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Determinants of diagnostic investigation sensitivities across the clinical spectrum of sporadic Creutzfeldt–Jakob disease

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To validate the provisional findings of a number of smaller studies and explore additional determinants of characteristic diagnostic investigation results across the entire clinical spectrum of sporadic Creutzfeldt–Jakob disease (CJD), an international collaborative study was undertaken comprising 2451 pathologically confirmed (definite) patients. We assessed the influence of age at disease onset, illness duration, prion protein gene (*PRNP*) codon 129 polymorphism (either methionine or valine) and molecular sub-type on the diagnostic sensitivity of EEG, cerebral MRI and the CSF 14-3-3 immunoassay. For EEG and CSF 14-3-3 protein detection, we also assessed the influence of the time point in a patient's illness at which the investigation was performed on the likelihood of a typical or positive result. Analysis included a large subset of patients ($n = 743$) in whom molecular sub-typing had been performed using a combination of the *PRNP* codon 129 polymorphism and the form of protease resistant prion protein [type 1 or 2 according to Parchi *et al*. (Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichemberger N, Julien J, Vital C, Ghetti B, Gambetti P, Kretzschmar H. Classification of sporadic Creutzfeldt–Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46: 224–233.)] present in the brain. Findings for the whole group paralleled the subset with molecular sub-typing data available, showing that age at disease onset and disease duration were independent determinants of typical changes on EEG, while illness duration significantly influenced positive CSF 14-3-3 protein detection; changes on brain MRI were not influenced by either of these clinical parameters, but overall, imaging data were less complete and consequently

conclusions are more tentative. In addition to age at disease onset and illness duration, molecular sub-type was re-affirmed as an important independent determinant of investigation results. In multivariate analyses that included molecular sub-type, time point of the investigation during a patient's illness was found not to influence the occurrence of a typical or positive EEG or CSF 14-3-3 protein result. A typical EEG was most often seen in MM1 patients and was significantly less likely in the MV1, MV2 and VV2 sub-types, whereas VV2 patients had an increased likelihood of a typical brain MRI. Overall, the CSF 14-3-3 immunoassay was the most frequently positive investigation (88.1%) but performed significantly less well in the very uncommon MV2 and MM2 sub-types. Our findings confirm a number of determinants of principal investigation results in sporadic CJD and underscore the importance of recognizing these pre-test limitations before accepting the diagnosis excluded or confirmed. Combinations of investigations offer the best chance of detection, especially for the less common molecular sub-types such as MV2 and MM2.

Keywords: sporadic CJD; diagnostic investigation results; molecular sub-typing

Abbreviations: CJD = Creutzfeldt–Jakob disease; PrP^{res} = protease-resistant prion protein; PSWC = periodic sharp wave complexes

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Introduction

Pre-mortem diagnosis of the rare, transmissible neurodegenerative disorder, sporadic Creutzfeldt–Jakob disease (CJD), largely relies on an appropriate clinical profile supported by characteristic findings on routine investigations such as the EEG and CSF analysis (Collins *et al.*, 2004). Neuropathological examination of the brain remains necessary to achieve a definite diagnosis. Cerebral MRI may also demonstrate highly suggestive changes and is assuming a greater role in CJD evaluation but is not currently included in diagnostic criteria for surveillance purposes (Collie *et al.*, 2001; Meissner *et al.*, 2004; Shiga *et al.*, 2004; Tschampa *et al.*, 2005). Although the diagnostic utility of these investigations (particularly the EEG and CSF 14-3-3 protein detection) has been supported by a number of studies (Steinhoff *et al.*, 1996, 2004; Zerr *et al.*, 2000*a*), they remain imperfect surrogate markers, with apparent non-uniform sensitivity across the clinical spectrum of sporadic CJD (Parchi *et al.*, 1999).

Different clinical and pathological sub-types of sporadic CJD have been linked to polymorphism status at codon 129 of *PRNP* combined with the type (1 or 2) of protease-resistant prion protein (PrP^{res}) found in the brain, and together these probably define different human prion strains (Parchi *et al.*, 1999; Hill *et al.*, 2003; Korth *et al.*, 2003). The most widely used scheme distinguishes only two PrP^{res} types (Parchi *et al.*, 1999), while another commonly employed nomenclature delineates a greater number (Hill *et al.*, 2003). Employing this nosology to sub-type sporadic CJD, the EEG has been shown to vary widely between the specific molecular sub-types in demonstrating characteristic or typical changes (Parchi *et al.*, 1999; Zerr *et al.*, 2000*b*). Generally, the likelihood of characteristic changes on the EEG was reported to be highest in MM homozygotes, reduced in heterozygotes and lowest in VV carriers. Immunoassay for 14-3-3 proteins in CSF, increasingly utilized in the diagnostic evaluation of patients with suspect CJD (Zerr *et al.*, 2000*a*), has also been shown to vary in rates of positivity according to molecular sub-type, with the likelihood of noticeably reduced detection rates in heterozygous patients with type 2 PrP^{res} (MV2) (Zerr *et al.*, 2000*b*; Otto *et al.*, 2002; Castellani *et al.*, 2004). A notable difficulty with all previous reports assessing rates of positive diagnostic investigations in molecular sub-types of sporadic CJD is the small numbers of patients studied, especially the less common phenotypic sub-types. Such small patient numbers militate against the reliability of the observations and have precluded multivariate analyses to assess for covariates and probable confounding factors. A corollary, unexplored so far, is whether within each of the molecular subgroups and across the phenotypic spectrum of sporadic CJD there are additional independent variables influencing diagnostic test results. Consequently, there remains a need to more confidently establish the diagnostic utility of these investigations across the various molecular sub-types of sporadic CJD and define further determinants of their sensitivity and specificity across the entire clinical range. More clearly defining additional factors influencing the sensitivity and specificity of these principal investigations has important practical implications when investigating individual patients.

