Age relationships of postmortem observations in Portuguese Water Dogs

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Abstract A dog model has been used to evaluate histological changes arising from senescence. Autopsies of 145 Portuguese Water Dogs have been used to evaluate the individual and group “state of health” at time of death. For each dog, weights or dimensions of organs or tissues were obtained, together with histological evaluation of tissues. Twenty-three morphological metrics correlated significantly to age at death. Many of these involved muscles; others were associated with derivatives of embryonic foregut. The latter included lengths of the small intestine and trachea as well as weights of the stomach and some lung lobes. Nearly all of the dogs

examined had histological changes in multiple tissues, ranging from two to 12 per dog. Associations among pathologies included inflammatory bowel disease with osteoporosis and dental calculus/periodontitis with atherosclerosis and amyloidosis. In addition, two clusters of histological changes were correlated to aging: hyperplasia, frequency of adenomas, and hemosiderosis constituted one group; inflammation, plasmacytic and lymphocytic infiltration, fibrosis, and atrophy, another. Heritability analysis indicated that many of the changes in tissue/organ morphology or histology could be heritable and possibly associated with *IGF1*, but more autopsies will be required to substantiate these genetic relationships.

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Introduction

During the past decade, the dog (*Canis familiaris*) has become increasingly useful as a model organism for research in mammalian genetics (Sutter and Ostrander 2004; Fleischer et al. 2008). Dog breeds represent genetic isolates in which different *body plans* and behaviors have been fixed, and in which elevated frequencies of genetic disease may be found. Dogs have undergone selection for different goals, resulting in morphological and behavioral changes, along with

the biochemical and physiological modifications required to maintain function.

Selection has been targeted primarily towards specialized function and form through early growth, maturity, and reproduction, but not towards traits of later life (longevity and senescence). This has had unintended consequences on the health and longevity of purebred dogs, the most striking of which is the dramatic impact of breed size on longevity, such that small breeds live longer than large ones (Galis et al. 2007; Jones et al. 2008). This stands in stark contrast to the trend of longer life associated with larger species (e.g., elephants live longer than mice).

We are using Portuguese Water Dogs (PWDs) as a model system to analyze the genetic basis of senescence. PWDs were reconstituted from approximately 30 dogs during the mid-1900s (Molinari 1993). Careful pedigree records have been kept, relating the current US population to these founders. A large amount of heritable variation in the PWD population, together with the high degree of owner cooperation, has made this breed a model system for canine genetic analysis (Chase et al. 1999; Lark et al. 2006a, b). Our approach has been to use autopsy to establish the state of health of these dogs at time of death.

Dissection of human cadavers has revealed a range of variation in organ size and weight, suggesting the potential for genetic analysis of organ metrics (de la Grandmaison et al. 2001; Haddad et al. 2001) and ultimately relating such information to diseases of senescence. The declining frequencies of human autopsies has made this tool ineffective in genetic studies of human populations, but the ability to undertake extensive postmortem analysis on animal populations promises to open new avenues for investigating the genetics of disease and senescence in model systems such as the dog. In long-range studies of animal health, autopsy has yielded an all-encompassing picture of organ metrics, tumor characterization, and other tissue histopathology (e.g., Lawler et al. 2006; Aupperle et al. 2007; Pavlica et al. 2008).

Here, we report gross autopsy and histopathological findings resulting from the autopsy of 145 cadavers of deceased Portuguese Water Dogs. We relate these to the age at death of each individual. Preliminary results indicate that genetic variation is present and that a detailed genetic basis for morphol-

ogies and pathologies of senescence (identification of loci and eventually genes) can be established using this, or similar, dog models.

Materials and methods

PWD population The USA population of PWDs, as registered by the American Kennel Club, numbers approximately 20,000 individuals at present. The derivation of this population consists of 31 founders that trace their lineage to just two founding kennels, a structure well-suited for studies of quantitatively inherited phenotypes, such as those that relate to senescence (Molinari 1993; Chase et al. 1999). Within the PWD breed, a wide range of values for the coefficient of inbreeding provides subpopulations that are highly inbred (homozygous, expressing recessive genes), as well as heterozygotes expressing phenotypes that result from interactions between haplotypes. Accurate and complete pedigree records (validated by DNA markers) have established consanguinities in the current PWD population that average 0.2 and range from 0.0 to 0.6 (Chase et al. 1999).

