Epidemiology of Creutzfeldt-Jakob disease

Incidence, risk factors and survival in European studies

Cover design: Iris de Jong
Layout: Bon Mot, Rotterdam
Printed by: Grafisch bedrijf Ponsen & Looijen b.v., Wageningen

ISBN 90-9010536-0

The European studies described in this thesis were funded by the European Union. The Dutch study was supported by a grant from the Netherlands Institute for Health Sciences (NIHES). In this project the Department of Epidemiology & Biostatistics and the Department of Neurology of Erasmus University Medical School, Rotterdam, collaborated with the National Institute of Public Health and Environmental Protection (RIVM).
The author gratefully acknowledges the contribution of the Stichting Het Remmert Adriaan Laan Fonds and the Product Boards for Livestock and Meat, to the publication of this thesis.

Epidemiology of Creutzfeldt-Jakob disease

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Epidemiologie van de ziekte van Creutzfeldt-Jakob

Incidentie, risicofactoren en prognose in Europese studies

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr P.W.C. Akkermans M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 7 mei 1997 om 11:45 uur

door

Dorothée Petronel Wilhelmien Maria Wientjens

geboren te Empel en Meerwijk.

Promotores	:	Prof. dr A. Hofman Prof. dr F.G.A. van der Meché
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Publications and manuscripts based on the studies described in this thesis

Chapter 2

Wientjens DPWM, Davanipour Z, Hofman A, Kondo K, Matthews WB, Will RG, van Duijn CM. Risk factors for Creutzfeldt-Jakob disease: a re-analysis of case-control studies. Neurology 1996;46:1287-1291.

Chapter 4

Delasnerie-Lauprêtre N, Poser S, Pocchiari M, Wientjens DPWM, Will RG. Creutzfeldt-Jakob disease in Europe. Lancet 1995;346:898.

The EU collaborative study group on Creutzfeldt-Jakob disease. Descriptive epidemiology of Creutzfeldt-Jakob disease: the EU collaborative studies of 1993-1995 (submitted).

Chapter 5

The EU collaborative study group on Creutzfeldt-Jakob disease. Risk factors for Creutzfeldt-Jakob disease: the EU collaborative studies of 1993-1995 (submitted).

Chapter 6

Wientjens DPWM, van Duijn CM, Delasnerie-Lauprêtre N, Poser S, Pocchiari M, Will RG, Hofman A, for the EU collaborative study group on Creutzfeldt-Jakob disease. Prognosis of Creutzfeldt-Jakob disease: the EU collaborative studies of 1993-1995 (submitted).

Chapter 7

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CHAPTER 1

Introduction

CREUTZFELDT-JAKOB DISEASE (CJD) is a rare neurodegenerative disorder with a highly interesting aetiology and potentially important public health implications.[1] In aetiological terms, CJD is one of the human prion diseases, characterised by rapid neurodegeneration leading to a characteristic spongiform encephalopathy.[2] From a public health perspective, CJD is of interest because of the possible link between the bovine spongiform encephalopathy (BSE) and CJD.[3] In this thesis studies on the incidence, risk factors and prognosis of CJD are reported. It is based on a collaborative European study in which the incidence of CJD was investigated in various European countries on the basis of national registries. In addition a collaborative case-control study of determinants of CJD was performed in the European countries with a CJD register. And finally, the survival of CJD patients was estimated on the basis of patients in the registers.

The outline of this thesis is as follows. Chapter 2 presents a reanalysis of former case-control studies on determinants of CJD conducted in the seventies and early eighties in the US, UK and Japan. In chapter 3 the methods of the various studies are described. In particular, details on the build-up of the register for CJD in the Netherlands and Belgium are given, with particular emphasis on the ascertainment of patients. Chapter 3 also describes the methodology of the case-control study that was conducted in various European countries to investigate risk factors for CJD. Chapter 4 presents the incidence of CJD in Europe from 1993 to 1995 based on registers in Belgium, France, Germany, Italy, the Netherlands, Slovakia, and the United Kingdom. In chapter 5 the risk factors for CJD are described, based on the collaborative case-control study of CJD conducted in various European countries between 1993 and 1995. In chapter 6 the survival of CJD and prognostic factors for survival are described based on CJD patients in the European registers. In the last chapter the methodological aspects of the studies of incidence, risk factors and prognosis are discussed, and the implications of the findings and suggestions for future research are presented.

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CHAPTER 2

Review

Abstract

To review the evidence for risk factors of Creutzfeldt-Jakob disease (CJD), we pooled and re-analyzed the raw data of three case-control studies. The pooled data set comprised a total of 178 cases and 333 controls. The strength of association between CJD and putative risk factors was assessed by computing the odds ratio as estimate of the relative risk. The risk of CJD was statistically significantly increased for subjects with a family history of CJD (odds ratio=19.1;95% confidence interval 1.1 - 348.0). Further, there was a significant association between the risk of CJD and a history of psychotic disease (odds ratio=9.9;95% confidence interval 1.1 - 86.1). Although not significantly increased, there was an elevated risk of CJD for subjects with a family history of dementia, a history of poliomyelitis, subjects employed as health professionals and subjects ever exposed to cows and sheep. No association could be shown with organ meat consumption including brain. The negative results of this re-analysis re-emphasises the absence of a common risk factor in all CJD patients. However, the ongoing epidemiological surveillance of CJD in several European countries may provide more evidence to exclude any environmental exposure early in childhood.

Introduction

Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy that may occur in an inherited, infectious, or sporadic form. CJD may be caused by various mutations in the prion protein gene (PRNP) on chromosome 20.[1] However, these mutations are found only in familial CJD patients, comprising not more than 15% of all CJD patients.[1] The infectious form of CJD appears to be rare[2] and limited to iatrogenic transmission through neurosurgery and electro-encephalographic electrode implantation[3-6], corneal transplantation[7] and human growth[8-10] and gonadotrophin[11] hormone. For the large majority of patients with sporadic CJD, the cause remains unknown. Studies on risk factors for CJD have yielded controversial results and did not show evidence for a common risk factor.[12-17] An increased risk of CJD has been reported for subjects with a history of infection[12,17], surgery of the head[13,14], trauma to the head or body[13,14], tonometry[14], consumption of pork [15], and contact with various animals[16,17] in some but not all studies. The recent infection of British bovine cattle through protein supplements derived from animal carcasses has fueled speculations on the possibility of infection of humans through meat consumption and animal contact. Other than the established transmission of Kuru through cannibalistic rituals[1,2], findings of studies on (organ) meat consumption including brain have not yielded consistent results.[12,15]

Some of the conflicting results may be explained by the small size of the individual studies yielding only limited statistical power to detect an association. To review the evidence for the risk factors previously reported, we pooled and re-analyzed the raw data of three case-control studies of CJD. As no meta-analysis of the existing case-control studies on Creutzfeldt-Jakob disease has been performed, we present here our results.

Patients and methods

All formal case-control studies of CJD were ascertained through MEDLINE search and personal communications. Thus, four studies

were identified as eligible for the re-analysis.[12-17] To increase the comparability between studies, we have restricted the analysis to the three studies comprising patients diagnosed with CJD according to the criteria of Masters et al.[18] One study was performed in Japan[13], one in the United States of America[14,15,16] and one in the United Kingdom.[17] The pooled data set comprised a total of 178 cases (mean age at diagnosis 61 years; SD=9.5; range 26-81 years) and 333 controls. For each study, ascertainment of cases and controls and data-collection will be discussed here briefly.

Japan

The study conducted in Japan comprised 60 cases.[13] According to the criteria of Masters et al,[18] 50% were classified as probable, 47% as definite and 3% as transmissible CJD. For this study, neurological and psychiatric institutions throughout Japan were requested to report patients diagnosed with CJD in the period 1975-1977. Patients were compared to a total of 109 age-matched controls (+/- 5 years), including 50 spouses and 59 neighborhood controls. In some instances, public health personnel served as neighborhood control. The response rate was 80% in cases, 78% in spouses and 93% in neighborhood controls. Data were collected by a structured interview. For cases the data were obtained from the spouse. Control subjects were questioned directly.

USA

The study performed in the United States comprised 26 cases of which 23% were classified as probable CJD, 46% as definite and 31% as transmissible.[14,15,16] Patients were ascertained through records submitted to the Laboratory of Central Nervous System Studies of the National Institutes of Health from Pennsylvania, New Jersey, Maryland, New York and Delaware. Patients were diagnosed with CJD in the period 1970-1981. Two control groups were selected: hospital controls diagnosed with a non-neurological disease at the hospital where the patient was diagnosed (n=22; matching sex and age +/- 5 years) and relative controls (n=18; matching sex and age +/- 10 years). The response rate was 96% in cases, 85% in hospital controls and 86% in relative controls. Data were collected by a structured interview of the spouse (58%),

child (27%), sibling (11%) or parent (4%) of the case. Control subjects were questioned directly.

UK

The study conducted in the United Kingdom comprised 92 cases of whom 24% were classified as probable and 76% as definite CJD.[17] Patients diagnosed with CJD in the period 1980-1984 were ascertained through neurological centers in England and Wales and through death certificates of the Office of Population, Censuses and Surveys. For each case, two age- and sex-matched controls were derived from the hospital where the patient was diagnosed (n=184; matching age +/- 4 years). Of the two controls, one suffered from a neurological disorder other than CJD, the other from a non-neurological disorder. The response rate was 75% for cases and is unknown for controls. For cases as well as controls the data were collected by interviewing an informant. The relationship to the informant was matched in 87% for cases and controls. In 78% of the subjects it was the spouse, in 16% a child and in 6% a sibling.

Analysis

This re-analysis was based on the original raw data of the three studies. We only included risk factors assessed comparably in at least two out of three studies. The risk factors studied were: *(1)* family history of CJD, dementia and Parkinson's disease; *(2)* medical history, including head trauma, hospitalization for psychotic disease, history of poliomyelitis, hepatitis, jaundice, allergy and blood transfusion; *(3)* employment as health professional; *(4)* exposure to animals; *(5)* living in a rural area; and *(6)* consumption of organ meat. Exposures of cases, relatives and spouses may be similar for family and occupational history,animal exposure, place of residence and diet, due to the shared environment. As this may bias the results towards the null hypothesis, relative and spouse controls were excluded in the analysis of these putative risk factors. Thus, the number of subjects included in the study may vary according to the number of studies and control subjects included, as well as to the missing values within a study. For each risk fac-

tor, we report the number of subjects exposed and the total number of subjects for whom data on the risk factor were available.

The strength of association between CJD and putative risk factors was assessed by computing the odds ratio (OR) as an estimate of the relative risk.[19] Relative risks were estimated by maximum likelihood methods and the 95% confidence intervals (95% CI) were based on the asymptotic standard errors. Confounding by age, sex and study center was taken into account by including these variables into the logistic regression model.

Results

Family history of CJD, dementia and Parkinson's disease for cases and controls are reported in the table. A positive family history of CJD was found only in CJD cases. A total of seven (4%) cases had a relative with CJD. An increased risk of CJD was also observed for cases with a positive family history of dementia in any relative, which was borderline significant (OR =1.9; 95% CI 0.9-4.1). When restricting the analysis to first degree relatives, the OR remained increased (OR=1.8; 95% CI 0.8-4.0 not in table).

With regard to the medical history (table), significantly more CJD patients than controls had a history of hospitalization for psychotic disease. Three of five CJD cases were hospitalized 15 years or more before the onset of CJD. No association was found with head trauma, hepatitis, jaundice, allergy or blood transfusion. The risk of CJD associated with poliomyelitis was increased (OR 3.9; 95% CI 0.3-43.4). However, the significance is difficult to interpret as the number of exposed subjects is very small. Fewer CJD patients than controls had a history of surgery. The difference was statistically significant for abdominal surgery.

A non-significantly increased risk of CJD was found for subjects exposed to patient tissue through their employment as a health professional, i.e., medical doctors, nurses, dentists, laboratory workers, ambulance employees (OR 1.5; 95% CI 0.5-4.1) (table). Exposure to cows and sheep was associated with a borderline significant increased risk of CJD (OR 1.7; 95% CI 0.9-3.1 and OR 1.6; 95% CI 0.9-2.9, respectively). Also, there was a non-significantly increased risk of CJD for subjects who lived in a rural area

Table — Family history of CJD, dementia and Parkinson's disease, medical history, and occupational, animal and dietary exposures and the risk of Creutzfeldt-Jakob disease

	Cases†	Controls†	Odds ratio (95% confidence interval)	
			Crude	Adjusted‡
Family history				
CJD[13-17]*§	7/166	0/285	18.8 [1.0-341.2]¶	19.1 [1.1-348.0]¶
Dementia[14-17]*	15/126	17/212	1.6 [0.7-3.2]	1.9 [0.9-4.1]
Parkinson's disease[14-17]*	7/117	7/194	1.7 [0.6-5.0]	1.7 [0.6-5.0]
Medical history				
Psychotic disease[13-17]*	5/111	1/219	10.3 [1.2-89.8]¶	9.9 [1.1-86.1]¶
Head trauma[13-17]*	27/176	55/329	0.9 [0.6-1.5]	0.9 [0.5-1.5]
Poliomyelitis[13-17]*	2/168	1/332	4.0 [0.4-44.6]	3.9 [0.3-43.4]
Hepatitis/jaundice[14-17]*	9/118	18/222	0.9 [0.4-2.2]	0.9 [0.4-2.1]
Allergy[13-17]*	39/174	64/330	1.2 [0.8-1.9]	1.2 [0.8-2.0]
Blood transfusion[13-17]*	17/174	45/328	0.7 [0.4-1.2]	0.6 [0.4-1.2]
Surgery:				
central nervous system[13,17]*	0/149	10/301	0.3 [0.1-1.2]	0.3 [0.1-1.2]
eye[13-17]*	6/176	23/332	0.5 [0.2-1.1]	0.5 [0.2-1.1]
abdominal[13-17]*	39/176	96/331	0.7 [0.5-1.1]	0.7 [0.4-1.0]¶
tonsillectomy [13-17]*	13/171	32/331	0.8 [0.4-1.5]	0.7 [0.4-1.4]
Occupational, animal and dietary exposure				
Health professional[14-17]*	8/119	7/206	1.5 [0.6-4.2]	1.5 [0.5-4.1]
Cow exposure[13-17]*	26/95	26/145	1.7 [0.9-3.2]	1.7 [0.9-3.1]
Sheep exposure[13-17]*	26/171	25/262	1.6 [0.9-2.9]	1.6 [0.9-2.9]
Organ meat: brain[13-17]*	14/177	33/261	0.6 [0.3-1.2]	0.6 [0.3-1.8]
liver[13-17]*	104/117	179/206	1.2 [0.6-2.5]	1.3 [0.6-2.6]
kidney[13-17]*	61/86	135/179	0.8 [0.4-1.4]	0.8 [0.3-1.4]

* Studies with data on risk factor
† Number of subjects with a positive history or subjects exposed/total number of subjects in analysis
‡ adjusted for age, sex and study site
§ CJD: Creutzfeldt-Jakob disease
¶ $p<0.05$

at diagnosis (OR 1.4; 95% CI 0.8-2.4; not in table). Consumption of organ meat including brain, liver and kidney was not associated with an increased risk of CJD (table).

Discussion

In this re-analysis of case-control studies, the risk of CJD was statistically significantly increased for subjects with a family history of CJD. We also found a statistically significant association between the risk of CJD and the patient's history of psychotic disease. No statistically significant association could be shown for medical history other than psychosis or with organ meat consumption. Although not significantly increased, more CJD patients than controls had a family history of dementia, a history of poliomyelitis, an occupational history as health professional, history of exposure to cows and sheep, and had lived in a rural area.

The internal validity of this re-analysis depends on the validity of the individual studies. Selection bias may have occurred through non-response in cases and controls in the individual studies. Further, control selection differed considerably across studies. However, to distort the findings, non-response and control selection should be associated with the risk factors studied. This seems unlikely for most risk factors. However, in the case of medical history, risk factor exposure may have been associated with control selection in the largest study conducted in the UK,[17] which was hospital based. The use of hospital controls may have led to high exposure frequencies for medical disorders, surgery, and blood transfusion. This may explain the lower frequency of surgery in CJD patients compared to controls. Unfortunately, the a priori statistical power of an analysis limited to the two small studies from Japan[13] and the US [14-16] was too low to yield a meaningful solution to this problem. Another source of information bias might have been biased recall of putative risk factors. Also, the data collection through informants for cases and personal interviews for controls might have biased the data in two out of three studies. To take this bias into account, we restricted the analysis to exposures not easily forgotten by controls and informants. For this reason, family history of dementia was restricted to first degree relatives and the

patient's history of psychotic disease to those requiring hospitalization. Further, we checked whether the association with a risk factor was found consistently across studies. In each of the studies, a family history of dementia, history of psychotic disease, employment as a health professional and exposure to cows and sheep was associated with an increased CJD risk. This suggests that if bias occurred, it must have occurred in all studies similarly despite the differences in the design among studies.

Another issue is that the statistical power of our analysis may have been limited for rare exposures. Although we pooled the data of all case-control studies in which the cases met the criteria for CJD by Masters et al,[18] the number of patients included in this re-analysis was still small which is reflected in the width of the 95% confidence intervals. Given an exposure frequency in controls of 10%, a significance level of 5% (two-sided) and statistical power of 90%, the smallest detectable relative risk in a study of 200 cases and 200 controls is 2.5.[19] If the exposure frequency is only 1%, the smallest detectable relative risk under these conditions is 8.0,[19] which is a strength of association seldom observed in epidemiologic research.

Despite the limitations, our analysis showed a significant association between history of psychotic disease and the risk of CJD. Whether psychotic disease is a risk factor for CJD or is merely an early symptom of CJD pathology is undetermined. Future studies on the relationship between CJD and psychotic disease may be relevant. Our study shows familial aggregation of CJD, which may be explained by mutations in the prion protein (PRNP) gene that segregate in the families.[1,20] Also, homozygosity at codon 129 may be associated with an increased susceptibility to prion diseases.[21,22] Our re-analysis showed a borderline significant association between CJD and family history of dementia. In a number of patients with familial forms of dementia, there have been PRNP mutations[23] and the familial aggregation of CJD and dementia may therefore be related to PRNP mutations segregating in these families. However, other genetic factors, e.g. the apolipoprotein E gene (associated with both the risk of CJD[24] and Alzheimer's disease[25]) or non-genetic factors, may underlie the relationship;

studies of families in which CJD and dementia aggregate may be of interest.

Our re-analysis did not support a number of previously reported associations. When pooling the data of the three studies, there was no association of the CJD risk with head trauma or the consumption of organ meat including brain. The latter was associated with an increased risk of CJD in the study of Bobowick[12], which is not included in the present re-analysis. Based on our findings, blood transfusion did not increase the risk of CJD in the period studied. There was no significant evidence in our study of transmission of CJD among humans or from cattle to human. However, there was a non-significant increase in risk of CJD for subjects employed as health professionals, subjects ever exposed to cows and sheep, and living in a rural area. In our re-analysis the power to assess a statistically significant relationship for exposure to patients and exposure to animals was low. Further, the individual studies were performed in the seventies and early eighties, when information in particular on inherited prion diseases among humans and animals was not yet available, which has limited the possibility to separate genetic and non-genetic patients in the present re-analysis. The negative results of our re-analysis are important in the light of public health to reassure the population that there are no common risk factors for Creutzfeldt-Jakob disease.

Our data cannot exclude an environmental source of infection that might have occurred early in childhood. The ongoing systematic epidemiological surveillance of CJD in several European countries might provide evidence of such exposure as residential histories throughout life are studied.[26] These studies with similar design will be highly comparable and will therefore be able to resolve the problem of selection bias and of the limited statistical power of our re-analysis.

