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Special Issue: Sex, Gender and Aging: Review in Depth

# Sex Differences in Aging: Genomic Instability

**Kathleen E. Fischer* and Nicole C. Riddle***

Department of Biology, The University of Alabama at Birmingham.

*These authors contributed equally to this work.

Address correspondence to: Nicole C. Riddle, Department of Biology, The University of Alabama at Birmingham, Birmingham, AL. E-mail: riddlenc@uab.edu

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## Abstract

Aging is characterized by decreasing physiological integration, reduced function, loss of resilience, and increased risk of death. Paradoxically, although women live longer, they suffer greater morbidity particularly late in life. These sex differences in human lifespan and healthspan are consistently observed in all countries and during every era for which reliable data exist. While these differences are ubiquitous in humans, evidence of sex differences in longevity and health for other species is more equivocal. Among fruit flies, nematodes, and mice, sex differences in lifespan vary depending on strain and treatment. In this review, we focus on sex differences in age-related alterations in DNA damage and mutation rates, telomere attrition, epigenetics, and nuclear architecture. We find that robust sex differences exist, eg, the higher incidence of DNA damage in men compared to women, but sex differences are not often conserved between species. For most mechanisms reviewed here, there are insufficient data to make a clear determination regarding the impact of sex, largely because sex differences have not been analyzed. Overall, our findings reveal an urgent need for well-designed studies that explicitly examine sex differences in molecular drivers of aging.

**Keywords:** DNA damage—Telomeres—Gender—Epigenetics—Senescence

Worldwide women live longer than men—currently and in all eras where there are reasonable demographic data. Paradoxically, women have a higher prevalence of chronic, age-related degenerative diseases than men. Sex differences in aging exist in many organisms. However, they are not universal, suggesting that how sex influences aging biology differs among taxa. Interspecific differences in the relationship between sex and aging and intraspecific sex differences in aging can provide crucial insights into the underlying mechanisms of aging.

Theories concerning the proximate causes of aging have proliferated in the last 25–30 years. Recently, two landmark papers that attempt to organize the state of aging research have been published. Lopez-Otin *et al.* (1) defined nine "hallmarks of aging", including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Similarly, Kennedy and colleagues (2) defined seven "pillars of aging": macromolecular damage, epigenetics, proteostasis, metabolism, stem cells and regeneration, adaptation to stress, and inflammation. While the "hallmarks" and "pillars" differ somewhat in their exact nature, they focus on similar themes, and both highlight the complex network of interactions between the different processes. To date, no extensive review exists that investigates sex differences in the genomic hallmarks/pillars of aging.

Several of the hallmarks/pillars of aging can be generally categorized as part of genome instability. Genomic instability in the form of DNA damage and mutations (nuclear and mitochondrial), telomere erosion, epimutations, alterations in chromosome structure and number, and changes in nuclear architecture all contribute to age-related declines in genomic function. It is generally accepted that as organisms age, genomic stability, and the efficacy of repair mechanisms decrease (3,4). In this review, we specifically address how aspects of genomic instability might contribute to sex differences in aging.

## DNA Damage and Mutations—Nuclear DNA

Sex differences in age-associated increases in mutations, mutation frequency, and chromosomal mosaicism are well-documented in humans. Less is known about whether sex differences exist in other species. Generally speaking, mosaicism and aberrant clonal expansion are more prevalent in elderly humans (5). Mosaic loss of the

Y chromosome in peripheral blood cells increases with age in men (6) and is correlated with increased risk of cancer mortality and Alzheimer's disease (6,7). X chromosome mosaicism in women also increases with age (8), as does autosomal mosaicism in both sexes (9,10). Recent studies have shown that the prevalence of age-related mosaic abnormalities is greater in men than women (9,10); however, mechanisms underlying the sex differences observed in chromosomal mosaicism in humans are unknown.

As with chromosomal abnormalities, human somatic mutation rate increases with age (3). Podolskiy and colleagues report that in cancers common to both sexes, total somatic mutation load and age-adjusted mutation load are greater in men than in women, consistent with sex differences in age-adjusted cancer incidence (11). Interestingly, this study found that somatic mutation accumulation began a decade earlier in men than in women (11), suggesting that the age at which defects in damage repair and/or altered mutation rates occur differs between men and women. Thus, available data suggest that both somatic mutation rate and mutation load are higher in men than in women.

