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Title: APP processing and β-amyloid deposition in sporadic Creutzfeldt-Jakob patients is dependent on Dab1.

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Running title: β-amyloid and prion protein in CJD disease

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ABSTRACT

Alzheimer's disease and prion pathologies (e.g., Creutzfeldt-Jakob disease) display profound neural lesions associated with aberrant protein processing and extracellular amyloid deposits. For APP processing, emerging data suggest that the adaptor protein Dab1 plays a relevant role in regulating its intracellular trafficking and secretase-mediated proteolysis. Although some data have been presented, a putative relationship between human prion diseases and Dab1/APP interactions is lacking. Therefore, we have studied the putative relation between Dab1, APP processing and Aβ deposition, targets in sCJD cases. Our biochemical results categorized two groups of sCJD cases, which also correlated with PrP^{sc} types 1 and 2 respectively. One group, with PrP^{sc} type 1 showed increased Dab1 phosphorylation, and lower βCTF production with an absence of Aβ deposition. The second sCJD group, which carried PrP^{sc} type 2, showed lower levels of Dab1 phosphorylation and βCTF production, similar to control cases. Relevant Aβ deposition in the second sCJD group was measured. Thus, a direct correlation between Dab1 phosphorylation, Aβ deposition and PrP^{sc} type in human sCJD is presented for the first time.

Keywords: prionopathies, amyloid plaques, Alzheimer's disease, dab1.

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) and Alzheimer's disease (AD) are different proteinopathies sharing some neuropathological and molecular features (Aguzzi and Haass, 2003; Checler and Vincent, 2002; DeArmond, 1993; Price et al., 1993). In TSEs, such us Bovine spongiform encephalopathy (BSE), or Creutzfeldt-Jacob Disease (CJD) in humans, the cellular prion protein (PrP^{c}) is abnormally converted into a protease-resistant form termed PrP^{sc} (Prusiner, 1991; Prusiner, 1998). In Alzheimer's disease, sequential proteolytic cleavage by β-(generating βCTF fragment) and γ-secretases of the amyloid precursor protein (APP) leads to an extracellular overproduction of β-amyloid (Aβ) peptides of 40-42 amino acids that compose the characteristic senile plaques (Mattson, 2004; Selkoe, 2001). Deposition of both PrP^{sc} and Aβ in the central nervous system (CNS) results in neurodegeneration. Moreover, the coexistence of Aβ and PrP^{sc} amyloid deposits in affected brains (AD or prionopathies) has been widely reported, although it is, to some extent, controversial (Barcikowska et al., 1995; Debatin et al., 2008; Ferrer et al., 2001; Hainfellner et al., 1998; Leuba et al., 2000; Tsuchiya et al., 2004). In addition, a recent study report that PrP^{c} may participate activively in segresting Aβ oligomers suggesting active role of PrP^{c} in AD (Charveriat et al., 2009).

CJD is the most common of the human prion diseases; it is classified as sporadic (sCJD), familial (fCJD), iatrogenic (iCJD) and variant (vCJD) (Glatzel et al., 2003). Although sCJD is rare, with an incidence of 0.6-1.2 per million, it represents approximately 85% of all recognized human prion diseases (Brandel et al., 2000; Hill et al., 2003). Two predominant PrP^{sc} types have been identified, based on the gel mobility of the PrP^{sc} fragments resistant to proteinase K (PK) treatment, and they are

associated with different CJD phenotypes (Cali et al., 2006; Gambetti et al., 2003; Monari et al., 1994; Petersen et al., 1994). Parallel to this, *PRNP* polymorphism at codon 129 (Met^{129}→Val) is a susceptibility factor for CJD, considering methionine homozygosis as a risk factor for sporadic and variant CJD (Collinge, 1999; Ladogana et al., 2005; Palmer et al., 1991). However, although a systematic meta-analysis of AD genetic association studies revealed *PRNP* as a susceptible gene (see (Bertram and Tanzi, 2008) for recent review), conflicting results have been reported about relationships between polymorphism in codon 129 and the appearance of the histopathological events and clinical features of AD (see (Hooper and Turner, 2008) and (Poleggi et al., 2008) for reviews).