As part of a collaborative multi-national CJD surveillance program (EUROCJD) initiated in 1993, we assessed the effect of patient clinical features such as age at onset, illness duration and *PRNP* codon 129 polymorphism on the diagnostic sensitivity of EEG, CSF 14-3-3 immunoassay and cerebral MRI in a large, population-based sample of 2451 pathologically confirmed (definite) sporadic cases of CJD, employing harmonized case definitions and data sets. Further, similar analysis was undertaken in a subset of patients (n = 743) in whom molecular sub-typing using brain PrP^{res} isotype and codon 129 polymorphism had been determined. Inclusion of this data allowed detailed assessment of the

variation in positive test results across the molecular sub-types, as well as whether patient parameters such as age at onset, illness duration and the specific time point at which the investigation was performed during a patient's illness were additional independent determinants of investigation results.

Patients and methods

The present study was undertaken as part of the ongoing activities of the prospective CJD surveillance programme (EUROCJD) conducted by the European Union and allied countries. In 1993, national surveillance registers commenced in France, Germany, Italy, The Netherlands, Slovakia and the UK, with the aim of ascertaining all patients diagnosed with probable or definite CJD in the respective countries. The study was extended in 1997 and again in 1998 to include Australia, Austria, Canada, Spain and Switzerland. EUROCJD collaborative study methods utilizing standardized case definitions and centralized harmonized demographic data sets were as described previously (Pocchiari *et al.*, 2004; Ladogana *et al.*, 2005*a*).

The study comprised all patients (n = 2451) with definite sporadic CJD who died between 31 December 1992 and 31 December 2002: 136 cases from Australia, 68 from Austria, 146 from Canada, 491 from France, 450 from Germany, 342 from Italy, 100 from The Netherlands, 438 from the UK, 18 from Slovakia, 183 from Spain and 79 from Switzerland. A diagnosis of definite sporadic CJD required neuropathological confirmation most commonly through post-mortem but occasionally through brain biopsy. Analysis of the *PRNP* open reading frame was performed in the majority of patients to exclude genetic CJD, as clinical differentiation from sporadic CJD is not always possible (Collins *et al.*, 2000; Ladogana *et al.*, 2005*b*). Of the 2451 patients, 746 had their brain PrP^{res} isotype determined, which in combination with *PRNP* codon 129 genotyping allowed molecular sub-typing according to the system of Parchi *et al.*, (1999). Whenever possible, PrP^{res} typing was based on analysis of a number of brain regions, including cerebral cortex, striatum or thalamus and cerebellum, although the preferred number and precise location of sampling sites varied between participating countries.

Whenever possible, all EEGs and MRI brain scans were reviewed by a member of the surveillance system and scored for the presence or absence of typical or characteristic diagnostic features. EEG records were scored positive or characteristic when they fulfilled validated criteria (Steinhoff *et al.*, 2004): sustained periodic sharp wave complexes (PSWC) with a variability of <500 ms, with the periodic complexes (lasting 100–600 ms) demonstrating a bi- or triphasic morphology and seen in a generalized or lateralized distribution. The CSF 14-3-3 immunoassays were performed in each of the national surveillance centres using western blotting, with conformity of testing methods and results interpretation confirmed by blinded sample exchange programme. On cerebral MRI, high signal in the putamen and caudate nucleus when using long-repetition time pulse sequences was considered a positive finding for sporadic CJD (Collie *et al.*, 2001); however, there was no systematic use of particular techniques such as fluid attenuated inversion recovery (FLAIR) or diffusion weighted imaging (DWI).

For assessment of the influence of the timing of the investigation during a patient's illness on the test result, data sets were of sufficient size for EEG and CSF 14-3-3 protein detection to allow this type of analysis. Acknowledging that the median total illness duration was 5 months, we chose to divide each patient's symptomatic phase into thirds (first, middle and final) rather than into a greater number of epochs. This was to ensure avoiding the creation of illness periods of such brevity, especially at the onset of symptoms, that they would be unlikely to correspond to a clinically meaningful phase of a patient's illness during which investigations would usually be undertaken, and if undertaken because of the development of symptoms suggesting neurological dysfunction, that these features were sufficiently developed that the investigations had a reasonable likelihood of displaying abnormalities. The median times (range in months) from the onset of symptoms for the analyses of each of the first, middle and final thirds of patient's illnesses for assessing CSF 14-3-3 protein detection were 1 (0–10), 2.5 (1–17) and 4 months (1–41), respectively. With respect to EEG findings, the median times (range in months) from the onset of symptoms for the analyses of each of the first, middle and final thirds of patient's illnesses were 1 (0–18), 2 (1–42) and 4 months (1–41), respectively.

All data were centralized and analysed collaboratively.

Statistical analysis

Descriptive statistics were calculated for the whole sample and for every molecular sub-type of the disease. Fisher's exact and Mann–Whitney tests were used to assess differences between qualitative and quantitative variables, respectively. The number of positive findings (acknowledging that patients may have undergone multiple tests) over the total number of tested CJD cases for EEG, MRI and the CSF 14-3-3 immunoassay was determined for each stratum. The frequency of positive results (number of positive test results in total number of patients) was then calculated for EEG, MRI and the CSF 14-3-3 test for each stratum.

