

# The Dog Aging Project: Can Old Dogs Teach Us New Tricks?

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## Abstract

Aging is an extremely complex phenotype, influenced by genes, the environment and the interaction between the two. While studies from laboratory-based model organisms have taught us much about the genetic and environmental determinants of lifespan and healthspan, we have had much less success translating these findings to a “real world” population. Companion dogs offer an ideal organism in which to study aging and its determinants. Dogs vary not only in morphological and behavioral traits but also in lifespan and the effect of age on disease risk. The Dog Aging Project (DAP) will study aging and age-related disease in thousands of companion dogs throughout the country, with the goal of identifying the genetic and environmental factors that shape variation in healthspan and lifespan, and will ask whether we can increase canine healthspan through pharmacological interventions.

## Introduction

Domestic dog breeds vary dramatically not only in shape, size and behavior but also in patterns of aging and age-related disease.<sup>1</sup> Taking advantage of that extraordinary variation, the DAP will study thousands of companion dogs to identify the genetic and environmental factors that shape lifespan and healthspan, the period of a dog’s healthy lifespan.<sup>2</sup> The project also includes a pharmacological study to determine the potential to safely increase healthspan or lifespan in companion dogs.

As its overarching goal, the DAP aims to identify the genetic and environmental factors that shape healthy aging and to determine the mechanisms by which they do so. However, to identify genetic or environmental determinants of healthy aging, we need to define healthy aging. In the follow-

## Glossary of Abbreviations

**BMI:** Body Mass Index  
**CCDS:** Canine Cognitive Dysfunction Syndrome  
**CCDRS:** Canine Cognitive Dysfunction Rating Scale  
**CCI:** Canine Comorbidity Index  
**CFS:** Canine Frailty Score  
**CIP:** Canine Inflammaging Panel  
**DAP:** Dog Aging Project  
**HRQL:** Health-Related Quality of Life  
**GWAS:** Genome-Wide Association Studies  
**VMDB:** Veterinary Medical DataBase

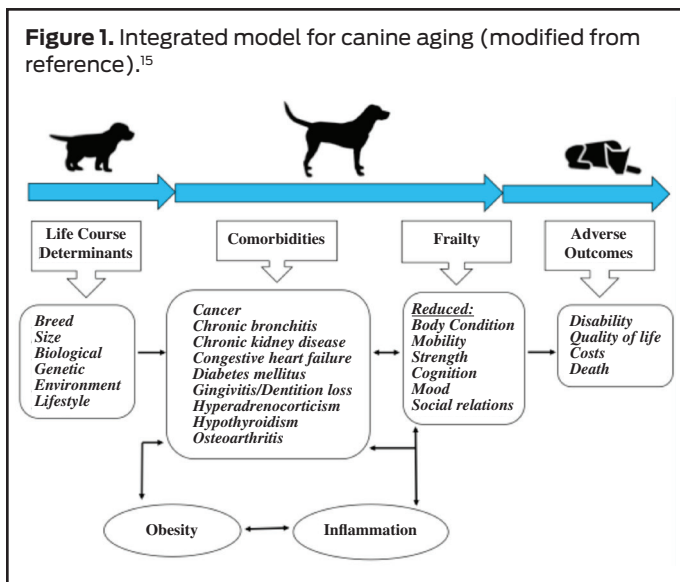
ing paper, we will first discuss ways the DAP will attempt to meet that challenge by developing a functional model of healthy aging in dogs. We then will briefly present the conceptual framework that the DAP will use to better understand the underlying causes of variation in functional aging.

## A Functional Model for Measuring Healthy Aging in Dogs

Small-animal veterinary practitioners know that geriatric dogs are an increasingly important dimension of their practice, though canine aging is complex to define because dogs vary in size-based life expectancy<sup>3</sup> and breed-based disease

risk.<sup>1</sup> Practitioners can readily recognize dogs that are “aging well” or “aging poorly,” but such observations are challenging to document in a manner easily understood by colleagues. By defining a canine aging phenotype, the DAP will develop immensely valuable tools to facilitate the description of, and therefore the care of, aging dogs. Additionally, shared objective descriptors will enhance veterinary research into mechanisms underlying healthy longevity in dogs. The canine aging phenotype will be described in the following ways:

- 1) Comorbidity: This is the coexistence of two or more chronic diseases and is a common phenomenon in older people.<sup>4,5</sup> Strong evidence indicates that the coexistence of multiple chronic conditions increases mortality risk,<sup>6,7</sup> causes a decline of physical and mental functioning,<sup>6,8</sup> and negatively influences quality of life.<sup>9,10</sup> Veterinarians routinely document comorbid disease in aging dogs but do not have a comorbidity instrument to compile these measures into a unified assessment of healthy aging.
- 2) Frailty: The concept of healthy human aging has traditionally focused on the prevention of disease and debili-



inflammaging will be applied to dogs, using validated clinical assays.

