Molecular Neurobiology (2020) 57:3143–3149
https://doi.org/10.1007/s12035-020-01951-0

ORIGINAL ARTICLE

Plasma Neurofilament Light Chain as a Translational Biomarker of Aging and Neurodegeneration in Dogs

Wojciech K. Panek[1] · Margaret E. Gruen[1] · David M. Murdoch[2] · Robert D. Marek[2] · Alexandra F. Stachel[1] · Freya M. Mowat[1,3] · Korinn E. Saker[1] · Natasha J. Olby[1]

Received: 19 February 2020 / Accepted: 22 May 2020 / Published online: 30 May 2020

Abstract

Age is a primary risk factor for multiple comorbidities including neurodegenerative diseases. Pet dogs and humans represent two populations that have experienced a significant increase in average life expectancy over the last century. A higher prevalence of age-related neurodegenerative diseases has been observed across both species, and human diseases, such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), have canine analogs, canine cognitive dysfunction (CCD), and degenerative myelopathy (DM) respectively. In humans, protein biomarkers have proved useful in the prediction and diagnosis of neurodegeneration. Molecular signatures of many proteins are highly conserved across species. In this study, we explored the potential of the neuronal cytoskeletal protein neurofilament light chain (NfL) as a biomarker of neuro-aging in dogs using an ultrasensitive single-molecule array assay to measure plasma concentrations. Healthy dogs of different ages and dogs affected with CCD and DM were evaluated. The mean plasma NfL concentrations in the different age groups of the healthy population were as follows: 4.55 ± 1.70 pg/mL in puppy/junior group (0.43–2 years), 13.51 ± 6.8 pg/mL in adult/mature group (2.1–9 years), and 47.1 ± 12.68 pg/mL in geriatric/senior group (9.3–14.5 years). Concentrations in dogs with DM (7.5–12.6 years) and CCD (11.0–15.6 years) were 84.17 ± 53.57 pg/mL and 100.73 ± 83.72 pg/mL, respectively. Plasma NfL increases in an age-dependent manner and is significantly elevated in dogs diagnosed with neurodegenerative disease. This work identified plasma NfL as a key clinical index of neuro-aging and neurodegeneration in pet dogs. Our findings mirror recent reports from human neurodegenerative diseases.

Keywords Canine · Cognitive dysfunction · Alzheimer's disease · Amyotrophic lateral sclerosis · Degenerative myelopathy

Introduction

Improvements in healthcare, nutrition, and lifestyle have contributed to an increased life expectancy in both dogs and humans [1–3]. Unfortunately, extended lifespan has been paralleled by a concurrent increase in clinical diagnosis of neurodegenerative diseases [4]. Disorders, such as Alzheimer's disease (AD), significantly impact quality of life in the elderly and have high mortality rates [5]. Proper understanding of the pathophysiology of age-related neurodegenerative processes requires reliable disease models, which is challenging in these complex diseases in which the passage of time also plays an important role [6]. Coupled with this is a need for non-invasive, reliable biomarkers that indicate particular disease stage, ideally the preclinical onset disease stage [7]. Among human companion animals, pet dogs enjoy unique privileges within human society and, in many cases, are considered family members. As a result, pet dogs and their owners share the same living environment with exposure to the same potential neurotoxins. Neurodegenerative diseases such as AD and superoxide dismutase–associated amyotrophic lateral

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-020-01951-0) contains supplementary material, which is available to authorized users.