Several mechanisms have been investigated as potential causes of sex-specific mutation accumulation patterns reported in aging humans. One possibility is that the efficacy of DNA repair mechanisms differs between males and females. Gaum and colleagues found that age-associated changes in DNA-damage response and double-strand break repair in human peripheral blood mononuclear cells (PMBCs) showed no sex differences among the 40–77-year-olds studied (12). Similarly, a meta-analysis of DNA damage studies published since 2004 detected a positive correlation between age and increasing DNA damage but no sex differences (13). Deficiencies in DNA repair underlie some of the progeroid syndromes, leading to symptoms that are similar to aging. The literature contains relatively little information, but there appears to be no consistent sex-bias in patients suffering from xerodema pigmentosum (14), Cockayne syndrome (14), or Hutchinson–Gilford progeria (15). Together, these findings suggest that the efficiency of DNA damage repair is not different between men and women.

Animal studies also show patterns of age-related increases in DNA damage (16–19). Consistent with this observation, DNA repair genes are expressed at higher levels in long-lived compared to shorter lived animals (20); however, the influence of sex on age-related changes in DNA damage and repair is less clear. For example, spontaneous DNA damage in PMBCs showed a significant effect of age but no influence of sex in Swiss Albino mice (18). In contrast, the somatic mutation rate at the *Aprt* locus in mouse kidney epithelial cells was significantly higher in female than male B6D2F1 mice (C57Bl/6 × DBA/2) (17). On the other hand, overexpression of *BubR1*, a mitotic spindle assembly checkpoint gene, decreased age-related aneuploidy and increased lifespan in both sexes, but the effect was more profound in males than females (21). Similarly, mouse models of progeroid syndromes provide mixed evidence of sex differences. For instance, in a mouse model of Werner's syndrome, females exhibit a more severe cardiac and metabolic phenotype than do males (22). Thus, in mice, some studies find sex differences and some do not, and results may depend on the tissue, the mouse strain and the source of genome instability measured.

Similar to rodent studies, sex differences in DNA damage and mutation accumulation in *Drosophila melanogaster* are complex and offer opportunities to gain mechanistic insights into the biology of aging. An investigation of the origin of intestinal stem cell (ISC) neoplasias in aging in *Drosophila* illustrates the utility of sex differences (16). Siudeja and colleagues placed a transgene reporter on the X chromosome to assess the importance of recombination-based mechanisms involved in loss of heterozygosity (LOH) and ISC neoplasias. Because recombination-based mechanisms rely on a pair of homologous chromosomes, recombination-based LOH on the X could only occur in females. LOH was greater in old than in young animals of both sexes. Crucially, females showed a much higher rate of LOH than males at both ages (young 12.7 vs 0% and old 68.5 vs 2%), suggesting that mitotic homologous recombination-based mechanisms play an important role in LOH and genetic mosaicism in aging female flies (16). Spontaneous male-specific ISC neoplasias associated with inactivation of the X-linked tumor suppressor gene Notch in wild-type flies increased with age. The frequency of neoplasia differed among strains (5–25%) and was correlated with the strain's ISC proliferation rate (16). Thus, this study demonstrates the importance of genetic background and sex differences for understanding mechanisms associated with increased genomic instability during aging.

Other *Drosophila* studies hint at similar levels of complexity for other measures of age-associated genome instability. In *Drosophila*, loss of the RecQ helicase gene, which causes Bloom syndrome in humans, increased somatic mutation rate throughout the lifespan, with higher mutation rates observed in females than in males. Interestingly, despite females' higher mutation rate, mean female lifespan does not differ significantly from male lifespan in these mutants (23). Thermal and chemical stressors are known to increase mutation rates. Garcia and colleagues showed that somatic mutation rate in *Drosophila* is positively correlated with temperature in both sexes, but females had a higher mutation rate than males in all conditions examined (24). In contrast, another study found that, while mutation rate increased with age in female and male *Drosophila*, it differed by treatment: control females had higher mutation rates than males, but mutation rates were greater in males than females after paraquat exposure (25). These results illustrate the intricate interactions of sex, genotype, and environment that impact lifespan.

