Neuroscience Letters 744 (2021) 135593

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Neurofilament light plasma concentration positively associates with age and negatively associates with weight and height in the dog

Jackie Perino [a], Margaret Patterson [a], Mehdi Momen [a], Mina Borisova [b,c], Amanda Heslegrave [b,c], Henrik Zetterberg [b,c,d,e], Jordan Gruel [a], Emily Binversie [a], Lauren Baker [a], John Svaren [f], Susannah J. Sample [a,*]

[a] Comparative Genetic Research Laboratory, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53706, USA
[b] Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United Kingdom
[c] UK Dementia Research Institute at UCL, London, United Kingdom
[d] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden
[e] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
[f] Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53706, USA

ARTICLE INFO

Keywords:
Neurofilament
Light Chain
Neuropathy
Dog

ABSTRACT

Plasma neurofilament light chain (pNfL) concentration is a biomarker for neuroaxonal injury and degeneration and can be used to monitor response to treatment. Spontaneous canine neurodegenerative diseases are a valuable comparative resource for understanding similar human conditions and as large animal treatment models. The features of pNfL concentration in healthy dogs is not well established. We present data reporting basic pNfL concentration trends in the Labrador Retriever breed. Fifty-five Labrador Retrievers were enrolled. pNfL concentration was measured and correlated to age, sex, neuter status, height, weight, body mass index, and coat color. We found increased pNfL with age ($P < 0.0001$), shorter stature ($P = 0.009$) and decreased body weight ($P < 0.001$). These are similar to findings reported in humans. pNfL concentration did not correlate with sex, BMI or coat color. This data further supports findings that pNfL increase with age in a canine population but highlights a need to consider weight and height when determining normal pNfL concentration in canine populations.

1. Introduction

Neurofilaments (Nf) provide structural support for axons and contribute to regulation of myelinated axonal diameter. Neurofilaments are comprised of 4 subunits, including neurofilament light chain (NfL), neurofilament middle chain, neurofilament heavy chain, and α-internexin [1]. Of these, NfL is the most abundant and soluble, and is stable in biofluids [2]. Low levels of NfL are constantly released from axons, and levels of NfL release increase with age [3,4]. Higher levels of NfL are also released in neurodegenerative and neuroinflammatory diseases, as well as in cases of trauma or vascular disease [1].

NfL is of interest as a biomarker for neurodegenerative disease due to ease of acquisition in blood samples. Use of NfL concentration in human neurodegenerative disease has focused on improvement of diagnostic accuracy [5,6], as a prognostic biomarker [3,5], and as a means for monitoring disease progression and potentially response to treatment. NfL measurements in blood have substantial promise for evaluating response to novel disease-modifying therapies in clinical trials [7].

Canine models of neurodegenerative diseases are valuable for investigation of pathologic disease progression and for studies of disease-modifying therapy. For example, degenerative myelopathy (DM) is a relatively common motor neuron disease in dogs that is genetically and clinically similar to ALS, and is currently used for drug treatment trials [8,9]. Canine models of Duchenne muscular dystrophy [10], globoid cell leukodystrophy [11], and Alzheimer's disease [12] are also under investigation as neurodegenerative disease models.

Use of plasma NfL (pNfL) concentration in dog models of neurodegenerative disease has potential for evaluation of response to therapy. Only one study is available reporting on the relationship between pNfL concentration and age in normal dogs, which included 34 healthy dogs of various breeds, and found pNfL concentration increased with age [7]. The objective of the present study was to determine the relationship between pNfL concentration and age, sex, coat color, height, weight and body mass index in a large homogeneous population of healthy purebred

* Corresponding author. Postal address: University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706, USA.
E-mail address: susannah.sample@wisc.edu (S.J. Sample).

https://doi.org/10.1016/j.neulet.2020.135593
Received 15 August 2020; Received in revised form 19 November 2020; Accepted 20 December 2020
Available online 24 December 2020

Labrador Retrievers. We hypothesized that in Labradors Retrievers, pNfL concentration would increase with age and body mass index (BMI) but would not associate with the other variables measured.

2. Materials and methods

Owners of purebred Labrador Retrievers were recruited from the UW Veterinary Care Hospital at the University of Wisconsin-Madison between 2016 and 2019. All procedures were conducted with the approval of the Animal Care & Use Committee, School of Veterinary Medicine, University of Wisconsin-Madison (V005453). Dogs were recruited with informed written consent from each owner.

