Association between deposition of beta-amyloid and pathological prion protein in sporadic Creutzfeldt-Jakob disease

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Abstract

BACKGROUND: Alzheimer's disease (AD) and prion diseases such as sporadic Creutzfeldt-Jakob disease (sCJD) share common features concerning their molecular pathogenesis and neuropathological presentation and the coexistence of AD and CJD in patients suggest an association between the deposition of the proteolytically processed form of the amyloid precursor protein, beta-amyloid (Abeta), which deposits in AD, and the abnormal form of the prion protein, PrP(Sc), which deposits in sCJD.

METHODS: We have characterized sCJD patients (n = 14), AD patients (n = 5) and nondemented controls (n = 5) with respect to the deposition of PrP(Sc) and Abeta morphologically, biochemically and genetically and correlated these findings to clinical data.

RESULTS: sCJD-diseased individuals with abundant deposits of Abeta present with a specific clinicopathological profile, defined by higher age at disease onset, long disease duration, a genetic profile and only minimal amounts of PrP(Sc) in the cerebellum.

CONCLUSION: The co-occurrence of pathological changes typical for sCJD and AD in combination with the inverse association between accumulation of Abeta and PrP(Sc) in a subgroup of sCJD patients is indicative of common pathways involved in the generation or clearance of Abeta and PrP(Sc) in a subgroup of sCJD patients.
Association between Deposition of Beta-Amyloid and Pathological Prion Protein in Sporadic Creutzfeldt-Jakob Disease

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\section*{Key Words}
Sporadic Creutzfeldt-Jakob disease • Alzheimer’s disease • Deposition of \(\beta\)-amyloid • Prion protein

\section*{Abstract}
\textbf{Background:} Alzheimer’s disease (AD) and prion diseases such as sporadic Creutzfeldt-Jakob disease (sCJD) share common features concerning their molecular pathogenesis and neuropathological presentation and the coexistence of AD and CJD in patients suggest an association between the deposition of the proteolytically processed form of the amyloid precursor protein, \(\beta\)-amyloid (A\(\beta\)), which deposits in AD, and the abnormal form of the prion protein, \(\text{PrP}^{\text{Sc}}\), which deposits in sCJD.

\textbf{Methods:} We have characterized sCJD patients (n = 14), AD patients (n = 5) and nondemented controls (n = 5) with respect to the deposition of \(\text{PrP}^{\text{Sc}}\) and A\(\beta\) morphologically, biochemically and genetically and correlated these findings to clinical data.

\textbf{Results:} sCJD-diseased individuals with abundant deposits of A\(\beta\) present with a specific clinicopathological profile, defined by higher age at disease onset, long disease duration, a genetic profile and only minimal amounts of \(\text{PrP}^{\text{Sc}}\) in the cerebellum.

\textbf{Conclusion:} The co-occurrence of pathological changes typical for sCJD and AD in combination with the inverse association between accumulation of A\(\beta\) and \(\text{PrP}^{\text{Sc}}\) in a subgroup of sCJD patients is indicative of common pathways involved in the generation or clearance of A\(\beta\) and \(\text{PrP}^{\text{Sc}}\) in a subgroup of sCJD patients.

