The "faulty male" hypothesis: implications for evolution and disease

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Summary

Biological differences between males and females lead to many differences in physiology, disease, and overall health. One of the most prominent disparities is in the number of germline mutations passed to offspring: human males transmit three times as many mutations as do females. While the classic explanation for this pattern invokes differences in post-puberty germline replication between the sexes, recent whole-genome evidence in humans and other mammals has cast doubt on this mechanism. Here, we review recent work that is inconsistent with a replication-driven model of male-biased mutation, and propose an alternative, "faulty male" hypothesis. Importantly, we suggest that the new model for male-biased mutation may also help to explain several pronounced differences between the sexes in cancer, aging, and DNA repair. Although the detailed contributions of genetic, epigenetic, and hormonal influences of biological sex on mutation remain to be fully understood, a reconsideration of the mechanisms underlying these differences will lead to a deeper understanding of evolution and disease.

Main text

Haldane (1947) was the first to suggest a higher per-generation mutation rate in males compared to females, using data on the appearance of hemophilia in the offspring of unaffected parents. This paper is also often cited for its proposed explanation for the observed male bias: since the male germline is continuously dividing and the female germline is not, "if mutation is due to faulty copying of genes at a nuclear division, we might expect it to be commoner in males than females" (Haldane 1947). Haldane's germline-replication hypothesis is also consistent with a paternal age effect, whereby older males leave more mutations to their offspring. Both male-biased mutation and a paternal age effect have been firmly established by whole-genome sequencing of human pedigrees (Kong et al. 2012; Goldmann et al. 2016; Rahbari et al. 2016; Wong et al. 2016; Jónsson et al. 2017) and pedigrees of multiple mammals (Venn et al. 2014; Thomas et al. 2018; Besenbacher et al. 2019; Lindsay et al. 2019; Wang et al. 2020, 2022a, 2022b; Wu et al. 2020; Bergeron et al. 2021; Bergeron et al. 2023). Male-biased mutation across mammals is also supported by data from comparative studies (e.g. Wilson Sayres et al. 2011; de Manuel et al. 2022), though it is not possible to detect a paternal age effect from phylogenetic data.

What is often overlooked is that Haldane (1947) proposed a second hypothesis to explain male-biased mutation: male chromosomes may not be as well protected as female chromosomes. If the female germline was "relatively invulnerable to radiation and other influences, the difference is explicable." Unfortunately, Haldane did not know of any biological mechanism that could offer such protection, and could only note in the end: "On either of these hypotheses we should expect higher mutability in the male to be a general property of human and perhaps other vertebrate genes. It is difficult to see how this could be proved or disproved for many years to come."

In this Essay, we consider the data on mutation rates that have accrued in the 75+ years since Haldane's original hypotheses. We focus on many aspects of recent whole-genome sequencing projects that are inconsistent with the germline-replication hypothesis, with respect to both male-bias and the paternal age effect. In order to reconcile these observations, we introduce a new hypothesis—the "faulty male" model—that proposes a general inability of males to protect their germline as well as females do. This model and associated mechanistic data reflect a modern interpretation of Haldane's overlooked hypothesis for differences in germline mutation between the sexes. Further, we highlight patterns of male-biased DNA repair, cancer, and aging that are consistent with the somatic tissues of males also being more liable to damage. These data suggest the possibility of a shared basis for male-biased mutation in the germline and soma.

Genome-scale data are inconsistent with the germline-replication model

The germline-replication hypothesis originally proposed by Haldane (1947) focuses on the mitotic cell divisions needed to maintain continuous spermatogenesis, and the errors that result from these replication events. While this framework has occasionally been questioned (Hurst and Ellegren 1998; Gao et al. 2019; de Manuel et al. 2022), post-puberty mitotic cell division in the male germline has become *the* textbook explanation for male-biased mutation (e.g. Lynch 2007; Jobling et al. 2014; Strachan and Read 2018). However, multiple results from recent whole-genome studies are inconsistent with the germline-replication hypothesis. Below we consider six observations that strongly conflict with this model.

A maternal age effect. While much weaker than the paternal age effect (Figure 1a), studies with large numbers of sequenced pedigrees have now been able to detect an effect of maternal age on the number of transmitted *de novo* mutations (Wong et al. 2016; Goldmann et al. 2016; Jónsson et al. 2017). In the absence of ongoing replication in the female germline, this pattern points to accumulating exogenous damage as a likely source of mutations. Presumably, such damage must also accumulate in the male germline, and must contribute to the paternal age effect.