For inclusion, dogs had to be systemically healthy pure-bred Labrador Retrievers with no evidence of neuropathy on examination. Dogs were excluded if they had a history of chemotherapy, steroid administration, uncontrolled endocrine disease, any other condition associated with neuropathy, or were found to develop clinical evidence of neuropathy within 6 months of sample collection. Features of neuropathy that were deemed worthy of exclusion included: change in mentation, alterations in cranial nerve reflexes or reactions, development of ataxia, paraparesis or tetraparesis, loss of normal myotatic reflexes in the thoracic or pelvic limbs, decreases in withdrawal reflexes, absence of the perineal reflex, alterations in the cutaneous trunci reflex, or development of spinal pain on palpation. Blood samples were obtained from qualifying animals and processed within 1 h. Blood was collected from a peripheral vein into EDTA-containing tubes and centrifuged at 1372 RCF for 10 min at 20 °C. Plasma was then aliquoted and stored at −20 °C.

Each dog's weight, withers height, coat color, sex and neuter status were recorded. Body mass index (BMI) was calculated as: [(weight (Kg)) / (withers height (m))^2] [13]. After plasma collection, dogs over 12 years of age were screened annually for development of neuropathy. For animals with multiple plasma samples obtained over time, the first plasma sample was used to measure pNfL concentration and its association with other measured variables.

Plasma NfL concentration was measured using the NF-Light Advantage kit by Single molecule array (Simoa) on an HD-1 analyzer, according to manufacturer's instructions (Quanterix, Billerica, MA). Briefly, samples were thawed at 21 °C, vortexed, and centrifuged at 10,000 RCF for five min at 21 °C with sample diluent. Plasma was diluted 1:4 with sample diluent then bound to paramagnetic beads coated with a capture antibody specific for human NfL. NfL bound beads were then incubated with a biotinylated NfL detection antibody in turn conjugated to streptavidin-β-galactosidase complex that acts as a fluorescent tag. The subsequent hydrolysis reaction with a resorufin β-D-galactopyranoside substrate produces a fluorescent signal proportional to the concentration of NfL present. Duplicate measurements were taken of each sample. Sample concentrations were extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. Intra and inter-assay CVs were less than 15 % as determined by two quality controls according to ISO 5725-2 standards. Samples were run on the same day by the same operator. Data was analyzed for normality using the D'Agostino & Pearson normality test. The Spearman Rank correlation test was used to determine correlation between pNfL concentration and age, sex, height, weight, BMI, and coat color in the entire sample population of dogs. This analysis was then broken down into subpopulations by sex and age groups. For age groups, subpopulations of dogs under 11 years of age and over 11 years of age were evaluated. Younger dogs were then grouped into young adult (<5 years of age) and adult (>5 years of age) groups. For sex, variables were evaluated for all males and all females, regardless of neuter status. The Spearman Rank correlation test was then used to evaluate correlations between age and other clinical variables obtained. Correlations were considered strong (S_R>0.7), moderate (S_R>0.5), or weak (S_R>0.3). Data were reported as mean ± standard error, or median (range) as appropriate. A bivariate regression model was undertaken to infer the relationship between pNfL concentration with age and weight.

3. Results

Plasma was collected from 59 Labrador Retrievers. Four dogs were excluded due to development of clinical neuropathy within 6 months of initial plasma sample collection. Of the 55 Labrador Retrievers included in the final analysis, 3 were intact males (5%), 17 were castrated males (31 %), 5 were intact females (9%), and 30 were ovariectomized females (55 %). All three primary coat colors were represented, including yellow: n = 23 (42 %), chocolate: n = 11 (20 %), and black: n = 21 (38 %). Median age was 9.1 years (range 1.4–14.8 years). Median pNfL level was 42.8 pg/mL (range 5.2–129.0 pg/mL). Median weight was 32.8 kg (range 22.0–56.0 kg) (Fig. 1A). Mean withers height was 0.57 ± 0.005 m (Fig. 1B). Median BMI was 98.8 kg/m^2 (range 64.5–154.8 kg/m^2) (Fig. 1C).