✉ Natasha J. Olby
njolby@ncsu.edu

[1] Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 1060 William Moore Dr., Raleigh, NC 27607, USA

[2] Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA

[3] Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA

sclerosis (SOD1-ALS) have canine analogs, canine cognitive dysfunction (CCD) and degenerative myelopathy (DM) respectively [8, 9]. Dogs with CCD exhibit signs of dementia, such as disorientation, anxiety, loss of learned behaviors, and changes in sleep cycles associated with neuronal loss and corresponding cortical atrophy within brain areas such as the prefrontal and frontal cortex, hippocampus, and limbic system [10–12]. The marked similarity of amyloid beta pathology between dogs and humans has led to the adoption of the dog as a model of early Alzheimer's disease [13–19]. As another parallel, dogs with DM develop an upper motor neuron paraparesis that progresses to generalized lower motor neuron tetraplegia over a period of 18–24 months. Onset of signs is in senior dogs and is associated with a mutation in the SOD1 gene that results in accumulation of SOD1 protein within neurons [9]. This naturally occurring model of SOD1-ALS is now being used to evaluate novel ALS therapies [20, 21].

Within these models, there is a need for biomarkers of neurodegenerative disease for use in the diagnosis and monitoring of disease and outcome assessment in clinical trials. Ideally, these biomarkers will be reliable, sensitive (particularly to the early stage of disease), non-invasive, and inexpensive. To this end, we were interested in examining plasma concentrations of neurofilament light chain (NfL). Neurofilament light chain is a neuronal structural protein that is genetically conserved across human and canine species and is released into the CSF and blood upon neuronal degeneration or axonal damage. Recent scientific reports in the field of AD and ALS in humans suggest that plasma NfL concentrations are a sensitive biomarker of neurodegeneration, when assessed with ultra-sensitive immune assays [22, 23]. In this study, we explored the potential application of measuring canine plasma NfL concentrations using single-molecule array immunoassay (Simoa, Quanterix) as a translational diagnostic biomarker for the aging and neurodegeneration of the CNS. The advantage of this technology lies in the ability to trap single molecules into the femtoliter-sized microwells, which then allows for the detection of substantially low concentrations of target molecules in plasma. We sought to analyze plasma NfL concentrations in a healthy population of pet dogs in different life stages and a population of dogs with CCD and dogs with DM. We found that NfL is detectable in canine plasma samples, and that its concentration increases in an age-dependent manner. In addition, dogs with CCD or with DM had higher concentrations of plasma NfL. Our novel canine data mirrors the dynamics of plasma NfL in human neurodegenerative diseases, thereby highlighting the translational potential of the aging pet dog. Clinical application of plasma NfL level testing may represent a highly sensitive, minimally invasive, and cost-effective means of monitoring neuro-aging in pet dogs.

Methods

Animals

This study included 63 dogs recruited from the staff of the North Carolina (NC) State College of Veterinary Medicine and NC State Veterinary Hospital neurology service caseload, 36 healthy and 27 suffering from neurodegenerative conditions. Because of differences in life expectancy between different dog breeds, dogs were stratified using life stage categories based on the American Animal Hospital Association (AAHA) canine life stage guidelines combined with breed lifespan by the American Kennel Club (AKC) [24]. Inclusion criteria for the cohort of healthy dogs were normal physical and neurological examinations. Exclusion criteria were history of or ongoing neurological conditions and use of drugs that might alter behavior (e.g., behavior-modifying drugs such as fluoxetine or anti-neuropathic pain medications such as gabapentin).

Dogs classified as having CCD were participating in a longitudinal study of neuro-aging in pet dogs. All dogs underwent physical, orthopedic, and neurological examinations and had no significant comorbidities and no evidence of focal neurological deficits. All dogs were visually intact and could hear, and were able to walk without assistance. Exclusion criteria were coexisting metabolic or neurological conditions such as epilepsy and use of drugs that might alter results of behavioral testing. Questionnaire-based assessment of cognitive status was performed using the Canine Dementia Scale (CADES) (Suppl. Fig. 1) [25]. The Canine Dementia Scale evaluates seventeen items, distributed into four domains associated with behavioral changes (spatial orientation, social interactions, sleep-wake cycles, and house soiling) and grades (normal, mild, moderate, and severe) are assigned for each item. The CADES score was used to classify dogs as normal (0–7), mild (8–23), moderate (24–44), or severe (45–95) cognitive impairment.

