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The changing understanding of ageing

Part 3: Diseases of ageing

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Summary

This third and final paper in this series considers ageing mechanisms across species, with emphasis on conserved metabolic pathways that relate to disease. The growth hormone (GH)-insulin-like growth factor (IGF-1)-insulin axis continues as an example of how critical pathways might relate to longevity and senescence. Aligning theory, research outcomes and clinical investigations at the levels of the cell, organism and population, is suggested as a means by which to consider the many complexities of the ageing process in an orderly fashion. A contentious debate revolves around whether ageing is purely a combined effect of stochastic events on residual programming relating to reproductive robustness, or whether ageing itself is programmed by natural selection. Emerging data indicate that the influence of genetic programming on specific late-life diseases, and even individual tissue pathologies, will probably need to be reconsidered in the light of newer theoretical possibilities. In particular, the evidence that late life and its diseases are objects of considerable investment of energy challenges theory that couples longevity with reproduction. Furthermore, the author suggests that ageing may have evolved at least partly as a means of niche preservation for contemporaries and for progeny.

Keywords

Ageing, Chaperone, Disease, Germ-soma conflict, IGF-1, Insulin-like growth factor, Natural selection, Portuguese water dog, Telomere.

L'evoluzione del concetto di invecchiamento
Parte 3: Malattie relative all'invecchiamento

Riassunto

Questo terzo contributo prende in considerazione i meccanismi dell'invecchiamento in alcune specie, soffermandosi sulle vie metaboliche conservate e correlate al processo patologico. Riprende l'esempio dell'asse GH-IGF-1-insulina per dimostrare come vie di importanza critica potrebbero essere correlate a longevità e invecchiamento. Comparando teorie, risultati di ricerche e indagini cliniche a livello di cellula, organismo e popolazione è possibile ponderare con sistematicità la complessità del processo di invecchiamento. Resta da chiarire, in quanto aspetto controverso e dibattuto, se l'invecchiamento sia il risultato dell'effetto combinato di eventi stocastici sulla programmazione relativa alla capacità riproduttiva o se l'invecchiamento stesso sia programmato dalla selezione naturale. Alla luce dei dati emergenti e delle più recenti ipotesi sarà probabilmente necessario riconsiderare l'influsso della programmazione genetica sulle malattie specifiche in età avanzata e, addirittura, sulle singole patologie tissutali. In particolare, le evidenze che l'età avanzata e le malattie ad essa correlata siano oggetto di un notevole investimento di energie da parte della selezione naturale confuta la teoria che associa longevità e riproduzione. L'autore avanza l'ipotesi che l'invecchiamento possa essere, almeno in parte, una sorta di modalità di preservazione di nicchia per i contemporanei e la loro progenie.

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Parole chiave
Cane d'acqua portoghese, Cellule germinali e somatiche, Chaperone molecolare, IGF-1, Malattia, Selezione naturale, Telomero.

Introduction

This is the third of three papers that examine a changing understanding of ageing. These discussions continue to approach why post-reproductive life span is common among animals, stochastic versus programmed ageing, and the disease-related implications. A key message of this series is the importance of aligning ageing research observations at the levels of the cell, the organism and the population. These three papers are written for progressive community clinicians and for academicians, and are not intended to be comprehensive reviews. The choices for emphasis reflect my career experience in population biology, reproduction and ageing.

In this third manuscript, I discuss conserved physiological pathways and mechanisms that relate to diseases of ageing. Parts of this discussion necessarily involve other life phases, from which many age-related disorders originate. Additional relevant post-mortem data are described from an ongoing investigation of canine genetics and diseases of late life. The growth hormone (GH)-insulin-like growth factor (IGF-1, IGF-2)-insulin pathway is continued as an example of underlying bio-chemistry to place the discussion in a setting that offers continuity among these three papers. However, biological processes that influence ageing are many and varied, as are theoretical and practical views of ageing. Herein is just one view.

Practical thinking about theory

Evolutionary theory closely links reproduction with resources and reproduction with longevity. Historically, a primary population role for ageing has been dismissed. Based solely on historical thinking, semelparity would probably be a maximally energy-efficient 'reproductive lifestyle'. This idea would also demand that consumption of resources, and population density prior to reproduction and death, do not damage the local environment. Interestingly, semelparity has not emerged as an important pathway for natural selection in complex species and post-reproductive life span is common, even among 'simple' species. This conundrum partly explains the emergence of new theories of ageing (28, 30).