\section*{Introduction}
In a subset of neurodegenerative diseases, deposition of abnormally processed proteins in the central nervous system constitutes the hallmark morphological characteristic. Alzheimer’s disease (AD) and sporadic Creutzfeldt-Jakob disease (sCJD) are prototypes of nontransmissible (AD) and transmissible (sCJD) cerebral proteinopathies \cite{1–3}. In sCJD, generation or deposition of \(\text{PrP}^{\text{Sc}}\), a misfolded isoform of the glycosylphosphatidylinositol-anchored, physiologically expressed prion protein (PrP\(^{\text{C}}\)), results in neurodegeneration. In AD, proteolytic processing of the amyloid precursor protein (APP) by \(\beta\)- and \(\gamma\)-secretases generates a peptide termed \(\beta\)-amyloid (A\(\beta\)). A\(\beta\) is mainly composed of the shorter A\(\beta\)\(_{40}\) and the longer A\(\beta\)\(_{42}\), the latter representing the neu...
rrototoxic species. Although the two diseases are clinically and epidemiologically diverse, AD presenting as a slowly progressing disease, affecting one third of octogenarians, sCJD presenting with a rapidly progressing course, affecting one individual per million per year, there is considerable neuropathological and pathophysiological overlap [4–7]. Both diseases are caused by extracellular deposition of respective proteins in the form of amyloid and the generation of PrPSc and Aβ leads to neurodegeneration. Furthermore, PrP immunoreactivity can be found within Aβ plaques and several studies on AD and sCJD patients have reported combinations of pathological features typical for AD and sCJD [8–11]. Evidence for yet undefined common molecular mechanisms for both diseases comes from studies demonstrating common genetic risk factors: homozygosity for valine or methionine on codon 129 PrP [12, 13]. The status of the latter-mentioned polymorphism leads to neurodegeneration during aging [14]. Recent in vitro and in vivo studies show that PrPC levels may control Aβ formation [15, 16], whereas other studies have postulated similar mechanisms of seeded protein aggregation for Aβ and PrPSc [17].

The goal of our study was to further investigate putative interactions of Aβ and PrPSc in sCJD patients. To this extend, we analyzed PrPSc, β-site APP-cleaving enzyme (BACE), Aβ40 and Aβ42 expression in patients suffering from sCJD or AD, and in nondemented control patients using immunohistochemistry, Western blot analysis and ELISA for respective proteins. In identical patient cohorts, we collected clinical data, performed genetic analysis of disease-associated genes and correlated this information to biochemical data.

### Materials and Methods

**Patients**

All patients were referred to the Swiss National Reference Center for Prion Diseases. The definite diagnosis of sCJD was established according to the WHO criteria [18], whereas the definite diagnosis of AD was made according to the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease [19]. Samples were processed according to established guidelines regarding safety and ethics. Table 1 summarizes the demographic characteristics of these patients.

**Immunohistochemistry**

Brain tissue (frontal cortex) was fixed with 4% buffered formalin, inactivated by 98% formic acid treatment for 1 h, and embedded in paraffin (tissue specimens from nondemented patients were formalin fixed only). Sections (3 μm) were subjected to conventional staining and to immunostaining for Aβ protein (A4, Sigma) and PrP (monoclonal antibody 3F4) according to standard protocols [20].

**Neuropathological Methods**

For quantification of the Aβ load, the frontal cortex was immunostained for Aβ protein and 3 representative regions were analyzed by quantifying the area immunoreactive for Aβ as a percentage of the total area for each image with the analySIS 5.0 program (Soft Imaging System GmbH, Münster, Germany) using published methods [21].

**Western Blot Analysis**

Brain homogenates were treated with proteinase K where indicated (specific activity of 41.3 U/mg; Roche, Basel, Switzerland) for 30 min at 37°C with a concentration of 0.03 U (40 μg/ml sample). Total protein (50 μg, measured prior to proteinase K treatment) was electrophoresed through an SDS-PAGE gel (12%). Proteins were transferred to nitrocellulose by wet blotting. Membranes were blocked with Tris/HCl-buffered saline/Tween 20 containing 5% top block, pH 7.4 (Sigma), incubated with antibodies to PrP (3F4; Signet, Dedham, Mass., USA; 1:2,000) or BACE [anti-BACE/Asp2 (CT); ProSci, Poway, Calif., USA; 1:500] and visualized by enhanced chemiluminescence (Amersham Biosciences). Relative quantification of the signal was performed em-
ploving a VersaDoc 5000 imaging station. To quantify PrPSc, serial dilutions of an identical standard sCJD brain homogenate (33, 16.5, and 8.25 μg of protein) were used on each Western blot to generate a standard curve according to published protocols [22].

ELISA Quantification of Aβ Peptides
For extraction of formic acid-soluble Aβ, a 10% homogenate was prepared in 70% formic acid using a RiboLyser (Hybaid, Ashford, UK). Following a centrifugation for 15 min at 16,000 g at 4°C, supernatants were neutralized with a ×20 excess of 1 M Tris.