Acquisition of cadavers An excellent relationship exists between PWD owners on the one hand and the University of Utah on the other (see www.georgieproject.com as well as Davis 2007). Currently, PWD owners are supplying cadavers of deceased dogs for analysis of their state of health at time of death. Immediately following death, the intact cadavers are air shipped on ice to the University of Utah, Salt Lake City, UT.

The data described here were obtained from postmortem evaluation of the first 145 submitted cases. The ages of these dogs at death ranged from 2.7 to 16.8 years, distributed around a median value of 11.7 years (Fig. 1).

Autopsy Upon arrival in Utah, complete anatomical and pathological evaluation is conducted, including 51 whole body and organ weights or dimensions (e.g., weights of the heart or lungs; lengths of the intestines or trachea), and one-to-several histological analyses of each of 27 tissues, including samples from the adrenal gland, bladder, bone, brain, colon, eye, heart, intestine, kidney, liver, lung, lymph nodes, muscle,

Fig. 1 Age at death of PW dogs received for autopsy. Dogs are ranked in order of increasing age

pancreas, pituitary gland, salivary gland, skin, spleen, stomach, thyroid, tongue, and tonsils (for details, see supplementary Tables 1 and 2). All data were collected uniformly, with an experimental design that was developed prior to study initiation, implemented by a dedicated autopsy team (SM, MN). Additional samples were collected for histology where gross lesions were visible in nondesignated tissues.

Dental analysis All skulls were evaluated by two people. Each tooth was scored for tooth loss, decay, bone loss, wear, and calculus. Decay, bone loss, and wear were scored on a scale 0 (none), 1 (mild), 2 (moderate), 3 (severe); missing teeth were recorded as “score unknown.” For each dog, dental data are presented as the mean of scores for all teeth.

Histological evaluation For histopathology, tissue samples were preserved in 10% buffered formalin, sectioned routinely, stained with hematoxylin and eosin, and evaluated blindly by an American College of Veterinary Pathologists certified pathologist (LM).

Table 1 Significant correlations between gross autopsy measurements and age of death

Trait	r =correlation with age at death	P for age effect
Gluteus medius	−0.46	<1.00E-05
Hamstrings ^a	−0.45	<1.00E-05
Gastronemus and planteras ^a	−0.41	<1.00E-05
Quadriceps ^a	−0.40	<1.00E-05
Triceps ^a	−0.38	<1.00E-05
Small intestine ^a	−0.34	2.00E-05
Adductor	−0.32	3.00E-05
Body weight ^a	−0.29	0.0002
Trunk circumference 2 ^a	−0.28	0.0004
Trunk circumference 1 ^a	−0.26	0.0005
Right accessory lobe	−0.26	0.0009
Temporalis ^a	−0.25	0.0010
Trachea width ^a	−0.25	0.0010
Left cranial lobe	−0.22	0.0040
Tongue weight ^a	−0.20	0.0069
Right kidney	−0.19	0.0105
Stomach	−0.19	0.0103
Left medial lobe	−0.19	0.0123
Tail length	−0.18	0.0148
Heart weight ^a	−0.15	0.0369
Achilles length ^a	−0.15	0.0403
Trachea length	0.15	0.0378
Adrenal weight	0.23	0.0024

Lengths, widths, and masses of various organs or muscles are indicated (see supplementary Table 1 for more details). The Pearson correlation coefficients and the significance (P) of this correlation are shown for each metric

^aDemonstrable heritability

Table 2 Frequency of histological changes in each organ

Organ/tissue	Percentage ^b
Skin	97
Spleen	85
Kidney	79
Liver ^a	92
Heart	60
Lung	82
Pancreas ^a	64
Adrenal	54
Skeletal ^a	41
GI tract ^a	50
Thyroid ^a	36
Hemolymphatic	21
Brain	16
Salivary gland ^a	17
Parathyroid	14
Eye	5
Urinary	4

For each organ, the percentages of dogs showing some histological changes in that organ are shown

^aOrgans with heritable pathologies

^b% of all PW dogs examined

Most tissues were scored for observed changes (methods supplementary Table 2) on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). Data were corrected as follows: Pathologies observed in less than 1% of the dogs were removed. Extreme values for gross metrics due to tumor growth were removed (e.g., the organ was marked as missing data for that individual). Histological observations that were obscured by autolysis were marked as missing.