Results of Spearman Rank correlations between pNfL concentration and recorded variables are displayed (Table 1). pNfL concentration had a significantly and moderately positive correlation with age (S_R = 0.68, P < 0.0001) (Fig. 2). Height and weight had weak and moderate negative correlations, respectively, with pNfL concentration (S_R=-0.35, P < 0.01; S_R =-0.51, P < 0.0001, respectively) (Fig. 2), with height and weight being correlated with each other (S_R = 0.58, P < 0.001). BMI did not correlate significantly with pNfL concentration (S_R=-0.23, P = 0.09) (Table 1, Fig. 2). There was no significant correlation between pNfL concentration and sex (S_R = 0.13, P = 0.36), coat color (S_R = 0.19, P = 0.17), neuter status (S_R=-0.08, P = 0.55), neuter status in males (S_R=-0.23, P = 0.33), or neuter status in females (S_R = 0.02, P = 0.93) (Table 1, Table S1).

When the population was segregated by sex, the association between age and pNfL concentration remained significant and positive (Table S1). Sex differences were seen between correlations with weight, height and BMI. In females, weight and height both negatively and moderately correlated with pNfL concentration (S_R= -0.55, P = 0.006; S_R= -0.55, P = 0.006), although BMI did not (S_R=-0.03, P = 0.87). In males, neither height nor weight correlated with pNfL concentration, while BMI had a moderate negative correlation (S_R=-0.52, P = 0.02).

When pNfL concentration was evaluated against variables obtained in a subset of dogs under 11 years of age (n = 39), the same significant associations were noted. pNfL concentration strongly correlated with age (S_R = 0.74, P < 0.0001) and moderately correlated with weight and height (S_R=-0.45, P = 0.005; S_R=-0.45, P = 0.004, respectively). Other variables did not correlate with pNfL concentration, including sex (S_R = 0.22 P = 0.17), coat color (S_R = 0.36, P = 0.15) and BMI (S_R=-0.24, P = 0.14) (Table 1). Results from analysis of dogs under 5 years of age and dogs between 5–11 years of age are presented in Table S2.

Correlations between variables obtained and age were determined (Table 1, Fig. 1). Weight was negatively and moderately correlated with age (S_R=-0.44, P < 0.001), as was BMI (S_R =-0.39, P = 0.004). No other significant correlations with age were noted.

Due to the individual correlations between age and weight, as well as both age and weight correlating to pNfL concentration, a bivariate linear regression model was used to further investigate the interactions between these variables. pNfL concentration was influenced by both increasing age (P < 0.0001) and decreasing weight (P = 0.04) (Table S3). This analysis was repeated to further investigate the relationship between age, BMI and pNfL concentration, confirming that increasing age (P < 0.001), but not decreasing BMI (P = 0.09), was associated with pNfL concentration (Table S3).

4. Discussion

The purpose of this study was to define the relationship between pNfL concentration with age, sex, body mass index, height, weight and coat color in a normal homogeneous population of purebred Labrador Retriever dogs. It is established that plasma and CSF NfL concentration

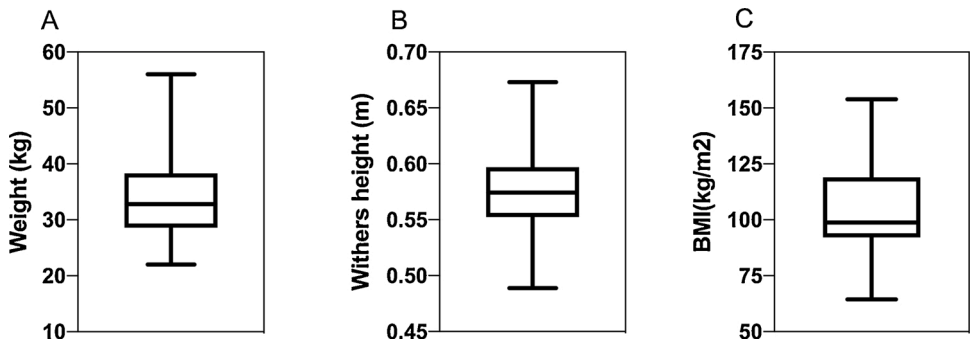

Fig. 1. Distribution of weight, withers height, and BMI in study population.
Box and whiskers plots showing the distribution of A) weight, B) withers height, and C) body mass index (BMI). BMI was thus calculated as BMI = (body weight / (withers height^2)). [13]. n = 55 dogs.