Manipulation of DNA repair mechanisms results in similarly complex outcomes. Loss of TDP1 (tyrosyl-DNA phosphodiesterase 1), an enzyme involved in DNA repair, shortened lifespan in female but not male flies (26). More recently a study assessing the effects of increased expression of a variety of DNA repair genes in *Drosophila* found that lifespan was increased, decreased, or unchanged, depending on the gene in question, the sex of the animal, and where/when overexpression was initiated (27). Together, these studies illustrate that, in *Drosophila* like in humans and mice, sex influences the extent and type of genome instability that occurs during aging and has complex effects on healthspan and lifespan.

## DNA Damage and Mutations—Mitochondrial DNA

In addition to nuclear DNA, mitochondrial DNA (mtDNA) also is affected by aging. Alterations in mitochondrial function and mitochondrial-nuclear signaling occur during aging and have been linked to sex biases in aging and age-related diseases (28). Due to their role in energy production, mitochondria are at high risk of oxidative damage. Not surprisingly, accumulation of oxidative lesions is an important source of age-related mtDNA damage (29). In aged Wistar rats' brains, DNA oxidation, as measured by the ratio of 8-oxo-dG to total DNA, is higher in male tissue, while mitochondrial function is better preserved and antioxidant levels are higher in female tissue (30). Overall, oxidative damage increases and antioxidant activity decreases in aging mouse brains, but not always. For example, while glutathione peroxidase levels declined with age in

CBA mice, they were higher in 18-month-old females compared to age-matched males and catalase activity actually increased with age in females, but declined in males (31).

Although there are multiple transgenic mouse models that have been used to investigate the relationship between aging and mtDNA damage, these studies contain scant data on the presence or absence of sex differences in these mice. In fact, the majority of papers on mitochondrial function and aging we reviewed failed to indicate the sex of the mice used in the study. For instance, of the papers cited in a 2011 review (32), we found that 88% of the studies included failed to indicate the sex of animals used in the experiments. Explicit comparisons between the sexes in studies of other model organisms such as *D. melanogaster* and *C. elegans* are similarly lacking. Thus, we know far less than we might about sex differences in mtDNA damage during aging.

## Telomere Attrition

Telomeres are specialized structures that protect the ends of linear chromosomes. They shorten during aging due to the unidirectional activity of DNA polymerase, which leaves a section of DNA unreplicated on the lagging strand. Telomeres also are subject to shortening by genotoxic stress, such as oxidative damage (33). Among many eukaryotes, the enzyme telomerase maintains telomere length; but telomerase activity varies over the lifespan and between cell types, tissues, and species (34). In most human cells, telomerase is not expressed, leading to telomere shortening and altered cellular signaling that contributes to "replicative aging" and cellular senescence. However, replicative aging and age-related telomere shortening are not universal features of aging, and mechanisms for maintaining telomere integrity are diverse, even among closely related species (35).

Upregulation of telomerase activity is characteristic of most human cancer cells; however, other mechanisms (eg, alternative telomere lengthening) also are known be activated in tumors (33). Nonetheless, short telomeres and greater telomere attrition rates are associated with increased morbidity and mortality in humans (33,36) and disappearance from study populations in some, but not all, wild birds and mammals [see (36) and references therein]. In organisms, such as laboratory mice, that experience negligible telomere shortening with age, individuals engineered to be deficient in telomere repair experience reduced lifespan, genetic instability, and decreased capacity to respond to stressors (37,38). Thus, it appears that telomere attrition is associated with aging in some species, but these studies have been largely correlational.