Levels of Aβ1–42 and Aβ1–40 in the central nervous system of sCJD, AD and control cases were measured analyzing brain homogenates (100 μl) with a high-sensitivity ELISA for the Aβ1–42 peptide (Imnotest Aβ1–42, high-sensitivity test, Innogenetics, Gent, Belgium) and with ELISA for the Aβ1–40 Peptide (The Genetics Company, Schlieren, Switzerland) according to manufacturer’s instructions. Samples were analyzed in duplicates and tests were performed twice.

Genetic Analysis
Genomic DNA was extracted from whole blood or brain tissue using standard protocols. Mutation screening was performed by direct sequencing of both strands of PCR-amplified coding exons of presenilin 1 (PSEN1; exons 2–12), presenilin 2 (PSEN2; exons 3–12), and APP (exons 16 and 17), and the single coding exon of PRNP according to published protocols [23]. Apolipoprotein E genotypes were assessed using a LightCycler® (Roche Diagnostics Corporation) according to published protocols [24].

Results

High Levels of Aβ42 in a Subgroup of sCJD Patients
In order to quantify levels of Aβ in frontal cortices, we analyzed Aβ1–42 and Aβ40 amounts by ELISA. While Aβ40 brain loads, ranging between 20 and 25 pg/g wet brain in all groups, revealed uniform levels in sCJD patients [n = 14, mean 24.8 pg/g wet brain, standard deviation (SD) 1.9 pg/g wet brain, data not shown], we found a significant increase in Aβ42 in a subset of sCJD patients [n = 14, mean 79.9 pg/g wet brain, SD 88.3 pg/g wet brain]. In order to compare neuropathological, biochemical, genetic and clinical data, we decided to group sCJD patients into 2 groups. The first group of patients (n = 6) showed cortical Aβ42 levels ranging from 80 to 193 pg/g wet brain (n = 6, mean 170.8 pg/g wet brain, SD 54.2 pg/g wet brain); this cohort was termed ‘high Aβ42’. The second group of patients (n = 8) did not show significant Aβ42 loads (below 18 pg/g wet brain) (fig. 1A). These sCJD patients constituted the sCJD cohort with ‘low Aβ42’ (n = 8, mean 11.8 pg/g wet brain, SD 2.6 pg/g wet brain). We compared Aβ42 and Aβ40 amounts in frontal cortices of sCJD patients to those of clinically and neuropathologically confirmed AD patients and age-matched nondemented control patients. Similarly to sCJD patients, levels of Aβ40 were low in both groups (n = 10, mean 21.6 pg/g wet brain, SD 1.9 pg/g wet brain, 5 samples were not analyzed for Aβ40, data not shown). As expected, Aβ42 levels were high in the AD group (n = 5, mean 122.5 pg/g wet brain, SD 28.6 pg/g wet brain) and low in nondemented controls (n = 5, mean 22.3 pg/g wet brain, SD 24.5 pg/g wet brain).

We further investigated deposition of Aβ in the above-mentioned cohorts by quantifying the amount of Aβ immunohistochemically. sCJD patients categorized in the high Aβ42 group showed abundant deposits of Aβ in the form of diffuse and neuritic, rarely cotton wool plaques (n = 6, mean 10.6% of investigated area occupied by immunoreactivity for Aβ, SD 9.5%; fig. 1B), whereas we could only detect scarce or no deposits of Aβ in sCJD patients categorized in the low Aβ42 group (n = 8, mean 1.0% of investigated area occupied by immunoreactivity for Aβ, SD 1.2%). As anticipated, AD patients harbored extensive Aβ deposits mainly in the form of neuritic plaques (fig. 1C; n = 5, mean 10.6% of investigated area occupied by immunoreactivity for Aβ, SD 6.1%) and nondemented controls exhibited limited or no Aβ deposits (n = 5, mean 0.2% of investigated area occupied by immunoreactivity for Aβ, SD 0.5%).

