Of mice, genes and aging.

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doi:10.3324/haematol.2019.238683

Why do we get old? How much of aging is genetic? And in what genes? There is clearly a genetic basis of aging, as demonstrated from yeast to worms to humans.[1] As one example, different mouse strains have different potential lifespans. Much effort has been invested in understanding the genetic underpinnings of lifespan differences between the long-lived C57Bl/6 strain and the short-lived DBA/2 strain, with 50% mortality in captivity by 914 and 687 days, respectively.[2] Quantitative trait loci mapping in C57Bl/6 X DBA/2 (BXD) recombinant inbred strains identified a locus on chromosome 11 that is linked to lifespan, narrowing the trait conferring region to 18.6 Mb.[3] These previous studies have also shown that the fraction of mouse hematopoietic stem and progenitor cells (HSPC) that lose function in response to hydroxyurea (HU) treatment is inversely correlated with lifespan across BXD strains, including for the chromosome 11 locus. In this issue of Haematologica, Brown *et al.* explored the genetic differences within this locus that contribute to both HU sensitivity and longevity.[4]

The authors show that a relatively small region on chromosome 11 is tightly linked to HU sensitivity of HSPC, as BXD recombinant strains that possessed this region displayed the high or low sensitivity of the DBA/2 or C57Bl/6 HSPC, respectively, even when almost all other genes were from the other strain. Previous work had demonstrated that this region suffices to confer the short or long lifespan of the donor strain.[3] Notably, the authors demonstrated that HU sensitivity and longevity differences mediated by this locus did NOT coincide, surprisingly, with differences in cell cycling, telomere length, HSPC number, DNA damage responses, senescence or viability. Thus, simple explanations for HSPC sensitivity to HU, which could also account for earlier stem cell exhaustion and aging, such as increased cycling, impaired DNA damage responses or precocious senescence, do not appear to account for strain differences.

Their subsequent analyses revealed that this locus confers differential expression of the pituitary tumor-transforming gene-1 *(Pttg1)/Securin* gene, with substantially higher expression conferred by the DBA/2 locus. Interestingly, the yeast homolog of *Pttg1* is *Pds1p*, shown to regulate the intra-S-phase checkpoint and responses to HU in yeast,[5] which is consistent with PTTG1 regulation of HU sensitivity. Also, intriguingly, PTTG1 is an inhibitor of separase, the cysteine protease that opens cohesin rings during the metaphase to anaphase transition, suggesting a role for PTTG1 in the cell cycle. Still, while PTTG1 overexpression has been shown to lower progression through S phase and increase senescence and DNA damage in human fibroblasts,[6] Brown *et al.* demonstrate that these phenotypes are not observed to differ for HSPC from the congenic strains.[4] Notably, genetic variation in *Pttg1* (together with other mitotic checkpoint genes) is associated with chromosomal aberrations in healthy humans.[7] Given that inherited defects in genome stability often result in premature aging,[8] PTTG1 level-dependent impacts on chromosomal segregation during mitosis could influence longevity.

They further demonstrate that the DBA/2 locus displays an apparent duplication that results in a longer promoter for the *Pttg1* gene, and this longer promoter confers greater transcriptional activity in reporter assays. Much of evolutionary change is associated with alterations of gene expression (without necessarily changing the activity of the encoded protein), involving mutations in cis-regulatory elements.[9] The evolution of lifespan may similarly involve changes in gene expression, rather than the activity of the gene products. Finally, they showed that ectopic PTTG1 expression in C57Bl/6 HSPC to levels approximating those in DBA/2 cells was sufficient to increase their susceptibility to HU, and downregulation of PTTG1 in HSPC with the DBA/2 locus resulted in a trend towards reduced sensitivity to HU. While more research is needed, variation in the *Pttg1* gene is a strong candidate as a regulator of aging.

