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ORIGINAL COMMUNICATION

Heightened incidence of sporadic Creutzfeldt-Jakob disease is associated with a shift in clinicopathological profiles

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M. Glatzel and A. Aguzzi coordinated the design and operation of the study. Katharina Stoeck and Klaus Hess were involved in clinical assessment of patients. M. Glatzel and Dieter Zimmermann were involved in assessment of specimen. All authors contributed to the manuscript and approved the final version. M. Glatzel and A. Aguzzi had full access to all data in the study and had final responsibility for the decision to submit for publication.

The study was performed according to established ethical guidelines

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Abstract Incidences of human transmissible spongiform encephalopathies are monitored by national registries in the majority of countries in Western Europe. During the past 13 years incidences for Creutzfeldt-Jakob disease (CJD) in Switzerland fluctuated between 0.4 and 2.63 cases/10^6 inhabitants. We have compared clinicpathological patient profiles including geographic and gender distribution, age at disease onset, duration of disease, clinical symptoms, and recognized or hypothetical risk factors for CJD, genetic risk factors, biochemical and histopathological data for two cohorts of Swiss sporadic CJD patients from years of regular sporadic CJD incidence (1996–2000, mean incidence 1.3 cases/10^6 inhabitants, n = 47) to Swiss sporadic CJD patients from years of elevated sporadic CJD incidence (2001–2004, mean incidence 2.3 cases/10^6 inhabitants, n = 73). Sporadic CJD patients from the cohort with elevated sporadic CJD incidence presented with a higher frequency of rare sporadic CJD subtypes. Patients of these subtypes were significantly older and showed a skewed male/female ratio when compared to published patients of identical sporadic CJD-types or to patients from the 1996–2000 cohort and indicates that improved detection of rare sporadic CJD subtypes may have contributed to increased incidence.

Key words Creutzfeldt-Jakob disease · prions · dementia · epidemiology

Introduction

Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative diseases affecting both humans and animals [23]. Neuropathologically, they are characterized by spongiosis, gliosis, neuronal loss and the accumulation of an aberrantly folded isoform of the normal cellular prion protein, termed PrP^{Sc}, which is an essential component of the infectious agent [22]. The most common human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD) comprises about 85% of all human prion diseases and is of unknown origin. sCJD may present with a marked clinical heterogeneity. By combining clinical features with histopathological analysis, the status of a polymorphism on the gene encoding the prion protein as well as biochemical analysis of PrP^{Sc}, several sCJD types may be differentiated [14, 19].

About 15% of human prion diseases are caused by known mutations in the prion protein gene (*PRNP*) and may be inherited as autosomal dominant traits [15]. A further subset of human prion diseases are acquired by exposure to infectious prions, in the framework of neurosurgical interventions or through hormone substitution, and are referred to as iatrogenic CJD [2]. A novel human prion disease, variant CJD (vCJD), is thought to be caused by exposure to BSE prions via uptake of BSE-contaminated material or by transfusion of vCJD contaminated blood products [3, 13, 20, 29].

The appearance of vCJD and its development into an epidemic in the UK has led to the establishment of CJD surveillance centres among European countries which carry out active surveillance according to standardized protocols in order to identify and monitor the epidemiology of human prion diseases (http://www.eurocjd.ed.ac.uk/).

In 1995, the Swiss National Reference Centre of Prion Diseases (NRPE) was established and active CJD surveillance has bee conducted since 1996. This includes clinical and epidemiological assessment of patients, genetic analysis, as well as pathological and biochemical analysis of tissue specimens [25].

From 1996 to 2000, sCJD affected between seven to 11 patients per year, corresponding to an annual incidence of 1.0 to 1.4 patients per million per year which is well in line with the presumed global incidence of sCJD, about one patient per million per year [11]. However, in 2001 the number of sCJD patients increased to 18, translating to an incidence of 2.5 patients per million per year [10]. In the subsequent three years, the incidence remained elevated, ranging from 1.4 to 2.0 per million per year (Fig. 1).

The aim of the present study was to compare clinical, genetic, biochemical and pathological features of sCJD in two cohorts of patients. The first cohort comprises patients from the years 1996 to 2000 with an average incidence of sCJD of 1.3 per million per year, whereas the second cohort comprises patients from the years 2001 to 2004 with an average incidence of sCJD of 2.3 per million per year.

