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# An Aged Canid with Behavioral Deficits Exhibits Blood and Cerebrospinal Fluid Amyloid Beta Oligomers

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Many of the molecular and pathological features associated with human Alzheimer disease (AD) are mirrored in the naturally occurring age-associated neuropathology in the canine species. In aged dogs with declining learned behavior and memory the severity of cognitive dysfunction parallels the progressive build up and location of A $\beta$  in the brain. The main aim of this work was to study the biological behavior of soluble oligomers isolated from an aged dog with cognitive dysfunction through investigating their interaction with a human cell line and synthetic A $\beta$  peptides. We report that soluble oligomers were specifically detected in the dog's blood and cerebrospinal fluid (CSF) via anti-oligomer- and anti-A $\beta$  specific binders. Importantly, our results reveal the potent neurotoxic effects of the dog's CSF on cell viability and the seeding efficiency of the CSF-borne soluble oligomers on the thermodynamic activity and the aggregation kinetics of synthetic human A $\beta$ . The value of further characterizing the naturally occurring Alzheimer-like neuropathology in dogs using genetic and molecular tools is discussed.

**Keywords:** canine cognitive dysfunction, Alzheimer, canine, A $\beta$  oligomers, neuropathology, aggregation, neurotoxicity

## INTRODUCTION

Aging dogs spontaneously deposit human-type amyloid  $\beta$  (A $\beta$ ) peptide (Selkoe et al., 1987; Johnstone et al., 1991) and thus are a natural higher mammalian model of aging. The canine A $\beta$  precursor protein (APP) is virtually identical to human APP (~98% homology). In parallel with progressive A $\beta$  pathology, aged dogs show decline in measures of learning and memory that are correlated with the extent and location of A $\beta$  (Cotman and Head, 2008). However, a recent report by Borghys and colleagues demonstrated that high levels of A $\beta$  in the cerebrospinal fluid (CSF) of young and middle-aged dogs correlated with impaired learning (Borghys et al., 2017), in contrast

with previous reports showing that CSF A $\beta$  content decreases in the aged/aging dog (Head et al., 2010). Of note, cognitive decline occurs prior to the accumulation of A $\beta$  plaques in the canine brain, suggesting that earlier assembly states of A $\beta$  (e.g., oligomers, protofibrils) may be the toxic species in the canine brain as in the human brain (Head et al., 2010).

In this study, we describe the presence of A $\beta$  soluble oligomers in serum and CSF of a 12-year-old Samoyed (referred to as “the Subject” throughout this report). Upon neurological examination, this subject displayed signs of cognitive impairment and magnetic resonance imaging (MRI) showed diffuse cortical atrophy. A $\beta$  immunostaining demonstrated extensive diffuse plaques in the neocortex and hippocampal regions; but no tau staining. Of importance, CSF and serum from this subject exhibited neurotoxic effects following treatment of a human neuroblastoma cell line and led to efficient aggregation of synthetic human A $\beta$  peptides.