Mammalian species that do reproduce only once prior to death, such as the shrew-like marsupial genus *Antechinus*, occupy ecological niches characterised by extreme resource seasonality. Among their evident adaptations are strict alignment of reproductive season with more predictably available resources in the environment and seasonal suppression of appetite mediated by the hypothalamic-pituitary-adrenal axis (15). One lesson of *Antechinus* in a resource-extreme ecology points to stress response physiology as a potential driver of reproductive adaptation. Are stress responses similarly involved in ageing, beyond the idea of being residuals created by other activities of natural selection? A first approach might be to evaluate whether recognisable ageing patterns appear across phylogenetic boundaries.

Regulation of ageing mechanisms

Regulation of entire life span is implied, although not independently proven, by similar organism-level observations across phylogeny. For example, Kenyon has reviewed studies of the nematode *Caenorhabditis elegans*, *Drosophila* fruit fly species, and yeast, pointing out that life-extending mutations occurring across these species support a fundamental genetic capacity for regulating the post-reproductive period (21). The fact that insulin-IGF-1 species-appropriate pathways are involved in the longevity of all three species supports the idea of programmed cellular ageing responses (21). By inference, the possibility of evolutionary selection for these traits (and thus possibly others) must also be considered.

On the other hand, Olshansky has argued that senescence properly should be viewed as a secondary outcome of robust fixed traits that

support early reproduction (32). Furthermore, on an equivalent timeline, intrinsic mortality patterns reveal a statistically identical alignment of survival curves across humans, dogs and mice (9). The latter observations suggest that longevity-related programming exists at species and population levels, but do not define whether an independent purpose exists. If ageing-related traits are only secondary residuals of natural selection, then viewing ageing-related diseases as residuals of reproduction-related programming would clearly be possible.

At first consideration, the ideas of purposeful selection and biological residuals each seem reasonable, but mutually opposed. The ideas, simply stated, also seem to be amenable to new and clarifying research. However, the matter of new studies is complicated by newer research technologies and human activities pose practical problems for researchers who are attempting to resolve conflicting theoretical perspectives. Selective breeding of domestic animals as companions, work partners, and food producers, are examples of genetically manipulated outcomes (by humans, be they successful or not). Civilization-related diseases couple with advances in sheltering and nutrition-health on the positive side, and with environmental toxicities and other effects of crowding and industrialisation on the negative side (44). Some of these types of effects can also be evident in laboratory animal populations.

While the above-described influences are usually viewed from the perspective of the adult organism (smoking, industrial toxicity, alcoholism, diet, encroachment), foetal effects often direct post-natal susceptibilities. One of the best known examples of the latter is small foetal size that predisposes to diabetes mellitus, hypertension and heart disease in a nutritionally replete postnatal environment (3).

Thus, classifying culture-related diseases as fundamentally intrinsic or extrinsic is likely to be progressively more challenging, as seemingly straightforward ideas become complicated in layers across the cell, organism and population. As a result, new studies of ageing will need to be designed with progressively greater care and some types of studies may be interpreted within a progressively more narrow scope. Finally, the relationship of laboratory experiments to natural biology of the same species will be an ongoing concern with experimental design.

With respect to selection or non-selection specifically for ageing, a (presently) viable alternative hypothesis in my view might be that purposeful programming and residual effects of reproduction both could be at least partly correct as ageing theories. For example, extended occupation of an ecological or micro-ecological niche might promote natural selection for long-term survival characteristics to preserve that niche for one's own relatives and future progeny. At the population level, some cohort of the surviving group would need to be very robust in terms of reproduction and some cohort would require robustness in terms of longevity.

For the present, the core theoretical debate will remain whether post-reproductive life span is itself programmed, or is an inevitable outcome of early robustness, especially in light of the relative rarity of senescence-related disease in nature (influenced by predation as much as anything else) (32). Even more basically, it is a question of whether reproductive life span is *the* obligate endpoint of natural selection. If not, other mechanisms and outcomes may be embodied in the same or different individuals. Again by inference, multiple-cohort segregation then may relate to diseases as well. In the first paper of this series, I argued that wholly dismissing or wholly accepting any of the various ageing theories is premature at this time. The preceding discussion is one example of why I hold this view.

It is further my view that the resolution of these theoretical debates requires addressing a commonly confused influence within theoretical discussions: Post-reproductive life span, senescence, and longevity are not inter-changeable. Post-reproductive life span is not unusual in nature. Lengthy and healthy post-productive life span can be observed readily, as in *Homo sapiens*, even given the shorter-term confusion caused by various new forms of sheltering over the last few hundred years.

Longevity in laboratory studies can be influenced by choice of subjects and experimental environments (it is more difficult to extend life span in a long-lived species than a short-lived species) (32), but extrinsic influences on life span can be quite powerful in field populations. In addition, definitions of maximum longevity can vary, the most common examples being the survival of the last individual or survival of the last 10% of the population.