Table 1
Results of correlations between variables measured, pNfL concentration, and age are presented. pNfL = plasma neurofilament light concentration.

Variable	pNfL All Dogs (n = 55)	pNfL Dogs <11 years (n = 39)	Age(n = 55)
pNfL (pg/mL)			*S_R = 0.68, P < 0.0001*
Age (years)	*S_R = 0.68, P < 0.0001*	*S_R = 0.74, P < 0.0001*	
Sex	S_R = 0.13, *P* = 0.36	S_R = 0.22, *P* = 0.17	S_R = 0.10, *P* = 0.46
Neuter status	S_R=-0.08, *P* = 0.55	S_R = 0.0, *P* = 0.99	S_R=-0.01, *P* = 0.92
Coat Color	S_R = 0.19, *P* = 0.17	S_R = 0.36, *P* = 0.15	S_R=-0.03, *P* = 0.81
Weight (kg)	*S_R=-0.51, P < 0.001*	*S_R=-0.45, P = 0.005*	*S_R=-0.44, P < 0.001*
Height (m)	*S_R=-0.35, P = 0.009*	*S_R=-0.45, P = 0.004*	S_R=-0.12, *P* = 0.40
BMI (kg/m^2)	S_R=-0.23, *P* = 0.09	S_R=-0.24, *P* = 0.14	*S_R -0.39, P = 0.004*

are biomarkers for neurodegenerative diseases in humans [5,14]. This work is important because pNfL levels are an informative biomarker that can be used to study dog models of neurodegenerative diseases. Specific considerations associated with dog phenotypic attributes must be defined. The correlation of pNfL and age has been shown in human samples, indicating that age corrections need to be optimally employed to assess pNfL concentration as a biomarker in neurodegenerative disease [14,15]. Our results support the hypothesis that pNfL concentration increases with age and is not influenced by sex or coat color. Results did not support our second hypothesis, as no significant association was seen between pNfL concentration and BMI, although associations were seen between pNfL concentration and height and weight.

Dogs are an important large animal model for numerous neurodegenerative diseases, providing insight into progression of disease and response to novel treatment therapies [12]. While many dog models are laboratory-bred, it is becoming increasingly common to study spontaneous models of neurodegenerative diseases through use of client-owned animals. This results in a larger degree of variance regarding age, sex and body condition [7]. For spontaneous genetic diseases in dogs, breed is also an important consideration; disease risk is highly breed-dependent, and phenotypic and genomic variance between breeds is substantial [16].

Use of a single purebred population helped minimize variance due to genetics, improving insight into phenotypic variables on pNfL concentration in dogs. We found that in the Labrador Retriever, pNfL concentration significantly increased with age, which is a well-documented feature of pNfL levels in humans. This finding is in agreement with recent work evaluating pNfL concentration in 34 clinically normal dogs of varying breeds [7]. Our method for measuring pNfL differed from this earlier dog study, preventing direct comparison of plasma concentrations. Another previous study investigating the relationship between age and pNfL concentration in dogs found a lack of significant changes in pNfL concentration within the first 2 years of life [17].

We chose to analyze data from all dogs as well the subset of dogs under 11 years of age. The average life expectancy for Labrador Retrievers in the USA is not known, although limited data has reported an average life expectancy of 13.2 years [18]; we, therefore, chose 11 years of age to be a conservative cut off for "aged" status. As with human beings, dogs can develop cognitive dysfunction in their senior years, and dogs with cognitive dysfunction have increased pNfL levels [7]. The dogs recruited for this study were not extensively evaluated for cognitive dysfunction, and thus evaluating a younger subset of dogs independently was deemed prudent. Results from the younger subset of dogs mirrored the results seen when all dogs were analyzed, suggesting that if any aged dogs were experiencing cognitive dysfunction it did not substantially influence results.

We undertook evaluation of dogs broken into more specific age groups, including young adults, adults and aged dogs. Correlations seen in the entire population were present in the adult dog group. However, the same correlations with pNfL did not hold for all variables when only dogs <5 years of age (1.4–3.8 years) or dogs >11 years of age (11.2–14.8 years) were considered. This is likely due to the limited age span of these subgroups and low sample sizes (Table S2).

