



# Preliminary insights into $\beta$ -Amyloid, phospho-tau and inbreeding in Labrador Retriever dogs

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**Abstract:** Serum concentrations of  $\beta$ -amyloid peptides (A $\beta$ 40 and A $\beta$ 42) and phosphorylated tau (p-tau) are emerging as potential biomarkers of age-related neurodegenerative processes in dogs, particularly in relation to canine cognitive dysfunction. At the same time, the genetic background, especially inbreeding, may influence aging trajectories and brain pathology. This paper reports preliminary observations on the relationship between plasma A $\beta$ 40, A $\beta$ 42, and p-tau levels and the inbreeding coefficient in 24 healthy Labrador Retriever dogs. Blood samples were collected and analyzed using ELISA kits specific for canine A $\beta$  and phosphorylated tau, and inbreeding coefficients were calculated based on pedigree data. In addition, the Canine Dementia Scale (CADES) was administered. Although the small sample size limits the strength of statistical inference, initial findings suggest potential associations between age, biomarker concentrations, and sex. However, the inbreeding coefficient was too low to detect possible correlations with biomarker levels. These results primarily provide preliminary insight into the effects of neurobiological aging in dogs, while the contribution of genetic factors remains to be clarified in larger cohorts.

**Key Words:** dog; labrador; inbreeding coefficient; pTau; Amyloid  $\beta$ ; CADES

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## Introduction

Dogs are increasingly recognized as a valuable model for studying age-related cognitive decline and neurodegeneration, due to similarities with human aging processes. Among the most studied biomarkers are amyloid-beta (A $\beta$ ) peptides and Tau proteins, which show age-dependent changes in both healthy and cognitively impaired dogs. In particular, plasma A $\beta$ 40 and A $\beta$ 42 levels tend to increase with age in healthy dogs, while significantly lower concentrations have been reported in dogs affected by canine cognitive dysfunction syndrome (CDS) (Panek et al., 2020). Tau-related synaptic impairment and neuroinflammation have also been associated with cognitive decline (Smolek et al., 2016), and recent studies have further characterized age-related behavioral and cognitive changes in dogs (Ciurli et al., 2023a; 2023b). Despite this growing knowledge, the possible influence of genetic factors, especially inbreeding, on these neurobiological aging markers remains poorly understood. Inbreeding is commonly used in selective breeding to stabilize desirable traits, but it can also lead to increased expression of recessive genetic disorders (Comparini et al., 2019; Cecchi et al., 2020; Bannasch et al., 2021; Barsotti et al., 2024), reduced genetic diversity, and inbreeding depression, a condition increasingly recognized as relevant to animal welfare (Keller & Waller, 2002; Brzeski et al., 2014; Cecchi et al., 2016) that has been linked to reduced fertility (Langlois & Blouin, 2004; Gonzales-Recio et al., 2007), changes in morphological traits in dog (Cecchi et al., 2018) and in other species (Bussiman et al., 2018; Hossein-Zadeh, 2012; Gómez et al., 2009), and a lower response to selection. In humans, inbreeding has also been associated with an increased risk of Alzheimer's disease (Vardarajan et al., 2015; Moreno-Grau et al., 2021; Ghani et al., 2015), suggesting a potential role of genetic background in cognitive decline.

Based on this framework, the present study aimed to evaluate serum concentrations of A $\beta$ 40, A $\beta$ 42 and phosphorylated Tau (pTau) in a small cohort of Labrador Retrievers, and to explore their relationship with inbreeding coefficients and cognitive status, as assessed through the Canine Dementia Scale (CADES) (Madari et al. 2015). Although preliminary, these findings may offer useful insights into the complex interplay between genetics and brain aging in dogs.

## Material and methods

The study included 24 clinically healthy Labrador Retriever dogs (7 males and 17 females), aged 6 to 15 years, obtained from family-owned breeders and currently living as privately owned pets. For each dog, the following parameters were considered:

*Tau and Beta.* From each dog, a blood sample of 5 ml was collected to evaluate, in serum,  $\beta$  amyloid 40 and 42, and phosphotau 181, by using ELISA kits, respectively: Canine Amyloid Beta Protein 40 (cat.No: MBS013429), Canine Amyloid Beta Peptide 1-42 (Cat.No: MBS742661) and Canine Phosphorylated Tau 181 (Cat.No: MBS006469), MyBioSource, San Diego, CA 92195-3308 USA.

