The relative abundance of APOE and A1-42 associated with abnormal prion protein differs between Creutzfeldt-Jakob disease subtypes

THE RELATIVE ABUNDANCE OF APOE AND Aβ1-42 ASSOCIATED WITH ABNORMAL PRION PROTEIN DIFFERS BETWEEN CREUTZFELDT-JAKOB DISEASE SUBTYPES

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ABSTRACT

Aggregated and protease-resistant mammalian prion protein (PrP^{Sc}) is the primary protein component of infectious prions. Enriched PrP^{Sc} preparations are often used to study the mechanisms underlying prion disease. However, most enrichment procedures are relatively non-specific and tend to yield significant amounts of non-PrP^{Sc} components, including various proteins that could confound functional and structural studies. It is thus important to identify these proteins and assess their potential relevance to prion pathogenesis. Following proteinase K treatment and phosphotungstic acid precipitation of brain homogenate, we have used mass spectrometry to analyze the protein content of PrP^{Sc} isolated from prion-infected mice, multiple cases of sporadic Creutzfeldt-Jakob disease (sCJD), and human growth hormone associated cases of iatrogenic CJD (iCJD). Creatine kinase was the primary protein contaminant in all PrP^{Sc} samples while many of the other proteins identified were also found in non-CJD controls, suggesting that they are not CJD specific. Interestingly, the Alzheimer's disease associated peptide amyloid β 1-42 (Aβ1-42) was identified in the majority of the sCJD cases as well as non-CJD age-matched controls while apoliprotein E was found in greater abundance in the sCJD cases. By contrast, while some of the iCJD cases showed evidence of higher molecular weight Aβ oligomers, monomeric Aβ1-42 peptide was not detected by immunoblot and only one case had significant levels of apolipoprotein E. Our data are consistent with the age-associated deposition of Aβ1-42 in older sporadic CJD and non-CJD patients and suggest that both apolipoprotein E and Aβ1-42 abundance can differ depending upon the type of CJD.

INTRODUCTION

Prion diseases are a group of neurodegenerative disorders that affect various mammals including humans[1]. A critical event in prion pathogenesis is the misfolding of normal host-encoded prion protein (PrP^{C}) into a pathological isoform, termed PrP^{Sc}, that is more protease resistant and higher in β-sheet structure than its precursor, PrP^{C} [2]. Prion disease is usually confirmed postmortem by the deposition of protease-resistant PrP^{Sc}, brain tissue vacuolation, and gliosis[3]. The most common form of human prion disease is sporadic Creutzfeld-Jakob disease (sCJD)[4, 5], which occurs at a yearly rate of approximately 1-2 cases per million people[6, 7] and is believed to result from the spontaneous misfolding of PrP^{C} into PrP^{Sc}. Acquired forms of CJD such as kuru, variant CJD, and iatrogenic CJD (iCJD) represent a relatively small percentage of all CJD cases[5, 8]. In multiple countries around the world including the United Kingdom, France and the United States, iCJD has been associated with exposure to CJD contaminated, cadaver-derived human growth hormone preparations as well as other contaminated medical products or procedures[9].

Prions display a wide range of observable phenotypes indicative of distinct strains[10]. Strains in CJD are defined genetically by a methionine or valine polymorphism at residue 129 in PrP^{C}, biochemically by the molecular mass of PrP^{Sc}, and neuropathologically by characteristic patterns of PrP^{Sc} deposition, neuronal vacuolation, and gliosis[4]. Using these criteria, 5 strains of CJD have been defined: MM1/MV1, MV2/VV2, MM2c, MM2t and VV1[4]. The precise molecular basis of prion strains remains poorly defined but differences in PrP^{Sc} conformation are thought to be a primary source of strain variability[2].

Given the central importance of PrP^{Sc} in prion pathogenesis, highly enriched PrP^{Sc} preparations are often used to study the mechanisms underlying prion disease. Several methods for the enrichment of PrP^{Sc} from infected brain tissue, most often from laboratory animal models, have emerged over the last three decades[11-18]. However, the procedures used are relatively non-specific and often yield variable concentrations of diverse molecular species including proteins other than PrP^{Sc}. Polysaccharides[19], fatty acids[20], metal ions[21], nucleic acids[11, 22] and ferritin[11, 23, 24] are known to co-purify with PrP^{Sc}. Whether or not all of these compounds represent artifacts of the enrichment procedure or are functionally associated with PrP^{Sc} is unclear. Indeed, a comparison of multiple studies profiling the proteins in PrP^{Sc} preparations shows that they can be

widely variable[23-27]. Thus, it is likely that most of these proteins are present due to the particular enrichment protocol used rather than any mechanistically significant affinity with PrP^{Sc}. Identification of the non-PrP^{Sc} proteins in these preparations is of critical importance, especially since it has been shown that such contaminants can interfere with structural studies of PrP^{Sc} [24].

A more recent, yet widely used, method for PrP^{Sc} isolation from prion-infected tissue is sodium phosphotungstic acid (PTA) precipitation[28-30]. Isolation of PrP^{Sc} with PTA is much faster and generally requires less sample than many of the more conventional methods that have been developed and its utility and convenience has been well documented[28-30]. It has been proposed that the affinity between the sodium salt of PTA and PrP^{Sc} is based upon multivalent electrostatic interactions between the polyoxometalate anions and PrP^{Sc} aggregates[30, 31]. Such interactions might lead to other multivalent or aggregated molecules co-purifying with PrP^{Sc} that could differ significantly from those contaminants derived from more conventional PrP^{Sc} enrichment techniques which are based upon repeated centrifugation. However, the identity and prevalence of non-PrP impurities in PTA-purified PrP^{Sc} is unknown.

In this study, we used tandem mass spectrometry to identify the major protein components that were PTA-precipitated from the brains of mice infected with the 22L prion strain or from brain samples of previously characterized[32] heterozygous cases of MV1 and MV2 sporadic and human-growth hormone associated cases of CJD. We found that the major PrP^{Sc}-associated proteins isolated using PTA differed from those isolated using a different method developed by Bolton et al. which involves repeated centrifugation of the prion-infected material[17, 18, 24]. A notable exception was apolipoprotein E (apoE), which was present in PrP^{Sc} isolated using both the PTA and the Bolton procedure, suggesting that apoE might have more than a coincidental association with PrP^{Sc}. However, apoE was present at significantly higher abundance in MV1 sCJD when compared to either MV2 sCJD or iCJD. A similar difference in abundance was observed for the Alzheimer's disease-associated peptide amyloid β 1-42 (Aβ1-42). These data suggest that the relative abundance of Aβ1-42 and apoE co-precipitating with PrP^{Sc} can differ depending upon the CJD type.

EXPERIMENTAL PROCEDURES

Supplies and Reagents. Dithiothreitol (DTT), iodoacetamide, ethylenediaminetetracetic acid (EDTA), detergent SB3-14, formic acid (FA) ampules and protein extraction buffer were all purchased from Sigma. Trypsin was from Promega. Imperial Coomassie blue stain was purchased from Thermo-Fisher Scientific. Protease inhibitors and proteinase K (PK) were purchased from Roche Diagnostics. Burdick & Jackson brand acetonitrile (ACN) and water were purchased from VWR. SDS-PAGE gels were run using reagents and gels from Life Technologies-Invitrogen Corporation with NuPAGE Bis-Tris gels and 4X lithium dodecyl sulfate (LDS) sample buffer. The PlusOne Silver Stain kit was from GE Healthcare. Phosphotungstic acid (PTA, $2Na_2O{\cdot}P_2O_5{\cdot}12WO_3{\cdot}18H_2O$) and protein extraction reagent (7M urea, 2M thiourea, 1% C_7BzO) were purchased from Sigma.

Tissue samples. Mouse PrP^{Sc} was isolated from the brains of clinically positive C57Bl/6 mice infected with the 22L strain of mouse prions. The experimental protocol was reviewed and approved by the Rocky Mountain Laboratories Animal Care and Use Committee (Animal Study Protocol 2003-06). The Rocky Mountain Laboratories are fully accredited by the American Association for Laboratory Animal Care and this study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Brain tissue samples of heterozygous sCJD cases (MV1 and MV2), heterozygous United Kingdom human growth hormone-associated iCJD cases (MV2), and non-CJD age matched controls were obtained from the National CJD Research & Surveillance Unit Brain and Tissue Bank in Edinburgh, UK. Samples were pseudo-anonymized using a Brain Bank reference number and no patient identifiable data was transferred to the NIH. Cerebellar cortex (CbC) and cerebral cortex (CC) tissue from two additional sCJD patients were obtained from the University of Verona, Italy. These tissues were obtained at autopsy and sent to the Neuropathology Unit at the University of Verona for statutory definite diagnosis of CJD. Strain typing and clinical and neuropathological features of all but two of the cases examined have been previously reported[32]. All UK cases had consent for research and their supply and use in this study was covered by LREC 2000/4/157 (National Creutzfeldt-Jakob disease tissue bank: acquisition and use of autopsy material for research on human transmissible spongiform encephalopathies, Professor

James Ironside, amended date: 9th October 2007). Ethical approval for the acquisition and use of human brain material was obtained from the National Institutes of Health (NIH) Office of Human Subject Research (Exempt #11763 and #12725).

Enrichment of PrP^{Sc} from brain homogenate by PTA precipitation. PrP^{Sc} enrichments using PTA precipitation were done using 10% brain homogenates (50mg brain equivalents) prepared in PBS and mixed with an equal volume of 4% sarkosyl/phosphate buffered saline (PBS) and treated with 50 units of benzonase. Brain lysate was cleared by a 5 min centrifugation at 5,000 *g* and then digested with 50 µg/mL PK for 1 hr at 37 °C. Following the addition of PTA at a final concentration of 0.3%, the samples were incubated at 37 °C for 2 hr and subjected to centrifugation at 16,000 *g* for 30 min. The resultant pellets were twice washed: first with 200mL PBS containing 200mM EDTA and then PBS alone at pH 8 for 30 min and subjected to centrifugation as before. Each sCJD, iCJD or non-CJD control sample was subjected to 3 or more individual PTA precipitations, each of which represented a single technical replicate.