Analysis of data Using the corrected data, histological outcomes, whole body metrics, and organ metrics were evaluated for relationship to age at death. Correlations were evaluated using the “cor” function of R (R Development Core Team 2006). Significance was established using 10,000 permutation tests. Heritability was estimated with the “regress” (Clifford and McCullagh 2006) package of R using the call: regress($y \sim 1$, $\sim A$, identity= T , fraction=0.1, pos= T) where y is the trait value for each individual, A is the additive genetic covariance matrix; twice the coefficient of consanguinity (Falconer and Mackay 1996) between each pair of dogs. The “regress” function fits

the model by maximizing the residual log likelihood (REML) using a Newton–Raphson algorithm and returns an estimate of the additive genetic variance. Significance is established using permutation tests (Manly 1997).

Broader histological categories were generated as the means of common histological observations across all tissues (e.g., fibrosis in the kidney, pancreas, heart, liver). These were used in a principal component analysis (generated using the precomp function of R) to search for clusters of traits related to age of death. Clusters presented in Fig. 3 are means from broad categories that had high loadings for PC1 and PC2. These were evaluated for their relationship to age of death as described above.

IGF-1 haplotype The IGF-1 small dog haplotype is represented here by the 502 bp allele of the SSR marker FH2295 (Sutter et al. 2007). Correlations between dosage of this allele (e.g., 0, 1, 2) and pathologies were tested using the correlation procedure described above.

Results

Morphological metrics

Among the 145 postmortem examinations, 23 morphological metrics correlated significantly to age at death, with some approaching $r=0.5$ (Table 1). Several that were negatively correlated involved skeletal muscles and were among the highest correlations found. This is consistent with previous results from a study on the effects of diet restriction in the dog, in which high and stable fat mass and declining lean mass both (although not correlated) were predictive of the time of death (Lawler et al. 2008).

Other potential predictors in the PWD were tissues derived from the embryonic foregut (Table 1): the lengths of the small intestine and trachea, the trachea width, as well as the weights of the stomach and several specific lung lobes.

Dentition

Teeth were scored for calculus, decay, wear, and the degree of periodontitis (bone loss) surrounding each

tooth. Only calculus formation could be significantly correlated with age of death ($r=0.28$), although periodontitis and caries were correlated ($r=0.42$), and caries are also correlated with calculus formation ($r=0.56$). Periodontitis correlated with histological changes in the kidney, and calculus formation correlated with changes in the heart (see below).

Histological traits (non-neoplastic)

Almost all of the 145 dogs examined had histological changes in multiple tissues and organs (Fig. 2), ranging from as few as two to as many as 12 in a single individual, with a median value between 7 and 8. Organs that yielded non-neoplastic histological traits are summarized by frequency ($\geq 4\%$) in the population (Table 2).

From available pedigrees (Chase et al. 1999), we estimated heritability of histological traits in the autopsied population. Heritable traits included (a) fibrosis of the pancreas and liver, (b) lymphocytic infiltration of the gastrointestinal tract and salivary gland, (c) osteoporosis of the shoulder and humeral head, and (d) atrophy of the thyroid gland (Table 2). For some processes (hemosiderosis, atherosclerosis, and age of death), heritability was also detected when

all tissues or organs were combined into a single category to increase statistical power. In the future, heritability estimates will become more robust as the number of autopsies increases.

Individual traits by organ

Individual histological traits also were summarized across all sampled tissues. A number of these traits correlated significantly to age at death (Table 3; for added detail see Supplementary Table 2). Correlations (r) ranged from -0.25 to 0.51 . Highly significant ($P < 0.001$) positive correlations to age at death were found for renal fibrosis and glomerulosclerosis, spleen hemosiderosis, and adrenal lymph node hyperplasia. Specific non-neoplastic organ pathologies are summarized in Table 3 and in the following comments on that table.