APP processing is a highly regulated process. Disabled-1 (Dab1) is a cytosolic adaptor protein that is phosphorylated by members of the Src family of tyrosine kinases (SFK) in response to extracellular molecules during neural development (Howell et al., 1999b). Several studies point to this molecule as a relevant factor in regulating APP processing (Hoe et al., 2006a; Parisiadou and Efthimiopoulos, 2007; Trommsdorff et al., 1998), and our group has recently examined whether APP processing and Dab1 phosphorylation are targets of the intracellular changes mediated by the synthetic peptide PrP(106-126) *in vitro* and by PrP^{sc} *in vivo* in 263K scrapie-inoculated hamsters (Gavin et al., 2008). PrP(106-126) induces APP accumulation in cultured neurons (Gavin et al., 2008; White et al., 2003) but also leads to decreased levels of βCTF and Aβ production. However, these data need further corroboration in human CJD patients. With this in mind, in the present study we analyze the putative correlation between PrP^{sc} type (1 and 2) and the Aβ plaque formation in a population of sCJD patients. Our findings point to Dab1 regulation as a key factor in the relations between the appearance of the neuropathological features of sCJD and AD.

PATIENTS AND METHODS

Cases

The brains of seventeen patients with sporadic CJD were obtained from 3 to 8 h after death and were immediately prepared for morphological and biochemical studies. The main clinical and pathological characteristics are summarized in Table I. For biochemical studies, samples of the parietal cortex were frozen in liquid nitrogen and stored at -80ºC until use. For neuropathological diagnosis, 4% formalin-fixed, formic acid-treated samples were embedded in paraffin. The neuropathological study was carried out on de-waxed 4-µm-thick paraffin sections of the frontal (area 8), primary motor, primary sensory, parietal, temporal superior, temporal inferior, anterior gyrus cinguli, anterior insular, and primary and associative visual cortices; entorhinal cortex and hippocampus; caudate putamen and globus pallidus; medial and posterior thalamus; hypothalamus; Meynert nucleus; amygdala; midbrain (two levels), pons and bulb; and cerebellar cortex and dentate nucleus. The sections were stained with haematoxylin and eosin, Klüver Barrera, and, for immunohistochemistry to glial fibrillary acidic protein (Dako, Glostrup, Denmark, dilution 1:250), CD68 for microglia (Dako, dilution 1:100), β-amyloid (Boehringer, Ingelheim, Germany, dilution 1:50), tau AT8 (Innogenetics, Ghent, Belgium, dilution 1:500), αB-crystallin (Abcam, Cambridge, MA, USA, dilution 1:100), α-synuclein (Millipore, Buillerica, MA, USA, dilution 1:500), ubiquitin (Dako, dilution 1:200), and PrP, (clone 3F4, (Dako, dilution 1:1000) with and without proteinase K pre-incubation). Immunohsitochemical controls including omission of the primary antibody or incubation with non-preimmune serum avoid immunostaining.

PrP typing

Samples of the frontal cortex (0.1 g) were homogenized in a bounce homogenizer containing 9 volumes of ice-cold lysis buffer (ICLB) (100 mM Tris, 100

mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, pH 6.9). Homogenates were centrifuged at 2,000g for 5 min. The resulting supernatant (100 µl) was incubated with 100 µg/ml of Proteinase K for 1 h at 37ºC. The reaction was stopped by the addition of 5 µl of PMSF; the solution was then mixed with 2X sample buffer and boiled at 96ºC for 5 min. 15 µl of the different samples was loaded side by side with the corresponding total homogenates, and electrophoresed in a 12% SDS-PAGE gel. Electrophoresed gels were transferred to nitrocellulose membranes, which were subsequently blocked with 5% non-fat dried milk in TTBS and incubated with the 3F4 mouse monoclonal anti-PrP antibody at 4ºC overnight. The membranes were washed with TTBS, incubated with the secondary anti-mouse antibody and developed with the ECL system. In PrP^{sc} type 1 the PK resistant domain has a gel mobility of about 21 KDa and commonly has its N-terminus, corresponding to the main PK cleavage site, at residue 82. In PrP^{sc} type 2, the corresponding PK-resistant domain migrates on gel to approximately 19 KDa, and its N-terminus starts most often at residue 97 (Parchi et al., 2000)(Fig. 1A).