SDS-PAGE and in-gel tryptic digestion. Pellets containing enriched PrP^{Sc} were suspended in 25 µL of protein extraction buffer (Sigma) and incubated at 37 ºC for 30 min. The sample was reduced with 14mM DTT prior to alkylation with 75mM iodoacetamide for 30 min at room temperature in the dark with orbital rotation. The reaction was quenched by dilution of 1M DTT to a final concentration of ~200mM. Life Technologies 4X LDS sample buffer was added, resulting in a final sample volume of approximately 50µL. Each sample was heated to 100 °C using a heating block and subjected to centrifugation (22,000 *g*) for 5 min prior to loading onto an SDS-PAGE NuPAGE 10% Bis-Tris 1.5mm gel. Gels were run in 2N-morpholinoethanesulfonic acid (MES) running buffer at 175V for 20 min, stained with Coomassie blue Imperial stain (Thermo-Fisher Scientific), and then destained with water prior to band selection for in-gel trypsin digestion. For every PTA preparation, one lane of a gel was cut into 8 slices each containing Coomassie blue stained material. Trypsin digestion of each band was conducted overnight in a mixture of 5% trifluoroethanol in 50mM ammonium bicarbonate, pH 8.0 at 37 ºC. The digests were quenched 16 hrs later with 10µL of 8% aqueous FA, then transferred to a fresh vial. The original gel slices were further extracted with 60% acetonitrile in water, then added to the fresh vials. The resulting peptide solutions were dried by centrifugal vacuum. Each digest was dissolved in 20µL of HPLC buffer A (water/3% ACN/0.1% FA),

subjected to centrifugation at 22,000 *g* and the upper 16µL transferred to an autosampler vial for LC-MS/MS analysis.

***Western blotting*.** Proteins were separated by SDS-PAGE as described above and transferred to polyvinylidene fluoride (PVDF) membrane by wet transfer using Towbin's buffer. For mouse tissues, the samples were probed with the mouse monoclonal anti-PrP antibody 6D11 (Covance, Inc.) at a dilution of 1:5,000. 6D11 recognizes residues 93-109 of mouse PrP. Human-derived samples were probed with the mouse monoclonal anti-PrP antibody 3F4, which recognizes residues 109-112 of human PrP, at a dilution of 1:2,000. The secondary antibody was horseradish peroxidase (HRP) labeled anti-mouse IgG secondary antibody (GE Healthcare) at a dilution of 1:100,000 and the blot was developed on film with ECL Plus Femto according to the instructions from the manufacturer. For the detection of the amyloid β 1-42 (Aβ1-42) peptide, samples were first suspended in denaturing buffer (7M urea, 2M thiourea, 1% C_7BzO) and then diluted 1:1 with 2X sample buffer (Life Technologies) for SDS-PAGE as detailed above. Membranes were developed with the anti-amyloid precursor protein mouse monoclonal antibody 6E10, which recognizes residues 1-16 of Aβ1-42, at a dilution of 1:5,000.

HPLC chip-based nanospray LC-MS/MS. Proteins were identified by LC-MS/MS using an Agilent 1200 connected to an XCT Ultra 6300 Ion Trap via a nanospray source and a microfluidic HPLC chip interface. Tryptic digests were loaded onto the chip (Agilent, Zorbax 300SB-C18, 5µm, 75µm x 43mm) with an autosampler and washed with Buffer A prior to elution at 300nL/min. The step gradient was 3 - 30% Buffer B (90% ACN/0.1% FA) over 30 min, to 50% B by 40 min, 90% B by 60 min, 97% B by 70 min, and back to 3% B by 78 min. Runs were followed by 4 min of post-run equilibration with Buffer A. External calibration was done using a tuning mix (Agilent) specific for the ion trap. Data-dependent MS acquisition was performed with air flow set to 4L/min at 350 ºC, MS capillary voltage 1800-1850V, maximum accumulation time 150ms, with a target maximum of 100,000. The MS scan range was set to 300 – 1400 m/z in the Ultrascan mode. Four parent ions were selected for each MS/MS cycle with a fragmentation amplitude of 1.0V and the SmartFrag setting on.

***Data analysis and protein identification*.** Raw data were processed into peak lists using MASCOT Distiller v2.4.3.0. The MGF files were searched against a target database filtered for human or mouse taxonomy (www.uniprot.org) using MASCOT Daemon. The search parameters

for MASCOT protein identification[33] utilizing trypsin/P search parameters consisted of one missed tryptic cleavage allowed with a fixed carbamidomethylation (+57, Cys) and a variable oxidation (+16, Met). Mass tolerances of 2.0 and 1.0 Daltons were used for parent and monoisotopic fragment ions, respectively. The DAT result files from MASCOT were used as input files for further analysis using the ProteoIQ v2.6.02 bioinformatics platform (Premier Biosoft Inc)[34]. A comprehensive list of protein identifications was derived using ≥2 peptides with unique *m/z* values, MASCOT ion scores of ≥ 30 and protein group probabilities of ≥ 0.9[35]. A more stringently filtered set of identifications for the purpose of listing only the most abundant proteins was derived from a MASCOT ion score threshold of ≥40, at least 2 peptides with unique *m/z* values, and protein and peptide probabilities of at least 0.99 or 0.9, respectively according the Protein and Peptide Prophet algorithms[35, 36]. In the case of amyloid beta A4 protein, hereafter referred to as Aβ, only one tryptic peptide (KLVFFAEDVGSNK) corresponding to residues 17-28 in the Aβ1-42 portion of the protein was consistently identified. When the trypsin-only constraints were removed from the search parameters another peptide (GAIIGLMVGGVVIA) corresponding to Aβ1-42 residues 29-42 was confirmed. Each peptide was allowed only a single protein identification according to criteria used in the ProteoIQ bioinformatics platform[34]. Thus, the strongest identifications were based upon the highest number of spectral counts, the highest cumulative MASCOT ion scores, the highest protein/peptide prophet probabilities, and the greatest number of unique peptides. Keratin was considered an environmental contaminant and was removed from the final protein lists. Spectral counts were used to semi-quantify the amount of each protein identified in the final PrP^{Sc} preparation. Percentages were calculated by dividing the number of mass spectra observed for a given protein by the total number of mass spectra multiplied by 100.

Statistics. Mean and standard deviation (SD) or standard error of the mean (SEM) were derived using the individual technical replicates described above. A 1-Way ANOVA with Dunnett's post-test or a Tukey's multiple comparisons test was used to compare multiple data sets. All calculations were done using the GraphPad Prism software package, version 6.04.

RESULTS

LC-MS/MS analysis of PrP^{Sc} PTA-precipitated from 22L-infected mouse brains.

Western blot analysis of total PrP in 22L versus normal brain homogenate (NBH) inoculated mice showed a significantly higher concentration of PrP in the 22L infected brain homogenate (Fig. 1A, lanes 1-2). Treatment of the brain homogenates with PK followed by PTA precipitation led to an almost complete loss of PrP signal in the NBH-inoculated sample but not the 22L-infected sample, indicating that the vast majority of PrP present in the PTA precipitate from 22L-infected brain was PrP^{Sc} (Fig 1A). In the absence of PK treatment, a very small amount of aggregated PrP^{C} from mice inoculated with NBH was detected in the PTA precipitate (Fig. 1A, lane 7). This is consistent with previous reports that uninfected mammalian brain may contain small amounts of detergent-insoluble PrP aggregates susceptible to precipitation with PTA[37, 38] which may comprise up to 5% of the PrP recovered[39].

Staining of the samples with either silver nitrate (Fig. 1B, lanes 1-4) or Coomassie blue (Fig. 1B, lanes 5-8) revealed that numerous non-PrP proteins were also precipitated by PTA. However, because PK treated PTA precipitates from prion infected brain are known to be highly infectious[29] and therefore must contain all of the critical components that constitute an infectious prion, we analyzed the proteins present in the less heterogeneous PK-treated PTA precipitates using LC-MS/MS. Since it is known that the protein concentration in a sample correlates reasonably well with the number of mass spectra assigned to that particular protein[40], spectral counting was used as a semi-quantitative method to estimate the relative abundance of each protein. We detected a limited number of PrP peptides in the uninfected NBH inoculated sample (Table 1). However, in samples derived from 22L-infected mouse brain, PrP peptides were present at much higher levels than in the uninfected samples and PrP was the most abundant PTA-precipitated protein, represented by an average over two independent experiments of 54% of the peptides found (Table 1).

We next compared the purity of PrP^{Sc} isolated by PTA precipitation to PrP^{Sc} isolated from the same starting brain homogenate using an enrichment protocol derived from the centrifugation-based method of Bolton et al.[18]. The relative PrP^{Sc} enrichment levels were similar for both the PTA and Bolton preparations (Figure 1C). The protein composition of the material isolated using the method of Bolton et al. (Supplemental Table 1) was comparable to what we have previously reported[24, 25] with ferritin heavy and light chains comprising the major impurities

(Figure 1C). By contrast, dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (Dlat) and creatine kinase (CK) were the major non-PrP^{Sc} proteins associated with the PTA precipitate. Creatine kinase was also the most abundant protein in uninfected, PK-treated and PTA precipitated mouse brain homogenate (Table 1) indicating that its presence in PTA-precipitated PrP^{Sc} was not related to prion infection. Neither Dlat nor CK were detected in the Bolton-derived preparations (Figure 1C). Overall, the data show that a single round of PTA precipitation and PK treatment leads to an enrichment of 22L mouse PrP^{Sc} similar to that from centrifugation-based techniques but with different protein contaminants.

LC-MS/MS analysis of PrP^{Sc} PTA-precipitated from sCJD brain tissue. We next examined the protein content of PrP^{Sc} isolated following PK treatment and PTA precipitation from 19 samples of heterozygous MV1 or MV2 sCJD (Fig. 2A). The clinical and molecular characteristics of these cases, the majority of which have been described in a previous study[32], are briefly summarized in Table 2. A comprehensive list of protein identifications is shown in Supplemental Table 2 and a stringently filtered list showing only the most abundant proteins is given in Table 3. As with mouse prion derived PTA-precipitated PrP^{Sc}, CK was a major impurity in all of the PTA precipitated human samples including three non-CJD age matched controls (Table 3). A total of 5 PrP peptides were detected in one of the three non-CJD cases and this was set as the background threshold for PrP^{C}. Consistent with the presence of PrP^{Sc}, PrP peptides were detected at well above the threshold level of $\geq$5 spectral counts in all of the sCJD samples (Fig. 2C). Some sCJD samples, however, had much lower levels of PrP^{Sc} relative to other sCJD samples (Figure 2A). In these latter samples, the contribution of CK and other co-precipitating proteins as a percentage of the mixture was much higher.