Purebred Labrador Retrievers have a wide range of heights, making body weight an inaccurate method to estimate body condition and a poor proxy for body size. We determined BMI as a measure of each dogs' body condition. In humans, neuropathy is an identified risk factor associated with obesity [19], and pNfL concentration correlated with BMI and blood volume (BV) when age and sex were considered in an analysis of 662 human controls [20]. We did not find pNfL concentration correlated with BMI (Fig. 2). This may reflect relatively low sample size and presence of differing hormonal sex status (intact male, intact female, castrated male and ovariectomized female) in the sample population, making detailed analysis of sex effects on pNfL concentrations difficult to interpret. However, when considered alone, male dogs had a moderate negative correlation with BMI while female dogs did not. Future work with increased sample sizes across all sexes would allow for more detailed analysis of the relationship between

pNfL levels and BMI with adjustments made for sex and age.

In this Labrador population, dogs with a shorter stature had increased pNfL concentration. The relationship between height and pNfL concentration has not previously been investigated in dogs or people. However, both BMI and BV were found to correlate with pNfL concentration in humans [20]; both BMI and BV calculations include height and weight. This is relevant as the height correlation seen in this study may similarly be the result of decreased blood volume in shorter dogs. Alternatively, this correlation with height may be due to shorter

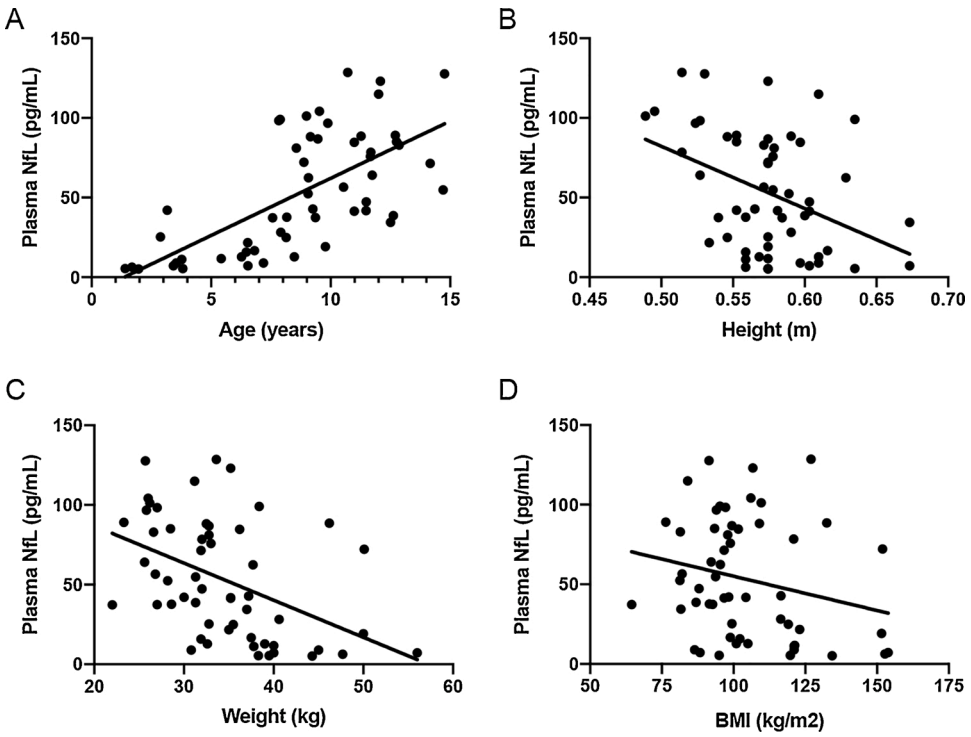

Fig. 2. Relationship between plasma neurofilament light chain concentration and age.
Plasma neurofilament light chain (pNfL) concentration was A) significantly and positively associated with age, B) significantly and negatively associated with height, C) significantly and negatively associated with weight and D) did not significantly correlate with BMI in the Labrador Retriever population. n = 55 dogs.

Labradors having a more chondrodysplastic phenotype, which predisposes dogs to Hanson Type II intervertebral disc degeneration [21]; magnetic resonance imaging would be needed to evaluate this further. Evaluation of multiple breeds with varying chondrodystrophic status would be prudent given the potentially heterogenous populations of dogs used for clinical studies.