In order to analyse the independent effect of each factor on positive test results, taking into account possible confounders, we fitted two multiple logistic regression models with the test result as output. Both models included age at onset, and disease duration as predictors, and included country of origin, year of death and gender as covariates.

In the first model *PRNP* codon 129 genotype was entered as a predictor, while in the second model we used the molecular sub-type (combination of *PRNP* codon 129 genotype and PrP^{res} type). Age at onset was categorized into clinically meaningful groups, with patients younger than 50 years serving as the reference group. Disease duration was also categorized with the same criteria into three groups: duration <6, 6–12 and >12 months. *PRNP* codon 129 genotype was entered in the model using two dummy variables, with MM as the reference group; for molecular sub-types we created eight dummy variables (MM2, MM1/2, MV1, MV2, MV1/2, VV1, VV2 and VV1/2) with MM1 group as reference. Adjusted odd ratios (ORs) and 95% confidence intervals (CIs) were generated. All statistical analyses were performed using SPSS 11.0 for Windows 2000 (SPSS Inc., Chicago, IL).

Results

Table 1 summarizes the salient features of all definite sporadic CJD cases. Of the 2451 patients identified, 1329 were females (54.2%). The median age at death was 68 years (range: 20–95), while the median disease duration was 5 months (range: 1–81).

Table 1 Summary of clinical features of all patients, including the subset undergoing molecular sub-typing

Clinical feature	All patients	Patients with molecular sub-typing
Male (%)	1122 (45.8)	351 (47.2)
Female (%)	1329 (54.2)	392 (52.8)
Median age at onset (range)	67.2 (15.6–94.9)	66.2 (15.6–90.0)
Age at onset < 50 years (%)	116 (5.1)	39 (5.5)
Age at onset 50–59 years (%)	412 (18.2)	141 (19.7)
Age at onset 60–69 years (%)	867 (38.3)	286 (40.0)
Age at onset 70–79 years (%)	729 (32.2)	212 (29.7)
Age at onset > 80 years (%)	137 (6.1)	37 (5.2)
Median duration of illness in months (range)	5 (1–81)	5 (1–62)
Patients with duration < 6 months (%)	1332 (58.8)	404 (56.5)
Patients with duration 6–12 months (%)	611 (27.0)	179 (25.0)
Patients with duration > 12 months (%)	321 (14.2)	132 (18.5)
Codon 129 genotype		
Met-Met/total (%)	1061/1604 (66.1)	504/743 (67.8)
Met-Val/total (%)	272/1604 (17.0)	117/743 (15.7)
Val-Val/total (%)	271/1604 (16.9)	122/743 (16.4)
Patients with *PRNP* gene sequenced (%)	1492 (63.6)	650 (87.5)
PrP isotype		
Type 1	495/743 (66.6)	495/743 (66.6)
Type 1 and 2	44/743 (5.9)	44/743 (5.9)
Type 2	204/743 (27.5)	204/743 (27.5)
EEG		
Typical/total (%)	1216/2083 (58.4)	371/666 (55.7)
14-3-3 protein in CSF		
Positive/total (%)	1340/1521 (88.1)	486/554 (87.7)
MRI		
Characteristic findings/total (%)	405/1036 (39.1)	150/387 (38.8)

An EEG was performed on 2083 patients (85%). Of those undergoing EEG, 1216 cases showed typical PSWCs (58.4%) (Table 1). The codon 129 genotype had a significant effect on the likelihood of a positive result of the EEG in sporadic CJD patients (Table 2), which ranged from 73.2% in MM homozygotes to 21.5% in VV homozygotes. After adjusting for possible confounding variables (country, sex, year of death, age at onset and disease duration), the prevalence of PSWC on the EEG was significantly decreased in heterozygotes ($P < 0.001$) and VV homozygotes ($P < 0.001$). The median age of death of sporadic CJD patients with a typical EEG was 69.0 (range: 34–92) versus 66 years (range: 20–88) in patients with an atypical EEG ($P < 0.001$). The presence of PSWC was further analysed by age at onset and disease duration (Table 3). The likelihood of a typical EEG steadily increased with age ($P < 0.001$, adjusted for the same variables), while there was an inverse correlation with disease duration ($P = 0.001$).

CSF 14-3-3 protein analysis was performed on 1521 cases (62.1%) of sporadic CJD. Of these, 1340 cases were positive (88.1%) (Table 1). Although the frequency of a positive CSF 14-3-3 immunoassay was high for all three codon 129 genotypes, heterozygotes had a significantly lower rate (77.0%) in adjusted analyses than VV ($P < 0.001$) and MM ($P < 0.05$) (Table 2). Age at disease onset did not influence CSF 14-3-3 protein detection but as for the EEG a positive result was less likely with longer disease duration (Table 3) ($P < 0.001$).

Table 2 Summary of principal investigation findings for all patients who underwent *PRNP* codon 129 genotyping

	EEG	MRI	14-3-3 protein in CSF
	Typical/total (%)	Typical/total (%)	Positive/total (%)
PRNP genotype			
M/M	710/970 (73.2)	172/474 (36.3)	709/796 (89.1)
M/V	99/245 (40.4)	65/134 (48.5)	147/191 (77.0)
V/V	50/233 (21.5)	71/139 (51.1)	184/196 (93.9)
P-value	<0.001	0.004	0.002

Adjusted by age at onset, disease duration, sex, country of origin and year of death.