The majority of precipitated proteins from both MV1 and MV2 sCJD were identical, with 11 of the 16 most abundant proteins (69%) present in both sCJD types (Table 3). Many of them were found not only in the majority of the sCJD cases examined but also in the age-matched non-CJD neurological controls (Table 3 and Fig. 3), indicating that they were not prion disease specific. Proteins common to all four groups included CK, Dlat, mammalian ependymin-related protein 1 (MERP-1), and collagen α chain (Table 3). The handful of top proteins that were present in a single group, such as complement C1q in MV1 CJD or versican and glial fibrillary acid protein (GFAP) in MV2 CJD, have also been found in PrP^{Sc} preparations from prion

infected mice and are likely related to the response of the brain to damage during the late stages of prion disease[25, 41,42]

LC-MS/MS analysis of PrP^{Sc} PTA-precipitated from iCJD brain tissue. In order to determine if there were relevant differences in the proteins co-precipitating with PrP^{Sc} between sporadic and iatrogenic CJD cases, 7 cases of heterozygous MV2 iCJD (Table 2) associated with exposure to contaminated human growth hormone in the UK were also analyzed. PrP^{Sc} was detectable in all of the iCJD cases by both western blot (Figure 2B) and MS (Table 3), although the relative abundance varied from case to case (Fig. 2C). The diversity of proteins identified in PrP^{Sc} isolated from iCJD brain tissue was low (Supplemental Table 2). Only seven high abundance proteins were identified in these samples, including CK and Dlat (Table 3). None were unique to the iCJD cases and most were found in the non-CJD controls (Table 3 and Fig. 3). Thus, many of the proteins found in PrP^{Sc} preparations from the sCJD and non-CJD controls were not found in the iCJD samples (Table 3 and Fig. 3).

Comparison of PrP^{Sc} PTA-precipitated from different brain regions. Our initial analysis of PTA precipitated PrP^{Sc} from sCJD brain tissue suggested that most of the proteins identified were not prion disease specific. To determine whether the same was true of PrP^{Sc} isolated from different regions of the same sCJD brain, PrP^{Sc} was PTA-precipitated from both the cerebellar (CbC) or cerebral (CC) cortex from 4 sCJD cases (Fig. 4). PrP^{Sc} was found in similar abundance in the CbC and CC for all of the cases examined except case 7, where the CbC had twice as much PrP^{Sc} when compared to the CC. For each case examined, there were some proteins that were found in one region of the brain and not the other. For example, in case 7 apoE was detected in the CbC and not the CC and contributed 5% of the total peptides identified. Other proteins that varied from case to case were also of relatively low abundance, for example myelin proteolipid protein (PLP) in case 9. The low abundance of these proteins suggests that their regional variability may be related to the level of sensitivity of the MS/MS assay rather than to region-specific differences in the proteins that consistently co-precipitate with PrP^{Sc}.

Identification of proteins associated with Alzheimer's disease in CJD brain tissue. We and others have shown that apolipoprotein E (apoE), a protein associated with Alzheimer's disease pathogenesis, consistently co-purifies with PrP^{Sc} isolated from prion-infected brain[23-26] suggesting that the two proteins may interact. In PrP^{Sc} precipitated from CJD brain tissue, apoE

was present in 15 out of the 26 total CJD brains analyzed and was detected at very low levels in 2 of the 3 non-CJD controls (Figure 5A). Interestingly, the prevalence of apoE differed depending upon the type of CJD. In PrP^{Sc} isolated from MV1 cases of sCJD, 6 of the 7 samples had relatively high amounts of apoE while only 6 of the 12 samples of PrP^{Sc} from MV2 cases of sCJD were apoE positive (Fig. 5A). The difference in apoE abundance between sCJD MV1 and the non-CJD control samples was statistically significant ($p=0.01$, Fig. 5C). Only one of the 7 iCJD MV2 PrP^{Sc} samples had significant numbers of apoE peptides (Fig. 5A, case 19) and the average abundance of apoE peptides in the iCJD samples more closely resembled that of the sCJD MV2 samples (Fig. 5C). The differences in apoE abundance between MV1 and MV2 CJD was likely not due to PrP^{Sc} sample variability since the relative average abundance of PrP^{Sc} was similar between sample groups (Fig. 2C). Thus, our data suggest that the association of apoE with human PrP^{Sc} may vary by CJD subtype.

In 15 out of 19 (79%) of the sCJD brain samples and in all 3 of the non-CJD controls, peptides derived from the Aβ were detected (Fig. 5B). Aβ peptides were present at a similar abundance in both sCJD MV and the non-CJD age-matched control samples (Fig. 5C). By contrast, the abundance of these peptides was low in PTA-precipitates from sCJD-MV2 and they were not detected in the 7 iCJD cases examined (Fig. 5B). The relative average abundance of Aβ was thus significantly lower in these cases when compared to the non-CJD controls. Interestingly, Aβ and apoE were not always detected in the same sample (compare Fig. 5A and B) suggesting that the presence of one is not indicative of the presence of the other. Overall, our data suggest that, like apoE, the presence of Aβ in human PrP^{Sc} preparations may vary by CJD type.

Alzheimer's disease-associated Aβ1-42 peptide in PTA-precipitates from CJD brain. A recent study has found Alzheimer's disease-associated Aβ peptide 1-42 (Aβ1-42) positive plaques in some human growth hormone-associated cases of iCJD in the UK[43]. Aβ1-42 positive plaques were not found in age-matched sCJD controls, raising the possibility of iatrogenic transmission of Aβ1-42 pathology[43]. Our MS analysis of PTA-precipitated PrP^{Sc} used samples from the same cohort of iCJD cases[32] but we only detected peptides from the Aβ protein in the sCJD samples. If these peptides were from regions of the Aβ protein outside of Aβ1-42, it could help to explain the discrepancy between our results and those of Jaunmuktane et al.[43]. However, the mass spectrum of the most common tryptic Aβ peptide identified corresponded specifically

to residues 17-28 of the Aβ1-42 peptide (Fig. 6A). Furthermore, a second non-tryptic peptide corresponding to residues 29-42 of Aβ1-42 was also identified (Figure 6B). Thus, residues 17-42 of Aβ1-42 were found in sCJD MV1 and MV2 as well as in the non-CJD neurological controls but not in any of the iCJD samples.

The consistent identification of Aβ1-42 peptides and the corresponding lack of peptides corresponding to full length Aβ protein strongly suggested that Aβ1-42 was present in the sCJD samples. In order to determine if the MS results correlated with the presence or absence of Aβ1-42 in PTA precipitates from CJD brain, we analyzed non-PK treated, PTA precipitates for the presence of the Aβ1-42 peptide by western blot. In the 3 representative sCJD samples tested, a peptide corresponding to Aβ1-42 as well as a high molecular weight smear consistent with the presence of Aβ oligomers[44], were detected (Fig. 7). A high molecular weight smear was also detected in 4 of the 7 iCJD samples, although a band consistent with monomeric Aβ1-42 was not found (Fig. 7). The immunoblot results are consistent with the MS data and confirmed the presence of Aβ1-42 in PrP^{Sc} preparations isolated from the sporadic, but not iatrogenic, cases of CJD analyzed. However, the presence of Aβ1-42 positive high molecular weight species in the iCJD samples suggests that SDS insoluble aggregates of Aβ1-42 are likely present in at least some cases of human-growth hormone associated iCJD.

DISCUSSION

The presence of Aβ1-42 in the majority of PTA precipitates from the sCJD samples tested is consistent with studies showing that Aβ1-42 can co-localize with PrP^{Sc} in amyloid plaques in the brains of sCJD patients[45, 46]. Thus, the co-precipitation of Aβ1-42 with PrP^{Sc} may indicate that the two proteins can interact in vivo. However, we also detected residues 17-42 of Aβ1-42 in PTA precipitates from all 3 of the age-matched, non-CJD brain samples examined, indicating that Aβ1-42 was also present in these samples. While one of these cases was diagnosed pathologically as Lewy body dementia, the other two died of non-neurological causes[32]. It is not surprising that that there was positivity to Aβ in the Lewy body dementia case as co-occurrence of aggregated proteins is not unusual in neurodegeneration as well as aging. This suggests that the co-occurrence of Aβ1-42 and PrP^{Sc} in prion infected brain may be more an age related phenomenon than a direct consequence of either prion infection or interaction with

PrP^{Sc}. A large scale study comparing the frequency of the co-occurrence of Alzheimer's pathology in over 100 cases of sCJD with age-matched, non-CJD control brain samples reached a similar conclusion[45]. If age is a driving factor for the deposition of Aβ1-42 in sCJD, then its presence in PTA precipitates from sCJD brain may be primarily due to its physical properties.

A recent study of several cases from the same cohort of human growth hormone-associated cases of iCJD as the current study found Aβ1-42 positive plaques and Aβ1-42 peptide in 4 out of 8 of the brain samples examined[43], and there have been several cases of iCJD with coincident Alzheimer's pathology associated with dura mater transplant[47-49]. The co-occurrence of Aβ1-42 and PrP^{Sc} has been suggested in these cases to be an incidental finding[47], a result of iatrogenic transmission of Alzheimer's disease[43], or the consequence of seeding of underlying brain tissue by Aβ present in the dura mater graft[49]. We were unable to detect the Aβ1-42 peptide, by either MS or immunoblot, in 7 samples of iCJD brain. However, we cannot rule out the possibility that we failed to detect monomeric Aβ1-42 due to an inability of PTA to quantitatively precipitate Aβ1-42 aggregates from brain tissue. In 4 of the 7 iCJD cases examined by western blot, we did detect a high molecular weight smear consistent with the presence of SDS insoluble Aβ oligomers[44]. The presence of higher molecular weight Aβ species in some of the iCJD brain samples assayed is consistent with the conclusion of Jaunmuktane et al.[43] that Aβ amyloid can be present in human growth hormone associated cases of iCJD from the UK.

