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Predictors of survival in sporadic Creutzfeldt–Jakob disease and other human transmissible spongiform encephalopathies

M. Pocchiari,[1] M. Puopolo,[1] E. A. Croes,[2] H. Budka,[3] E. Gelpi,[3] S. Collins,[4] V. Lewis,[4] T. Sutcliffe,[5] A. Guilivi,[5] N. Delasnerie-Laupretre,[6] J.-P. Brandel,[6] A. Alperovitch,[6] I. Zerr,[7] S. Poser,[7] H. A. Kretzschmar,[8] A. Ladogana,[1] I. Rietvald,[2] E. Mitrova,[9] P. Martinez-Martin,[10] J. de Pedro-Cuesta,[10] M. Glatzel,[11] A. Aguzzi,[11] S. Cooper,[12] J. Mackenzie,[12] C. M. van Duijn[2] and R. G. Will[12]

[1]*Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences Rome, Italy,* [2]*Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands,* [3]*Austrian Reference Centre for Human Prion Diseases (OERPE) and Institute of Neurology Department of Cell Biology and Neuro Sciences, Vienna, Austria,* [4]*Department of Pathology, The University of Melbourne, Victoria, Australia,* [5]*Blood Safety Surveillance and Health Care Acquired Infections Division, Centre for Infectious Disease Prevention and Control, LCDC Building, Ontario, Canada,* [6]*U.360 INSERM, Hôpital de la Salpetriere, Paris, France,* [7]*Department of Neurology, Georg-August-Universität Göttingen,* [8]*Institute of Neuropathology, University of Munich, Munich, Germany,* [9]*Institute of Preventative and Clinical Medicine, National Reference Centre of Slow Virus Neuroinfections, Bratislava, Slovakia,* [10]*Instituto de Salud Carlos III, Centro Nacional de Epidemiologia, Departamento de Epidemiologia Aplicada, Madrid, Spain,* [11]*Swiss National Reference Centre for Prion Diseases, University Hospital of Zurich, Zurich, Switzerland and* [12]*National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK*

Correspondence to: Professor R. G. Will, National Creutzfeldt–Jakob disease Surveillance Unit, Western General Hospital, Edinburgh EH4 2XU, UK
E-mail: r.g.will@ed.ac.uk

Summary

A collaborative study of human transmissible spongiform encephalopathies has been carried out from 1993 to 2000 and includes data from 10 national registries, the majority in Western Europe. In this study, we present analyses of predictors of survival in sporadic (*n* = 2304), iatrogenic (*n* = 106) and variant Creutzfeldt–Jakob disease (*n* = 86) and in cases associated with mutations of the prion protein gene (*n* = 278), including Gerstmann–Sträussler–Scheinker syndrome (*n* = 24) and fatal familial insomnia (*n* = 41). Overall survival for each disease type was assessed by the Kaplan–Meier method and the multivariate analyses by the Cox proportional hazards model. In sporadic disease, longer survival was correlated with younger age at onset of illness, female gender, codon 129 heterozygosity, presence of CSF 14-3-3 protein and type 2a prion protein type. The ability to predict survival based on patient covariates is important for diagnosis and counselling, and the characterization of the survival distributions, in the absence of therapy, will be an important starting point for the assessment of potential therapeutic agents in the future.

Keywords: survival; sporadic CJD; variant CJD; iatrogenic CJD; genetic prion diseases

Abbreviations: CI = confidence interval; FFI = fatal familial insomnia; gCJD = genetic forms of Creutzfeldt–Jakob disease; GSS = Gerstmann–Straüssler–Scheinker disease; gTSEs = genetic transmissible spongiform encephalopathies; iCJD = iatrogenic Creutzfeldt–Jakob disease; iDM = iatrogenic Creutzfeldt–Jakob disease following dura mater implant; ihGH = iatrogenic Creutzfeldt–Jakob disease following human growth hormone therapy; MM = methionine/methionine; MV = methionine/valine;

PRNP = prion protein gene; PrP^{Sc} = protease-resistant prion protein; PSWC = periodic sharp wave complexes; RR = relative risk; sCJD = sporadic Creutzfeldt–Jakob disease; TSEs = transmissible spongiform encephalopathies; vCJD = variant Creutzfeldt–Jakob disease; VV = valine/valine.

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Introduction

Human transmissible spongiform encephalopathies (TSEs) or prion diseases comprise a number of conditions with varying aetiology and include sporadic Creutzfeldt–Jakob disease (sCJD), iatrogenic CJD (iCJD), variant CJD (vCJD) and cases associated with mutations of the prion protein gene (*PRNP*) (gTSE). Within the latter group are genetic forms of CJD (gCJD), for example cases linked to mutations at codon 200 and codon 210 of *PRNP*, Gerstmann–Sträussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI) (Pocchiari, 1994). The clinical phenotypes of the subtypes of human TSE vary, and this variation is determined in part by the polymorphism at codon 129 of *PRNP* (Collinge *et al*., 1991; Palmer *et al*., 1991; Kovacs *et al*., 2002) and the type of prion protein deposited in the brain (Parchi *et al*., 1996). One important variable is the total duration of clinical illness, also termed survival, and this parameter can be useful in clinical diagnosis of the various forms of human TSE, including sCJD (Brown *et al*., 1994). In genetic cases, iCJD cases following human growth hormone therapy (ihGH) and vCJD, survival is more prolonged than in sCJD, but considerable variation in survival is seen within all subtypes of human TSE.

Variables that are thought to influence survival are age (with a reduction in survival with increasing age in sCJD; Puopolo *et al*, 2003), the codon 129 genotype and the type of prion protein deposited in the brain. There have been suggestions of a gender effect on the incidence of sCJD (Will *et al*., 1986; Brown *et al*., 1987), but this has been inconsistent between studies (Galvez *et al*., 1980; Lundberg, 1998). The identification of significant and consistent predictors/determinants of survival is a difficult task because of the rarity of human TSEs, but establishing definitive factors that influence disease phenotype, including survival, may lead to clues to the underlying pathogenic mechanisms and to improved case recognition and clinical discrimination between the various disease subtypes.

The renewed interest in developing specific therapies for human TSEs (Brown, 2002; Korth *et al*., 2002) has highlighted the importance of clearly delineating the natural history of these disorders. Establishing the expected survival and the variables that influence survival in a cohort of untreated cases is likely to provide a crucial baseline to assessing the effectiveness of novel therapies. If, for example, age and gender are important determinants of survival, it may be crucial to include these variables in the assessment of whether or not an intervention actually prolongs survival. It is known that survival is variable in sCJD, with some cases presenting acutely (McNaughton and Will, 1994) and others surviving for many years (Brown *et al*., 1984). The aim of this paper is to define predictors of survival in all forms of human TSE and to quantify these effects as accurately as possible.