The correlations between increased pNfL concentration and decreased weight are also interesting. We found that both age and weight independently influenced pNfL concentration. Given that shorter dogs typically weigh less, the association between pNfL concentration and weight likely reflects the association between pNfL concentration and height. The only prior study to evaluate weight and pNfL concentration in dogs used multiple breeds and did not identify this relationship [7]. An association between weight and pNfL levels has not been defined in humans, although individuals with metabolic syndrome have a higher prevalence of peripheral neuropathy [19]. The correlation we found between weight and pNfL concentration was negative. A positive correlation would be expected if obesity and associated peripheral neuropathy was the underlying mechanism driving this association.

We did not find an association between pNfL concentration and sex, which is in agreement with earlier work [7]. The relationship between sex and progression of human neurodegenerative disease is not well-defined, although recent work indicates sex affects the relationship between BMI and cognitive aging in humans [22]. The effect of neuter status on pNfL concentration could not be robustly evaluated in this study due to low numbers of intact dogs. However, it should be considered in future work as sex hormones could be influential to NfL concentration in the dog model.

In Labrador Retrievers, coat color is associated with a number of disease conditions, likely through positive selection pressure resulting from artificial selection [23,24]. We found no association with pNfL concentration and coat color in this study population.

Purebred dogs are genetically and phenotypically diverse populations. A single purebred dog population was chosen for this work to mitigate confounding factors associated with this extensive phenotypic and genetic diversity. This approach enabled targeted evaluation of relationships between factors such as height, weight and BMI. However, further work would be necessary to understand how these Labrador specific data relate to other breeds. Given that the trends reported in this Labrador population are also present in humans, we suspect that similar findings will be seen across breeds. Dogs were screened for clinical evidence of neurodegenerative disease using neurologic and physical examination; nerve conduction velocities or advanced imaging were not used as these require sedation and/or general anesthesia in canine patients.

Measurement of pNfL concentration provides a rapid and repeatable sample collection method to monitor for neurodegenerative disease progression or response to novel therapies. pNfL concentration has been established as a useful biomarker for a number of neurodegenerative diseases in humans and is starting to be established as an important biomarker for disease states in canine models.

5. Conclusions

We found that in dogs, pNfL concentration increases with aging and decreasing weight and height. The use of a single breed of dog was advantageous to correlate phenotypic measures to baseline pNfL concentration in this population, providing important insight into variables that can affect pNfL concentration in the canine model.

Funding

This work was supported by the National Institutes of Health [K01 OD019743/OD/NIH, T32OD10999/NH/NIH), the United States National Library of Medicine (NLMT15LM007359), grants from the Swedish Research Council(#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF) USA(#201809-2016862) and the UK Dementia Research Institute at UCL. The funding sources were not involved in study design, data

collection or analysis, or in writing of the manuscript.

CRediT authorship contribution statement

Jackie Perino: Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Margaret Patterson:** Data curation. **Mehdi Momen:** Formal analysis. **Mina Borisova:** Investigation. **Amanda Heslegrave:** Investigation, Supervision. **Henrik Zetterberg:** Funding acquisition, Resources, Supervision. **Jordan Gruel:** Data curation, Investigation. **Emily Binversie:** Data curation, Investigation. **Lauren Baker:** Data curation. **John Svaren:** Conceptualization, Methodology. **Susannah J. Sample:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing.

Declarations of Competing Interest

Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The other authors declare no competing interests.

Acknowledgements

The authors would like to acknowledge Alexander Rossor of the Department of Neurodegenerative Disease, UCL Institute of Neurology, for his input and support in this work.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.neulet.2020.135593.

References

[1] A.R. Gafson, N.R. Barthélemy, P. Bomont, R.O. Carare, H.D. Durham, J.P. Julien, J. Kuhle, D. Leppert, R.A. Nixon, R.O. Weller, H. Zetterberg, P.M. Matthews, Neurofilaments: neurobiological foundations for biomarker applications, Brain 143 (2020) 1975–1998, https://doi.org/10.1093/brain/awaa098.

[2] A. Keshavan, A. Heslegrave, H. Zetterberg, J.M. Schott, Stability of blood-based biomarkers of Alzheimer's disease over multiple freeze-thaw cycles, Alzheimer's Dement, Diagnosis, Assess. Dis. Monit. 10 (2018) 448–451, https://doi.org/10.1016/j.dadm.2018.06.001.