Patients and methods

Patients

In this study, 120 sporadic CJD patients, who were reported to the Swiss National Reference Centre of Prion Diseases (NRPE) and to the Swiss Federal Office of Health between 1996 and 2004, were included. Clinical information including assessment was carried out according to standardized protocols. We collected tissues at necropsy from patients according to established safety and ethical guidelines [4]. The group comprises 109 'definite', neuropathologically-proven, and 11 'probable' CJD patients, corresponding to a proportion of 'definite

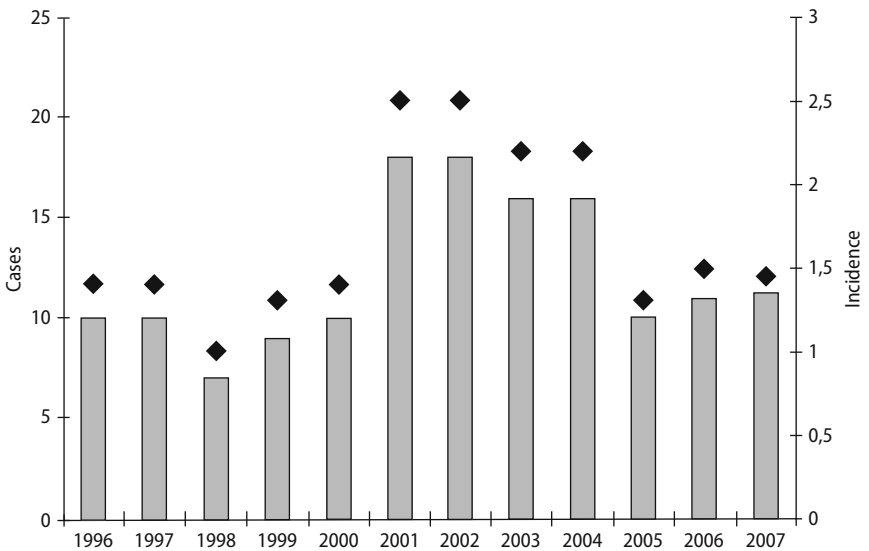

Fig. 1 Incidence of sCJD from 1996–2007 in Switzerland. From 1996 to 2000, the number of sporadic CJD patients (grey bars) ranged from 8 to 11 per year, corresponding to an annual incidence of sCJD deaths (black diamonds) of 1.3–1.4 cases per million. From 2001 to 2004, the incidence has increased to 2.2–2.5 cases per million per year

CJD' of 91 %. Clinical diagnosis of 'probable CJD' was carried out according to established criteria [8].

Technical investigation

Cerebrospinal fluid (CSF) samples from suspected CJD patients were sent from notifying hospitals. The 14-3-3 test was performed according to an established protocol [31]. Original electroencephalogram (EEG) recordings were provided from notifying hospitals and evaluated for the presence of periodic sharp wave complexes (PSWC) using standardized criteria [26]. Magnet resonance imaging (MRI) scans were analysed for detection of hyperintense basal ganglia on T2-/FLAIR and diffusion-weighted images (DWI).

Genetic analysis

Genetic analysis of *PRNP* was performed on genomic DNA isolated from blood or brain tissue according to standard procedures [30]. In order to exclude disease-associated mutations and to determine the methionine/valine polymorphism on codon 129 the entire open reading frame of the protein was sequenced.

Neuropathological examination

Specimen from the following brain regions were examined: frontal, parietal, occipital, and temporal cortex, putamen, thalamus, midbrain, medulla oblongata and cerebellum. Neuropathological examination included assessment of spongiosis, neuronal loss, gliosis and detection of PrP by immunohistochemistry.

Immunohistochemistry

Conventional immunohistochemical staining was performed on formalin-fixed, paraffin embedded tissue. Sections were cut (3 µm), following deparaffinization and pre-treatment including hydrolytical autoclaving for 30 min at 121 °C, formic acid for 2.5 min and guanidinium thiocyanate for 30 min at 4 °C. For detection of PrP^{Sc}, sections were then probed with monoclonal anti-PrP antibody 3F4 according to published methods [25].

Western blot analysis

Western blot analysis for detection of PrP^{Sc} in brain samples of sCJD patients was performed according to published protocols [21]. PrP^{Sc} was typed according to the size of protease-resistant unglycosylated PrP^{Sc} fragment and designated as either PrP^{Sc} type 1 (unglycosylated fragment running at 21 kDa) or PrP^{Sc} type 2 (unglycosylated fragment running at 19 kDa) [5, 7, 19].

Triplot of glycoform profiles

Glycoform ratios were compared in a triangular plot correlating the intensities of diglycosylated, monoglycosylated and unglycosylated bands of PrP^{Sc} according to published protocols [25]. Densitometric analysis of PrP glycoforms was performed using a one-dimensional software analysis program (Quantity One, Biorad).