Dogs diagnosed with DM were participating in a longitudinal study or a clinical trial. They had progressive tetraparesis with no evidence of compressive or inflammatory myelopathy on magnetic resonance imaging (MRI) of the spine and cerebrospinal fluid (CSF) analysis and were homozygous for the SOD1c.118A allele on genetic testing [8].

All owners were provided with details of the study, were given the opportunity to ask questions, and signed an informed consent before participating. All of the study participants underwent physical and neurological examinations followed by blood draw. All procedures were performed in accordance with the North Carolina State University Institutional Animal Care and Use Committee. Data gathered on the dogs included age, breed, sex, and health status. Table 1

Table 1 Demographic and clinical features of study participants. Mean (SD) and median (range) have been used to present the NfL concentrations. *CCD*, canine cognitive dysfunction; *DM*, degenerative myelopathy; *AAHA*, American Animal Hospital Association; *CADES*, Canine Dementia Scale; *plasma NfL*, plasma neurofilament light chain; *SD*, standard deviation

	Number of dogs (*n*)	Life stage (AAHA)	Age at the evaluation (years)	Dementia score (CADES)	Plasma NfL conc. (pg/mL) mean SD	Plasma NfL conc. (pg/mL) median (range)
Healthy controls	$n = 12$	Puppy/junior	0.40–2	Normal	4.55 ± 1.70	4.41 (1.8–7.5)
	$n = 14$	Adult/mature	2.1–9	Normal	13.51 ± 6.8	11.88 (5.8–22.0)
	$n = 10$	Senior/geriatric	9.3–14.5	Normal	47.1 ± 12.68	42.67 (31.2–68.04)
Canine cognitive dysfunction (CCD)	$n = 11$	Senior/geriatric	11.0–15.6	Mild, $n = 4$ Moderate, $n = 2$ Severe, $n = 5$	100.73 ± 83.72	69.72 (30.6–327.2)
Degenerative myelopathy (DM)	$n = 16$	Senior/geriatric	7.5–12.6	N/A	84.17 ± 53.57	71.77 (21.4–184.3)

summarizes the demographic and clinical information for participants grouped according to life stage and disease status.

Measurement of Plasma NfL Concentrations

Blood samples were taken into purple-top (EDTA) tubes and centrifuged at 2000×*g* at 4 °C for 8 min within 2 h of collection. Plasma supernatant was collected, divided into aliquots, and frozen at − 80 °C until further use. Samples were thawed on ice before analysis and the concentration of neurofilament light chain (NfL) was measured using Single-Molecule Array Assay kit (NF-light, Quanterix, Lexington, MA), following the manufacturer's instructions using the Sr-X ultrasensitive biomarker detection system (Quanterix, Lexington, MA). This system has a reported coefficient of variation of < 10% and analytical sensitivity of 0.62 pg/mL.

Statistical Analysis

Statistical analyses were performed using JMP14 (SAS Institute, Cary, NC). Dogs were grouped according to life stage and disease status, and summary data were prepared for each group on age, weight, sex, and breed. The influence of age, body weight, and sex on plasma NfL concentrations was examined using linear regression. Plasma NfL concentrations were compared between life stage groups using the Wilcoxon test for each pair. Given the influence of age on plasma NfL concentrations, a model was built to examine the effect of disease status (healthy, CCD, or DM) and age on plasma NfL concentration using logistic regression. The relationship between CADES score and pNfL concentrations was examined in healthy senior/geriatric dogs and dogs diagnosed with (CCD) using logistic regression. All reported *p*-values were considered to be statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$.