Pulmonary congestion was found in 65% of the dogs, with an inverse correlation to age at death. The inverse correlation between age at death and pulmonary congestion is somewhat surprising. Periternal pulmonary congestion and edema may occur both in acute and chronic physiological states and may be associated with immobility, inflammation, perfusion abnormalities, hypoxia, vasculopathy, many systemic metabolic abnormalities, or the agonal process (Dungworth 1993) and thus might have been expected to show a positive or neutral correlation. However, evaluation of pulmonary histological outcomes demonstrated surprisingly few additional pulmonary findings.

Renal changes (glomerular, tubulointerstitial) were associated positively with age at death. These changes are observed frequently in the kidneys of midlife to older dogs. Causes of histological change in the kidney frequently cannot be identified with certainty. Malignancy and chronic inflammation, such as pancreatitis or inflammatory bowel disease (both observed frequently in our study see below) can trigger immunopathies that may lead to secondary glomerular involvement.

Tubulointerstitial changes usually are inflammatory or degenerative, followed by localized vascular compromise that in turn appears to present a compounding fibrosing insult (Lin et al. 2008). Two other major glomerular changes in dogs are immune complex deposition and amyloidosis (Grauer and DiBartola 2000). We found a correlation between amyloidosis

Fig. 2 Non-neoplastic histological changes in multiple organs. The frequency of dogs in which two or more organs/tissues affected by non-neoplastic histological changes were found is shown. The number of dogs (y axis) with 2, 3, 4, etc. organ/tissues affected (x axis) are shown. Data are from histological score sheets as in supplemental Table 1

Table 3 Most frequent non-neoplastic histological changes

Organ	Observation	# of dogs	<i>r</i>
Heart	Atrioventricular valve	45%	ns
	Endocardiosis cardiomyopathy	12%	ns
Lung	Congestion	65%	−0.24**
	Edema	52%	ns
Pancreas	Fibrosis	37%	0.27**
	Pancreatitis	30%	ns
	Hemorrhage	20%	−0.25**
	Acinar hyperplasia	10%	0.25**
Kidney	Glomerulosclerosis	63%	0.51**
	Fibrosis	24%	0.38**
	Tubulointerstitial nephritis	21%	ns
	Congestion	19%	ns
	Glomerulopathy	16%	0.19*
	Amyloid	12%	0.25**
Spleen	Hemosiderosis	73%	0.30**
	Congestion	43%	ns
Hemolymphatic	Lymph node hemosiderosis	16%	0.17*
Adrenal	Cortical hyperplasia	38%	0.44**
	Atrophy, degeneration	19%	ns
Skeletal	Osteoporosis	41%	0.27**
Digestive	Inflammatory bowel disease	37%	0.18*
	Sialadenitis	11%	ns
Liver & Gall bladder	Congestion	61%	ns
	Hemosiderosis	49%	0.20*
	Fibrosis	28%	ns
	Vacuolative hepatopathy	25%	ns
	Hepatitis, cholangitis	18%	ns
Thyroid	Atrophy	35%	0.23*
Parathyroid	Hyperplasia	11%	0.18*
Skin	Follicular dysplasia	97%	ns

Histological observations are grouped according to organ. For each histological change, the percentage of dogs showing the change and the significant correlations with age of death are shown. Traits in bold were correlated with age at death

ns not significant

* $p < 0.05$, ** $p < 0.005$

and periodontitis ($r=0.33$). Additional autopsies confirming this association would support a more detailed investigation of this relationship.

Liver, spleen, and lymph nodes The most prominent change observed was hemosiderosis, which correlated significantly with age of death. ($P < 0.001$ in the case of splenic hemosiderosis; Table 3). Ferritin breakdown results in formation of hemosiderin, an insoluble product that is deposited in body tissues, especially in liver and spleen. Hemosiderin is an end product resulting primarily from breakdown of senescent erythrocytes, but excessive iron intake and diseases that increase erythrocyte breakdown rates

can contribute as well (Jubb et al. 1993). Deposited hemosiderin, although a part of the body's iron stores, is not readily mobilized under normal physiological circumstances.

Endocrine organs The thyroid, parathyroid, and adrenal glands showed significant correlations with age of death (Table 3). These included 35% frequency of *thyroid* atrophy, 11% frequency of parathyroid hyperplasia, 38% frequency of adrenal cortical hyperplasia, and 19% frequency of adrenal cortical atrophy or complete absence of adrenals. Hyperplasia of the adrenal lymph nodes also correlated highly ($P < 0.001$) to age at death.

