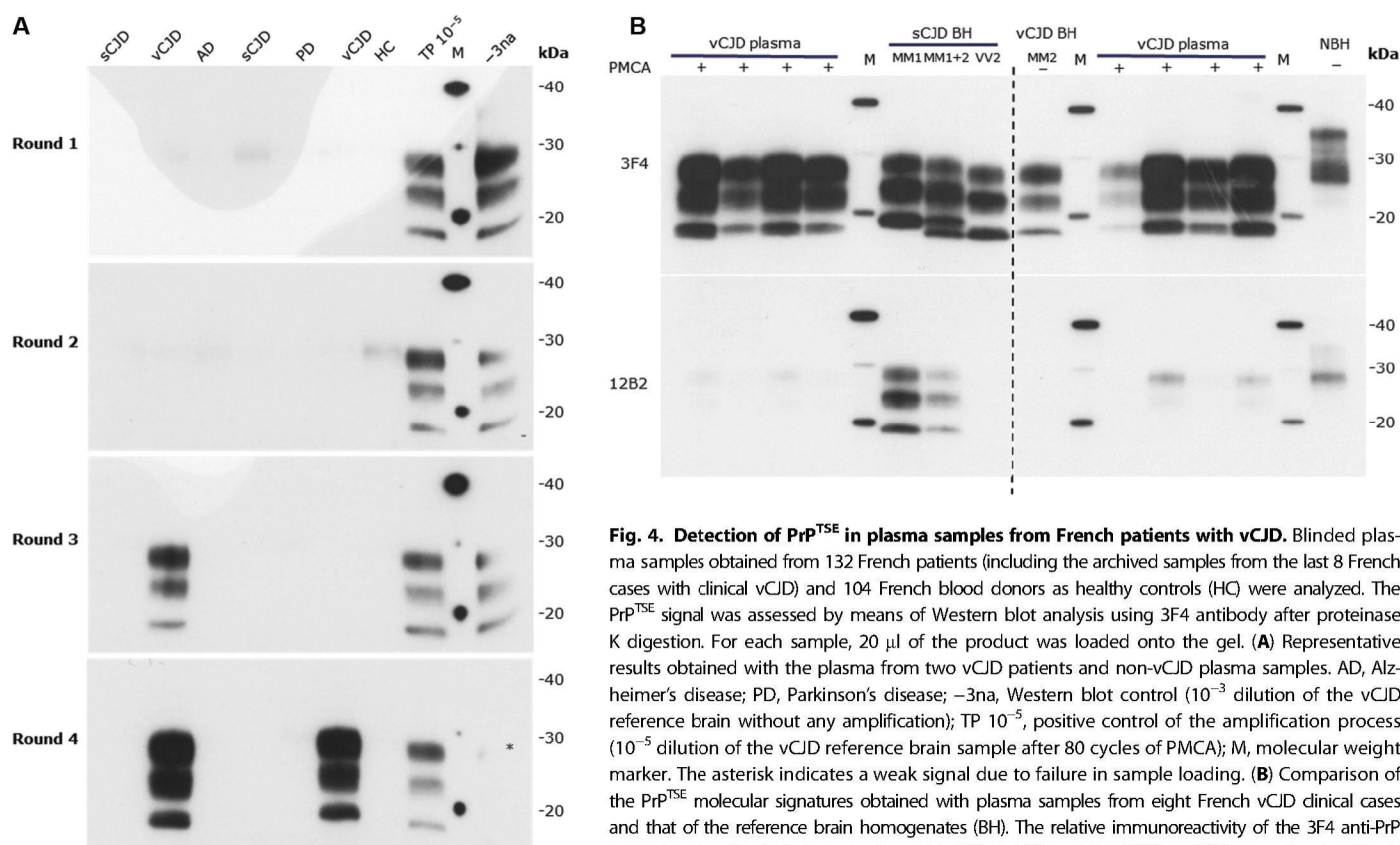
There are some limitations to our study. Only a limited number of blood samples from vCJD patients with clinical disease were tested, but the number of samples in this study nonetheless fulfilled the requirements of the European Commission Directive (25) because vCJD is a very rare disease. In addition, the number of 18 symptomatic vCJD patients is within the range of recent international studies in the field of vCJD diagnosis (18, 24). Our study demonstrated the presence of PrP<sup>TSE</sup> in the plasma in only two presymptomatic vCJD cases, but samples of such presymptomatic blood donors are rare, and their

Table 2. Characteristics of the 18 vCJD clinical cases included in this study.