Statistical analysis

The two cohorts were compared by Student's t-test for independent samples with unequal variances and chi square test. P-values of < 0.05 were considered significant. As statistical software for calculations, Epi Info(tm) (Version 3.4) and SPSS® (Version 13) were used.

Results

Incidence of sCJD in Switzerland from 1996 to 2007

Supplementary Table 1 shows the yearly incidence of sporadic, genetic and iatrogenic CJD from the years 1996 to December 2007. The mean incidence of sCJD in the period 1996 to 2000 was 1.290 cases/10^6 inhabitants (95 % C.I. 1.062–1.517) which was significantly higher (p-value 0.000; Wilcoxon rank-sum test) than the mean incidence in the period 2001 to 2004 of 2.318 cases/10^6 inhabitants (95 % C.I. 2.037–2.599). By including the most recent sCJD incidence data into this analysis, the differences between mean incidences of sCJD from 1996–2000 to 2001–2007 were still statistical significante (from 2001 to 2007, the mean incidence of sCJD was 2.009 cases/10^6 inhabitants (95 % C.I. 1.592–2.426), p-value of 0.0185 (Wilcoxon rank-sum test)).

Patient characteristics of the cohorts 1996–2000 and 2001–2004

From 1996–2000, 47 sCJD patients were identified, 46 patients were classified as definite and one as probable sCJD. 26 of them were male and 21 were female resulting in a male to female ratio of 1.1:1. From 2001–2004, 73 sCJD patients were diagnosed, 63 definite and 10 probable sCJD (Fig. 1). 45 of them were male and 28 were female resulting in a male to female ratio of 1.6:1 (Fig. 2 A, B). The overrepresentation of male sCJD patients for the years 2001–2004 is statistically significant ($p < 0.036$, chi square test) when compared to the age-matched normal population of Switzerland (Odds ratio 2.05; 95 % CI 1.22–3.46; $p = 0.037$, chi square test).

Mean age at onset and disease duration

Table 1 presents collective data on age at onset and disease duration of both patient cohorts substratified by sCJD subtypes. From 1996–2000, in depth data on molecular CJD subtypes was available in 25 of 47 patients and from 2001–2004 in 60 of 73 patients.

From 1996–2000, the mean age at onset of disease in sCJD patients was 66 years ($n = 47$, standard deviation (SD) ± 9 years). Age at onset varied between CJD subtypes: MM1 patients presented the youngest group with a mean age at onset of 65 years ($n = 18$, SD ± 8 years). Patients of the other groups were older: VV2 and MV2 subtypes showed a mean age at disease onset of 68 ($n = 1$) and 69 ($n = 3$) years, whereas for patients of the MM2 and MV1 subtype, age at onset was 72 ($n = 1$) and 77 ($n = 2$) years.

Mean disease duration in this time period was 6 months (SD ± 5 months). Disease duration varied among