Results

Clinical Characteristics

Sixty-three pet dogs (39 males and 24 females) of various breeds (Beagle $n = 8$, Boxer $n = 8$, Border Collie $n = 4$, Cairn Terrier $n = 2$, Jack Russell Terrier $n = 3$, German Shepherd Dog $n = 8$, Golden Retriever $n = 1$, Hound $n = 1$, Labrador Retriever $n = 7$, Mix Breed $n = 13$, Pembroke Welsh Corgi $n = 5$, Rhodesian Ridgeback $n = 1$, Rottweiler $n = 2$) and life stages (puppy/junior; adult/mature; senior/geriatric) were enrolled into this prospective study. Of these, 36 were healthy and 27 were diagnosed with canine cognitive dysfunction (CCD, $n = 11$) or degenerative myelopathy (DM, $n = 16$). The ages of dogs ranged from 0.40–15.6 years. The population of healthy pet dogs included puppy/junior dogs (0.40–2 years, $n = 12$), adult/mature dogs (2.1–9 years, $n = 14$), and senior/geriatric dogs (9.3–14.5 years, $n = 10$). All dogs with a neurodegenerative condition were senior/geriatric (CCD 11.0–15.6 years; DM 7.5–12.6 years). Demographic features of study participants and CADES dementia scores are presented in Table 1.

Plasma NfL Concentrations in Healthy Pet Dogs Increase with Age

Plasma NfL concentrations for dogs in different life stages are presented in Table 1. Using logistic regression to examine the effect of age, weight, and sex, we determined there was a direct relationship between plasma NfL concentrations and age in healthy pet dogs ($r^2 = 0.75$, $p < 0.0001$) (Fig. 1). However, plasma NfL concentrations were not affected by body weight ($r^2 = 0.07$, $p = 0.19$) or sex ($r^2 = 0.1$, $p = 0.64$). When healthy dogs were grouped according to life stage, and compared using Wilcoxon test for each pair, there was a significant difference between each group (Table 1) ($p < 0.0001$ for senior/geriatric versus both other groups, and $p < 0.0004$

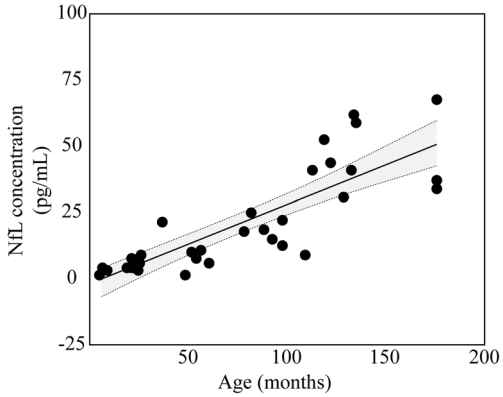

Fig. 1 Plasma NfL concentrations increase with age in the population of healthy pet dogs. A multiple linear regression model was fitted using plasma NfL concentration as response variable and age, sex and body weight as dependent variables. There is a positive correlation between age and plasma NfL, ($r^2 = 0.75$, $p < 0.0001$)

for puppy/junior compared with adult/mature) (Fig. 2). Our results mirror the gradual increase in plasma NfL concentrations that occur in humans as they age.

Neurodegenerative Disease Is Associated with High Plasma NfL Concentrations

Plasma samples from dogs affected with CCD and DM (analogs of Alzheimer's disease and SOD1-ALS respectively) were tested and the results are reported in Table 1. When plotted against age using logistic regression, dogs with these neurodegenerative diseases combined had significantly higher plasma NfL concentrations than healthy dogs ($p < 0.01$). The rate of change of NfL with age appears to be accelerated in dogs with CCD and DM when compared with healthy dogs (Fig. 3). While plasma NfL concentrations in dogs with CCD

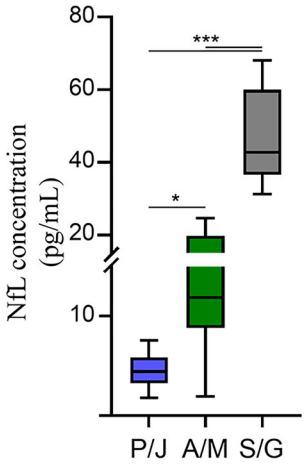

Fig. 2 Plasma NfL concentrations increase in an age-dependent manner in the population of healthy pet dogs stratified into different life stage groups; P/J, puppy/junior ($n = 10$); A/M, adult/mature ($n = 14$); S/G, senior/geriatric ($n = 12$). There was statistically significant difference between the groups and when each group was compared pairwise with the Wilcoxon test. *$p < 0.001$; ***$p < 0.0001$