No Influence of CJD- or AD-Associated Mutations
Both CJD and AD may be inherited in an autosomal dominant fashion. In the case of AD, mutations in the genes encoding APP, PSEN1 and PSEN2 have been shown to be associated with genetic AD, whereas mutations in PRNP are accountable for genetic sCJD. Analysis of APP, PSEN1, PSEN2, and PRNP did not reveal any pathogenic mutations in the subgroup of patients characterized by high Aβ42 levels (table 1).

sCJD Patients with High Aβ42 Are Genetically Diverse
The development of AD and sCJD is modulated by genetic risk factors such as homozygosity for methionine on polymorphic codon 129 of PRNP in the case of sCJD, or the presence of at least one allele of apolipoprotein E ε4 in the case of AD [25]. Patients of the high Aβ42 group were more likely to carry the uncommon polymorphism PRNP 129 valine/valine or methionine/valine. Of the 6 sCJD patients belonging to the high Aβ42 group, 3 were methionine homozygotes, 2 were valine homozygotes and 1 was heterozygous. Of the 8 sCJD patients belonging to the low Aβ42 group, 7 were methionine homozygotes and 1 was a valine homozygote (table 1). Furthermore, sCJD patients of the high Aβ42 group were more likely to carry a least one allele of apolipoprotein E ε4 [50% apoli-
Apoprotein E e4 carriers in the high Aβ42 group (n = 6), compared to 12.5% apolipoprotein E e4 carriers in the low Aβ42 group (n = 8).

sCJD Patients with Abundant Aβ Harbor Low Amounts of PrPSc

We have recently developed a method that enables us to compare the PrPSc load in defined central nervous system regions of CJD patients [22]. Using this method, termed PrPSc profiling, we compared PrPSc loads of sCJD patients belonging to the above-mentioned groups (high and low Aβ42). This analysis showed that patients belonging to the high Aβ42 group (n = 5, in one individual this analysis was not possible due to insufficient sampling) display significantly less PrPSc when compared to sCJD patients belonging to the low Aβ42 group (n = 8) (fig. 2).
These differences are visible in all central nervous system regions that were investigated, but are most prominent in the putamen, thalamus and cerebellum.

**sCJD Patients with Abundant Aβ Present with a Particular Clinical Profile**

Since sCJD patients belonging to the high Aβ42 group show particular neuropathological, genetic and biochemical features, we were interested in the clinical presentation of these patients. We compared age of disease onset and disease duration between the above-mentioned cohorts. Patients of the high Aβ42 group were on average 74.7 years old (SD 5.5 years), whereas patients of the low Aβ42 group were younger (68.6 years, SD 8.7 years; p = 0.070, Student’s t test). Disease durations were statistically significantly longer in the high Aβ42 group (10.1 months, SD 9.4 months) when compared to the low Aβ42 group (3.8 months, SD 2.2 months; p = 0.044, Student’s t test). There were no differences regarding female-to-male ratios between the groups (3/3 for the high Aβ42 group, 3/5 for the low Aβ42 group).

**No Significant Difference in Cortical β-Secretase Expression Between Groups**

Elevated BACE protein levels and increased BACE activity have been shown to be a prominent feature in the central nervous system of AD patients [26]. This finding is in line with the fact that BACE is involved in the generation of Aβ [27]. In an effort to further investigate molecular pathways underlying enhanced generation of Aβ in the high Aβ42 group of sCJD patients, we quantified BACE expression by Western blot analysis in frontal cortices in sCJD (n = 6) and AD patients (n = 2), and in controls (n = 1) (fig. 3). Although there was some heterogeneity in the expression of BACE between the groups, there was no significant difference in BACE expression between high Aβ42 and low Aβ42 sCJD patients.