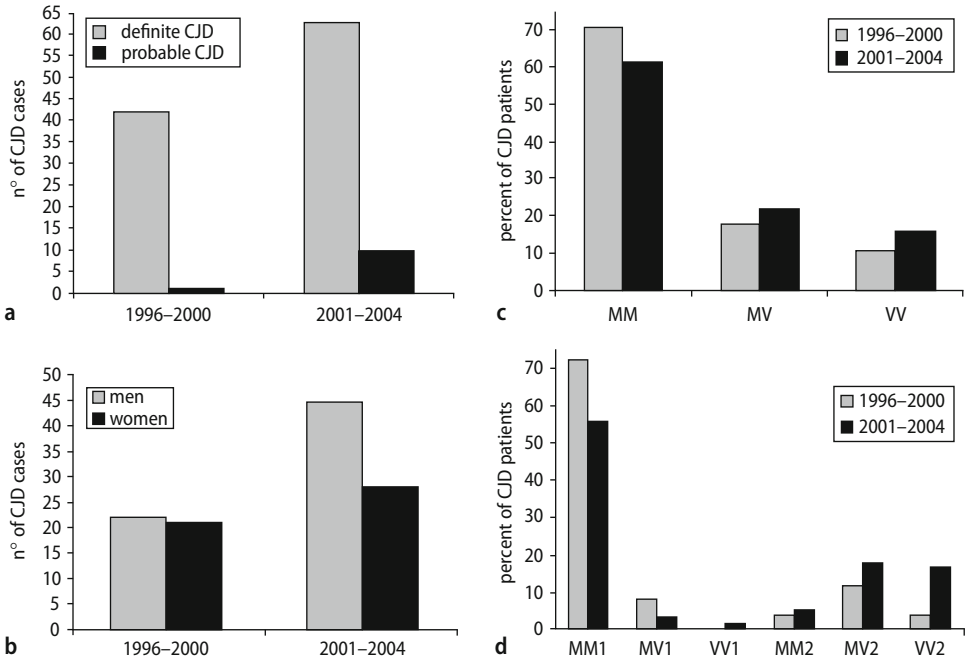

Fig. 2 Clinical characterization of sCJD patients from 1996–2000 and 2001–2004 (n = 120). Shown are demographic characteristics of two sCJD cohorts (1996 to 2000 and 2001 to 2004). **A** Ratios of identified probable to definite sCJD patients from 1996–2000 (n = 47) compared to 2001–2004 (n = 73). **B** Gender ratios of CJD patients from 1996–2000 and 2001–2004. Note: Increase in male to female ratio (1.6:1) 2001–2004 when compared to the 1996–2000 cohort or other published cohorts. **C** Distribution of the polymorphism at *PRNP* codon 129 (n = 91). sCJD patients are predominantly homozygous for methionine (70 %) or valine (18 %) [1]. Note: Higher frequency of MV and VV patients in the years 2001–2004 when compared to the 1996–2000 cohort or other published cohorts. **D** Analysis of the molecular CJD subtype based on PrP^{Sc} type and codon 129 genotpye (n = 90): Higher frequency of the MV2 and VV2 subtype in 2001–2004 when compared to 1996–2000 or other published cohorts [12, 19]

Table 1 Clinical characteristics of Swiss CJD patients from 1996–2000 and 2001–2004

Time range	Subtypes	n°of cases (% of total)			Mean age at disease onset (years) (range; ± SD)			Mean disease duration (month) (range; ± SD)		
		total	m	f	total	m	f	total	m	f
1996–2000	MM1	18 (72)	6	12	65 (48–80; 8)	67	64	5 (2–27; 6)	3	5
	MV1	2 (8)	1	1	77 (77; 0)	77	77	8 (3–12; 6)	12	3
	MV2	3 (12)	3	0	69 (54–81; 14)	69	–	10 (2–21; 10)	10	–
	VV2	1 (4)	1	0	68	68	–	4	4	–
	MM2	1 (4)	0	1	72	–	72	5	–	5
2001–2004	MM1	33 (55)	18	13	70 (41–82; 9)	69	70	4 (1–11; 2)	4	4
	MV1	2 (3)	1	1	71 (60–83; 16)	83	60	3 (3–4; 1)	4	3
	VV1	1 (1.7)	1	0	63	63	–	4	4	–
	MV2	11 (18.3)	8	3	69 (48–84; 11)	66	76	11 (2–28; 7)	11	11
	VV2	10 (16.7)	7	3	66 (47–81; 10)	68	62	7 (4–14; 3)	7	9
	MM2	3 (5)	2	1	64	71	49	7 (49–72; 13)	9	4

CJD subtypes. The shortest disease course was observed in the MM1 (5 months, SD ± 6 months) and VV2 subgroup. Patients with MV1 and MV2 phenotype had longer disease durations of 3 and 12 months for MV1 and 10 months for MV2.

In the 2001–2004 cohort, the mean age at disease onset for sCJD patients was 68 years (n = 73, SD ± 10 years). Patients of the MM1 subgroup had a mean age at disease onset of 70 years (n = 33, SD ± 9 years), whereas patients belonging to the MV2 and VV2 subgroup of sCJD patients had a mean age at disease onset of 69 years (n = 11, SD ± 11 years) and 66 years (n = 10, SD ± 10 years), respectively.

Mean disease duration in the years 2001–2004 was 6 months (SD ± 6 months). MM1 and VV2 patients had short disease durations of 4 months (SD ± 2 months)

and 7 months (SD ± 3 months), whereas MV2 patients presented with the longest disease duration of 11 months (SD ± 7 months).

Among all atypical cases combined (i.e. VV1, MM2, MV2 and VV2 subtypes), mean age at onset was 67.3 years (n = 30). In the 1996–2000 cohort (n = 5) it was 69.4 years, and in the 2001–2004 cohort (n = 25) it was 67.0 years.

Detailed patients characteristics of the sCJD cohort 2001–2004

A detailed analysis, including clinical signs and results of technical investigations was carried out for the 2001–2004 cohort. Collective data were available in 51 of 73 patients on clinical signs (neurological and psychiatric) and in 53 of 73 patients on results of technical investigations. We did not find evidence for regional clustering or iatrogenic and zoonotic exposure in this group of patients when assessing recognized or hypothetical risk factors for CJD (data not shown).