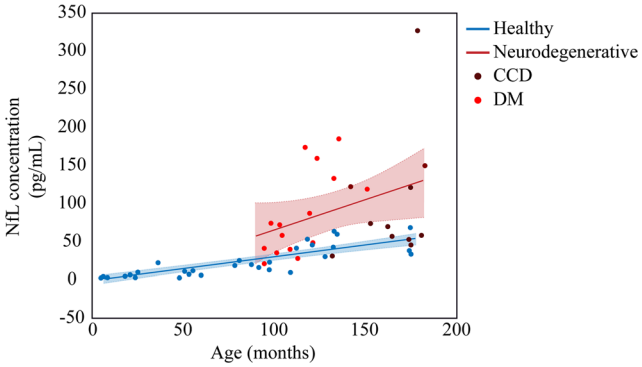

Fig. 3 The relationship between age and plasma NfL concentration in a population of healthy dogs ($n = 36$) and dogs affected with neurodegenerative disease ($n = 27$). There was a significant difference between these groups of dogs when evaluated using logistic regression ($p < 0.01$)

were higher than dogs with DM (Table 1), there was no significant difference between these groups when compared with a Wilcoxon test ($p = 0.35$). Using logistic regression, a positive correlation was found between plasma NfL concentrations and CADES scores in senior and geriatric dogs (dogs with degenerative myelopathy were not included in this evaluation) (r^2 0.27; $p < 0.05$) (Fig. 4).

Discussion

This study provides the first report of plasma NfL measurement in healthy pet dogs at different ages and in dogs with naturally occurring neurodegenerative disease. The main findings are that (1) plasma NfL can be successfully detected in pet dogs; (2) plasma NfL concentration increases in an age-dependent manner and is not influenced by body weight and sex; (3) neurodegenerative disease is associated with a significant increase in plasma NfL concentration; and (4) plasma NfL concentration positively correlates with CADES scores. Our findings support the use of canine plasma NfL concentration as a biomarker of neuro-aging and neurodegeneration.

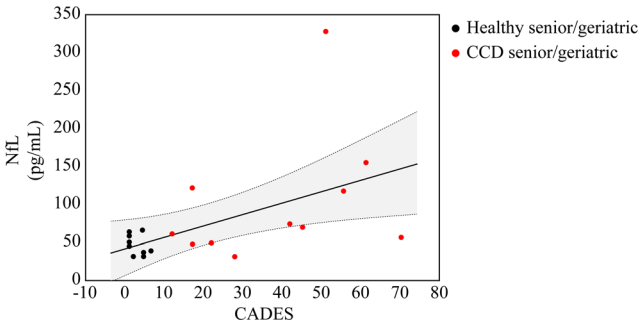

Fig. 4 The relationship between plasma NfL concentrations and Canine Dementia Score (CADES) in healthy senior/geriatric dogs ($n = 10$) and dogs with CCD ($n = 11$). Plasma NfL concentrations are associated with the CADES score when evaluated using logistic regression ($r^2 = 0.27$, $p < 0.05$)

Moreover, our findings mirror the dynamics of the plasma NfL changes that occur in human aging and neurodegenerative diseases, highlighting the promising translational potential of the aging pet dog as a model of human aging (Suppl. Fig. 2).