Previous work based on either genotyping[50, 51] or the allotype ratio of M129 to V129 in PrP^{Sc} [32] has suggested that human growth hormone related cases of iCJD in the UK are the result of exposure to the MV2/VV2 strain of sCJD. In the current work, the relative average abundance of apoE and Aβ1-42 peptides was statistically indistinguishable in PrP^{Sc} preparations from the sporadic and iatrogenic MV2 samples and clearly differentiated these cases from the MV1 cases examined (Fig. 5C). These data are consistent with the hypothesis that human growth hormone-associated cases of iCJD in the UK are related to exposure to the MV2 strain of sCJD. Moreover, the fact that the number of Aβ1-42 and apoE peptides associated with PrP^{Sc} is higher in MV1 versus MV2 cases of sCJD suggests that their abundance may differ depending upon CJD subtype which may be related to variables such as age at infection, PrP^{Sc} conformation, degree of prion spread, or rate of prion accumulation.

With the exception of PrP^{Sc} itself, apoE was the only other protein specific to both PrP^{Sc} enrichment procedures. This is the case not only in the current studies but also in other PrP^{Sc} samples that have been profiled[23-26]. ApoE has been implicated as a biomarker for prion disease[27, 52], co-localizes with PrP^{Sc} deposits in vivo[53], and is present at significantly elevated levels in mice infected with different prion strains[41]. The consistent presence of apoE in enriched PrP^{Sc} samples coupled with the fact that it is overrepresented in MV1 cases of sCJD when compared to non-CJD controls (Fig. 5C), could suggest a potential interaction with PrP^{Sc}. However, if PrP^{Sc} and apoE are directly interacting, the stoichiometry of that interaction is unclear. In each CJD case we examined, the relative apparent abundance of PrP^{Sc} was much higher than that of apoE. It is plausible that the PK treatment step utilized during purification is digesting a higher proportion of apoE than PrP^{Sc} itself. Alternatively, the presence of apoE in PrP^{Sc} samples may be related to its involvement in cholesterol and lipid transport which appear to be altered during clinical prion disease[41].

The utility and convenience of PTA-based precipitation has been well documented[28-30, 39, 54] and PTA precipitated PrP^{Sc} has been used to study various aspects of prion pathogenesis including PrP^{Sc} uptake[55]. Our MS analysis shows that PK treatment followed by PTA precipitation of 22L-infected mouse brain homogenate yields an approximate 50% abundance of PrP^{Sc} relative to other proteins, similar to that seen when using the centrifugation-based enrichment protocol first developed by Bolton et al. (Fig. 1)[18]. Thus, there are significant levels of non-PrP proteins in both preparations that could potentially confound molecular, biochemical, or structural studies. We have identified the primary protein contaminant in PTA-purified PrP^{Sc} as creatine kinase, an enzyme active in the brain and other tissues that effects the conversion of creatine to phosphocreatine. The primary contaminant previously identified by us and others in PrP^{Sc} preparations has been ferritin rather than creatine kinase[23-26]. The fact that different contaminants are consistently associated with PrP^{Sc} enriched using different methods suggests that their presence in these samples is a function of their physical properties rather than any functional association with PrP^{Sc}.

SUPPORTING INFORMATION:

The following files are available free of charge at ACS website http://pubs.acs.org:

Moore et al Supplemental Table 1.docx. Proteins identified in PrP^{Sc} purified from 22L-infected and uninfected brain homogenate using the method of Bolton et al.

Moore et al Supplemental Table 2.xlsx. Proteins identified in PrP^{Sc} PTA-precipitated from CJD and non-CJD brain samples.

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CONFLICT OF INTEREST. The authors declare no conflict of interest.

Table 1. Proteins identified after PTA precipitation of 22L-infected and uninfected mouse brain homogenate.

ID[a]	PTA associated proteins (22L)	Prep1	Prep2	SpC[c]	Unique peptides[d]	Total score[e]	% total[f]
P04925	Major Prion protein	✓	✓	545	15	1015.5	53.6
Q8BMF4	Dlat[b]	✓	✓	195	10	747.8	19.2
P30275	Creatine kinase U-type, mitochondrial	✓	✓	97	10	540.3	9.5
P55066	Neurocan core protein	✓	✓	47	6	352.7	4.6
P60202	Myelin proteolipid protein		✓	24	5	285.1	2.4
P08226	Apolipoprotein E	✓	✓	22	6	363.5	2.2
Q9ES97	Reticulon-3	✓	✓	15	2	156.5	1.5
P11087	Collagen alpha-1(I) chain		✓	14	3	172.9	1.4
Q99104	Myosin-Va	✓		12	3	159.7	1.2
P10493	Nidogen-1	✓		8	3	155.2	0.8
Q9R171	Cerebellin-1		✓	8	2	134.3	0.8
Q60994	Adiponectin	✓	✓	7	3	168.5	0.7
P62702	40S ribosomal protein S4, X isoform		✓	5	2	89.1	0.5
Q80SY4	E3 ubiquitin-protein ligase MIB1		✓	4	3	153.8	0.4
P63082	V-type proton ATPase 16 kDa proteolipid subunit		✓	4	2	134.4	0.4
Q5SV85	Synergin gamma	✓		3	2	91.7	0.3
Q9D2P8	Myelin-associated oligodendrocyte basic protein	✓		3	2	89.1	0.3
P03893	NADH-ubiquinone oxidoreductase chain 2	✓		2	2	94.4	0.2
Q64521	Glycerol-3-phosphate dehydrogenase, mitochondrial	✓		2	2	87.3	0.2
	PTA associated proteins (uninfected)						
P30275	Creatine kinase U-type, mitochondrial	✓	---	32	8	461.1	76.2
P04925	Major Prion protein	✓	---	8	3	151.4	19.0
P60202	Myelin proteolipid protein	✓	---	2	2	89.3	4.8

[a]Protein identifiers are from www.uniprot.org.

[b]Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex

[c]The total number of assigned spectra (spectral counts or SpC) from two independent PTA purifications.

[d]The number of unique peptides from two independent PTA purifications.

[d]The sum of the highest MASCOT peptide scores for each unique peptide.

[f]The average percent relative abundance of each protein based upon the sum of spectral counts from two independent replicates. Positive protein identifications required at least 2 peptides with unique *m/z* values and MASCOT ion scores of ≥ 40.

Table 2. Clinical and molecular features of heterozygous (MV) CJD cases analyzed.

sCJD						
Case ID Numbers	Gender[a]	Age of Onset (yrs)[b]	Duration (mo)[c]	Brain Region[d]	Molecular Subtype	Reference
1-5	4M and 1F	53-77	4-14	CC, CbC	MV1	32
21	M	86	6	CC	MV1	This paper
6-14	4M and 5F	57-75	7-21	CC, CbC	MV2	32
iCJD						
15-20	3M and 3F	27-33	8-32	CC	MV2	32
22	M	28	15	CC	MV2	This paper

[a]Number of male (M) and female (F) cases.

[b]Age of onset in years (yrs).

[c]Disease duration in months (mo).

[d]CC = cerebral cortex; CbC = cerebellar cortex.

Table 3. The most commonly observed proteins PTA-precipitated with PrP^{Sc} from PK-treated sporadic, iatrogenic and non-CJD brain tissue.

Group	Gene[a]	Major proteins[b, c]	Commonly observed peptide	Total ion score[d]	Unique peptides[e]
CJD MV1 (n=7)	CKMT1A	Creatine kinase U-type (P12532)	TVGMVAGDEETYEVFADLFDPVIQER	1306	17
	PRNP	Prion protein (P04156)	HMAGAAAAGAVVGGLGGYMLGSAMSR	1545	19
	DLAT	Dlat (P10515)	GVETIANDVVSLATK	607	8
	APP	Amyloid β A4 protein or Aβ[f](P05067)	LVFFAEDVGSNK	100	2
	LTF	Lactotransferrin (P02788)	LADFALLCLDGK	1408	21
	APOE	Apolipoprotein E (P02649)	GEVQAMLGQSTEELR	587	8
	COL1A2	Collagen α-2 (CP08123)	GETGPSGPVGPAGAVGPR	238	3
	MERP-1	MERP-1 (Q9UM22)	ALLSYDGLNQR	265	3
	PLP1	Myelin proteolipid protein (P60201)	MYGVLPWNAFPGK	371	6
	TNXB	Tenascin-X (P22105)	CVCDPGYTGDDCGMR	537	8
	APCS	Serium amyloid P-component (P02743)	AYSLFSYNTQGR	196	3
	C1QTNF5	Complement C1q TNF (Q9BXJ0)	VLVNEQGHYDAVTGK	339	6
	CBLN1	Cerebellin-1 (P23435)	TMIIYFDQVLVNIGNNFDSER	303	5
sCJD MV2 (n=12)	PRNP	Prion protein (P04156)	HMAGAAAAGAVVGGLGGYMLGSAMSR	1650	20
	CKMT1A	Creatine kinase U-type (P12532)	TVGMVAGDEETYEVFADLFDPVIQER	1284	17
	DLAT	Dlat (P10515)	GVETIANDVVSLATK	745	10
	LTF	Lactotransferrin (P02788)	LADFALLCLDGK	1954	28
	COL1A2	Collagen α-2 (P08123)	GETGPSGPVGPAGAVGPR	306	4
	GFAP	Glial fibrillary acidic protein (P14136)	LADVYQAELR	968	14
	APP	Amyloid β A4 proteinor Aβ[f] (P05067)	LVFFAEDVGSNK	100	2
	PLP1	Myelin proteolipid protein (P60201)	MYGVLPWNAFPGK	384	6
	MERP-1	MERP-1 (Q9UM22)	ALLSYDGLNQR	322	5
	APOE	Apolipoprotein E (P02649)	GEVQAMLGQSTEELR	659	8
	COL1A1	Collagen α-1 (P02452)	DGEAGAQGPPGPAGPAGER	231	3
	CBLN1	Cerebellin-1 (P23435)	TMIIYFDQVLVNIGNNFDSER	285	4
	APCS	Serium amyloid P-component (P02743)	AYSLFSYNTQGR	261	4
	VCAN	Versican core protein (P13611)	VSVPTHPEAVGDASLTVVK	276	4
iCJD MV2 (n=7)	PRNP	Prion protein (P04156)	HMAGAAAAGAVVGGLGGYMLGSAMSR	1588	23
	CKMT1A	Creatine kinase U-type (P12532)	TVGMVAGDEETYEVFADLFDPVIQER	1238	17
	DLAT	Dlat (P10515)	GVETIANDVVSLATK	720	10
	TNXB	Tenascin-X (P22105)	CVCDPGYTGDDCGMR	758	12
	APOE	Apolipoprotein E (P02649)	GEVQAMLGQSTEELR	283	4
	MERP-1	MERP-1 (Q9UM22)	ALLSYDGLNQR	192	3
	PLP1	Myelin proteolipid protein (P60201)	MYGVLPWNAFPGK	176	3
Non-CJD (n=3)	CAMK2A	CAMK2A (Q9UQM7)	ITQYLDAGGIPR	382	6
	CKMT1A	Creatine kinase U-type (P12532)	TVGMVAGDEETYEVFADLFDPVIQER	735	13
	APP	Amyloid β A4 proteinor Aβ[f] (P05067)	LVFFAEDVGSNK	100	2
	COL4A2	Collagen α-2-IV (P08572)	GEQGFMGNTGPTGAVGDR	479	7
	COL1A2	Collagen α-2 (P08123)	GEIGAVGNAGPAGPAGPR	213	3
	MERP-1	MERP-1 (Q9UM22)	ALLSYDGLNQR	197	3
	NID1	Nidogen (P14543)	QDLGSPEGIAVDHLGR	336	5
	HSPG2	Proteoglycan core protein (P98160)	EHLLMALAGIDTLLIR	468	8
	RPL18	60S ribosomal protein (Q07020)	ILTFDQLALDSPK	259	4
	LAMC1	Laminin γ-1 (P11047)	QDIAVISDSYFPR	375	6
	DLAT	Dlat (P10515)	GVETIANDVVSLATK	404	7
	FTH1	Ferritin heavy chain (P02794)	IQNVVTSFAPQR	228	4