Case no.	Duration of the clinical phase (months)	Time at which the tested sample was harvested (months before death)	No. of rounds of serial PMCA to detect PrP <sup>TSE</sup> in the plasma samples
FR-20	14.0	2.6	3
FR-21	8.7	2.8	3
FR-22	15.8	9.8	3
FR-23	9.8	1.9	4
FR-24	13.5	5.5	3
FR-25	13.4	5.5	3
FR-26	26.7	10.1	3
FR-27	27.1	21.2	4
UK-1	14	4.3	4
UK-2	15	10.5	3
UK-5	14	0.9	3
UK-6	10	3.4	4
UK-8	18	9.7	4
UK-11	18	6.0	4
UK-15	13	2.8	2
UK-16	24	0.4	4
UK-17	10	6.7	4
UK-19	15	2.0	4



**Fig. 3. Detection of PrP<sup>TSE</sup> in plasma samples from U.K. patients with CJD.** Blinded plasma samples obtained from 20 U.K. CJD patients were analyzed. The PrP<sup>TSE</sup> signal was assessed by means of Western blot analysis using 3F4 antibody after proteinase K digestion. For each positive signal, the equivalent of 7 to 10  $\mu$ l of the product obtained after four rounds of PMCA was loaded onto the gel, whereas for negative signals, 20  $\mu$ l of the product obtained after five rounds of PMCA was loaded onto the gel. UK-1, UK-2, UK-5, UK-6, UK-8, UK-11, UK-15, UK-16, UK-17, and UK-19 refer to vCJD patients. UK-3, UK-4, UK-7, UK-9, UK-10, UK-12, UK-13, UK-14, UK-18, and UK-20 refer to sCJD patients. NBH refers to a negative control brain homogenate from a non-CJD individual without any proteinase K digestion. M, molecular weight marker. The dashed lines indicate separate Western blots.



**Fig. 4. Detection of PrP<sup>TSE</sup> in plasma samples from French patients with vCJD.** Blinded plasma samples obtained from 132 French patients (including the archived samples from the last 8 French cases with clinical vCJD) and 104 French blood donors as healthy controls (HC) were analyzed. The PrP<sup>TSE</sup> signal was assessed by means of Western blot analysis using 3F4 antibody after proteinase K digestion. For each sample, 20  $\mu$ l of the product was loaded onto the gel. **(A)** Representative results obtained with the plasma from two vCJD patients and non-vCJD plasma samples. AD, Alzheimer's disease; PD, Parkinson's disease; -3na, Western blot control (10<sup>-3</sup> dilution of the vCJD reference brain without any amplification); TP 10<sup>-5</sup>, positive control of the amplification process (10<sup>-5</sup> dilution of the vCJD reference brain sample after 80 cycles of PMCA); M, molecular weight marker. The asterisk indicates a weak signal due to failure in sample loading. **(B)** Comparison of the PrP<sup>TSE</sup> molecular signatures obtained with plasma samples from eight French vCJD clinical cases and that of the reference brain homogenates (BH). The relative immunoreactivity of the 3F4 anti-PrP monoclonal antibody (epitope amino acids 109 to 112) and the 12B2 anti-PrP monoclonal antibody (epitope amino acids 89 to 93) is shown. 12B2 is specific for type 1 PrP<sup>TSE</sup> because its epitope is cleaved during proteinase K digestion in type 2 PrP<sup>TSE</sup>. NBH refers to a negative control brain homogenate from a non-CJD plasma sample without any proteinase K digestion. The dashed line indicates separate Western blots.

inclusion provided a unique opportunity to assess the kinetics of PrP<sup>TSE</sup> presence in blood during the incubation period. Until recently, all patients with clinical vCJD worldwide, including those enrolled in the present study (table S1), have been methionine-homozygous at codon 129 of the *PRNP* gene. The potential for infection to occur in lymphoid tissues and in the central nervous system in the other genotypes at codon 129 is supported by the observation of PrP<sup>TSE</sup> accumulation in the lymphoid tissues of an asymptomatic heterozygous recipient of a potentially contaminated blood transfusion (12) and the recent report of the first vCJD case in a heterozygous patient (4). In addition, 50% of the asymptomatic carriers identified in the prevalence study on appendix tissues (16) were not homozygous for methionine at codon 129. Although the present study is an important first step for the systematic detection of infected individuals, with a significant potential impact on transfusion safety, whether the assay could detect PrP<sup>TSE</sup> in the blood of vCJD patients with other genotypes has yet to be confirmed.