Finally, fully understanding senescence requires a greater depth of effort to align late-life diseases with theoretical considerations. Diseases of senescence often are quite complex within the individual *and* across the population. There is an irreducible uncertainty of dealing with mutually complicating influences of multiple diseases occurring simultaneously. The latter are not obscure happenstance: Any clinician who has dealt with concurrent complications of cardiac and renal failure in the same patient (or among the same types of patient) readily understands these difficulties.

Considering the preceding discussion, it seems reasonable to conclude that regulatory capacity for ageing mechanisms does exist, with recognisable similarities across species. Is this conclusion sufficient to also conclude purposeful selection, compared to biological residuum? In order for these discussions to aid clinicians and clinical scientists, some beginning must be made to translate research findings to clinically oriented environments.

Translating in clinical terms: comparing ageing research and clinical diseases

If the theoretical debate on regulation of reproduction, senescence and longevity, has an impact on the clinically oriented understanding of ageing-related diseases, why and how is this so? Answering this question requires exactly what has become more difficult, in particular:

- accurate segregation of extrinsic and intrinsic causes of death, and
- designing laboratory experiments and population studies with great caution; experimental approaches can be neither excessively reductionist or environmentally misconceived.

It seems to me that investigating ageing diseases with all life levels in mind is more likely to yield the necessary genetic, physiological, and population alignment, both conceptually and actually. Recent illustrative examples follow.

Considering ageing-related diseases at multiple biological levels

Example: Telomeres and the cell

A report by Sahin and colleagues provides an excellent example of evaluating data at multiple biological levels (40). The authors begin with the well-recognised concept that telomere shortening (and therefore dysfunctional protection of chromosomes) is associated with earlier onset of cellular replicative senescence. They then studied specifically altered mouse models and observed that telomere dysfunction:

- represses peroxisome proliferator-activated receptor gamma co-activators 1α and 1β (PGC-1α and PGC-1β) and mitochondrial biogenesis, resulting in fewer mitochondria
- reduces DNA and energy production in mitochondria
- induces organ pathologies compatible (in the model) with decreased mitochondrial energetics, including cardiomyopathy and reduced gluconeogenesis
- induces P53-mediated repression of co-activators PGC-1α and PGC-1β.

At the 4th generation, mice that were genetically telomerase-deficient had total body mass declines associated with declining fat mass *but preserved lean mass*, with no change in activity or food intake. Exercise endurance, blood triglyceride and blood cholesterol were lower in affected 4th generation mice. Circulating free fatty acids were increased. These observations are consistent with disrupted energy production and metabolism at cell and systemic levels (40). This report underscores the fact that cellular ageing mechanisms can extend into interwoven messaging networks. In this example, continued maintenance of specific genetic

dysfunctions predisposed to clinically evident heart disease and altered energy metabolism. In effect, the authors directly connected loss of telomere protection at the cellular level to disease states at the multi-organ level and the whole organism level.

Example: Chaperones and the cell

Chaperone proteins represent another example of intracellular protection systems that change with ageing. Chaperones are highly conserved proteins that function to prevent aggregations of misfolded proteins, and to assist in their degradation (18). Stable proteins, especially intracellular enzymes, are normally subjected over time to post-translational modifications (18). Some of these modifications alter their conformation and function and may cause them to aggregate to the degree that cellular functions are compromised and cell life span is shortened. The chaperone proteins function to associate with dysfunctional proteins and prevent aggregation until they can be degraded by normal intracellular processes (44). Chaperone molecular functions thus support the idea of late-life disease-related genetic programming, as it involves cellular capacity for stress response.

Chaperone functions also have a general similarity to intracellular oxygen radical scavenging. Chaperone molecules appear to serve a control and removal function for altered proteins, with outcomes that generally parallel control and removal of oxygen radicals by the cell's complex antioxidant system (24, paper 2 of this series).

Example: Reflecting the cell in the organism and population

The observation of preserved lean mass in the telomere-related report by Sahin and colleagues (40) is thought-provoking in another way. Other studies have revealed that lean mass decline (muscle cell loss) in advanced life relates variably to death trajectories. Previously (24, paper 2 of this series), I noted two studies wherein male Sprague-Dawley rats maintained stable lean body mass into late life (25, 26), and a third study wherein Fischer 344 (F344) rats had stable lean mass until after the onset of terminal ageing diseases (47). Similarly, mass loss and disease can be shown to be mutually independent (2, 36, 49). Ageing-related changes, whether they are pathological or not, reflect underlying biological plasticity that can be directed quite variably by cellular biochemistry (19, 20). The effects themselves are often circumstantial and species- or strain-related, especially in research studies.

