X-linked competition - implications for human development and disease

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Abstract

During early mammalian female development, X-chromosome inactivation leads to random transcriptional silencing of one of the two X chromosomes. This inactivation is maintained through subsequent cell divisions, leading to intra-individual diversity, whereby cells express either the maternal or paternal X chromosome. Differences in X chromosome sequence content can trigger competitive interactions between clones that may alter organismal development and skew the representation of X-linked sequence variants in a cell-type-specific manner — a recently described phenomenon termed X-linked competition in analogy to existing cell competition paradigms. Skewed representation can define the phenotypic impact of X-linked variants, for example, the manifestation of disease in female carriers of X-linked disease alleles. Here, we review what is currently known about X-linked competition, reflect on what remains to be learnt, and map out the implications for X-linked human disease.

Introduction

Male and female humans differ in the number of their X chromosomes: biologically female individuals have two X chromosomes (XX), whereas biologically male individuals have one X and one Y chromosome (XY). Expressing two doses of X-linked genes would be lethal, and specific mechanisms ensure that the dosage of X-linked genes is compensated between male and female individuals [1]. In early XX embryos, each cell randomly chooses one of its two X chromosomes for inactivation [2-4]. This choice occurs prior to cell-type specification and is then clonally propagated through subsequent cell divisions. In other words, daughter cells and grand-daughter cells maintain the original choice of which X chromosome to express and which X chromosome to keep inactive [2-4]. This pattern of inheritance through mitotic divisions is epigenetic, because X-chromosome inactivation changes the functional state of an X chromosome, rather than its DNA sequence. Thus, X-chromosome inactivation generates two families of clones within each XX individual, one expressing the maternally inherited X chromosome and the other expressing the paternally inherited X chromosome (Fig. 1a). Clonal propagation of the active and the inactive X chromosome from mother cells to daughter cells is illustrated by cell patches that share the expression of the same X chromosome in transgenic mice with X-linked reporter genes [5] (Fig. 1b). Other examples include the familiar coat colour patches of Calico cats and the mosaic manifestation of skin defects in patients with incontinentia pigmenti and other X-linked disorders [6].

The existence of two clonal populations of cells in XX individuals has interesting implications for organismal development and the manifestation of X-linked disease. No two human X-chromosomes are identical in sequence: on average, the two X chromosomes in XX humans differ by ~150 non-synonymous coding variants in addition to thousands of non-coding variants [13-17]. Genetic variation between X chromosomes combines with X-

chromosome inactivation to generate intra-individual diversity, as the expanded progeny of individual cells give rise to clones that selectively express the unique sequence variants present on one or the other X chromosome. XX individuals therefore comprise two sets of clones that express different genetic sequences in a mutually exclusive fashion.