In conclusion, our results provide valuable data on circulating abnormal prion proteins during the incubation phase in humans and validate a new protein misfolding amplification-based assay that will reduce the potential risk of vCJD secondary transmission. Moreover, beyond these public health issues, our study provides evidence that a brain proteinopathy responsible for neurodegenerative disease can be diagnosed in asymptomatic patients through the detection of circulating abnormal protein aggregates. This finding is of interest for future translational research in neurological diseases caused by protein

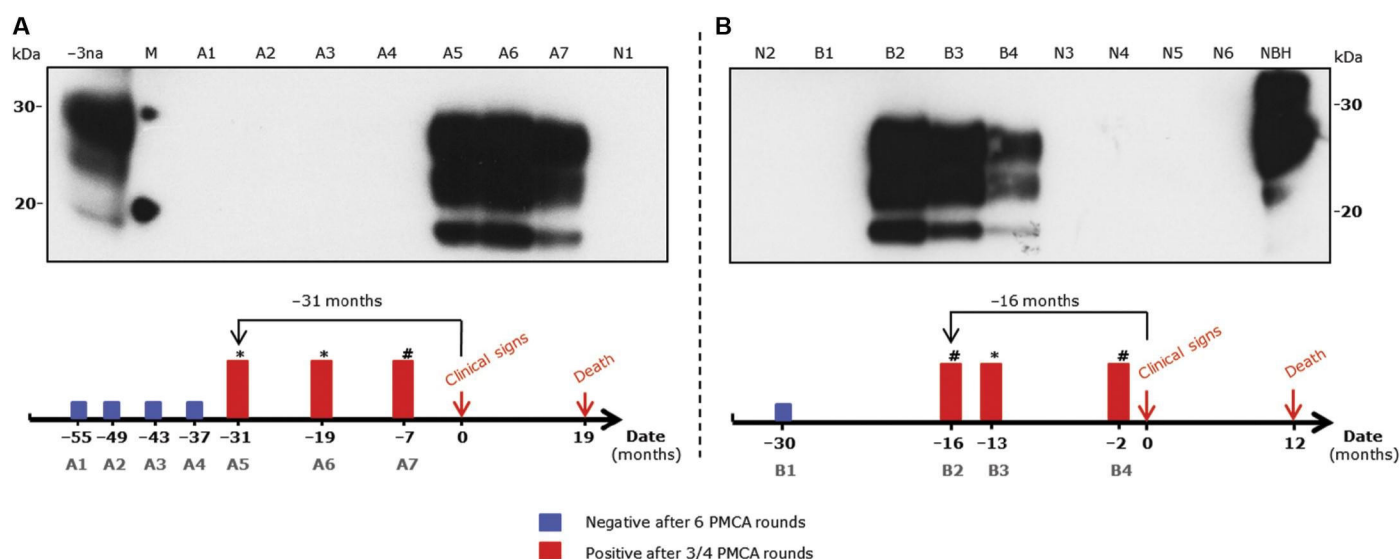
misfolding and aggregation, such as Alzheimer's disease and Parkinson's disease, for which there is a major need for a noninvasive early diagnostic test.

## MATERIALS AND METHODS

### Study design

The aim of the study was to investigate the suitability of our blood diagnostic assay comprising plasminogen-bead capture coupled with PMCA to identify vCJD-infected individuals. Compared with the handling of an emerging viral infection, development of a screening assay for infectious prions that in addition can be applied to blood samples from asymptomatic patients is challenging. In particular, only a small number of blood samples are available from clinical cases in France; no seroconversion panels are available nor a gold standard assay against which candidate-screening assays can be compared. From a regulatory point of view, vCJD assays for blood screening, diagnosis, and confirmation have been added to List A of Annexe II to Directive 98/79/EC by the Commission Directive 2011/100/EU of the EU In Vitro Diagnostics Directive, meaning that candidate assays must meet strict requirements with regard to sensitivity and specificity (25). Our study aimed to meet these acceptance criteria by testing plasma samples distributed in blinded panels from the U.K. and France. These panels included 18 plasma samples from vCJD patients, 134 potentially cross-reacting plasma samples from patients with neurological disorders, and 104 plasma samples from French blood donors. The detectability





**Fig. 5. Detection of PrP<sup>TSE</sup> in plasma from two blood donors in the preclinical phase of vCJD.** A set of 20 archived plasma vials stored at the Etablissement Français du Sang was tested. Seven plasma samples (labeled A1 to A7) had been collected from the eighth vCJD French case (donor A) during each of his last blood donations before he developed clinical signs of disease. Similarly, four plasma samples (labeled B1 to B4) had been collected from the 13th vCJD French case (donor B). Time of plasma sample collection (months) for each case is shown as the dates at which the first clinical signs appeared. Nine plasma control samples were obtained from blood donors who never developed CJD. Results obtained for all the plasma samples from donor A (A) and donor B (B) and six control plasma samples (N1 to N6) are shown. The PrP<sup>TSE</sup> signal was assessed by means of Western blot analysis with 3F4 antibody after proteinase K digestion. For each sample, 20  $\mu$ l of the product obtained after six rounds of PMCA was loaded onto the gel, except for samples labeled A5, A6, A7, B2, B3, and B4 for which the equivalent of 4  $\mu$ l of the product obtained after four rounds of PMCA was loaded. -3na, Western blot control ( $10^{-3}$  dilution of the vCJD reference brain tissue without any amplification); NBH, a negative control brain homogenate from a non-CJD plasma sample without any proteinase K digestion; M, molecular weight markers. "\*" indicates positive after three rounds and "#" indicates positive after four rounds.

of PrP<sup>TSE</sup> in the blood of presymptomatic carriers was also assessed on 11 archived plasma vials from two blood donors who subsequently developed vCJD.