Patients and methods

Patients with all forms of human TSEs were ascertained by national surveillance centres as part of a prospective CJD surveillance programme funded by the European Union. Those patients fulfilling the validated diagnostic criteria for definite or probable TSEs were included in a common database. The database contained core medico-demographic information on all TSE cases who died between 1993 and 2000 for Australia, France, Germany, Italy, The Netherlands, Slovakia and the UK, and between 1998 and 2000 for Austria, Spain and Switzerland. Detailed descriptions of the study methodology have been published (Will *et al*., 1998a).

TSE patients were classified as sCJD, iCJD, vCJD and gTSE according to previously published diagnostic criteria. This last group includes gCJD, GSS, FFI and cases carrying an insert mutation of the *PRNP* gene (although patients with gTSEs carry a mutation of the *PRNP* gene, it is not yet clear whether the mutation is the cause of disease or a predisposing factor). Iatrogenic cases were also divided into two categories according to whether they developed the disease following therapy with native human cadaveric growth hormone (ihGH) or after human dura mater implant (iDM). The protease-resistant prion protein type (PrP^{Sc}) found in the brain was classified according to the two-type system published by Parchi *et al*. (1999). Survival was defined as the interval between disease onset and death. Dates of death were available from direct notification by clinicians, from hospital records or from death certificates. The date of onset of clinical symptoms was defined in each case after review of hospital records, clinical correspondence and/or interview with the patient's family. In the great majority of cases, there was little difficulty in defining the date of onset, but in some cases a judgement was made based on information from a range of sources. In Germany, attribution of the date of onset was dependent solely on information from relatives.

Statistical analysis

We investigated the effect on survival of the following demographic, clinical and laboratory investigation variables: sex, age at onset, polymorphism at codon 129 of the *PRNP* gene, country participating in the study, EEG pattern, presence of protein 14-3-3 in the CSF and PrP^{Sc} type.

Survival curves were estimated by the Kaplan–Meier method, both overall and by stratifying for each of the above variables.

For each form of TSE, we report as descriptive statistics the median survival times overall and stratified for each variable; the comparisons of survival curves between groups were carried out by the generalized Wilcoxon test. The Cox proportional hazards model was used to assess the independent effects of the investigated factors by a multivariate analysis. Crude relative risk (RR) and adjusted RR with 95% confidence intervals (CIs) were generated. We used the 'cluster' function on the STATA program for the categorical variable 'country'. The 'cluster' function is used to specify non-independent observations, such as all patients observed in each country, in order to allow robust calculations of standard errors.

Age (in years) at onset was analysed as a continuous variable for 10 year increments. The relative risk for codon 129 was estimated by including two dummy variables with methionine/methionine (MM) as reference.

For sCJD, the multivariate model was used first by including only the gender, the age at onset and the codon 129 polymorphism, because this set contained the largest number of data ($n = 1452$). The Cox model was then used on smaller samples with available data for EEG characteristics and presence of the 14-3-3 protein in the CSF ($n = 893$) or PrP^{Sc} type ($n = 420$). The distributions of sex, age at onset and codon 129 status in these different data sets were compared by the χ^2 test to confirm that missing values were not selected for any of the considered variable. The χ^2 test was also adopted to assess dependency between categorical variables.

The Bonferroni correction for multiple testing was adopted within the five subgroups of gTSE (E200K and V210I gCJD, GSS, FFI and insert mutations) and the two subgroups of iCJD patients. This correction was not applied for all forms of human TSEs since sCJD, iCJD, vCJD and gTSEs are distinct diseases with different aetiology. Thus, the critical level of significance for gTSE was 0.01 (five subgroups) and for iCJD 0.025 (two subgroups).

Statistical analyses were performed using BMDP and STATA.

Results

The mean and median survival times for the different forms of TSEs are summarized in Table 1. SCJD, gCJD and iDM had the shortest median survival times of <6 months; ihGH, vCJD, FFI and gTSE with insert mutations had median clinical durations of ~1 year, while that for GSS patients exceeded 3 years.

Kaplan–Meier survival curves for sCJD, iCJD and vCJD are shown in Fig. 1A, and for gTSE in Fig. 1B. One year after clinical onset, only a few patients with sCJD (15%), iDM (15%) or gCJD (8%) were still alive, while about half the patients with ihGH (54%), vCJD (55%), FFI (48%) and gTSE with insert mutations (55%) survived. About 80% of GSS patients were still alive 1 year after clinical onset.

Sporadic CJD

sCJD patients from Germany had a significantly ($P < 0.0001$) longer median duration (6 months, $n = 568$) of illness than patients from all the other countries: 5 months for Spain ($n = 123$), 4.5 months for Switzerland ($n = 43$), and 4 months for Australia ($n = 149$), Austria ($n = 29$), France ($n = 543$), Italy ($n = 379$), Slovakia ($n = 12$), The Netherlands ($n = 70$) and the UK ($n = 388$).

Univariate survival analyses of sCJD cases with available data (see Table 2) showed differences in the duration of illness by gender (Fig. 2A), age at onset (Fig. 2B) and the polymorphism at codon 129 of the *PRNP* gene (Fig. 2C). The multivariate analysis confirmed that survival is longer in females compared with males and showed that increments of 10 years in the age at onset are associated with an ~30% increase in risk of death (Table 3A). Survival was shorter in patients who were MM at codon 129 of the *PRNP* gene (Table 3A) compared with that seen in valine/valine (VV) and methionine/valine (MV) cases.

When we include a dummy variable in the Cox multivariate model indicating whether patients were from Germany or other countries, the RR for all the above parameters did not change, but the clinical duration of German sCJD patients still remained significantly longer (adjujsted RR = 0.78; CI 0.74–0.83, $P < 0.001$) than that in other countries.

Two investigational features were considered as possible predictive markers of survival: periodic sharp wave complexes (PSWC) in the EEG, the 'typical' pattern in sCJD, and the presence of the 14-3-3 protein in the CSF. In the univariate analysis, survival was significantly shorter in patients with PSWC than in patients without PSWC and in patients with a positive versus negative 14-3-3 test (Table 2). When the EEG pattern and the 14-3-3 test were included in

Table 1 *Clinical duration of disease in different forms of TSEs*

	Mean survival time months (SEM)	Median survival time months
Sporadic CJD ($n = 2304$)	7.3 (0.2)	5
Genetic TSE		
Genetic CJD ($n = 191$)	6.3 (0.5)	4
FFI ($n = 41$)	15.6 (2.2)	12
GSS ($n = 24$)	42.7 (6.1)	39
Insert ($n = 22$)	46.9 (12.8)	14
Iatrogenic CJD*		
hGH CJD ($n = 85$)	14.7 (0.9)	13
DM CJD ($n = 20$)	9.4 (2.4)	5.5
Variant CJD ($n = 86$)	15.3 (0.8)	13

FFI = fatal familial insomnia; GSS = Gerstmann Straussler–Scheinker syndrome; hGH = human growth hormone; DM = dura mater. *A single case of iatrogenic CJD following corneal transplant had a survival of 11 months.