Neurological and psychiatric signs

Dementia was the most common clinical sign in all subgroups (not listed) followed by focal neurological features such as cerebellar signs, myoclonus, pyramidal and eyxtapyramidal signs, presence of primitive reflexes and akinetic mutism (Table 2). MM1 patients presented mainly with rapid progressive dementia, cerebellar signs, myoclonus and akinetic mutism. MV2 patients showed dominantly extrapyramidal and early cerebellar signs, whereas VV2 patients were mainly characterized by late-onset dementia and early cerebellar signs.

Psychiatric symptoms were frequent in all subtypes. Patients of all subgroups were similarly affected by visual hallucination and anxiety. Depression, low mood and apathy were more frequent in MM1 patients than in MV2 or VV2 patients, whereas these patients showed a higher frequency of delusion and aggressive behaviour than MM1 patients.

Results from technical investigations

A variety of technical investigations support the clinical diagnosis of sCJD. However, the sensitivity of CJD-typical findings in different investigations, such as MRI (hyperintense basal ganglia), cerebrospinal fluid analysis (elevation of the 14-3-3 protein) or EEG (PSWC) is limited. In our cohort, CSF analysis was the most sensitive test in all investigated groups with an overall sensitivity of 91.5 % (Table 3). The overall sensitivity of EEG was limited. The highest sensitivity for this examination was 66.7 % for the MM1 group of patients. Sensitivities for patients of the MV2 and VV2 subtype of sCJD were 18 % and 10 %, respectively. Typical MRI changes were mainly observed in MV2 (75 %) and in MM1 (60 %) patients, respectiely, whereas in VV2 patients, CJD-typical MRI changes were less frequently (40 %).

By analysing CJD-typical MRI signs in CJD patients from 2001–2004 including novel imaging methods, such as diffusion-weighted imaging (DWI), we observed an increase of the ratio of CJD-typical MRI in the years 2001–2004 (Fig. 3).

Table 2 Neurological and psychiatric signs in Swiss sCJD patients 2001–2004

sCJD subgroup	MM1 (n = 30)	MV2 (n = 11)	VV2 (n = 10)
Frequency of neurological signs (%)			
cerebellar signs	83.3	81.8	80
myoclonus	76.7	54.5	80
primitive reflexes	71.4	36.4	70
extrapyramidal signs	60	90.9	60
akinetic mutism	63.3	54.5	50
visual disturbances	43.3	36.4	30
dizziness	40	63.6	60
other involuntary movements	33.3	45.5	20
oculomotoric signs	30	9.1	50
pyramidal signs	23.3	18.2	40
seizures	13.3	0	10
headache	16.7	9.1	30
pain	10	9.1	10
other sensory disturbances	16.7	9.1	10
pseudobulbar signs	7.1	45.5	30
neurogenic muscle wasting	3.6	0	10
Frequency of psychiatric signs (%)			
visual hallucination	41.9	54.5	40
anxiety	29.0	36.4	30
depression	25.8	9.1	10
low mood and apathy	25.8	9.1	20
delusion	19.4	36.4	20
aggression	16.1	27.3	40
social withdrawal	12.9	18.2	10

Genetic analysis of sCJD patients

Genetic analysis, which included sequencing of *PRNP* and determination of a polymorphism on codon 129, was carried out in 91 patients (1996–2000 cohort, 28 of 47 sCJD patients; 2001–2004 cohort, 63 of 73 patients).

In the group of patients from 1996–2000, 20 patients (71.4 %) were homozygous for methionine (MM), 5 patients (17.9 %) were heterozygous for methionine and valine (MV) and 3 patients (10.7 %) were homozygous for valine (VV).

In the group of patients from 2001–2004, 39 patients (62 %) were MM, 14 patients (22.2 %) were MV and 10 patients (15.9 %) were VV (Fig. 2 C).