A low prevalence of *hyperparathyroid* changes in our database (11%) may represent (a) sequelae to renal disease, (b) association with mild osteoporotic changes (see below), or (c) a physiological response to chronic antigenic stimulation associated with inflammatory bowel disease (see below) symptoms of which were commonly observed in our histological evaluations.

Cortical *adrenal* hyperplasia occurred with a frequency of 38% and was correlated with age of death. Hyperplasia of the adrenal cortex is a frequent finding in older dogs, although its significance is not well understood. Addison's disease, or hypoadrenocorticism, has been reported in the PWD (Gough and Thomas 2004; Clark and Stainer 1994) at a breed-specific incidence of 1.5%, and quantitative trait analysis has identified two loci that regulate late-onset Addison's disease in the breed (Chase et al. 2006). Eleven dogs (7%) had clinical and autopsy data that were consistent with Addison's disease. These data support previous observations in PWDs. The incidence, ~7%, of the cadavers examined is high and probably reflects the selective desire of owners to have dogs autopsied that are believed to be Addisonian.

Pancreas Pancreatic fibrosis was frequent (37%) and significantly correlated to age of death; pancreatic hemorrhage, however, was inversely correlated with age of death (Table 3). Both pathologies often relate to pancreatitis. In most instances of canine pancreatitis, the proximate cause remains unidentified. A growing body of studies of humans with various forms of inflammatory bowel disease has demonstrated an association between inflammatory bowel disease (IBD) and pancreatitis that could be more causal than associative (Seyrig et al. 1985; Tromm et al. 1992; Triantafyllidis et al. 2004; Barthet et al. 2006). The intermittent and sometimes mild nature of IBD in dogs may suggest other health problems in the clinical setting and, as a consequence, definitive diagnosis remains elusive in many instances (Hall and Simpson 2000). The relationship of IBD to this and other tissue pathologies remains the subject of future research.

Osteoporosis Histological changes consistent with a degree of bone loss were noted in 41% of the dogs and were significantly correlated with age at death (Table 3). The histological mass loss was confined to

trabecular thinning. In our study, the prevalence of osteoporosis in dogs with histological evidence of inflammatory bowel disease was 54%, compared to 32% in dogs without histological evidence of inflammatory bowel disease ($p=0.02$). Thus, inflammatory bowel disease in the dog may be associated with a degree of bone mass decline, similar to effects of inflammatory bowel disease on bone mass in humans (Arden and Cooper 2002).

Heart and skin Neither heart nor skin pathologies were correlated with age at death, although these histological changes were observed with the greatest frequency. Endocardiosis of atrioventricular (AV) heart valves was identified in 45% of the cadavers. AV endocardiosis is common in the dog, with prevalence approaching 75% by age 16 years. However, from early- to midlife, the lesion may be found frequently (Robinson and Maxie 1993a, b) so lack of correlation to age at death is not exceptional.

A strong correlation was observed between dental calculus accumulation and atherosclerosis ($r=0.43$), in line with reports for humans (Slavkin and Baum 2000; Renvert 2003). If this association is strengthened by additional autopsies, the dog model could become an excellent system for pursuing the causative basis for this relationship as well as for establishing preventive treatment (not unexpectedly, calculus accumulation is significantly correlated with age of death).

In the skin, follicular dysplasia, found in 97% of cadavers, was not associated with hair loss but appeared to be a histological anomaly that has become fixed in the breed.

Neoplasms

A number of malignant and benign tumors were found and analyzed. Twenty-five different malignant and 16 different benign tumors were identified. The most frequent of these (found in three or more dogs) are presented in Table 4. Of these, two were related to age of death: Lymphosarcomas (malignant, $r=-0.30$) and lipomas (benign, $r=0.21$). When all types of benign adenomas (43) were considered as a group, adenomas were positively correlated with age ($r=0.26$). Finally, in a number of instances, autopsy revealed more than one type of malignancy within a single dog—e.g., melanoma and hemangiosarcoma.