Codon 129 genotyping

PRNP sequencing was carried out in every case. The putative amino acid polymorphism at codon 129 of the PrP gene, a methionine (M) to valine (V) substitution resulting from an adenine to guanine transition was evaluated. We investigated by allele specific oligonucleotide (ASO) hybridization to amplified genomic DNA as described in Owen *et al.* (Owen et al., 1990). Homozygoism of codon 129 was examined in every case.

Immunoprecipitation and Western immunoblotting

Samples were categorized in the Neuropathological Institute of the Bellvitge Hospital as PrP^{sc} type 1, 2 or control. However, the cases were analyzed in blind since a

initial nomenclature A, B, C was used in all the experiments per each group. After finishing the biochemical experiments and the quantification the PrP^{sc} type was determined in each group. Thus all the biochemical experiments and the quantification were developed in a bind manner. Samples of the parietal cortex (0.1 g) were homogenized in a glass homogenizer containing nine volumes of ICLB and then centrifuged. The resulting supernatant was normalized for protein content. After that, one fraction was mixed with twice-concentrated Laemmli sample buffer and boiled at 96º C for 5 minutes, and 30 µg of the different samples were electrophoresed for immunoblotting. The second fraction was used for immunoprecipitation. Thus, 1000 µg total protein was incubated with αDab1 (Exalpha Biologicals, Watertown, MA, USA) at 4 µg in 500 µl total volume overnight at 4º C. Afterwards, immune complexes were precipitated using Protein-G-Sepharose (Amersham-Pharmacia Biotech; GE Healthcare, Barcelona, Spain) at 4º C for 90 minutes. After centrifugation, proteins were eluted in twice-concentrated Laemmli sample buffer at 96º C for 5 minutes, followed by 10% SDS-PAGE and immunoblotting using the anti-phosphotyrosine 4G10 (Upstate Biotechnology Inc., Lake Placid, NY, USA). Membranes were reprobed with ab7522 antibody for total Dab1 detection (Abcam, Cambridge, MA, USA). βCTF fragment was detected using the ab2073 antibody (Abcam) and reprobed with an antibody against Actin (Sigma, Saint Louis, MO, USA). For immunoblotting, proteins were electrotransferred to nitrocellulose membranes for 6 hours. Membranes were then blocked with 3% BSA or 5% not-fat milk in 0.1M Tris-buffered saline (pH. 7.4) for 2 hours and incubated overnight in 0.5 % blocking solution containing primary antibodies. After incubation with peroxidase-tagged secondary antibodies, membranes were revealed by ECL-plus chemiluminescence Western blotting kit (Amershan-Pharmacia Biotech). In our experiments, each nitrocellulose membrane was used to detect both phosphorylated and total Dab1 levels or βCTF and Actin. Membranes were incubated in 25 ml of stripping solution (2% SDS, 62.5 mM Tris pH 6.8, 100 mM 2-mercaptoethanol) for 30 min. at 65º C and then

extensively washed before reincubation with blocking buffer and antibodies for re-blotting.

Gel electrophoresis and Western blotting for Aβ species identification

In order to determine different Aβ species in processed samples. After PrP^{sc} identification selected brain samples (0.2 g) of some cases were homogenized separately in a glass homogenizer in 1 ml of RIPA Buffer (50 mM Tris-HCl ph 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxicholate, 1% NP40 and 0.1% SDS) and rotated for 30 min at 4ºC. After a brief centrifugation at 12,000 rpm (4ºC for 10 min), the pellet was re-suspended with 200 µl of formic acid, sonicated for 30 min at 4ºC and centrifuged at 27,256 rpm for 20 min. The supernatant was discarded and formic acid was left to evaporate for no less than two hours. Then the pellet was re-suspended in 200 µl SB2X, sonicated for 10 min at 4ºC, boiled and sonicated again for 30 min at 4ºC.