## CASE REPORT

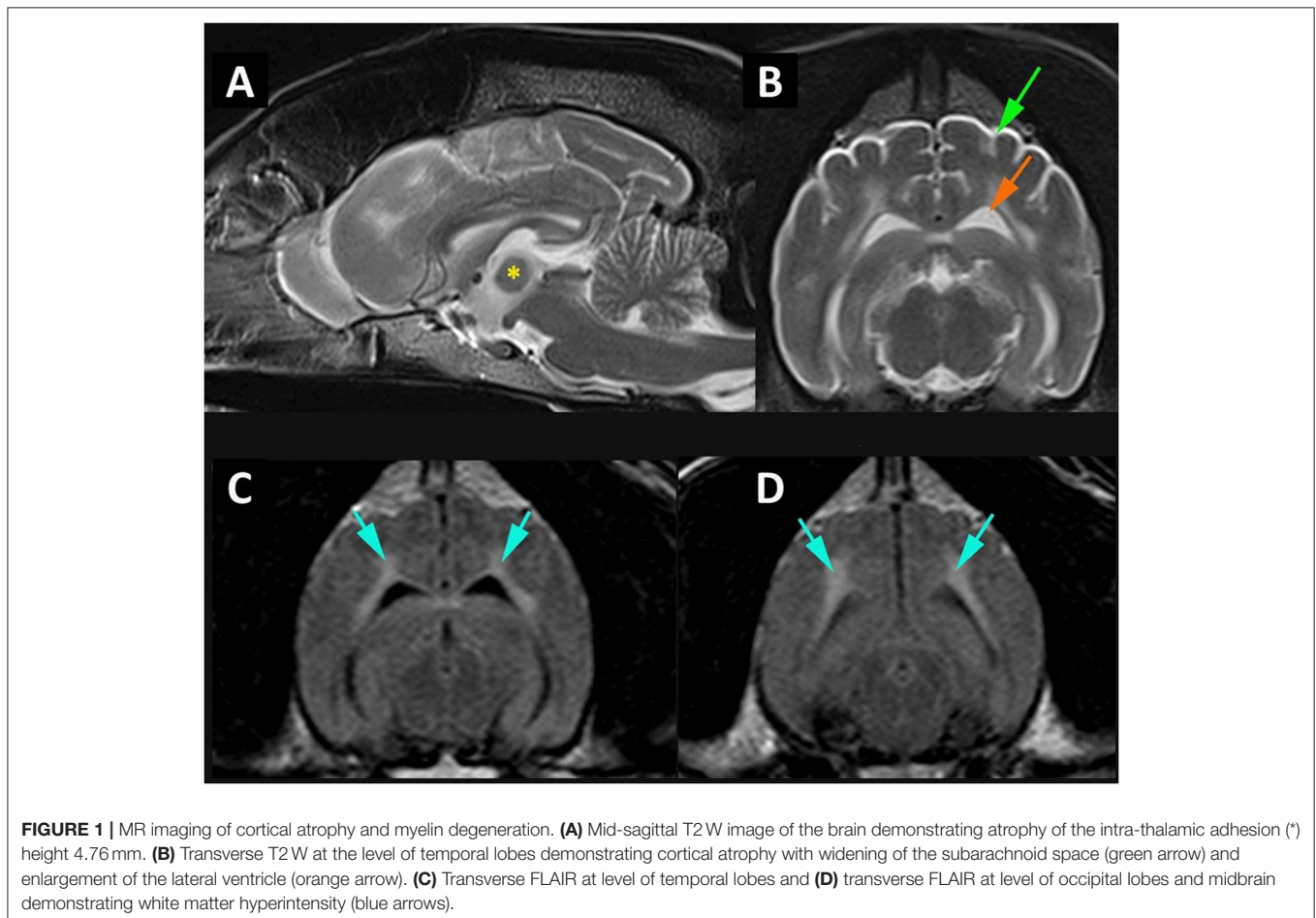
### Ethics Statement

This project was reviewed by the University of Surrey Ethics Committee and verified that the aspects of the study pertaining

to the Samoyed dog including use of body fluids excess and post mortem material did not come under the auspices of Animals (Scientific Procedures) Act 1986 (ASPA). The subject's owner gave informed consent to participation in the study. Full clinical and neurological examination and presentation, including MR imaging assessment are found in Supplementary Materials. Negative control CSF was obtained from a 10-year-old male Rottweiler suffering from nodular granulomatous episclerokeratitis following submission for routine teaching post-mortem and not subject to animal ethics guidelines. CSF derived from a 79-year-old patient with advanced sporadic AD (pos1-CSF) and from a 65 year old patient with advanced sporadic AD (pos2-CSF) were provided by The UK Multiple Sclerosis Tissue Bank.

### Clinical Presentation

A 12-year-old neutered male Samoyed dog was presented for pain management and evaluation of difficulty in rising. Neurological examination revealed tetraparesis and reduced spinal reflexes and muscle tone consistent with a polyneuropathy. The difficulty rising was attributed to this, complicated by the sedative polypharmacy. The historical and consulting