[3] G. Disanto, C. Barro, P. Benkert, Y. Naegelin, S. Schädelin, A. Giardiello, C. Zecca, K. Blennow, H. Zetterberg, D. Leppert, L. Kappos, C. Gobbi, J. Kuhle, Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis, Ann. Neurol. 81 (2017) 857–870, https://doi.org/10.1002/ana.24954.

[4] L. Nyberg, A. Lundquist, A. Nordin Adolfsson, M. Andersson, H. Zetterberg, K. Blennow, R. Adolfsson, Elevated plasma neurofilament light in aging reflects brain white-matter alterations but does not predict cognitive decline or Alzheimer's disease, Alzheimer's Dement, Diagnosis, Assess. Dis. Monit. 12 (2020) 1–9, https://doi.org/10.1002/dad2.12050.

[5] B. Olsson, E. Portelius, N.C. Cullen, Å. Sandelius, H. Zetterberg, U. Andreasson, K. Höglund, D.J. Irwin, M. Grossman, D. Weintraub, A. Chen-Plotkin, D. Wolk, L. McCluskey, L. Elman, L.M. Shaw, J.B. Toledo, J. McBride, P. Hernandez-Con, V. M.Y. Lee, J.Q. Trojanowski, K. Blennow, Association of cerebrospinal fluid neurofilament light protein levels with cognition in patients with dementia, motor neuron disease, and movement disorders, JAMA Neurol. 76 (2019) 318–325, https://doi.org/10.1001/jamaneurol.2018.3746.

[6] F. Verde, P. Steinacker, J.H. Weishaupt, J. Kassubek, P. Oeckl, S. Halbgebauer, H. Tumani, C.A.F. Von Arnim, J. Dorst, E. Feneberg, B. Mayer, H.P. Müller, M. Gorges, A. Rosenbohm, A.E. Volk, V. Silani, A.C. Ludolph, M. Otto, Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis, J. Neurol. Neurosurg. Psychiatry. 90 (2019) 157–164, https://doi.org/10.1136/jnnp-2018-318704.

[7] W.K. Panek, M.E. Gruen, D.M. Murdoch, R.D. Marek, A.F. Stachel, F.M. Mowat, K. E. Saker, N.J. Olby, Plasma neurofilament light chain as a translational biomarker of aging and neurodegeneration in dogs, Mol. Neurobiol. 57 (2020) 3143–3149, https://doi.org/10.1007/s12035-020-01951-0.

[8] T. Awano, G.S. Johnson, C.M. Wade, M.L. Katz, G.C. Johnson, J.F. Taylor, M. Perloski, T. Biagi, I. Baranowska, S. Long, P.A. March, N.J. Olby, G.D. Shelton, S. Khan, D.P. O'Brien, K. Lindblad-Toh, J.R. Coates, Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resemblesnamyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 2794–2799, https://doi.org/10.1073/pnas.0812297106.

[9] Fighting Canine ALS | Tufts Now, (n.d.). https://now.tufts.edu/articles/fighting-canine-als (accessed August 12, 2020).

[10] P.P. Nghiem, J.N. Kornegay, Gene therapies in canine models for Duchenne muscular dystrophy, Hum. Genet. 138 (2019) 483–489, https://doi.org/10.1007/s00439-019-01976-z.

[11] A.M. Bradbury, M.A. Rafi, J.H. Bagel, B.K. Brisson, M.S. Marshall, J. Pesayco Salvador, X. Jiang, G.P. Swain, M.L. Prociuk, P.A. Odonnell, C. Fitzgerald, D.S. Ory, E.R. Bongarzone, G.D. Shelton, D.A. Wenger, C.H. Vite, AAVrh10 gene therapy ameliorates central and peripheral nervous system disease in canine globoid cell leukodystrophy (Krabbe disease), Hum. Gene Ther. 29 (2018) 785–801, https://doi.org/10.1089/hum.2017.151.

[12] B.D. Story, M.E. Miller, A.M. Bradbury, E.D. Million, D. Duan, T. Taghian, D. Faissler, D. Fernau, S.J. Beecy, H.L. Gray-Edwards, Canine Models of Inherited Musculoskeletal and Neurodegenerative Diseases, Front. Vet. Sci. 7 (2020), https://doi.org/10.3389/fvets.2020.00080.