The relationship between age, sex, and telomere length in humans is complex. A recent meta-analysis suggested that women have longer telomeres than men irrespective of cell type or age. However, there were differences associated with the techniques used to measure telomeres (real-time PCR, Flow-FISH, and Southern blotting), and only Southern blotting showed significant differences in mean telomere length between the sexes (39). A second meta-analysis of leukocyte telomere length (LTL) in humans found that women either had longer telomeres than men or no difference was reported, regardless of the telomere assay used (36). Most of these studies used mean or median telomere length, and in none of the 46 studies examined were men reported to have longer telomeres than women (36). Similarly, two recent studies measured relative telomere length using quantitative PCR and found that women had greater mean telomere length than men did at comparable ages (40,41). Interestingly, Lapham *et al.* (40) found that sex differences in median telomere length from saliva samples were significant only among individuals over the age of 50, when the linear decline in telomere length decelerates in women but not men. Contrasting results come from Berglund and colleagues, who examined longitudinal changes in LTL mean telomere length over 20 years in older (≥50 years of age) twins. They found that women had longer LTLs at baseline, but the slopes describing LTL attrition rate were similar and nearly linear for men and women, with men having a marginally lower intercept and a late-life acceleration of attrition (40). Whether these differences reflect the distinct tissues, differences in measurement techniques, differences in populations studied, or differences between longitudinal and cross-sectional samples is unclear. While there are exceptions (42), most studies find that adult women have longer telomeres than men, but the mechanisms associated with sex differences remain unclear.

Telomere attrition rates in humans are not constant, and when sex differences in telomere length first appear is unclear. Telomere attrition occurs rapidly from birth, slowing around 4 years of age, and the subsequent trajectory of telomere attrition continues to change in an age- and sex-specific fashion (43). In some studies, neonates show no sex differences, regardless of tissue used [eg, (44)]. But in others, female newborns are reported to have longer telomeres than males [eg, (45)]. Interestingly, a twin study comparing adults reported that women had longer mean LTL than men when same-sex twin pairs (mono- and dizygotic) were compared. In contrast, men and women from opposite-sex twin pairs had similar telomere lengths, a difference that the authors attributed to antenatal influences of opposite-sex twins on one another (46). Results from studies of LTL in prepubescent children are mixed, reporting mean LTL either greater in females than in males (47) or not different (48). In two studies of adolescents (ages 13–18 years old), mean LTL was greater in females than in males (49,50), suggesting that sex differences in telomere length may arise during sexual maturation. A longitudinal study of Danish twins found that women had longer LTLs at baseline and displayed decelerated LTL attrition following menopause (51). Crucially, while LTL in women declined with age, the relationship between LTL attrition and age was no longer significant if menopausal status was included as a covariate. These examples illustrate that while many studies find greater telomere length in females, this trend is not universal.

In sum, as adults men have shorter telomeres than women in most populations sampled (39). Whether the sex difference in telomere length appears shortly after conception or later in life is unclear. Similarly, whether the sex difference in telomere lengths observed in adult humans results from slower attrition rates, differential telomere length at earlier ages, sex differences in the effects of telomere length on survival, sex differences in telomere maintenance, or other factors in not clear. Additional, carefully controlled longitudinal studies on the dynamics of telomere length and attrition rates in multiple tissues using standardized methods are needed to better evaluate the mechanisms creating sex differences in human telomere attrition during aging.

Comparative studies of age-related telomere attrition in other species also reveal a variety of patterns. Barrett and Richardson (36) recently summarized the comparative data available on sex differences in telomere length. They found a strong correlation between male-biased mortality and either shorter telomeres or greater telomere attrition in males across bird and mammal taxa. However, telomere length did not differ between males and females in species where females are shorter-lived than males (36), suggesting that telomere shortening is not associated with species-specific

longevity in a simple linear fashion. These studies generally suffer from relatively small sample sizes and are largely cross-sectional. Further, the use of diverse assays, different tissues (eg, leukocytes in mammals vs erythrocytes in birds), and lack of standardized benchmarks for accuracy makes comparisons between studies difficult.

Several long-term, longitudinal studies of wild and free-ranging animals exist, which suggest that the relationship between sex, age, and telomere dynamics over the life course in these species is likely to be as complex as in humans. A study on free-ranging Soay sheep found adult LTL was positively correlated with early life survival in females but not males (52). In addition, sex differences in LTL were significant only in older animals (≥3 years), with adult males having shorter telomeres than females (53). A longitudinal study of wild European badgers (*Meles meles*) examined LTL and documented within individual age-related telomere attrition but no sex differences in mean LTL or LTL attrition rate with age (54).