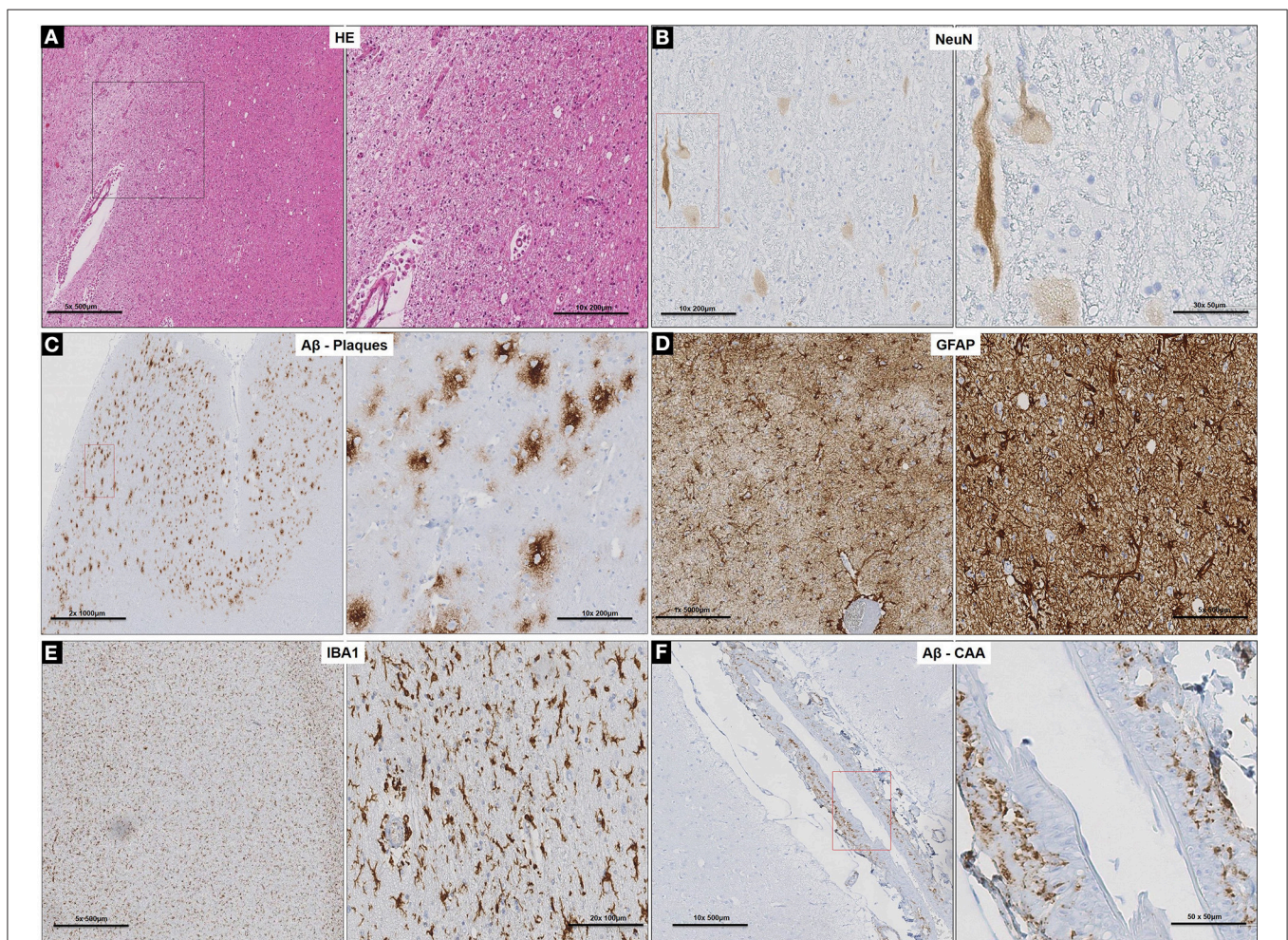




room behavior suggested a cognitive function deficit possibly complicated by a urinary tract infection. The brain MRI scan revealed a diffuse cortical atrophy and a hyperintensity in the white matter on T2W, particularly in the corona radiata (**Figure 1**); changes consistent with age-associated cognitive decline (Hasegawa et al., 2005). Signs were controlled for the next 6 months after which the dog deteriorated and described as being extremely agitated and distressed during the night which was unresponsive to changing dose of medication and resulting in significant sleep deprivation for the owners. A full post mortem examination was undertaken at the pathology facility at the University of Surrey and the brain and other organs were removed for further analysis. A more extensive description of the clinical and neurological examination and presentation is included as supplementary results.

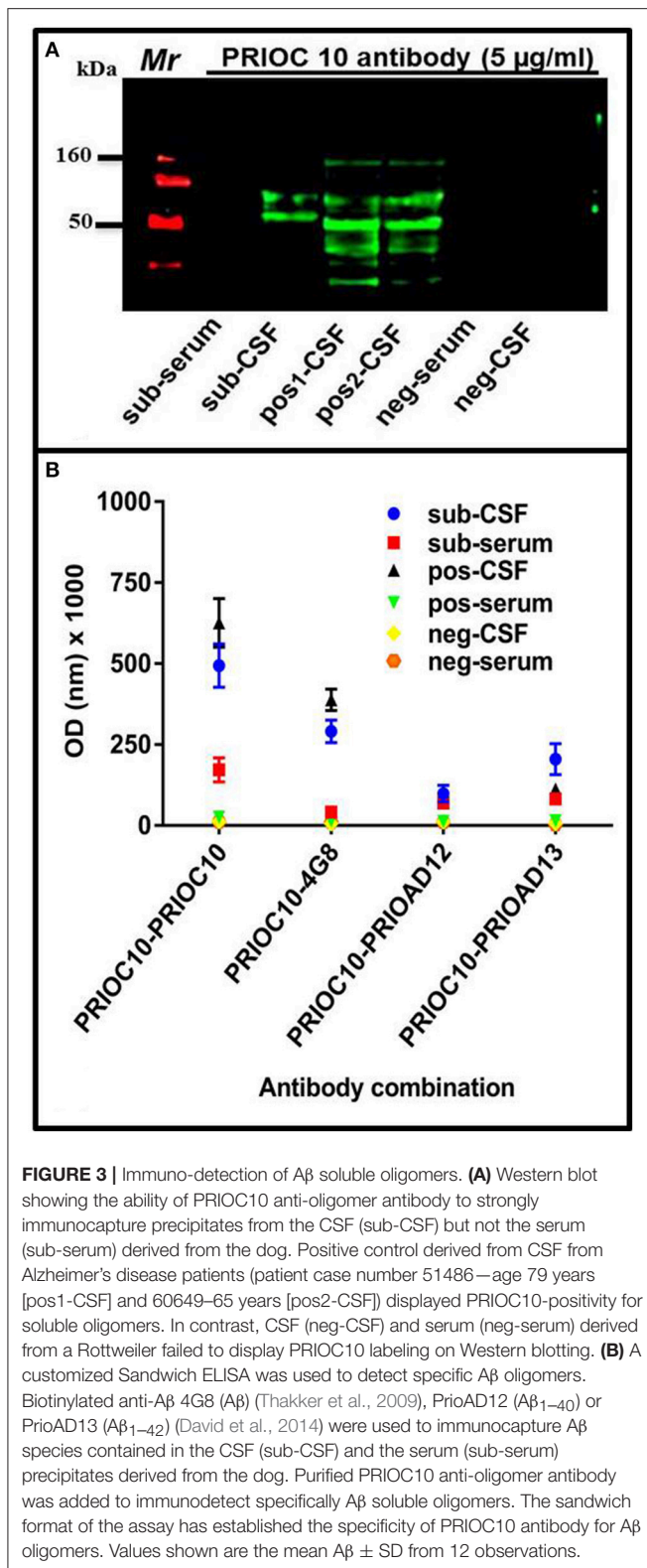
## Detection of A $\beta$ Species in the Subject's Serum, CSF, and Brain