Table 3 Sensitivity of diagnostic tests in CJD subtypes from 2001–2004 (in %)

CJD subtype	n	typical EEG[a]	abnormal EEG[b]	normal EEG	14-3-3 positive	14-3-3 trace	14-3-3 negative	typical MRI[c]	abnormal MRI[d]	normal MRI
MM1	27	66.7	33.3	0	85.2	3.7	11.1	62.1	20.7	17.2
MV1	2	0	100	0	100	0	0	0	100	0
VV1	1	0	0	100	100	0	0	100	0	0
MM2	2	0	100	0	100	0	0	50	50	0
MV2	11	18.2	63.6	18.2	100	0	0	75.0	25.0	0
VV2	10	10.0	50.0	10	100	0	0	44.4	33.3	22.2
Total	53	40.4	51.9	7.7	91.5	2,1	6.4	60.0	26.0	14.0

[a] generalized PSWC; [b] mild to moderate EEG changes, diffuse slowing; [c] hyperintense signal in basal ganglia and /or cortical; [d] cerebral atrophy

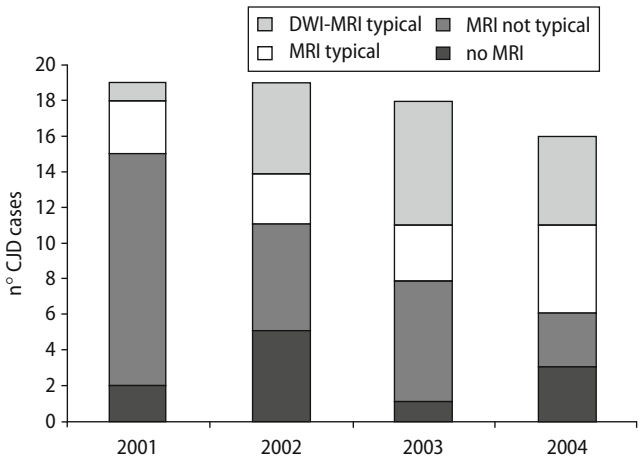

Fig. 3 Ratios of MRI results in the years 2001–2004. Shown is the temporal development of imaging results in sCJD patients. Note: increase in CJD-typical MRI over time may be attributed the introduction of (DWI)-MRI

Molecular sCJD subtypes

The molecular sCJD subtype, based on the combination of codon 129 genotype and PrP^{Sc} type in Western blot and neuropathological analysis, could be assessed in 85 sCJD patients (25 of 47 for the 1996–2000 cohort, 60 of 73 for the 2001–2004 cohort, Table 1).

The most prominent sCJD subtype in the patient group from 1996–2000 was MM1 (72 %, n = 18), followed by MV2 (12 %, n = 3), MV1 (8 %, n = 2) and solitary cases of MM2 (4 %) and VV2 (4 %). From 2001–2004, the ratio in the subtype distribution changed. MM1 was again the most frequent subtype with 55 % (n = 33) of the patients, followed by MV2 (8.3 %, n = 11), VV2 (16,7 %, n = 10), MM2 (5 %, n = 3) as well as cases of MV1 (3 %, n = 2) and VV1 (1,7 %, n = 1). Interestingly, there was a significant increase of atypical cases (VV1, MM2, MV2 and VV2) in the 2001–2004 cohort (43 %) when compared to the 1996–2000 cohort (20 %; n = 83 odds ratio 3.03; 95 % CI 2.47–3.72; $p < 0.001$, chi square test). Results were similar when taking into account all patients (including the non-specified sCJD subtypes): the ratio increased from 11 % atypical vs. 43 % typical cases in the 1996–2000 cohort to 34 % atypical vs. 45 % typical cases in the 2001–2004 cohort (n = 120; odds ratio 3.04; 95 % CI 2.34–3.96; $p < 0.001$, chi square test).

Neuropathological analysis of above mentioned patients confirmed published data [14]. Patients belonging to the MM1 group showed synaptic and perivacuolar PrP deposition, whereas in MV1 and MV2 patients synaptic and plaque like deposits could be identified. In patients belonging to the VV2 group, PrP was deposited in band-like arrangements, perineurally and plaque-like (Fig. 4).

Triplot analysis of glycoform ratios

Glycoform ratios of sCJD patients from 1996–2000 and from 2001–2004 were compared in a triangular plot correlating the intensities of the diglycosylated, monoglycosylated and unglycosylated bands of PrP^{Sc} (Fig. 5). Patients with sCJD from 1996–2000 and 2001–2004 cluster in the same area of the plot. Non-Swiss control patients of various sCJD subtypes show similar glycoform ratios, whereas patients with vCJD segregate in a distinct region of the plot.