*Behavioural examination* - In this study, the CADES assessment scale, developed and validated by Madari et al. (2015), was chosen. This scale evaluates four behavioral domains associated with cognitive changes in dogs: spatial orientation (Domain A), social interactions (Domain B), sleep-wake cycle (Domain C), and house soiling (Domain D). Its use allows for the quantification of cognitive decline in dogs and the classification of individuals into different cognitive states based on their scores: Normal Aging (NA, 0–7 points), Mild Cognitive Impairment (MiCI, 8–23 points), Moderate Cognitive Impairment (MoCI, 24–44 points), and Severe Cognitive Impairment or Canine Dementia (CD, 45–95 points).

Due to its high sensitivity in detecting early cognitive changes, the CADES scale is considered a valuable tool for both diagnosing and monitoring cognitive decline in dogs.

*Inbreeding coefficient (F)* - the pedigree of each dog was also collected, and a database was built. We used Pedigree Viewer software (Kinghorn, 1994) to verify whether there were any errors left in the data files. The individual and average inbreeding coefficient (F), both overall and by sex, was computed using the CFC software (Sargolzaei et al., 2006). The inbreeding coefficient is the probability that at any randomly drawn locus of a given individual has two identical by-descent alleles (Wright, 1922). This parameter was calculated by the tabular method described by Meuwissen & Luo (1992).

## Statistical Analysis

To highlight the relationships between F and both  $\beta$ -amyloid 40 and phospho-tau 181, the non-parametric Spearman's rank correlation test was performed using Jamovi version 2.5 (The Jamovi Project, 2024), retrieved from <https://www.jamovi.org> and accessed on 7 July 2025. The Spearman test was chosen after verifying, through the Shapiro–Wilk test, that the data were not normally distributed. All parameters were also evaluated for differences between male and female dogs. Regarding CADES scores, to reduce the impact of outliers, scores were winsorized, and a small amount of noise was added to zero values.

Moreover, a cluster analysis, an exploratory multivariate statistical method, was performed with JMP software version 5.0 (SAS, JMP) to identify possible groups of animals sharing similar characteristics across variables such as age, F, A $\beta$ 40, tau, and scores obtained from the CADES assessment. This type of analysis allows for the grouping of individuals based on patterns of similarity, facilitating the detection of naturally occurring subgroups within the dataset. A one-way analysis of variance (ANOVA) was used to identify potential differences between the groups,

with a sufficiently large number of dogs included to support statistical comparison. To assess whether there was a significant association between cluster membership and the sex of the dogs, a chi-square test of independence was performed. The number of males and females within each cluster was tabulated, and expected frequencies were calculated based on the overall sex distribution in the sample. Due to the limited sample size and small expected cell counts, Fisher’s exact test was also considered to validate the robustness of the results.

Results and discussion

Results showed that, out of the 24 purebred animals, only 5 females were found to be inbred (mean inbreeding coefficient  $F = 0.046$ ;  $\min = 0.002$ ,  $\max = 0.125$ ), with one individual having  $F = 0.125$  and the remaining four with  $F < 0.05$ .

An inbreeding coefficient of 0.05 is considered the maximum acceptable threshold (Ciampolini et al., 2013). As Beuchat (2015) explains, while adverse effects of inbreeding typically begin to appear at around 5%, a marked decline in offspring vitality and an increased risk of expressing deleterious recessive mutations are observed when the coefficient exceeds 10% (Marín Navas et al., 2021).

Low inbreeding levels have also been reported in previous studies, such as the one conducted on the same breed at the Guide Dog School for the Blind in Scandicci (Cecchi et al., 2009).

Mean values, both overall and by sex, for age (in years), and for Aβ40, pTau, and CADES scores in the analyzed dogs are reported in Table 1. The dogs were all clinically healthy and privately owned, with an overall mean age of  $11.54 \pm 1.87$  years. The age distribution was similar between sexes, with no substantial difference between females and males. Although some sex-related trends were observed in biomarker levels and cognitive scores, no statistically significant differences were found. In particular, pTau concentrations and CADES scores were higher in males, suggesting a possible tendency toward greater cognitive impairment in this group. However, these trends did not reach statistical significance and should be interpreted cautiously. Further research with a larger sample size may help clarify these observations.