Here, I suggest that pre-disease body composition phenotypes also probably involve stress responses at the organism level (see below) (24, paper 2 of this series). Genetic programming that supports either cell protection or cell loss is present across many species. For example, as evolutionary outcomes, these response programmes could have multiple possible origins, including those described by Kenyon and Soti and Csermely (21, 44):

- convergent from different beginnings
- pre-existing mutations that are exposed to cell processes by uncorrected protein misfolding
- genes that formerly served one purpose that are recruited to new purposes, even if they had been silenced as 'junk DNA'.

Does finding various possible routes of programming origin affect the decision relative to selected versus stochastic origin of ageing? Multiple genetic pathways to similar endpoints are well-known facets of operation for natural selection. Thus, the observed genetic variability most likely has little impact on the basic argument. However, it seems to me that emerging research evidence begins to suggest that some cross-species observations may fit classical 'reproduction versus longevity trade-offs' less well than they might fit the idea that trade-offs need not be obligatory.

How do the foregoing discussions relate to ageing and diseases of ageing? Over these three papers, I have used a number of examples to illustrate the complex, inter-woven, and sometimes 'Janus-faced' nature of ageing-related changes. These have included apoptosis, oxygen radical scavenging, telomere biology, chaperones and body composition. Ageing-related changes in these and other cell

protection or removal mechanisms are recognised widely in nature and have been described extensively in the literature. What I find remarkable is that so much energy-requiring complexity might exist to begin with, apparently having the essential purpose of either preserving the individual cell (organism) or removing it quickly.

Consequently, one may take the view that these mechanisms represent a significant continuing energy investment in late life, brought about by natural selection. One then may take the corollary view that repair or removal strategies may be considered as cellular responses to cellular 'illness' (1). The latter again argues for purposeful selection of ageing that aligns the cell with the organism and population. Once again, perhaps the 'purpose' involves something like niche preservation in somewhat the same way as complex organisms might be programmed in cohorts for reproductive versus ageing robustness.

Would 'purpose' also enable selection of cohorts within populations to serve different species-preserving functions in macro-ecologies? Certainly this occurs in insect populations. If so, could this biological scenario reflect in species-related late-life diseases at the organism level? Post-mortem body metrics and histology (24, paper 2 of this series) could reflect genetic programming and abnormalities that result from underlying regulation and dysregulation of the gene and cell. Is stress-response physiology an (or *the*) important underlying factor that supports translation of cellular changes to diseases of ageing, considered at the population level?

Modest stress and diseases

Across wide evolutionary distances and across phylogeny, the modest stress of diet restriction has extended longevity. In addition, the *onset of species- and strain-specific late-life diseases are postponed or prevented* (29). Diet restriction studies in rodents and primates have revealed a delay in or prevention of late-life diseases that include renal disease, cardiomyopathy, hypertension, gastric ulcer, osteodystrophy, autoimmunity, cataract, mammary neoplasia, lymphoproliferative malignancy, pulmonary malignancy, leukaemias, lymphomas, pituitary adenoma, pancreatic adenoma, islet cell carcinoma and adenoma, fibrosis of islet cells, testicular neoplasia, obesity and diabetes mellitus (6, 17, 22, 29). These observations strongly reinforce the idea that genetic programming influences late-life diseases. The effects that can be noted again raise the hormesis idea that mild chronic stress of extrinsic origin can extend average longevity (28).

The fact that so many body systems can be involved in diet restriction responses further implies that the operating mechanisms may be conserved across species and are most likely quite fundamental in their biological nature. Therefore, the clear associations among diet restriction, longevity, diseases and highly conserved stress-response pathways are collective evidence that cannot be dismissed. How does the 'Age of genomics' contribute to exploring and understanding ageing and diseases in fundamentally new ways?

Age of genomics and ageing-related diseases

A first step in bringing newer genetically based ageing disease research to clinical environments actually involves clinicians directly: phenotypes (traits *and* diseases) must be established carefully and with great accuracy, and DNA for analysis must be collected properly from affected subjects and carefully selected controls (41). Within a genetically well-defined population, heritability may then be calculated, accompanied by linkage analysis based on how the phenotype (trait) segregates through a related population of known ancestry. Comparison with the known chromosomal positions of many micro-satellite markers within a described genome then facilitates identifying suspected locations of governing genes (34, 35, 41).

Additionally, single nucleotide polymorphisms (SNPs) chips are now available and are useful for linkage analyses and genome-wide association scans (GWAS), comparing populations of affected and unaffected individuals. The dog is an attractive model for

such studies because fewer genetic markers are required (27, 41, 45), and many dog breeds may be assigned to genetic clusters to facilitate analyses (37, 38, 41). For example, Shearin and Ostrander (41) have summarised data involving several canine neoplasms that have been analysed by these techniques, including the following:

- transitional cell carcinoma; renal cystadeno-carcinoma and dermatofibrosis (German Shepherd dog)
- malignant histiocytosis (Bernese Mountain dog)
- appendicular sarcoma; and chronic myelogenous leukaemia.