Neuronal loss and degeneration was marked in the cortical region of the subject (**Figures 2A,B**) while intense binding of large extra-cellular diffuse A $\beta$  plaques, recruitment, and activation of astrocytes and microglial cells (**Figures 2C–E**) were observed in the neocortex and hippocampus. Pronounced cerebral amyloid angiopathy (CAA) was observed in several cortical blood vessels (**Figure 2F**), but was less intense in the tunica media of leptomeningeal arteries. Of note, PHF and the signaling adapter p62 were not detected (data not shown) (Babu et al., 2005). Furthermore, white matter degeneration took the form of myelin vacuolation and isomorphic gliosis (**Figure 2A**) with macrophages containing pale yellow cytoplasmic material evident as small clusters of cells and as perivascular aggregates (**Figure 2A**). Iba1



**FIGURE 2 |** Cortico-neuropathological microscopic lesions. **(A)** Cortical degeneration (vacuolation) and neuronal death observed on routine H&E stained sections of prefrontal cortex and diffuse cerebral periventricular white matter degeneration (vacuolation and pallor); **(B)** Neuronal degeneration and loss (arrows) confirmed with neuron-specific marker, NeuN. **(C)** Specific labeling of diffuse A $\beta$  plaques with anti-A $\beta$  specific antibody in the prefrontal cortex. **(D)** Extensive gliosis in prefrontal cortex revealed by GFAP stain and associated with **(E)** microglia activation demonstrated with Iba1 staining. **(F)** Specific labeling of CAA with anti-A $\beta$  specific antibody in the cortical blood vessels in the prefrontal cortex.





stain demonstrated more widespread microglial/macrophage activation while GFAP confirmed the brisk gliosis (data not shown).

The ability of PRIOC10 mAb to bind to A $\beta$  soluble oligomers in sub-serum and sub-CSF was assessed by Western blotting and Sandwich ELISA. Western blotting results indicate that PRIOC10 mAb bound to soluble oligomers in sub-CSF but not in sub-serum or negative control CSF (neg-CSF) derived from the Rottweiler (Figure 3A). Further, PRIOC10 mAb pattern of recognition revealed several bands ranging between 50 and 160 kDa (Figure 3A); and positive control CSF (pos-CSF) isolated from two patients with AD also led to detection of soluble oligomers (Figure 3A).

We then set out to investigate the specificity of the soluble oligomers and confirm that the PRIOC10-specific bands detected in the sub-CSF were mainly composed of A $\beta$  using our customized sELISA as described previously (Tayebi et al., 2011). PRIOC10, 4G8 (A $\beta$ ) (Thakker et al., 2009), PrioAD12 (A $\beta$ <sub>1–40</sub>), or PrioAD13 (A $\beta$ <sub>1–42</sub>) (David et al., 2014) were used as immunocapture antibodies to detect soluble oligomers in the sub-CSF and sub-serum, followed by immunodetection with biotinylated PRIOC10 (Figure 3B). We show that the 4G8 vs. biot-PRIOC10 combination detected highest levels of A $\beta$  in sub-CSF as expressed in OD-values ( $p = 0.0001$ ) and almost matched CSF levels detected with the PRIOC10 vs. biot-PRIOC10 combination. This was followed by the PRIOAD13 vs. biot-PRIOC10 combination that displayed higher CSF levels of detection compared with the PRIOAD12 vs. biot-PRIOC10 combination ( $p = 0.0279$ ). Of note, sELISA lead to detection of A $\beta$  in the sub-serum (Figure 3B) in contrast with western blotting, albeit with significantly lower OD intensity as compared to levels detected in the sub-CSF.

## Mutations in Presenilin 1, Presenilin 2, and Amyloid Precursor Protein Genes Were Not Identified

Genome assembly CanFam3.1 and transcripts ENSCAFT00000013599.4 for *APP*, ENSCAFT00000026626.1 for *PSEN1*, and ENSCAFT00000025451.3 for *PSEN2* were used for primer design (See Supplementary Results: Table S2) and as the reference for sequence analysis. The subject's DNA was used to sequence the genes that when mutated are known to cause AD in humans. No variants expected to be pathogenic were identified. Synonymous variants were found in *APP* (p.G120G; p.K178K; p.A242A; p.T266T), and *PSEN2* (p.P436P).