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CHAPTER 3

Methods

3.1 Introduction

Creutzfeldt-Jakob disease (CJD) is a rare neurodegenerative disorder. Its infectious nature was demonstrated in 1968 through successful transmission to primates.[1] Since the outbreak of bovine spongiform encephalopathy (BSE) in cattle in the United Kingdom in 1986, the hypothetical risk of transmission of the infectious agent to humans prompted the UK Ministry of Health to reinstitute the national prospective epidemiological surveillance of CJD.[2] Also in other European countries concern was expressed about a possible link between animal spongiform encephalopathy (among others scrapie and BSE) and human spongiform encephalopathy including CJD. From the European perspective, a collaborative study aimed to provide comparative epidemiological data that would enable baseline epidemiological parameters to be established which might be crucial to the assessment of the risk of CJD in relation to animal spongiform encephalopathies.[3] In this chapter we will address the methods and general design of the Dutch register and the collaborative study on CJD conducted in Europe from 1993 until 1995.[3,4,5]

3.2 Diagnostic criteria

All patients described in this thesis were classified according to common diagnostic criteria adapted from those originally proposed by Masters et al, shown in tables 1 and 2 (overleaf).[6,7] Whenever possible the classification upon inclusion into the study was reviewed after new results of clinical or post mortem examinations became available.

- **Definite CJD** included those patients that were confirmed by neuropathology with the classic triad comprising spongy degeneration of the cerebral grey matter, neuronal loss and the proliferation and hypertrophy of astrocytes and/or by immunocytochemical staining of prion protein.
- **Probable CJD** included patients who had a classical clinical course defined as a rapidly progressive dementia and a typical electroencephalogram (generalised periodic sharp wave activ-

Table 1 — Diagnostic criteria for CJD in the collaborative European studies.[6]

Sporadic

1. Definite	• Neuropathologically confirmed and/or • Immunocytochemically confirmed PrP^{sc} positive/ Western blot) and/or • Scraple associated fibres	
2. Probable	• Progressive dementia • Typical EEG • At least 2 out of 4 clinical features listed	*Clinical features:* *1. Myoclonus* *2. Visual or cerebellar signs* *3. Pyramidal or extra-pyramidal signs* 4. *Akinetic mutism*
3. Possible	• Progressive dementia • At least 2 out of 4 clinical features • No EEG or atypical EEG • Duration less than 2 years	

Iatrogenic

1. Progressive cerebellar syndrome in a pituitary hormone recipient, or
2. Sporadic CJD with a recognized exposure risk.

Genetic

1. Definite or probable CJD plus definite or probable CJD in a first degree relative, or
2. Neuropsychiatric disorder plus CJD-specific PRNP mutation.

Table 2 — Diagnostic criteria for CJD by Masters et al.[7]

1. Transmissible virus dementia

Experimentally transmitted to nonhuman primates and/or other animals, producing an experimental spongiform encephalopathy.

2. Definite or probable CJD

2a Definite	• Neuropathologically confirmed, and • At least 1 of the following 5 clinical features listed	*Clinical features:* *1. Myoclonus* *2. Pyramidal signs* *3. Characteristic EEG* *4. Cerebellar signs* 5. *Extrapyramidal signs*
2b Probable	• Progressive dementia, and • At least 2 out of 5 clinical features listed, and • No neuropathological confirmation available	

3. Possible CJD

Progressive dementia with:
- Myoclonus and duration less than three years, or
- A relative with a transmissible, definite or probable CJD, or
- At least 2 out of 5 clinical features listed, together with signs of lower motor neuron involvement

ity occurring at 1-2Hz) and at least two out of the following specific clinical features: myoclonus, visual or cerebellar signs, extra-pyramidal or pyramidal signs, akinetic mutism.
- **Possible CJD** included those patients who had a history of a progressive dementia with a duration less than two years and at least three of the above-mentioned clinical features but without the typical EEG changes or neuropathological confirmation.

Furthermore, cases were classified according to the pathogenesis or aetiology of the disease. Three types were distinguished.

- **Iatrogenic CJD** was defined as *(1)* progressive cerebellar syndrome in a pituitary hormone recipient or *(2)* sporadic CJD with a recognized exposure risk.
- **Genetic CJD** was defined as *(1)* definite or probable CJD plus definite or probable CJD in a first degree relative or *(2)* neuropsychiatric disorder plus disease-specific PRNP mutation.
- **Sporadic CJD** was defined as a case of unknown origin, i.e. non genetic and non iatrogenic.

3.3 Ascertainment study population

For the collaborative studies on incidence, risk facors and survival different study populations, comprising sporadic, genetic and iatrogenic CJD patients, were derived from the national registries. The incidence study comprised all definite and probable patients with year at onset (n=570) or death (n=606) in the three year period 1993 to 1995. For the case control study all definite and probable CJD patients with disease onset in the four year period 1992-1996 were eligible. However, per country only a proportion of those eligible, were finally included (n=405) in the case-control study. For the survival analysis all definite and probable patients with year at onset in the three year time period 1993 to 1995 (n=606) were eligible and included.

3.3.1 Netherlands and Belgium

The data collection was coordinated by the Department of Epidemiology & Biostatistics of the Erasmus University Medical School in Rotterdam, the Netherlands and the Department of Neurology of the University Hospital Rotterdam, Dijkzigt in Rotterdam, the Netherlands. The study population comprised two countries, the Netherlands (study period January 1, 1992 to December 31, 1995) and the Dutch speaking part of Belgium (Flanders; study period January 1, 1994 to December 31, 1995). In the Netherlands neurologists, (neuro)pathologists and those in training, in total 835 physicians, were sent requests to notify us all known or suspected cases of CJD with onset of disease or death from January 1, 1993 onwards (see appendix). Each centre was asked to assign a contact person, in total 160 neurologists, whom we repeatedly (two to three times a year) contacted by mail and telephone. The Flemish speaking part of Belgium was included in the study from January 1, 1994. For Flanders 250 neurologists, (neuro)pathologists and those in training were sent requests to notify suspected cases of CJD. A total of 191 neurologists were assigned as contact persons. Referred patients were seen by a research physician, sometimes repeatedly, and their clinical and EEG records were reviewed. During the course of the illness the clinical records, EEG records, vital status and pathology records of the patients were reassessed. We recommended recording of serial EEG's and performing investigations as CT and MRI scan to exclude other possible causes of dementia. We encouraged and discussed the importance of post mortem examination and the precaution methods to be taken to reduce the potential risk of contamination. After obtaining informed consent from the patient's relatives, blood was taken for prion protein gene analysis. The genetic screening for coding mutations or non-encoding polymorphism at codon 129 of the PRNP gene was performed in St Mary's Hospital, in London, (Prof. J. Collinge). When the patient was known to us only through neuropathology, hospital notes where obtained and EEG recordings were examined. In most cases the post mortems were performed in the hospital where the patient was diagnosed. A number of patients were referred to a university hospital for second opinion. Neuropathology was reviewed by one neuro-

pathologist (Dr G.H. Jansen) with immunohistochemical staining techniques.

The Dutch and Belgian registries ascertained 48 newly diagnosed cases and 44 mortality cases in the three year period from 1993 to 1995. A total of 49 patients were eligible for the case-control study, of whom 43 (88%) were included. Reasons of non-response to the case-control study were lack of cooperation of relatives (four) and no informed relative available (two). One control subject was selected for each case. Control subjects were recruited by questioning ward staff. Patients with a neurodegenerative disorder were excluded. Matching criteria were sex, age (+/- 5 years), and hospital of admission. Information on cases and control subjects was obtained through a structured interview with an informant. In each matched set relatives of an identical degree were interviewed whenever possible.

3.3.2 EU collaborative study

In 1993 a project for coordination of national CJD surveillance programmes was funded by the European union, linking already established national registries in France and the UK and proposed national registries in Germany, Italy, the Netherlands and Flanders (project-leaders: Dr R.G. Will and Prof. A. Hofman).
The objectives of the European collaborative study were *(1)* to study the frequency of CJD in Europe in relation to the occurrence of BSE, *(2)* to assess the risk of CJD in relation to genetic, occupational and nutritional factors, with particular emphasis on animal spongiform encephalopathies and *(3)* to study the clinical course of CJD.
For this purpose the European collaborative study was carried out in three parts: *(1)* registries of CJD were established in 6 European countries, *(2)* a case-control study of risk factors for CJD was performed on the basis of cases in the registries and *(3)* molecular genetic studies of CJD with material collected in the registries were conducted.
The method of case ascertainment was through direct notification, mainly from neurologists and was similar to previous epidemiological surveys of CJD.[8-14] The registries included sporadic, genetic and iatrogenic CJD. The aim of the registries was to achieve a

complete ascertainment of all cases emerging in a defined geographic area and to serve as a basis for further epidemiologic and molecular genetic studies. Prion protein gene analysis on blood or frozen brain tissue was performed in national genetic laboratories to screen for mutations and a polymorphism at codon 129 of the PRNP gene.

France

The data collection was coordinated by Unit 360 of the Institute National de la Santé et la Recherche (INSERM), Paris. The French registry ascertained 148 newly diagnosed cases and 150 mortality cases in the three year period from 1993 to 1995. For the case-control study 194 patients with definite or probable CJD were eligible of whom 75 (39%) were included in the case-control study. One control subject was selected for each case, matched for age (+/- 5 years) and sex. Information on cases was obtained from an informant, mainly a next of kin. A relative of a non-CJD patient admitted at the same hospital served as a control subject, with the non-CJD patient acting as the informant for the control.

Germany

The data coordinating centre for the study was the Department of Neurology of the Georg August University of Göttingen, Germany. The German registry ascertained 155 newly diagnosed cases and 123 mortality cases in the three year period from 1993 to 1995. For the case-control study 162 patients with definite or probable CJD were eligible of whom 136 (84%) were included in the case-control study. One control subject was selected for each case. Matching criteria were sex, age (+/- 5 years) and hospital of admission. Excluded as control subjects were patients with dementia or any other disorder of the central nervus system. Information on cases and control subjects was obtained through a structured interview with an informant.

Italy

The general case ascertainment for the registry was coordinated by the Laboratorio di Virologica, Instituto Superiore di Sanità, Roma. Coordination of the case-control study was carried out by the Department of Neurology of the Universita Cattolica del Sacro

Cuore, Rome. The Italian registry ascertained 114 newly diagnosed cases and 98 mortality cases in the three year period from 1993 to 1995. For the case-control study 123 patients with definite or probable CJD were eligible of whom 63 (51%) were included in the case-control study. Matching criteria were sex, age (+/- 5 years) and hospital of admission. Information on cases and control subjects was obtained through a structured interview with an informant. Information on cases and control subjects was obtained through a structured interview with an informant.

United Kingdom

The data collection was coordinated by the national CJD surveillance Centre at the Western General Hospital, Edinburgh, UK. The British registry ascertained 138 newly diagnosed cases and 141 mortality cases in the three year period from 1993 to 1995. For the case-control study 153 patients with definite or probable CJD were eligible of whom 88 (58%) were included in the case-control study. One control subject was selected for each case, matched for age (+/- 5 years), sex and hospital. Control subjects were selected from the neurological ward with exclusion of any neurodegenerative disorder or from a non-neurologic ward. Control subjects were recruited by questioning ward staff excluding those with a neurodegenerative disorder. For cases data were obtained from a relative. For controls, this procedure was followed whenever possible.

Slovakia

The data collection was coordinated by the Institute of Preventive and Clinical Medicine in Bratislava. The registry of Slovakia ascertained ten newly diagnosed cases and seven mortality cases in the three year period from 1993 to 1995. Cases from Slovakia were not included in the collaborative case-control study.

Table 3 — Clinical, genetic and pathological characteristics of CJD studied

Neurological signs and symptoms at onset / during course		Investigations & prion protein gene analysis	Pathological post mortem / biopsy
Dementia	Sensory	EEG	Nerve cell loss
Cerebellar	Vertigo/dizziness	CT-scan	Gliosis
Visual/oculomotor	Pseudobulbar	MRI scan	Spongiform change
Pyramidal	Neurogenic muscle wasting	CSF	Immunostaining
Extrapyramidal	Akinetic mutism	LFT	Western blot
Seizures	Gait disturbances	PRNP mutation	SAF
Involuntary movements	Speech disturbances	PRNP polymorphism 129	
Myoclonus	Visual disturbances		
Headache	Forgetfulness		

3.4 Data collection

3.4.1 Clinicopathologic features

For all patients the clinical course of disease was studied. Neurological signs at onset and during the course, and symptoms reported by patient's relatives were assessed from medical records. In table 3 the clinical features, investigations and pathological characteristics assessed are summarised.

Sporadic, genetic and iatrogenic patients with definite or probable CJD with onset of disease during the period January 1, 1993 to December 31, 1995 were eligible for studies on survival and clinical course (n=606). Duration of illness was defined from first symptoms till death or censoring date at May 31, 1996. Using data and material collected in the national registries, clinical and molecular genetic studies were carried out with the aim to improve diagnostic criteria[15-17] for CJD and to study the role of the PRNP gene.[18-22]

Table 4 — Risk factors studied for CJD

Medical history	Familial history	Exposure	Animal (product) exposure	Consumption
Surgery *Overall* *Brain* *Vertebral column* *Other surgery central nervous sytem* **Blood transfusion** **Organ transplantation** **Disease history** *Epilepsy* *Treatment psychiatrist/ psychologist* *Jaundiced* *Glandular fever* *Polio* *Herpes Zoster* *Herpes simplex* *Rheumatoid arthritis* *Diabetes* *Allergies/atopy* *Head injury* **Medical treatments/tests** *EMG* *Lumbar punction* *Acupuncture* *Vaccination* *Hormone suppl.* *Opthalmologist/ optician test* *Dentist visit*	**CJD** *Overall* *1st degree relative* *Grandparents* **Dementia** *Overall* *1st degree relative* *Grandparents* **Parkinson's disease** *Overall* *1st degree relative* *Grandparents*	**Occupational exposure to patients/patient material** *Overall exposure to patients/ body tissue* *Physician* *Neuropathologist* *Nurse* *Laboratory technician* *Dentist* *Ambulance worker* **Occupational exposure to animal products** *Butcher* *Slaughter* *Veterinary doctor* *Meat/food processor* *Leather worker* *Animal/animal products* **Occupational exposure to animals** *Husbandry* *Cows* *Sheep* *Pigs* *Horses* *Other cattle* *Mink/ferrets* *Other fur animals* *Deer/elk* *Other animals*	**Domestic exposure to animals** *Cats* *Dogs* *Rodents* *Birds* *Fish* *Snakes* *Other pets* **General exposure to animals and animal products** *Contact with fur/ leather other than through clothes* *Lived on a farm* *Fertilizer* *- artificial f.* *- hoofs + horns* *Leather worker* *Bone meal* *Dried blood of animals* *Ever bitten by animal*	**Meat and milk products** *Sausage* *Raw meat* *Raw fish* *Products animal blood* *Milk* *Cheese* **Organ meat** *Tripe* *Kidney* *Liver* *Brain* *Eye* **Meat** *Beef* *Veal* *Lamb* *Pork*

3.4.2 Case-control study

To study risk factors of CJD a case-control study was performed with cases from the registries including cases with onset of disease from 1992 onwards. Definite and probable patients with sporadic, genetic or iatrogenic CJD were eligible for the case-control study. Data collection on possible risk factors was obtained through a structured interview by a research physician with an informant, in most cases a next of kin. To achieve symmetrical data collection, information on risk factors of the control subject was also obtained through an interview with an informant.
For the collaborative study a standardised core questionnaire on risk factor exposure was developed to be used in all participating countries. The core questionnaire was adapted from a questionnaire used in the ongoing epidemiological surveillance in the UK.[23] Data collected using the former questionnaire (in Italy and the UK) were mapped to the European format. A computer data-entry programme was developed based on the core questionnaire. Data were centralised and analyzed at the Department of Epidemiology & Biostatistics of the Erasmus University in Rotterdam. The risk factors that were investigated are summarised in table 4 (page 27).

3.5 Statistical analysis

3.5.1 Incidence

Cases were included according their diagnostic status on May 31, 1996. Sporadic, iatrogenic and genetic cases classified as definite or probable CJD have been included in the analysis. Mortality rates were estimated using all deaths from CJD in the period 1993 to 1995. Mortality rates (cases/million/year) were estimated for each country and overall for the collaborative study. Incidence was estimated based on patients newly diagnosed with CJD in the period 1993 to 1995. The demographic data used for estimating the denominators are shown in table 5.[24]

Table 5a — Population denominators (in millions)†

Belgium	France	Germany	Italy	Nether-lands	Slovakia	UK
7.1	57.7	81.2	57.1	15.3	5.3	58.2

† Denominator derived from Population Trends, winter 1996;issue 33:table 5a

Table 5b — Population in 10 year age bands (in thousands)†

	France	Germany	Italy	Netherlands	UK
< 39	32759	42517	32332	8942	31687
40–49	7303	10166	7448	2128	7591
50–59	5754	10572	7121	1521	6021
60–69	5453	7691	6341	1302	5011
70–79	3197	5059	3669	871	4091
> 80	2145	3008	1866	434	2021

† Denominator figures derived from Demographic Year Book by the United Nations 1991;33: table 5b

3.5.2 Case-control study

This analysis was based on the original raw data of the five participating centres: Netherlands/Belgium, France, Germany, UK, and Italy. Principal investigators of the studies were invited to Rotterdam to coordinate the strategy of analysis in the four major topics and reported their results at the fifth meeting of the collaborative EU study on CJD, held in Edinburgh in May 1996. The strength of association between CJD and putative risk factors was assessed by computing the odds ratio (OR) as an estimate of the relative risk. Relative risks were estimated by maximum likelihood methods and the 95% CI were based on the asymptotic standard errors.

Two different strategies were followed in the analysis. First, all analyses were conducted using an unconditional logistic regression model. Confounding by age, sex and study centre was taken into account by including these variables into the regression model. Second, we performed an analysis using a conditional logistic regression model which took into account the matched case and control pairing. This design is in accordance with the matched design of the study however at the expense of statistical power. A major problem resulting from the second approach is the loss of information in that a matched set of case and control was dropped out of the analysis if data were missing on a variable in either case or control. Therefore risk estimates may be biased due to selection. As both strategies gave similar findings judged upon the relative risk estimates, findings of the method with the highest statistical power, the unconditional analyses, are given. In order to test for effect modification by significant differences between study centre, in both strategies an interaction term with the risk factor studied was included into the model.
Stratified analyses were performed based on sex, age at onset, diagnosis (probable versus definite), family history of CJD, PRNP mutations and polymorphism at codon 129 of the PRNP gene. For each risk factor, we report the number of subjects exposed and the total number of subjects for whom data on the risk factor were available.

3.5.3 Survival

In the studies of survival, only patients with definite and probable CJD were included. The aim was to estimate *(1)* median duration of disease from onset to death and *(2)* the death risk ratios for different clinical and genetic subgroups. Kaplan-Meier survival curves and log rank tests were used to estimate median survival (in months) and to compare survival.[25,26] A Cox's proportional hazards model was used to compute crude and adjusted death rate ratios.[27] We adjusted for possible confounders as sex, age, classification (probable versus definite) and study centre. Both methods take into account the fact that not every patient was followed until death due to termination of the follow-up of patients on May 31, 1996. Stratified analysis was performed for the pres-

ence of a PRNP mutation and for diagnosis (probable versus definite).

Appendix

Appendix

ERASMUS UNIVERSITEIT ROTTERDAM

FACULTEIT DER
GENEESKUNDE EN
GEZONDHEIDS-
WETENSCHAPPEN
dr. Molewaterplein 50

Instituut
Epidemiologie
en Biostatistiek

Uw Brief	Ons kenmerk	Datum
	AH/dw 93.2704	26 mei 1993
Onderwerp	Faxnummer	Doorkiesnummer
Jakob-Creutzfeldt	Fax 010-408 7494	7481

Zeer geachte collega,

In sommige Europese landen is vrees geuit voor een mogelijk verband tussen de ziekte van Jakob-Creutzfeldt bij de mens en de spongioforme encephalopathie bij dieren zoals scrapie en BSE. Het is daarom van belang de risico-factoren van deze zeldzame ziekte te bestuderen. Zoals u wellicht weet wordt in Groot-Brittanië al enkele jaren epidemiologisch onderzoek naar de ziekte van Jakob-Creutzfeldt verricht. Nu zal in Europees verband een patiënt-controle onderzoek worden uitgevoerd waarin ook Nederland zal participeren, om zo in twee jaar een voldoende aantal patiënten te verkrijgen. De uitvoering hiervan in Nederland zal geschieden door de afdelingen Neurologie en Epidemiologie van de Erasmus Universiteit en het Academisch Ziekenhuis Dijkzigt te Rotterdam, in samenwerking met het R.I.V.M. te Bilthoven.
Graag zouden wij uw medewerking willen vragen:

- om een register op te zetten van patiënten met de ziekte van Jakob-Creutzfeldt gezien vanaf 01-01-93 door neurologen in Nederland.
- om bij deze patiënten een patiënt-controle onderzoek uit te voeren (hiervoor verwijzen wij u naar bijgaand artikel).