Neurofilaments (NFs) are cytoskeletal proteins that are particularly abundant in the axons of neurons and highly conserved across species. Within the central nervous system (CNS), there are four different NF subunits, light, medium, heavy chains, and α-internexin, which are replaced with peripherin in the peripheral nervous system [26]. They play important functions during the neurogenesis and are responsible for the radial growth and maintenance of axon caliber in later life as well as supporting axonal conduction [27, 28]. Upon neuro-axonal breakdown, the NF subunits are released into the CSF and both phosphorylated NF heavy chain (pNfH) and NfL have been detected in the plasma [29, 30]. Measurement of plasma NfL has generated a lot of interest due to its stability and long half-life (approximately 3 weeks) in the blood [31]. There have been numerous reports of the association between plasma NfL and severity of acute CNS injury such as stroke, as well as the presence and stage of neurodegenerative disease in humans [32, 33].

Due to extended life expectancy, age-related neurodegenerative diseases pose a major health crisis. More than 40 million people worldwide are affected by AD, and this number is growing dramatically [34, 35]. Similarly, the number of people affected with ALS constantly increases and it is expected to rise across the globe by approximately one-third from 80,162 in 2015 to 105,693 in 2040 [36]. Development of effective treatments to prevent the onset or to delay progression of the disease course is an international multidisciplinary research priority [34, 37]. The assessment of the efficacy of novel therapies for AD and ALS requires proper models and sensitive biomarkers that would reflect particular life stage and presymptomatic phases of the disease in a condensed period of time. Aging pet dogs represent a valuable model of spontaneously occurring neurodegenerative diseases due to the similarities in neurobiology and molecular cascades, the shared environmental risk factors between pet dogs and humans, comparable clinical phenotypes and pathology, the ability to provide advanced medical care in a manner comparable with human medical care, the beneficial genetic makeup of dog breeds, and the relatively truncated canine lifespan [8, 16, 18, 38].

The use of single-molecule array assay (SIMOA) technology allowed detection of plasma NfL in the picomolar range in healthy dogs. There was a clear relationship between plasma NfL and age with plasma concentrations ranging from 1.8 pg/mL in puppies to 68 pg/mL in healthy senior dogs. Given the different life spans of different breeds of dog, and the short life span of dogs when compared with humans, dogs were then grouped according to life stage. Grouping in this manner allowed us to control for breed-specific lifespan and showed an even stronger relationship between plasma NfL concentration and life stage, suggesting that aging of the CNS is influenced by the breed-specific aging factors rather than simply reflecting the passage of time. This suggests that examination of the genetic basis of different rates of aging in different breeds will uncover important regulators of aging of the nervous system, highlighting again the potential of study of pet dogs to expand our understanding of the pathophysiology of aging in humans [39]. Plasma NfL concentrations are elevated in humans with AD and ALS, and the range of concentrations reported correlates well with the ranges we report here [23]. While individuals may vary considerably in their NfL concentration, the rate of change of concentration shows considerable promise in predicting onset of AD in humans [22]. Similarly, we show that the rate of change of NfL with age is accelerated in dogs with CCD and DM when compared with life stage–matched healthy individuals. We also found that plasma NfL concentration correlates with CADES scores reported by owners of dogs with CCD. Additional longitudinal work is needed to define the rate of change of NfL and the ability to predict onset of disease. This is particularly important in neurodegenerative diseases, where early detection is difficult but likely to be extremely important when considering therapeutic intervention before significant irreversible damage has been done.

This report provides additional evidence of shared pathophysiology of age-related neurodegenerative diseases between dogs and humans by providing a common plasma biomarker that reflects health status in the similar fashion. Our findings suggest that the dynamics of plasma NfL are comparable between dogs and humans and that plasma NfL is a sensitive biomarker for the existence of age-related normal and pathological neurodegenerative processes. We conclude that measurement of plasma NfL concentration represents a promising biomarker that can be monitored in clinical trials for newly developed therapies against age-related neurodegenerative disorders in humans and dogs.

Acknowledgments We would like to acknowledge Quanterix company representatives for technical support. We want to thank Jackie Blake and Beth Case for their help with obtaining the biological samples and the dogs and their owners for taking part in the study.