### Normal plasma samples and reference brain tissues

To assess the diagnostic specificity, anonymized blood samples were collected from consenting French blood donors in compliance with French regulations (code de la santé publique article L.1243-3). Whole-blood samples ( $n = 104$ ) were collected in EDTA-containing tubes. Plasma was isolated after centrifugation at 1500g for 15 min at 21°C and stored at -80°C until use.

For the study of analytical sensitivity, WHO reference brain samples (10%, w/v) were provided by the NIBSC (U.K.) under the reference number NHBX0/0003 for vCJD and number NHBX0/0001 for sCJD [methionine-homozygous at codon 129 with PrP<sup>TSE</sup> type 1 + 2 (MM1 + 2)]. Two additional sCJD brain samples were provided by CHU-Lyon (France), including one methionine-homozygous case with PrP<sup>TSE</sup> type 1 (MM1) and one valine-homozygous case with PrP<sup>TSE</sup> type 2 (VV2). NIBSC also provided a negative control WHO reference brain homogenate (number NHBZ0/0005).

### Blind panels from the French CJD surveillance network

Since 1993, all CJD suspects across France have been notified to the national CJD surveillance network (43). Most suspects are notified by the laboratories that perform cerebrospinal fluid examination for detection of the 14-3-3 protein. Other sources of notification are neurological clinics, neuropathology laboratories, and the French national institute for public health surveillance [Institut de Veille Sanitaire (InVS)], which collects data on all notifiable diseases. For each suspected case, the CJD surveillance network collects all avail-

able medical data to classify each case as sCJD, genetic CJD, iatrogenic CJD, vCJD (definite, probable, or possible), or non-CJD, using internationally recognized criteria (44, 45). Only definite and probable CJD cases are reported to the EuroCJD network, and all vCJD cases are reported to the European Centre for Disease Prevention and Control.

Blood sampling from cases with a suspected diagnosis of CJD notified to the surveillance network was initiated in June 2006. This collection is dedicated to the assessment of novel diagnostic procedures for CJD. An informed and signed consent from the patients or their relatives was obtained for each sample. The procedure was approved by the ethics committee of the Pitié-Salpêtrière Hospital (CCPBRB/130-05). Blood was sampled in tubes containing sodium citrate, heparin, or EDTA. After centrifugation at 2000g for 15 min at 4°C, plasma, buffy coat, and red blood cells were sampled in aliquots of 150  $\mu$ l and stored at -80°C. Here, only EDTA plasma samples were used. Samples from 132 patients including 8 cases with vCJD (the last 8 cases on a total of 27 French cases who have died of vCJD), 57 cases with sCJD, and 67 cases with other neuropsychiatric diagnosis (see Table 1) were split up into six consecutive groups and provided blindly to undergo our testing procedure.

### Blind panels from the U.K. National CJD Research and Surveillance Unit

The National CJD Research and Surveillance Unit was established in 1990 and receives referrals of all suspect cases of CJD in the U.K. Whenever possible, staff from the unit visit the referred cases for assessment and collection of detailed medical data before classifying the cases using internationally recognized criteria (44, 45). Only definite and probable CJD cases are reported to the EuroCJD network, and



all vCJD cases are reported to the European Centre for Disease Prevention and Control.

Blood samples have been obtained in all cases since 1990. Informed and signed consent was provided by the patient or relatives. The study was approved by the National Health Service Lothian Ethics Committee. The blood samples were collected in EDTA or citrate. After centrifugation at 400g for 10 min, plasma, buffy coat, and red blood cells were stored at  $-80^{\circ}\text{C}$ . Here, samples stored in citrate or EDTA were used. In eight vCJD samples, the type of anticoagulant, either EDTA or citrate, was not recorded. Samples included 10 from vCJD cases (of 178 cases of vCJD in the U.K.) and 10 from sCJD cases. The 20 samples were provided blinded to undergo the testing procedure.