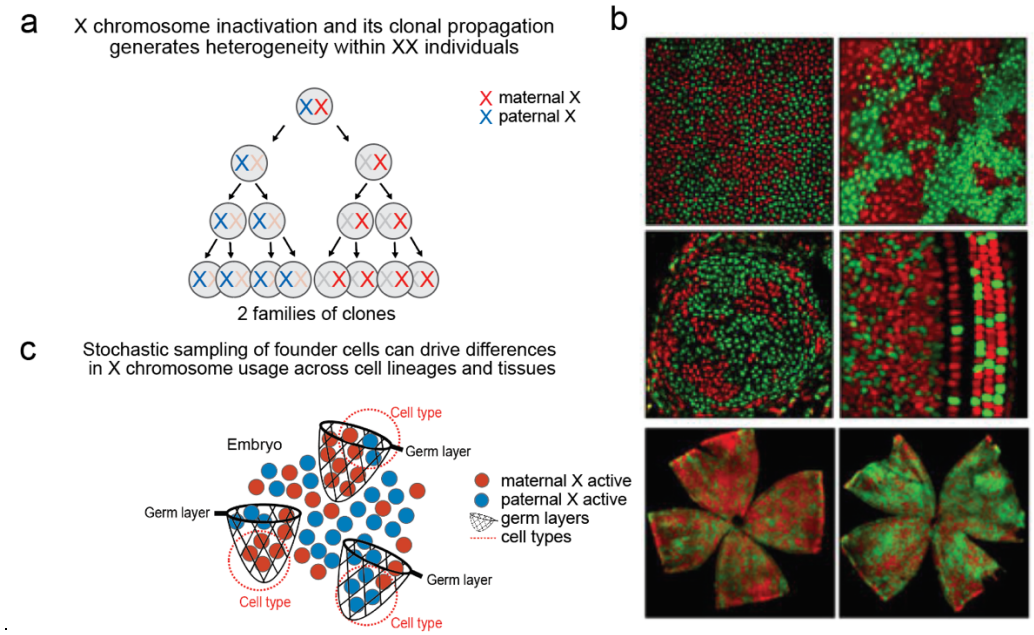


Figure 1. X-chromosome inactivation and its clonal propagation. a, X-chromosome inactivation and its epigenetic propagation mean that XX individuals comprise a mosaic of clones expressing either the maternal or the paternal X chromosome [1-4]. **b,** Visualization of X-chromosome usage in mouse tissues [5]. Expression of the two X chromosomes is marked by either red or green fluorescent reporter proteins. Top and middle: patterns of clonal mixing in different cell types and tissues. Bottom: skewed X-chromosome usage in the retina of two individual mice. Skewing in this case is random, as it differs between individual mice that are genetically identical. These images are from transgenic mice, and X-chromosome activity patterns have not yet been directly visualized in humans. **c,** Stochastic differences in maternal versus paternal X-chromosome usage between germ layers and cell types of the same XX individual. The ratio of cells expressing the maternal versus the paternal X chromosome in undifferentiated cells of the early embryo is not necessarily 50:50 and can be further skewed by stochastic sampling of small numbers of progenitors to form each of the three germ layers, which can each show different X-chromosome usage. Within each germ layer, small numbers of progenitors give rise to each cell type and tissue, which may skew X-chromosome usage further. This results in considerable variation in X-chromosome usage between cell types and tissues. **c,** Genetic variation on the X chromosome generates an added layer of intra-individual variation in XX individuals. The human X chromosome exhibits substantial genetic variation, with on average approximately 150 protein-coding variants found between any two human X chromosomes. Individual cells in tissues selectively express the unique sequence content of one or the other X chromosome owing to X-chromosome inactivation followed by clonal propagation, leading to mosaicism at the cellular level. Part b reproduced with permission from Ref. [5].

Although the chance of expressing either the maternal or the paternal X is theoretically equal, at least three distinct processes can skew X-chromosome usage. First, sequence variation in the genetic region that controls X-chromosome inactivation [2-4,7,8] (including the non-coding *XIST* RNA), X:autosome translocations [8] or familial skewing of unknown cause [9] can bias the choice which X chromosome to inactivate. Second, X chromosome inactivation occurs early in development when the number of cells in the embryo is still relatively small: the estimated cell number at the time of human X chromosome inactivation is as low as 6–16

cells [10,11]. Comparable to taking a handful of marbles from a bag that contains two different colours, a small sample size causes variation in the representation of each colour. The extent of skewing towards one or the other X chromosome varies not only between individuals, but also between cell types within the same individual. Variation of X chromosome usage has been harnessed to estimate how many cells give rise to each of the three germ layers (endoderm, ectoderm and mesoderm) and to cell types within each germ layer. High variability of X chromosome usage in blood contrasts with low variability in brain, suggesting that blood is formed by fewer founder cells (~20 cells) than brain (~40–140 cells) [10] (Fig. 1c). Third, the resulting pattern of X chromosome usage can be shaped further by deterministic processes that act to favour or disfavour clones expressing sequence variants encoded by one or the other X chromosome [12, 13]. For example, as discussed in detail below, intrinsically deleterious variants can impair the ability of clones to expand, survive or differentiate towards a particular lineage, and cells expressing such variants may therefore be selected against. Recent evidence suggests that intraindividual diversity is subject not only to selection but also competition, whereby clones expressing distinct genetic variants are intrinsically competent to form particular cell types or tissues but fail to do so in the presence of wild-type competitors [13]. This phenomenon, termed X-linked competition, can alter the representation of clones expressing maternal or paternal X chromosome sequences in different cell types and tissues[13]. This has important potential implications not only for normal development but also the manifestation of disease in XX individuals.

Here, we review what is known about X-linked competition and discuss the questions it raises in the context of disease, since processes that alter the representation of cells expressing disease-associated variants have the potential to affect the manifestation of X-linked disease alleles. The molecular underpinnings of X-chromosome inactivation in early development are covered by other excellent reviews [2-4].