After refining and validating assessments of aging in companion dogs on these three separate axes – comorbidity, frailty and inflammaging – we also will investigate the genetic and environmental factors, and underlying mechanisms, that influence these aging phenotypes (Figure 1).

### Comorbidity and Aging

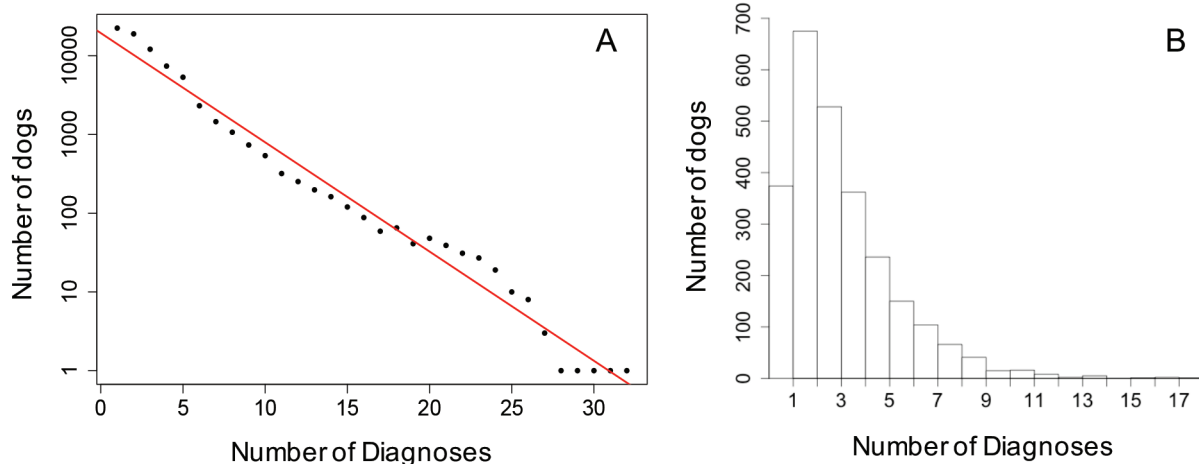
Dogs receive sophisticated individualized medical care and may have more than one disease managed for extended periods of time. Investigation into comorbidities among dogs in the Veterinary Medical DataBase (VMDB)<sup>1,26</sup> dataset from U.S. veterinary teaching hospitals (VTHs) has revealed that 43.9% of dogs have three or more diagnoses at the time of death, and of these, 2.7% have 10 or more diagnoses (Figure 2A). The VetCompass database of the Royal Veterinary College in the U.K.<sup>27</sup> contains dogs being seen exclusively at private primary-care practices, and the records of 2,586 canine veterinary visits over three and a half years demonstrated that most dogs have only a few comorbidities, while a few dogs, even in general practice, exhibit many (Figure 2B).

Several comorbidity indices for humans exist, with diagnoses based on prevalence, chronicity and morbidity in older adults. These indices range from 5 to 20 components.<sup>28-30</sup> Canine-specific diseases for inclusion in the canine comorbidity index (CCI) have been selected based on these same parameters. Initial diseases for inclusion are allergic and inflammatory conditions (sites specified), cancer (any malignant neoplasm), chronic bronchitis, chronic kidney disease, cognitive dysfunction syndrome, congestive heart failure, diabetes mellitus, gastrointestinal chronic conditions, hyperadrenocorticism, hypothyroidism, obesity, osteoarthritis, periodontal disease, and seizure disorders, as well as an “other, please define”