### Archived plasma panels from the Etablissement Français du Sang (French blood service)

Since 1999, two aliquots of nonleukodepleted plasma from all blood donations are archived for 5 years by the Etablissement Français du Sang in liquid nitrogen to allow additional controls with regard to blood safety. These aliquots, each of 500  $\mu\text{L}$ , are prepared from whole blood collected in EDTA-containing tubes. Eleven sequential archived plasma vials from two donors who later developed vCJD were traced and specifically archived for long-term conservation (over 5 years). These vials correspond to the last seven blood donations for the 8th vCJD French case (donor A) and the last four donations for the 13th vCJD French case (donor B). Nine archived plasma samples from blood donors who never developed CJD were added to this study.

### Testing procedure

The testing procedure is a three-step assay (30) that captures  $\text{PrP}^{\text{TSE}}$  from infected blood using plasminogen-coated magnetic nanobeads before serial amplification of  $\text{PrP}^{\text{TSE}}$  via PMCA and finally specific detection by Western blot (Fig. 1). Plasma samples were thawed in a water bath for 10 min at  $37^{\circ}\text{C}$  and clarified at 1500g for 5 min at  $21^{\circ}\text{C}$ . In the preanalytical capture step, 10  $\mu\text{L}$  of 1% (w/v) beads (Ademtech) at 10  $\mu\text{g}$  of plasminogen per milligram of beads was added to a mix (1:1) of plasma samples (400 to 500  $\mu\text{L}$ ) and ligation buffer [0.1 M phosphate, 0.5% sodium chloride, and 0.1% sarkosyl (pH 7.4)] before being incubated for 90 min at  $25^{\circ}\text{C}$ . After washing steps with phosphate-buffered saline, beads were directly resuspended in the PMCA substrate.

For the PMCA amplification, the source of  $\text{PrP}$  used as substrate was obtained from brain homogenates of healthy transgenic mice overexpressing sixfold more human  $\text{PrP}$  with a methionine at codon 129 (tg650 line) (46). Brains were prepared at 10% (w/v) homogenate in converting buffer (phosphate-buffered saline containing 150 mM sodium chloride and 1% Triton) and clarified at 2000g for 20 s before freezing at  $-80^{\circ}\text{C}$  in single-experiment aliquots of PMCA substrate.

The captured prion protein was mixed with 90  $\mu\text{L}$  of PMCA substrate in PCR tubes containing three Teflon beads. Amplification (80 cycles) was performed by three different technicians using two different types of sonicators (S4000, Misonix, and Q700; Qsonica). Each cycle is composed of an incubation step (29 min 40 s at  $37^{\circ}\text{C}$ ) and a sonication step (20 s at 240 W). Successive rounds of 80 cycles were performed by diluting the amplified material 1:10 in fresh PMCA substrate. To avoid any cross-contamination, experiments were carried out under strict quality-controlled PCR conditions. In addition, positive samples were not systematically subjected to an additional PMCA round.

After amplification, protease-resistant prion protein was detected by Western blot, as previously described (47). Samples were incubated

with proteinase K (200  $\mu\text{g}/\text{mL}$ ) for 60 min at  $45^{\circ}\text{C}$  before denaturation at  $100^{\circ}\text{C}$  in denaturing buffer. Samples were run on 12% polyacrylamide gel electrophoresis before being electrotransferred onto a polyvinylidene difluoride membrane and revealed using 3F4 or 12B2 monoclonal antibodies against prion protein.

### Statistical analysis

Calculations of 95% Clopper-Pearson CIs for sensitivities and specificities were performed according to the exact binomial test (48) using the free software R (49).

### SUPPLEMENTARY MATERIALS

[www.sciencetranslationalmedicine.org/cgi/content/full/8/370/370ra182/DC1](http://www.sciencetranslationalmedicine.org/cgi/content/full/8/370/370ra182/DC1)  
Methods

Table S1. Codon 129 genotype of the patients included in this study (including presymptomatic cases).

Reference (50)