## CSF and Serum Derived from Subject Was Toxic to Neuron-Like SH-SY5Y Cell Line

The toxic effects of monoA $\beta$ <sub>1–40</sub>, monoA $\beta$ <sub>1–42</sub>, scamA $\beta$ <sub>25–35</sub>, oligoA $\beta$ <sub>1–40</sub>, oligoA $\beta$ <sub>1–42</sub>, fibA $\beta$ <sub>1–40</sub>, fibA $\beta$ <sub>1–42</sub>, sub-serum, and sub-CSF on differentiated human neuroblastoma cell line RA-SH-SY5Y viability was investigated using the MTT assay. To achieve similar concentrations of synthetic A $\beta$  and CSF/serum-borne A $\beta$ , standard curves of all synthetic A $\beta$  was generated and the subject's CSF and serum A $\beta$  oligomers values were determined by comparison to the appropriate standard curve. MonoA $\beta$ <sub>1–40</sub>, monoA $\beta$ <sub>1–42</sub>, scamA $\beta$ <sub>25–35</sub>, and sub-serum displayed no toxicity toward RA-SH-SY5Y neuroblastoma cells as compared to untreated cells ( $p \leq 0.05$ ) (Figure 4). In

contrast, treatment with oligoA $\beta_{1-40}$ , oligoA $\beta_{1-42}$ , fibA $\beta_{1-40}$ , fibA $\beta_{1-42}$ , and sub-CSF lead to significant cell death as compared with untreated cells, resulting in  $\leq 61\%$  cell viability for treatment with both oligoA $\beta_{1-40}$  and fibA $\beta_{1-40}$  ( $p \leq 0.05$ ) and  $\leq 44\%$  cell viability for treatment with oligoA $\beta_{1-42}$ , fibA $\beta_{1-42}$ , and sub-CSF ( $p \leq 0.05$ ) (Figure 4).

Cell viability was significantly affected following treatment with oligoA $\beta_{1-42}$  compared with treatment with the fibrillary species of A $\beta_{1-42}$  (17 vs. 27%;  $p \leq 0.05$ ), while treatment with sub-CSF lead to 44% cell death. These results show that the subject's CSF induced RA-SH-SY5Y cell death and confirmed the potent toxic effects of A $\beta$  soluble oligomers previously shown to affect neurons (Bate et al., 2008).

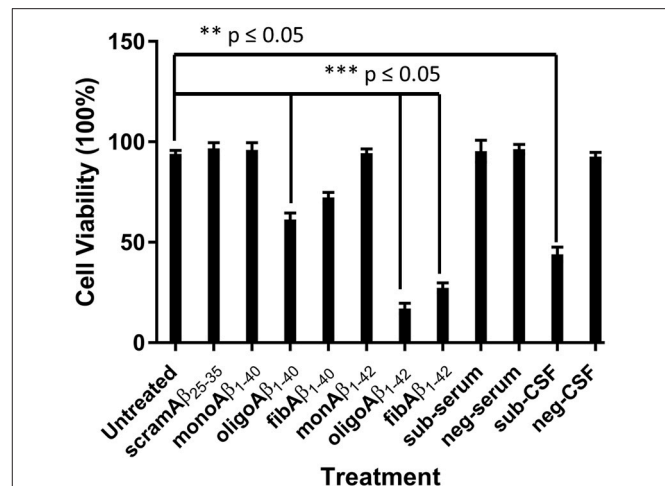
## CSF but Not Serum Derived from the Subject Accelerates A $\beta$ Aggregation Kinetics *in Vitro*

We first demonstrated that PRIO10 immunodetected A $\beta$  soluble oligomer species derived from monoA $\beta_{1-40}$  and monoA $\beta_{1-42}$  peptides (Figure 5A). Secondly, ThT fluorescence intensity of the fibrillar species was measured following conversion of monoA $\beta_{1-40}$ , monoA $\beta_{1-42}$ , and scramA $\beta_{25-35}$  peptides was assessed and was shown to be inversely proportional to levels of PRIO10-specific oligomer species (Figure 5B). ThT did not bind to scramA $\beta_{25-35}$  peptide before and after being incubated in conversion buffer and to monoA $\beta_{1-40}$  and monoA $\beta_{1-42}$  peptides before conversion. Of note and as shown previously, PRIO10 failed to bind the fibrillar species (Tayebi et al., 2011).

We then assessed aggregation kinetics of “seed-free” synthetic monomeric A $\beta$  peptide following addition of A $\beta$  oligomers or fibrils (Figures 5C,D). A known concentration of A $\beta$  prepared by conversion during 12 h ( $t_{12}$ ) and 72 h ( $t_{72}$ ), as described above, was used in the seeding reaction; as  $t_{12}$  represents maximal optical density (OD) expression of A $\beta$  soluble oligomers immunodetected with PRIO10 (Figure 5A) and  $t_{72}$  represents maximal fluorescence expression of A $\beta$  fibrils bound to ThT (Figure 5B).

Here, we added 10 pmol oligoA $\beta_{1-40}$ /oligoA $\beta_{1-42}$  ( $t_{12}$ ), fibA $\beta_{1-40}$ /fibA $\beta_{1-42}$  ( $t_{72}$ ), or scramA $\beta_{25-35}$  ( $t_{12}$  and  $t_{72}$ ) prepared by conversion during  $t_{12}$  and  $t_{72}$  to 2 mM monoA $\beta_{1-40}$ , or monoA $\beta_{1-42}$  in order to assess their effects on the “lag-phase” kinetic as measured by ThT fluorescence. We report that  $t_{12}$  oligoA $\beta_{1-40}$ , oligoA $\beta_{1-42}$  but not post-conversion scramA $\beta_{25-35}$  led to substantial reduction of the “lag-phase” ( $p \leq 0.05$ ) compared to  $t_0$  A $\beta$  (Figures 5C,D). Importantly, we show that oligoA $\beta_{1-42}$  was more efficient in shortening the “lag-phase” compared to oligoA $\beta_{1-40}$  ( $p \leq 0.05$ ). Both  $t_{72}$  fibA $\beta_{1-40}$  and fibA $\beta_{1-42}$  but not post-conversion scramA $\beta_{25-35}$  affected the A $\beta$  aggregation kinetic by shortening the reaction's “lag-phase,” albeit the effect was limited when compared to the addition of the oligomers (Figures 5C,D), reflecting a weaker seeding ability of the fibrils.