Mutation is male-biased just after puberty. Pedigree-based studies can only observe transmitted *de novo* mutations among individuals that have had children: this means that our knowledge about male-bias typically begins at the age of reproductive maturity. Nevertheless, studies including young parents reveal that many more paternally inherited mutations are already present shortly after puberty, in both humans (Figure 1a; Forster et al. 2015; Gao et al. 2019) and domestic cats (Wang et al. 2022a). Under the assumption that the male and female germlines have approximately the same number of cell divisions before puberty (Drost and Lee 1995), the same degree of male bias at this stage is not consistent with an important role for post-puberty mitotic cell division.

Spermatogenic cycle length is not predictive of mutation accumulation rates. The production of sperm follows a highly synchronized cycle of cell division, with a duration that varies between species (Luetjens et al. 2005). If mutations in the male germline are largely driven by mitotic replication, we would expect the number of mutations to increase at a rate that is proportional to the length of the spermatogenic cycle (i.e. the slope of the line after puberty in Figure 1b). However, comparisons between species have revealed highly similar rates of paternal mutation accumulation (~1.5 mutations/year), even when there is a two-fold difference in the spermatogenic cycle length (Jónsson et al. 2017; Wu et al. 2020; Wang et al. 2020). While there are a number of reasons why cycle length might not exactly correspond to the degree of male bias (Ségurel et al. 2014; Scally 2016), the observation of a relatively constant bias across species (see also de Manuel et al. 2022) suggests that replication rate is unlikely to be the major factor influencing this bias.

CpG mutations accumulate in a male-biased fashion. C-to-T mutations at CpG dinucleotides occur an order of magnitude more frequently than other mutations due to the deamination of methylated cytosines (Coulondre et al. 1978; Bird 1980; Duncan and Miller 1980). Importantly, deamination occurs spontaneously, and is not driven by polymerase errors during replication. This suggests that mutations at CpG sites should be free from the male-bias and paternal age effect that would be observed in replication-driven mutations. However, C-to-T mutations at CpG sites demonstrate both of these effects (Supplementary Figure 1). These patterns at CpGs are difficult to reconcile with a replication-driven model for mutation.

Hibernating species do not show a lower degree of male bias. Many seasonally breeding animals undergo testicular regression, whereby testis size decreases by up to 95% (Young and Nelson 2001). In addition to an overall reduction in size, spermatogenesis is greatly reduced or absent for a large fraction of the year (Tsubota et al. 1997; Young and Nelson 2001). A reasonable prediction from the germline-replication model might then be a reduction in the degree of male bias, and a diminution of the paternal age effect, among seasonal breeders. However, a study of germline mutation rates in grizzly bears found the same level of male bias as in non-hibernating species, as well as a match with the predicted number of transmitted

mutations given paternal ages (Wang et al. 2022b). These results further suggest a disconnect between male mutation bias and spermatogenic cycling.

Somatic mutation accumulation is not correlated with number of cell divisions. The somatic mutation rate varies greatly among tissues, and is consistently higher in all somatic cell types than the germline mutation rate (Milholland et al. 2017; Moore et al. 2021). Nonetheless, variation in somatic mutation rate among tissues is not associated with replication activity (Abascal et al. 2021). For example, mutation rates in neurons and smooth muscle, two cell types that are thought to rarely divide, are similar to those in frequently dividing cells. In fact, for many tissues, there is no observed difference in the rate of mutation accumulation between terminally differentiated cells and their progenitor stem cells (Abascal et al. 2021). Although there are clearly differences in mutation rates in the germline and soma, the limited effect of differences in replication rates between somatic tissues suggests that replication may be playing a more limited role in the germline as well.

The "faulty male" hypothesis for higher male germline mutation rates

Biological sex influences many different aspects of phenotype and physiology (Mauvais-Jarvis et al. 2020). These effects are driven by genetic, epigenetic, hormonal, and exogenous mechanisms, or some combination of all these (Khramtsova et al. 2018; Bernabeu et al. 2021). Here, we propose that mutation rates in male mammals are higher than in females because males are generally worse at protecting and repairing DNA. The "faulty male" hypothesis invokes physiological and molecular differences between the sexes as the main cause of the difference in mutation rates, rather than post-puberty germline replication. While this does not preclude a role for continuing cell division in the male germline as a source of mutation, it reduces the explanatory role that it plays.