### REFERENCES AND NOTES

1. M. E. Bruce, R. G. Will, J. W. Ironside, I. McConnell, D. Drummond, A. Suttie, L. McCordle, A. Chree, J. Hope, C. Birkett, S. Cousens, H. Fraser, C. J. Bostock, Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389**, 498–501 (1997).
2. R. G. Will, J. W. Ironside, M. Zeidler, S. N. Cousens, K. Estibeiro, A. Alperovitch, S. Poser, M. Pocchiar, A. Hofman, P. G. Smith, A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**, 921–925 (1996).
3. J.-P. Brandel, C. A. Heath, M. W. Head, E. Levavasseur, R. Knight, J.-L. Laplanche, J. P. M. Langeveld, J. W. Ironside, J.-J. Hauw, J. Mackenzie, A. Alperovitch, R. G. Will, S. Haik, Variant Creutzfeldt-Jakob disease in France and the United Kingdom: Evidence for the same agent strain. *Ann. Neurol.* **65**, 249–256 (2009).
4. National Creutzfeldt-Jakob Disease Research and Surveillance Unit, *Variant Creutzfeldt-Jakob Disease Cases Worldwide* (2016); [www.cjd.ed.ac.uk/documents/worldfigs.pdf](http://www.cjd.ed.ac.uk/documents/worldfigs.pdf).
5. A. F. Hill, R. J. Butterworth, S. Joiner, G. Jackson, M. N. Rossor, D. J. Thomas, A. Frosh, N. Tolley, J. E. Bell, M. Spencer, A. King, S. Al-Sarraj, J. W. Ironside, P. L. Lantos, J. Collinge, Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet* **353**, 183–189 (1999).
6. J. D. F. Wadsworth, S. Joiner, A. F. Hill, T. A. Campbell, M. Desbruslais, P. J. Luthert, J. Collinge, Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* **358**, 171–180 (2001).
7. O. Andréoletti, C. Litaize, H. Simmons, F. Corbière, S. Lugan, P. Costes, F. Schelcher, D. Vilette, J. Grassi, C. Lacroux, Highly efficient prion transmission by blood transfusion. *PLoS Pathog.* **8**, e1002782 (2012).
8. N. Bons, S. Lehmann, N. Mestre-Francès, D. Dormont, P. Brown, Brain and buffy coat transmission of bovine spongiform encephalopathy to the primate *Microcebus murinus*. *Transfusion* **42**, 513–516 (2002).
9. F. Houston, J. D. Foster, A. Chong, N. Hunter, C. J. Bostock, Transmission of BSE by blood transfusion in sheep. *Lancet* **356**, 999–1000 (2000).
10. C. Lacroux, D. Bougard, C. Litaize, H. Simmons, F. Corbière, D. Darnis, R. Tardivel, N. Morel, S. Simon, S. Lugan, P. Costes, J. L. Weisbecker, F. Schelcher, J. Grassi, J. Coste, O. Andréoletti, Impact of leucocyte depletion and prion reduction filters on TSE blood borne transmission. *PLoS ONE* **7**, e42019 (2012).
11. C. A. Llewellyn, P. E. Hewitt, R. S. G. Knight, K. Amar, S. Cousens, J. Mackenzie, R. G. Will, Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* **363**, 417–421 (2004).
12. A. H. Peden, M. W. Head, D. L. Ritchie, J. E. Bell, J. W. Ironside, Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient. *Lancet* **364**, 527–529 (2004).
13. S. J. Wroe, S. Pal, D. Siddique, H. Hyare, R. Macfarlane, S. Joiner, J. M. Linehan, S. Brandner, J. D. F. Wadsworth, P. Hewitt, J. Collinge, Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: A case report. *Lancet* **368**, 2061–2067 (2006).
14. A. Peden, L. McCordle, M. W. Head, S. Love, H. J. T. Ward, S. N. Cousens, D. M. Keeling, C. M. Millar, F. G. H. Hill, J. W. Ironside, Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia* **16**, 296–304 (2010).
15. K. Holada, J. G. Vostal, P. W. Theisen, C. MacAuley, L. Gregori, R. G. Rohwer, Scrapie infectivity in hamster blood is not associated with platelets. *J. Virol.* **76**, 4649–4650 (2002).