Thus, the genetics- and disease-related value of these methodologies is established.

Even initially, from a veterinary perspective, these genetic observations have a direct impact on breeding programmes. Clinicians become alerted to the levels of complexity that can be involved in breeding to avoid some quantitatively inherited diseases. Regional and distant (i.e. by insemination) breeding may differentially emphasise popular sires or particular family lines. Existing clinical marker phenotypes may be inadequate for various reasons, including time of disease development, variable disease phenotype expression and contributing epigenetic events, or poor choice of phenotypic markers. Equally important for veterinary clinicians is the fact that many of these research models can be reciprocal, with species serving as models for one another, as noted for animal body composition phenotypes (24, paper 2 of this series).

Continued elaboration of relevant genetic data will enable more specific identification of genetic components of anatomic variation and diseases, better recognition of 'high-risk matings', improved elimination efforts and contributions to therapy. Partly to these ends, an intensive research project is presently in progress, with the goal of understanding the genetics of the canine body plan and the relationships to disease.

Genetics of body plans and of diseases: Portuguese water dog model

Skeletal metrics

An interesting application of the foregoing ideas is an ongoing investigation of the canine body plan and its relationship to diseases that is being conducted at the University of Utah. As a part of these studies, skeletal radiographs (skull, pelvis, forelimb, hind limb) were made and supplied by community clinicians who also collected samples for DNA harvest, initially from 330 Portuguese water dogs (PWD) in North America (10).

One hundred metric skeletal traits were measured carefully from the radiographs, and the data were evaluated for association with approximately 500 established DNA markers. The principal component (PC) analysis that followed revealed that ten skeletal trait groupings (principal components) accounted for 75% of the within-breed variation, independent of factors such as age and gender. Among the groupings of skeletal traits, the first trait group (PC1) accounted for 44% of the population total variation and 10% of the heritable variation. PC1 heritability was 0.23 and was highly significant ($p = 5.9 \times 10^{-5}$). The contributions to PC1 (called 'loadings') related to skeletal size. *Quantitative trait loci (QTLs) regulating PC1 included FH2295 on chromosome 15, which links closely with a gene coding for IGF-1*. In fact, 91 of the 100 measured traits were significantly associated with FH2295 (10).

These observations are reminders that the same locus (FH2295) explains approximately half of the size variation among dog breeds (46). Quoting directly from Sutter *et al.*, 'A single *IGF-1* single-nucleotide polymorphism haplotype is common to all small breeds and nearly absent from giant breeds, suggesting that the same causal sequence variant is a major contributor to body size in all small dogs' (46). The studies conducted by Sutter and colleagues included first a group of 463 PWDs, followed by 526 dogs of 43 different breeds, ruling out the possibility that the IGF-1-size observation is breed-

specific. Of considerable and unexplained interest is the fact that larger dogs live less long on average, whilst across Mammalia, larger species tend to live much longer. This observation implies that other factors are also operative. Defining these related genetic variables will represent a major biomedical advance.

Skeletal diseases

If skeletal metrics can be genetically traced in the PWD population, what about skeletal diseases? Ventrodorsal, legs-extended hip radiographs that were made by community clinicians and submitted to the University of Utah study group (Project Georgie) were evaluated. Initially, a group of 286 radiographs were scored for subluxation of coxofemoral joints using the Norberg angle metric (11). Observations from these data included a significant non-heritable asymmetry (more laxity in the left hip) that has been documented in humans as well (43). Norberg Angles indicating laxity (33) were highly heritable for mean ($h^2 = 0.73$), left ($h^2 = 0.46$) and right ($h2 = 0.46$) PWD hips. Given the low mean consanguinity 0.2 (0.0-0.6) among the dogs, it is clear that these observations did not result from inbreeding depression (12). Moreover, two genes on the first chromosome were involved principally but independently, with one explaining 14% of the heritable variation in the left hip and the other explaining 16% of the variation in the right hip. The latter observation also indicates that other genetic factors, having smaller but collective effects, are involved in hip laxity, probably along with epigenetic effects that must include the controversial impact of the legs-extended radiographic phenotype.