For Western blot studies, 30 µg was mixed with sample buffer processed in a gradient gel of tris-tricine 10-20% (Bio-Rad, Madrid, Spain). Electrohoresis of samples was carried out in parallel with the positive control Aβ protein fragment 1-40 (Sigma, cat no A-1075). Proteins were transferred to nitrocellulose membranes., incubated with methanol and boiled in PBS for 5 min. Once at room temperature, the membranes were incubated with 5% skimmed milk in TBS-T buffer (100 mM Tris-buffered saline, 140 mM NaCl and 0.1% Tween 20, ph 7.4) for 30 min at room temperature, and then incubated with the β-amyloid 4G8 monoclonal antibody (Sigma) diluted 1:1,000 in TBS-T containing 3% BSA (Sigma) at 4ºC overnight. Subsequently, the membranes were incubated for 45 min at room temperature with the secondary antibody labeled with horseradish peroxidase at a dilution of 1:1,000, and washed with TBS for 30 min. Protein bands were visualized with the chemiluminescence ECL method (Amershan-Pharmacia Biotech).

Densitometry and statistical processing of processed films

For quantification, developed films were scanned at 2400 x 2400 dpi (i800 MICROTEK high quality film scanner) and the densitometric analysis was performed using Quantity One Image Software Analysis (Biorad). Statistical analysis of the obtained data was performed using STATGRAPHICS plus 5.1 and Origin 8™ programs using a ANOVA test. Asterisks in the histograms indicate the following *p* values of significance: (*) $p < 0.05$; (**) $p < 0.01$

RESULTS

Criteria for the neuropathological, molecular and phenotypic diagnosis of CJD used in the present series are those currently accepted and detailed elsewhere (Parchi P, Budka et al., 2003). Neuron loss, spongiform degeneration, astrocytic gliosis and microgliosis involving the cerebral neocortex, striatum and cerebellum, occurred in every case. Synaptic-like PrP^{sc} deposits were also found in the cerebral cortex and striatum in every case. In addition, PrP^{sc} plaques were common in three VV cases. Neurofibrillary tangles were absent except for a very few neurons in the entorhinal cortex in a few cases (corresponding to stages I and II of Braak). No Lewy bodies or αB-crystalline-immunoreactive ballooned neurons occurred in any cases. Ubiquitin deposition was restricted to scattered small dots in the cerebral cortex and white matter (not shown).

Analysis of Dab1 phosphorylation defined two groups of sCJD cases

Levels of Dab1-phosphorylation in protein samples of sCJD cases were studied by immunoprecipitation, as previously described (Gavin et al., 2008). Dab1 protein levels were also analyzed in the same samples by Western blot using the ab7522

antibody. In these cases, the amount of the cytoskeletal protein Actin was used as an internal loading control of the experiments. In the first experiments, densitometric analysis of phosphorylated Dab1 (80 KD band) showed the presence of two groups of sCJD cases compared to controls. Further determination of PrP^{sc} type showed that also correlated with PrP^{sc} types 1 and 2 respectively. Then, we grouped the samples into these groups for further analysis (Fig. 2A-D). One group of 8 sCJD cases showed increased Dab1 phosphorylation levels (0.85 ± 0.13; mean ± sem) compared to controls (0.59 ± 0.17) and the rest of sCJD cases (0.62 ± 0.16, n = 9) (Fig. 2B). These results correlate with total levels of Dab1 in protein samples (0.74 ± 0.11 (sCJD group 1); 0.93 ± 0.16 (controls) and 0.86 ± 0.12 (sCJD group 2) (Fig. 2D).