The faulty male hypothesis follows the logic laid out by Haldane's (1947) alternative model: males are worse at protecting and/or repairing their gametes from DNA damage, resulting in male-biased mutation and a paternal age effect. While these general patterns are also predicted by the germline-replication hypothesis (Figure 1b), only the faulty male model—in which mutation is uncoupled from cell division—accounts for the additional patterns laid out above (Figure 1c).

What mechanism(s) might explain differences in the germline mutation rate between the sexes? There is some direct evidence for the differential action of DNA repair machinery between males and females. For example, researchers have found that polymerase theta is more effective in the female germline; this is likely explained by the inaccessibility of mature sperm to repair by this polymerase due to chromatin structure (Wang, Meyers, Schumacher 2023). Indeed, DNA in sperm is packaged in a distinct way compared to oocytes, using protamines rather than histones (Moritz and Hammoud 2022). There is, however, much indirect evidence for different mechanisms of mutation between the sexes (Broestl and Rubin 2021). For instance, the frequency of each type of single-nucleotide mutation differs in the male and female germlines (Goldmann et al. 2016; Jónsson et al. 2017; Wang et al. 2023), as does the amount of gross DNA damage experienced (Bajpayee et al. 2002; Slyskova et al. 2011).

More generally, there are a number of molecular mechanisms that differ between the sexes, many of which are modulated by sex hormone regulation, that likely contribute to the

disparity in mutation rates. Almost 37% of human genes show sex-biased expression in at least one tissue (Oliva et al. 2020), including many genes in the germline. Differences in germline gene expression are likely due in part to epigenetic marks, especially differential methylation (Stewart et al. 2016), and chromatin accessibility is known to be sex-biased in many tissues (Kukurba et al. 2016). Additionally, sex-biased differences in metabolite concentrations (Mittelstrass et al. 2011) and macroscopic differences in protective organs (such as skin; Giacomoni et al. 2009) could plausibly contribute to sex-biased mutation.

Finally, note that levels of sex hormones vary throughout mammalian development and adulthood—for both males and females—and are absent prior to embryonic sexual differentiation (Broestl and Rubin 2021). The absence of male-female hormonal differences early in development might explain why there is no male-bias in the mutations arising during this period (Rahbari et al. 2016; Sasani et al. 2019).

Is the male soma "faulty"?

By deemphasizing the role of germline replication as a major driver of male-biased mutation, we raise the possibility that the underlying causes of male bias may be acting similarly outside the germline. Many of the mechanisms invoked in the previous section to explain differences in mutation rates between the sexes are not specific to the germline, and may have similar effects on somatic mutation rates. Mutational variants in common between germline and somatic datasets support the idea of a mechanistic link (Meyerson et al. 2020). Such a connection between germline mutation rates and somatic mutation rates would open many new avenues of research.

Male-biased somatic mutation rates. The most straightforward question to ask is whether somatic nucleotide mutation rates male-biased. However, this question is surprisingly difficult to answer, as many studies either do not have the power to address the question or do not consider the possibility of a difference between male and female samples. The largest source of data is from studies of cancer tissues. A male bias in the number of nucleotide mutations (often referred to as "mutation load") is observed across cancer datasets. This is consistent across whole-genome sequencing data—which includes both coding and non-coding changes (Podolskiy et al. 2016; Li et al. 2020)— and the targeted sequencing of protein-coding genes (e.g. Li et al. 2018). While data from cancer sequencing supports the generality of male-bias in mutation, it is possible that such samples do not represent mutation processes in healthy somatic tissues. It is also important to note that the observed somatic male-bias is both weaker than that observed among germline mutations and is not observed in every tissue (Podolskiy et al. 2016; Li et al. 2020).

Male-biased cancer. The vast majority of cancers in tissues present in both sexes are male-biased (Lopes-Ramos et al. 2020). Figure 2 summarizes recent worldwide data on cancer incidence (Sung et al. 2021), illustrating a higher incidence of most cancers in males. While lifestyle choices associated with gender roles may explain some of these disparities, differences between the sexes remain after controlling for multiple risk factors (Jackson et al. 2022). In addition, childhood cancers are also highly male-biased (Liu et al. 2019; Radkiewicz et al. 2022), which suggests sex as the fundamental biological factor driving this pattern.