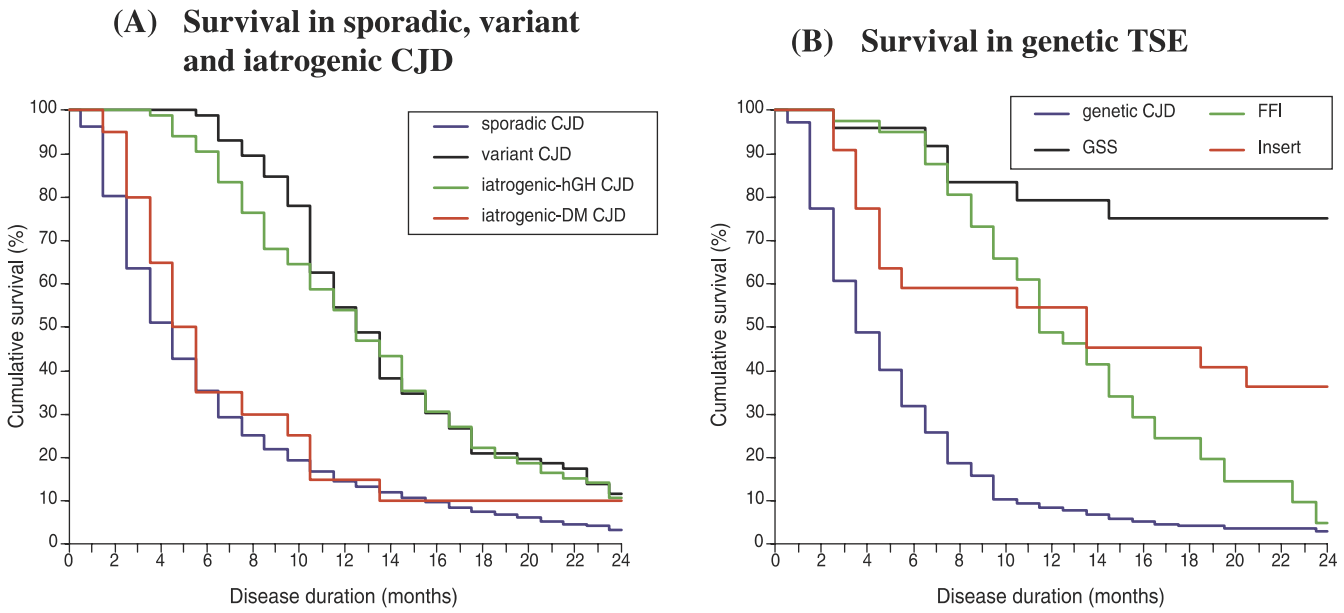

Fig. 1 Kaplan–Meier survival curves in (**A**) sporadic CJD, variant CJD, dura-mater related CJD and human growth hormone-related CJD; (**B**) genetic TSEs, including genetic CJD, GSS, FFI and insert mutations.

Table 2 *Survival time for sporadic CJD*

Features	Median (months)	*P* (generalized Wilcoxon test)
Overall (n = 2304)	5	
Gender		
Male (n = 998)	4	<0.0001
Female (n = 1304)	5	
Age at onset		
11–20 (n = 2)	54, 58*	<0.0001
21–30 (n = 6)	21	
31–40 (n = 14)	23	
41–50 (n = 95)	7	
51–60 (n = 425)	6	
61–70 (n = 937)	5	
71–80 (n = 703)	4	
81–90 (n = 120)	3	
91–100 (n = 2)	2, 3*	
Codon 129		
MM (n = 993)	4	<0.0001
MV (n = 227)	9	
VV (n = 233)	6	
EEG		
PSWC (n = 1429)	4	<0.0001
No PSWC (n = 683)	6	
14-3-3 in CSF		
Positive (n = 1254)	5	<0.0001
Negative (n = 104)	9	
PrP type		
Type 1 (n = 319)	4	<0.0001
Type 2a (n = 131)	8	

*Observed values.

the Cox regression model with sex, age at onset and codon 129 polymorphism, the 14-3-3 test, but not the EEG, retained significance in determining clinical duration of disease together with the other three parameters (Table 3A and B). The reason for the lack of significance in the multivariate analysis for the EEG variable is likely to be because a typical EEG is more frequently observed in patients with late clinical onset (78% of patients >80, 73% in age class 71–80, 69% in 61–70, 58% in 51–60 and 47% of patients ⩽50 years, $P < 0.0001$, χ^2 test), and in patients homozygous for methionine at codon 129 (80% of MM, 51% of MV and 31% of VV patients, $P < 0.0001$, χ^2 test), representing groups of patients with the shortest survival. The missing data for EEG and 14-3-3 were randomly distributed (χ^2 tests for the comparison of the distributions in the two data sets: $P = 0.97$ for gender, $P = 0.54$ for age at onset and $P = 0.66$ for codon 129).

PrP^{Sc} type analysis was available for 450 subjects. Univariate analyses (Fig. 2D) showed that patients with the PrP^{Sc} type 1 had a significantly shorter survival than PrP^{Sc} type 2a (Table 2). When PrP^{Sc} type was included with sex, age at onset and *PRNP* polymorphism at codon 129 in the Cox regression model, the genotype lost its effect on survival (Table 3A and C). This was mostly due to the concomitant fact that there was an uneven distribution of codon 129 polymorphism between PrP^{Sc} type 1 (MM = 87.9%, VV = 3.7%, MV = 8.4%) and type 2a (MM = 21.1%, VV = 47.2%, MV = 31.7%) ($P < 0.0001$, χ^2 test), and an increased proportion of MV and VV patients (with longer clinical duration) in PrP^{Sc} type 2a with respect to type 1. The missing data for PrP^{Sc} type were randomly distributed (χ^2 tests for the comparison of the distributions in the two data sets: $P = 0.23$ for gender, $P = 0.80$ for age at onset and $P = 0.97$ for codon 129).

The crude RR for gender, age at onset and codon 129 obtained in the univariate Cox regression models (Table 3) did not vary when estimated in different subgroups of patients where data on EEG and 14-3-3 test (n = 893) or on PrP^{Sc} type (n = 420) were available.