Additionally, examining a larger group of 431 ventrodorsal, legs-extended hip radiographs, coxofemoral osteoarthritis (OA) was estimated in a continuation of skeletal disease observations of the PWD model (12). Each hip was scored using an ordinal scale of 0 to 3, covering normal to severe OA, at four joint sites, namely: osteophytes at cranial and caudal acetabular margins, subchondral sclerosis at the cranial acetabular margin and femoral head osteophytes. OA-affected dogs constituted 50% of the study population, with OA heritability estimated at 0.30 (30%). While laxity was greater for the left hip, OA did not reflect right-left differences. The identified QTL (FH2320 on the third chromosome) accounts for about 16% of the total variation, characterised primarily by acetabular osteophytes.

Coxofemoral joint OA correlated to skeletal metrics of the pelvis and limb bones through the FH2320 QTL that accounted for 16% of OA variation, demonstrating relationships between bone metrics and disease. Note, however, that these associations are not defined as a linear cause-effect relationship. Given that 16% of the OA variation in the study population is accounted for by one QTL, it is important to realise that other and perhaps numerous individual genes each will contribute less than 16% to variation. Thus, it is much more likely that the aforementioned networks of genes are actively and collectively involved, and the authors hypothesised that this particular QTL may include a haplotype that influences abnormal growth in joints (12). It may be noteworthy that other studies of PWDs and genetics of disease revealed QTLs associated with autoimmune Addison's disease. Surprisingly, one of these QTLs was also associated with OA; the biochemical and biological nature of that relationship requires further study (12).

In more recent studies, Chase and colleagues evaluated dogs from 147 breeds, using diagnosis data obtained from the Veterinary Medical Data Program (13). Significant ($p<0.01$) negative correlation to the small haplotype at the IGF-1 locus was found for hip dysplasia, while significant positive correlations were found for patellar luxation and pancreatitis, indicating that the small size haplotype was less common among dogs with hip dysplasia and more common among dogs with patellar luxation and pancreatitis. Veterinary clinicians will readily recognise that these findings align with size-related frequencies of these diseases in the clinical arena and also imply that non-skeletal diseases may be examined in the same way (13).

Physiological traits and diseases of ageing

Example: Growth hormone-insulin-like growth factor 1-insulin axis

Since IGF-1 is associated with growth, skeletal development, inflammation (stress response) and insulin metabolism (stress-response), the heritable relationships that were observed initially from PWD data created interest in the heritability of post-mortem pathologies (14). Several heritable pathological traits were found (Table I), along with several traits that correlated to the small IGF-1 haplotype (Table II). Remaining mindful of size duality in the PWD breed (independent of gender), the fact that the significant correlations were negative (one exception) indicates less of those traits were associated with the small IGF-1 haplotype.

Table I
Heritabilility of post-mortem traits at death in 145 Portuguese water dogs

Trait	Heritability*	*p*-value
Body weight	0.59	0.001
Liver fibrosis	0.62	0.001
Thyroid atrophy	~1	0.003
Pancreas tail hyperplasia	~1	0.003
Pancreas tail fibrosis	0.91	0.006
Salivary gland lymphocytosis	0.84	0.011
Age at death	0.65	0.022
Sarcoma (all tissues)	0.47	0.069
Spleen lymphoid hyperplasia	0.97	0.069

* heritabilites are preliminary and are expected to change with increasing size of the data set; they are included here only to document that relationships were observed

The growth hormone-insulin-like growth factor 1-insulin-energy pathway and 'diseases'

The GH-IGF-1-insulin pathway is among the many physiological responses to diet restriction across species. Increased body fat correlates to increased insulin resistance, which signals defective glucose regulation (4, 5, 7). In just one example relating to longevity and disease specifically, increased insulin resistance in primates resulted in hyperinsulinaemia and 3.7-fold increased risk for earlier death (6). Sinclair has suggested that findings involving the GH-IGF-1-insulin pathway might signal that conserved stress-response genes are important in the diet restriction-longevity outcome (42). The latter would, of course, include disease phenotypes.

Table II
Tissue correlations to canine insulin-like growth factor 1 small haplotype

Tissue	Correlation	*p*-value
Adrenal weight	–0.28	0.01
Small bowel length	–0.27	0.01
Brain oedema	-0.27	0.01
Tail length	–0.26	0.01
Brain weight	–0.24	0.02
Trachea length	–0.23	0.03
Oesophagus length	–0.22	0.03
Right kidney weight	–0.22	0.03
Tongue weight	–0.22	0.03
Atherosclerosis (all tissues)	–0.20	0.03
Infarction (all tissues)	0.21	0.04
Triceps weight	–0.20	0.05
Kidney congestion	–0.20	0.05

If disease modulation in diet restriction is related to stress-response capacity, the next logical question is how this might occur. Specifically stated with respect to the example pathway: how can a single gene or family of genes that basically govern growth regulation become involved so widely in physiological processes? Not surprisingly, the answer probably lies in the innate plasticity of the genome and its response capacity (3). Kenyon has examined the family of genes that includes GH, IGF-1, IGF-2, insulin (INS), along with various receptors and related signalling pathways that affect energy metabolism, immune regulation, inflammation and oxidative stress. It is evident that evolutionary conservation of species-appropriate versions of this gene family and function is found from yeast to humans, influencing longevity in all of them (21).