Several biological mechanisms have been proposed to explain male-biased cancer, including differences between the sexes in hormones, metabolism, immunity, X-linked tumor

suppressors, and general DNA repair (Dorak and Karpuzoglu 2012; Clocchiatti et al. 2016; Rubin 2022). There is increasing evidence for sex-specific differences in the DNA damage response pathway, defects in which are thought to fuel carcinogenesis (Cardano et al. 2022). In addition, some experimental evidence points to differing responses in, for example, double-strand break repair (Rall-Scharpf et al. 2021). Finally, there is indirect evidence that DNA repair in males is relatively inferior in populations already susceptible to DNA damage. For instance, males are more likely to develop secondary cancers when radiation is used to treat a primary cancer, and are more likely than females to develop cancers when they have inherited germline mutations in tumor suppressor genes (Rubin 2022).

Given the evidence presented above for higher somatic mutation rates in males, we propose that a faulty male soma may also play a role in driving cancer rates between the sexes. Differences in somatic mutation rates should not be considered the only cause of differences in cancer rates—especially as not every tissue shows male-biased mutation—but should be considered alongside other commonly proposed mechanisms. While the invocation of sex-bias in somatic mutation rates overlaps with previous hypotheses about differences in DNA repair between the sexes, the underlying causes may be quite different; this also suggests that approaches used to study this mechanism could be expanded, for instance by whole-genome sequencing of somatic tissues.

Male-biased aging. As with cancer, there is a clear sex bias in human aging, with females consistently living longer than males (Austad and Fischer 2016; Bronikowski et al. 2022). A higher mortality rate in males is present from birth and extends well into old age: only 10% of super-centenarians are male (Austad and Fischer 2016). Lower longevity in males arises from many causes, with male-bias in 14 of the top 15 causes of death in the United States (Xu et al. 2021)—only Alzheimer's disease has an age-adjusted death rate that is female-biased.

There are multiple proposed mechanisms to explain sex differences in aging and senescence (Hägg and Jylhävä 2021; Bronikowski et al. 2022). These mechanisms include differences in sex hormones, mitochondria, telomeres, epigenetic marks, proteostasis, cellular senescence, metabolism, immunological factors, and general genomic instability. “Genomic instability” covers many different types of mutations, and generally minimizes the role of point mutations, but is commonly invoked as a factor driving sex differences in aging (Fischer and Riddle 2017). On the other hand, there is now a large literature on the accumulation of somatic single-nucleotide variants with age, regardless of sex (see Ren et al. 2022 for a review). Indeed, the somatic DNA damage theory of aging (Jin 2010) posits that deleterious mutations occurring throughout a lifetime are a major determinant of mortality and senescence (Kinzina et al. 2019; Vijg and Dong 2020; Schumacher et al. 2021).

We propose that a faulty male soma contributes to male-biased aging. Somatic nucleotide mutations would not explain all differences in aging between the sexes, but are perhaps one important contributor to faster aging and higher mortality in males. An additional intriguing link between somatic mutation and aging comes from a study that found reduced longevity in families with higher germline mutation rates (Cawthon et al. 2020). If, as we have posited here, there is an underappreciated relationship between germline and somatic mutation rates, then the aging process may be amenable to study via more-easily measured germline mutations.

Discussion and conclusions

Uncovering the molecular basis for evolution and disease is key to understanding the mechanisms driving both. Here, we have proposed that differences in germline mutation rates between the sexes are driven by "faulty" males—the reduced ability of males to repair and/or protect germline cells from mutation. There are multiple lines of evidence that favor this model over the dominant germline-replication model. More speculative is the proposal that the male soma is similarly faulty. While such a model could explain many aspects of male-biased cancer and aging, we do not yet have enough data to properly evaluate it relative to previously proposed explanations. If mutational mechanisms act very differently between the sexes, this would significantly impact our study of human health, influencing the diagnosis and treatment of congenital disease, fertility management, and our understanding of the aging process. Such differences may also change how we think about the processes driving evolution, especially the molecular basis for many evolutionary differences among species.

Given that we do not yet know the molecular basis underlying faulty males in either the germline or soma, it will be important to explore possible mechanisms. One intriguing possibility is found in the DREAM complex, a repressor of DNA repair active in somatic tissues (Bujarrabal-Dueso et al. 2023). DREAM is a cell cycle regulator that—directly or indirectly—increases the number of mutations in tissues where it is active, such that its inhibition restores germline-like mutation rates to somatic tissues (Bujarrabal-Dueso et al. 2023). An obvious potential mechanism for sex-biased mutation is therefore sex-biased expression of the DREAM complex: higher expression in male tissues would lead to higher mutation rates.