βCTF production and Aβ deposition in sCJD groups

Next we aimed to determine the contents of the product of the β-secretase activity in sCJD samples to further correlate the data with the presence of Aβ deposits in parallel histological sections. Western blotting analysis of sCJD samples revealed that the first group of sCJD cases showed lower levels of βCTF (1.48 ± 0.37) compared to controls (1.93 ± 0.36). In contrast, the second sCJD group displayed no significant reduction in βCTF content (1.65 ± 0.28) compared to both controls and the first sCJD group (Fig. 3). The presence or absence of β-amyloid was examined in the parietal cortex of every sCJD case. Histological examples of the semi-quantitative study (no plaques: 0; a very few diffuse plaques: +; several diffuse, some neuritic plaques: ++) are illustrated in Fig. 4A-C. However, histopathological correlation of the present results with the appearance of Aβ plaques in the parietal cortex of the first and second sCJD groups revealed a clear correlation between individuals in the number of Aβ deposits and the biochemical data (Table 1). Thus Aβ deposits in the first sCJD group were rare, while occurring more frequently in the second group (Table 1).

Western blots to recombinant β-amyloid 1-40 showed a band of about 4-6 kDa and several weak bands of high molecular weight (the more pronounced between 37 and 50 kDa) corresponding to aggregates presumably resulting from sample processing (Fig. 4D lane 1). Tissue samples with large numbers of plaques (i.e. case CJD16, Fig 4D lane 2) showed a robust pattern characterized by two bands of about 4-6 and 12-13 kDa and several bands of higher molecular weight, three of them more pronounced between 25 and 50 kDa, and another at about 200 kDa. No β-amyloid immunoreactivity was seen in brain homogenates of cases with no β-amyloid plaques (i.e. case CJD4) (Fig. 4D lane 3).

Correlation between codon 129 polymorphism with PrP^{sc} type and Aβ deposits in sCJD groups

Lastly we performed a correlation between the genomic characterization of codon 129 polymorphims of every sCJD case with the results obtained from the biochemical analysis, the histopathological study (Aβ deposits) and the PrP^{sc} typing. Results indicated that most members of the first sCJD group (n = 7), with PrP^{sc} type 1, showed an absence of Aβ deposits and MM polymorphism. Only one case of 8 from the first sCJD group displayed a VV polymorphism. In contrast, the second sCJD group, with PrP^{sc} type 2, showed increased numbers of Aβ deposits (ranked from + to ++) but only in VV (n = 5) and MV (n = 1) polymorphism. All these correlated data are summarized in Table 1. In conclusion, PrP^{sc} type 1 largely correlated with MM polymorphism, high Dab1 phosphorylation, low βCTF production and an absence of Aβ deposits. In contrast, PrP^{sc} type 2 did not fully correlate with a clear polymorphism, although MM cases of type 2 also showed an absence of Aβ deposits, in contrast to other polymorphisms (MV or VV).

DISCUSSION

The molecular mechanisms underlying prion pathology stand out as an intriguing unsolved issue (Nicolas et al., 2009; Weissmann, 2004). Considerable effort has been made to solve the multiple aspects of this pathology, and recently information about intracellular responses in affected neurons and glia has been revealed (e.g., (Aguzzi et al., 2008) for review). In this respect, most intracellular mechanisms induced by proteinopathies displaying amyloid deposits should be, to some extent, similar (e.g., (Gaggelli et al., 2006)). Therefore, a rise in crosstalk points between these amyloid diseases (e.g., CJD and AD) has been described in recent years and some of them are controversial. One recent example, among others, is the recent discovery that PrP^{c} may bind Aβ oligomers decreasing Aβ deposition and long term potentiation (LTP) changes independently of PrP^{sc} (Charveriat et al., 2009) while other authors have indicated that PrP^{c} increases Aβ deposition (Schwarze-Eicker et al., 2005). Dab1 was described initially as a cytosolic adaptor protein phosphorylated by members of the Src family of tyrosine kinases (SFK) in response to extracellular signals, and by experimentally induced oxidative stress *in vitro* (e.g., (Howell et al., 1999a)). Although Dab1 is normally stable, the degradation of Dab1 occurs after tyrosine phosphorylation by SFK via the proteasome pathway (Arnaud et al., 2003). Although with some modifications, it has been proposed that Dab1 is relevant for APP-processing, since the short cytosolic domain of APP contains a YENPTY motif that interacts with several PTB-containing adaptor proteins, such as Fe65, X11, Dab1 and Jip (e.g., (Trommsdorff et al., 1998)). Concerning Aβ–processing, it has been reported in conflicting studies that overexpression of Dab1 may decrease or increase Aβ production in cell lines (Hoe et al., 2006b; Parisiadou and Efthimiopoulos, 2007). In addition, it has been determined that the overexpression of Dab1 or Jip has no evident effect upon APP cleavage (Hoe et al., 2006b).