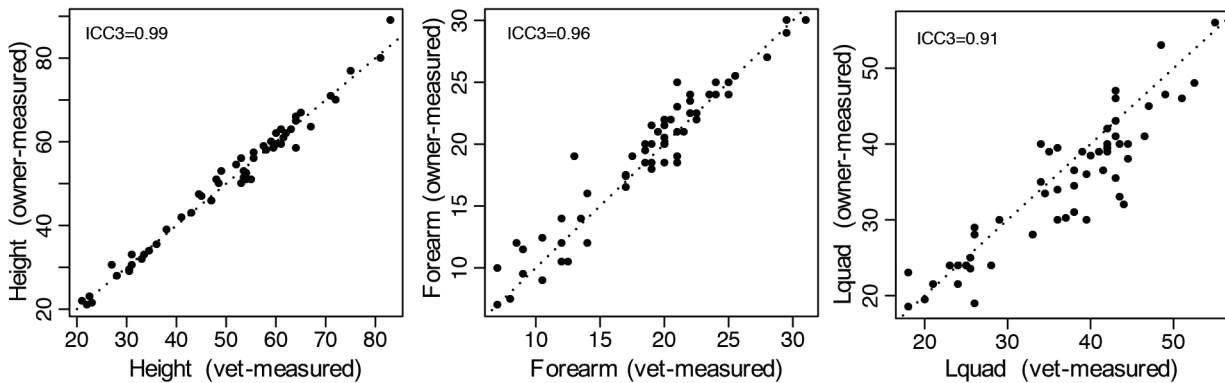
tation, but expanded definitions of “active” aging require maintenance of independent physical function, cognitive ability, mental health, and well-being.<sup>11,12</sup> The opposite of active aging is “frailty.” As described by Malmstrom,<sup>13</sup> models of a frailty phenotype have been developed using three domains: functional, deficit accumulation and biological.<sup>14-20</sup> Many components of frailty are measurable in dogs, and validating these measurements will allow early detection of the nuances of canine aging.

- 3) **Inflammaging:** An emerging theory of aging focuses on the activation of subclinical, chronic inflammation that occurs with aging, called “inflammaging.”<sup>21-23</sup> Mounting evidence reveals that chronic oxidative and inflammatory stress mediate the aging process by damaging proteins, lipids and DNA, resulting in the age-related decline of physiological function.<sup>24,25</sup> The emerging concept of

**Figure 2.** Frequency of comorbidity among dogs. A) Number of diagnoses recorded for each dog at time of death in a dataset of 74,556 dogs at veterinary teaching hospitals (VTHs). Note that the Y-axis is on a log-scale, with the majority of dogs having 1-3 comorbidities. Comorbidity number at death is approximately exponentially distributed. B) Morbidity counts for dogs in general practice. As with dogs at VTHs, most dogs show a small number of comorbidities while a few experience many comorbidities.



**Figure 3.** Morphometric values obtained from veterinarians and owners. Dashed lines indicate isometric value (vet=owner). Inset value, ICC3, refers to the intraclass coefficient for k=two judges rating each target. All ICC values are significant at  $P < 0.001$ . The third panel illustrates a bias toward larger quadriceps measures from veterinarians (paired  $t_{55}=3.81$ ,  $P < .001$ ).



category. Comorbidities will be collected annually on all dogs in the study, and the prospective nature of a longitudinal cohort will enable discovery of any trends in the order of accumulation of comorbidities.

### Frailty

Similar to the experience of the human population, improvements in veterinary health care over recent decades have also greatly expanded the U.S. population of geriatric companion dogs. It is clear to dog owners and their veterinarians that aging dogs experience a phenomenon of frailty, including declining energy levels, limitations in mobility and changes in cognition, but this experience has been poorly described in the literature.<sup>31-34</sup> We will create a canine frailty score (CFS) to describe this dimension of the canine aging process. To be effectively deployed among companion dogs, new instruments to assess frailty must be simple to perform within common household or veterinary clinic conditions, painless, apply to dogs of all sizes and breed backgrounds, and result in an outcome that is easily measured.

Mobility is relevant to frailty, but dog variation in morphology adds a complicating factor in any measurement of mobility. A recent pilot study investigated dog morphometrics and mobility on a leash along a flat 10-meter distance, off leash along the same 10-meter distance at its chosen pace, and off leash up a flight of stairs. We determined that minimally trained owners could acquire similar morphologic measurements and movement times as trained staff regardless of the size, shape or breed of their dogs (Figure 3), that movement speed did vary with weight as expected, and that time to complete mobility trials varied by quartile of life expectancy regardless of size, shape or breed.