Voor het opbouwen van het register verzoeken wij u het bijgaand antwoordkaartje te retourneren of contact op te nemen met de onderzoekcoördinator, mevr. D.P.W.M. Wientjens, arts (telefonisch te bereiken onder telefoonnummer 010-4087481 tussen 10 en 12 uur 's ochtends).
Wij vragen u iedere patiënt die u verdenkt van de ziekte van Jakob-Creutzfeldt aan te melden. Vanzelfsprekend zullen wij u van de resultaten van het onderzoek op de hoogte houden en zijn wij te allen tijde bereid eventuele vragen van uw kant te beantwoorden.
Bij voorbaat onze hartelijke dank voor uw medewerking.

Met vriendelijke groet,

Prof. Dr. A. Hofman
Afd. Epidemiologie

Prof. Dr. F.G.A. van der Meché
Afd. Neurologie

Patiënt-controle onderzoek naar de ziekte van Jakob-Creutzfeldt

In een patiënt-controle onderzoek zullen mogelijke oorzaken en risico-factoren van de ziekte van Jakob-Creutzfeldt worden bestudeerd. Het onderzoek zal twee jaar duren. Iedere patiënt die u verdenkt van de ziekte van Jakob-Creutzfeldt komt in aanmerking voor het onderzoek. Wij verzoeken u daarom alle patiënten die u verdenkt, aan te melden! De onderzoekcoördinator zal vervolgens uw kliniek bezoeken en een vragenlijst afnemen bij een familielid van de patiënt. Er zal ondermeer gevraagd worden naar vleesconsumptie, het houden van dieren en doorgemaakte operaties. Voor elke patiënt zal een controle-patiënt geselecteerd worden uit dezelfde kliniek. Daarnaast zal in overleg met u eventueel bloed voor verder onderzoek worden afgenomen. Daar de ziekte van Jakob-Creutzfeldt een uiterst zeldzame ziekte is en mogelijk niet alle verdachte patiënten met deze ziekte spontaan aangemeld zullen worden, willen wij voorstellen dat wij elke twee maanden een contactpersoon in uw kliniek telefonisch benaderen om te informeren of u een patiënt heeft aan te melden.
Gelieve het bijgaande strookje te retourneren, ook indien u geen patiënt met de ziekte van Jakob-Creutzfeldt heeft gezien.

Antwoordstrookje

Vanaf 1 januari 1993 heb ik patiënten gezien verdacht van de ziekte van Jakob-Creutzfeldt

Ja / neen

Voor onze kliniek treedt op als contactpersoon voor het patiënt-controle onderzoek

Naam: _____

Ziekenhuis: _____

Adres: _____

Plaats _____

Tel./doorkiesnr.: _____

Opmerkingen:

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CHAPTER 4

Incidence

Abstract

National registries for Creutzfeldt-Jakob disease (CJD) were established in 1993 (or earlier) in France, Germany, Italy, The Netherlands, Slovakia and the United Kingdom (UK). In 1994/1995 registries were also established in Spain and part of Belgium. Between 1993-1995 cases of CJD were identified in participating countries using identical methodologies and were classified according to agreed diagnostic criteria. 570 cases of definite or probable CJD died in the study period with an overall annual mortality rate of 0.73 cases/million. The incidence and mortality rates for CJD are almost similar because of the short median duration of illness of about four months. The incidence rates for CJD were similar in all participating countries despite variations in post-mortem rates, and age-specific incidence rates were also relatively consistent, with the exception of an increased incidence of CJD in patients aged <39 years in the UK. In relation to etiological subtypes of CJD, 90% of cases were sporadic, 8% genetic and 2% iatrogenic. Genetic forms of CJD accounted for 80% of all cases in Slovakia and iatrogenic forms of CJD occurred most frequently in France and the UK. The data in this paper establish baseline epidemiological parameters for CJD in participating European countries, which may be important in the assessment of any future change in the characteristics of CJD as a result of the epidemic of bovine spongiform encephalopathy (BSE).

Introduction

Epidemiological research into Creutzfeldt-Jakob disease (CJD) has shown that the disease is rare, with a worldwide distribution. A causal link with scrapie in sheep or goats is unproven[1]. The possibility that bovine spongiform encephalopathy (BSE) might represent a greater risk than scrapie to the human population has led to a range of legislative measures in the UK and elsewhere in Europe and the recommendation of the Southwood committee[2] for a systematic study of CJD in the UK. Although BSE has occurred only at low incidence in other European countries, including France, Germany and Italy, there has nonetheless been extensive concern about the potential implications for public health.

Historical data on the epidemiological and other characteristics of CJD are available in the UK[3], France[4], Italy[5] and Slovakia[6], but comparison with contemporary data in order to identify any change related to BSE might be compromised by improved case ascertainment and scientific advances, for example prion protein (PRNP) gene analysis. In order to obtain contemporary comparative data on CJD, a system for harmonising the research methodologies of national surveillance systems for CJD was established in 1993 and included France, Germany, Italy, the Netherlands, Slovakia and the United Kingdom (UK). The primary aim of this programme was to identify any change in the characteristics of CJD that might correlate with exposure of the human population to BSE and thereby indicate a causal link between animal and human prion diseases.

Methods

The European Union (EU) collaborative study of CJD was initiated in 1993. National registers for CJD had been established in the UK in 1990, in France in 1992 and in Slovakia in the 1970s. Similar registers were set up in Italy and the Netherlands in January 1993 and in Germany in May 1993. In 1994 and 1995 registers were established in Spain and in part of Belgium (Flanders).

The aim of the national registers was to identify all incident cases of CJD, to obtain detailed clinical information on all cases and to study putative risk factors for CJD through a case-control study. Cases were ascertained primarily by direct referral from targeted professional groups including neurologists, neurophysiologists and neuropathologists. In each country all members of these professional groups were circulated at the start of the study and at regular intervals through the study period and asked to notify any suspect case of CJD. Detailed information was obtained in the great majority of cases by a research worker who visited the referral centre and in cases notified after death by review of the case-notes. Cases were classified according to common diagnostic criteria[7] adapted from those originally proposed by Masters et al.[8] Whenever possible blood was obtained for DNA analysis and details of the PRNP genotype were available in 68% of all cases.

As this was a prospective study, the time at which case classification is possible varies according to the availability on information on clinical features and in particular on neuropathological confirmation of the diagnosis, which may not be available for months after death. For the analyses in this paper cases were included according to the diagnostic status on May 31, 1996. Cases classified as definite or probable have been included in analysis. Incidence rates were calculated based on patients diagnosed with CJD in the period 1993-1995. Mortality rates were estimated using all deaths from CJD in the period 1993-1995.

Results

Incidence / mortality rates of CJD

The incidence and mortality rates for CJD are almost similar because of the short median duration of illness of about four months. However in the first year of this prospective study, mortality rates may have been underestimated because clinically incident cases were identified and some deaths may have been missed. In the last year of the study some incident cases will not be included in analysis because of inevitable delays in obtaining neuropathological information. This effect has been minimised by

Table 1a — Total numbers of cases per year, by date of death.

	Belgium	France	Germany	Italy	Neth.	UK	Slovakia	Total
1993	0	39	16*	32	11	44	3	145
1994	5	53	56	35	18	56	5	228
1995	6	58	51	31	8	41	2	197
Overall	11	150	123	98	37	141	10	570

* Extrapolated from part year data

Table 1b — Total numbers of cases per year, by date of onset of disease.

	Belgium	France	Germany	Italy	Neth.	UK	Slovakia	Total
1993	1	43	45*	40	13	47	2	191
1994	7	55	54	35	15	56	4	226
1995	2	50	56	39	6	35	1	189
Overall	10	148	155	114	34	138	7	606

* Extrapolated from part year data

delaying final classification of cases incident in 1995 to May 31, 1996. Table 1 lists the number of cases of CJD classified by year. One hundred and ninety-one cases of CJD were identified with disease onset in 1993, compared with 145 deaths. There were 197 deaths from CJD in 1995 in comparison to 189 cases with disease onset in that year. Overall there were a lower number of deaths from CJD (570) than clinically incident cases (606). All further analyses of this chapter are based on mortality from CJD.

Mortality rates for CJD are listed in Table 2 (overleaf). The overall annual mortality rate for CJD in Europe is 0.73 cases/million and the rates in individual countries range from 0.57 (Italy) to 0.87 (France). There is a greater range of mortality rates in analyses by year and study site with a high figure of 1.18 cases/million in 1994 in the Netherlands and a low figure of 0.38 in 1995 in Slovakia.

Table 2 — Mortality rates (cases/million/year) in Europe from 1993-1995†

	Belgium	France	Germany	Italy	Neth.	UK	Slovakia	Total
1993		0.68	0.79*	0.56	0.72	0.74	0.57	0.68
1994	0.71	0.92	0.68	0.61	1.18	0.95	0.94	0.85
1995	0.86	1.00	0.57	0.54	0.52	0.67	0.38	0.65
Overall	0.78	0.87	0.67	0.57	0.81	0.78	0.63	0.73

† Denominators figures derived from Population Trends, winter 1996 issue;33:table 5a
* Extrapolated from part year data

This may reflect the small population size in the Netherlands and Slovakia, resulting in unstable risk estimates. Overall, however, there is relative consistency in mortality rates both with time and between countries. The proportion of cases of suspect CJD in which necropsy is performed might influence mortality rates for CJD. The post mortem rate varied among countries with 41% in France, 52% in Italy, 65% in the Netherlands, 66% in Germany, 73% in Belgium, 86% in the UK and 100% in Slovakia. Furthermore, there was marked variation between countries in the ratio definite to probable cases of CJD: in Italy and France there was an excess of probable cases (70% and 63%, respectively) in contrast to Germany, the Netherlands, Belgium, the UK and Slovakia, with an excess of definite cases (53%, 65%, 70%, 88% and 100%, respectively). There was an overall excess of female cases (ratio female:male = 1.4:1) with little variation between countries.

Table 3 — Total cases by aetiological subtype in Europe, 1993–1995.

	Sporadic	Genetic	Iatrogenic
1993	127	13	5
1994	205	19	4
1995	179	13	5
Overall	511 (90%)	45 (8%)	14 (2%)

Cases of CJD can be classified according to three clinical subtypes: sporadic, genetic/familial and iatrogenic. The distribution of cases according to aetiology is listed in Table 3. The proportions of aetiological subtypes are computed from the entire sample of patients with CJD (=570). Ninety per cent of cases were classified as sporadic,

8% as genetic and 2% as iatrogenic. The proportion of genetic cases was 12% if they are computed from the sample with PRNP analysis. The proportions of subtypes of CJD was heterogeneous between countries. There were no iatrogenic cases identified in the study period in Italy, the Netherlands, Belgium or Slovakia, while 6% of cases in the UK were iatrogenic. A relative excess of genetic cases were identified in France (12%), Italy (13%) and Slovakia (80%) and no such cases were identified in the Netherlands or Belgium. The percentage of cases with DNA analysis also varied between countries, with 90% cases analyzed in Slovakia, 80% in Germany, 78% in France, and 66% in Italy, compared to 58% or less in other countries.

PRNP codon 129 genotype was available in 341 cases: 74% were homozygous for the methionine allele, 16% were heterozygous and 10% were homozygous for the valine allele. The relative proportions of genotype at codon 129 of the PRNP gene were consistent between countries with the exceptions of the Netherlands (n=18) with 22% homozygous and 22% heterozygous for the valine allele, and Slovakia (n=7) with 43% homozygous and 57% heterozygous for the methionine allele.

Age at onset of disease was available in 560 cases. The age-specific mortality rates overall and by country are shown in table 4 and figure 1 (overleaf). The peak mortality of CJD occurred in the 70-79 year age group with a decline in the >80 year age group. The age distribution of cases was similar in all countries with the exception of a peak mortality in the 60-69 year age group in Italy, a high rate in the 40-49 and 50-59 year age groups in the Netherlands, and a relative increase in the mortality rate in the UK in cases aged less than 39 years, with a rate of 0.14 cases/million representing at least twice the mortality of CJD in this age group in relation to any other country. With regard to the small numbers of patients in Belgium and Slovakia, the age-specific rates for these countries are not computed nor included in table 4 and figure 1.

Table 4 — Age specific mortality rates/year/million in Europe, 1993–1995†

	<39	40–49	50–59	60–69	70–79	>80
France	0.03	0.27	2.03	3.30	4.48	1.39
Germany	0.02	0.10	0.90	1.69	2.44	0.55
Italy	0.03	0.18	1.17	2.21	1.20	0.36
Netherlands	0.07	0.63	1.54	2.56	4.21	2.33
UK	0.14	0.26	1.16	3.26	3.42	0.99
Overall	0.05	0.22	1.25	2.53	3.06	0.88

† Denominator figures derived from Demographic Year Book by the United Nations 1991;33: table 5b

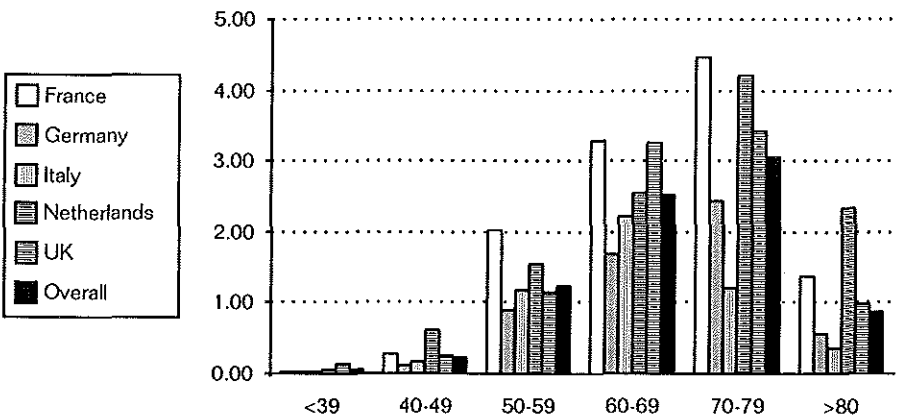

Figure 1 — Creutzfeldt-Jakob disease in Europe 1993–1995. Age-specific incidence rates (cases/million)

Discussion

This is the largest systematic survey of CJD, including data from national registers in Belgium, France, Germany, Italy, the Netherlands, Slovakia and the UK. Participating countries have wide variations in the provision of health care, notably in relation to the numbers of professionals who have been targeted to provide noti-

fication of cases of suspect CJD. An important question is whether the data from these countries is truly comparable. An important assumption in CJD surveillance is that incident cases of CJD will be referred for a neurological opinion and/or will undergo investigation including electroencephalogram and necropsy. Available evidence suggests that the great majority of cases of CJD can be identified by the study methodology utilised by all countries in this study[9] and that case-identification rests on the overall level of awareness of targeted professional groups rather than simply the overall numbers of such individuals.[10] Of particular concern is the wide variation in necropsy rates between countries. This would tend to result in a relative increase in apparent incidence in countries with higher necropsy rates because a higher proportion of cases in which a diagnosis could not be made on clinical grounds would be reported. This effect is minimised if, as in this study, active efforts are made to obtain detailed clinical information on every suspect case and it is of interest that cases of CJD have been identified throughout all regions of participating countries.

The most important finding in this study is the remarkable consistency in the mortality rates for CJD in participating countries. There is some variation from year to year and the high incidence of CJD in 1994 may reflect a higher level of case ascertainment than in other years due to the inclusion of cases incident in 1993 and 1994 and with necropsy information available in the great majority by May 31st, 1996. The continuing study of CJD (funded for 1997-1999) will allow the systematic accumulation of data on incidence and mortality which will remove year-on-year biases. The incidence of BSE is orders of magnitude higher in the UK than in any other participating country and there is no correlation in this study between the overall mortality rates for CJD and the incidence of BSE. However, significant but minor changes in the characteristics of CJD might not be reflected by analysis of mortality rates alone.

Other analyses in this study, including the frequency of clinical subtypes of CJD, the sex distribution of cases and the frequency of genotypes at codon 129 of the PRNP gene are consistent with

previous data on CJD.[3,11-14] Analysis by country has shown some differences between countries in these variables. The relative excess of genetic forms of CJD in France and Italy may reflect, at least in part, the higher proportion of cases with available genetic information in these countries.[12,13] The very high frequency of genetic cases in Slovakia has long been recognised and may be due to genetic isolation.[15] The gene frequency at codon 129 of the PRNP gene confirms a great excess of methionine homozygotes in comparison to the normal genotype distribution at this locus. The unusual codon 129 distribution in the Netherlands and Slovakia is unexplained but may reflect the small numbers of cases analyzed in these countries.

Overall, the age-specific mortality rates for CJD are as expected[3,4] with an increase in incidence up to middle age and highest mortality rates in the 60-69 and 70-79 year age-groups. The mortality rates in the very elderly are higher than expected in all countries and it is possible that this reflects improved ascertainment of cases in this population. Another important finding is the high relative incidence rate in the youngest age group (<39 years) in the UK, which is even more marked if the age distribution is analyzed by age at onset of disease. This excess is due to the occurrence of iatrogenic cases of CJD at a higher rate than in other countries and to the occurrence of new variant CJD.[16] Indeed the availability of contemporary comparative data on the incidence of CJD in the younger population was crucial to the hypothesis that these cases might be causally linked to BSE.

The collaborative study of CJD in Europe has allowed comparative information on the clinical, pathological and epidemiological characteristics of CJD to be defined. While the evidence for a causal link between BSE and CJD is weak in this study, the prolonged incubation periods in prion diseases indicate that BSE-related changes in mortality of CJD could still occur. Recent evidence[17] has strengthened the hypothesis that there may be a causal link between BSE and new variant CJD and the availability of this baseline information may be of crucial importance in the interpretation of data from continuing epidemiological research in Europe.

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CHAPTER 5

Risk factors

Abstract

We have studied risk factors for Creutzfeldt-Jakob disease (CJD) as part of the 1993-1995 EU collaborative studies of CJD in Europe. Patients with definite or probable CJD (N=405) were derived from population-based studies conducted in the period 1993 to 1995 in Belgium, France, Germany, Italy, the Netherlands and the United Kingdom. Patients were compared to 405 hospital controls. We found significant evidence for familial aggregation of CJD as well familial aggregation of CJD with dementia due to other causes than CJD. A significant increased risk of CJD was found for subjects exposed to domestic birds, leather products and fertilizer consisting of hoofs and horns. Of the dietary factors studied, the consumption of brain and raw meat were associated with an increased risk of CJD. In the period studied, no association could be shown between the risk of CJD and to the consumption of beef, veal, lamb, cheese or milk.