16. O. N. Gilli, Y. Spencer, A. Richard-Loendt, C. Kelly, R. Dabaghian, L. Boyes, J. Linehan, M. Simmons, P. Webb, P. Bellerby, N. Andrews, D. A. Hilton, J. W. Ironside, J. Beck, M. Poulter, S. Mead, S. Brandner, Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: Large scale survey. *BMJ* **347**, f5675 (2013).
17. D. A. Hilton, A. C. Ghani, L. Conyers, P. Edwards, L. McCauley, D. Ritchie, M. Penney, D. Hegazy, J. W. Ironside, Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J. Pathol.* **203**, 733–739 (2004).
18. J. A. Edgeworth, M. Farmer, A. Sicilia, P. Tavares, J. Beck, T. Campbell, J. Lowe, S. Mead, P. Rudge, J. Collinge, G. S. Jackson, Detection of prion infection in variant Creutzfeldt-Jakob disease: A blood-based assay. *Lancet* **377**, 487–493 (2011).
19. G. S. Jackson, J. Burk-Rafel, J. A. Edgeworth, A. Sicilia, S. Abdilahi, J. Korteweg, J. Mackey, C. Thomas, G. Wang, S. Mead, J. Collinge, A highly specific blood test for vCJD. *Blood* **123**, 452–453 (2014).
20. G. S. Jackson, J. Burk-Rafel, J. A. Edgeworth, A. Sicilia, S. Abdilahi, J. Korteweg, J. Mackey, C. Thomas, G. Wang, J. M. Schott, C. Mummery, P. F. Chinnery, S. Mead, J. Collinge, Population screening for variant Creutzfeldt-Jakob disease using a novel blood test: Diagnostic accuracy and feasibility study. *JAMA Neurol.* **71**, 421–428 (2014).
21. C. Lacroux, E. Comoy, M. Moudjou, A. Perret-Liaudet, S. Lugan, C. Litaise, H. Simmons, C. Jas-Duval, I. Lantier, V. Béringue, M. Groschup, G. Fichet, P. Costes, N. Streichenberger, F. Lantier, J. P. Deslys, D. Vilette, O. Andréoletti, Preclinical detection of variant CJD and BSE prions in blood. *PLoS Pathog.* **10**, e1004202 (2014).
22. C. D. Orrú, J. M. Wilham, L. D. Raymond, F. Kuhn, B. Schroeder, A. J. Raeber, B. Caughey, Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. *MBio* **2**, e00078-11 (2011).
23. P. Saá, J. Castilla, C. Soto, Presymptomatic detection of prions in blood. *Science* **313**, 92–94 (2006).
24. F. Moda, P. Gambetti, S. Notari, L. Concha-Marambio, M. Catania, K.-W. Park, E. Maderna, S. Suardi, S. Haik, J.-P. Brandel, J. Ironside, R. Knight, F. Tagliavini, C. Soto, Prions in the urine of patients with variant Creutzfeldt-Jakob disease. *N. Engl. J. Med.* **371**, 530–539 (2014).
25. European Commission, *Technical specifications for in vitro diagnostic medical devices* (Official Journal of the European Union, 2011).
26. G. P. Saborio, B. Permann, C. Soto, Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411**, 810–813 (2001).
27. M. B. Fischer, C. Roeckl, P. Parizek, H. P. Schwarz, A. Aguzzi, Binding of disease-associated prion protein to plasminogen. *Nature* **408**, 479–483 (2000).
28. M. Maissen, C. Roeckl, M. Glatzel, W. Goldmann, A. Aguzzi, Plasminogen binds to disease-associated prion protein of multiple species. *Lancet* **357**, 2026–2028 (2001).
29. C. E. Mays, C. Ryou, Plasminogen stimulates propagation of protease-resistant prion protein in vitro. *FASEB J.* **24**, 5102–5112 (2010).
30. C. Segarra, D. Bougard, M. Moudjou, H. Laude, V. Béringue, J. Coste, Plasminogen-based capture combined with amplification technology for the detection of PrP<sup>TSE</sup> in the pre-clinical phase of infection. *PLoS ONE* **8**, e69632 (2013).
31. L. Concha-Marambio, S. Pritzkow, F. Moda, F. Tagliavini, J. Ironside, P. Schulz, C. Soto, Detection of prions in blood from patients with variant Creutzfeldt-Jakob disease. *Sci. Transl. Med.* **8**, 370ra183 (2016).
32. J. Y. Douet, S. Zafar, A. Perret-Liaudet, C. Lacroux, S. Lugan, N. Aron, H. Cassard, C. Ponto, F. Corbière, J. M. Torres, I. Zerr, O. Andreoletti, Detection of infectivity in blood of persons with variant and sporadic Creutzfeldt-Jakob disease. *Emerg. Infect. Dis.* **20**, 114–117 (2014).
33. R. Atarashi, J. M. Wilham, L. Christensen, A. G. Hughson, R. A. Moore, L. M. Johnson, H. A. Onwubiko, S. A. Priola, B. Caughey, Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. *Nat. Methods* **5**, 211–212 (2008).
34. J. M. Wilham, C. D. Orrú, R. A. Bessen, R. Atarashi, K. Sano, B. Race, K. D. Meade-White, L. M. Taubner, A. Timmes, B. Caughey, Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog.* **6**, e1001217 (2010).
35. R. Atarashi, K. Satoh, K. Sano, T. Fuse, N. Yamaguchi, D. Ishibashi, T. Matsubara, T. Nakagaki, H. Yamanaka, S. Shirabe, M. Yamada, H. Mizusawa, T. Kitamoto, G. Klug, A. McGlade, S. J. Collins, N. Nishida, Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat. Med.* **17**, 175–178 (2011).