Discussion

From 2001–2004, Switzerland has been reporting an increased incidence of sCJD patients, when compared to earlier years of active CJD surveillance, raising questions about the origin of this phenomenon [9, 10]. In this study, we analysed two sCJD cohorts. The first cohort comprised sCJD patients from a period with "regular" sCJD incidence (1996 to 2000). The second cohort com-

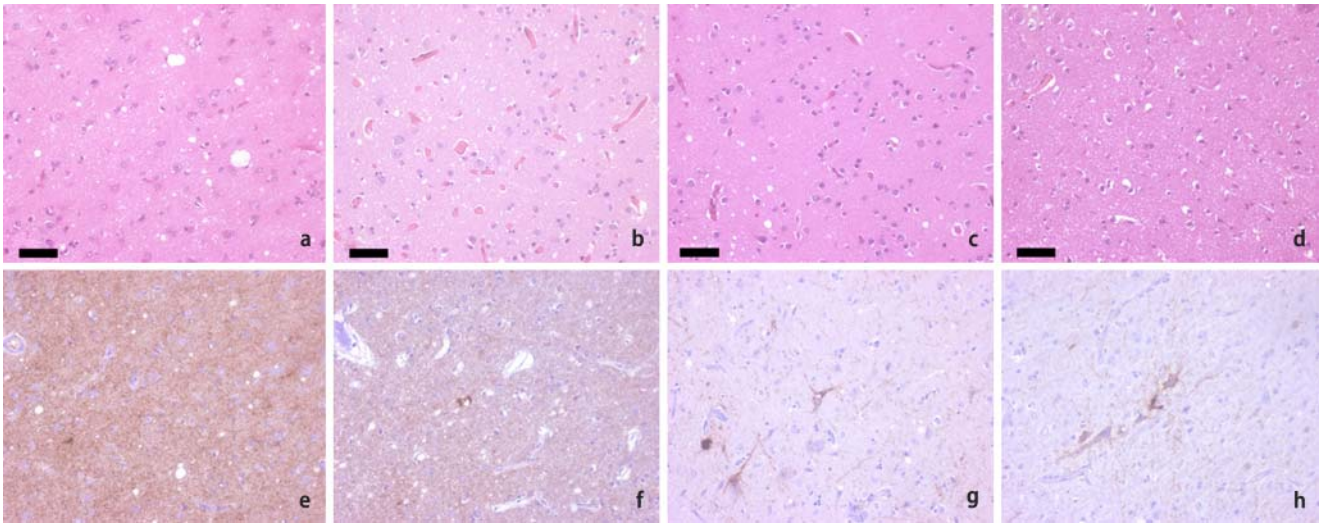

Fig. 4 Histopathological findings in frontal cortex of sCJD patients. HE stain (**A–D**) and PrP immunostain (**E–H**) shows spongiform changes and CJD-type specific PrP deposits. sCJD patients of the MM1 group show synaptic PrP deposition (**E**), whereas MV1 patients and MV2 patients show additional plaque like or perineural PrP deposits (**F, G**). In patients belonging to the VV2 group, PrP deposits in band-like arrangements and perineurally (**H**)

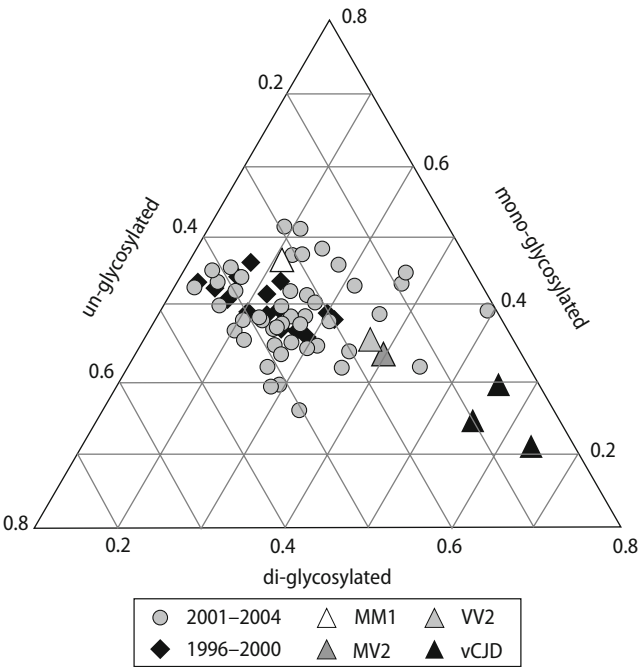

Fig. 5 Glycoform profiles of patients with sCJD. The triangular plot correlates the intensities of the diglycosylated (upper), monoglycosylated (middle), and unglycosylated (lower) bands of PrP^{Sc}. Patients with sCJD from 1996–2000 (black diamond) and 2001–2004 (gray circles) as well as controls (MM1, MV2 and VV2 patients: white, dark grey and light grey triangle) cluster in the same area of the plot. Instead, control patients with vCJD (black triangle) are segregated in a distinct region of the plot

prised sCJD patients from a period with elevated sCJD incidence (2001–2004). We compared differences in clinicopathological profiles including CJD subtypes between both cohorts and to published data [14, 19]. In an initial analysis, we formulated several hypotheses concerning the increase in sCJD in Switzerland [9]. One hypothesis comprised that it might be due to statistical fluctuation. We consider this an unlikely explanation for the observed increase in incidence since the rise in incidence between the 1996–2000 and 2001–2004 cohorts was statistically significant. Yet, this possibility cannot be dismissed unequivocally given that the statistical significance is marginal if most recent incidence data (2001–2007) are included in the analysis.