In the present study, we have checked whether APP processing and Dab1 phosphorylation might also be targets of the neural changes observed in sCJD cases. In order to avoid mixed responses due to coexisting prion species in brain regions (Polymenidou et al., 2005), we selected the parietal cortex of sCJD cases because it is a region of study with clear content of type 1 or 2 PrP^{sc}, which was corroborated in every case. Unfortunately, phospho-Dab1 detection in post-mortem sections is not possible with current antibodies. Thus we used the Dab-1 characterization by using the well established method (e.g., (Howell et al., 1997)). Our results demonstrate that, in some sCJD cases (first group) the tyrosine phosphorylation of Dab1 is increased, decreasing total Dab1 protein levels, which correlates with low Aβ production and the absence of Aβ plaques in histological sections. These data also corroborate those obtained using *mdab1*-deficient neuronal cultures showing less Aβ production compared to wild-type neurons (Gavin et al., 2008). Thus, it is reasonable to consider that intracellular effects mediated by PrP^{sc} presence may affect Dab1 phosphorylation and Aβ production. PrP^{sc} inoculation gives rise to a myriad of molecular responses in the brains of infected mice (Xiang et al., 2004) including a relevant increase in oxidative stress (Choi et al., 1998; Martin et al., 2007; Yun et al., 2006) also described in sCJD cases (Freixes et al., 2006; Pamplona et al., 2008; Petersen et al., 2005). Indeed, sCJD patients with PrP^{sc} type 1 and MM polymorphism are characterized by faster evolution and decreased life expectancy compared with PrP^{sc} type 2 with other polymorphisms (Uro-Coste et al., 2008). In addition, Petersen and coworkers determined that vCJD and Gerstmann-Sträussler-Scheinker disease (GSS) patients do not display Aβ deposits, which correlates with long time duration of the neuropathology compared to the sCJD analyzed (Petersen et al., 2005). Thus, in this scenario, the high degree of oxidative abnormalities observed in these studies in affected brains of sCJD patients with PrPsc type 1 may alter Dab1 function, impairing normal APP processing and decreasing Aβ production. However, we cannot exclude other