Table 4 Frequency of malignant and benign tumors

Malignant	% dogs	Correlation with age of death	Benign	% dogs	Correlation with age of death
Hemangiosarcoma	27%	0.02	Lipoma	36%	0.21
Lymphosarcoma	10%	0.3	Adrenal adenoma	11%	0.13
Round cell tumor	4%		Pituitary adenoma	6%	
Malignant melanoma	3%		Thyroid adenoma	4%	
Pheochromocytoma	2%		Parathyroid adenoma	4%	
Islet cell carcinoma	2%		Any adenoma	23%	0.26
Bronchiogenic carcinoma	2%		Ocular melanoma	3%	
No malignant tumors	47%	0.07	Skin fibroma/papilloma	2%	
			No benign tumors	51%	-0.26

The frequencies of observed malignant and benign tumors are indicated as a percentage of the total number of autopsied dogs ($n=145$). Only tumors observed in $\geq 2\%$ are shown. Correlations with age of death are shown for all categories $>10\%$. Correlation coefficients in bold are significant at $p<0.01$

Correlations between histological changes

Several significant correlations were found, in which changes in one organ were associated with histological change in a different organ (Table 5). We have already noted the increased prevalence of histological osteoporosis in dogs that present changes consistent with inflammatory bowel disease as well as the correlation between the accumulation of calculus on the teeth (as well as increased periodontitis) and atherosclerosis in the heart. Changes in kidney weight were inversely correlated with atrophy of the adrenals; increase in spleen weight (presumably due to congestion as seen histologically) was inversely associated with amyloidosis of the kidney, which itself was correlated with increased periodontitis;

stomach weight was correlated with congestion in the spleen and inversely with metastatic hemangiosarcoma of the lung.

Age dependence of multiple histological changes

We have used cluster analysis (see “[Materials and methods](#)”) to identify multiple pathological processes, which as a group are correlated with aging. That is, as an animal ages, it will tend to accumulate increasing numbers of the pathologies that comprise the group. Figure 3 presents two trait clusters that we have identified. One group comprises inflammation, plasmacytic, and lymphocytic infiltration as well as atrophy and fibrosis; in the other, we find hyperplasia, adenomas, and hemosiderosis.

Table 5 Correlations between organs and histological traits

Organ (trait)	Histology (organ)	<i>r</i>	<i>p</i> Value
Kidney weight	Atrophy (adrenals)	-0.27	0.0006
Spleen weight (congestion)	Amyloidosis (kidney)	-0.21	0.001
Stomach (weight)	Hemangiosarcoma (lung)	-0.26	0.0008
Stomach (weight)	Spleen (Congestion)	0.34	<0.0001
Teeth (Tartar/periodontitis)	Atherosclerosis (heart)	0.44	0.003
Teeth (periodontitis)	Amyloidosis (kidney)	0.33	0.002
Small intestine (length)	Age of death	-0.26	0.001

Correlations between gross organ metrics and histological observations are presented along with the significance (p value) of each correlation. For each histological observation, the organ is indicated in parenthesis. The correlation between age at death and the length of the small intestine also is included (bottom line)

Fig. 3 Clusters of histological changes correlated with age of death. Histological changes clustered such that as age of death increases the probability of finding multiple changes also increases. Cluster one: inflammation; plasmacytic infiltration, lymphocytic infiltration, fibrosis, and/or atrophy are histological changes that occur together at autopsy in increasing frequency with age of death. The animals with 0, 1, 2, 3, 4,

or 5 of these pathologies are graphed on the *x* axis as a function of their age at time of death (*y*-axis). Cluster two: Animals showing adenomas, hyperplasia, and/or hemosiderosis at autopsy are histological changes that occur together at autopsy in increasing frequency with age of death. The animals with 0, 1, 2, or 3 of these pathologies are graphed on the *x* axis as a function of their age at time of death (*y*-axis)

Discussion

Because dogs share a large number of genetic diseases with man (Sutter and Ostrander 2004) as well as common environments that may trigger disease, they present an excellent model system for the study of intrinsic diseases and the factors that predispose toward the diseased state. We have begun an in-depth study of the genetic basis of diseases associated with aging using gross autopsy and histopathology to define the state of health of a dog at death. We have chosen the Portuguese Water Dog as our model system to demonstrate the power of this approach.