Another idea implied that the heightened incidence might be related to ascertainment bias due to a temporarily heightened awareness of the disease. In the most recent years, the observed incidence in sCJD deaths (2005: 10 cases; 2006: 11 cases, 2007: 15 cases) dropped to levels just slightly above the 1996–2000 period (http://www.eurocjd.ed.ac.uk/allcjd.htm). The recent development may reflect decreased awareness of prion diseases in the general public due to decreased media coverage in recent years. The hypothesis of ascertainment bias is further supported by data from European countries reporting increased CJD incidences related to heightened awareness due to the introduction of novel diagnostic tools, such as measurement of protein 14-3-3 in the CSF [18, 24]. Typically, increased incidence of CJD attributed to heightened awareness goes along with an increase in the median age of CJD patients [28]. This is believed to be caused by more frequent recognition of CJD in elderly demented patients. Although the mean age at onset for the Swiss sCJD cohort 2001–2004 is similar when compared to that of 1996–2000, it is obvious that Swiss sCJD patients of the rare, and clinically-atypical, sCJD subtypes were on average 5 years (VV2) to 10 years (MV2) older than those from large published sCJD co-

horts [19, 32]. Although these data do not clearly speak in favour of an ascertainment bias, the fact that the 2001–2004 cohort of sCJD patients showed a higher frequency of the less common MV2 subtype when compared to the 1996–2000 cohort and to previous publications, in concert with the fact that there is a significant increase in the median age of this patient group is remarkable [12, 19, 32]. This observation might be of particular clinical interest as it highlights the possibility of less common variants such as the MV2 subtype, in older patients with atypical clinical presentations.

A genetic origin for the rise in CJD incidence seems unlikely as there is no increased incidence of genetic CJD from 2001 onwards. Finally, an acquired origin of the rise in incidence, in the framework of iatrogenic or zoonotic transmission which had initially been considered, remains an unlikely option, since neither a iatrogenic nor a zoonotic exposure is evident and we did not observe regional clustering.

Other differences between the two cohorts which do not support or dismantle any of the hypotheses mentioned above, relate to gender distribution. In 2001–2004 the male to female ratio was (1.6:1), whereas the male to female ratio in the years 1996–2000 was (1.1:1) which is similar to that of published studies [32]. Interestingly, when analysing the gender distribution in different CJD subtypes from 2001–2004, we observed an over-representation of the MV2 and VV2 subtype in male (70 %) versus female patients (30 %), whereas this phenomenon could not be observed in MM1 patients.

Results in routine investigations in patients from 2001–2004 were similar to those previously described [6, 17]. CSF analysis was the most sensitive test in all investigated groups with an overall sensitivity of 91.5 %. Interestingly, all MV2 and VV2 patients showed a positive 14-3-3 test. This is surprising, as lower 14-3-3 test sensitivities have been described in these subtypes in recent publications [6, 16]. Typical MRI changes were mainly observed in MV2 (75 %) and in MM1 patients (60 %), while in VV2 patients, CJD-typical MRI changes were less frequent (40 %). These results are again comparable to published data and support the importance of MRI as a diagnostic tool for the detection of less common CJD subtypes such as MV2 [16, 27]. Additionally, we show a tendency towards improved diagnostic specificity in sensitive imaging methods, such as DWI-MRI, for the years 2001–2004. This is in agreement with recent studies stressing the importance of sensitive imaging methods in the early diagnosis of sCJD [27]. In summary, this analysis confirms our initial finding of an increased incidence of sCJD in Switzerland. Although, the reason for this phenomenon remains unexplained to date, our analysis demonstrates that patients from the years 2001–2004 with increased sCJD incidence differ in several aspects from published sCJD cohorts. The fact that the MV2 subgroup of patients showed an increase in mean age at disease onset when compared to published cohorts, together with the fact that these patients demonstrate distinct features in sensitive imaging methods, may indicate that improved detection of these patients has contributed to the rise in sCJD incidence. Further studies investigating biochemical and genetic aspects will contribute to our understanding of the mechanisms underlying sCJD.

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