**Discussion**

In a wide range of dementias, generation and subsequent deposition of abnormally processed proteins is thought to be causally involved in the pathophysiology of the disease and assessment of protein deposition can be employed as a diagnostic tool for the classification of these entities [28]. AD and CJD are two examples of this group of diseases, where generation and deposition of abnormally processed proteins, PrPSc, in the case of sCJD and Aβ in the case of AD, are involved in the pathophysiology. Coexistence of AD-type neuropathology in sCJD has been repeatedly reported, yet the interpretation of these findings is highly controversial. Some investigators surmise unspecific age-related changes [11], others assume that this points to similarities in the pathogenesis of AD and CJD [29]. In this study, we characterized deposition of Aβ in sCJD patients by immunohistochemistry, Western blotting, ELISA and genetic investigations. Using biochemical methods we were able to identify a subgroup of sCJD patients, which is defined by high cerebral Aβ42 loads. These patients were on average more than 6 years older than those with minimal or no depos-
its of Aβ42, presented with significantly longer disease durations, were more likely to carry an uncommon polymorphism on PRNP codon 129 or deposit PrP Sc type 2 [30] and had a high likelihood to carry at least one allele of apolipoprotein E e4. Interestingly, these patients harbor only minimal amounts of PrP Sc in the cerebellum. This clinicopathological signature suggests that this group of patients represents a subgroup of sCJD.

A study focusing on the assessment of genetic profiles of sCJD demonstrated that the apolipoprotein E e4 allele is an independent risk factor for developing sCJD [31, 32]. In this study, we provide evidence that the apolipoprotein E e4 status may be linked to the development of a subtype of sCJD. The above-mentioned study did not investigate Aβ loads in apolipoprotein E e4-positive sCJD patients. One could hypothesize that patients identified by van Everbroeck et al. [32] belong to the subgroup of sCJD patients characterized by abundant Aβ and scarce PrP Sc deposits.

A study focusing on the influence of PrP Sc expression on Aβ plaque formation suggested that overexpression of PrP Sc promotes Aβ plaque formation [15]. Given the fact that PrP Sc expression is unchanged during the course of prion disease [33], PrP Sc upregulation is an unlikely explanation for enhanced Aβ deposition in certain sCJD patients. Since accumulation of malprocessed proteins in the brain is the result of the differential between its de novo generation and its clearance, it is conceivable that Aβ deposition in sCJD may be the result of a saturation of common clearance mechanisms [34, 35]. This hypothesis is supported by a wealth of data suggesting that similar processes of protein degradation are in place for PrP Sc and Aβ [36–38].

In an attempt to delineate possible molecular pathways explaining the abundant generation of Aβ42 in a subset of sCJD patients, we measured central nervous system expression of Aβ-secretase, a key protease for the generation of Aβ [27]. Several studies have shown that Aβ-secretase activity and protein expression are increased in the cortex of patients suffering from AD [26, 39, 40]. Furthermore, BACE activity seems to be increased in the cerebrospinal fluid of sCJD patients [41]. The fact that we did not find any significant differences in BACE expression is in agreement with published studies and may indicate alternative pathways for Aβ generation in these patients [41].

The group of sCJD patients with high cerebral levels of Aβ42 showed minimal deposits of PrP Sc. PrP Sc profiling allowed us to directly compare PrP Sc levels between sCJD cohorts [22]. PrP Sc levels showed the most drastic differences in the cerebellum. PrP Sc levels in low Aβ42 patients uniformly showed high cerebellar PrP Sc loads, whereas PrP Sc levels in high Aβ42 patients uniformly showed low or nondetectable cerebellar PrP Sc levels. Interestingly, cerebellar involvement is a rarity in AD [42]. It has been speculated that the descriptive classification
of AD may camouflage CJD [43]. Although one could argue that the subgroup of sCJD we have identified could have been misdiagnosed as AD, thus supporting the above-mentioned hypothesis, the sCJD typical clinical presentation of these patients argues against the theory that sCJD is commonly misdiagnosed as AD. Taking into account that the majority of cerebral proteinopathies are characterized by deposition of more than one abnormally processed protein [28], neuropathological diagnosis of dementia should only be carried out in centers equipped to monitor deposition of all disease-associated proteins.

In conclusion, the present study provides evidence for the existence of a subgroup of sCJD characterized by abundant Aβ42 deposits, high age at onset of disease, a specific genetic profile and only marginal deposits of PrPSc. These data are in line with common molecular mechanisms leading to the deposition of Aβ and PrPSc.

Their delineation will be the focus of future studies. The fact that this newly defined group of patients harbors only minimal amounts of PrPSc is intriguing. On the one hand, this represents a challenge to neuropathologists, and on the other hand, the possibility that minimal PrPSc deposits may go undetected could lead to the misdiagnosis of AD.

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References