These dogs present a picture of morphological (Table 1) as well as histological (Table 3) changes, many of which are correlated with age of death. Perhaps the most notable aspect we have observed is the large number of autopsies (exclusive of those with neoplasms) in which numerous histological changes are found that involve multiple organs or tissues (Table 2, Fig. 2). As yet, we do not know if these are independent events or related to some underlying systemic change. However, several of the changes in different organs appear to be correlated (Table 5)

suggesting some common etiology. Additionally, we have identified clusters of pathological processes that point to a common denominator dependent on aging (Fig. 3). Thus, in one cluster, we find traits that are characteristic of immune processes. Subacute and chronic inflammations are accompanied by plasmacytic and lymphocytic infiltration. The continued inflammatory process can result in either resolution or atrophy and fibrosis. With increasing age, incidents involving the immune system increase and result in accumulated damage across tissues or organs, an observation that aligns well with current concepts of the aging process (Cheville 1983; Amenta et al. 1990). The second cluster suggests that processes that lead to increased cell growth, adenomas, and hyperplasia are connected with iron storage. This association was unexpected. It is not surprising that factors giving rise to hyperplasias might also favor growth of benign tumors; however, the connection with hemosiderosis requires further exploration.

Several of the morphological and histological changes that we have observed appear to be heritable. In the PWD breed, two major *IGF1* haplotypes are segregating (Chase et al. 2002; Sutter et al. 2007). Identification of these haplotypes led to the discovery

that fixation of these two types differentiate large from small breeds of dogs (Sutter et al. 2007). IGF1 has been implicated in regulating longevity in a number of organisms (Kenyon 2001; Bartke 2005), and genome comparisons between different dog breeds have demonstrated that IGF1, a major determinant of variation in size between breeds (Sutter et al. 2007), also plays an important role in determining the difference in longevity between breeds (Jones et al. 2008).

In a recent review, Rodriguez et al. (2007) summarized much of the evidence implicating the IGF1 pathway in disease concluding that “The constellation of genes in this key pathway contains

potential candidates in a number of complex diseases, including growth disorders, metabolic syndromes, diabetes, cardiovascular disease, central nervous system diseases, as well as longevity, aging and cancer.” Preliminary data, comparing genomes of dogs from large and small breeds (differentiated by their *IGF1* haplotypes) demonstrated that the breed incidence of specific diseases could be associated with specific haplotypes of *IGF1* (Chase et al. 2009). Thus, the Portuguese Water Dog, as a model system, presents an unusual opportunity to assess the impact of *IGF1* on disease and senescence.

As a preliminary analysis (Table 6), we have examined the correlation of parameters of morpholo-

Table 6 The correlations between the “small dog” IGF-1 allele (FH2295) and various organ metrics or histological observations in specific tissues

Age corr. ^a	Organ/tissue	Metric	FH2295	<i>p</i> Value
(—)	sm intest	Length	−0.27	0.01
+	Adrenal.wt	Weight	−0.28	0.01
(−)	Tail	Length	−0.26	0.01
	Brain	Weight	−0.24	0.02
+++	Trachia	Length	−0.23	0.03
	Esophogus	Length	−0.22	0.03
(−)	Right.kidney	Weight	−0.22	0.03
(−)	Tongue	Weight	−0.22	0.03
(—)	Triceps	Weight	−0.20	0.05
(—)	Hamstrings	Weight	−0.19	0.06
	Neck	Length	−0.19	0.07
(—)	quadriceps	Weight	−0.19	0.07
	Left.kidney	Weight	−0.19	0.07
(−)	Left.med.lung	Weight	−0.18	0.09
	rt.caud.lung	Weight	−0.17	0.09
	Gastro and plant	Weight	−0.17	0.10
Histological change	Process	Tissue		
	Edema	Brain	−0.27	0.01
	Congestion	Kidney	−0.20	0.05
	Atherosclerosis	All tissues	−0.20	0.03
	Edema	All tissues	−0.19	0.07
	Hemangiosarcoma	Kidney	−0.18	0.10
	Atherosclerosis	Heart	−0.18	0.06
	Inflammatory.bowel	Jejunum	−0.18	0.08
	Fibrosis	Liver	0.18	0.09
	Necrosis	All tissues	0.18	0.08
	Vacuolization	Liver	0.19	0.07
	Carcinoma	All tissues	0.19	0.07
	Infarction	All tissues	0.21	0.04