36. L. I. McGuire, A. H. Peden, C. D. Orrú, J. M. Wilham, N. E. Appleford, G. Mallinson, M. Andrews, M. W. Head, B. Caughey, R. G. Will, R. S. G. Knight, A. J. E. Green, Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann. Neurol.* **72**, 278–285 (2012).
37. C. D. Orrú, M. Bongiovanni, G. Tonoli, S. Ferrari, A. G. Hughson, B. R. Groveman, M. Fiorini, M. Pocchiari, S. Monaco, B. Caughey, G. Zanusso, A test for Creutzfeldt-Jakob disease using nasal brushings. *N. Engl. J. Med.* **371**, 519–529 (2014).
38. A. H. Peden, L. I. McGuire, N. E. J. Appleford, G. Mallinson, J. M. Wilham, C. D. Orrú, B. Caughey, J. W. Ironside, R. S. Knight, R. G. Will, A. J. E. Green, M. W. Head, Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J. Gen. Virol.* **93**, 438–449 (2012).
39. M. Gillies, G. Chohan, C. A. Llewellyn, J. MacKenzie, H. J. T. Ward, P. E. Hewitt, R. G. Will, A retrospective case note review of deceased recipients of vCJD-implicated blood transfusions. *Vox Sang.* **97**, 211–218 (2009).
40. S. Mead, J. D. F. Wadsworth, M.-C. Porter, J. M. Linehan, W. Pietkiewicz, G. S. Jackson, S. Brandner, J. Collinge, Variant Creutzfeldt-Jakob disease with extremely low lymphoreticular deposition of prion protein. *JAMA Neurol.* **71**, 340–343 (2014).
41. B. Chen, R. Morales, M. A. Barria, C. Soto, Estimating prion concentration in fluids and tissues by quantitative PMCA. *Nat. Methods* **7**, 519–520 (2010).
42. S. el Tawil, G. Mackay, L. Davidson, D. Summers, R. Knight, R. Will, Variant Creutzfeldt-Jakob disease in older patients. *J. Neurol. Neurosurg. Psychiatry* **86**, 1279–1280 (2015).
43. J. P. Brandel, A. Welaratne, D. Salomon, I. Capek, V. Vaillant, A. Aouba, S. Haik, A. Alpérovitch, Can mortality data provide reliable indicators for Creutzfeldt-Jakob disease surveillance? A study in France from 2000 to 2008. *Neuroepidemiology* **37**, 188–192 (2011).
44. M. Zeidler, C. Gibbs, F. Meslin, WHO manual for strengthening diagnosis and surveillance of Creutzfeldt-Jakob disease. (1998).
45. I. Zerr, K. Kallenberg, D. M. Summers, C. Romero, A. Taratuto, U. Heinemann, M. Breithaupt, D. Varges, B. Meissner, A. Ladogana, M. Schuur, S. Haik, S. J. Collins, G. H. Jansen, G. B. Stokin, J. Pimentel, E. Hewer, D. Collie, P. Smith, H. Roberts, J. P. Brandel, C. van Duijn, M. Pocchiari, C. Begue, P. Cras, R. G. Will, P. Sanchez-Juan, Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. *Brain* **132**, 2659–2668 (2009).
46. V. Béringue, A. Le Dur, P. Tixador, F. Reine, L. Lepourry, A. Perret-Liaudet, S. Haik, J.-L. Vilotte, M. Fontés, H. Laude, Prominent and persistent extraneural infection in human PrP transgenic mice infected with variant CJD. *PLoS ONE* **3**, e1419 (2008).
47. C. Segarra, S. Lehmann, J. Coste, Prion protein expression and processing in human mononuclear cells: The impact of the codon 129 prion gene polymorphism. *PLoS ONE* **4**, e5796 (2009).
48. C. J. Clopper, E. S. Pearson, The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* **26**, 404–413 (1934).
49. R. C. Team, R: A language and environment for statistical computing. (2014).
50. K. Peoc'h, P. Manivet, P. Beaudry, F. Attane, G. Besson, D. Hannequin, N. Delasnerie-Lauprêtre, J.-L. Laplanche, Identification of three novel mutations (E196K, V203I, E211Q) in the prion protein gene (*PRNP*) in inherited prion diseases with Creutzfeldt-Jakob disease phenotype. *Hum. Mutat.* **15**, 482 (2000).

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## Detection of prions in the plasma of presymptomatic and symptomatic patients with variant Creutzfeldt-Jakob disease

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### A new blood test for prion diseases

Variant Creutzfeldt-Jakob disease (vCJD) is a human prion disease that can be transmitted from person to person through medical procedures. The development of a diagnostic blood test is an urgent priority. Bougard *et al.* describe a sensitive and specific blood test based on a prion capture step and an amplification method. This test for vCJD was very accurate and worked not only for blood samples from patients suffering from vCJD but also for samples taken from two individuals 1.3 and 2.6 years before they developed clinical symptoms. This blood test has important implications for transfusion medicine and public health.

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