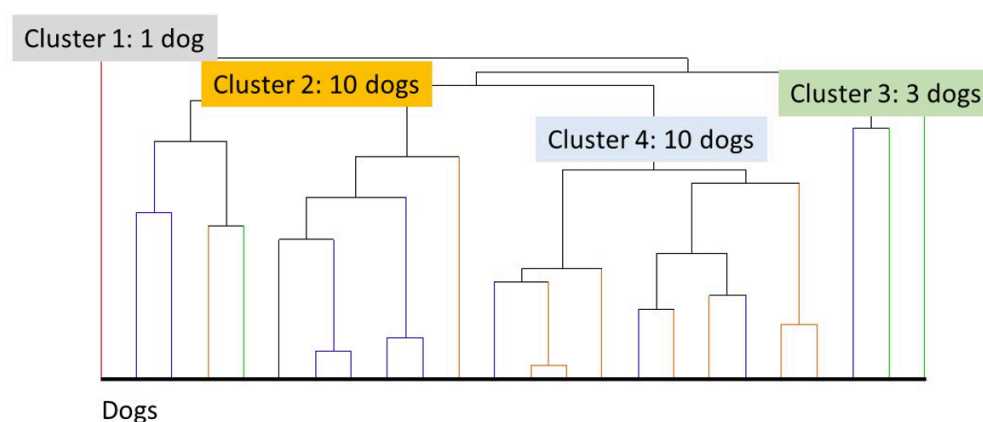
It is also noteworthy that Aβ42 was not detected or quantifiable in any of the analyzed samples, a finding that may suggest either a limitation of detection sensitivity or genuinely low circulating levels in this population. A similar result was reported in aged horses, where Aβ42 was undetectable in all subjects despite the presence of measurable Aβ40 and pTau181 levels, and no signs of cognitive decline were observed. This absence of Aβ42, considered a key isoform in initiating Tau phosphorylation, was hypothesized to reflect species- or population-specific differences in amyloid metabolism (Gazzano et al., 2025). These findings raise the possibility that undetectable Aβ42 might be associated with the preservation of cognitive function, although further studies are needed to clarify its biological significance in dogs.

**Table 1.** Mean values, both overall and by sex, for age (in years), and the levels of Aβ40, pTau, and CADES scores in the analyzed dogs. Aβ42 was not detected or quantified in the analyzed samples.

	Total sample	Females	Males
N°	24	17	7
Age (years)	11.54±1.87	11.53±0.47	11.57±0.73
Aβ40 (pg/ml) T0	21.63±2.85	23.81±3.36	16.35±5.22
Tau pg/ml	11.80±2.66	9.25±3.07	18.00±4.79
CADES score	4.29±2.14	2.53±2.11	8.57±3.91

When investigating possible correlations among the analyzed parameters, statistical analysis revealed only one significant association, between age and the CADES score ( $r = 0.614$ ;  $P < 0.01$ ), suggesting a possible age-related cognitive decline. However, given the limited sample size, this finding should be interpreted with caution.

Using multivariate analysis, four distinct clusters of dogs were identified based on age, inbreeding coefficient (F), A $\beta$ 40 and Tau concentrations, and CADES scores (Figure 1). The differences among dogs belonging to the different clusters are reported in Table 2.



**Figure 1.** Multivariate clustering of dogs based on age, F coefficient, A $\beta$ 40 and Tau levels, and CADES score. The figure shows the distribution of individual dogs within the four identified clusters, highlighting differences in biomarker profiles and cognitive outcomes.

**Table 2.** Descriptive data for each cluster, including number and sex of dogs, mean age, inbreeding coefficient (F), plasma A $\beta$ 40 and Tau concentrations, and CADES scores.

Cluster	Dogs (n°)	Sex	Age (years)	F	A $\beta$ 40 (pg/ml) T0	Tau pg/ml	CADES score
1	1	Female	6	0.125	0	13.76	0
3	3	2 Females and 1 Males	14.30	0.001	13.30	1.88	27.00
2	10	5 Females and 5 Males	10.9 B	0.006	21.92 B	24.95 A	1.40 B
4	10	9 Females and 1 Males	11.9 B	0.008	25.98 B	1.43 B	0.88 B

Letters (A, B) on the same column indicate statistically significant differences ( $p < 0.01$ ) between clusters, based on one-way ANOVA and post hoc comparisons.

Cluster 1 included a single young dog (6 years old) with a high inbreeding coefficient ( $F = 0.125$ ), undetectable A $\beta$ 40, elevated Tau levels (13.76 pg/ml), and a CADES score of 0. The unique biomarker and cognitive profile of this dog prevents meaningful comparisons with the other clusters. Its distinctive pattern likely reflects individual variability rather than group-level trends, as this subject was the youngest and had the highest inbreeding coefficient, with a markedly different profile compared to the others. In the remaining clusters (2, 3, and 4), inbreeding coefficients (F) were generally low and showed limited variability. This narrow distribution hampers the assessment of the potential influence of inbreeding on biomarker expression or cognitive outcomes. While F was included in the clustering process, its contribution to group differentiation appears minimal in this dataset.