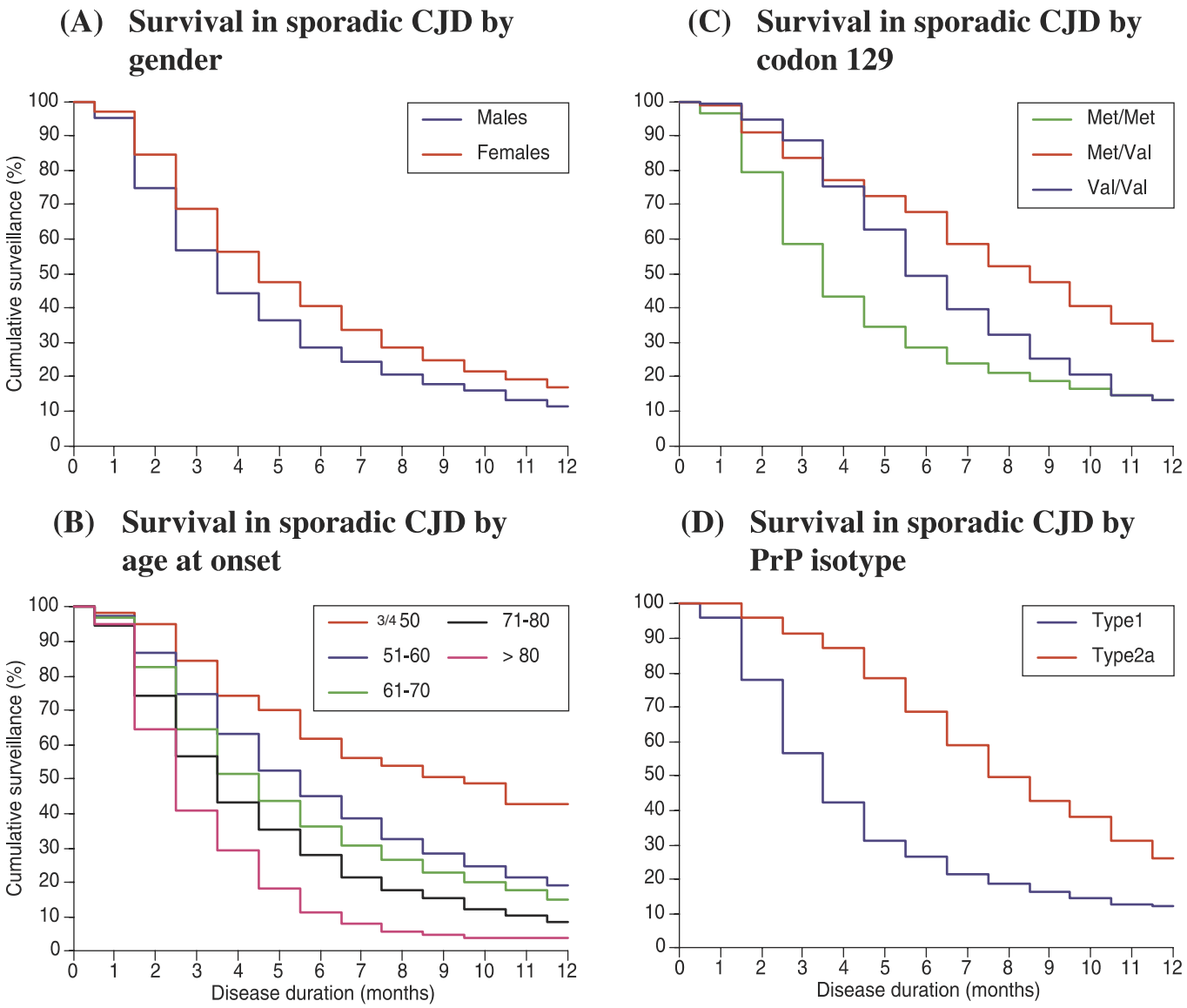

Fig. 2 Kaplan–Meier survival curves in sporadic CJD by (**A**) gender, (**B**) age at onset, (**C**) codon 129 genotype and (**D**) prion protein isotype.

Genetic TSEs

Survival in gCJD patients differed between the various mutations of the *PRNP* gene (Table 4). An analysis was only performed for the two most frequent *PRNP* mutations (i.e. E200K and V210I). Median clinical duration (Table 5) for E200Kand V210I patients was 4 months. In the univariate Cox analysis, survival of both E200K and V210I patients was shorter in males than in females. Though both mutations co-segregate with methionine at codon 129, methionine homozygosity was associated with a shorter survival in E200K patients, but a long survival in V210I patients (Table 5, Fig. 3). Survival was significantly shorter in patients with late age at onset only in V210I patients (Table 5). Multivariate analyses confirmed the significance of all variables for V210I patients (Table 5). In E200K patients, the only significant predictor of survival remained the polymorphism at codon 129. The effect of gender just missed statistical significance when correcting for multiple testing (Table 5).

In FFI (D178N) patients, only the polymorphism at codon 129 of the *PRNP* gene significantly affected survival (Table 6). MV patients showed a longer survival than MM patients.

In gTSE with insert mutations, survival was influenced by the age at onset and codon 129 polymorphism (Table 6). The multivariate analysis with age at onset and codon 129 showed that only MV patients had a significantly longer survival than MM patients (adjusted RR for age at onset = 1.88, CI 0.82–4.30, $P = 0.133$; adjusted RR for MM versus MV, 0.31, CI 0.20–0.48, $P < 0.001$; adjusted RR for MM versus VV = 0.81, CI 0.38–1.71, $P = 0.583$). We did not analyse the relationship between survival and the size of the insert because of the small numbers of cases.

Table 3 *Cox regression model for survival in sporadic CJD patients*

Features	Crude RR (95% CI)	Adjusted RR (95% CI)		
		for A (n = 1452)	for A and B (n = 893)	for A and C (n = 420)
A				
Gender				
Male	1	1	1	1
Female	0.85 (0.73–0.99)* (P = 0.045)	0.74 (0.63–0.88) (P = 0.001)	0.74 (0.67–0.82) (P < 0.001)	0.76 (0.62–0.94) (P = 0.010)
Age at onset [+]	1.34 (1.28–1.40)* (P < 0.001)	1.34 (1.28–1.40) (P < 0.001)	1.30 (1.20–1.40) (P < 0.001)	1.39 (1.34–1.44) (P < 0.001)
Codon 129				
MM	1	1	1	1
MV	0.55 (0.44–0.70)* (P < 0.001)	0.55 (0.45–0.67) (P < 0.001)	0.61 (0.46–0.80) (P < 0.001)	0.80 (0.49–1.31) (P = 0.379)
VV	0.70 (0.61–0.80)* (P < 0.001)	0.77 (0.67–0.88) (P < 0.001)	0.82 (0.71–0.94) (P = 0.005)	1.34 (0.86–2.08) (P = 0.197)
B				
EEG				
PSWC	1	–	1	–
No PSWC	0.79 (0.65–0.96) (P = 0.020)	–	1.01 (0.86–1.19) (P = 0.905)	–
14-3-3 in the CSF				–
Positive	1	–	1	
Negative	0.62 (0.43–0.88) (P = 0.007)	–	0.71 (0.52–0.96) (P = 0.027)	–
C				
PrP^{Sc} glycotype				
Type 1	1	–	–	1
Type 2a	0.54 (0.43–0.68) (P < 0.001)	–	–	0.53 (0.43–0.64) (P < 0.001)

*Crude RR did not change when estimated for A and B and A and C; [+]continuous for increment of 10 years.