Previous studies have shown that CpG DNA methylation profiles across tissues for selected genes can be used as an aging clock, able to predict chronological age as well as "biological age" (a measure of physiological aging, and thus the risk of aging-associated diseases and death for older ages).[10] These clocks have been extensively validated in humans, and epigenetic deviation from the age-average profile for one's chronological age has been shown to predict various hallmarks of physiological aging including immunosenescence, diseases from cancer to heart disease to Alzheimer's disease, frailty, and, grimly, time to death. Your clock-predicted biological age is determined by factors such as smoking status, diet, body mass index, exercise, and sleep. For C57Bl/6 mice, CpG sites within three genes have been shown to serve as markers of chronological aging,[11] with accelerated changes in methylation in DBA/2 mice coinciding with their reduced longevity. Here, the authors show similar accelerated aging in the congenic mice with the DBA/2 chromosome 11 locus in the C57Bl/6 background. Thus, the DBA/2 version of this locus is sufficient to promote epigenetic aging. While hypothetical, this could be more than an association - given roles for PTTG1 in chromosome cohesin, a known regulator of higher-order chromatin organization and gene expression profiles[12] important for stem cell and differentiation programs, differential expression of PTTG1 could lead to changes in these programs and thus the tissue maintenance which is critical for staying young.

Let's consider our original question - why do we get old? - at an even higher level. Natural selection only acts