Cluster 3 ( $n = 3$ ) included the oldest dogs (mean age: 14.3 years), characterized by the lowest F values (0.001), low A $\beta$ 40 (13.30 pg/ml), low Tau (1.88 pg/ml), and the highest CADES scores (27.00), indicating pronounced cognitive impairment. This cluster appears to differ from the two largest clusters with respect to age, A $\beta$ 40 concentrations, and CADES scores. Nevertheless, the limited number of subjects did not allow for a statistical significance evaluation.

Cluster 2 ( $n = 10$ ) comprised slightly younger dogs (mean age: 10.9 years), with low F (0.006), elevated A $\beta$ 40 (21.92 pg/ml), high Tau (24.95 pg/ml), and low CADES scores (1.40), suggesting preserved cognitive function despite elevated biomarker levels.

Cluster 4 ( $n = 10$ ) included dogs with a mean age of 11.9 years, low F (0.008), high A $\beta$ 40 (25.98 pg/ml), low Tau (1.43 pg/ml), and the lowest CADES scores (0.88), indicating intact cognitive abilities. This Cluster consisted of 9 females and only 1 male, whose values were similar to those of the females.

The only significant difference between the two largest clusters (2 and 4) concerns Tau levels; all other variables included in the multivariate analysis are comparable, thus making it challenging to interpret this finding.

No significant association was found between the four clusters and the sex of the animals. Although this distribution did not reach statistical significance, the pattern observed in Cluster 4, where females predominated and the only male showed values comparable to those of the females, could tentatively point to sex-related influences on cognitive preservation or biomarker expression. Further research with larger and more balanced samples is needed to explore potential sex-related effects on cognitive aging in dogs (Schütt et al. 2015). These findings highlight the heterogeneity of cognitive aging in dogs and suggest a potential dissociation between biomarker profiles and behavioral outcomes. It is possible that individual cognitive reserve, lifestyle, or unmeasured genetic and environmental factors modulate this relationship.

Future studies involving larger and genetically more diverse populations, with longitudinal follow-up, are needed to clarify the trajectories of biomarker changes and their clinical relevance. Additionally, the role of inbreeding should be further explored in samples with a broader F distribution to better assess its potential impact on neurodegeneration and cognitive resilience.

## Conclusions

This preliminary study represents an initial attempt to explore potential correlations between inbreeding and biomarkers of cognitive degeneration in dogs. In the present population, the inbreeding coefficients derived from pedigree analysis were relatively low, limiting the ability to detect clear associations with pTau and  $\beta$ -amyloid (A $\beta$ 40) levels. Expanding the sample to include a greater number of dogs with higher inbreeding coefficients would allow for more robust and informative analyses. This approach is more feasible in the canine model than in human studies, where identifying inbred individuals is considerably more difficult.

Moreover, increasing the number of older dogs is equally important. The biomarker and cognitive profiles observed in Cluster 3, characterized by advanced age, low A $\beta$  and Tau levels, and high CADES scores, suggest a distinct neurocognitive trajectory that warrants further investigation. Including more aged subjects may help clarify the progression of cognitive decline and its relationship with neurodegeneration in dogs, enhancing our understanding of canine aging and its translational relevance to human conditions. Our findings also highlight the need to further investigate potential sex-related influences on cognitive aging and biomarker profiles in dogs.

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### Osservazioni preliminari su $\beta$ -Amiloide, fosfo-tau e consanguineità nel Labrador Retriever

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### Sintesi

Le concentrazioni plasmatiche di  $\beta$ -amiloide (A $\beta$ 40, A $\beta$ 42) e tau fosforilata (p-tau) sono potenziali biomarcatori di neurodegenerazione nel cane. Questo studio ha esaminato 24 Labrador Retriever sani per valutare l'associazione tra

questi biomarcatori e il coefficiente di consanguineità, calcolato dai dati genealogici. I campioni di sangue sono stati analizzati con kit ELISA e i cani valutati tramite *Canine Dementia Scale* (CADES). I dati preliminari indicano possibili correlazioni tra età, sesso e livelli dei biomarcatori, ma non tra questi ultimi e il coefficiente di consanguineità, risultato troppo basso per evidenziare legami significativi. Questi risultati confermano l'interesse di approfondire il rapporto tra invecchiamento cerebrale e fattori genetici in studi su campioni più ampi.