Regardless of the specific actors, as the sequencing of somatic tissues becomes more prevalent it will be imperative to ensure that future studies include sex as a biological variable. Currently, patient cohorts in such studies are not selected with sex-specific effects in mind, but this will be crucial for uncovering the sources of mutational differences between males and females. Similarly, studies of methylation and other epigenetic marks that may drive differences in mutation rates must be carried out in cells or tissues from both sexes—simply knowing the methylation state in one sex is insufficient for understanding associated phenotypic differences. Such studies may also help us to understand the source of male-bias in cancers (Figure 2) and many other diseases. In carrying out research for this Essay, it also became clear that there is considerably more work on DNA repair and packaging in human sperm than in oocytes. While much of this difference could be due to the relative accessibility of each cell type, a fundamental understanding of the differences in germline mutation will require additional efforts in studying the oocyte.

Uncovering the mechanisms underlying sex differences will also likely require a comparative approach, both among species and among types of mutations. Comparisons among species allow us to observe variation in many biological parameters that do not vary within humans (e.g. average age at puberty, average age at reproduction, and maximum lifespan) or that show a different pattern than in humans (e.g. male *C. elegans* live longer than females; Austad and Fischer 2016). Comparative sequencing has also revealed the degree to which germline mutational sex bias varies among vertebrates (Bergeron et al. 2023). While comparative somatic sequencing studies have only recently appeared (e.g. Cagan et al. 2022), future work that includes both sexes from each species will be invaluable. Such studies may also provide independent tests of the correlations discussed here. For instance, birds exhibit a less male-biased germline mutation rate than humans (de Manuel et al. 2022), as well as little-to-no male bias in either aging (Bronikowski et al. 2022) or cancer (Kapsetaki et al. 2023). Finally, understanding

the mechanisms driving male-biased nucleotide mutations will be helped by studying different types of mutations. In humans, small insertions and deletions show the same major patterns as nucleotide mutations (Jónsson et al. 2017). In contrast, evidence suggests that larger structural variants are male-biased (Belyeu et al. 2021), but these do not appear to be age-dependent in either humans or macaques (Thomas et al. 2021; Belyeu et al. 2021). Understanding the differences between these mutation types—and whether the same patterns appear in somatic tissues and across species—will help us to uncover the processes leading to male-biased mutation.

Acknowledgements

We thank Jeff Rogers and Josh Rubin for helpful comments on the manuscript. This work was supported by National Institutes of Health grant R01-HD107120.

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Figure Legends

Figure 1. Models and data for germline mutation.

(a) The accumulation of germline mutations in human males and females (colored points and solid lines) with parental age (from Jónsson et al. 2017). Each point represents the average number of total mutations transmitted from male and female parents of the specified age. Lines show the linear regression of these points with parental age. Cell divisions with age (dashed lines) come from calculations in Ségurel et al. (2014). For clarity, dashed lines pre-puberty are shifted so as not to overlap between males and females.

(b) The germline replication model proposes that the number of mutations transmitted by a parent should be proportional to the number of germline cell divisions. Here, the expected number of mutations from male and female parents is drawn to exactly track the cell divisions shown in panel (a).

(c) The faulty male model proposes that the number of mutations is consistently higher in males than females after germline sex differentiation, but that mutations accumulate in both sexes through time. Here, the slopes of the mutation-accumulation lines after differentiation are identical to those from the linear regression of mutation data in panel (a).

Figure 2. Male bias in the incidence of cancer.

Male and female age-standardized incidence rates for 27 types of cancer per 100,000 people. Rates reported are worldwide numbers for the year 2020 (all data from Sung et al. 2021). The dashed line represents equal incidence rates in males and females (i.e. no sex bias); points above the line are male-biased, while those below are female-biased. Note that the y-axis is artificially shortened to include results for lung cancer incidence on the plot.

Supplementary Figure 1. Patterns of CpG mutations in human males and females.

The accumulation of germline mutations in human males and females with parental age for only mutations at CpG sites. All elements of the plot are the same as in Figure 1a, except that only mutations at CpG sites are included.