Additional frailty factors that will be measured as components of the CFS include:

- **Weight Loss and Body Condition:** Body weight is a standard part of the physical examination of a canine patient. Body condition score (BCS) on a nine-point scale ranging from cachexic (1) to obese (9) is also customarily recorded at

each exam. Changes in these parameters can be individually tracked over time.

- **Physical Activity, Behavior, Anxiety/Nervousness, and Social Avoidance:** The canine cognitive dysfunction rating scale (CCDRS) is a validated instrument developed to facilitate diagnosis of canine cognitive dysfunction syndrome (CCDS), a nonspecific syndrome of senile dementia among dogs.<sup>33-35,36</sup> We will use CCDRS annually as a continuous measure of the range of cognitive changes that occur with aging, even those not compatible with a diagnosis of senile dementia.
- **Activity Monitoring:** The use of accelerometer-based activity monitors has become commonplace in dogs. We will place accelerometers on collars to be worn at scheduled intervals to develop descriptors of typical activity patterns of dogs within and across breed, age, sex, and size groups.
- **Cognitive Performance:** Executive functions are the higher-order processes such as inhibitory control, working memory and discrimination choices that govern goal-directed action and adaptive responses to situations. Measurement systems for these processes have been validated in companion dogs by Dognition™ and have been shown to vary across the aging trajectory.<sup>37-43</sup> This will be assessed annually.
- **Attitude:** We also will use the validated health-related quality of life (HRQL) instrument<sup>44,45</sup> annually in study dogs. The HRQL assesses dogs on four attitudinal axes [energetic/enthusiastic (E/E), happy/content (H/C), active/comfortable (A/C), calm/relaxed (C/R)], and has been shown to detect changes over time that parallel medical evaluations.

### Inflammaging

While inflammaging is not as well-studied in canines as in humans,<sup>46</sup> reports of its significance in dogs have begun to appear.<sup>47,48</sup> Inflammatory cytokines and markers of white-blood cell activation that have proven valuable in the study of this phenomenon in humans and that are validated in the dog will be investigated as components of a canine inflammaging

ing panel (CIP).<sup>49-60</sup> Adipose tissue is increasingly recognized as a contributor to an inflammatory state that promotes other adverse events,<sup>61</sup> but measurements of adiposity are not standardized in dogs. We will attempt to develop a body mass index (BMI) calculation suitable for diverse dogs using our morphologic measurements. Lipoprotein profiling may allow for a metabolic assessment of adiposity that circumvents the challenge of diverse canine morphologies and, if so, will also be utilized.<sup>62-65</sup>

## Genetic Determinants of Healthy Aging

The long-term goal is to identify the genetic and environmental determinants of healthy aging in companion dogs. In fact, age is the single greatest risk factor for many human diseases.<sup>66</sup> However, we have abundant evidence from lab-based studies that longevity is affected by evolutionarily conserved genetic pathways<sup>67-73</sup> and environmental processes.<sup>74</sup> Despite these advances, we are far from understanding the underlying mechanisms of these genetic pathways,<sup>75</sup> the extent to which they explain variation in natural populations, and the relative role of environmental variation. The DAP aims to fill this gap.

We think of the relationship between genes and environment, and the traits they influence, not in terms of mean values, but rather in terms of variances. To do this, quantitative genetics give us a single, powerful equation:  $P=G+E+G\times E$ . In words, the variation in phenotype (P) is the sum of genetic

variation (G), environmental variation (E), and the interaction between the two. While the equation is simple, genome-wide association studies (GWAS) that try to identify specific genes that determine the G→P relationship in humans typically find single-nucleotide polymorphisms that explain no more than 0.1-0.5% of the total variance.<sup>76</sup> This leads to the so-called “missing heritability” problem.