[a] Gene abbreviations and protein names are from www.uniprot.org.

[b]Keratin was removed from the protein list and the remaining proteins were filtered based upon a MASCOT ions score of ≥40, protein and peptide probabilities of at least 99% and 90% respectively, according to the protein prophet and peptide prophet algorithms, and at least 2 peptides with unique *m/z* values required for a protein identification. Each peptide was allowed only a single protein match according to the settings of the ProteoIQ bioinformatics platform. The strongest protein identifications, based upon the highest number of spectral counts, the highest cumulative MASCOT ion scores, the highest protein/peptide prophet probabilities and the greatest number of unique peptides are listed in the table above. A more comprehensive list containing additional lower abundance protein identifications is provided as Supplemental Table 2.

[c]For simplicity, the proteins Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, Lactotransferrin, Ca/calmodulin-dependent kinase-II-subunit α and Mammalian ependymin-related protein 1 are listed in the table by their either their gene names Dlat, LTF, CAMK2A, respectively, or abbreviated names (MERP-1).

[d]The sum of the highest MASCOT ion scores derived from each unique peptide.

[e]The number of tryptic peptides with unique *m/z* values.

[f]The amyloid β A4 protein, abbreviated as Aβ throughout the text, was consistently identified by the single tryptic peptide LVFFAEDVGSNK. However, additional MASCOT searches without the constraints of trypsin specificity resulted an additional peptide within the amyloid β A4 protein sequence.

FIGURE LEGENDS

Figure 1. **Proteins which co-purify with PrP^{Sc} from the brain homogenate of 22L-infected mice differ depending upon the purification protocol used**. (A) Immunoblot of 22L-infected and NBH-inoculated mouse brain homogenate. The blot was developed using the anti-PrP monoclonal antibody 6D11 which recognizes PrP residues 95-105. PTA precipitation, PK-treatment, and whether or not the samples were from 22L-infected mice is indicated under each lane. Lanes 7-10 represent a longer exposure of lanes 3-6. The major PrP^{Sc} bands are indicated by the bracket on the right. (B) Silver stain (lanes 1-4) and Coomassie blue stain (lanes 5-8) of PTA-precipitated brain homogenates with and without protease treatment. The major PrP^{Sc} bands are indicated by the bracket on the right. (C) Relative percentages of PrP^{Sc} and other proteins in 22L-infected brain homogenates precipitated with PTA (left) or isolated using a centrifugation technique based on Bolton et al. (right, modified Bolton)[18]. apoE=apolipoprotein E; CK=creatine kinase; Dlat=dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex.

Figure 2. Variable PrP^{Sc} content in PK-treated sCJD, iCJD and non-CJD brain homogenates. Samples were analyzed to estimate the relative concentrations of PrP^{Sc} in each brain homogenate and each lane represents 50µg brain equivalents of brain homogenate that has been PK-treated. (A) PrP^{Sc} from 19 sCJD cases. Three separate gels are shown (lanes 1-7, lanes 8-14, and lanes 15-23) with excised irrelevant lanes indicated by a thin white line. Exposure times were matched based on the intensity of the signal in case 1 (lanes 2 and 22) or case 12 (lanes 11 and 23). Standards for Type 1 sCJD (MV1, lane 1) and type 2 sCJD (MV2, lane 8) are shown. Precise PrP^{Sc} molecular subtyping for the cases as MV1 or MV2 has been previously reported[32] and is indicated in Table 2. All samples are CC unless otherwise noted. Case numbers are given above each blot and lane numbers are given below. (B) PrP^{Sc} from 7 iCJD cases. A single gel is shown with excised irrelevant lanes indicated by a thin white line. All iCJD cases were type MV2 (Table 2). The three non-CJD controls analyzed are also shown. All samples are CC unless otherwise noted. Case numbers or letters are given above each blot and lane numbers are given below. (C) The average spectral counts and standard error of the mean for PrP^{Sc} PTA-precipitated from the 26 samples of CJD and 3 samples of non-CJD brain shown in panels A and

B. Data are derived from Supplemental Table 2. There were no significant differences in the average number of spectral counts between the sample groups (p=0.2, Tukey's multiple comparisons test). Black bars: sCJD MV1; gray bars: sCJD MV2; white bars: iCJD; hatched bars: non-CJD.

Figure 3. Differences in protein content between PTA-precipitated material from CJD and non-CJD brain homogenates. (A) sCJD MV1, (B) sCJD MV2, (C) iCJD MV2, and (D) non-CJD controls. Creatine kinase and Dlat are present in all samples, while PrP is found at high abundance only in the CJD samples. Aβ protein was found in all samples except iCJD. For all panels, the data shown are derived from Table 3. Gene and protein names are the same as in Table 3.

Figure 4. Regional differences in proteins co-purifying with PrP^{Sc}. Proteins identified from PK-treated, PTA precipitates from the cerebellar cortex (CC) and cerebral cortex (CbC) of four cases of sCJD. (A) Case 3, sCJD MV1, (B) Case 7, sCJD MV2, (C) Case 9, sCJD MV2, and (D) Case 14, sCJD MV2. Gene and protein names are the same as in Table 3.

Figure 5. Relative abundance of apoE and Aβ in PK-treated PTA precipitates from CJD brain differs by CJD type. The average spectral counts and standard error of the mean (SpC $\pm$ SEM) for (A) apoE and (B) Aβ . (C) The average SpC $\pm$ SEM for all technical replicates for each CJD group (sCJD MV1 n=22, sCJD MV2 n=45, iCJD MV2 n=27, non-CJD n=9). Using a 1-way ANOVA multiple comparisons test with Dunnett's post-test with non-CJD set as the control group, the average abundance of apoE in cases of sCJD MV1 was significantly different from that of the non-CJD cases (**, $p < 0.01$). The relative abundance of Aβ in sCJD MV2 (***, $p<0.001$) and iCJD (****, $p<0.0001$) was significantly different from that of the non-CJD control. For all panels, the data shown are derived from Supplemental Table 2 and represent the average percent abundance from 2 independent LC-MS/MS runs. Black bars: sCJD MV1; gray bars: sCJD MV2; white bars: iCJD; hatched bars: non-CJD.

Figure 6. Aβ1-42 peptides are present in PK-treated, PTA-precipitated sCJD and non-CJD brain homogenate. Representative LC-MS/MS spectra of the two unique peptides derived from Aβ that were found in the majority of the sCJD and non-CJD cases examined. (A) LVFFAEDVGSNK represents C-terminal residues 17-28 of the Alzheimer's disease associated Aβ1-42 peptide and was identified using standard trypsin search parameters. (B) GAIIGLMVGGVVIA represents residues 29-42 of the Aβ1-42 peptide and was found by removing the trypsin-specific restraints for MASCOT database searching. Designations in blue denote b fragment ions and red denotes y fragment ions.

Figure 7. The Aβ1-42 peptide is present in PTA-precipitates from sCJD brain. Samples of brain tissue from 3 representative cases of sCJD and all 7 samples of human-growth hormone associated iCJD were PTA-precipitated without PK. Aβ1-42 and higher molecular mass Aβ oligomers were detected in PTA pellets from the sCJD cases while high molecular mass Aβ oligomers were detected in 4 of the 7 iCJD cases. The first 2 lanes represent 5 and 10 ng amounts of purified Aβ1-42 peptide (arrow). Case numbers for the sCJD and iCJD samples analyzed are shown above each lane. Molecular mass markers in kilodaltons are shown on the left.