Introduction

Creutzfeldt-Jakob disease (CJD) is the most important prion disorder in humans.[1,2] CJD has been transmitted from man to man through human growth and gonadotropin hormone,[3-6] neurosurgery and EEG electrode implantation,[7-10] and corneal transplantation.[11] However, iatrogenic transmission has been found to be rare in case series[2] as well as in a re-analysis of case-control studies of risk factors for CJD conducted in Japan,[12] the United States[13-14] and the United Kingdom.[15,16] Prion disease may be endemic in livestock including sheep (scrapie) and cows (bovine spongiform encephalopathy [BSE]). The aforementioned re-analysis of case-control studies conducted in the period 1975 to 1984 failed to show statistically significant evidence for transmission from cattle to human.[16] However, the discovery of a new variant of early-onset CJD in the United Kingdom following the BSE epidemic has reopened the discussion whether prion disorders may be transmitted from cattle to human through animal contact or the consumption of meat (products). In a number of cases CJD is inherited.[1] Various dominant mutations in the prion protein gene (PRNP) on chromosome 20 have been identified that may cause CJD.[1,17] Furthermore, subjects homozygote for the codon 129 polymorphism of PRNP have been shown to be at increased risk of CJD.[18,19] In addition to familial aggregation of CJD, there is also evidence for familial clustering of CJD with dementias other than CJD and with Parkinson's disease.[16]

In the period 1993-1995, we have conducted a collaborative study of risk factors for CJD in Europe. Here, we report the findings of the risk factors studied, including genetic factors, medical and occupational history, animal exposure and diet.

Patients and methods

The EU collaborative studies of CJD were initiated in 1993. National registries for CJD had been established in the UK, France, and Slovakia before 1993. Similar registries were set up in other European countries in 1993 and 1994. The aim was to monitor the incidence of CJD and to study risk factors. Population-based

studies were started in Belgium, France, Germany, Italy, the Netherlands and the United Kingdom.[20] A standardised diagnostic work-up as well as a standardised core questionnaire on risk factor exposure was used. The studies aimed to ascertain all patients diagnosed with definitive or probable CJD according to the criteria adapted from those described by Masters et al,[21] living in a defined geographical area.[20] Here, we present the overall findings of the case-control study conducted in the period 1993-1995. The study population differs from that of the incidence studies in that we included patients diagnosed with CJD in the period 1992-1995.[20] A total of 405 (199 definite and 206 probable CJD) were included in the case-control studies presented here. The patients series comprised 154 men and 251 women. In 9 patients, the onset of CJD was below the age of 40 years, in 190 between 40 and 64 years and in 206 at age 65 years or later. Below, for each site data-collection for the case-control studies of risk factors will be discussed briefly. Studies are in alphabetical order based on the name of the country.

Belgium/Netherlands

The data coordinating centre for the study was the Department of Epidemiology & Biostatistics of the Erasmus University Rotterdam, the Netherlands. The study population comprised two countries, the Netherlands (study period January 1, 1992 to December 31, 1995) and the Dutch speaking part of Belgium (Flanders; study period January 1, 1994 to December 31, 1995). A total of 49 patients with probable or definite CJD were ascertained,[20] of whom 43 (88%) were included in the case-control study. The 43 patients were matched according to sex and age (+/- 5 years) to controls. Control subjects were derived from the neurological ward at which the matched patient was diagnosed. Excluded subjects were controls with a history of dementia or other neurodegenerative disorder. Data were collected by the standardised questionnaire of the collaborative EU studies. For cases and controls the data were obtained from a next of kin.

France

The data collection was coordinated by Unit 360 of the Institut National de la Santé et de la Recherche (INSERM), Paris. In the period January 1, 1992 to December 31, 1995, 194 patients with probable or definitive CJD were ascertained.[20] Included in the case-control study are 75 (39%) patients. The patients were matched according to sex and age (+/- 5 years) to controls. For the data collection, a structured interview based on the standardised protocol of the collaborative EU CJD studies was used. For cases the data were obtained from a next of kin. A relative of a non-CJD patient at the hospital served as a control subject, while the non-CJD patient served as the informant for this control subject. Thus, for controls data on risk factor exposure were also obtained from an informant.

Germany

The data coordinating centre for the study was at the Department of Neurology of the Georg August University of Göttingen, Germany. In the period January 1, 1992 to December 31, 1995, 162 patients with probable or definitive CJD were ascertained.[20] Of these patients, 136 (84%) were included in the case-control study. The 136 patients were matched according to sex and age (+/- 5 years) to controls. Control subjects were derived from the same hospital as the matched case. Excluded were patients with a history of dementia or other neurodegenerative disorder. Data were collected by a research physician using a structured interview based on the standardised protocol of the collaborative EU CJD studies. For cases and controls the data were obtained from a next of kin.

Italy

The data coordinating centre for the study was at the Department of Neurology of the Universita Cattolica del Sacro Cuore, Rome. In the period January 1, 1992 to December 31, 1995, 123 patients with probable or definite CJD were ascertained from the study region,[20] of whom 63 (51%) were included in the case-control study. The 63 patients were matched according to sex and age (+/- 5 years). Control subjects were derived from the same

hospital where the patient was diagnosed. Data were collected by an interview based on the standardised protocol of the collaborative EU CJD studies. For cases and controls the data were obtained from a next of kin.

United Kingdom

The data collection was coordinated by the CJD Surveillance Centre based at Western General Hospital Edinburgh, Scotland. In the period January 1, 1992 to December 31, 1995, 153 patients with probable or definite CJD were ascertained,[20] of whom 88 (58%) were included in the case-control study. The patients were matched to controls according to sex and age (+/- 5 years). Control subjects were derived from the neurological ward where the CJD patient was diagnosed. Data were collected by a structured interview by a physician. These data were mapped to the standardised questionnaire of the collaborative EU CJD studies. For cases the data were obtained from a next of kin. For controls, this procedure was followed whenever possible.

Statistical Analysis

This analysis is based on the original raw data of the five participating centres: Netherlands/Belgium, France, Germany, Italy, and the UK. The data were centralised at the Department of Epidemiology & Biostatistics of the Erasmus University Rotterdam. Principal investigators of the studies were invited to Rotterdam to coordinate the strategy of analysis in the four major topics, *(1)* genetic factors (Drs Alpérovitch and Lauprêtre, France), *(2)* medical history (Dr Zerr, Germany), *(3)* occupational history and animal exposure (Dr Will, UK), and *(4)* diet (Dr Masullo, Italy).

The strength of association between CJD and putative risk factors was assessed by computing of the odds ratio (OR) as an estimate of the relative risk.[23] Relative risks were estimated by maximum likelihood and the 95 confidence intervals (95% CI) were based on the asymptotic standard errors. We followed two different strategies in the analyses. First, all analyses were conducted using an unconditional logistic regression model, taking the matching

variables age, sex and study centre into account by including these variables in the regression model. In this way, maximum use was made of the data available. Secondly, we performed an analysis using a conditional logistic regression model in order to take the matching variables into account. Although this analysis is in accordance with the matched design of the study, a major problem was the loss of information in that a matched set of cases and controls was dropped out of the analysis if data were missing on a variable in either the case or the control. As a consequence, the statistical power of the conditional analysis was low and risk estimates may be biased due to selection. As both strategies gave similar findings judged upon the relative risk estimates, findings of the method with the highest statistical power, the unconditional analyses, are presented here.

In both strategies, significant differences between study centres were tested for by including an interaction term with the risk factor studied into the model. Furthermore, stratified analyses were performed based on sex, age at onset, diagnosis (probable versus definite), family history of CJD, PRNP mutations and polymorphism at codon 129 of the PRNP gene. However, details on these stratified analyses and on specific exposures are not discussed here but will be published separately.

Results

Family history of CJD, dementia and Parkinson's disease was studied in siblings, parents and grandparents (table 1, overleaf). For seven patients included in the case-control study, a positive family history of CJD was reported. A positive family history of dementia other than CJD was found significantly more often in CJD patients than in controls. The risk of CJD for those with a first degree relative with dementia due to antoher cause than CJD was found to be 2.26 (95% CI: 1.31-3.90). Although a family history of Parkinson's disease was reported more often for CJD cases, the difference between cases and controls was not significant (p=0.07).

Table 1 — Family history of CJD, dementia and Parkinson's disease and the risk of CJD

Family history	Exposure frequency		Odds Ratio	
	cases	controls	crude	adjusted[1]
CJD				
Overall	7/358	0/311	12.0* [0.70-219.48]	—
First degree relatives	7/358	0/311	—	—
Dementia other than CJD				
Overall	54/358	22/311	2.33+ [1.39-3.93]	2.35+ [1.38-3.99]
First degree relatives	50/358	21/311	2.24+,* [1.31-3.83]	2.26+,* [1.31-3.90]
Parkinson's disease				
Overall	13/289	5/285	2.64 [0.93-7.50]	2.79 0[.97-8.05]
First degree relatives	13/289	5/285	2.64 [0.93-7.50]	2.79 [0.97-8.05]

[1] Adjusted for age, gender,country, 95% CI in parentheses
+ $p < 0.05$
* $p < 0.01$ by Fisher exact test

Medical histories of patients and controls are compared in table 2. Significantly fewer cases than controls had a history of surgery and blood transfusion. Specifically a history of surgery of the vertebral column was found less often among CJD patients, while there was a non-significant increase in the risk of CJD associated with surgery of the brain and organ transplantation. There was no significant evidence for an association of CJD to any of the disorders studied in the medical history including psychiatric history, infectious disorders and head trauma (see table 2). A history of EMG and lumbar punction was found significantly less in CJD cases than in controls.

With regard to occupational history, the exposure to human tissue through contact with patients and patient materials as well as the exposure to animal products and animals was evaluated (table 3, p. 59). None of these factors were significantly associated to the risk of CJD.

Table 2 — History of surgery and the risk of CJD

History	Exposure frequency (at CJD diagnosis)		Odds ratio	
	cases	controls	crude	adjusted[1]
Surgery				
Overall	315/400	342/405	0.68* (0.48-0.98)	0.69* (0.49-0.99)
Brain	12/400	7/405	1.76 (0.69-4.51)	1.77 (0.68-4.61)
Vertebral column	17/400	31/405	0.54* (0.29-0.98)	0.53* (023-0.98)
Other surgery central nervous system	2/400	2/405	1.01 (0.14-7.22)	1.03 (0.14-7.40)
Eye	33/401	34/406	0.98 (0.59-1.62)	0.96 (0.57-1.59)
Blood transfusion	38/341	71/378	0.54* (0.35-0.83)	0.56* (0.37-0.97)
Organ transplantation	6/284	2/282	3.02 (0.60-15.1)	3.01 (0.61-15.22)
Disease history				
Epilepsy	14/398	17/400	0.82 (0.40-1.69)	0.87 (0.42-1.69)
Treatment psychiatrist/ psychologist	69/399	55/401	1.32 (0.89-1.93)	1.38 (0.92-2.07)
Jaundiced	44/364	43/374	1.06 (0.68-1.66)	1.06 (0.67-1.67)
Glandular fever	9/306	13/317	0.71 (0.30-1.68)	0.53 (0.21-1.35)
Polio	3/367	4/367	0.75 (0.17-3.37)	0.74 (0.16-3.35)
Herpes zoster	54/330	63/340	0.86 (0.58-1.28)	0.86 (0.55-1.36)
Herpes simplex	64/167	65/180	1.10 (0.71-1.70)	1.14 (0.71-1.84)
Rheumatoid arthritis	20/171	21/180	1.00 (0.52-1.92)	0.99 (0.50-1.95)
Diabetes mellitus	21/182	33/192	0.63 (0.35-1.13)	0.63 (0.34-1.14)
Allergies/Atopy	41/158	33/166	1.41 (0.84-2.38)	1.44 (0.84-2.46)
Head injury	66/394	54/400	1.29 (0.87-1.90)	1.33 (0.89-2.00)

Table 2 — continued

History	Exposure frequency (at CJD diagnosis)		Odds ratio	
	cases	controls	crude	adjusted[1]
Medical treatments/tests				
EMG	9/162	26/176	0.34* [0.15-0.75]	0.32* [0.14-0.72]
Lumbar punction	38/188	40/144	0.66 [0.40-1.10]	0.49* [0.28-0.87]
Acupuncture	59/393	76/400	0.75 [0.52-1.09]	0.74 [0.50-1.09]
Vaccination	109/379	114/385	0.96 [0.70-1.31]	0.95 [0.68-1.32]
Hormone supplements	41/313	30/320	1.46 [0.88-2.40]	1.50 [0.89-2.54]
Ophthalmologist/optician test	188/351	210/379	0.93 [0.69-1.24]	0.96 [0.68-1.35]
Dentist visit last year before onset CJD	173/339	164/343	1.14 [0.84-1.54]	1.15 [0.84-1.57]

[1] Adjusted for age, gender,country, 95% CI in parentheses
* $p < 0.05$

When studying domestic exposure to animals (table 4, p. 61), a significant increase in risk of CJD was found for subjects exposed to domestic birds (OR 1.55; 95% CI: 1.09-2.19). Of the general exposures to animals and animal products (table 4), contact with leather products other than through clothes was associated with a 1.87 (95% CI: 1.11-3.15) fold increase in the risk of CJD. Exposure to dried blood, animal bites or having lived on a farm was not associated with CJD. Exposure to the fertilizer consisting of hoofs and horns was reported significantly more often for patients than controls (OR 2.26; 95% CI: 1.35-3.77).

The relation between diet and the risk of CJD is summarised in tables 5 (p. 62) and 6 (p. 63). Table 5 shows the association between consumption of several animal products and the risk of CJD. The consumption of raw meat products was significantly associated with an increased risk of CJD. There was no significant

Table 3 — Occupational history and animal exposure and the risk of CJD

Exposure	Exposure frequency		Odds Ratio	
	cases	controls	crude	adjusted[1]
Occupational exposure patients or patient material				
Overall exposure to patients / body tissue	32/397	35/404	0.92 [0.56-1.53]	0.92 [0.55-1.53]
Physician	3/397	1/404	3.05 [0.32-29.47]	3.03 [0.31-29.50]
Neuropathologist	0/397	0/403	—	—
Nurse	16/394	21/402	0.77 [0.39-1.49]	0.75 [0.38-1.48]
Laboratory technician	3/397	2/404	1.54 [0.26-9.25]	1.53 [0.25-9.42]
Dentist	0/397	1/402	0.34 [0.01-8.29]	—
Ambulance worker	0/397	0/404	—	—
Occupational exposure animal products				
Overall exposure to Animals/animal products	73/395	75/402	0.98 [0.69-1.41]	0.98 [0.68-1.42]
Butcher	7/392	5/401	1.44 [0.45-4.58]	1.44 [0.45-4.62]
Slaughter	2/347	2/354	1.02 [0.14-7.28]	1.05 [0.14-7.87]
Veterinary doctor	0/393	0/401	—	—
Meat/Food processor	9/347	9/354	1.02 [0.40-2.60]	1.01 [0.39-2.61]
Leather worker	2/347	2/354	1.02 [0.14-7.28]	1.01 [0.14-7.33]
Occupational exposure animals				
Husbandry	57/393	51/349	1.16 [0.77-1.74]	1.17 [0.77-1.77]
Cows	43/344	38/354	1.19 [0.75-1.89]	1.20 [0.75-1.39]
Sheep	23/343	23/354	1.03 [0.57-1.88]	1.05 [0.57-1.93]
Pigs	26/330	28/346	0.97 [0.56-1.69]	0.97 [0.55-1.70]

Table 3 — continued

Exposure	Exposure frequency		Odds Ratio	
	cases	controls	crude	adjusted[1]
Horses	17/326	14/340	1.28 [0.62-2.64]	1.31 [0.63-2.75]
Other cattle	2/331	2/343	1.04 [0.15-7.40]	1.04 [0.14-7.48]
Mink/Ferrets	1/395	2/402	0.51 [0.05-6.62]	0.51 [0.05-5.65]
Other fur animals	2/336	3/349	0.69 [0.11-4.16]	0.68 [0.11-4.19]
Deer/elk	0/343	0/353	—	—
Other animals	20/395	14/398	1.46 [0.73-2.94]	1.45 [0.71-2.94]

[1] Adjusted for age, gender,country, 95% CI in parentheses

evidence for a dose response relation between the consumption of raw meat and the risk of CJD. For subjects for whom a consumption pattern was described as one time a year, the relative risk was 2.32 (95%CI:0.86-6.26), while the relative risk for consumption several times a year was 1.11 (95%CI:0.60-2.03), for once a month 2.08 (95% CI: 1.08-4.00), for once a week 2.08 (95% CI: 0.69-6.30) and for each day 0.58 (95% CI:0.05-6.42). The consumption of raw beef (45/391 in cases and 31/394 in controls) was more common than that of raw pork (18/405 in cases and 13/405 in controls). Raw beef consumption was associated to a 1.5 fold (95% CI: 0.94-2.46) increase in risk. The risk associated with the consumption of raw pork was very similar (OR 1.40; 95% CI: 0.68-2.80). Brain consumption was associated with an increased risk of CJD (OR 1.68;95% CI: 1.18-2.39). The relative risk of CJD associated with the consumption of bovine brain was 1.56 (95% CI: 0.68-3.51). None of the meat products studied, such as sausage and black pudding was associated to the risk of CJD. Finally, when analyzing the data on meat consumption, there was no evidence for a significant association of CJD to the consumption of beef, veal, lamb or pork (table 6), except for the consumption of pork once a week.

Table 4 — Domestic exposure and general exposure to animals and animal products exposure and the risk of CJD

Exposure	Exposure frequency		Odds ratio	
	cases	controls	crude	adjusted[1]
Domestic exposure to animals				
Cat	158/353	155/352	1.02 [0.77-1.39]	1.03 [0.77-1.40]
Dog	196/354	196/354	1.02 [0.76-1.38]	1.02 [0.76-1.39]
Rodent/Hamster	43/353	36/353	1.22 [0.76-1.95]	1.22 [0.76-1.96]
Bird	100/342	73/346	1.55[+] [1.09-2.19]	1.56[+] [1.09-2.23]
Fish	44/355	32/352	1.41 [0.87-2.29]	1.44 [0.87-2.39]
Snakes	14/355	8/354	1.78 [0.74-4.29]	1.91 [0.75-4.84]
Other pets	27/339	20/344	1.38 [0.76-2.52]	1.40 [0.75-2.60]
General exposure animals and animal products				
Contact with fur/leather other than through clothes	42/392	24/398	1.87[+] [1.11-3.15]	1.94[+] [1.13-3.33]
Lived on farm	153/395	142/399	1.14 [0.86-1.53]	1.15 [0.85-1.54]
Fertilizer	116/381	108/390	1.14 [0.84-1.56]	1.15 [0.83-1.60]
- Artificial fertilizer	24/302	32/318	0.77 [0.44-1.34]	0.76 [0.43-1.37]
- Hoofs and horns	48/366	24/383	2.26[+] [1.35-3.77]	2.32[+] [1.38-3.90]
Bone meal	57/358	46/374	1.35 [0.89-2.05]	1.41 [0.90-2.21]
Dried blood of animals	28/403	32/405	0.87 [0.51-1.47]	0.96 [0.56-1.64]
Ever bitten by animal	29/128	35/151	0.97 [0.55-1.70]	0.97 [0.55-1.70]

[1] Adjusted for age, gender,country, 95% CI in parentheses
[+] $p < 0.05$

Table 5 — Consumption of organ, meat and milk products and the risk of CJD.

Consumption animal products	Exposure frequency		Odds ratio	
	cases	controls	Crude	Adjusted[1]
Meat and milk products				
Sausage	382/400	381/403	1.23 [0.65-2.32]	1.22 [0.64-2.34]
Raw Meat	143/391	107/394	1.55[+] [1.14-2.09]	1.63[+] [1.18-2.23]
Raw fish	24/131	20/143	1.38 [0.72-2.64]	1.53 [0.72-3.23]
Products animal blood	206/269	190/262	1.24 [0.84-1.84]	1.24 [0.80-1.92]
Milk	292/307	290/313	1.54 [0.79-3.02]	1.57 [0.79-3.12]
Cheese	321/332	329/339	0.89 [0.37-2.12]	0.89 [0.37-2.14]
Organ meat				
Tripe	152/387	155/393	0.99 [0.75-1.32]	1.00 [0.70-1.42]
Kidney	176/383	175/398	1.08 [0.82-1.44]	1.10 [0.80-1.51]
Liver	317/391	310/395	1.18 [0.83-1.66]	1.18 [0.83-1.69]
Brain	49/115	37/118	1.63 [0.95-2.78]	1.68[+] [1.18-2.39]
Eye	4/316	2/332	2.12 [0.38-11.63]	2.12 [0.38-11.90]

[1] Adjusted for age, gender,country, 95% CI in parentheses
[+] $p < 0.05$

Discussion

In this collaborative study of risk factors of CJD, we found evidence for familial aggregation of CJD as well as familial aggregation of CJD with dementia due to other causes. With regard to the exposures to animals and animal products studied, a significant increase in risk of CJD was found for subjects exposed to domestic birds, leather products and fertilizer consisting of hoofs and horns. Of the dietary factors studied, the consumption of brain and raw meat were associated with an increased risk of CJD. No

Table 6 — Consumption of meat and the risk of CJD.