possibilities. For example in a recent study, termed PrP^{sc} profiling by Schoch and coworkers showed greater amounts of PrP^{sc} in cortical regions (frontal, parietal, temporal and occipital) of cases with PrP^{sc} type 1 (Schoch et al., 2006). The increased presence of PrP^{sc} in these regions may decrease endogenous PrP^{c} levels and its natural functions. It is well known that PrP^{c} participates in the control of cooper homeostasis and redox maintenance (Brown, 2001; Nicolas et al., 2009; Vassallo and Herms, 2003). In addition, copper imbalance is also a hallmark of neurodegenerative diseases such as ALS and Alzheimer's disease (Gaggelli et al., 2006). Although it warrants further study, perhaps the increased presence of PrP^{sc} in type 1 increased the total amount of oxidative radicals in these regions which in turn may affect Dab1 phosphorylation. In contrast, in type 2 patients Dab1 becomes less phosphorylated and regulates APP processing in a more efficient manner, and Aβ production and further deposition could take place. However, another interesting scenario derives from the recent description that PrP^{c} removes Aβ oligomers from the brain parenchyma impairing Aβ deposition in a PrP^{sc} independent way (Lauren et al., 2009). Taken together, the effects of the conversion of PrP^{c} to PrP^{sc} might have a dual effect, first increasing Dab1 phosphorylation which reduces APP processing and Aβ production, but also reducing Aβ removal by PrP^{c} which may increase the evolution of the disease due to the combined effect of PrP^{sc} and unremoved toxic Aβ. The relevance of each process in Aβ deposition in sCJD patients warrants further study, even if we take into account that a recent study reported increased formic acid-extractable Aβ(1–42) levels in mouse model of AD after prion innoculation, which may support this last possibility (Baier et al., 2008). However, a recent study by Debatin and colleagues described a relation between PrP^{sc} and Aβ deposition similar to that reported in the present study, with only Aβ deposition in the cerebellum of sCJD cases with higher age at disease onset and long disease duration (Debatin et al., 2008). Moreover they showed, using PrP^{sc} profiling (Schoch et al., 2006), that sCJD patients with abundant Aβ harbor low

amounts of PrP^{sc} in the cerebellum. Despite these observations, the study does not analyze a putative intracellular factor/s mediating these differences. The present results, although pointing to Dab1 as a key factor, raise a number of unsolved questions (see above). In this respect, it is well known that PrP^{c} also inhibits BACE1-dependent APP cleavage, which may lend support to the loss of function hypothesis (Hooper and Turner, 2008) but the above described results of Lauren et al., introduce additional views (Lauren et al., 2009). Knowledge of the differential presence of Aβ oligomers in different sCJD patients (type 1 or 2) may help to solve this puzzle. However, the present study introduces the cytosolic adaptor protein Dab1 in the context of the sCJD and Aβ deposition scenario as a key molecule participating in the parallel evolution of these diseases.

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FIGURE LEGENDS

Figure 1.

Examples of the patterns of PrP^{sc} type 1 and type 2 (PK: proteinase K pre-treatment) of some cases used in the present study (see material and methods for technical details).

Figure 2.

Example of Western blotting determination of pDab1 **(A-B)** and total Dab1 protein levels **(C-D)** in sCJD cases. sCJD cases were categorized as described above. Protein samples from different groups of sCJD (first and second groups) are shown. **B)** The densitometric results of the analysis of revealed films are shown. Each data item corresponding to an sCJD case is displayed in the histograms. A significant increase in the pDab1/Dab1 ratio is observed in the first group of sCJD cases compared to the second sCJD group and controls. **C-D)** Parallel determination of total Dab1 levels in the same sCJD protein samples. The increased phosphorylation of Dab1 in the first sCJD cases correlates with decreased levels of total protein. Each dot corresponds to a single case. Asterisks indicate statistical differences between sCJD groups and controls in **(B and D)**. * $p < 0.05$; ** $p < 0.01$ (ANOVA test).

Figure 3.

Example of Western blotting determination of βCTF **(A-B)** in sCJD cases compared to controls. sCJD cases were categorized as described above. Decreased levels of βCTF can be seen in the first sCJD group compared to controls. **B)** Histograms showing the densitometric study. Asterisks indicate statistical differences between sCJD groups and controls. * $p < 0.05$; (ANOVA test).

Figure 4.

Low power photomicrographs illustrating examples of amyloid plaques in some of the sCJD cases used in the present study after Aβ immunocitochemistry. **A)** no plaques (score 0); **B)** a few diffuse plaques (score +); **C)** many diffuse plaques, some neuritic plaques (score ++). **D)** Gel electrophoresis and western blotting to β-amyloid in cases CJD16 (lane 2) and CJD4 (lane 3) run in parallel with recombinant β-amyloid 1-40 (lane 1). Several bands of variable molecular weight, corresponding to β-amyloid aggregates are seen in the CJD case with a large burden of amyloid plaques (case 16). Scale bar A = 500 μm pertains to B-C.