Figure 1

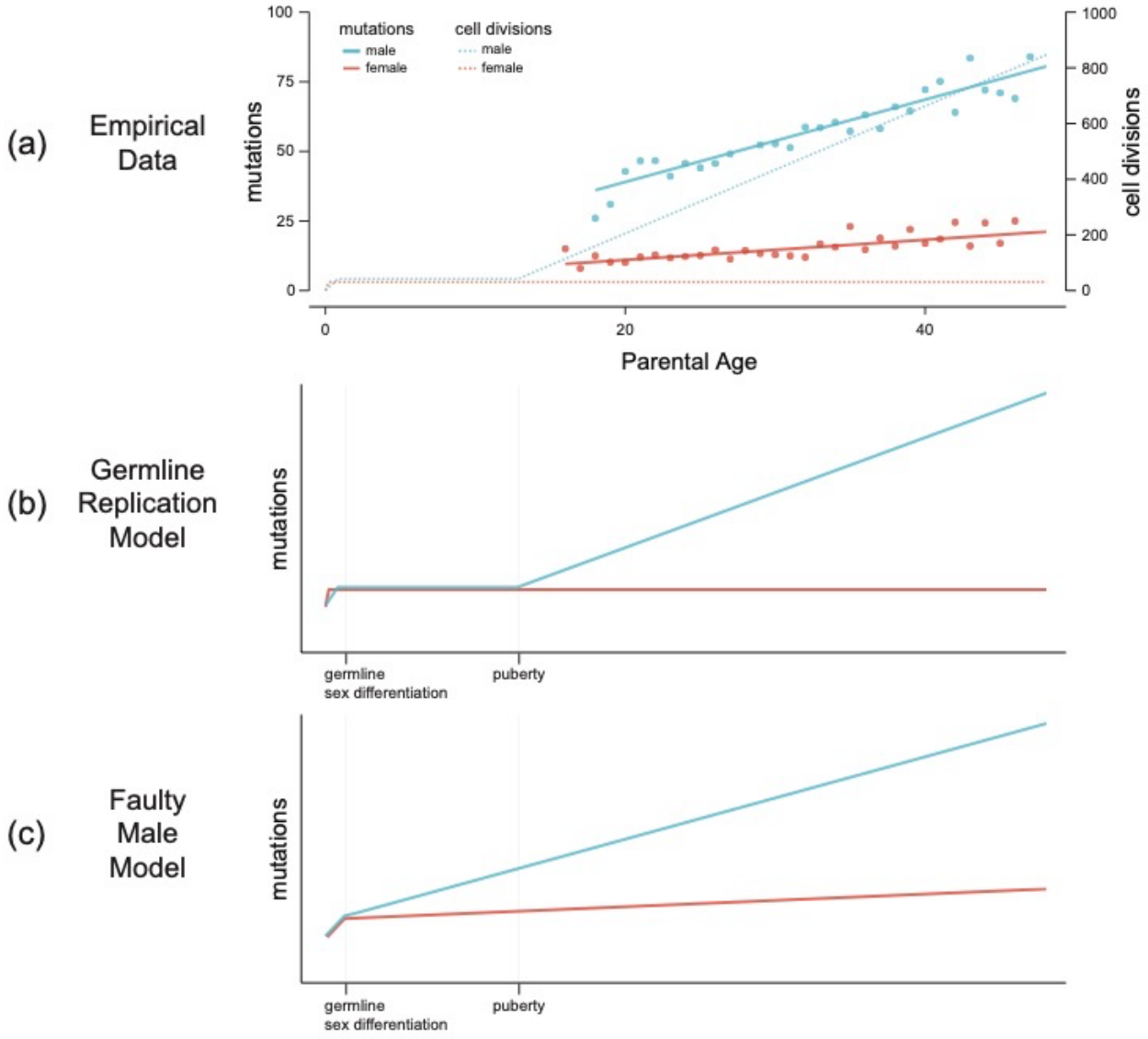
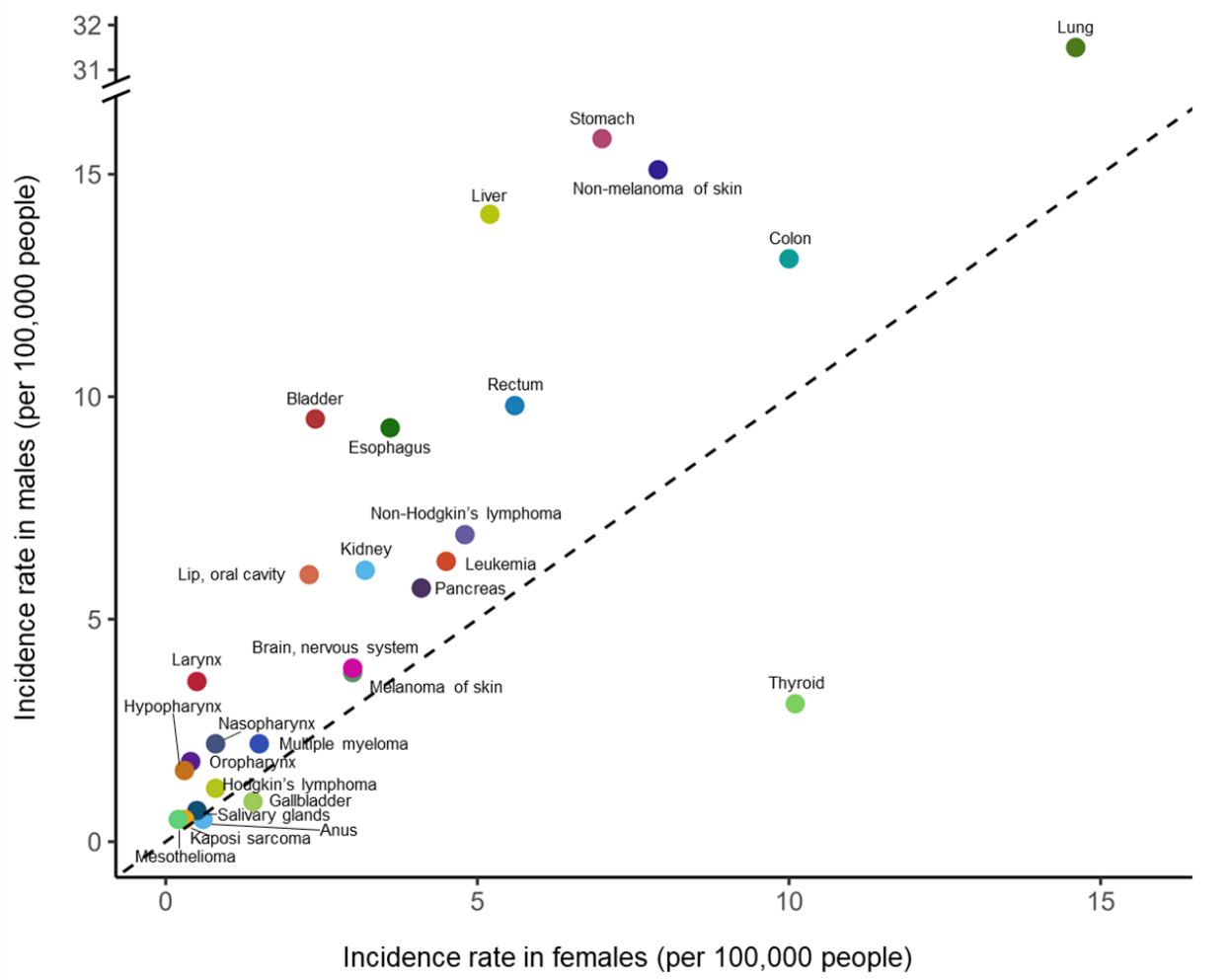

(a)
Empirical Data
mutations
male
female
cell divisions
male
female
100
75
50
25
0
1000
800
600
400
200
0
0
20
40
Parental Age
cell divisions
(b)
Germline Replication Model
mutations
germline sex differentiation
puberty
(c)
Faulty Male Model
mutations
germline sex differentiation
puberty

Figure 2


Lung
Stomach
Non-melanoma of skin
Liver
Colon
Rectum
Bladder
Esophagus
Non-Hodgkin's lymphoma
Kidney
Leukemia
Lip, oral cavity
Pancreas
Brain, nervous system
Larynx
Melanoma of skin
Thyroid
Hypopharynx
Nasopharynx
Multiple myeloma
Oropharynx
Hodgkin's lymphoma
Gallbladder
Salivary glands
Anus
Kaposi sarcoma
Mesothelioma
32
31
15
10
5
0
0
5
10
15
Incidence rate in males (per 100,000 people)
Incidence rate in females (per 100,000 people)

Supplementary Figure 1