In birds, where erythrocyte telomere length (ETL) is measured, the majority of species sampled have shown no sex difference (36). Nonetheless, bird telomere dynamics are complex and, as with humans, may be affected by environment and stress. For example, a longitudinal study of black-tailed gulls (*Larus crassitostris*) over 2–5 years found no correlation between ETL and age or sex. Rather, ETL attrition was correlated with reduced food availability and environmental stressors (55). In a captive zebra finch (*Taeniopygia guttata*) population, male and female mean telomere length decreased with increasing age of the animals, but did not differ between sexes (56). As these examples illustrate, the relationship between telomere length, lifespan, and sex is likely to be complex in other vertebrates.

In some organisms, there is no clear relationship between telomere length and lifespan. Age-related telomere attrition could not be detected in *Daphnia pulex* (57) or sea urchin species (*Strongylocentrotus franciscanus* and *Lytechinus variegatus*) (58). Studies in *C. elegans* examining natural variation in telomere length and experimentally manipulated telomere length detect no correlation with lifespan (59,60), and in *Drosophila*, which uses a telomerase-independent mechanism for telomere maintenance, there is a similar lack of correlation between longevity and telomere length (61). Similarly, data on sex differences in age-related telomere shortening are mixed. For example, in the ant species *Lasius niger*, the rate of telomere shortening is more rapid in short-lived males compared to longer-lived females. But, mean telomere length does not differ between the two types of females, queens and workers, despite the fact that queens live much longer than workers (up to 28 years vs 2–3 months) (62). These findings suggest that the question of how telomere shortening affects aging across species and how sex affects telomere attrition rates are complex.

These examples illustrate that, while sex differences exist, the rates of age-associated telomere attrition, the impact of sex on telomere length, and the mechanisms associated with age-related changes in telomere length vary between species. The available data do not explain the origins of or mechanisms involved in sex differences in telomere length during aging or why such differences are seen in some species and not in others. Given this diversity, it may be possible to use the many differences in life histories, breeding systems, and sex determination systems to address this question. Comparative studies across multiple species that carefully control for these parameters will be of great use in delineating the sources of sex differences in telomere biology during aging.

## Epigenetics

In addition to increased DNA damage, mutations, and telomere attrition, large-scale epigenetic changes have been associated with increased age in a number of species. The epigenetic changes seen in old compared to young animals are quite diverse and include changes in histone modifications, DNA methylation, and levels of chromatin remodeling and modifying enzymes [for recent reviews see (63) or (64)]. Heterochromatin, the silent form of chromatin required for proper centromere and telomere function and repression of transposable elements, is often lost during aging. Increased transcriptional noise associated with epigenetic changes during aging has been proposed to cause at least some of the degenerative phenotypes observed with increased age. While a variety of epigenetic changes occur with age, the relative importance of each of these changes and the impact of sex and genetic background on these changes is poorly understood.

### DNA Methylation

In mammalian systems, much of the work has focused on DNA methylation dynamics during the aging process. A variety of studies have indicated that overall, DNA methylation levels decrease with age (65), which is thought to lead to spurious loss of gene silencing and increased transcriptional noise. However, the notion that aging is associated with global decline in DNA methylation levels is not universally accepted. Much recent work in humans has focused on the idea of the epigenetic clock. Based on analysis of over 8000 DNA methylation datasets, Horvath proposed that a subset of DNA methylation sites exist that behave in a predictable way with age and can thus be used to deduce chronological and biological age (66). The "clock CpGs" include sites that gain as well as those that lose DNA methylation with age, and Horvath proposes that the DNA methylation dynamics observed with aging reflect the action of a postulated "epigenetic maintenance system" (66). Horvath and colleagues investigated the role of sex in this epigenetic clock model of aging and found increased rates of epigenetic aging in men relative to women in blood, saliva, and brain but not cerebellum (67). In contrast, a longitudinal (10 years) study of elderly twins (age at start of study 73–82 years) found no sex differences, but did find that age-related changes in methylation at CpG sites were consistent in the twins studied (68). Thus, data from epigenetic clock analyses and other studies suggest that age-related epigenetic changes in humans may be associated with either gain or loss of DNA methylation. In the case of human clock CpGs, this gain/loss may occur at a greater rate in males than in females in some tissues, suggesting that this difference might contribute to the different aging pattern seen in men and women but further studies are needed.