A study of the GH-IGF-1-insulin homologue pathway in the microscopic nematode

Caenorhabditis elegans revealed that strong inhibition of the insulin-like receptor *daf-2* in the juvenile form results in the quiescent state (dauer state) that survives but does not grow, mature or reproduce (1). By contrast, partial mutation-caused inhibition of *daf-2* function resulted in extended adult life span. Further increased life span resulted when the weaker mutation was augmented with *daf-2* RNA interference (RNAi). Finally, when gonad ablation was added as well, a 6× increase in mean life span was observed. The location and nature of a mutation can also have a pronounced effect. RNAi + gonadal ablation of worms with the described mutation resulted in greatly increased longevity but relative inactivity. However, the same treatment given to another *daf-2*-weakening mutant resulted in very long life, along with greater activity (1).

From a disease perspective, if the inactive 'treated' mutant could be interpreted loosely as an 'ill' individual, then it is possible to envisage that these species- and mutation-specific pathways might represent a primitive version of a health concept (1), as I suggested previously for cellular repair or removal mechanisms. How does this scenario translate to species and diseases of ageing that are more familiar to clinicians in human and veterinary medicine?

Rodriguez and colleagues have presented a list of candidate diseases and disease groups for IGF-1 associations (39). These include growth disorders, metabolic syndromes, diabetes mellitus, cardiovascular disease, central nervous disturbances and neoplasia (also recognising these associations with the ageing process and with longevity) (39). One implication is that similar and complex stress-associated responses may associate with both orderly and disorderly late-life events across species. Implied also is the idea that other genes and gene families are probably involved in diseases in similar ways, along with environmental factors that help to bring about context-specific outcomes in a fashion not greatly dissimilar from those often seen in laboratory settings.

The theoretical aspect of these observations suggests that the concept of pleiotropy needs to be redefined, especially as new studies appear across species. Described historically as disparate early- and late-life effects of naturally selected genes, antagonistic pleiotropy may actually represent plasticity that allows different haplotypes of a given gene to govern expression of traits within more complicated gene networks. Furthermore, the clear involvement of the micro- and macro-environment in disease phenotypes (supported by histology and moderate heritabilities) (14) again indicates diverse and non-linear gene-disease relationships.

Another confusing thought about pleiotropy is that energy restriction and delay of late-life diseases suggest that a reproduction-longevity trade-off is not necessarily obligatory. However, obligatory trade-off has been implicit in both mutation accumulation and pleiotropic theory. What other aspects of reproduction might be involved in species- and strain-specific diseases of ageing? To continue this line of thinking, examples in the embryo could be considered next.

Reproductive patterns and ageing-related diseases

Early embryonic aspects of development that may establish a scope for later-life disease traits have been reviewed by Monk (31). Describing the changing patterns of DNA methylation from the 8-cell mouse (model) embryo to the blastocyst, Monk pointed out some mechanisms by which differential expression of maternal and paternal genes is accomplished in germ line and soma cells. DNA expression can be silenced or regulated by:

- changing its conformation or chromatin structure
- cytosine methylation
- selective transcription of DNA strands
- nuclear compartment modification.

Moreover, regulation may involve specific genes, or include large blocks of genes. These regulatory mechanisms have been termed 'epigenetic modifications' for many years (48), although recently new interest has emerged with respect to how epigenetic modifications to DNA may be inherited (31) and contribute

to later-life diseases. Many 'modern' diseases may reflect epigenetic influences on these aspects of gene expression.

Diseases of modern civilisations

Many diseases are influenced by the development of cultures among humans and subsequent rapid evolution of surrounding species can occur as a result of intervention by humans. Stress-response pathways appear to have evolved biological plasticity that facilitates efficient adaptive responses to such changes, while these capacities are clearly much older than modern cultural stress generators. Continuing with the example of the GH-IGF-1-insulin pathway, Camidge and colleagues point out that the IGF-1 axis has endocrine, paracrine and autocrine functions, and therefore can function in circumstances that may be regarded as 'positive' or 'negative' (8). Centrally, the axis is involved with normal development and growth (and size in the dog) (46). While core signalling is driven through IGF-1 receptors in complexes with insulin receptors, a large number of upstream and downstream factors are influential, including insulin substrates and stimulatory ligands. A modern example is recognised in the relationship among diet, obesity, diabetes mellitus and metabolic syndrome (8).