Table 4 *Clinical duration of disease in genetic CJD patients carrying different point mutations of the PRNP gene*

PRNP mutations	Median (months)
D178N-129V (n = 8)	9
T188R (n = 1)	2*
E196K (n = 1)	13*
E200K (n = 123)	4
V203I (n = 2)	2, 4*
R208H (n = 1)	10*
V210I (n = 48)	4
E211Q (n = 5)	4

Observed values.

In GSS, methionine homozygous patients at codon 129 showed a median survival time 36 months longer than heterozygous patients. However, due to the small sample size and to the correction for multiple testing in the gTSE group, the difference did not reach statistical significance (Table 6).

Iatrogenic CJD

In ihGH CJD cases, a significantly lower risk was found for females compared with males and a higher risk for patients with late versus early age at onset. Survival curves were different in patients carrying distinct polymorphisms at codon 129 (Table 7, Fig. 3). However, this parameter was not significant in the univariate Cox analysis when MM patients were considered as the reference group. The multivariate Cox model with gender and age at onset confirmed these results (adjusted RR for gender = 0.75, CI 0.63–0.89, P = 0.001; adjusted RR for age at onset = 1.56, CI 1.35–1.81, P < 0.001). It is likely that the loss of significance for codon 129 was because the median survival of MM cases was between those of MV and VV cases. When we used VV cases as the reference group (those with the shortest median survival), the MV cases showed a lower risk than VV cases (crude RR = 0.41; 95% CI = 0.26–0.65, P < 0.001).

In iDM CJD cases, no significant effect of gender or age at onset was found by the Cox univariate model (Table 7). We did not analyse the effect of the codon 129 polymorphism because there were only two MV and one VV patients.

Variant CJD

Since all vCJD patients were MM, only the effect of gender and age at onset as predictors of survival was analysed. A significantly longer median illness duration was observed in females compared with males and in patients with early compared with late age at onset. These findings were confirmed by the Cox univariate and multivariate analyses (Table 8).

Table 5 *Survival times for genetic CJD (E200K and V210I)*

Features	E200K		V210I	
	Median in months	*P**	Median in months	*P**
Overall	4 (n = 123)		4 (n = 48)	
Gender				
Male	3 (n = 48)	0.020	3 (n = 22)	0.060
Female	5 (n = 75)		5 (n = 26)	
Age at onset				
31–40	14, 20$^+$ (n = 2)	0.067	5$^+$ (n = 1)	0.0047
41–50	6 (n = 20)		4 (n = 10)	
51–60	5 (n = 36)		5 (n = 14)	
61–70	3 (n = 41)		3 (n = 15)	
71–80	3 (n = 20)		2 (n = 7)	
81–90	3 (n = 4)		4y (n = 1)	
Codon 129				
MM	4 (n = 88)	0.002	5 (n = 38)	0.04
MV	8 (n = 23)		3 (n = 9)	
VV	4, 7$^+$ (n = 2)		– (n = 0)	

Cox regression model for survival in genetic E200K and V210I CJD patients

Features	E200K (n = 111)		V210I (n = 47)	
	Crude RR (95% CI)	Adjusted RR (95% CI)	Crude RR (95% CI)	Adjusted RR (95% CI)
Gender				
Male	1	1	1	1
Female	0.75 (0.60–0.92) (P = 0.007)	0.78 (0.63–0.95) (P = 0.015)	0.76 (0.64–0.90) (P = 0.002)	0.79 (0.67–0.93) (P = 0.004)
Age at onset†	1.13 (1.01–1.27) (P = 0.048)	1.13 (0.98–1.29) (P = 0.090)	1.39 (1.24–1.56) (P < 0.001)	1.51 (1.42–1.62) (P < 0.001)
Codon 129				
MM	1	1	1	1
MV	0.57 (0.50–0.65) (P < 0.001)	0.56 (0.45–0.70) (P < 0.001)	2.17 (1.51–3.11) (P < 0.001)	2.67 (2.10–3.39) (P < 0.001)

*Generalized Wilcoxon test; $^+$observed values; †continuous for increment of 10 years.

Discussion

The findings presented in this paper are generally consistent with previous studies of survival in sCJD (Wientjens, 1997; Will *et al*., 1998*a*; Parchi *et al*., 1999; Puopolo *et al*., 2003), but the large number of cases included in our analysis allows a more detailed description of predictors that influence survival in all forms of human TSE. There are a number of noteworthy and novel findings, including the influence of gender on survival in a number of subtypes of human TSE and the effect of prion protein PrP^{Sc} type on survival in sCJD. A reduction in survival with increasing age has been confirmed in sCJD, gCJD, cases with insert mutations and vCJD.

In order to allow comparability of data, it is essential to achieve consistency of data collection between countries. Details of the methodology of the study have been described previously (Will *et al*., 1998*a*). In this paper, there is a remarkable consistency in the results from country to country, for example in the median duration of illness in sCJD. The exception is a significant increase in the median survival in sCJD in Germany in comparison with the other countries (4 months in the majority compared with 6 months in Germany). The reason for this finding may be because of a systematic difference in classification of the time of disease onset. However, the analyses are not affected by this anomaly. The numbers of cases in this study are larger than any previous series, allowing firm conclusions on the major predictors of survival in sCJD. However, the numbers of cases in the subtype analyses are smaller, and this may result in insufficient power to assess some parameters properly.

Assessment of survival by disease type (Fig. 1A and B) demonstrates differences in illness duration between the various forms of human TSE. The shortest survival is in sCJD, iDM and, perhaps surprisingly, in some forms of gCJD. It is important to stress that the surveillance system depends on referral of suspect cases from neurologists, and this may result in a bias in the identification of genetic cases with a phenotype similar to sporadic cases (Will *et al*., 1998*b*). In vCJD, ihGH and FFI, a small proportion of patients survive to 24 months, which contrasts with GSS and cases associated with insert mutations in which a significant proportion survive beyond 24 months. In sCJD, about one in seven cases survives to 1 year and one in 30 to 2 years.