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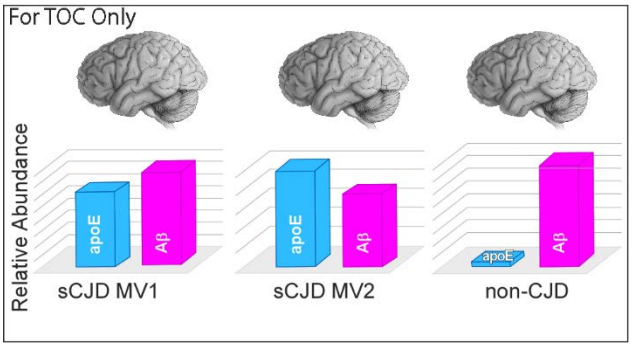
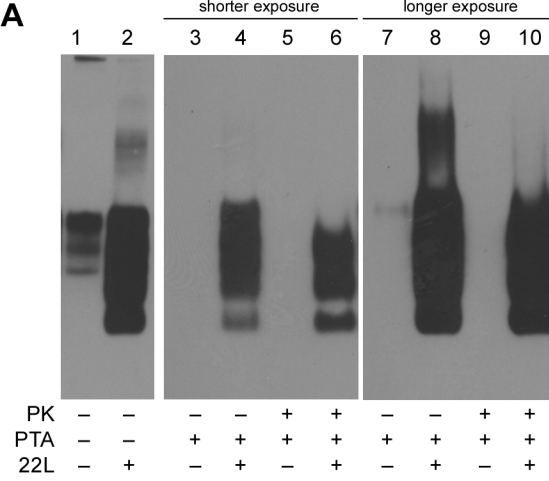
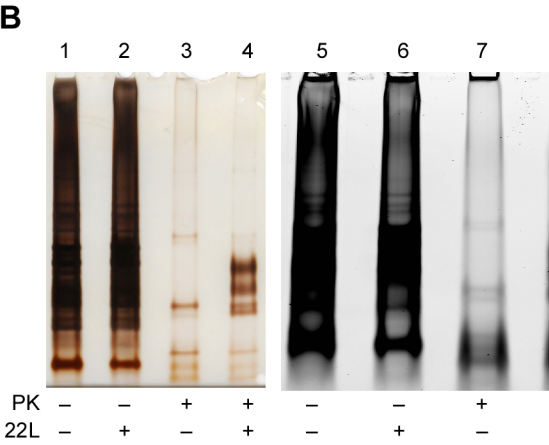
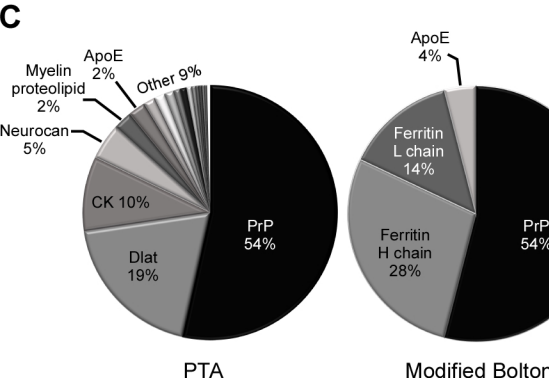
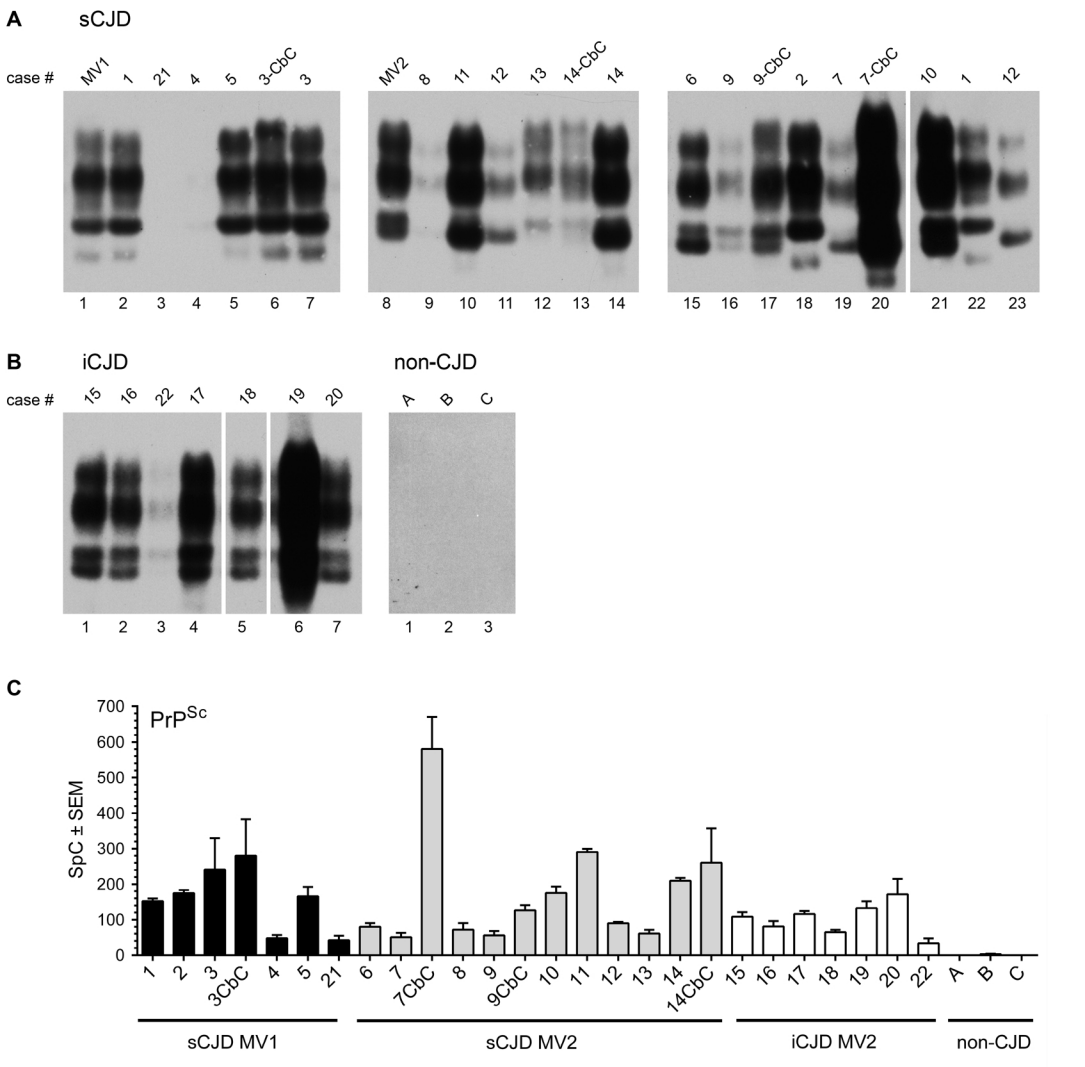
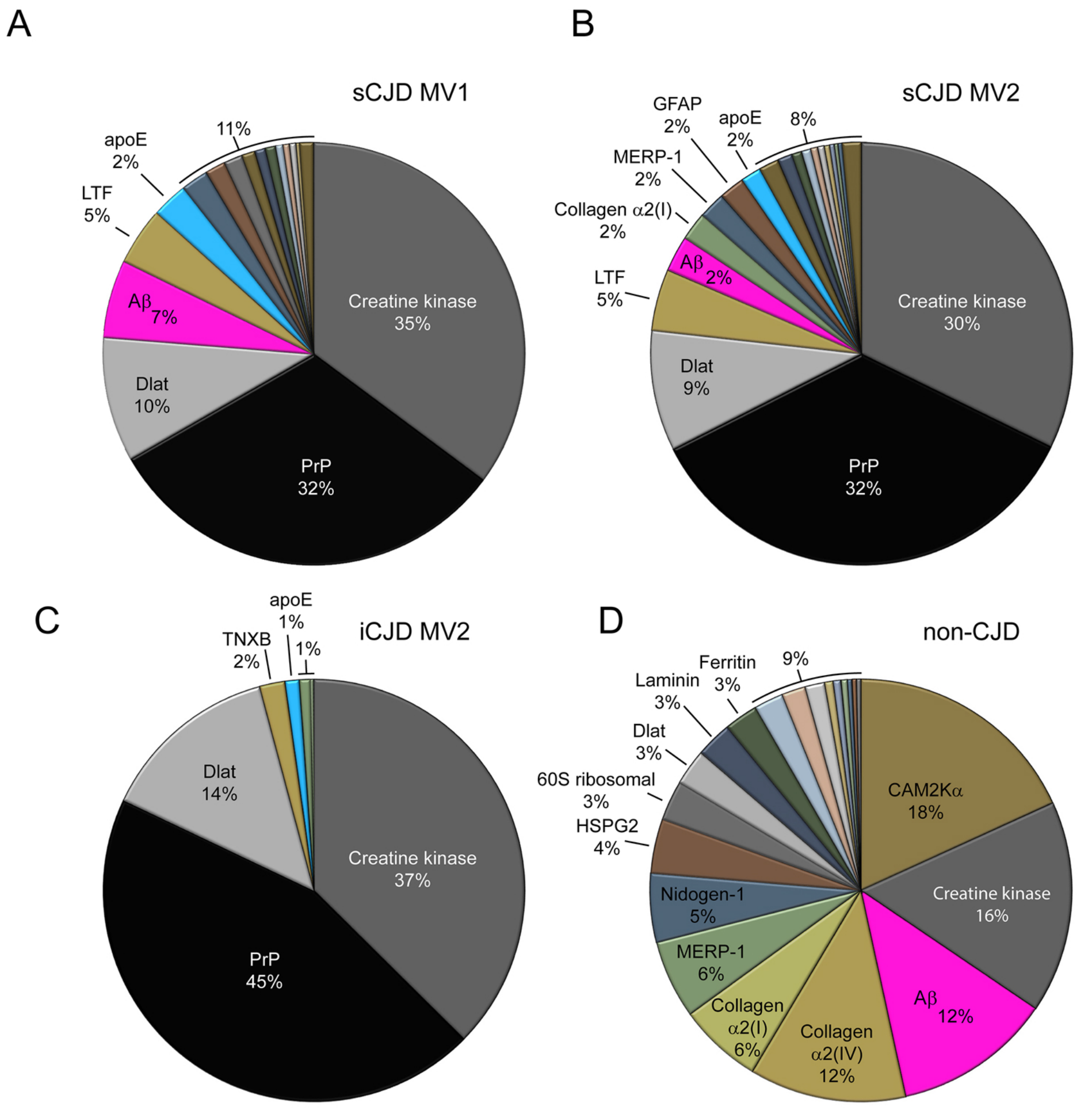

For TOC Only
Relative Abundance
apoE
Aβ
sCJD MV1
apoE
Aβ
sCJD MV2
apoE
Aβ
non-CJD