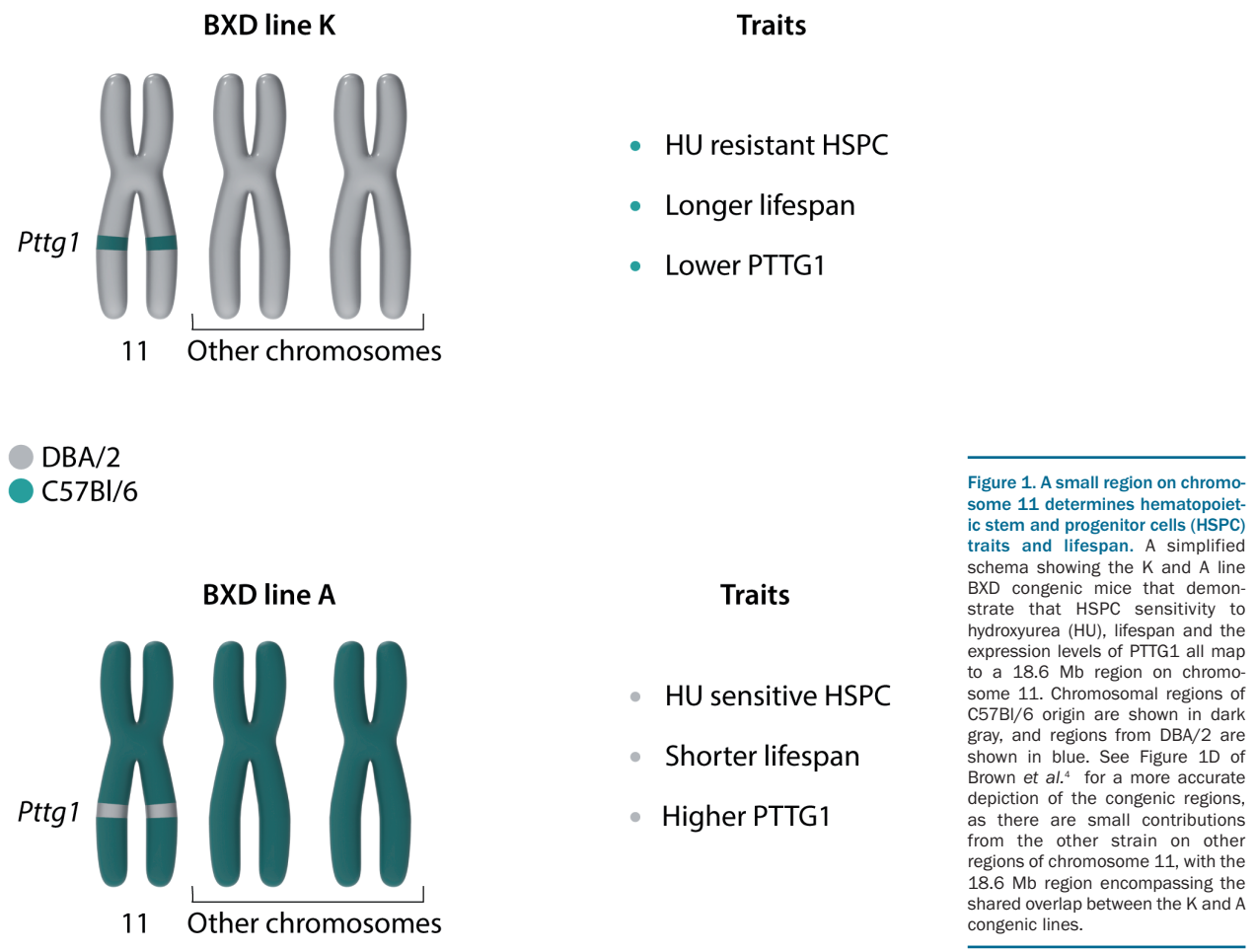

Figure 1. A small region on chromosome 11 determines hematopoietic stem and progenitor cells (HSPC) traits and lifespan. A simplified schema showing the K and A line BXD congenic mice that demonstrate that HSPC sensitivity to hydroxyurea (HU), lifespan and the expression levels of PTTG1 all map to a 18.6 Mb region on chromosome 11. Chromosomal regions of C57Bl/6 origin are shown in dark gray, and regions from DBA/2 are shown in blue. See Figure 1D of Brown *et al.*[4] for a more accurate depiction of the congenic regions, as there are small contributions from the other strain on other regions of chromosome 11, with the 18.6 Mb region encompassing the shared overlap between the K and A congenic lines.

to promote longevity to the extent that it benefits the passage of genetic material to subsequent generations.[13] Different animals have evolved different strategies for somatic maintenance that maximize reproductive success, and the extension of youth through additional investment in tissue maintenance would be disfavored if the costs (often manifested through reduced investment in reproduction) outweigh benefits. As concisely noted by George Williams,[14] "natural selection may be said to be biased in favor of youth over old age whenever a conflict of interests arises." For a small vulnerable animal like a field mouse that faces high extrinsic hazards (such as predation), natural selection has favored a "fast" life history – a breed early, breed often strategy with little investment in longevity. For larger animals like humans, elephants and whales, or for animals like tortoises, moles, bats and birds that have evolved other strategies to greatly reduce extrinsic hazards, natural selection has favored a "slow" life history, with greater and/or prolonged tissue maintenance leading to longer potential lifespans. While we understand how natural selection has shaped the pathways that control longevity, we know less about what these pathways actually are. Studies from model organisms have clearly demonstrated that modulation of the insulin-like growth factor-1 (IGF-1) pathway, which positively regulates the mTOR pathway and negatively regulates autophagy, can significantly impact longevity.[1,15] Decreases in IGF-1 and mTOR, or increases in autophagy, have been shown to prolong lifespans in organisms ranging from yeast to mammals. Additional studies have shown how inflammation can contribute to aging-associated phenotypes, and polymorphisms in genes controlling the IGF-1 pathway and inflammation are enriched in human centenarians,[16] but the extent to which these polymorphisms and their impact on inflammation are contributing to differences in longevity has not been established.

While genetic screens in model organisms have revealed key pathways that regulate lifespan, the mechanisms employed by natural selection in the evolution of lifespans largely remain a mystery. Although one could argue that the selective breeding to generate different mouse strains over the last couple of centuries may not qualify as "natural" selection, the studies of Brown *et al.* reveal at least one potential (and novel) mediator of lifespan control. Key questions remain: Do variations in PTTG1 expression or activity contribute to lifespan differences across species, and perhaps within a species (including variability in the human population)? Would modulation of PTTG1 expression or activity promote the extension of healthspan or lifespan? How do activities known to modulate lifespan, such as dietary restriction and exercise, influence PTTG1 activity? Are there links between known aging pathways such as *via* IGF-1 and PTTG1? Good science generates good questions, leading to new insights (and sometimes even solutions). As a sen-

ior colleague once told me after I had told him that I worked on aging – "Hurry up".

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