A reduction in survival with increasing age in sCJD is well known (Wientjens, 1997) but is not well documented. In this study, survival was found to decrease significantly with

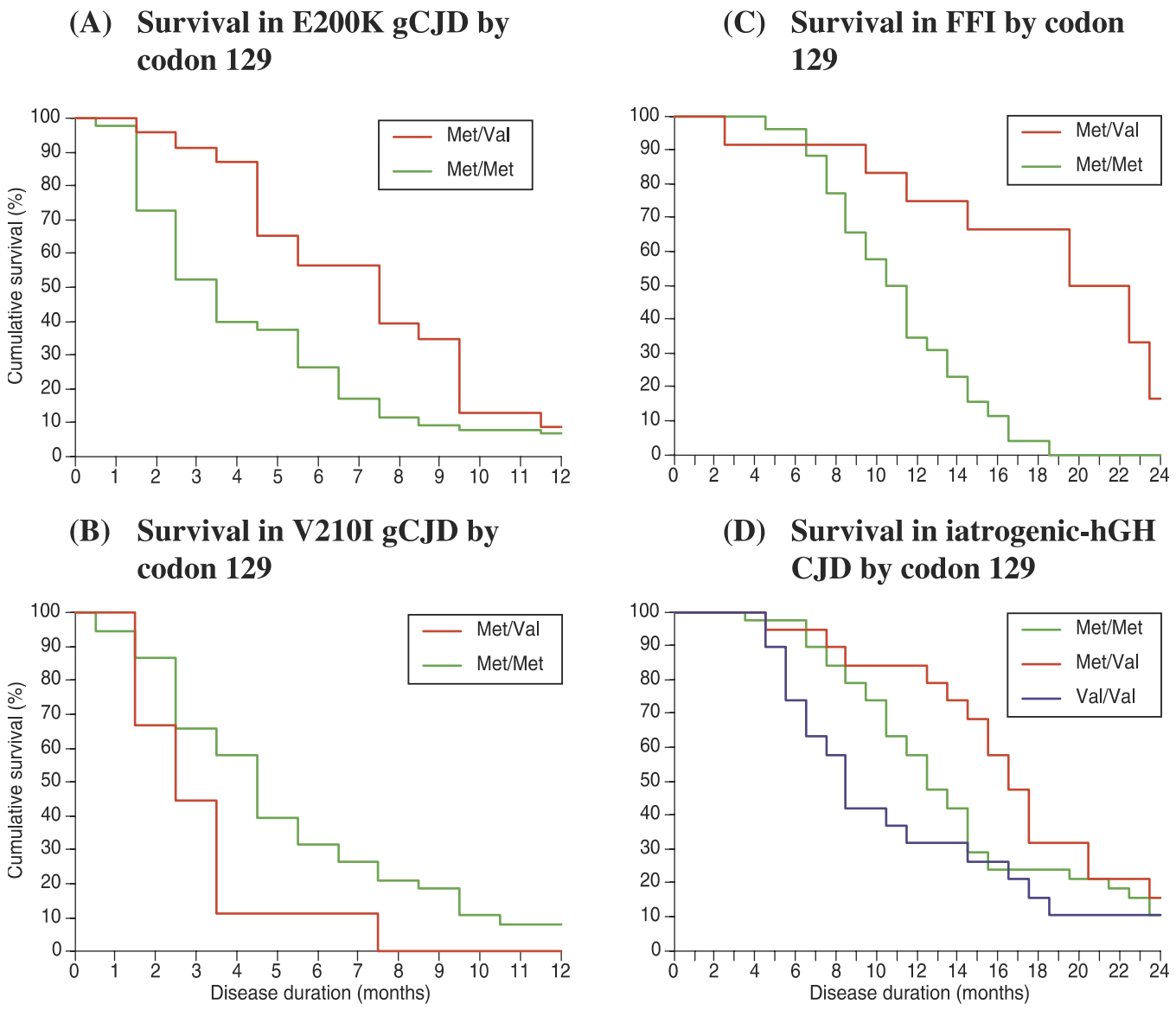

Fig. 3 Influence of codon 129 genotype on Kaplan–Meier survival curves in (**A**) E200K CJD, (**B**) V210I CJD, (**C**) FFI, (**D**) human growth hormone-related CJD.

increasing age in sCJD (Fig. 2B) with, for example, ~10% of cases aged >80 years surviving at 6 months in comparison with ~60% of cases aged <50 years. A similar age effect on survival is seen in cases associated with mutations of *PRNP* at codon 210 and in insert mutations. In ihGH, there is also a borderline relationship between prolonged survival and younger age, and there is a trend in a similar direction with vCJD. The most obvious difference between the ihGH cases and vCJD in comparison with the other subtypes is the relatively young age at death (Brown, 1988; Will *et al*., 2000). It is possible that should cases of iatrogenic or vCJD occur in the older age groups, the relationship between survival and age would become more significant as in the other forms of human TSE. The single case of vCJD in the older age group died at the age of 74 years and had one of the shortest illness durations yet seen of 7 months (Henry *et al*., 2002). The reason for the inverse relationship between age and survival in the majority of subtypes of human prion disease is unknown and, although age-related variations in care or resistance to terminal infection may play a role, there is evidence from laboratory studies of age-related effects on pathogenesis (Bruce and Fraser, 1982; Manolakou *et al*., 2001).

The overall female : male ratio in sCJD in this study, based on sex-specific mortality rates, is 1.24, representing a significant excess of female cases. A female preponderance in sCJD has been documented previously, but this finding is not consistent between studies, even in those carried out systematically (Brown, 1998). Because sCJD is predominantly a disease in the older age groups (60–79 years) and life expectancy is longer in females, population demographics may influence the overall sex distribution. However, this ratio may be affected by the uneven distribution of cases in different age classes, and a better estimate is given by the average female to male