Another important clinical implication of these observations is that the IGF-1 axis can be involved in maintaining non-neoplastic and neoplastic diseases of various types. From the pharmacological perspective, anti-IGF-1 axis therapies are being examined and are especially directed at influencing IGF-1 receptors (8). However, the most important idea, once again, is the underscoring of physiological capacity for plasticity that is conserved across phylogeny.

Genetic plasticity and disease

Heininger has produced an exhaustive review of the physiology of cellular stress responses, with an emphasis on ageing (19). From these writings, one possible view of ageing-related diseases emphasises plasticity and efficiency among the array of stress responses available to cells. A partial list of these response capacities includes naturally selected phenomena, such as sporulation, sexual reproduction (separating germ and soma cell lines), dauer formation (in simple life forms), apoptosis induction, stress resistance (tolerance), mutagenic adaptation, DNA repair, differentiation – dedifferentiation (coupled with apoptosis) and many signalling pathways (19, 20).

Theoretically, natural selection favours traits that are part of successful cell programming in a way that brings about both diversification and uniformity within specific contexts (19, 20). Each of these capacities is biochemically complex and ambiguity in their functions may occur in different given situations (19, 20). This capacity for ambiguity is necessary and at least partly related to challenge outcomes, such as necrosis, mutagenesis, oncogenesis, a number of chronic diseases of ageing and sometimes death (19, 20).

In complex organisms, Heininger refers to reproductive characteristics and ageing biology generally as the outcome of a 'germ-soma' *conflict* that may give rise to the many aspects of cellular ageing (20). From the perspective of a biologist and clinician, I suggest that these observations support ageing-related genetic programming, but need to be moulded to observations at the individual and population levels. In particular, the idea of ageing as a purposefully selected phenomenon, perhaps related to niche preservation from cell to population, now seems a powerful argument that must be researched further. The argument can be strengthened when one realises the amount of energy that is invested in these activities, which is inconsistent with historically accepted theory about natural selection.

In the foregoing context, at least some ageing-related diseases of complex animals could result from a fixed germ-soma conflict and a disposable soma (19, 20). Other diseases may represent adaptations that become 'weaknesses' when unadapted individuals or populations are suddenly allowed a longer life span. Still other late-life diseases possibly reflect genetic events that bring about differential capacity of individuals in populations in much the same

way that differential robustness appears at the cellular level. The new question then becomes: 'What ageing-related intrinsic diseases might represent *'don't die yet'* early- or mid-life signals, in the sense of prolonging life to eventually help maintain an ecological niche or micro-niche?'.

Conclusions

The argument that ageing may be a selected genetic programming, rather than a by-product of robust natural selection for other traits, is a newer evolutionary controversy. While the controversy will not be resolved in the near future, available evidence suggests that to dismiss the idea would be equally unproductive as would total dismissal of other ageing theories (23, paper 1 of this series). Natural selection for ageing is supported by several bodies of information about stress-response metabolic pathways, as follows:

- the relationship of stress physiology to disease at all levels and stages of life, from cell to population
- the hormesis-like longevity response to diet restriction
- the disease-independent nature of age-related body mass loss
- heritable components of chronic pathologies and diseases of late life that may begin relatively early in life
- the evident continued investment of energy in chronic late-life diseases that can often be detected initially during reproductive and pre-senescent life stages.

These ideas support the possibility that maintenance and preservation of a population- or species-related eco-niche is a selectable and distinct evolutionary advantage.

For veterinary clinicians, whether in communities or in academia, the central ideas of these discussions are:

- new understanding of post-reproductive life span, longevity and senescence
- the nature of stochastic versus programmed influences on age-related diseases
- the disease-related implications for the future.

The 'Age of genomics' will increasingly influence the way we look at diseases of late life and, ultimately, the way that we approach them, particularly in the preventive sense.

What are the means by which to uncover new ways to understand the role of ageing at multiple life levels and across species? It has become clear that advancing knowledge and resolving questions and problems relative to ageing will not be possible without greatly increasing the level of interdisciplinary research and especially collaborations between research scientists and practising clinicians. I anticipate that the One Health-One Medicine Initiative will have a unifying effect on work in ageing and many other biomedical disciplines. The goal of the initiative to bring together broad spectra of scientists and clinicians for the purpose of resolving ongoing health issues and threats (16) is precisely what must be applied to further understanding the complex nature of ageing and elaborating specific preventions and remedies beyond the historical posture of within-discipline disease cataloging.

It is my hope that these three manuscripts will give clinicians a perspective from which to evaluate the emerging complexities in the study of ageing, remaining grounded in the knowledge that the best research will make sense at the levels of the cell, organism and population.

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