MR brain imaging was performed on 1063 patients (42.3%). Of these patients, 405 patients showed characteristic changes (39.1%) (Table 1). In contrast with the EEG, the likelihood of a positive MRI scan was higher in cases with the MV ($P < 0.05$) and VV genotypes ($P = 0.002$) in comparison to those with an MM genotype (Table 2). MRI results were not significantly associated with age at onset or disease duration (Table 3).

Table 3 Principal investigation findings for all patients according to age at disease onset and duration of illness

	EEG Typical/total (%)	MRI Typical/total (%)	14-3-3 protein in CSF Positive/total (%)
Age at onset			
Age at onset < 50 years (%)	35/104 (33.7)	18/58 (31.0)	62/77 (80.5)
Age at onset 50–59 years (%)	197/371 (53.1)	83/196 (42.3)	222/260 (85.4)
Age at onset 60–69 years (%)	457/796 (57.4)	166/415 (40.0)	472/545 (86.6)
Age at onset 70–79 years (%)	438/675 (64.9)	126/328 (38.4)	452/492 (91.9)
Age at onset > 80 years (%)	81/124 (65.3)	12/37 (32.4)	73/83 (88.0)
P for trend	<0.001	0.3	0.4
Disease duration			
Patients with duration < 6 months (%)	814/1228 (66.3)	203/587 (34.6)	779/840 (92.7)
Patients with duration 6–12 months (%)	283/558 (50.7)	125/287 (43.6)	358/408 (87.7)
Patients with duration > 12 months (%)	115/288 (39.9)	77/160 (48.1)	146/211 (69.2)
P for trend	0.001	0.4	<0.001

Adjusted by *PRNP* codon 129 genotype, sex, country of origin and year of death.

Table 4 Summary of age at disease onset and illness duration for all patients undergoing molecular sub-typing

	Sporadic CJD molecular sub-types								
	MM1	MM2	MM1/2	MV1	MV2	MV1/2	VV1	VV2	VV1/2
Total number (%)	444 (59.8)	31 (4.2)	29 (3.9)	37 (5.0)	73 (9.8)	7 (0.9)	14 (1.9)	100 (13.5)	8 (1.1)
Median age at onset (range)	67.8 (31.1–89.4)	60.3 (37.7–82.3)*	65.6 (48.9–89.9)	65.5 (15.6–84.2)	63.6 (40.8–81.1)*	66.3 (56.7–77.9)	47.2 (23.6–72.7)*	66.3 (40.2–86.3)	59.7 (18.7–72.7)
Age at onset < 50 years (%)	12 (2.8)	5 (16.7)†	1 (3.8)	1 (2.8)	2 (2.9)	0 (0.0)	7 (53.8)†	9 (9.3)†	2 (25.0)†
Age at onset 50–59 years (%)	78 (18.2)	9 (30.0)	3 (11.5)	9 (25.0)	17 (24.6)	1 (14.3)	2 (15.4)	20 (20.6)	2 (25.0)
Age at onset 60–69 years (%)	172 (40.1)	11 (36.7)	12 (46.2)	10 (27.8)	37 (53.6)†	3 (42.9)	3 (23.1)	35 (36.1)	3 (37.5)
Age at onset 70–79 years (%)	143 (33.3)	4 (13.3)*	7 (26.9)	13 (36.1)	12 (17.4)*	3 (42.9)	1 (7.7)	28 (28.9)	1 (12.5)
Age at onset > 80 years (%)	24 (5.6)	1 (3.3)	3 (11.5)	3 (8.3)	1 (1.4)	0 (0.0)	0 (0.0)	5 (5.2)	0 (0.0)
Median duration (months) (range)	4.0 (1.0–39.0)	12.5 (3.0–38.0)†	6.0 (2.0–33.0)	5.0 (1.0–54.0)†	12.0 (3.0–62.0)†	13.0 (2.0–30.0)	11.0 (2.0–29.0)†	6.0 (2.0–25.0)†	5.5 (3.0–62.0)
Duration < 6 months (%)	307 (71.6)	9 (30.0)*	11 (42.3)*	21 (58.3)	5 (7.2)*	3 (42.9)	4 (30.8)*	40 (41.2)*	4 (50.0)
Duration 6–12 months (%)	78 (18.2)	4 (13.3)	11 (42.3)†	7 (19.4)	28 (40.6)†	0 (0.0)	3 (23.0)	47 (48.5)†	1 (12.5)
Duration > 12 months (%)	44 (10.3)	17 (56.7)†	4 (15.4)	8 (22.2)†	36 (52.2)†	4 (57.1)†	6 (46.2)†	10 (10.3)	3 (37.5)†
Female (%)	239 (53.8)	15 (48.4)	14 (48.3)	18 (48.6)	35 (47.9)	5 (71.4)	5 (35.7)	56 (56.0)	5 (62.5)

*Significantly lower than MM1 ($P < 0.05$); †Significantly higher than MM1 ($P < 0.05$).

Analysis of sporadic CJD patients according to molecular sub-type

In 743 patients the codon 129 polymorphism and brain PrP^{res} isotype were determined allowing molecular phenotypic sub-stratification (Table 1) (Parchi *et al.*, 1999). In 87% of these patients the *PRNP* gene was sequenced, ruling out pathological mutations associated with genetic CJD. In comparison to the entire group, the median age at onset, median disease duration and *PRNP* codon 129 polymorphism distributions were similar, and there were no significant differences in the overall rates of positive results for each of the investigations. The most common PrP^{res} isotype was type 1 (67%); type 2 was present in 27% of the sample, and in 44 cases (6%) there was coexistence of both type 1 and 2.