Table 6 *Survival times for FFI, GSS and patients with insert mutations*

Features	FFI			GSS			Insert		
	Median in months	*P**	Crude RR (95% CI)	Median in months	*P**	Crude RR (95% CI)	Median in months	*P**	Crude RR (95% CI)
Overall	12.0 (n = 41)			39.0 (n = 24)			14.0 (n = 22)		
Gender									
Male	12.0 (n = 24)	0.43	1	30.5 (n = 9)	0.59	1	32.5 (n = 11)	0.53	1
Female	12.5 (n = 17)		0.92 (0.69–1.24) (P = 0.590)	39.5 (n = 15)		0.66 (0.20–2.17) (P = 0.490)	8.5 (n = 11)		1.61 (0.62–4.20) (P = 0.326)
Age at onset [+]									
21–30	–	0.65	0.82 (0.60–1.13) (P = 0.227)	7, 70[†] (n = 2)	0.52	1.19 (0.85–1.66) (P = 0.305)	–	0.018	2.00 (1.62–2.47) (P < 0.001)
31–40	11.0 (n = 6)			8, 32[†] (n = 2)			93.0 (n = 4)		
41–50	13.5 (n = 9)			46.0 (n = 9)			86.0 (n = 4)		
51–60	12.5 (n = 19)			20.0 (n = 5)			5.0 (n = 4)		
61–70	12.0 (n = 6)			29.5 (n = 3)			8.0 (n = 7)		
71–80	–			3, 67[†] (n = 2)			4, 5[†] (n = 2)		
81–90	–			–			4[†] (n = 1)		
Codon 129									
Met/Met	11.0 (n = 26)	0.0026	1	44.0 (n = 6)	0.19	1	4.0 (n = 7)	0.13	1
Met/Val	20.0 (n = 12)		0.15 (0.06–0.39) (P < 0.001)	8.0 (n = 6)		2.56 (1.03–6.39) (P = 0.044)	40.0 (n = 5)		0.28 (0.12–0.66) (P = 0.003)
Val/Val	–			–			5.0 (n = 5)		0.52 (0.16–1.71) (P = 0.280)

*Generalized Wilcoxon test; [+] continuous for increment of 10 years; [†] observed values.

Table 7 *Survival times for iatrogenic-hGH and iatrogenic-DM CJD*

Features	Iatrogenic-hGH CJD			Iatrogenic-DM CJD		
	Median in months	*P**	Crude RR (95% CI)	Median in months	*P**	Crude RR (95% CI)
Overall	13.0 (n = 85)			5.5 (n = 20)		
Gender		0.19			0.38	
Male	13.0 (n = 62)		1	5.0 (n = 12)		1
Female	14.0 (n = 23)		0.61 (0.60–0.63) (P < 0.001)	6.0 (n = 8)		0.78 (0.34–1.76) (P = 0.546)
Age at onset [+]		0.014	1.69 (1.41–2.02) (P < 0.001)		0.0008	1.17 (0.90–1.53) (P = 0.242)
10	36[†] (n = 1)			–		
11–20	25.0 (n = 8)			–		
21–30	11.5 (n = 59)			7.0 (n = 5)		
31–40	12.5 (n = 17)			4.0 (n = 3)		
41–50	–			5.0 (n = 4)		
51–60	–			4.5 (n = 5)		
61–70	–			2[†] (n = 1)		
71–80	–			3, 11[†] (n = 2)		
Codon 129		0.0065			0.70	
Met/Met	13.0 (n = 38)		1	6.0 (n = 9)		–
Met/Val	17.0 (n = 19)		0.59 (0.30–1.19) (P = 0.141)	5, 8[†] (n = 2)		–
Val/Val	9.0 (n = 19)		1.46 (0.46–4.63) (P = 0.522)	5[†] (n = 1)		–

*Generalized Wilcoxon test; [+] continuous for increment of 10 years; [†] observed values.

Table 8 *Survival times for vCJD patients*

Features	Median in months	*P**	Crude RR (95% CI)	Adjusted RR (95% CI)
Overall	13.0 (n = 86)			
Gender		0.035		
Male	12.0 (n = 45)		1	1
Female	14.0 (n = 41)		0.69 (0.61–0.79) ($P < 0.001$)	0.76 (0.68–0.85) ($P < 0.001$)
Age at onset $^{+}$		0.005	1.26 (1.25–1.28) ($P < 0.001$)	1.22 (1.18–1.26) ($P < 0.001$)
11–20	14.0 (n = 18)			
21–30	13.0 (n = 39)			
31–40	12.0 (n = 20)			
41–50	7.0 (n = 4)			
51–60	11.0 (n = 4)			
61–70	–			
71–80	7† (n = 1)			

*Generalized Wilcoxon test; $^{+}$continuous for increment of 10 years; †observed values.

ratios of each 10 year age class interval, weighting for the number of cases in each age class. The weighted female to male ratio is 1.08, but this still represents an 8% excess of female cases. The excess of female cases in sCJD does not explain the significant difference in survival in sCJD, with females surviving a median of 1 month longer than males. Similar gender effects on survival were found in cases associated with mutations of *PRNP*, with the exception of FFI, and in vCJD.

The effect of gender has been studied in mouse models, with varying results. One study has shown a major influence of gender in experimental transmission of bovine spongiform encephalopathy (BSE), with extended incubation periods in females (Abiola *et al*., 2002). In contrast, a recent study of non-*PRNP* genetic influences on incubation period showed a significant reduction in the incubation period in females (Manolakou *et al*., 2001), consistent with previous studies (Bruce and Dickinson, 1985; McLean and Bostock, 2000). The excess of female cases and the prolonged female survival in a number of types of human TSE suggest that there are sex-specific factors that influence the clinical phase of human disease. Possibilities include genetic determinants outside the *PRNP* gene, hormonal effects or neuroanatomical factors.

Prolonged survival is one of the variables that distinguish the subtypes of CJD classified according to the seminal work by Parchi and colleagues, using PrP^{Sc} type and codon 129 genotype (Parchi *et al*., 1991). The findings in our study support the hypothesis that the type of protein deposited in the brain influences the clinical phenotype. There is a clear and significant effect on survival in sCJD according to PrP^{Sc} type, with more prolonged survival in cases with the type 2a prion protein. One caveat to this conclusion is that there is evidence in some cases of sCJD of deposition of more than one PrP^{Sc} type in the brain (Puoti *et al*., 1999) and in our study there was no consistency in the brain area from which protein was extracted. Whether the PrP^{sc} type correlates with different 'strains' of infectious agent remains unproven.

There has been a recent resurgence of interest in identifying potential therapies for human TSEs, including CJD (Brown, 2002; Knight, 2002). Drug trials are likely to pose many difficulties, not least because of the rarity of CJD, and comparison of outcome, including survival, in treated cases in relation to the natural history may be an important strategy in assessing the efficacy of potential treatments. The detailed analyses in this study are likely to constitute important considerations for the assessment of treatment efficacy in human TSEs because of the identification and quantification of variables that influence survival in various TSE subtypes. This study documents the influence of a range of variables on survival in a population of cases that were not treated with any therapy known to influence the underlying disease process. A number of variables have been identified that have an influence, and often a major influence, on survival, including age, gender and codon 129 genotype. Although variables such as the PrP^{Sc} type may be of clinical use in the future by using nervous tissue taken from the biopsy of the olfactory epithelium (Zanusso *et al*, 2003), the results of investigations including the 14-3-3 CSF immunoassay correlate with survival. A 'typical' EEG is associated with shortened survival (Zerr *et al*., 2000) and a negative 14-3-3 test with relatively prolonged survival. In this study, the multivariate regression analysis has shown that only the 14-3-3 result is an independent predictor of survival, with a positive result probably reflecting rapid neuronal damage. In the univariate analysis, patients with a typical EEG had a significantly shorter survival than patients with non-specific EEG findings. This significance was lost in the multivariate analysis because the EEG pattern strongly correlates with the polymorphism at codon 129 of the *PRNP* gene. A possible explanation is that a typical EEG is likely to be related to the site of lesions in the brain, which in turn depends on the polymorphism at codon 129 (Tschampa *et al*., 2002).