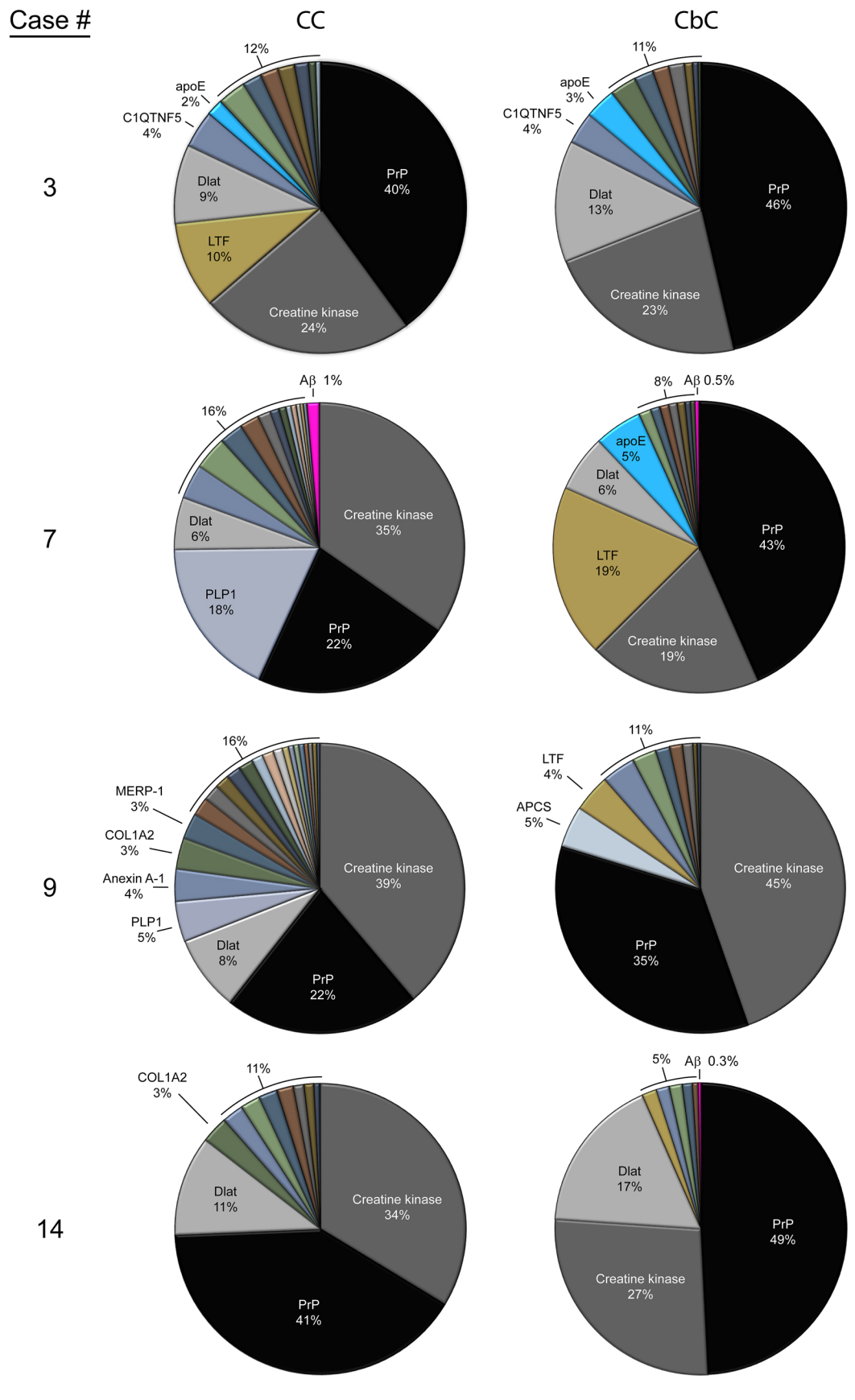

A
shorter exposure
longer exposure
1 2 3 4 5 6 7 8 9 10
PK – – – – + + – – + +
PTA – – + + + + + + + +
22L – + – + – + – + – +

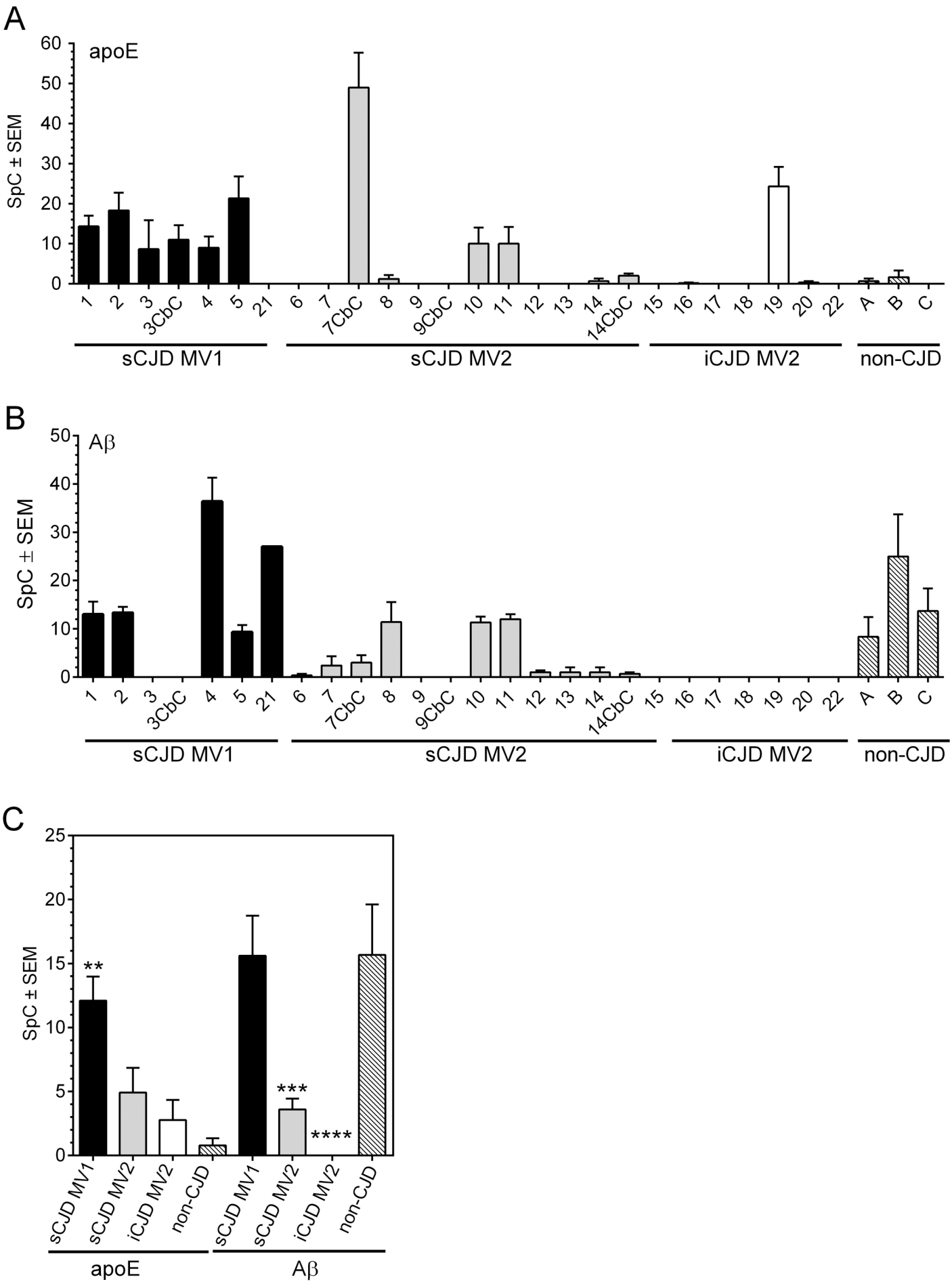

B
1 2 3 4 5 6 7 8
PK – – + + – – + +
22L – + – + – + – +

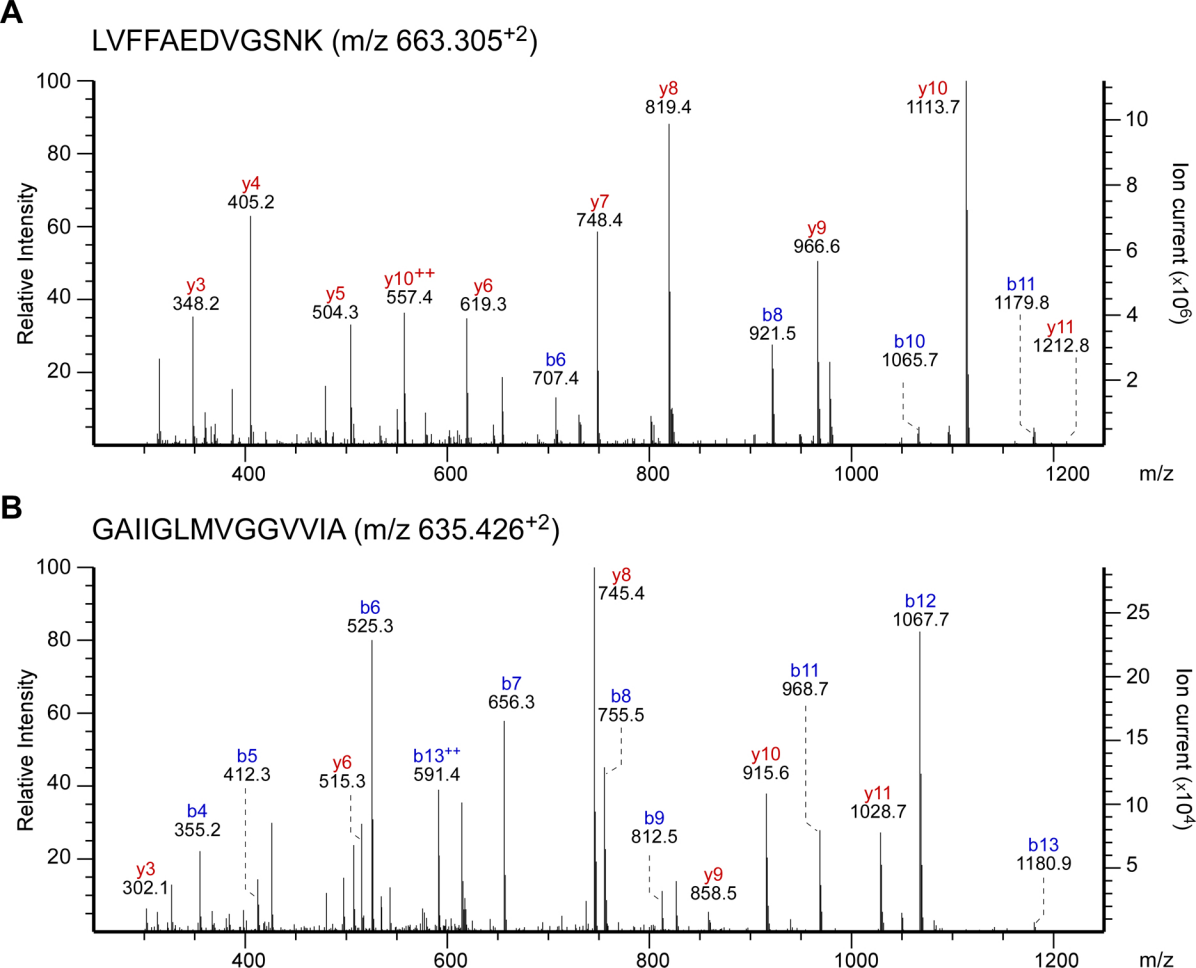

C
ApoE 2%
Myelin proteolipid 2%
Other 9%
Neurocan 5%
CK 10%
Dlat 19%
PrP 54%
PTA
ApoE 4%
Ferritin L chain 14%
Ferritin H chain 28%
PrP 54%
Modified Bolton