[13] F.H. Comhaire, F. Snaps, Comparison of two canine registry databases on the prevalence of hip dysplasia by breed and the relationship of dysplasia with body weight and height, Am. J. Vet. Res. 69 (2008) 330–333, https://doi.org/10.2460/ajvr.69.3.330.

[14] Å. Sandelius, H. Zetterberg, K. Blennow, R. Adiutori, A. Malaspina, M. Laura, M. M. Reilly, A.M. Rossor, Plasma neurofilament light chain concentration in the inherited peripheral neuropathies, Neurology. 90 (2018), https://doi.org/10.1212/WNL.0000000000004932 e518–e524.

[15] H. Wang, M. Davison, K. Wang, T.H. Xia, M. Kramer, K. Call, J. Luo, X. Wu, R. Zuccarino, C. Bacon, Y. Bai, J.J. Moran, L. Gutmann, S.M.E. Feely, T. Grider, A. M. Rossor, M.M. Reilly, J. Svaren, M.E. Shy, Transmembrane protease serine 5: a novel Schwann cell plasma marker for CMT1A, Ann. Clin. Transl. Neurol. 7 (2020) 69–82, https://doi.org/10.1002/acn3.50965.

[16] J. Plassais, J. Kim, B.W. Davis, D.M. Karyadi, A.N. Hogan, A.C. Harris, B. Decker, H. G. Parker, E.A. Ostrander, Whole genome sequencing of canids reveals genomic regions under selection and variants influencing morphology, Nat. Commun. 10 (2019) 1–14, https://doi.org/10.1038/s41467-019-09373-w.

[17] Y. Ru, C. Corado, R.K. Soon, A.C. Melton, A. Harris, G.K. Yu, N. Pryer, J.R. Sinclair, M.L. Katz, T. Ajayi, D. Jacoby, C.B. Russell, S. Chandriani, Neurofilament light is a treatment-responsive biomarker in CLN2 disease, Ann. Clin. Transl. Neurol. 6 (2019) 2437–2447, https://doi.org/10.1002/acn3.50942.

[18] S.J. Sample, A. Stilin, E.E. Binversie, L.A. Baker, R.J. Hardie, Late-onset laryngeal paralysis: owner perception of quality of life and cause of death, Vet. Med. Sci. 00 (2020) 1–8, https://doi.org/10.1002/vms3.240.

[19] P. Thaisetthawatkul, E. Lyden, J. Americo Fernandes, D.N. Herrmann, Prediabetes, diabetes, metabolic syndrome, and small fiber neuropathy, Muscle Nerve 61 (2020) 475–479, https://doi.org/10.1002/mus.26825.

[20] A. Manouchehrinia, F. Piehl, J. Hillert, J. Kuhle, L. Alfredsson, T. Olsson, I. Kockum, Confounding effect of blood volume and body mass index on blood neurofilament light chain levels, Ann. Clin. Transl. Neurol. 7 (2020) 139–143, https://doi.org/10.1002/acn3.50972.

[21] N. Bergknut, A. Egenvall, R. Hagman, P. Gustås, H.A.W. Hazewinkel, B.P. Meij, A. S. Lagerstedt, Incidence of intervertebral disk degeneration-related diseases and associated mortality rates in dogs, J. Am. Vet. Med. Assoc. 240 (2012) 1300–1309, https://doi.org/10.2460/javma.240.11.1300.

[22] L. Bohn, G.P. McFall, S.A. Wiebe, R.A. Dixon, Body mass index predicts cognitive aging trajectories selectively for females: evidence from the Victoria longitudinal study, Neuropsychology. 34 (2020) 388–403, https://doi.org/10.1037/neu0000617.

[23] P.D. McGreevy, B.J. Wilson, C.S. Mansfield, D.C. Brodbelt, D.B. Church, N. Dhand, R.J. Soares Magalhães, D.G. O'Neill, Labrador retrievers under primary veterinary care in the UK: demography, mortality and disorders, Canine Genet. Epidemiol. 5 (2018) 1–13, https://doi.org/10.1186/s40575-018-0064-x.

[24] H.M. Terhaar, P. Muir, L.A. Baker, E.E. Binversie, J. Chi, S.J. Sample, Contribution of habitual activity to cruciate ligament rupture in Labrador retrievers, Vet. Comp. Orthop. Traumatol. 33 (2020) 82–88, https://doi.org/10.1055/s-0039-3399527.