X-linked competition

How intra-individual clonal heterogeneity can drive X-linked competition and skew X-chromosome usage is exemplified by sequence variation in mammalian *STAG2*, an essential X-linked gene that encodes a subunit of the cohesin complex. *STAG2* missense variants — R370P, R370Q and W334A — disrupt the binding interface between cohesin and CTCF [13]. In mouse models, *Stag2* wild-type and variant clones interact with each other to modulate the contribution of X-linked diversity to organismal development [13]. *Stag2*^{variant} clones contribute equally to most tissues, including high-turnover tissues such as the gut, but are under-represented in skeletal muscle and fail to form lymphocytes in heterozygous *Stag2*$^{wild\text{-}type/variant}$ mice (Fig. 2a). *STAG2*^{variant} (R370P) clones are also undetectable in a polyclonal B cell line derived from the blood of a human *STAG2*$^{wild\text{-}type/variant}$ individual [13] (Fig. 2a). Interestingly, the same *Stag2* variants that fail to contribute to form lymphocytes in heterozygous *STAG2*$^{wild\text{-}type/variant}$ females prove perfectly capable of generating lymphocytes in the absence of *STAG2*$^{wild\text{-}type}$ cells: homozygous *Stag2*$^{variant/variant}$ females and hemizygous *Stag2*^{variant} males generate normal numbers of lymphocytes [13]. Of note, *Stag2*^{variant} lymphocytes undergo cohesin-dependent processes such as V(D)J recombination and immunoglobulin class switch recombination [13], presumably supported by wild-type STAG1, which is partially redundant with STAG2 (Ref. [18]). These data show that the exclusion of *Stag2*^{variant} clones from the lymphoid compartment of heterozygous *STAG2*$^{wild\text{-}type/variant}$ females is not explained simply by cell-intrinsic defects of *Stag2*^{variant} clones and suggests that competition occurs between *Stag2*$^{wild\text{-}type}$ and *Stag2*^{variant} clones.

There are substantial differences in X-chromosome inactivation and escape between human and mouse [2-4] and it will be important to complement mouse models for X-linked competition with direct observations in humans. Nevertheless, the absence of *STAG2*^{variant} clones from human blood-derived B cells suggests that X-linked competition likely operates in humans [13].

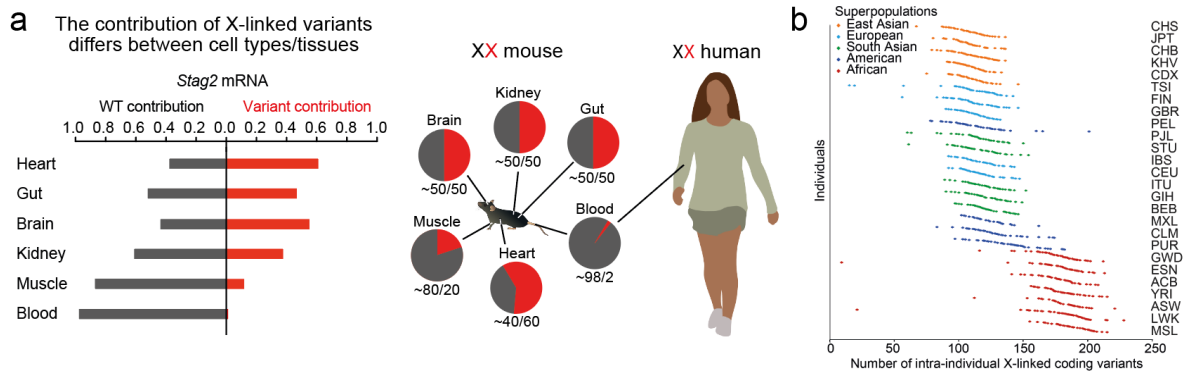


Figure 2. X-linked competition. a, The tissue-specific skewing of X-chromosome usage in specific cell types and tissues in mouse models of human sequence variation in the X-linked *Stag2* gene [13].-*STAG2* variant clones are absent from a polyclonal B cell line derived from the blood of a human XX individual[13]. **d,** The number of heterozygous missense variants (non-synonymous single-nucleotide variants (SNVs)) between the two X chromosomes in 1,271 XX individuals across human populations in phase 3 of the 1000 Genomes Project[123] and SNVs were filtered with bcftools v1.20 (Ref. [124]) to those with only one alternate allele present per individual. We removed SNVs in genes known to escape X-chromosome inactivation [10], identified missense variants with the Bioconductor package VariantAnnotation[125] using Gencode v19 (GRCh37.p13), and counted non-synonymous heterozygous X-chromosome variants for each individual. Ethnicities are from ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/working/20130606_sample_info/20130606_sample_info.xlsx. Colours indicate superpopulations. CHS: Southern Han Chinese South; JPT: Japanese in Tokyo, Japan; CHB: Han Chinese in Beijing, China; KHV: Kinh in Ho Chi Minh City, Vietnam; CDX: Dai Chinese in Xishuangbanna, China; TSI: Tuscan in Tuscany, Italy; FIN: Finnish in Finland; GBR: British in England and Scotland; PEL: Peruvian in Lima, Peru; PJL: Punjabi in Lahore, Pakistan; STU: Sri Lankan Tamil in the UK; IBS: Iberian populations in Spain; CEU: Utah residents with Northern and Western European ancestry; ITU: Indian Telugu in the UK; GIH: Gujarati Indian in Houston, TX; BEB: Bengali in Bangladesh; MXL: Mexican Ancestry in Los Angeles, California; CLM: Colombian in Medellin, Colombia; PUR: Puerto Rican in Puerto Rico; GWD: Gambian in Western Division, The Gambia; ESN: Esan in Nigeria; ACB: African-Caribbean in Barbados; YRI: Yoruba in Ibadan, Nigeria; ASW: African-American Ancestry in Southwest US; LWK: Luhya in Webuye, Kenya; MSL: Mende in Sierra Leone.