Finally, the subject's serum and CSF was added to “seed-free” synthetic monomeric A $\beta$  to investigate whether pre-existing

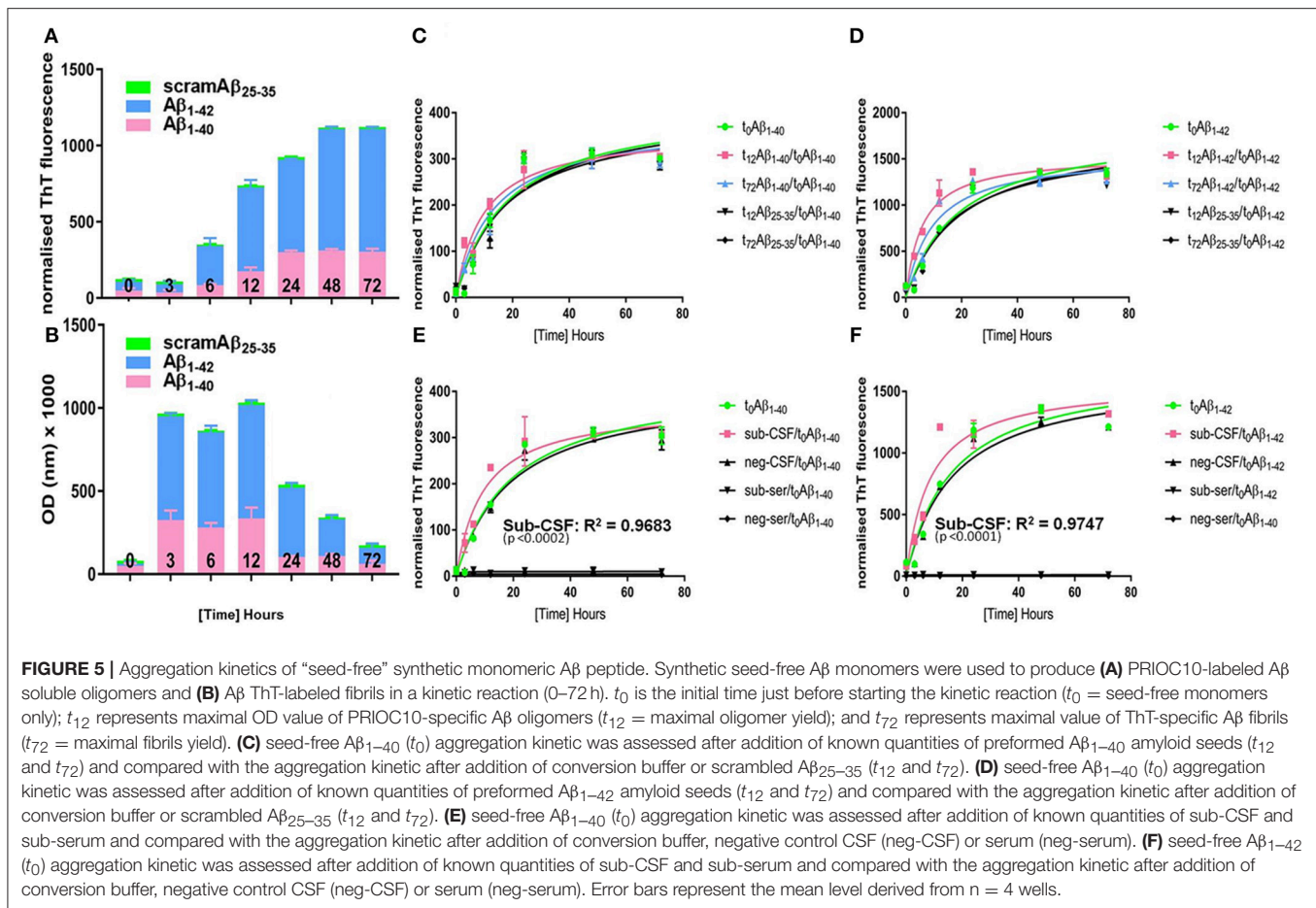


**FIGURE 4** | CSF but not serum derived from the aged dog leads to neurotoxicity of neuron-like SH-SY5Y cell line. The effect of CSF and serum on the survival of SH-SY5Y cell line was compared with monoA $\beta_{1-40}$ , monoA $\beta_{1-42}$ , scramA $\beta_{25-35}$ , oligoA $\beta_{1-40}$ , oligoA $\beta_{1-42}$ , fibA $\beta_{1-40}$ , fibA $\beta_{1-42}$  as well as CSF (neg-CSF) and serum (neg-serum) derived from a Rottweiler. Values shown are the mean cell survival  $\pm$  SD from 12 observations.