There remains considerable unexplained sex- and tissue-specific variation in the dynamics of DNA methylation during aging. An elegant 2016 mouse study illustrates this complexity. Hadad *et al.* (69) measured both DNA methylation levels and the levels of the enzymes regulating DNA methylation in aging mice in the hippocampus. This carefully controlled study involved animals housed under identical conditions at two institutions and utilized several different methods to assay DNA methylation levels. The authors found no age-dependent changes in gene expression of DNA methyltransferases or ten-eleven-translocation (TET) enzymes, nor did they detect consistent differences between the sexes. Neither were global, age-related changes in DNA methylation levels detected in the hippocampus for either 5mC or 5hmC. Males did show a small but significant increase in levels of 5mC compared to females when measured by ELISA.

Interestingly, a follow-up oxBS-seq experiment revealed that age and sex only affected DNA methylation levels in certain sequence contexts, whereas global effects were not detectable by these assays in the mouse hippocampus (69). Data from these human and mouse studies suggest that the simple and seductive notion that genome-wide loss of DNA methylation is a hallmark of aging needs to be revised to include the existence of age-associated increase and decrease of methylation levels and to reflect sex- and base-specific alterations in DNA methylation and hydroxymethylation.

### Histone Modifications

DNA methylation is mostly absent from the repertoire of epigenetic mechanisms used by the two common invertebrate models of aging *D. melanogaster* and *C. elegans*. Thus, research on epigenetic changes during aging in invertebrates has largely focused on a variety of histone marks and chromatin proteins. Unfortunately, the impact of sex and genetic background are rarely considered in these studies. Nonetheless, some data do exist suggesting that epigenetic changes may have sex-specific effects on lifespan. The cellular metabolite acetyl-coenzyme A (AcCoA) serves as the acetyl-donor for histone acetylation and, by modulating levels of AcCoA, has the potential to alter chromatin states and gene expression levels. In a recent study using RNAi knockdown of the AcCoA synthase enzyme in *Drosophila* brains, both males and females showed a significant increase in mean and maximum lifespan but the nature of the response to this treatment differed markedly between the sexes (70). Age-specific mortality was reduced among treated females at all ages compared to controls. In contrast, early life mortality was increased among treated males, followed by a deceleration of mortality rate at midlife compared to controls (70). While the mechanism(s) are unknown, these results suggest that the effect of altering histone acetylation via down-regulation of AcCoA is sex-specific and affects lifespan trajectories differently in males and females.

Sex differences in lifespan extension may also be influenced by the type intervention used to alter histone acetylation levels, the genetic background, and type of study animals. For example, SIR2, a histone deactylase, has been linked to longevity in a variety of organisms (71,72); however, the results of studies are inconsistent. In one study of *Drosophila,* dSir2 overexpression increased average lifespan across all experimental lines by 18% for males and 29% for females (71). However, a recent study (73) by Burnett and colleagues found that dSir2 overexpression did not increase lifespan in *Drosophila* or the nematode *C. elegans* when confounds due to genetic background and transgene effect were controlled. In yet another study, targeted, inducible overexpression of Sir2 in the adult fat body of adult *Drosophila* extended median lifespan in males and females equally, by about 13% (74). Clearly much work remains to be done to elucidate the role of SIR2 in aging, and investigating sex differences in response to interventions that affect SIR2 will be a critical part of this effort.

Other manipulations of histone acetylation levels also have unpredictable impacts on aging depending on sex. The transcriptional repressor SIN3a is a structural component of histone deacetylase complexes (75). Knockdown of Sin3A in *Drosophila* using RNAi reduced lifespan and stress resistance in both male and female flies to a comparable extent (76). In contrast, deacetylation of histones by feeding spermidine, a polyamine compound that has been shown to repress age-related histone acetylation changes, had distinct sex-specific effects when administered to mice and flies. In *Drosophila*, spermidine feeding induced a more robust lifespan extension in females than in males (77). In mice spermidine administered to older (18 months) retired male and female C57BL/6 breeders extended median lifespan in both sexes equally, by about 10% (78). In another study, flies fed 4-phenylbutyrate (PBA) had increased lifespan and showed a global increase in histone acetylation, but the effect was greater in females than males (79). Clearly, sex, study animal, and other aspects of genetic background contribute to organism's responses to the epigenetic changes that can occur with age.