The relative frequency of the various molecular sub-types is provided in Table 4. There were occasional significant differences in demographic characteristics between subgroups. MM1 patients were the oldest at disease onset (median: 67.8 years) and VV1 patients were the youngest (median: 47.2 years). The duration of the disease was significantly shorter in MM1 patients (median: 4 months) in comparison with the rest, especially the MM2 (median: 12.5 months) and MV2 (median: 12 months) subgroups, with these groups also

demonstrating a higher percentage of patients with disease lasting >1 year (57 and 52%, respectively). Of note, albeit the small patient numbers preclude statistically significant differences, patients with co-existence of PrP^{res} types 1 and 2 (MM1/2, VV1/2 and MV1/2) tended to have intermediate phenotypes but generally aligning more to the most common molecular PrP^{res} sub-type within each *PRNP* codon 129 genotype.

Overall, determinants of investigation findings in patients who underwent molecular sub-typing were the same as for the entire group. Independent of disease duration and molecular sub-type, age at disease onset correlated significantly with a positive EEG result ($P = 0.007$) (Fig. 1). Patients who developed symptoms <50 years had a lower rate of typical PSWCs (22%) than patients presenting after 60 years of age. The median age at onset of the patients with an atypical EEG was 64 years (range: 16–86), compared with 68 years (range: 31–90) in patients showing the typical findings. CSF 14-3-3 protein immunoassay and MRI results were not significantly correlated with patient's age at disease onset.

The duration of disease was independently associated with the likelihood of PSWCs. Patients with disease duration <6 months had a significantly higher rate ($P = 0.02$) of a typical EEG than patients with duration >12 months (65 versus 35%, respectively). The median disease duration of patients with an atypical EEG was 6 (range: from 1 to 62) versus 4 months (range: from 1 to 39) in patients with typical EEG. Disease duration significantly ($P = 0.004$) influenced the likelihood of a positive CSF 14-3-3 test result. Those patients with disease duration <6 months had a higher rate of positive test results than those with duration >12 months (91 versus 72%, respectively). On average, patients with 14-3-3 proteins detectable in their CSF had shorter disease durations (median: 5 months, range: 1–39) than patients with a negative test result (median: 11 months, range: 2–54). MRI results did not correlate with disease duration (Fig. 2).

Certain molecular sub-types showed significant correlations with investigation results. MM1 patients demonstrated the highest frequency of positive EEG results (73%) followed by MV1 patients (53%), although after adjusting for covariates the significance was reduced ($P = 0.01$). VV2 and MV2 patients had infrequent occurrence of a typical EEG, with a positive result only observed in 12.8 and 17.5% of the cases, respectively (Fig. 3). Of the sub-groups with coexistence of PrP^{res} types, only VV1/2 patients had a significantly lower frequency compared with the MM1 subgroup. CSF 14-3-3 immunoassay was the most frequently positive investigation, with an overall positive rate of 88%. However, there were some significant variations across molecular sub-types, with the test significantly more often positive (all *P*-values < 0.05) for MM1 and VV2 patients (91 and 95%) than for MM2 (61%) and MV2 (71%) (Fig. 3). Of the patient groups with coexistence of PrP^{res} types, only MM homozygotes had a significantly lower rate of positive results for this test (Fig. 3). For MRI, only the VV2 subgroup demonstrated a significantly increased likelihood of a typical positive result. Of note, within each molecular sub-type except VV1, occasional patients did not display typical or characteristic findings with any of the three investigations.

Assessment of the influence of the time point of the investigation during a patient's illness on the test result

For both EEG and the detection of CSF 14-3-3 proteins, sufficient data were available to analyse the influence of the time point at which the investigation was performed

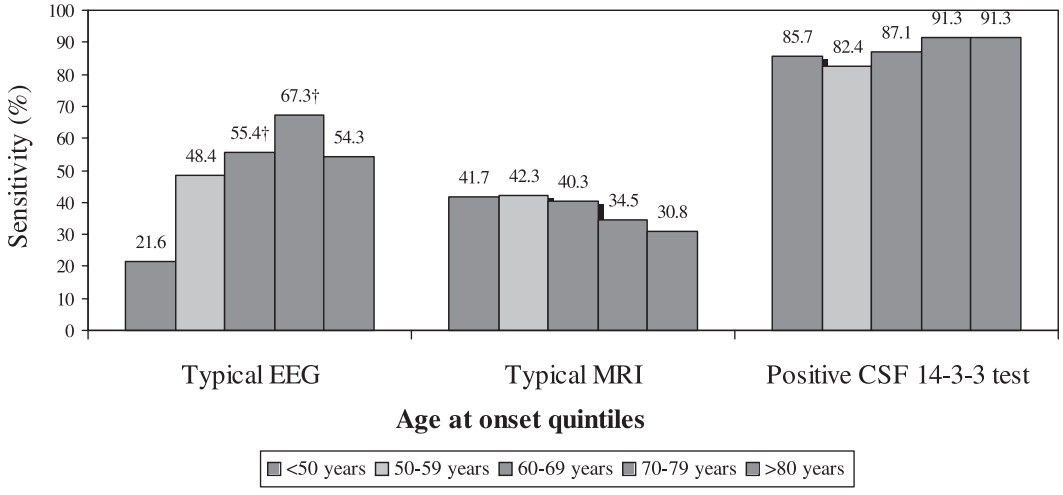

Fig. 1 The principal investigation results for EEG, brain MRI and CSF 14-3-3 protein analysis were correlated with age at disease onset in sporadic CJD for all patients undergoing molecular sub-typing. Patients were grouped by age at onset: <50, 50–59, 60–69, 70–79 and >80 years. Only the EEG showed a significant correlation with age at disease onset. †Significantly higher than in <50 years group ($P < 0.05$); adjusted by molecular sub-type, disease duration, sex, country of origin, year of death.