Frequency	Beef			Veal			Lamb			Pork		
	cases	controls	odds ratio	cases	controls	odds ratio	cases	controls	odds ratio	cases	controls	odds ratio
Never	7	12	reference	102	121	reference	91	91	reference	7	9	reference
<1 time/year	10	10		56	62	1.07 [0.68-1.67]	31	41	0.76 [0.44-1.31]	7	17	
Several times/yr	38	30	1.64 [0.74-3.62]	85	78	1.28 [0.85-1.91]	45	38	1.18 [0.71-1.88]	32	35	1.70 [0.76-3.81]
1 time/month	154	175	1.14 [0.58-2.22]	97	97	1.19 [0.81-1.74]	20	26	0.77 [0.40-1.48]	128	145	1.64 [0.82-3.27]
1 time/week	182	169	1.39 [0.72-2.71]	44	35	1.49 [0.89-2.50]	8	3	2.67 [0.68-10.37]	213	186	2.13* [1.08-4.14]
1 time/day	5	5	1.29 [0.32-5.21]	1	1	1.19 [0.07-18.20]	—	—	—	6	5	2.23 [0.58-8.62]

* $p<0.05$

association was found to the consumption of beef, veal, lamb, cheese or milk in these analyses. Surgery of the vertebral column, blood transfusion, EMG and lumbar punction were inversely associated to the risk of CJD.

When interpreting the findings, a number of methodological issues are to be kept in mind. Although the study aimed to be population based, the inclusion rate of cases in the case-control study of (known) patients at the participating sites varied from 39% to 88%. Response rates in controls are not known. Thus, findings may have been distorted if the response rate in cases and/or controls was associated to the factor(s) studied. Further, the use of hospital controls may have introduced selection bias in that exposure frequencies to surgery, medical disorders and/or treatments may not be representative for the general population. When excluding controls ascertained from neurological wards, there was no significant difference between CJD patients and controls found for surgery of the vertebral column (OR 0.82; 95% CI: 0.24-2.84), EMG (OR 5.36; 95% CI 0.38-74.70) or lumbar punction (OR 4.50; 95% CI 0.83-24.50) suggesting the inverse association may be explained in part by the control selection. When considering blood transfusion ten years before the interview, a period aetiologically relevant given the long incubation period of CJD, no significant difference was found between cases and controls in the history of blood transfusion (OR 0.76; 95% CI 0.40-1.26). This suggests the inverse association between a history of blood transfusion and CJD may be explained in part by the increased frequency for this putative risk factor in hospital controls, who may have been admitted specifically for surgery.

With regard to information bias, it is important to note that all data on risk factor exposures were collected retrospectively from a surrogate informant for cases. This may have reduced the validity of the information. In some instances, the control subject was interviewed in person, which may have introduced a systematic difference between the cases and controls. For all putative risk factors, the data were therefore analyzed including only patients and matched controls for whom the data were obtained from a next of kin. Another source of information bias may have been recall bias,

i.e., because of the devastating effects of the disease, informants of patients may have been more willing to consider putative exposures in the past than informants of controls. Important determinants of this type of bias are the time between the onset of CJD and the interview and the vital status of the patient at the time of the interview. Data were therefore stratified according to the vital status of the patient and the time between onset of CJD and the interview. None of these analyses change the conclusions of the analysis presented.

As expected given the dominant mutations that have been implicated in CJD,[1,17] our study shows familial aggregation of CJD. Our study confirms earlier findings of familial aggregation of CJD with other dementias.[16] A family history of Parkinson's disease was reported more often for CJD cases. However, no significant difference could be shown between cases and controls. Although PRNP mutations have been found in in a number of patients with familial forms of dementia[19,22], it is unlikely that the occurrence of dementia in the relatives of CJD patients can be fully explained by PRNP mutations. Other genetic or non-genetic factors may underlie the relationship. The most important candidate gene is the apolipoprotein E gene which has been associated to Alzheimer's disease and various types of dementia as well as CJD.[24,25]

In agreement with earlier findings, iatrogenic transmission of disease appears to be rare and no significant increased risk of CJD related to the medical history, including organ transplantation and blood transfusion, could be shown in this large population-based sample of CJD patients. However, there is reason to interpret these findings with caution because of the limited validity of our study due to the use of hospital controls. Both the magnitude as well as the direction of associations of CJD to surgery and blood transfusion could be explained for a large part by the use of hospital controls. These findings indicate that further studies on iatrogenic transmission using population-based controls and well defined incubations periods are necessary. The present study failed to confirm the association between history of psychotic disease and the risk of CJD observed in a pooled analysis of case-control studies conducted in the period 1975 to 1984.[16]

With regard to the exposure to animals and animal products, the consumption of brain as well as raw meat were associated with an increased risk of CJD. These findings may be explained by the fact that the concentration of prions in affected animals is expected to be highest in brain tissue and spinal cord and although prions are resistant to temperature, concentrations are expected to be higher in raw meat compared to cooked meat. The absence of a dose-response relationship between the consumption of raw meat and the risk of CJD makes a cause-effect relationship less likely. Furthermore, a significant increase in risk of CJD was found for subjects with domestic birds and those exposed to fertilizer consisting of hoofs and horns. For each of these findings, a plausible biological explanation is lacking and these findings remain to be confirmed in future experimental and observational studies. Findings on exposure to leather were inconsistent as no association was found to occupational exposure (table 3) while a significant association was found to general exposure (table 4).

No evidence was found for an increase in risk of CJD associated with the (occupational) exposure to cattle. One of the findings of our study with the most important public health implications is that in the period studied, there was no association of CJD to the consumption of beef, veal or lamb. Although this finding is compatible with the view of no transmission of prion disease from cattle to humans through the consumption of meat, our study is limited to patients diagnosed at or before 1995. As the first patients with new variant CJD[26], an atypical form of CJD that has been related to the BSE epidemic in the UK[27], were described in 1995, we have to await future studies to determine whether consumption of beef/veal from animals infected with prion disease may increase the risk of new variant CJD. In particular countries in which BSE occurred may yield crucial information when compared to those countries without BSE.

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CHAPTER 6

Prognosis

Abstract

The prognosis of patients with Creutzfeldt-Jakob disease (CJD) was studied in a series of cases identified from a European collaborative study on CJD. A total of 606 cases were ascertained through direct notification, mainly by neurologists and were classified as definite and probable according to common diagnostic criteria. The information on clinical features was obtained from examination and medical records. Prion protein gene (PRNP) coding sequence analysis was performed in 434 subjects with CJD. Duration of illness was defined from first symptoms to death or censoring date. Statistical comparisons were performed using Kaplan-Meier survival curves, log rank tests and Cox's proportional hazard analysis.

In our study of 606 CJD patients the median survival was four months and gender and early age at onset were important prognostic factors. Most clinical features were not related to survival. In contrast to earlier findings, mortality was significantly increased in patients with a PRNP mutation and/or a positive family history for CJD (death rate ratio (DDR) 1.23; 95% confidence interval (95% CI) 1.01-1.49). Among patients without a mutation in the prion protein gene (PRNP), mortality among carriers of valine at codon 129 was significantly lower (DRR 0.66; 95% CI 0.54-0.79 for heterozygous, and DRR 0.67; 95% CI 0.55-0.81 for homozygous). This study does not provide evidence for a clinical subgroup of CJD that relates to survival except for the new variant of CJD. The finding that sporadic CJD carriers of at least one valine allele at codon 129 of the PRNP gene had an increased survival, highlights the influence of genetic factors in the pathophysiology and prognosis of prion diseases.

Introduction

The term 'Creutzfeldt-Jakob disease' (CJD) was first used by Spielmeyer in 1922[1], bringing together the original case report by Creutzfeldt in 1920[2] with subsequent patients described by Jakob.[3] CJD belongs to the group of transmissible spongiform encephalopathies, which may occur in a sporadic, inherited or iatrogenic form. CJD is a neurodegenerative disorder, clinically recognized by the occurrence of a rapidly progressive dementia with myoclonus, which may be accompanied by cerebellar ataxia and other neurological signs. The electroencephalogram (EEG) may show characteristic pseudoperiodic sharp wave activity. The clinical course is usually rapid and many patients die within a few months of onset.[4] Although the vast majority of patients have a duration of illness of less than 12 months, around 10% of patients with CJD present atypically with a longer duration of illness.[5,6] A prolonged duration has been reported in CJD associated with PRNP mutations[7-10] and in the recently described new variant of CJD.[11,12]

Little is known about other factors predicting survival in CJD.[3,6,7,13,14] Clinical characteristics associated with survival may yield important clues for the definition of clinical subgroups in CJD including the new variant of CJD. The polymorphism at codon 129 of the PRNP gene plays a key role in the pathogenesis of, and susceptibility to, sporadic, inherited and iatrogenic CJD.[7,9,15-19] In this report we study 606 patients from six different European countries to evaluate the importance of various clinical and genetic features as predictors of mortality.

Materials and Methods

Patients selection

This study is based on a collaborative European study on CJD.[20,21] In 1993 a project for coordination of national CJD surveillance programs was started linking already established registries in France and the UK to new registries in Germany, the Netherlands, Italy and Belgium. Incident sporadic, genetic and iatrogenic CJD patients were ascertained with year at onset of disease from 1993

to 1995.[22,23] The method of case ascertainment was based on a common protocol in participating countries. All centres aimed to ascertain all patients with CJD in a defined population by direct notification of any clinically suspect case, mainly by neurologists and by the use of death certificates and neuropathology as a safety net. Pathologically confirmed cases were identified by direct notification from neuropathologists. CJD cases were classified as definite or probable according to diagnostic criteria adapted from Masters et al.[24] The diagnosis of definite cases was neuropathologically confirmed, exhibiting spongy degeneration of the cerebral grey matter, neuronal loss and proliferation and hypertrophy of astrocytes and/or by immunocytohistochemical staining for prion protein.[25] Probable cases had a rapidly progressive dementia, a typical electroencephalogram (EEG) with periodic sharp wave activity and at least two out of four specific clinical features (myoclonus, visual or cerebellar symptoms, extrapyramidal or pyramidal symptoms, akinetic mutism). Iatrogenic patients with a known source of accidental contamination as human pituitary growth hormone, corneal graft or dura mater graft recipients were also included in the analysis. Prion protein gene analysis on blood or frozen brain tissue was performed in national genetic laboratories to screen for mutations and polymorphism at codon 129 of the PRNP gene. We defined genetic CJD as cases with a neuropsychiatric disorder and a disease specific PRNP mutation and/or individuals with a positive family history of CJD.

Clinical assessment

CJD patients with an onset of disease during the period January 1, 1993 to December 31, 1995 were eligible for the study. Referred patients were visited and examined by a research physician and blood was taken for prion protein gene analysis. Duration of illness was defined as the period from first symptoms to death or the censoring date at May 31, 1996. The clinical and EEG records of the patients were reviewed in each case in order to obtain information on the clinical signs reported by the neurologist and for symptoms related to the present illness reported by the patient's relatives. During the course of the illness the clinical records, EEG records, vital status and pathology records of the patients were reviewed. We studied the concomitant occurrence

of clinical features earlier described in clinical subgroups of CJD.[4,6,11,12]

Statistical analysis

Kaplan-Meier survival curves and log rank tests were used to estimate and to compare survival.[26,27] Cox's proportional hazards model was used to compute crude and adjusted death rate ratios (DRR).[28] We adjusted for possible confounders, including gender, age, classification as probable and country of residence. Both methods take into account the fact that not every patient was followed until death due to termination of the follow-up of patients on May 31, 1996.

Table 1 — Descriptive summary of CJD patients from the collaborative European study.

	Number	%
Patients	606	
Country		
Belgium	10	1.7
France	148	24.4
Germany	155	25.6
Italy	114	18.8
Netherlands	34	5.6
United Kingdom	138	22.8
Slovakia	7	1.2
Definite	338	55.8
Typical EEG	471	77.7
Men	241	39.8
Age at onset		
mean (sd)	64.1 (11.6)	
median	65.1	
range	16.3–92.2	
Duration (months)		
mean (sd)	6.1 (5.7)	
median	4.0	
range	0–35	
Aetiology		
genetic patients	40	9.2*
iatrogenic	12	2.0

* percentage of total number tested (n=434)

Results

Descriptive summary

Numerical data for the total of 606 patients are presented in table 1. Of the 606 patients 365 were female (60.2%). Duration from first symptom to death or to censoring date ranged from less than one to 35 months; 50% of the patients died within four months. Age at onset of illness ranged from 16 to 92 years (mean 64.1 years). Genetic patients (n=40) accounted for 9.2% of the cases in which PRNP genotype was available. There were 40 genetic cases with a mutation of the PRNP gene of whom 33% had a positive family history of CJD. At the censoring date 63 of the 570 patients were still alive.

Table 2 — Survival in patients with Creutzfeldt-Jakob disease

	Number	Median (in months)	Death rate ratio[1]	
			crude	adjusted[1]
Overall	606	4		
Gender				
men	241	4	1.21* (1.01-1.44)	1.25* (1.04-1.49)
women	365	5	reference	reference
Age at onset				
<40 yrs	27	10	0.51* (0.42-0.61)	0.36*(0.30-0.43)
40–64 yrs	271	5	0.91* (0.76-1.09)	0.78* (0.65-0.93)
>64 yrs	308	4	reference	reference
Classification				
definite	338	4	reference	reference
probable	268	4	0.75* (0.63-0.89)	0.71* (0.59-0.85)
Typical EEG				
+	471	4	0.98 (0.82-1.17)	0.98 (0.83-1.18)
–	107	6	reference	reference

[1] Adjusted for age, gender, country, and class; 95% CI in parentheses
* $p<0.05$

Survival and general characteristics

The association between mortality and general characteristics is presented in table 2. Men had a significantly higher mortality rate; the median survival time for men was four months as compared to five months for women (fig. 1). Classification as probable CJD and age at onset below 40 years as well as between 40 and 64 years were related to lower mortality rates (fig. 2). Residence in Germany was statistically significantly associated with a longer survival of eight months as compared to four months for the other countries ($p< 0.05$, not in table). When analyzing the definite and probable patients separately, the differences between men and women and Germany versus other sites were not observed in definite CJD (DRR 1.09; 95% CI 0.90-1.32 and DRR 1.14: 95% CI 0.94-1.38 respectively, not in table). Age at onset below 40 years and between 40 and 64 years remained significantly reduced in

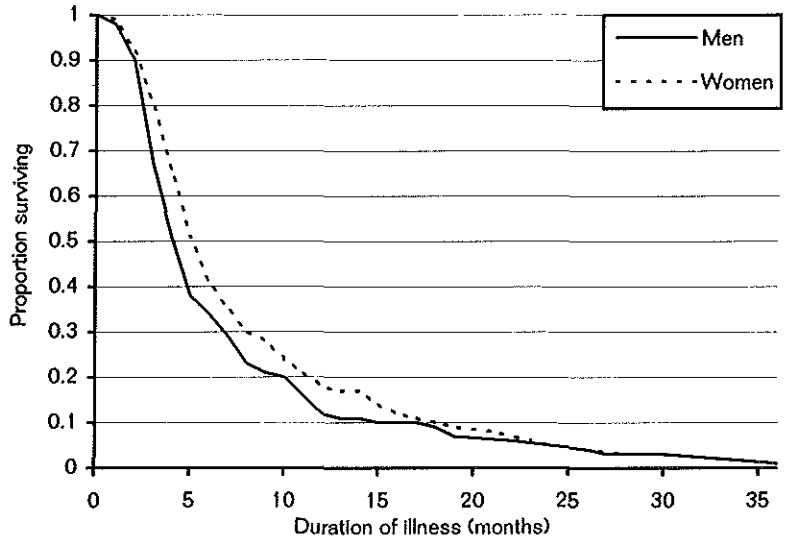

Figure 1 — Survival of Creutzfeldt-Jakob patients by gender.

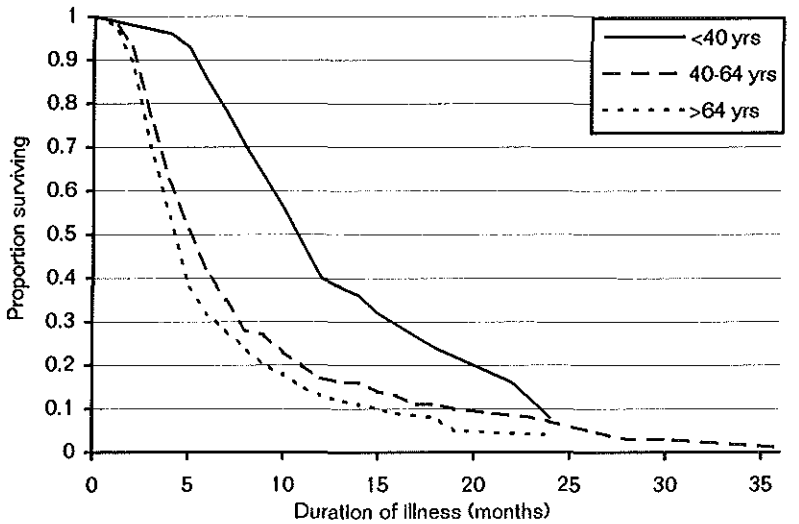

Figure 2 — Survival of Creutzfeldt-Jakob patients by age at onset.

Table 3 — Survival in patients with Creutzfeldt-Jakob disease

Symptoms at onset		Number	Median (in months)	Death rate ratio[1]	
				crude	adjusted[1]
Rapid cognitive decline	+	300	4	1.05	1.02
	–	228	4	(0.87-1.27)	(0.85-1.24)
Psychiatry	+	114	4	0.84	0.99
	–	330	4	(0.69-1.02)	0.83-1.21)
Sensory	+	77	5	0.93	1.07
	–	432	4	(0.77-1.12)	(0.88-1.29)
Vertigo/Dizziness	+	152	4	1.17	1.29*
	–	363	4	(0.96-1.41)	(1.07-1.56)
Headache	+	63	6	0.82*	0.93
	–	440	4	0.67-0.99)	(0.77-1.13)
Seizures	+	24	6	0.72*	0.66*
	–	478	4	(0.59-0.87)	(0.55-0.80)
Gait disturbances	+	315	4	0.94	0.99
	–	203	4	(0.77-1.13)	0.81-1.93)
Speech disturbances	+	178	4	0.99	1.03
	–	338	4	(0.81-1.19)	(0.85-1.25)
Visual disturbances	+	207	4	1.25*	1.29*
	–	315	5	(1.03-1.51)	(1.07-1.57)
Forgetfulness	+	310	4	1.12	1.06
	–	210	4	(0.92-1.35)	(0.87-1.28)

[1] Adjusted for age, gender, country, and class; 95% CI in parentheses
* $p<0.05$

definite CJD (DRR 0.39; 95% CI 0.33-0.48 and DRR 0.81; 95% CI 0.67-0.99 respectively, not in table).