Histone methylation also has been linked to aging. A 2015 genetic screen in yeast identified the histone 3 residue lysine 36 (H3K36), a known methylation site, as a key histone residue controlling replicative lifespan but the relationship is complex (80). Removing the demethylase (rph1Δ) and increasing H3K36 methylation levels increased replicative lifespan by 30%, while removing the methyltransferase (set2Δ) and lowering H3K36 methylation levels decreased the yeast's replicative lifespan by 15%. The loss of H3K36 methylation in old cells was found to be associated with cryptic transcription, which was also seen in aging *C. elegans* (80). In *C. elegans*, similar to yeast, loss of H3K36me decreased lifespan, while additional H3K36me extended lifespan (81). Unfortunately, no data are available that directly investigate the role of this mechanism for the suppression of cryptic transcription in both sexes, but a study of gene expression changes observed in older humans suggests that it might be. Variance in gene expression profiles tends to increase with age, and generally, it is thought that transcriptional noise increases. A 2008 study examined *post-mortem* samples of brain tissue from cognitively normal individuals (aged 20–99) and found that gene expression profiles change drastically between the sixth and seventh decades of life in males, whereas female gene expression changes most during the eighth and ninth decades (82). This finding suggests that the timing of age-related transcriptional changes differ between men and women and that therefore transcription-regulating mechanisms, such as those controlling H3K36me, might show sex-dependent patterns as well.

Together, the examples above provide strong evidence that epigenetics—both DNA methylation and histone modifications—influence aging and that these impacts can differ between the sexes. The data from human DNA methylation studies suggest that alterations to the epigenome occur at a slower pace in females than in males. The data from model organisms are limited; additional studies will be needed to get a clear picture of how age-associated epigenetic changes might contribute to the sex-differences in aging observed.

## Nuclear Architecture

Reports from several species indicate that the nuclear architecture undergoes large-scale changes with aging (83). These changes include shifts in the ratio of heterochromatin to euchromatin and alterations to the nuclear lamina. Most studies of nuclear architecture ignore sex in their study design, and thus the available data on sex differences in age-associated changes to nuclear architecture are very limited. One exception comes from a set of studies on dilated cardiomyopathy. Mutations in the lamin A/C gene (LMNA) are associated with approximately 8% of familial and sporadic dilated cardiomyopathy cases (DCM) (84). In human patients, DCM is more frequent and severe in males than in females (M:F ratio 2.5) (84). Similarly, a mouse DCM model carrying a missense mutation (H222P) has male biased morbidity and mortality and cardiomyocyte-specific nuclear accumulation of androgen receptor (AR) (85). Gonadectomy improved survival and cardiac function in $Lmna^{H222P/H222P}$ male mice, whereas treatment with testosterone enhanced nuclear accumulation of AR, exacerbated the DCM phenotype and reduced survival in both sexes. Finally, an AR antagonist suppressed nuclear

accumulation of AR in testosterone-treated mice. Taken together, these results suggest that testosterone interacts with the LMNA mutation to produce cardiomyocyte-specific nuclear accumulation of AR and the associated DCM phenotype (84). These studies illustrate that in the rodent models of aging, the impact of sex on genome instability is variable and might depend on the tissue examined and the genetic background.

## Conclusion

Currently available data thus indicate that sex influences measures of age-associated genomic instability. The levels of genomic instability increase in both males and females with age, in humans and in model organisms. However, how sex affects genome instability is less clear, as tissue studied, genetic background, and the method selected can influence results immensely. In humans, males experience higher levels of age-associated mosaicism and somatic mutations, possibly due to an earlier age at onset. In animal models, the results are more mixed; sometimes females experience a higher level of genomic instability with age, sometimes males. This complexity exists in both rodent and insect models. Likely, differences in tissues studied, the age of the study animals, and differences in assay methodologies contribute to the variability. In *Drosophila*, it appears that genetic background plays an important role in determining the interaction of sex and genomic instability; whether this is true in rodents remains to be determined, since the vast majority of studies are performed in a single inbred strain, C57BL/6. While humans represent an outbred species, the model systems that are currently in use tend to be highly inbred and thus might amplify genotype-dependent effects that are undetectable in the outbred human population.