oligomer seeds contained in the serum and CSF of the aged dog can affect the A $\beta$  aggregation kinetics through reduction of the “lag-phase” and to explore if cross-species interaction of dog A $\beta$  with human A $\beta$  synthetic peptide overcomes the so-called “species barrier” as applies for prion disorders (Hill and Collinge, 2002). First, we tested whether the precipitation protocol altered the conformation of A $\beta$  soluble oligomer content of sub-CSF and sub-serum. We show that PRIO10-specific A $\beta$  oligomers in TCA/acetone treated sub-CSF and sub-serum were preserved (Figure S1). Total protein (20  $\mu$ l of 10 mg/ml) derived from sub-serum and sub-CSF samples were incubated with solutions of monoA $\beta_{1-40}$ , and monoA $\beta_{1-42}$  peptides. Surprisingly and for the first time, we show that the dog's CSF led to a substantial shortening of the reaction's “lag-phase” ( $p \leq 0.05$ ) and acceleration of human synthetic monomeric A $\beta_{1-40}$  and A $\beta_{1-42}$  aggregation as compared to a negative control CSF derived from the Rottweiler or scramA $\beta_{25-35}$  (Figures 5E,F). Sub-CSF was more efficient in speeding up A $\beta_{1-42}$  than A $\beta_{1-40}$  aggregation. In contrast, sub-serum and negative control serum led to complete inhibition of the A $\beta$  kinetics and the “lag-phase” was not observed (Figures 5E,F). Of note, similar concentrations of synthetic A $\beta$  and CSF/serum-borne A $\beta$  were used.

## DISCUSSION

The neuropathological changes observed in the 12-year-old Samoyed dog were previously described in aged dogs (Youssef et al., 2016). The extra-cellular diffuse A $\beta$  deposits were observed throughout the cerebral cortex I-IV layers adhering to the typical staged distribution recognized in cognitively impaired dogs (Pugliese et al., 2006) and human AD (Schmidt et al., 2015). Several blood vessels of the cerebral cortex displayed severe



and pronounced CAA. Colle et al. have previously shown that A $\beta_{1-42}$ -positive and Congo red-A $\beta_{1-40}$ -negative deposits were predominant in the brain parenchyma of aged dogs while A $\beta_{1-40}$  deposited to the vasculature (Colle et al., 2000). White matter degeneration was also evident in our aged dog with vacuolation of myelinated tracts, accumulation of what appears to be lipofuscin-filled macrophages as perivascular aggregates and widespread microglial activation and gliosis. In human AD, the significance of white matter degeneration remains in dispute as its significance in disease pathogenesis remains uncertain (Ihara et al., 2010) mainly because these are considered as geriatric changes and recognized in cognitively normal individuals and dogs (Lintl and Braak, 1983; Chambers et al., 2012). Notably, in our behaviorally impaired dog, we have not been able to detect *APP*, *PSEN1*, or *PSEN2* gene autosomal dominant mutations which are known to directly influence accumulation of A $\beta$  plaques and CAA in human AD (Selkoe, 2001).

In human AD, A $\beta$  soluble oligomers are considered the neurotoxic species with the ability to affect cognitive ability and alter synaptic functions (Selkoe, 2002). Attempts to detect putative relatively low amount of CSF- and serum-borne A $\beta$  soluble oligomers in AD patients have been hampered by the lack of assays with sufficient sensitivity and specificity (Bruggink et al., 2013; Herskovits et al., 2013; Hölttä et al.,

2013; Tai et al., 2013; Savage et al., 2014). The importance of A $\beta$  soluble oligomers in the pathogenesis of cognitive deficits and their effects on synaptic activity and function have not been investigated in aged dogs with cognitive deficits; however a report by Head et al. demonstrated that levels of CSF-borne A $\beta$  soluble oligomers correlated inversely with total brain amounts of A $\beta$  in aged beagles (Head et al., 2010). However, a recent report by Borghys et al. showed that high levels of A $\beta$  in the CSF of young and middle-aged dogs were detected and correlated with impaired learning (Borghys et al., 2017). The report does not specify whether A $\beta$  measured in the CSF of these animals contains soluble oligomer species (Borghys et al., 2017). We have shown here that our anti-oligomer antibody displayed oligomer-specific bands ranging from 90 to 200 kDa in the CSF but not in serum derived from the behaviorally impaired dog, the latter probably reflecting low levels of A $\beta$  soluble oligomer concentrations in blood (Santos et al., 2008). Similarly, A $\beta$  was detected with our two-site sELISA in CSF; using PRIOC10 as immunocapture antibody and a biotinylated anti-A $\beta$  (4G8), anti-A $\beta_{1-40}$  (PrioAD12), or anti-A $\beta_{1-42}$  (PrioAD13) for immunodetection, and further confirms the A $\beta$  specificity of the soluble oligomers observed on western blotting. Of importance, the sELISA detected significantly higher levels of total A $\beta$  oligomers (PRIOC10 vs. 4G8), followed by A $\beta_{1-42}$



soluble oligomers (PRIOC10 vs. PRIOAD13 combination) then A $\beta_{1-42}$  soluble oligomers (PRIOC10 vs. PRIOAD12). In contrast with the result observed on Western blotting, we were able to detect soluble oligomers in the serum of our aged dog, albeit the levels were much lower than those observed in the CSF. Taken together, these results demonstrate the presence of different species of A $\beta$  soluble oligomers in the subject's CSF in agreement with the results reported by Head et al. (2010). The results also confirm the "hierarchy" of A $\beta_{1-42}$  over A $\beta_{1-40}$  as observed in human AD (Armstrong, 2014).