Author Contributions W.K.P. and N J.O. conceived and designed the study, analyzed data, and wrote the manuscript. W.K.P performed experiments. A.F.S., W.K.P, and R.D. M assisted with sample processing and testing. N.J.O., D.M. M., M.E. G., F.M. M, and K.E. S provided critical feedback and oversaw the research program. All authors listed reviewed the manuscript and provided feedback with writing and revisions.

Funding Information This work is supported by the Dr. Kady M Gjessing and Rhanna M Davidson Distinguished Chair of Gerontology. F.M. is funded by NIH/NEI K08 EY028628.

References

1. Landsberg GM, Nichol J, Araujo JA (2012) Cognitive dysfunction syndrome: a disease of canine and feline brain aging. Vet Clin North Am Small Anim Pract 42(4):749–768 **vii**
2. Inoue M, Kwan NCL, Sugiura K (2018) Estimating the life expectancy of companion dogs in Japan using pet cemetery data. J Vet Med Sci 80(7):1153–1158
3. Oeppen J, Vaupel JW (2002) Demography. Broken limits to life expectancy. Science 296(5570):1029–1031
4. Wyss-Coray T (2016) Ageing, neurodegeneration and brain rejuvenation. Nature 539(7628):180–186
5. James BD, Leurgans SE, Hebert LE, Scherr PA, Yaffe K, Bennett DA (2014) Contribution of Alzheimer disease to mortality in the United States. Neurology 82(12):1045–1050
6. Li C, Briner A, Gotz J (2019) The search for improved animal models of Alzheimer's disease and novel strategies for therapeutic intervention. Future Med Chem 11(15):1853–1857
7. Gitler AD, Dhillon P, Shorter J (2017) Neurodegenerative disease: models, mechanisms, and a new hope. Dis Model Mech 10(5):499–502
8. Awano T, Johnson GS, Wade CM, Katz ML, Johnson GC, Taylor JF, Perloski M, Biagi T et al (2009) Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 106(8):2794–2799
9. Prpar Mihevc S, Majdic G (2019) *Canine cognitive dysfunction and Alzheimer's disease - two facets of the same disease?* Front Neurosci **13**:604
10. Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW (2008) Region specific neuron loss in the aged canine hippocampus is reduced by enrichment. Neurobiol Aging 29(1):39–50
11. Simic G et al (1997) Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. J Comp Neurol 379(4):482–494
12. Cummings BJ, Head E, Ruehl W, Milgram NW, Cotman CW (1996) The canine as an animal model of human aging and dementia. Neurobiol Aging 17(2):259–268
13. Toepper M (2017) Dissociating Normal aging from Alzheimer's disease: a view from cognitive neuroscience. J Alzheimers Dis 57(2):331–352
14. Selkoe DJ et al (1987) Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. Science 235(4791):873–877
15. Johnstone EM, Chaney MO, Norris FH, Pascual R, Little SP (1991) Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. Brain Res Mol Brain Res 10(4):299–305
16. Head E (2013) A canine model of human aging and Alzheimer's disease. Biochim Biophys Acta 1832(9):1384–1389
17. Guy MK et al (2015) *The golden retriever lifetime study: establishing an observational cohort study with translational relevance for human health*. Philos Trans R Soc Lond Ser B Biol Sci **370**(1673)
18. Cotman CW, Head E (2008) The canine (dog) model of human aging and disease: dietary, environmental and immunotherapy approaches. J Alzheimers Dis 15(4):685–707
19. Ruehl WW et al (1995) Canine cognitive dysfunction as a model for human age-related cognitive decline, dementia and Alzheimer's disease: clinical presentation, cognitive testing, pathology and response to 1-deprenyl therapy. Prog Brain Res 106:217–225