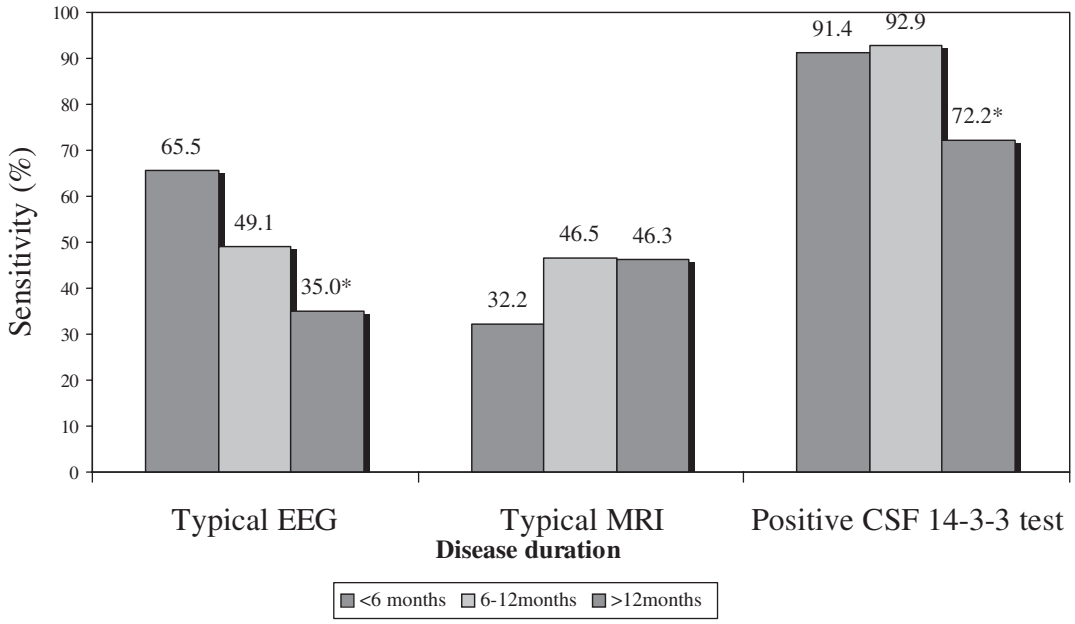

Fig. 2 The principal investigation results for EEG, brain MRI and CSF 14-3-3 protein analysis were correlated with disease duration in sporadic CJD for all patients undergoing molecular sub-typing. Disease duration was arranged as <6, 6–12 and >12 months. For both the EEG and CSF 14-3-3 protein detection there was a significant inverse correlation with disease duration >12 months. * Significantly lower than in <12-months group ($P < 0.05$); adjusted by molecular sub-type, age at onset, sex, country of origin, year of death.