Survival and clinical features

The association between mortality and the occurrence of clinical features is presented in tables 3 and 4 as crude and as adjusted death rate ratios (DRR). After adjustment for gender, age, pathological confirmation and country, the presence of vertigo, visual disturbances and pseudobulbar signs at onset of disease were

Table 4 — Survival in patients with Creutzfeldt-Jakob disease

Signs at onset		Number	Median (in months)	Death rate ratio[1]	
				crude	adjusted[1]
Cerebellar	+	240	4	0.89	0.96
	–	259	4	(0.74-1.08)	(0.79-1.16)
Visual/Oculomotor	+	182	4	1.16	1.17
	–	326	4	(0.97-1.41)	0.97-1.42)
Extrapyramidal	+	68	4	1.04	1.14
	–	437	4	(0.86-1.27)	(0.94-1.38)
Pyramidal	+	47	4	1.18	1.17
	–	457	4	(0.97-1.43)	(0.96-1.41)
Involuntary movements	+	103	4	0.90	0.95
	–	404	4	0.74-1.09)	(0.79-1.15)
Myoclonus	+	92	4	1.00	1.06
	–	421	4	(0.83-1.22)	(0.87-1.28)
Pseudobulbar	+	45	4	1.16	1.29*
	–	417	4	(0.96-1.41)	(1.06-1.56)
Neuromuscular wasting	+	8	4	1.23*	0.97
	–	493	4	(1.02-1.49)	(0.79-1.17)

[1] Adjusted for age, gender, country, and class; 95% CI in parentheses
* $p<0.05$

both significantly associated with a reduced survival (table 3). The presence of seizures at onset was after adjustment significantly associated with an increased survival. However, a significant increase in DRR in definite patients was only found for visual disturbances (DRR 1.46; 95% CI 1.21-1.77, not in table) and a significant decrease in DRR in definite patients for seizures (DRR 0.53; 95% CI 0.44-0.65, not in table).

The concomitant occurrence of rapid cognitive decline, myoclonus and typical EEG as described as the classical triad of CJD [4,6], was not significantly associated with a reduced survival as compared to absence of these features (DRR 1.14; 95% CI 0.95-1.39; not in table). The concomitant occurrence of psychiatric, cerebellar symptoms at onset of disease and the development of dementia during the course as described in the new variant of CJD, was

Table 5 — Survival in patients with Creutzfeldt-Jakob disease

Genetic factors		Number	Median (in months)	Death rate ratio[1]	
				crude	adjusted[1]
Aetiology					
Sporadic		554	4	reference	reference
Genetic		40	4	1.16 (0.96-1.41)	1.23*(1.01-1.49)
Iatrogenic		12	7	0.59*(0.49-0.72)	0.91 (0.76-1.11)
PRNP mutation	+	40	4	1.19 (0.98-1.44)	1.20 (0.99-1.45)
	–	313	4	reference	reference
PRNP mutation					
Codon 200	+	26	3	1.37*(1.13-1.66)	1.29*(1.07-1.57)
	–	313	4	reference	reference
Codon 'other'	+	14	5	0.91 (0.75-1.10)	1.05 (0.87-1.27)
	–	313	4	reference	reference

[1] Adjusted for age, gender, country, and class; 95% CI in parentheses
* $p<0.05$

not significantly associated with an increased survival as compared to absence of these features (DRR 0.82; 95% CI 0.68-1.01; not in table).

Survival and genetic factors of the PRNP gene

The survival of aetiological subtypes as computed from the whole sample is shown in fig. 3. The association between mortality and genetic factors is presented in table 5. After adjustment for sex, age, pathological confirmation and country, genetic cases were significantly associated with a reduced survival. The proportions of cases with or without mutations were computed only for patients with PRNP analysis available. Mutations of the PRNP gene were found in 40 patients; 26 patients had a mutation at codon 200, six at codon 210, three at codon 178, one at codon 117 and four had an insertion. The survival of patients with a PRNP mutation was significantly different from sporadic CJD patients. The median survival time of patients with a mutation at codon 200 of PRNP gene was three months, for patients with a mutation at an-

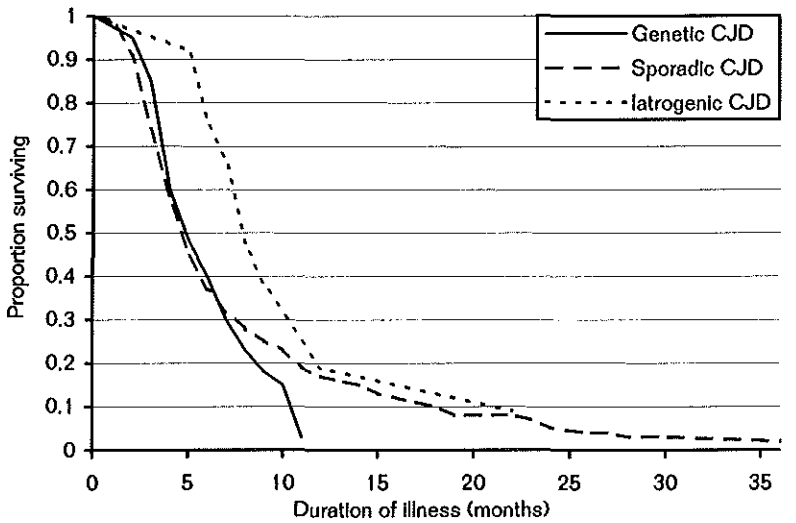

Figure 3 — Survival of Creutzfeldt-Jakob patients by aetiological subtype

other codon five months, as compared to four months for patients without a mutation. The genotype of 355 sporadic, genetic and iatrogenic CJD patients were assessed for the polymorphism at codon 129 of the PRNP gene: 77% were homozygous for the methionine allele, 13% were heterozygous and 10% were homozygous for the valine allele. The corresponding proportions were 78%, 12% and 10% for sporadic CJD patients, 77%, 18% and 5% for genetic CJD patients, and 50%, 33% and 17% for iatrogenic CJD patients, respectively.

Table 6 (overleaf) shows the association between polymorphism at codon 129 of the PRNP gene and mortality overall and stratified by the presence of a mutation. When adjusted for sex, age, pathological confirmation, and country, carriers of at least one valine allele had a statistically significant lower mortality in the overall analysis as well as in the analysis without a PRNP mutation. Among CJD patients without a mutation with CJD classified as definite a significantly prolonged survival was observed for carriers of the valine allele (DRR 0.63; 95% CI 0.52-0.77 for heterozygotes and DRR 0.77; 95% CI 0.64-0.94 for homozygotes, not in

Table 6 — Survival in patients with Creutzfeldt-Jakob disease

Genetic factors	Number	Median (in months)	Death rate ratio[1]	
			crude	adjusted[1]
Overall				
Polymorphism codon 129				
MM	273	4	reference	reference
VV	35	5	0.76*(0.63-0.93)	0.67*(0.55-0.81)
MV	47	6	0.72*(0.59-0.87)	0.67*(0.55-0.81)
No PRNP mutation				
Polymorphism codon 129				
MM	239	4	reference	reference
VV	31	5	0.80*(0.66-0.97)	0.67*(0.55-0.81)
MV	40	6	0.69*(0.57-0.83)	0.66*(0.54-0.79)
PRNP mutation				
Polymorphism codon 129				
MM	30	4	reference	reference
VV	2	4	0.28*(0.23-0.34)	0.37*(0.30-0.45)
MV	7	6	0.88 (0.73-1.07)	0.84 (0.69-1.01)

[1] Adjusted for age, gender, country, and class; 95% CI in parentheses. Overall analysis also adjusted for mutation.
* $p<0.05$

table). Although numbers were small, among the genetic cases we also found a significant correlation between mortality and polymorphism at codon 129 of the PRNP gene.

Discussion

In our study of 606 CJD patients, female gender, early age at onset and classification as probable CJD were important prognostic factors. The clinical features of CJD did not influence survival except for vertigo, visual disturbances and pseudobulbar signs, which were associated with a slight reduction in survival and seizures which were associted with an increased survival. In sporadic CJD

the presence of at least one valine allele at codon 129 of the PRNP gene was a predictor of a relative increase in survival. The effects on survival related to early age at onset, visual disturbances, seizures, PRNP mutation and the genotype at codon 129 of the PRNP gene were confirmed in analysis of definite cases only.

Regarding the validity of the data, the occurrence of clinical signs was assessed through examination of medical records. We consider it likely that neurological signs are accurately recorded particularly as CJD is usually a severe and rapidly progressive disease, although differences may exist between clinicians in different centres. However, the assessment of symptoms recorded in the medical notes, depends on information from the patient's relatives. Recall is liable to misclassification.[29] Although this type of misclassification is expected to be random, we cannot exclude the possibility that recall bias has resulted in an apparent spurious association between symptoms and survival time.
Age at onset below 40 years was associated with a significant increase in survival. The association between an early age at onset and long illness duration has been reported in the literature for cases with inherited prion diseases[7-10,17] and for cases with the new variant of CJD.[11,12] Our study included 14 new variant CJD patients from the UK (among them two patients with age at onset above 40) and one from France. If these 15 patients are excluded from the analysis, the median survival time for the age category below 40 decreased from ten to seven months (adjusted DRR 0.45; 95% CI 0.37-0.55; not in table). This suggests that the prolonged survival in early onset cases is predominantly but not fully explained by the patients with the new variant of CJD. Even if six iatrogenic cases with age at onset below 40 were excluded from the analysis, the age category below 40 remained associated with a significantly prolonged survival (adjusted DRR 0.58; 95% CI 0.48-0.70; not in table). However, the statistical power of this analysis was low given the small number of patients and the significant death rate ratio of 0.58 does not exclude the possibility that mortality may be reduced in younger patients in general. In general, younger subjects will have a higher life expectancy than older subjects. Furthermore, in relation to disease process it is possible that in younger persons subtle memory loss is recognized

and diagnosed at an earlier stage than in older patients, in whom subtle cognitive changes will be attributed to normal ageing. It has also been suggested that younger patients may have more relative brain reserve and have a higher clinical threshold for slowly progressive brain damage.[10] Another possibility is that the expression of disease is different in younger patients, particularly in the new variant of CJD.

We found a statistically significantly increased survival in probable cases. This is expected because 'long survivors' alive at censoring are per definition classified as probable. Misclassification could have occurred in patients who lack the subsequently neuropathological confirmation. We therefore stratified and verified our data for definite and probable CJD separately. Although all centres shared the same diagnostic protocol, there is evidence of heterogeneity among participating countries with regard to survival. The time period between disease onset and diagnosis was significantly longer in Germany than in other countries. This suggests that in Germany disease onset was defined more sensitively. Although a common protocol was used for diagnosis, the onset of disease was not strictly defined and personality disturbances at onset was more often reported for German patients than for patients from other sites. We therefore performed our analysis for the German patients by date of clinical diagnosis as the definition for onset of disease.

We found a statistically significant difference in the survival of genetic patients in comparison with sporadic patients. In this study of incident cases of CJD single patients with different PRNP mutations have been identified and not multiple patients from individual pedigrees. There may have been selective identification of genetic cases of CJD with a relatively short duration of illness. Various mutations of the PRNP gene have been associated with inherited prion diseases including familial CJD.[30,31] In this study, the PRNP mutation was located in codon 200 in 26 of the 40 patients with genetic CJD, of whom 50% were from France. It has been suggested that the codon 200 mutation has less influence on the phenotypic expression than other PRNP mutations with age at onset and duration of illness almost indistinguishable from spo-

radic CJD.[32,33] In contrast to these earlier findings, we found a significantly different, i.e. reduced, survival in carriers of the codon 200 mutation of the PRNP gene as compared to those without a mutation. The short survival time found in our study for genetic CJD patients could be ascribed to the high proportion of codon 200 mutations and by the fact that in the analysis we adjusted for sex, age and pathological confirmation, and took into account that not every patient was followed until death due to termination of the follow-up.

There are a range clinico-pathological phenotypes in the human transmissible spongiform encephalopathies or prion diseases including: 'ataxic', 'Heidenhain', 'myoclonic', 'long duration', 'panencephalopathic' and variants.[10,13,34-36] However, improved immunohistochemical and molecular genetic techniques suggest that the subclassification of human prion diseases needs to be reviewed. Beside the new variant of CJD[11,12], other forms of CJD have been postulated on molecular grounds and evidence of phenotypic variability.[37,38]
The most striking clinical features of the new variant of CJD are psychiatric symptoms at onset and the long duration of disease.[11,12] In our study we did not find evidence to support an association between psychiatric symptoms and increased survival time in patients with classical CJD. This suggests that the prolonged survival in the new variant of CJD, which presents predominantly with psychiatric symptoms, cannot be explained by a subgroup of patients with psychiatric presentation in patients with classical CJD. The identification of a subgroup of CJD cases with visual disturbance, associated with reduced survival is consistent with the Heidenhain's variant of CJD, with occipital lobe pathology.[34,35] The occurrence of this type of CJD may be more frequent than reported, because visual symptoms and signs may be missed in patients with dementia. The finding of the occurrence of seizures associated with a significantly increased survival is in contrast what one should expect as seizures indicate severe involvement of the cortex. Furthermore, it may sometimes be difficult to distinguish between involuntary jerky movements and epilepsy. Our data do not suggest any other clinical subtypes of CJD based on the concomitant occurrence of clinical features

except for the early onset patients with the new variant of CJD who have a longer survival.[4,11,12]

Considerable attention has been focused on the role of the common protein polymorphism at PRNP codon 129 in the pathogenesis of, and susceptibility to, prion diseases. The genotype frequencies found for sporadic patients in our study are consistent with earlier studies.[14,15,18] Among the inherited prion diseases the role of polymorphism at codon 129 of the PRNP gene in phenotypic expression of the disease is complex and depends on the type of mutation: among patients with a 144-bp insertion a significant correlation between an early age at onset with homozygosity at codon 129 of the PRNP gene has been reported.[17] Among patients with a codon 178 mutation those homozygous for the valine allele were associated with an early onset and longer duration[9,10]; among patients with codon 200 mutation no such correlation has been found.[7,33] Homozygosity at codon 129 of the PRNP gene either for the methionine or valine allele has been reported to predispose to sporadic or iatrogenic CJD.[15,16,19,30,39,40] In sporadic CJD the influence of the codon 129 genotype on clinical phenotype such as age at onset and duration of illness has been controversial.[18,19,41,42]

This study, the largest series of CJD patients with data on genotype at codon 129 of the PRNP gene, shows that among patients without a PRNP mutation heterozygosity and homozygosity for valine are associated with prolonged survival. Again, desrepancies may reflect differences in statistical analysis. In the present analysis we adjusted for censoring, age, sex, pathological confirmation, and country. Our finding is compatible with the view that the prion propagation and conversion into the abnormal isoform may therefore not only rely on identical copies of the prion protein but that also other genetic co-factors from the host may determine the progression of the disease. This finding highlights the important influence the genotype at codon 129 of the PRNP gene has on both the pathophysiology and the prognosis of prion diseases.

Conclusion

This study does not suggest that the clinical features of classical CJD relate to survival. Gender, early age at onset and pathological confirmation are important prognostic factors. Among CJD patients without a PRNP mutation homozygosity and heterozygosity for the valine allele are associated with an increased survival. This finding highlights the important influence of the genotype at codon 129 of the PRNP gene on the survival of prion diseases. Furthermore, this study indicates that the prolonged duration of illness in new variant CJD may not simply reflect the age at onset of disease or the predominantly psychiatric presentation, providing further evidence that this form of CJD is clinically distinct from classical CJD.

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CHAPTER 7

Discussion

In the previous chapters we reported the EU collaborative studies on Creutzfeldt-Jakob disease (CJD) conducted in a three year period 1993 to 1995 and a re-analysis of case-control studies that was conducted in preparation of the prospective collaborative studies. In this chapter, first a brief overview will be given on the major findings of the incidence, case-control and survival analysis. Subsequently the methodological aspects of the three studies will be discussed separately. Finally, implications of the findings of the individual studies will be addressed and directions for further research will be outlined.

7.1 Findings

In the European collaborative study the overall annual mortality rate in the three year period was 0.73 per million person years.[1] This finding is consistent with earlier systematic surveys (table 1). The incidence rates and age-specific-incidence rates were all relatively similar in all participating countries. The most important exception is the increased incidence in patients aged <39 years in the UK in particular in the year 1995 (0.14 per million person years).[2] In relation to aetiological subtypes, 90% of all cases were sporadic, 8% genetic and 2% iatrogenic. With regard to risk factors studied the predominantly negative results of the re-analysis of three studies conducted in the period 1975-1984 re-emphasise the absence of a common risk factor in CJD patients in the period studied.[25] In the case-control study of the collaborative EU studies on risk factors we found significant evidence for familial aggregation of CJD as well as evidence for familial clustering of CJD with dementia due to other causes.[26] A significantly increased risk of CJD was found for subjects exposed to domestic birds, leather products and fertilizer consisting of hoofs and horns. Of the dietary factors studied, the consumption of brain and raw meat were associated with an increased risk of CJD. No association could be shown between the risk of CJD and the consumption of beef, veal, lamb or milk.

Table 1 — Summary of national surveys of Creutzfeldt-Jakob disease.[2] Annual mortality rates (cases/year/million).

Country	Years	Number	Familial %	Mortality cases/million
Chile[3,4]	1955–1972	19	27	0.10
	1973–1977	16		0.31
	1978–1983	46		0.69
Czechoslovakia[5]	1972–1986	46	22	0.66
France[2]	1968–1977	178	6–9	0.34
	1978–1982	151		0.58
England/Wales[6-9]	1964–1973	46	7	0.09
	1970–1979	158	4–6	0.31
	1980–1984	120	6	0.50
	1985–1989			0.47
	1990–1994	185	5	0.76
Scotland/Wales[9,10]	1980–1985	10		0.21
	1985–1989	15		0.60
	1990–1994	24		0.90
United Kingdom[11]	1990–1993	100	9*	0.67
Hungary[12]	1960–1986	65	11	0.39
Israel[13,14]	1963–1972	23	4	1.07
	1963–1977			
Italy[15,16]	1958–1971	32	8	0.05
	1972–1985	87	0	0.11
Finland[17,18]	1974–1984	30	20	0.57
	1979–1984	26	20	0.91
	1974–1989	44	25	
Japan[19]	1975–1977	75	6	0.15
United States[20-21]	1973–1977	265		0.26
	1979	148		0.66
	1980	142		0.63
	1979–1990			0.90
	1986–1988			0.83
Libya[23]	1982–1986			0.48
Austria[24]	1969–1985	79	0	0.18
	1986–1994			0.67
	1995			1.50

* Familial were 9% of all cases and 13.4% of cases with DNA analysis.

In our study of survival of patients with CJD the median survival was four months. Female gender, early age at onset and a diagnosis of probable CJD were important prognostic factors.[27] Most clinical features were not related to survival. In contrast to earlier findings, mortality tended to be increased in patients with PRNP mutations and/or a positive family history for CJD. Among patients without a PRNP mutation, mortality among carriers of at least one valine allele at codon 129 op the PRNP gene was significantly lower.

7.2 Methodological aspects

The epidemiologic surveillance of CJD is an important instrument for monitoring incidence, risk assessment and clinical course of human prion diseases in relation to animal spongiform encephalopathies. However, when interpreting the findings it is crucial to review critically its weaknesses with regard to selection bias, information bias or confounding.

Incidence study

The EU collaborative studies of CJD started in 1993 with the aim to provide comparative epidemiological data that would enable baseline epidemiological parameters to be established which will be crucial to the assessment of the risk of human prion diseases, in particular CJD in relation to animal spongiform encephalopathies.