Unfortunately, many studies reviewed here have used one sex only, failed to analyze the effects of sex when using both males and females, or failed to report the sex of the animals used. Studies that included both sexes but did not analyze sex differences represent an untapped resource: data from those studies could be used to interrogate sex differences retrospectively if study design is appropriate. There is a clear need for additional studies, both in humans, model systems, and non-model organisms that have a population structure more similar to humans.

### A Robust Design for Studying Sex Differences

Given the urgent need for additional, well-designed studies to elucidate sex differences in aging, we want to highlight one such effort. The National Institute on Aging (NIA) Intervention Testing Program (ITP) was designed to provide rigorous, reproducible studies to identify pharmacological and nutritional interventions that extend lifespan and healthspan in mice. The ITP uses female and male genetically heterogeneous UM-Het3 mice in carefully controlled studies that compare median and maximum lifespan at three separate sites and explicitly compares survival and aspects of health between the sexes (86).

With this study design, six compounds out of the 20+ compounds tested to date have extended lifespan in UM-Het3 mice, and all show sex-specific effects: rapamycin had a larger effect in females than males, acarbose had a greater effect in males, and three robustly extended lifespan in male mice only (87). Nordihydroguaiaretic acid (NDGA) and aspirin have antioxidant and anti-inflammatory properties and are known to activate the histone deacetylase SIR2. Both increased median lifespan in male but not female mice (88). Similarly, 17 α-estradiol (17 α-ER) is a non-feminizing estrogen with antioxidant and anti-inflammatory properties that extends life in males only (89). Acarbose, a treatment for type 2 diabetes and purported dietary restriction mimetic, showed a more robust impact on male compared to female lifespan (median lifespan extension of 22 vs 5% respectively) (89). Rapamycin, a potent mTOR inhibitor, delays aging and extends lifespan in both male and female mice, in multiple genetic backgrounds, given early or late in life, administered enterically, or by injection (90–94). Inhibition of mTOR, whether via rapamycin, rapalogues, or genetic downregulation of mTOR activity, acts in a dose-, sex-, and tissue-specific fashion and illustrates the interconnectedness of all of the major pathways that influence aging (1,95,96).

The ITP has shown that with appropriate study design, sex differences in response to aging interventions can be detected, and

**Table 1.** Sex Differences in Age-associated Genome Instability

| Type of Genome Instability | Species | Sex Differences Detected? | Sex Most Affected |
|---|---|---|---|
| DNA damage/mutations | | | |
| Nuclear | Human | Yes | Male |
| | Mouse | Sometimes | Mixed |
| | Fly | Yes | Mixed |
| Mitochondrial | Rat | Yes | Male |
| | Mouse | ? | ? |
| | Fly | ? | ? |
| Telomere attrition | Human | Yes | Male |
| | Mammals | Sometimes | Male |
| | Birds | Sometimes | Male |
| | Fly | No | — |
| | Worm | — | — |
| Epigenetic | | | |
| DNA methylation | Human | Sometimes | Male |
| | Mouse | Maybe | ? |
| Histone modifications | Fly | Maybe | ? |
| | Worm | Maybe | ? |
| Nuclear architecture | Human | Maybe | ? |
| | Mouse | Maybe | ? |

based on these results, they are likely to be pervasive. Table 1 summarizes what this review has reported regarding sex differences in age-associated genome instability. Mechanisms involved in the maintenance of genomic integrity vary among species and, as the data reviewed here suggest, may differ between sexes of the same species. Clearly, there are many more questions than answers and for most mechanisms and most species, very little is known. Sex differences in aging humans are pervasive and that alone makes them worth studying. The biology of sex differences in aging is of interest in its own right and is important for the development of effective interventions. Lastly, sex differences and sex-specific responses to senescence-retarding therapies offer a tool for interrogating the basic biological mechanisms involved in aging. For most mechanisms reviewed here, there are insufficient data to make a clear determination regarding the impact of sex, largely because sex differences have not been analyzed or both sexes studied. Overall, our findings reveal an urgent need and terrific opportunities for well-designed studies that explicitly examine sex differences in molecular drivers of aging.

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