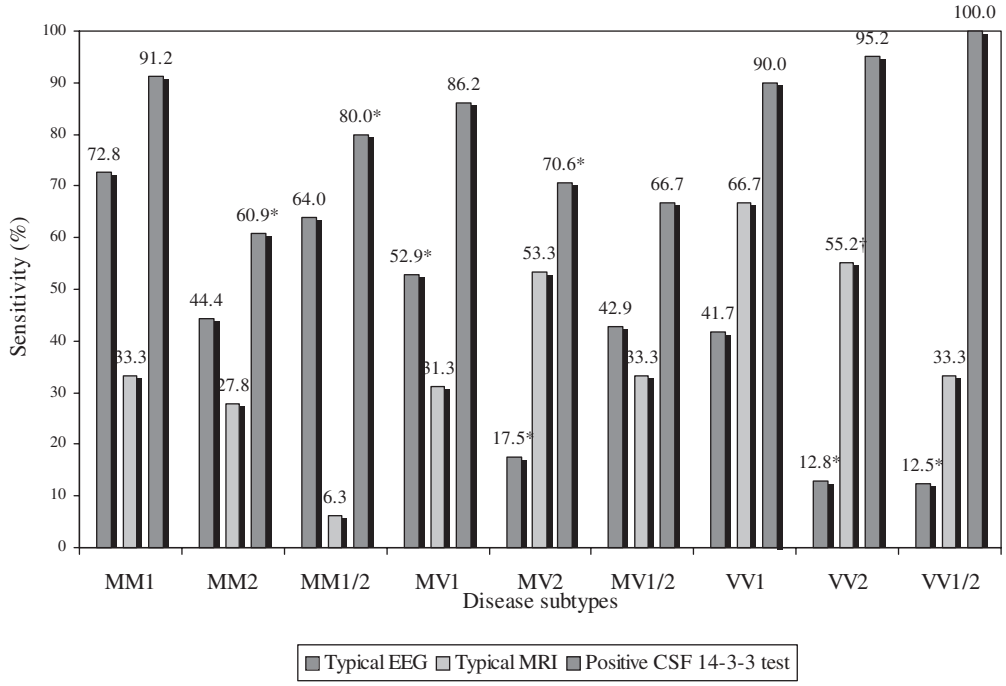

Fig. 3 The sensitivities of EEG, brain MRI and CSF 14-3-3 protein detection were assessed according to the sporadic CJD molecular sub-type: MM1, MM2, MM1/2; MV1, MV2, MV1/2; and VV1, VV2 and VV1/2. The MV1, MV2, VV2 and VV1/2 sub-types all showed a significantly reduced EEG sensitivity, while the VV2 sub-type displayed a significantly higher likelihood of a typical MRI result. The MM2, MM1/2 and MV2 sub-types all showed significantly reduced rates of CSF 14-3-3 protein positivity. * Significantly lower than MM1 ($P < 0.05$); †significantly higher than MM1 ($P < 0.05$); adjusted by age at onset, disease duration, sex, country of origin, year of death.

during the patient's illness on the likelihood of a typical or positive result. With the median duration of illness only 5 months, we chose to divide the symptomatic phase into thirds (first, middle and final) rather than into a greater number of epochs. This was undertaken to avoid the creation of illness phases of such brevity, especially at the onset of symptoms, that they would be unlikely to correspond to a clinically meaningful period of a patient's illness during which investigations would usually be undertaken and that the test would have a reasonable chance of showing typical abnormalities.

With respect to EEGs, 2425 recordings with a known time point in relation to the chronology of the patient's illness were available for analysis from 1728 cases. When analysed in isolation, the likelihood of a typical EEG was significantly less in the first third of a patient's illness compared with the last third ($P = 0.02$). Multivariate analysis (adjusted by country, gender, year of death, age at onset, disease duration and codon 129 polymorphism) also showed that a positive EEG was significantly more likely in the last third of a patient's illness compared with the first third ($P = 0.001$). When a patient's molecular sub-type was substituted for codon 129 polymorphism in the same regression model, however, a typical periodic EEG was no longer significantly more likely in any particular time period of the patient's illness. These results are consistent with the specific time point at which the diagnostic test is undertaken during the symptomatic period of a patient's illness not being independent of molecular sub-type in determining the likelihood of a positive EEG. With respect to detection of CSF 14-3-3 proteins, 1032 assays from 985 cases were available for analysis, showing no significant association between a positive result and the time point of sampling during a patient's illness, regardless of whether assessed in isolation or in analogous multivariate analyses. For each of the first, middle and final third time periods of patient's illnesses, the CSF 14-3-3 protein sensitivities were 88, 86.4 and 91.3%, respectively.

Discussion

The current study is by far the largest to date to assess the diagnostic sensitivity of investigations usually employed in the evaluation of suspected sporadic CJD. By virtue of the large number of definite sporadic CJD patients comprising our study we have been able to confidently delineate the sensitivities of the principal investigation results across the entire phenotypic spectrum, especially the less common molecular sub-types, as well as clarify and extend hitherto uncertain independent determinants of investigation results (Pocchiari *et al.*, 2004). Analysis of only neuropathologically confirmed CJD patients has allowed us to avoid the inherent difficulties that arise from analysis of investigations that also serve in case definition criteria for probable cases; however, it is noteworthy that separate analysis of all EUROCJD sporadic CJD patients from this time period, that is, inclusion of clinically probable cases, gave the same results as analysis of just definite cases (data not shown). Multivariate regression modelling demonstrated that age at disease onset and disease duration both act as independent determinants of some investigation results in sporadic CJD, in addition to the influence of molecular sub-type. Consequently, in the clinical evaluation of suspect CJD, our findings reinforce the importance of fully appreciating the limitations of specific investigations relative to these salient patient clinical features; otherwise the heightened likelihood of a negative test result may be underestimated and incorrectly interpreted as undue reassurance against the diagnosis. Specifically, the probability that an EEG will demonstrate PSWCs increases with age and conversely decreases with disease duration, especially if >12 months. Hence, in a patient <50 years it is most likely that an EEG will not show typical changes, and even in older patients, if their illness is >6 months, the probability of PSWCs is at best only ~50%. In contrast to a prior smaller study (Zerr *et al.*, 2000*b*) disease duration >12 months was also observed to significantly lessen the likelihood of a positive CSF 14-3-3 result. This underscores the importance of acknowledging illness duration when interpreting a negative result in an individual patient suspected to be manifesting sporadic CJD.

The present study re-affirmed that molecular sub-type, in addition to age at onset and illness duration, is also an important independent determinant of principal investigation results (Parchi *et al.*, 1999; Zerr *et al.*, 2000*b*; Otto *et al.*, 2002; Hill *et al.*, 2003; Castellani *et al.*, 2004; Meissner *et al.*, 2004). In contrast to the EEG, where the presence of valine alleles at codon 129 additively reduces the likelihood of PSWCs, valine homozygotes (especially VV2 patients) were significantly more likely to demonstrate a typical MRI scan than methionine homozygotes (particularly the MM1 subgroup). These data conform to a pattern whereby sub-types with 'classical' sporadic CJD and predominant cerebral cortical involvement (MM1 and MV1) are more likely to manifest PSWCs on EEG, while those with ataxia and subcortical neuropathological changes (VV2 and MV2) are more likely to demonstrate characteristic basal ganglia changes on MRI. However, in contrast to a prior report suggesting phenotypic homogeneity between the MM1 and MV1 sub-types (Parchi *et al.*, 1999), we found that the likelihood of a typical EEG was significantly lower and the average illness duration significantly longer in the MV1 patients. The most likely explanation for this difference in findings is the much larger sample of MV1 patients available for analysis in our study.