Case ascertainment

Surveillance of CJD is complicated by the rarity of the disease and the absence of a typical diagnostic test. To obtain meaningful results it is essential to achieve a high level of case ascertainment by systematic identification of incident cases.[28,29] In the EU collaborative studies case ascertainment has depended on two major mechanism: *(1)* direct referral from targeted professional groups: neurologists, neuropathologists and neurophysiologists and *(2)* death certificates in countries where they are accessible.[30] CJD is usually a dramatic illness which generally occurs in the 6th and 7th decade of life, but patients with onset in early adolescen-

ce and late senescence have been reported.[31-34] The median survival from first symptoms to death is approximately four months but may be as long as 13 years.[35-37] In countries with a developed medical system it is extremely unlikely that patients who become totally dependent will not be admitted to the hospital and subsequently referred to a neurologist. However the referral of CJD cases to a national registry depends fully on the cooperation of neurologists and other professional groups as well as the cooperation of relatives of the patients. In order to identify a high proportion of cases, including atypical variants, the referral of any suspect case is mandatory.[38]

Diagnosis

Diagnostic classification of cases in definite and probable CJD is standardized in the collaborative European study.[39] However we did find heterogeneity among countries regarding post mortem rates varying from 41% to 100%. Practical difficulties have risen in obtaining autopsies in CJD because of potential risks to personnel in pathology departments despite the availability of guidelines to minimize potential risks during postmortems.[40] Twenty-three patients underwent brain biopsies; 10 also had a post mortem. Light microscopic examination of the biopsy specimens showed spongiform change in 8 (80%) of the autopsy verified cases. Criteria for the diagnosis of CJD were first proposed in 1979 but have been adapted according to acquired scientific knowledge about iatrogenic CJD and genetic form of prion diseases.[20,39] The EEG is crucial to distinguish probable from possible cases where there is no pathological confirmation of the diagnosis. In some cases of CJD, no EEG is carried out and in others only a single tracing is obtained early in the course of the illness. The chances of obtaining a characteristic record are enhanced if serial recordings are carried out. A major problem with the use of EEG for the diagnosis of CJD is that there are no standardised established EEG criteria.[38] An EEG with generalised periodic sharp wave activity at 0.6-2 Hz supports the clinical diagnosis of CJD. Recently a sensitivity of 67% and specificity of 86% was computed for the typical EEG tracing in CJD.[41] Misclassification of EEGs may occur because of variation in EEG reports by clinicians. Therefore it is important for the national registries to build up expertise in reviewing EEG

of all referred cases.[38] A high degree of case ascertainment is crucially dependent on obtaining a high postmortem rate of all suspect cases. In this prospective study cases were included according to the diagnostic status on May 1st 1996. The case classification upon inclusion may change according to the availability of information especially on neuropathological information. Atypical cases, such as those with a stroke-like presentation or a long duration of illness, may not be clinically recognized as CJD and identification may only be possible through neuropathological examination.[42]

In the descriptive epidemiology of CJD mortality figures are often used to compute the annual and age-specific mortality rate per million person years (table 1). For the collaborative study on CJD we computed both incidence for cases with onset of illness in the three year time period 1993 to 1995 as well as mortality for cases with date of death in the three year period 1993 to 1995.[1] Difference between those two figures may have occurred due to the fact that in the first year of the prospective study mortality figures may be underestimated because emphasis in the new established registries may have been focused on incidence rather than mortality. Also, at the end of the three year period mortality data may underestimate cases with an atypical long duration. Despite these considerations the difference between the incidence and mortality is in most cases almost negligible because of the short median duration of illness of four months.

Studies of genetic forms of prion diseases have established marked phenotype variation in the clinical and pathological features of CJD.[43,44] A proportion of these cases do not fulfil current diagnostic criteria for CJD. In the UK systematic use of DNA analysis in CJD has demonstrated that about 13.4% of cases with DNA analysis are associated with PRNP gene mutations.[11] Approximately one third of these cases have no evident family history of CJD. The low percentage of genetic cases of eight per cent in the collaborative study is likely to be explained by the different percentages of DNA analysis performed in the participating countries varying from 100% of cases analyzed in Slovakia, 80% in Germany, 78% in France, and 60% in Italy compared to 58% or

less in other countries. Furthermore, the figure of eight per cent may be slightly underestimated as it remains possible that not all novel mutations within the PRNP open reading frame (ORF), nor PRNP mutations outside the ORF, may have been detected.[11]

Risk factors

Case-control studies have been carried out in the past in the United States, United Kingdom, Israel and Japan to assess potential risk factors for CJD.[45-51] Due to the low frequency of the disease all five studies included only small numbers of patients. The few positive associations from these studies were mutually inconsistent and even the re-analysis of three case-control studies[46-50], comprising patients diagnosed with CJD according to the criteria of Masters et al., lacked statistical power to provide significant positive associations for rare exposures, which is reflected in the width of the 95% confidence intervals.[25] However, the joint registries of the collaborative studies which share common methodologies, included 405 CJD cases and provided sufficient statistical power to study a number of putative risk factors for CJD.[26]

Selection bias may have occurred for several reasons. First through non response in cases and controls in the different countries. The percentage of cases from the national registries included in the case-control studies varies from 39% to 88% These differences may be caused by the major practical and geographic problem to conduct the studies of CJD on a nationwide basis because of its rarity and random occurrence. The vital status of cases at time of interview may have influenced the selection of cases into the case-control study. Especially for cases ascertained from neuropathologists and death certificates where there had been a prolonged delay in obtaining hospital notes or other conformation of the diagnosis, there might have been caution to approach relatives. They were therefore excluded from the case-control study. Controls were selected for each case using the following criteria: sex and age (+/- 5 years) matched, inpatient in the same hospital as the case, and excluding patients with a neurodegenerative disease. The response rate for controls is not known, but is generally lower than in cases. In general, findings may have been biased if

the response rate in cases and/or controls was associated to the risk factors studied like geographical distribution.
The use of hospital controls may have introduced selection bias in that exposure frequencies to surgery, medical disorders and or medical treatment may not be representative for the general population. The preference for neurological controls with exclusion of neurodegenerative disorders may have led to a potentially over-representation of common risk factors and therefore the association found may have been an underestimation of the real association. The validity of the case-control study may have been compromised as only one study centre selected control series from the general population.

Information bias has inevitably occurred as the information on possible risk factors for CJD cases was obtained through a structured interview with a surrogate informant, mainly a next of kin. To achieve symmetrical data collection, information on risk factors for the control subject was also obtained from a surrogate informant. In each case-control pair relatives of an identical degree were interviewed whenever possible. However in some instances control subjects were interviewed in person. This may have introduced a systematical difference between cases and controls that could have affected the validity of the findings. For all risk factors, the data were analyzed including only patients and matched controls for whom the data were obtained from a next of kin.
Another form of information bias may have been recall bias, as relatives of CJD cases are more likely to remember putative exposures in the past than informants of controls. Important determinants for this form of recall bias are time between onset of CJD and the interview and the vital status of the patient at time of the interview. Stratified analysis for these determinants did not change the results. As the incubation period in human prion diseases may be extremely long with a mean incubation period of 13 years in human growth hormone recipients and a range of incubation periods from 5 to over 30 years in Kuru, risk factors studied must include potential exposure in early childhood.[52] In general, information obtained from decades in the past may in itself introduce recall bias.

Another potential source of information bias which may have occurred non-differentially is the awareness of relatives of the hypothesis tested. Since the outbreak of BSE in the UK in 1986, there has been great public and media interest in a possible link between BSE and CJD.[53] As the collaborative studies were carried out in 1993 to 1995, so before the new variant CJD became front page news in Europe, it seems reasonable to assume that bias has mainly afflicted the UK up until 1995, given the media attention in the UK. However, given the size of the BSE epidemic in the UK compared to other European countries, one may argue that the exposure frequencies in the UK may have differed significantly from those at the European mainland. Also, the perception of the interviewer may have been influenced by the awareness of possible causes. In this respect it is important to note that interviewers were not blind to disease status of the subject interviewed.
We performed stratified analyses for definite and probable CJD separately. In the case-control study the risk estimates for putative risk factors from the stratified analysis did not statistically significantly differ form the unstratified analysis.

Confounding — Although putative confounders as age, sex and study centre were effectively controlled for, there may be several yet unknown risk factors including genetic factors not controlled for in the analysis. At present it is impossible to exclude this source of bias.

Survival

Survival analysis was carried out with incident cases from the registries with their onset of disease in the three year period of 1993 to 1995. Duration of illness was defined as the period from first symptoms to death or to censoring date at May 31 1996.[27] In contrast to earlier studies, we took into account in the analysis that not every patient was followed until death due to termination of the follow-up of patients on May 31 1996.

Selection

The majority of cases from the registries were included in the survival analysis. Patients lacking basic clinical information on clinical course as date of birth, date of first symptoms and date of

death had to be excluded. Similar to the possibilities of bias in the incidence studies bias may have occurred in the ascertainment of cases. For instance there may have been selective identification of genetic cases of CJD with a relatively short duration of illness.

Information

Data on the occurrence of clinical signs were assessed through examination of medical records. Neurological signs were assumed to be recorded accurately in the medical records due to the severity and rapid progression of the disease although differences may exist between clinicians from different countries. The assessments of symptoms recorded in the medical notes, depends on information from the patient's relatives. Although this type of misclassification is expected to be random, we cannot exclude that recall has caused an effect between occurrence of symptoms and survival time. In the survival analysis probable cases had a significantly longer survival as compared to definite cases. This may be explained by the misclassification of other neurological disorders as probable CJD. In the analyses we therefore stratified and verified our data for definite and probable cases. Although all centres shared the same diagnostic protocol, there is evidence for heterogeneity among participating countries with regard to survival. The onset of disease was not strictly defined and personality disturbances at onset were more often reported for German patients.

Confounding

We adjusted for possible confounders including gender, age, classification as probable and country of residence. Again, there may be yet unknown predictors including genetic factors not adjusted for.

7.3 Implications

Incidence

Variation in measurements

The mortality of the prospective collaborative study in the three year period of 1993 to 1995 was 0.73 per million person years.[1]

Earlier systematic surveys consistently reported a mortality of around 0.5 cases per million per annum (table 1). Most studies were done on a nationwide basis and cases were identified retrospectively through death certificates, neuropathology reports or medical records. However, higher mortality figures were occasionally reported for a number of countries for localized areas as Slovakia and Libyan born Israelis which could eventually be explained by a high frequency of mutations in the PRNP gene.[2] Surveillance in small countries leads to an unstable incidence/mortality estimate. Variation in incidence figures may occur by sampling error if incidence/mortality figures for a rare disease such as CJD are based on a small number of cases in a restricted area over a limited period of time. This could explain the fluctuation of the annual mortality rate of in particular Austria and the Netherlands in the period studied.[1,24] In the UK the geographical distribution of CJD over the past 20 years showed no evidence of spatio-temporal aggregation of CJD despite the occurrence of local areas of relatively high incidence over short periods.[30]

Age-specific incidence curve

The sharp decline of the incidence of CJD in all countries of the collaborative studies in the age group above 80 years is in contrast with observations in other neurodegenerative disorders such as Alzheimer's disease.[1,54] This may reflect a more difficult ascertainment of CJD in the older age-groups. In contrast to what should be expected based on this hypothesis, this decline is also seen in the UK despite their higher ascertainment in the elderly since 1990.[9] An alternative explanation may be that the genetic susceptibility determines the shape of the curve. Expression of the prion protein gene may be age dependent and highest at relatively early age and low at high age.

Despite the relatively similar overall incidence rates for the participating countries in the collaborative studies, there was a marked, approximately fourfold, increase in the age-specific incidence in the year 1995 for cases aged < 39 year in the UK compared to the other European countries.[1] A possible explanation of this increased incidence among young patients may be a better ascertainment in the younger age-groups by analogy of the increased overall incidence of CJD in the UK in the 1990s due to

improved ascertainment of CJD in the elderly.[9] However, this group comprises 14 cases with the new variant of CJD.[55] The occurrence of 14 cases with new variant CJD emerging in the UK in a relatively short time period with a distinctly different clinico-pathological presentation in a for classical CJD unusual age-group and in the absence of encoding mutations of the PRNP gene associated to CJD raised the question wether this was a new form of CJD in the UK.[55,56] The strikingly common neuropathological picture of these cases indicates infection with a common strain of the causative agent which shows similarity with the pathology seen in scrapie in sheep.[57] At least 20 different strains of scrapie prions can be distinguished in mouse models inoculated with scrapie brain on the basis of disease-incubation period and neuro-pathological profile. The most obvious explanation for this new type of CJD emerging in the UK is a link with BSE. BSE has most likely been caused by feeding cows scrapie infected brains. Recently, evidence in support of a causal link between BSE and a new variant CJD is provided by experimental transmission of BSE to macaques, revealing neuropathological similarity to new variant CJD. Furthermore, biomolecular studies provided evidence of a distinct strain of the causative agent associated to new variant CJD that is distinctly different from two strains of the causative agent in classical CJD and one strain in iatrogenic CJD. The similarity on Western blots between the prion protein of new variant CJD and BSE was striking.[58]

The exposure of the general population to the BSE agent through consumption of meat products containing high titres of BSE prions, is likely to have been greatest in the eighties which indicates incubation periods of between five to ten years. This is compatible with a relatively short incubation period as compared to a mean incubation period of 13 years in human growth hormone recipients and a range of 5 to 30 years in Kuru, because in these cases there is no so-called species barrier.[59,60] This may indicate a low threshold of the species barrier between cattle and humans for the BSE prions. However, we are presumably in a very early phase of a new variant 'epidemic' as the cases that have been identified so far represent minimum incubation periods and it is most likely that the mean/median latency time in patients with new variant CJD is to increase due to the cases still to

develop.[61] The question emerges why this new variant of CJD afflicts particularly young patients. One reason could be that younger age groups are more susceptible because of the age-dependent expression of the prion protein gene. The similarity in genetic make-up of new variant CJD cases, coding methionine/methionine for the polymorphism at codon 129 of the PRNP gene supports this hypothesis. Another possible explanation may be the misdiagnosis of the new variant in the older age-groups, especially in those in which dementia is more common.[54] Finally, the absence of the new variant in the older age-group may be associated to an aged related exposure of the causative agent in relation to food consumption patterns. However, there is no obvious reason to believe why the consumption of with BSE prions contaminated meat products was restricted to the younger age groups alone unless through the consumption of 'modern' food products such as hamburgers that are not consumed by elderly. As the first patient with new variant CJD aged fifty has been identified [RG Will, personal communication], it remains to be seen wether new cases of new variant CJD will afflict all age-groups equally, if they occur at all.

Risk factors

Case to case transmission of CJD has been proven to occur through accidental iatrogenic contamination via neurosurgical procedures, EEG electrode implantations, corneal transplantation and human and gonadotrophin hormone.[59] Non iatrogenic case-to case transmission remains speculative. Two major objections have been raised to reject case to case transmission as a mechanism of acquiring sporadic CJD[2]. First, examples of contact between cases are rare and the risk of acquiring CJD appears not to be greater in highly exposed individuals as spouses, friends, nurses and physicians. Iatrogenic transmission was rare in the re-analysis of case-control studies conducted in the period 1975 to 1984.[25] Also, this re-analysis failed to show statistically significant evidence for transmission from cattle to humans. However, the data on the presence of prion disorders in patients or animals to which patients were exposed were not available in the time period studied. In the light of the new variant CJD and the possible link with BSE, the prospective case-control study of the collaborative studies

conducted in 1993 to 1995 may yield important clues on the risk assessment of CJD in relation to BSE.[26] The availability of information on genetic factors as mutation of the PRNP gene and polymorphism at codon 129 of the PRNP gene has made separate analysis possible of genetic and sporadic cases.

Genetic factors

The finding of familial aggregation of CJD as a statistically significant risk factor for CJD is what one would expect as various dominant mutations in the PRNP gene on chromosome 20 have been linked with human prion diseases including particular familial CJD.[43,44] In the case-control study of the collaborative studies only a proportion of the genetic CJD cases with a PRNP mutation were included in the case-control study. It is known that a family history was not ascertained in 30 to 60 per cent of the cases harbouring PRNP mutations.[11,62] So the odds ratio of 12.0 found for familial aggregation of CJD may underestimate the true association. The analysis further showed a significant association between CJD and family history of dementia due to other causes than CJD. In a number of patients with a familial form of dementia, PRNP mutations have been found and the familial segregation of CJD and dementia may therefore be related to PRNP mutations segregating in these families.[63] However, other genetic factors (such as the apolipoprotein E gene, which is associated with both the risk of CJD and Alzheimer's disease) or non-genetic factors may underlie the relationships.[64-66]

Iatrogenic transmission

In agreement with earlier findings, iatrogenic CJD appears to be rare as no association of CJD related to medical history could be shown in this large population-based sample of CJD patients.[26] However, there are two reasons to interpret these findings with caution. First, the referral of iatrogenic cases to the national surveillances may have varied among countries and may furthermore have been excluded from the case-control study as the source of accidental contamination for these cases, such as human pituitary growth hormone, corneal graft or dura mater graft, was known. Furthermore, the findings should be interpreted with caution because of the limited validity of our study due to the use of

hospital controls. As mentioned earlier, the association found of CJD to surgery, blood transfusion, and lumbar puncture can largely be explained by the use of hospital controls. Further studies with population-based controls and precise definition of potential incubation periods should be carried out in the future.

Link between CJD and BSE within Europe

There was no significant evidence in the case-control study of transmission of CJD from cattle to humans. With regard to the occupational exposure to patients or patient material, there was a non significant increase in risk of CJD for subjects employed as health professionals. With regard to the consumption of meat or meat products, the consumption of brain and raw meat was associated with an increased risk of CJD. However the absence of a dose response relation between the consumption of raw meat and the risk of CJD makes a dose-effect relation less likely. In the light of a possible link between CJD and BSE, it is interesting to consider the PRNP codon 129 genotype of the CJD cases with raw meat exposure in analogy to the homozygosity for the methionine allele among the new variant CJD cases.[55]

As brain tissue, spinal cord and eyes are expected to contain the highest concentration of prions, the increased risk of CJD with the consumption of brains, in particular those from cows, would support the hypothesis of a link between CJD and BSE. However, the analysis showed an increased risk of CJD for the consumption of pig brains as compared to cow brains, which argues against a causal relationship as the probability of prion disease is much higher in cows. No evidence was found for an increased risk of CJD associated with (occupational) exposure to cattle or sheep (products) or with the consumption of beef, veal or lamb. The negative results of our analysis are important in the light of public health to re-emphasise that at present the case-control study of the EU collaborative studies do not provide evidence for a link between BSE and CJD. However these findings should be interpreted with caution as the study includes patients with onset of disease before or in 1995. So far the number of new variant CJD cases included in the case-control study was small and we will have to await future studies to see wether consumption of beef, veal or lamb may increase the risk of new variant CJD.

Survival

The survival analysis does not suggest that clinical features of classical CJD are related to survival except the new variant of CJD.[27] There is a significant difference in survival between men and women. This may be related to an earlier diagnosis of the disease in men. Also, mortality in the general population is higher in men than in women. The prolonged duration of illness in new variant CJD does not simply reflect the age at onset of disease or the predominantly psychiatric presentation providing further evidence that this form of CJD is clinically distinct from classical CJD. The collaborative studies provide the largest data set ever assembled with information on the genotype of the polymorphism at codon 129 of the PRNP gene. The study confirms the role of genetic factors in the pathophysiology and the prognosis in prion disease, in particular in sporadic CJD. The recent findings of four distinctly different strains in classic, new variant CJD and iatrogenic CJD illustrates the importance of the genotype of PRNP gene in the host for the monomorphic phenotypic expression of the different prion strains in CJD.[58]

7.4 Directions for further research

Continuing the surveillances of CJD in Europe

Continuation of the EU collaborative studies on CJD in Europe will provide important comparative epidemiological data to monitor any change in the incidence of CJD, and in particular new variant CJD, attributed to BSE. With the identification of the new variant of CJD in the UK it was noteworthy to have comparative data available that showed that in other European countries with different incidence figures of BSE, in the same period in particular in the year 1995, no increase has been observed in the incidence of CJD in young patients. In relation to the hypothetical risk of BSE and other animal prion diseases for humans an atypical form of prion disease in humans was considered possible, by analogy to the atypical clinical presentation of the first human growth hormone recipient.[67-68] Indeed, all cases with the new variant that have been linked to CJD presented with aspecific psychiatric

symptoms in an unusual age group for classical CJD.[55] The question emerges wether we have to adapt the ascertainment to this new knowledge and extend the targeted professional groups to psychiatrists and paediatrics, which will inevitably result in many more referrals of suspected cases who in the end will not be related to CJD. On current evidence a clinical diagnosis of new variant CJD cannot be made during the psychiatric phase of the illness and diagnosing of these cases depends on the evolution of neurological signs.[55] All cases with new variant CJD were promptly referred to a neurologist within eight weeks after the neurologic symptoms had developed [R.G. Will, personal communication]. Crucial for the ascertainment of CJD, especially with regard to atypical cases which are not clinically diagnosed with CJD, will be the maintenance of a high level of post mortem rates.