This study underlines the importance of collaborative research in a rare disease. Some of the findings are novel, but attaining statistical significance has depended on the pooling of results from 10 countries carrying out national surveillance for human TSEs. The detailed analyses of factors influencing survival raise a number of scientific questions, for example the mechanism by which gender influences

disease expression, and may be critical in assessing the efficacy of novel therapeutic strategies.

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References

Abiola OO, Iyegbe C, Lantos P, Plomin R, Anderton BH, Whatley SA. Profound sex-specific effects on incubation times for transmission of bovine spongiform encephalopathy to mice. Intervirology 2002; 45: 56–8.

Brown P. The clinical neurology and epidemiology of Creutzfeldt–Jakob disease, with special reference to iatrogenic cases. In: Bock GR, editor. Novel infectious agents and the central nervous system. Ciba Foundation symposium: 135. Chichester (UK): John Wiley; 1988. p. 3–18.

Brown P. Drug therapy in human and experimental transmissible spongiform encephalopathy. Neurology 2002; 58: 1720–5.

Brown P, Rodgers-Johnson P, Cathala F, Gibbs CJ Jr, Gajdusek DC. Creutzfeldt–Jakob disease of long duration: clinicopathological characteristics, transmissibility, and differential diagnosis. Ann Neurol 1984; 16: 295–304.

Brown P, Cathala F, Raubertas RF, Gajdusek DC, Castaigne P. The epidemiology of Creutzfeldt–Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. Neurology 1987; 37: 895–904.

Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, et al. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 1994; 35: 513–29.

Bruce ME, Dickinson AG. Genetic control of amyloid plaque production and incubation period in scrapie-infected mice. J Neuropathol Exp Neurol 1985; 44: 285–94.

Bruce ME, Fraser H. Effects of age on cerebral amyloid plaques in murine scrapie. Neuropathol Appl Neurobiol 1982; 8: 71–4.

Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt–Jakob disease. Lancet 1991; 337: 1441–2.

Galvez S, Masters C, Gajdusek DC. Descriptive epidemiology of Creutzfeldt–Jakob disease in Chile. Arch Neurol 1980; 37: 11–4.

Henry C, Lowman A, Will RG. Creutzfeldt–Jakob disease in elderly people. Age Ageing 2002; 31: 7–10.

Knight R. CJD: the promise of treatment. Br J Infect Control 2002; 3: 4.

Korth C, May BCH, Cohen FE, Prusiner SB. Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. Proc Natl Acad Sci USA 2001; 98: 9836–41.

Kovacs GG, Trabattoni G, Hainfellner JA, Ironside JW, Knight RSG, Budka H. Mutations of the prion protein gene: phenotypic spectrum. J Neurol 2002; 249: 1567–82.

Lundberg PO. Creutzfeldt–Jakob disease in Sweden. J Neurol Neurosurg Psychiatry 1998; 65: 836–41.

Manolakou K, Beaton J, McConnell I, Farquar C, Manson J, Hastie ND, et al. Genetic and environmental factors modify bovine spongiform encephalopathy incubation period in mice. Proc Natl Acad Sci USA 2001; 98: 7402–7.

McLean AR, Bostock CJ. Scrapie infections initiated at varying doses: an analysis of 117 titration experiments. Philos Trans R Soc Lond B Biol Sci 2000; 355: 1043–50.

McNaughton H, Will R. Creutzfeldt–Jakob disease presenting as stroke: an analysis of 30 cases. Neurol Infect Epidemiol 1997; 2: 19–24

Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease. Nature 1991; 352: 340–2.

Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt–Jakob disease. Ann Neurol 1996; 39: 767–78.

Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, et al. Classification of sporadic Creutzfeldt–Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46: 224–33.

Pocchiari M. Prions and related neurological diseases. Mol Aspects Med 1994; 15: 195–291.

Puopolo M, Ladogana A, Almonti S, Daude N, Bevivino S, Petraroli R, et al. Mortality trend from sporadic Creutzfeldt–Jakob disease (CJD) in Italy, 1993–2000. J Clin Epidemiol 2003; 56: 494–9.

Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, Tagliavini F. Sporadic Creutzfeldt–Jakob disease: co-occurrence of different types of PrP(Sc) in the same brain. Neurology 1999; 53: 2173–6.

Tschampa HJ, Herms JW, Schulz-Schaeffer WJ, Maruschak B, Windl O, Jastrow U, et al. Clinical findings in sporadic Creutzfeldt–Jakob disease correlate with thalamic pathology. Brain 2002; 125: 2558–66.

Wientjens DPWM. Epidemiology of Creutzfeldt–Jakob disease. Incidence, risk factors and survival in European studies [dissertation]. Rotterdam: Erasmus University; 1997.

Will RG, Matthews WB, Smith PG, Hudson C. A retrospective study of Creutzfeldt–Jakob disease in England and Wales 1970–1979. II: epidemiology. J Neurol Neurosurg Psychiatry 1986; 49: 749–55.

Will RG, Alperovitch A, Poser S, Pocchiari M, Hofman A, Mitrova E, et al. Descriptive epidemiology of Creutzfeldt–Jakob disease in six European countries, 1993–1995. Ann Neurol 1998a; 43: 763–7.

Will RG, Campbell MJ, Moss TH, Bell JE, Ironside JW. FFI cases from the United Kingdom. Brain Pathol 1998b; 8: 562–63.

Will RG, Zeidler M, Stewart GE, Macleod MA, Ironside JW, Cousens SN, et al. Diagnosis of new variant Creutzfeldt–Jakob disease. Ann Neurol 2000; 47: 575–82.

Zanusso G, Ferrari S, Cardone F, Zampieri P, Gelati M, Fiorini M, et al. Detection of pathologic prion protein in the olfactory epithelium in sporadic Creutzfeldt–Jakob disease. N Engl J Med 2003; 348: 711–9.

Zerr I, Schulz-Schaeffer W, Giese A, Bodemer M, Schroter A, Henkel K, et al. Current clinical diagnosis in Creutzfeldt–Jakob disease: identification of uncommon variants. Ann Neurol 2000; 48: 323–9.