The CSF 14-3-3 protein immunoassay was the most sensitive investigation across the sporadic CJD phenotypic spectrum, but the influence of molecular sub-type was still evident. Codon 129 heterozygotes, especially the MV2 sub-group, were significantly less likely to be associated with a positive result [Zerr *et al.*, 2000*a*, *b*; Otto *et al.*, 2002; Castellani *et al.*, 2004], as were the very uncommon MM2 and MM1/2 subgroups. In comparison with the simple

demographic features of age at disease onset and illness duration, the molecular sub-typing profile is much less likely to be available pre-mortem in individual patients. Nevertheless, if this information is available, investigation results would also need to be interpreted in this specific context for the most accurate estimation of excluding or confirming the likelihood of sporadic CJD. Having recognized these overall limitations and variations across the molecular sub-types, from a practical clinical perspective it would appear prudent to utilize all three principal investigations (brain MRI, CSF 14-3-3 protein analysis and EEG), as, irrespective of molecular sub-type, only very uncommonly (except the rare MM2 sub-group) will all three investigations not show characteristic findings in an individual patient with sporadic CJD (Shiga *et al.*, 2004).

Our analyses do not suggest that the time point at which the investigation is undertaken during a patient's illness is an independent determinant of the test result. This was particularly evident for CSF 14-3-3 protein detection, for which no association between time point of sampling and a positive result was found regardless of analytical approach. For EEG, the apparent reduced likelihood of a typical or periodic recording in the first third of a patient's illness compared with the final period, when examined in isolation or with one of the multivariate regression models, was no longer evident when the regression model included molecular sub-type. Collectively, our findings are consistent with the generally dominant influence of age at onset, illness duration and molecular sub-type as the key independent determinants of investigation results compared with other demographic variables, including the time point of test sampling during a patient's illness.

Although molecular sub-typing of sporadic CJD patients has proven a useful phenotypic classification system, there are some difficulties with this approach. Not uncommonly, more than one PrP^{res} type may be present in the brain of a patient dying from sporadic CJD (Parchi *et al.*, 1999), and it is likely that the frequency of occurrence increases with the number of regions assessed (Puoti *et al.*, 1999; Head *et al.*, 2004). At present, there is no consensus on how to classify such patients and whether it is generally better to adopt a dichotomized approach based on predominance of one type when several regions are studied or accept classification based on the analysis of only one or two brain sites. In our study, patients with more than one PrP^{res} type were a small minority, but such patients were allowed status as a separate group within each *PRNP* genotype without attempting to adjust for relative abundance of the two PrP^{res} types. Regardless of *PRNP* genotype, our patients with simultaneous occurrence of both PrP^{res} types tended to present a different phenotype, sometimes intermediate between those with a single PrP^{res} isotype and the same codon 129 status, but often extending beyond the range observed. This suggests that the simultaneous presence of two PrP^{res} types may evoke a third or unique phenotype within each codon 129 genotype, although our small patient numbers militated against achieving significance with these observations. Analysis of investigation results also generally suggests that subgroups with co-existence of PrP^{res} isotypes tended to appear different although not invariably. For CSF 14-3-3 results, the MM1/2 group behaved more like MM2 patients, and for the EEG the VV1/2 group were also more like the VV2 patients. The latter is in keeping with the predominant phenotypic influence of type 2 PrP^{res} on these investigations or may simply represent a sampling bias wherein type 2 PrP^{res} was under-appreciated as the dominant protease resistant isotype across the brain.

MRI brain imaging is now very commonly employed in the diagnostic evaluation of suspected sporadic CJD but its ultimate sensitivity and specificity is still being defined (Finkenstaedt *et al.*, 1996; Collie *et al.*, 2001; Meissner *et al.*, 2004; Shiga *et al.*, 2004; Tschampa *et al.*, 2005). For a number of reasons, our overall sensitivity for typical changes on MRI scan is lower than that reported in much smaller studies (Finkenstaedt *et al.*, 1996; Schröter *et al.*, 2000; Zerr *et al.*, 2000*b*; Meissner *et al.*, 2004; Shiga *et al.*, 2004; Tschampa *et al.*, 2005). Importantly, patient MRI data collected for this study extended over a decade, during which time this imaging technique was increasingly used in the evaluation of suspect sporadic CJD, and there were concurrent improvements in the diagnostic sensitivity of pulse sequences used for this illness (Collie *et al.*, 2001; Shiga *et al.*, 2004; Tschampa *et al.*, 2005). Consequently, the overall number of patients studied with MRI was relatively low (42.3%), and the utilization of optimized MRI techniques such as DWI and FLAIR mainly occurred only in the very latter phase of our study epoch and even then was not uniformly adopted by all neuroimaging services. Further, during the study period there was progressive appreciation that increased cerebral cortical signal using the more sensitive techniques of DWI and FLAIR is probably a reasonably common feature in sporadic CJD (Shiga *et al.*, 2004; Tschampa *et al.*, 2005). Consequently, another reason for our low MRI sensitivity relates to our restrictive definition of what changes we considered characteristic of the diagnosis (i.e. signal hyperintensity limited to the basal ganglia). Overall, this combination of factors has compromised our assessment of the true diagnostic sensitivity and clinical utility of MRI in the evaluation of sporadic CJD. This sub-optimal aspect of the present study is being redressed by a further prospective EUROCJD collaborative project.

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