Improvement of diagnosis

The accuracy of clinical diagnosis is over 95% in cases with a typical presentation and a characteristic EEG.[38] However neuropathological confirmation of the diagnosis remains an important component of the surveillance as a small proportion of cases may not be clinically suspected of CJD or do not undergo (serial) EEG recordings. In the atypical clinical course of new variant CJD it is important to critically review the diagnostic criteria. Some considerations to improve the diagnosis and case classification are briefly discussed.

Adaptation of diagnostic criteria currently used in the collaborative studies is mandatory. However, the majority of the 14 new variant cases in the UK were notified through the normal surveillance system and only one of these cases was identified solely by post mortem examination.[55] Further studies have to evaluate wether the clinical features of the 14 new variant patients represent the uniform phenotypic expression of new variant CJD in humans linked with BSE. It will be important to perform serial EEG recordings to enhance the chances of obtaining a characteristic recording of the EEG and to establish EEG criteria for the diagnosis of CJD.

Brain biopsies as a diagnostic investigation in CJD remain controversial as they carry a notable morbidity and may be non informative as an unaffected region of the brain is obtained.[69] Especially in young cases biopsies are occasionally performed if treatable alternatives of CJD are seriously considered. However, the predictive value of a negative finding at pathology is low.

Recently a diagnostic test of CJD has been reported based on immunoassay of 14-3-3 protein in the cerebrospinal fluid.[70] The test rapidly detects neuronal loss and false positive results have been reported in herpes encephalitis and carcinomatous meningitis with a primary small-cell carcinoma of the lung.[70,71] It will be crucial to derive large patient-series for the development of tests for which pooling of patients from different centres is necessary. Future research has to determine the specificity of the test in different stages of the disease and the specificity in animal spongiform encephalopathies as BSE and scrapie. At present there is no evidence suggesting an interaction of these proteins in the pathophysiology of prion diseases.

Collinge et al. recently reported for classical, iatrogenic, and new variant CJD specific patterns of protease-resistant prion protein on Western blot analysis.[58] They showed that this marker could be used to aid differential diagnosis on brain and tonsil biopsy samples as the prion protein is widely expressed outside the central nervous system in the lymphoreticular system.[72]
If new variant CJD is caused by BSE, early diagnostic markers such as the immunoassay of 14-3-3 protein in the cerebrospinal fluid and the protease-resistant PrP in tonsillar tissue will allow early clinical diagnosis of (new variant) CJD. However, large prospective studies are necessary to evaluate the clinical relevance of these tests. The ongoing collaborative studies of CJD will provide patient tissue to enable research in developing a diagnostic test ante mortem.

Exposure

An important issue to tackle in future research is the exposure assessment. Putative modes of transmission from cow to man in-

cludes nutrition, medication and animal exposure. The operalisation to assess exposure to BSE infected tissue is particulary difficult in the first above-mentioned fields. With regard to nutrition, mechanically derived meat may hypothetically be infected because of contact with brain and/or spinal cord tissue. Up until now it has been impossible to trace the products of BSE cows in food products and medication. The issue of exposure will be crucial in future epidemiological research.

Gene susceptibility

Animal studies as well as the EU collaborative studies suggest that genetic factors other than PRNP genes must be implicated. It will be crucial to find these genes. From a public health point of view this will enable us to detect subgroups of people (or animals) genetically susceptible for the disease. From a scientific point of view, identification of these genes will give new insight in the pathogenesis of CJD through its gene product (protein). From a clinical point of view these genes may eventually lead to the development of new diagnostic tests and perhaps subsequently lead to a therapy.

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CHAPTER 8

Summary

CHAPTER 1 gives a brief overview of various issues of the collaborative European studies on incidence, risk factors and survival of Creutzfeldt-Jakob disease (CJD) described in this thesis. In historical context, the epidemiological surveillance of CJD conducted by Professor WB Matthews in the UK throughout the seventies and early eighties served as a basis for the collaborative studies conducted from 1993 to 1995. The collaborative studies were possible through funding from the European Community for coordination of national CJD surveillance programmes to evaluate any changes in the pattern of CJD that might be attributable to BSE.

Chapter 2 reviews the evidence for risk factors of Creutzfeldt-Jakob disease by pooling and re-analyzing the raw data of three case-control studies, conducted in the United States, Japan and United Kingdom during the seventies and early eighties. Despite the difference in methodology between the individual studies, they shared similar diagnostic criteria as described by Masters et al. The pooled data set comprised a total of 178 cases and 333 controls. The risk of CJD was statistically significantly increased for subjects with a family history of CJD. Further, there was a significant association between the risk of CJD and a history of psychotic disease. Although not significantly increased, there was an elevated risk of CJD for subjects employed as health professionals and subjects ever exposed to cows and sheep. No association could be shown with organ meat consumption including brain. Our re-analysis did not support a number of previously reported associations. The negative results of this re-analysis re-emphasise the absence of a common risk factor in all Creutzfeldt-Jakob patients.

Chapter 3 examines in detail the shared methodologies and general design of the collaborative studies on incidence, risk factors and survival of CJD. First, the common diagnostic criteria applied to all CJD cases included in the collaborative studies are described. Subsequently, case ascertainment in the various participating countries, i.e. Belgium, France, Germany, Italy, the Netherlands and the UK, are addressed with emphasis on the design of

the register in the Netherlands and Belgium. Also the process of data collection, data processing and at last the statistical analysis are outlined for the three collaborative studies.

Chapter 4 presents the descriptive epidemiology of the collaborative studies on CJD in Europe. In the three year time period from 1993 to 1995 570 definite or probable cases of CJD were identified in the collaborative studies with an overall annual mortality rate of 0.73 cases per million person years. Both incidence and mortality figures are described which are approximately similar due to the short mean duration of illness. The mortality rates for CJD were similar in all participating countries despite variations in post mortem rates, and age specific mortality rates were also relatively consistent, with the exception of an increased mortality of CJD in patients aged < 39 years in the UK. In relation to the aetiological subtypes of CJD, 90% of cases were sporadic, 8% genetic and 2% iatrogenic. These incidence data establish baseline epidemiological parameters for CJD in participating European countries, which may be important in the assessment of any future change in the characteristics of CJD as a result of the epidemic of BSE.

In **chapter 5** the results and the analysis of the collaborative European study on risk factors for Creutzfeldt-Jakob disease are described. Four hundred and five patients with definite or probable CJD derived from the population-based studies conducted in the period from 1993 to 1995 were included in the case-control study and compared to 405 hospital controls. We found significant evidence for familial aggregation of CJD as well as familial aggregation of CJD with dementia due to other causes than CJD. A significant increased risk of CJD was found for subjects exposed to domestic birds, leather products and fertilizer consisting of hoofs and horns. Of the dietary factors studied, the consumption of brain and raw meat was associated with an increased risk of CJD. In the period studied, no association could be shown between the risk of CJD and to the consumption of beef, veal, lamb, cheese or milk.

Chapter 6 describes the prognosis of patients with CJD. Clinical characteristics associated with survival may yield important clues for the definition of clinical subgroups in CJD including new variant CJD. Six hundred and six cases with definite or probable CJD were derived from the collaborative European studies on CJD and were included in the survival analysis. Duration of illness was defined from first symptoms to death or censoring date (May 31, 1996). The overall mean survival of CJD patients was six months. Gender, early age at onset and a diagnosis as probable CJD were important prognostic factors. Most clinical findings were not related to survival. In contrast to earlier findings, mortality tended to be significantly increased in patients with a PRNP mutation and/or a positive family history of CJD. Among patients without a PRNP carriers of at least one valine allele were associated with a significantly erduced mortality. The findings do not provide evidence for a clinical subgroup of CJD that relates to survival except for new variant CJD but do highlight the influence of genetic factors in the pathophysiology and prognosis of prion diseases.

Chapter 7 gives a brief overview of the major findings of the collaborative studies on incidence, risk factors and survival of CJD. Subsequently, the methodological aspects of the three studies are discussed separately. Finally the implications of the findings of the individual studies are addressed and we tried to assess their design and analysis. The chapter ends with recommendations for future research, i.e. to continue the epidemiological surveillance of CJD in Europe for many years in order to assess the risk of BSE for humans in relation to the potentially long incubation period of the causative agent.

CHAPTER 9

Samenvatting

HOOFDSTUK 1 van dit proefschrift geeft een kort overzicht van de onderwerpen die zijn bestudeerd in een gemeenschappelijke Europese studie naar incidentie, risicofactoren en prognose van de ziekte van Creutzfeldt-Jakob. Als voorbeeld voor deze gemeenschappelijke Europese studie diende de epidemiologische studie naar de ziekte van Creutzfeldt-Jakob die in de jaren zeventig en het begin van de jaren tachtig door professor Matthews in Engeland en Wales werd uitgevoerd. De studies werden mogelijk gemaakt door subsidie die de Europese Unie in 1993 verleende voor coördinatie van nationale 'surveillances' in België, Frankrijk, Duitsland, Italië, Nederland, Slowakije en het Verenigd Koninkrijk, om eventuele veranderingen in het vóórkomen van de ziekte van Creutzfeldt-Jakob op te sporen die mogelijk zijn gerelateerd aan de 'dolle-koeienziekte'.

Hoofdstuk 2 beschrijft de meta-analyse van drie eerder uitgevoerde patiënt-controle onderzoeken naar risicofactoren voor de ziekte van Creutzfeldt-Jakob. Voor deze meta-analyse werden de ruwe data van de individuele studies, uitgevoerd in de Verenigde Staten, Japan en het Verenigd Koninkrijk, verkregen en opnieuw geanalyseerd. Ondanks de verschillen in methodologie tussen de individuele studies, gebruikten zij alle drie de diagnostische criteria van Masters et al. De gezamenlijke dataset bestond uit 178 patiënten en 333 controles. Het risico op de ziekte van Creutzfeldt-Jakob was statistisch significant verhoogd voor diegenen met een positieve familie-anamnese voor de ziekte van Creutzfeldt-Jakob en voor diegenen met psychose in de voorgeschiedenis. Verder was er een – weliswaar niet significant – verhoogd risico voor personen werkzaam in de medische sector en voor personen met blootstelling aan koeien en schapen. De consumptie van orgaanvlees was niet geassocieerd met een verhoogd risico op de ziekte van Creutzfeldt-Jakob. De overwegend negatieve resultaten van de meta-analyse zijn van belang omdat deze de afwezigheid bevestigen van een gemeenschappelijke risicofactor voor patiënten met de ziekte van Creutzfeldt-Jakob in de periode van 1970 tot 1984.

Hoofdstuk 3 beschrijft in detail de methodologie en de gemeenschappelijke opzet van het Europese onderzoek naar de incidentie, risicofactoren en prognose van de ziekte van Creutzfeldt-Jakob. Allereerst worden de gemeenschappelijke diagnostische criteria beschreven. Verder wordt ingegaan op het opzetten van de nationale registers in de participerende landen, waar alle patiënten met de ziekte van Creutzfeldt-Jakob werden aangemeld. Met name wordt uitgebreid ingegaan op de ontstaanswijze en het functioneren van het register van Nederland en België. Als laatste wordt ingegaan op dataverzameling, dataverwerking en statistische analyse van de drie studies naar incidentie, risicofactoren en prognose van de ziekte van Creutzfeldt-Jakob.

Hoofdstuk 4 geeft de incidentie van de ziekte van Creutzfeldt-Jakob weer. Van 1993 tot 1995 werden in Europa 570 patiënten verzameld met een zekere of waarschijnlijke diagnose van Creutzfeldt-Jakob, resulterend in een mortaliteitscijfer van 0.73 per miljoen persoonjaren. In dit hoofdstuk worden zowel de incidentie- als de mortaliteitscijfers beschreven, die nagenoeg gelijk zijn door de gemiddeld korte ziekteduur. De mortaliteitscijfers waren ook nagenoeg gelijk voor alle participerende landen, ondanks het verschil in obductiepercentage. Ook de leeftijdsspecifieke incidentiecijfers voor alle participerende landen zijn nagenoeg gelijk, met uitzondering van een sterk verhoogde incidentie van patiënten onder de veertig jaar in het Verenigd Koninkrijk. Met betrekking tot de onderverdeling naar etiologie behoorden 90% van de patiënten tot de 'sporadische', 8% tot de 'genetische' en 2% tot de 'iatrogene' vorm van de ziekte van Creutzfeldt-Jakob. De incidentiecijfers van de participerende landen zullen als epidemiologische uitgangswaarden dienen, welke in de toekomst van belang zullen zijn voor het registreren van een mogelijke verandering in het vóórkomen van de ziekte van Creutzfeldt-Jakob.

In **hoofdstuk 5** worden de resultaten van de gemeenschappelijke Europese studie beschreven naar risicofactoren voor de ziekte van Creutzfeldt-Jakob. Vierhonderdenvijf patiënten uit de populatiestudies van de participerende landen werden opgenomen in het patiënt-controle onderzoek en vergeleken met 405 ziekenhuiscontroles. Een statistisch significant verhoogd risico werd gevon-

den voor patiënten met een positieve familie-anamnese voor de ziekte van Creutzfeldt-Jakob en voor patiënten met een positieve familie-anamnese voor dementie van andere origine. Verder werd een verhoogd risico gevonden voor patiënten met blootstelling aan vogels, kunstmest en produkten van leer. Met betrekking tot risicofactoren naar eetgewoonten werd een verhoogd risico gevonden voor de consumptie van hersenen en rauw vlees. Echter de consumptie van rundvlees, kalfsvlees, lamsvlees, melk of kaas was niet geassocieerd met een verhoogd risico voor de ziekte van Creutzfeldt-Jakob.

Hoofdstuk 6 beschrijft de studie naar prognostische factoren van patiënten met de ziekte van Creutzfeldt-Jakob. Zeshonderdenzes patiënten met de zekere of waarschijnlijke diagnose van Creutzfeldt-Jakob werden opgenomen in de studie. De mediane ziekteduur, gedefinieerd van het moment van eerste symptomen tot de dood of 'censoring' datum op 31 mei 1996, was gemiddeld vier maanden. Geslacht, een leeftijd onder de 40 jaar en classificatie als een waarschijnlijksdiagnose van de ziekte van Creutzfeldt-Jakob waren belangrijke prognostische factoren. Klinische verschijnselen waren niet gerelateerd aan overleving. In tegenstelling tot eerdere bevindingen vonden wij een verhoogde mortaliteit bij patiënten met een mutatie van het prion-gen en/of een positieve familie-anamnese voor de ziekte van Creutzfeldt-Jakob. Tevens werd een langere overleving gevonden voor patiënten zonder mutatie van het prion-gen die drager zijn van ten minste één valine-allel op codon 129. Deze bevindingen tonen aan dat er behoudens de nieuwe variant van de ziekte van Creutzfeldt-Jakob geen andere klinische subgroep van de ziekte van Creutzfeldt-Jakob bestaat die is geassocieerd met overleving. Verder benadrukken deze resultaten de rol van genetische factoren in de pathofysiologie en prognose van de ziekte van Creutzfeldt-Jakob.

Hoofdstuk 7 geeft in het kort de belangrijkste bevindingen weer van de gezamenlijke studies naar incidentie, risicofactoren en prognose van de ziekte van Creutzfeldt-Jakob. Verder worden de methodologische aspecten van de drie studies afzonderlijk geëvalueerd. Tevens worden de implicaties van de bevindingen

van de afzonderlijke studies besproken en aanbevelingen gedaan voor verder epidemiologisch onderzoek naar de ziekte van Creutzfeldt-Jakob. Tot slot wordt nogmaals het belang uiteengezet van het continueren van het onderzoek, gezien de mogelijk lange incubatietijd van het infectieuze agens.

Dankwoord

De Europese studie zoals beschreven in dit proefschrift werd geïnitieerd door Dr. R.G. Will en Prof. A. Hofman. De Europese studie is tot stand gekomen dank zij de welwillende medewerking van neurologen en neuropathologen in het Verenigd Koninkrijk, Frankrijk, Duitsland, België, Italië, Nederland en Slowakije.

Allereerst zou ik mijn dank willen betuigen aan de familieleden van patiënten, die vaak op moeilijke momenten hun medewerking verleenden aan het onderzoek.

Mijn twee promotoren, Prof. dr A. Hofman en Prof. dr F.G.A. van der Meché, ben ik dank verschuldigd voor de wijze waarop zij mij hebben begeleid bij het uitvoeren van het onderzoek en het schrijven van het manuscript. Ondanks hun drukke werkzaamheden waren zij bereid tijd vrij te maken voor waardevolle opmerkingen en verbeteringen.

De dagelijkse begeleiding van het onderzoek was in handen van Cock van Duijn, die mij op sleeptouw nam in het land van manuscriptenschrijven en BMDP. Haar vele waardevolle suggesties en ideeën zijn overal in dit proefschrift terug te vinden.

The very first people to investigate the epidemiology on a nationwide basis were the neurologists Professor W.B. Matthews and his successor Dr R.G. Will. Their surveillances during the seventies and eighties in the UK enabled us to entertain the concept of the ongoing European study. Bob, you instilled in me my first enthusiasm for neurology and epidemiology of Creutzfeldt-Jakob disease. Thank you for your support in times of adversity and for your advice when needed.

Voor het totstandkomen van het Nederlands en Vlaams register ben ik veel dank verschuldigd aan Gerard Jansen, die immer bereid was voor een obductie te pleiten of deze desnoods zelf uit te voeren. Beste Gerard, ik hoop dat je ondanks je drukke werkzaamheden tijd overhoudt voor deze 'hobby'.

I am grateful to John Collinge for performing all prion protein analyses for the Dutch/Flemish registry.

Pim van Gool wil ik graag bedanken voor de levendige discussies over prionen.

I would like to thank all contributors from the various participating countries of the European study for the fruitful collaboration: Nicole Delasnerie-Lauprêtre, Annick Alpérovitch, and Jean-Philippe Brandel from France; Mauricio Pocchiari and Carlo Masullo from Italy; Sigrid Poser, Inga Zerr, Thomas Weber, and Maria Lantz from Germany; Eva Mitrovà from Slovakia; Jan Mackenzie, Rajith da Silva, Martin Zeidler, James Ironside, and Jane Bell from the UK, and – last but not least – Paul Brown from the US.

Voordat ik mijn naaste collega's van de afdeling Epidemiologie zal noemen, wil ik eerst Hanneke den Breeijen, René Vermeeren en Michael Koenders bedanken, omdat zij altijd beschikbaar waren bij netelige computer-, programmeer- of BMDP-problemen. Elly van der Heiden was stoïcijns in het corrigeren en invoeren van de data — dank je wel!

Bettina, Carolien en Carl wil ik bedanken voor de vele relativerende (telefoon-)gesprekken. De eindeloze thee- en koffie-uurtjes met de buren van Ee 2130 en 2132, Maarten, Sesmu, Daniëlle, Anske, Marianne, Paul, Sandra en Alewijn, mis ik al. Sandra, bedankt voor het kritisch doorlezen van het boekje. Ik hoop dat er nog vele buitenlandse congressen voor ons samen in het verschiet liggen.

Ik ben zeer blij met mijn twee paranimfen Tanneke en Pa, en met Sjoerd als 'usher', omdat zij de afgelopen jaren lief en leed met mij hebben gedeeld. Lida, met jou deel ik de dubbele geboorte van jullie twee kruimeltjes en mijn boekje.

Marieke Visser wil ik als dubbele collega noemen, omdat onze wegen elkaar in neurologenland al vaak hebben gekruist en, naar ik hoop, dat nog vaak zullen doen.

Hans van Krimpen en de verpleging van afdeling 6 Noord wil ik bedanken voor het begrip voor de vele onbereikbare uurtjes in de namiddag en voor het achterhouden van tompoezen.

Anna, Ed en Iris, door jullie betrokkenheid is dit proefschrift 'het boekje' geworden.

Pa, ma en tante Chris wil ik ten slotte bedanken voor de mogelijkheden die zij mij hebben geboden om steeds mijn keuzes te kunnen verwezenlijken.

Curriculum vitae

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