20. Katz ML, Jensen CA, Student JT, Johnson GC, Coates JR (2017) Cervical spinal cord and motor unit pathology in a canine model of SOD1-associated amyotrophic lateral sclerosis. J Neurol Sci 378:193–203
21. Stroud C, Dmitriev I, Kashentseva E, Bryan JN, Curiel DT, Rindt H, Reinero C, Henry CJ et al (2016) A one health overview, facilitating advances in comparative medicine and translational research. Clin Transl Med 5(Suppl 1):26
22. Preische O et al (2019) Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med 25(2):277–283
23. Verde F, Steinacker P, Weishaupt JH, Kassubek J, Oeckl P, Halbgebauer S, Tumani H, von Arnim CAF et al (2019) Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 90(2):157–164
24. Bartges J, Boynton B, Vogt AH, Krauter E, Lambrecht K, Svec R, Thompson S (2012) AAHA canine life stage guidelines. J Am Anim Hosp Assoc 48(1):1–11
25. Madari, A.F., Jana & Katina, Stanislav & Smolek, Tomas & Novak, Petr & Weissova, Tatiana & Novak, Michal & Zilka, Norbert, *Assessment of severity and progression of canine cognitive dysfunction syndrome using the CAnine DEmentia Scale (CADES).* Appl Anim Behav Sci, 2015. 171: p. 135–145.
26. Yan Y, Jensen K, Brown A (2007) The polypeptide composition of moving and stationary neurofilaments in cultured sympathetic neurons. Cell Motil Cytoskeleton 64(4):299–309
27. Friede RL, Samorajski T (1970) Axon caliber related to neurofilaments and microtubules in sciatic nerve fibers of rats and mice. Anat Rec 167(4):379–387
28. Jones SL, Svitkina TM (2016) Axon initial segment cytoskeleton: architecture, development, and role in neuron polarity. Neural Plast 2016:6808293
29. Benedet AL, Ashton NJ, Pascoal TA, Leuzy A, Mathotaarachchi S, Kang MS, Therriault J, Savard M et al (2019) *Plasma neurofilament light associates with Alzheimer's disease metabolic decline in amyloid-positive individuals*. Alzheimers Dement (Amst) 11:679–689
30. Olby NJ, Lim JH, Wagner N, Zidan N, Early PJ, Mariani CL, Muñana KR, Laber E (2019) Time course and prognostic value of serum GFAP, pNFH, and S100beta concentrations in dogs with complete spinal cord injury because of intervertebral disc extrusion. J Vet Intern Med 33(2):726–734
31. Barry DM, Millecamps S, Julien JP, Garcia ML (2007) New movements in neurofilament transport, turnover and disease. Exp Cell Res 313(10):2110–2120
32. Uphaus, T., et al., NfL (Neurofilament light chain) levels as a predictive marker for long-term outcome after ischemic stroke. Stroke, 2019. 50(11): p. 3077–3084.
33. Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobágyi T, Höglund K, Aarsland D, Lovestone S et al (2019) Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. Acta Neuropathol Commun 7(1):5
34. Collaborators GBDN (2019) Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the global burden of disease study 2016. Lancet Neurol 18(5):459–480
35. Prince M, Ali GC, Guerchet M, Prina AM, Albanese E, Wu YT (2016) Recent global trends in the prevalence and incidence of dementia, and survival with dementia. Alzheimers Res Ther 8(1):23
36. Arthur KC, Calvo A, Price TR, Geiger JT, Chiò A, Traynor BJ (2016) Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. Nat Commun 7:12408

37. Rakesh G, Szabo ST, Alexopoulos GS, Zannas AS (2017) Strategies for dementia prevention: Latest evidence and implications. Ther Adv Chronic Dis 8(8–9):121–136
38. Sandor S, Kubinyi E (2019) Genetic pathways of aging and their relevance in the dog as a natural model of human aging. Front Genet 10:948
39. Wang T, et al. (2019) Quantitative translation of dog-to-human aging by conserved remodeling of epigenetic networks. bioRxiv p. 829192.