One potential solution lies in identifying the genetic basis not of the downstream phenotypes, like lifespan, but rather of the molecular networks that lie between genotype and phenotype — the so-called “endophenotypes.” These include the transcriptome, proteome, microbiome, metabolome, and so forth. We seem to be better able to map relationships between genotype and endophenotype than between genotype and downstream phenotype. For example, metabolites, the building blocks of our physical and biochemical features, are sensitive to genetic variation, with GWAS variants accounting for up to 63% of the variance in a single metabolite.<sup>77</sup> As we and others have shown, the metabolome is strongly correlated with age in worms,<sup>78,79</sup> flies,<sup>80,82</sup> mice,<sup>83-89</sup> marmosets,<sup>90-92</sup> and humans.<sup>93-95</sup> The working model of the DAP takes advantage of the knowledge that we can gain from dogs about their genotype, their environment (e.g., air quality, social setting, activity level, etc.), and their endophenotypes, coupled with sophisticated measures of aging. The framework is illustrated in Figure 4. Importantly, this approach holds the promise of not only identifying stronger genetic and environmental signals associated with aging but also of explaining the underlying mechanisms that link genotype and environment to aging phenotype.

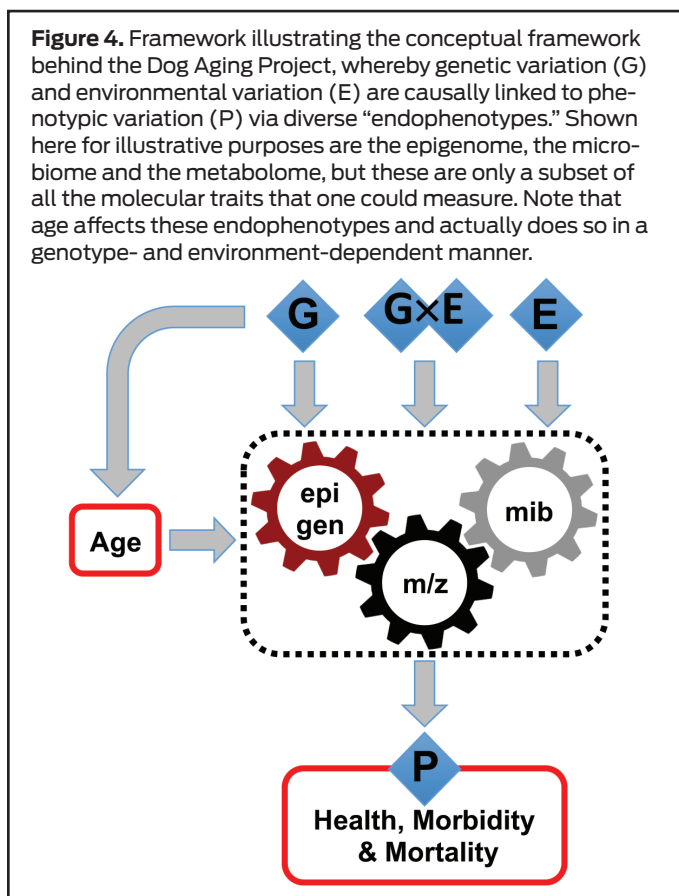
## Interventions to Improve Healthy Lifespan

The primary goal of the DAP is to understand the determinants of healthy aging in companion dogs, but we also want to know if we can delay the onset or reduce the severity of the age-related decline in vigor that affects all of us. With this in mind, the DAP has already begun safety testing of rapamycin, a drug that has been shown to be highly effective in increasing healthspan and lifespan in the laboratory setting.<sup>96</sup>

In mice, treatment with low doses of rapamycin not only increases lifespan but also decreases the deleterious effects of age on left ventricular heart function.<sup>97,98</sup> Our recent placebo-controlled pilot study in companion dogs found that rapamycin was not only safe but also that dogs on rapamycin showed improvement in multiple measures of left ventricular function.<sup>99</sup> The DAP will carry out the first double-blind placebo-controlled study testing the efficacy of a drug to improve healthspan outside a lab setting.

## Conclusion

To develop a complete model that gives us the power to predict, diagnose, treat, and prevent age-related disease, we need one that will provide answers in years, not decades, and that can benefit from the wealth of current methods and technologies available to scientists in the 21st



century. We think the Dog Aging Project offers just such a model. Our approach will generate a tremendous amount of data (on the order of a petabyte, no pun intended, that is  $10^6$  gigabytes). Importantly, to maximize the outcome of this enormous effort, the DAP will be an open science initiative, with all data made publicly available, other than that needed to protect confidentiality. Scientists, veterinarians, dog owners, and the general public will be welcome to explore all that dogs have to teach us, about dogs and about ourselves.

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