We then set out to investigate the toxic nature of CSF- and serum-borne A $\beta$  species derived from our behaviorally impaired dog by treating a neuron-like cell line and compare their effects with synthetic A $\beta$  oligomers and fibrils derived from synthetic monomeric A $\beta_{1-40}$  and A $\beta_{1-42}$ . Surprisingly, CSF but not serum derived from our behaviorally impaired dog significantly affected cell viability as measured by MTT. Several studies have reported that soluble oligomers accumulate in the CSF of AD patients and exhibit putative neurotoxic effects of homologous A $\beta$  *in vitro* (Bate and Williams, 2007; Shankar et al., 2007; Bate et al., 2008). To our knowledge, this is the first report showing that CSF-borne A $\beta$  oligomers from a dog with behavioral impairment affected viability of human-derived neuron-like cell lines; further proving the potent neurotoxic effects of A $\beta$  soluble oligomers, although other factors might be implicated in the observed toxic effect. The impact on cell viability of the CSF was compared to synthetic A $\beta_{1-40}$  and A $\beta_{1-42}$  oligomers and fibrils following treatment of the neuroblastoma cell line; CSF was shown to be more toxic than either A $\beta_{1-40}$  oligomers and fibrils but expectedly less so than A $\beta_{1-42}$  oligomers and fibrils. This is similar to our previous results showing that A $\beta_{1-42}$  was more potent at damaging neuronal synapses compared with A $\beta_{1-40}$  peptide (Bate et al., 2008). It is important to note that the oligomeric/fibrillar A $\beta$  content of the CSF derived from the behaviorally impaired dog is not known, hence a more accurate comparison can only be achieved through subjecting the cells to similar and known concentration of A $\beta$  species in biological fluids.

A central feature in AD is fibril biogenesis leading to senile plaques (Powers and Powers, 2008). The molecular mechanism underlying the formation of fibrils controls the speed and the degree of its formation and the kinetics of oligomers and protofibrils (Jarrett and Lansbury, 1993; Caughey and Lansbury, 2003) and directly influences AD pathogenesis. Here, we investigated the seeding efficiency of CSF- and serum-borne A $\beta$  oligomers and their influence on human synthetic A $\beta$  peptides. Initially, we established assay conditions and reproducibility by adding known concentrations of post-conversion  $t_{12}$ -A $\beta$  and  $t_{72}$ -A $\beta$  oligomers and fibrils into the reaction. As anticipated, we show that all forms of post-conversion A $\beta$ , except scrambled A $\beta_{25-35}$  peptide, led to a substantial reduction of the lag phase (oligoA $\beta_{1-42}$  > oligoA $\beta_{1-40}$  > fibA $\beta_{1-42}$   $\geq$  fibA $\beta_{1-40}$ ). Following addition of CSF derived from our behaviorally impaired dog, the A $\beta$  aggregation kinetic was substantially altered and led to reducing the lag phase, confirming both the presence but also the potent effect of soluble oligomers. In contrast, serum, negative control CSF and scrambled A $\beta_{25-35}$  peptide did not affect the kinetic of the reaction. The ability

of CSF-borne soluble oligomer to affect synthetic peptides derived from human  $\beta$  sequences was perhaps expected as the canine APP shares about 98% homology with human APP. Nevertheless, this result is important as it demonstrates inter-species molecular interaction but does not suggest nor does it demonstrate any interspecies transmission between humans and dogs. Finally, studies are underway to determine whether the above findings are a more universal phenomenon recognized in different breeds of aged and behaviorally impaired dogs.

## CONCLUDING REMARKS

We have comprehensively demonstrated that this behaviorally impaired dog exhibited A $\beta$  and A $\beta$  soluble oligomers in its blood, CSF, and brain. We also show that the dog's A $\beta$  affects the survival of human-derived neuron-like cell line and has a direct effect on the aggregation kinetics of human synthetic A $\beta$  peptides. The study failed to demonstrate the involvement of phospho-tau and more genetic and molecular studies are needed to decipher its role in the neuropathology underlying cognitive dysfunction, yet we would advocate that dogs with behavioral impairments should be studied and the disease mechanisms investigated in a similar fashion as is the case with AD.

## AUTHOR CONTRIBUTIONS

CR: Performed Clinical assessment of the dog, performed MRI and revised manuscript; FS: Performed experiments; MD: Performed experiments; KF: Performed experiments; JB: Performed experiments; RG: Performed experiments; AR-L: Performed experiments; DG: Performed experiments; EH: Conceived experiments and revised manuscript; SB: Conceived experiments and revised manuscript; BS: Conceived experiments and revised manuscript; JH: Conceived experiments and revised manuscript; MT: Designed experiments, performed experiments and wrote manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnagi.2018.00007/full#supplementary-material>



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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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