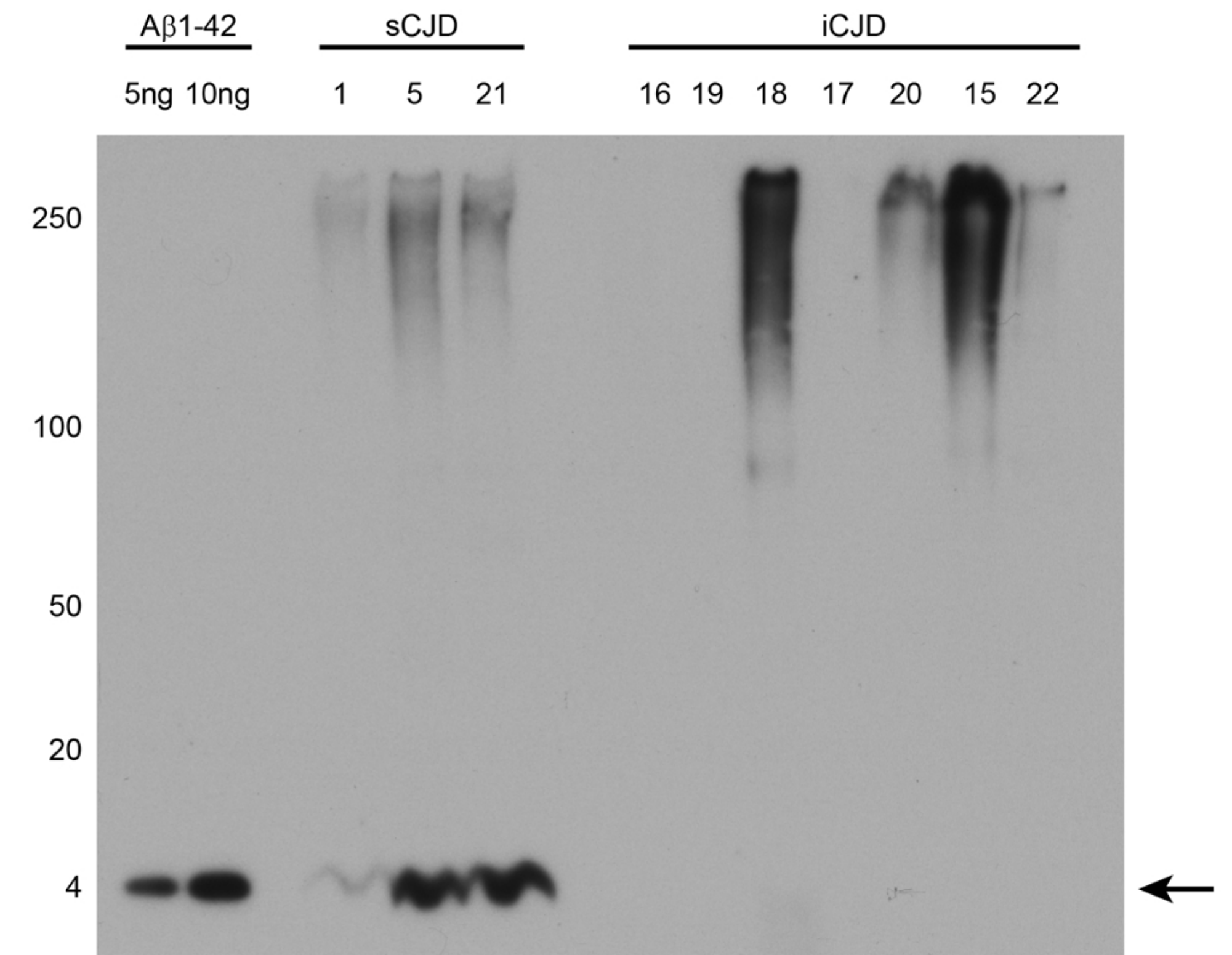

A
sCJD
case #
MV1
1
21
4
5
3-CbC
3
MV2
8
11
12
13
14-CbC
14
6
9
9-CbC
2
7
7-CbC
10
1
12
1 2 3 4 5 6 7
8 9 10 11 12 13 14
15 16 17 18 19 20
21 22 23
B
iCJD
non-CJD
case #
15
16
22
17
18
19
20
A
B
C
1 2 3 4 5 6 7
1 2 3
C
PrPSc
SpC ± SEM
700
600
500
400
300
200
100
0
1 2 3 3CbC 4 5 21 6 7 7CbC 8 9 9CbC 10 11 12 13 14 14CbC 15 16 17 18 19 20 22 A B C
sCJD MV1
sCJD MV2
iCJD MV2
non-CJD


A
sCJD MV1
Creatine kinase 35%
PrP 32%
Dlat 10%
Aβ 7%
LTF 5%
apoE 2%
11%
B
sCJD MV2
Creatine kinase 30%
PrP 32%
Dlat 9%
LTF 5%
Aβ 2%
Collagen α2(I) 2%
MERP-1 2%
GFAP 2%
apoE 2%
8%
C
iCJD MV2
Creatine kinase 37%
PrP 45%
Dlat 14%
TNXB 2%
apoE 1%
1%
D
non-CJD
CAM2Kα 18%
Creatine kinase 16%
Aβ 12%
Collagen α2(IV) 12%
Collagen α2(I) 6%
MERP-1 6%
Nidogen-1 5%
HSPG2 4%
60S ribosomal 3%
Dlat 3%
Laminin 3%
Ferritin 3%
9%


Case #
CC
CbC
3
12%
apoE 2%
C1QTNF5 4%
Dlat 9%
LTF 10%
PrP 40%
Creatine kinase 24%
11%
apoE 3%
C1QTNF5 4%
Dlat 13%
PrP 46%
Creatine kinase 23%
7
Aβ 1%
16%
Dlat 6%
PLP1 18%
PrP 22%
Creatine kinase 35%
8%
Aβ 0.5%
apoE 5%
Dlat 6%
LTF 19%
PrP 43%
Creatine kinase 19%
9
16%
MERP-1 3%
COL1A2 3%
Anexin A-1 4%
PLP1 5%
Dlat 8%
PrP 22%
Creatine kinase 39%
11%
LTF 4%
APCS 5%
PrP 35%
Creatine kinase 45%
14
COL1A2 3%
11%
Dlat 11%
PrP 41%
Creatine kinase 34%
5%
Aβ 0.3%
Dlat 17%
Creatine kinase 27%
PrP 49%


A
apoE
SpC ± SEM
60
50
40
30
20
10
0
1
2
3
3CbC
4
5
21
6
7
7CbC
8
9
9CbC
10
11
12
13
14
14CbC
15
16
17
18
19
20
22
A
B
C
sCJD MV1
sCJD MV2
iCJD MV2
non-CJD
B
Aβ
SpC ± SEM
50
40
30
20
10
0
1
2
3
3CbC
4
5
21
6
7
7CbC
8
9
9CbC
10
11
12
13
14
14CbC
15
16
17
18
19
20
22
A
B
C
sCJD MV1
sCJD MV2
iCJD MV2
non-CJD
C
SpC ± SEM
25
20
15
10
5
0
**
***
****
sCJD MV1
sCJD MV2
iCJD MV2
non-CJD
sCJD MV1
sCJD MV2
iCJD MV2
non-CJD
apoE
Aβ


A
LVFFAEDVGSNK (m/z 663.305+2)
Relative Intensity
Ion current (x10^6)
m/z
y8 819.4
y10 1113.7
y4 405.2
y7 748.4
y9 966.6
y3 348.2
y5 504.3
y10++ 557.4
y6 619.3
b11 1179.8
b8 921.5
y11 1212.8
b10 1065.7
b6 707.4
B
GAIIGLMVGGVVIA (m/z 635.426+2)
Relative Intensity
Ion current (x10^4)
m/z
y8 745.4
b6 525.3
b12 1067.7
b7 656.3
b11 968.7
b8 755.5
b5 412.3
y6 515.3
b13++ 591.4
y10 915.6
y11 1028.7
b4 355.2
b9 812.5
b13 1180.9
y3 302.1
y9 858.5


Aβ1-42
sCJD
iCJD
5ng 10ng
1 5 21
16 19 18 17 20 15 22
250
100
50
20
4

Supplemental Table 1. Proteins identified in PrP^{Sc} purified from 22L-infected and uninfected brain homogenate using the method of Bolton et al.

ID[a]	Centrifugation (22L-infected)	SpC[b]	Unique peptides[c]	Score[d]
P04925	Major prion protein	253	10	709.7
P09528	Ferritin heavy chain	107	10	647.2
P29391	Ferritin light chain 1	103	12	779.1
P49945	Ferritin light chain 2	51	6	369.5
P08266	Apolipoprotein E	18	6	291.8

[a]Protein identifiers are from www.uniprot.org.
[b]The total number of spectra assigned to that protein
[c]the number of unique peptides (*e.g*. with distinct *m/z* values).
[d]The sum of the highest MASCOT ion score for each unique peptide.