The significance of each correlation is shown (*p* value). The correlations with age at death from Table 1 are included

^aCorrelation with age (from Table 1): 0.15<+<0.2; +++>0.3; −0.15>c>−0.2; −0.2>(−)>−0.3; −0.3>(—)

gy and of histological change found at autopsy with the PWD *IGF1* haplotype that is common to small breeds (Sutter et al. 2007). Since small dogs live longer, and longer-lived dogs could have different frequencies and time courses of disease, there could be a potential bias in the correlations between the *IGF1* small dog haplotype and various pathologies. However, there was no significant age effect for the small dog haplotype in our dataset (the mean age of death of PW dogs with no copy of the small dog haplotype was 10.73 ± 0.71 years; with one copy 11.56 ± 0.42 year; with two copies 11.44 ± 0.51 years).

The correlations in Table 6 are only suggestive, given the number of traits tested and the somewhat low frequency of dogs homozygous for the small dog haplotype (18%). Because of the limited number of homozygotes in the current dataset, we could not analyze the correlations between age of death and specific pathologies within a specific genotype. Nevertheless, the analysis in Table 6 is instructive.

As expected, all of the morphological traits are negatively correlated with the small dog haplotype—i.e., their weight or length becomes smaller with the presence of one to two copies of this haplotype. Many, but not all, of these traits also are negatively correlated with age. Most interesting is the finding that the length of the small intestine is inversely correlated with age of death (Table 5). Although organs of *IGF1* small-haplotype dogs may be smaller because of loss of mass with age, an alternative may be that those dogs with smaller organs are longer lived. This latter explanation would be more likely for the left medial lobe of the lung, the length of the small intestine and the tail, as well as the weight of the right kidney and the tongue. It is interesting to note that although the weight of the adrenals and the length of the trachea are positively correlated with age, they are negatively correlated with the small dog *IGF1* haplotype that is associated with increased longevity. Data from other breeds should assist in clarifying the relationship of these two phenotypes to longevity.

There also is the suggestion in Table 6 that several histological changes could prove to be associated with *IGF1* as the database becomes more robust. Although the present state of the database makes it premature to draw conclusions, some relationships in Table 6 are worthy of comment: many of the observed tissue changes are negatively correlated with the small dog *IGF1* haplotype (i.e., fewer tissue changes may be

associated with the increased longevity haplotype). In contrast to humans, atherosclerosis is not considered to be an important source of tissue infarct in the dog (Maxie 1993; Robinson and Maxie 1993a, b). As a result, the effects of *IGF1* on these two histopathologies can be evaluated separately, and as can be seen in Table 6, they appear to respond differently to the small dog *IGF1* haplotype, which appears to increase the risk of infarcts despite decreasing atherosclerosis. It is also interesting that the frequency of carcinomas is positively correlated to the small dog *IGF1* haplotype, whereas the frequency of hemangiosarcomas is inversely correlated with this haplotype. MacEwen et al. (2004) suggest the *IGF1* receptor expression could regulate growth of different neoplasms, whereby those that express the receptor will respond to increased levels of *IGF1* (inverse correlation with PWD small dog *IGF1* haplotype). Previous studies of different rodent species concluded that distinct tumor suppressing mechanisms evolved in large, long-lived species as well as in small but long-lived species (Seluanov et al. 2008). In purebred dogs, such evolution is confounded by selection during breeding, which combines genes from different, more ancient, breeds without allowing subsequent counter-selection that would offset ill effects in later life.

In this paper, we have presented a first report on the distribution of phenotypes that were encountered. Several of these appear to be heritable. However, the number of dogs autopsied will need to be greatly increased before identification of genetic loci will be possible. Nevertheless, evidence of heritability implies that such identification eventually will be possible. When that occurs, we may be able to identify loci associated with senescence, e.g., for the reduction in muscle mass that accompanies aging as well as for the changes in bone (osteoporosis) or thyroid (atrophy) that take place. Most importantly, we should be able to clarify the role that *IGF1* plays in determining disease frequency and longevity in a large mammal.

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