Genetic variants that drive X-linked competition

Although X-linked genetic variation is ubiquitous [13-17], we currently do not know the spectrum of X-linked variants that drive X-linked competition, how multiple such variants may interact, and how frequently X-linked competition occurs in XX individuals. Analysis of Genotype-Tissue Expression (GTEx) project data from several hundred individuals did not identify a link between X-linked heterozygosity in protein-coding variants and X-chromosome usage [10,11]. This observation indicates that X-linked competition is unlikely to be driven by common genetic variants, which would have been detected in these studies. Analysis of larger sample sizes revealed a contribution of rare genetic variants to X-chromosome usage in non-human animals [11], which suggests that rare rather than common X-linked variants may drive competitive interactions in XX individuals. Consistent with such a scenario, female individuals with heterozygous mutations in the X-linked cohesin regulator *HDAC8* (Ref. [19-21]) as well as *STAG2* (Ref. [22-25]) often show heavily skewed X-chromosome usage in blood in favour of the X chromosome that harbours the wild-type allele. These patients have missense and nonsense mutations, and it is currently unknown whether other types of variants such as repeat expansions, partial X-linked aneuploidy or mutations in regulatory regions can give rise to X-linked competition. Variants that drive X-linked competition do not necessarily cause disease: *STAG2* variants that disrupt cohesin–CTCF interactions are classified as pathogenic by the AlphaMissense variant pathogenicity predictor [26], but the donor heterozygous for the

STAG2^{R370P} variant described above was healthy and fertile [13]. Similarly, mice that model *Stag2* variants show X-linked competition but do not display obvious disease phenotypes on an outbred genetic background [13]. This may point to the ability of common allelic variants present in laboratory mouse strains to buffer the potentially detrimental impact of *Stag2* variants, for example, by genetic interactions whereby autosomal variants modify the impact of X-linked mutations and their manifestation as X-linked disease [27-29].

Consistent with the origin of modern humans on the African continent, African populations show higher genetic diversity than non-African populations [14-17]. Analysis of X-linked coding variation between the two X chromosomes of XX individuals in the 1000 Genomes Project mirrors this trend (Fig. 2b). In contrast to common variants, which are mostly shared across global populations, rare variants are often restricted to specific populations or population groups [17]. Admixed individuals therefore have an increased likelihood of heterozygosity for rare X-linked variants [16,30]. If X-linked competition is indeed driven by rare variants, one could speculate that an increased likelihood of heterozygosity for such variants may indicate greater potential for X-linked competition.

Classic cell competition

X-linked competition is reminiscent of classic 'cell competition' paradigms in developmental biology, whereby cells with higher fitness assume ‘winner’ status and eliminate neighbouring 'loser' cells with lower fitness, usually by apoptosis. Importantly, cell competition is based not on absolute levels of fitness, but rather on fitness differentials between neighbouring cells, and loser cells are excluded from organismal development not because they are intrinsically unable to contribute, but because they are actively prevented from doing so by their neighbours [31-36] (Fig. 3a) . The principles and mechanisms of cell competition are summarized by several excellent reviews [32,37,38] (Fig. 3b). Of note, here, we use the term 'cell competition' in the sense of 'active' cell competition, which refers to cellular interactions that amplify fitness differences between winner and loser cells [38]. Active cell competition stands in contrast to random fluctuations in clonal contributions — or clonal drift — also called 'neutral' competition [20], whereas shifts in clonal balance driven by cell-intrinsic differences, such as different proliferation rates, without designated winner or loser states have been termed 'biased' competition [38].

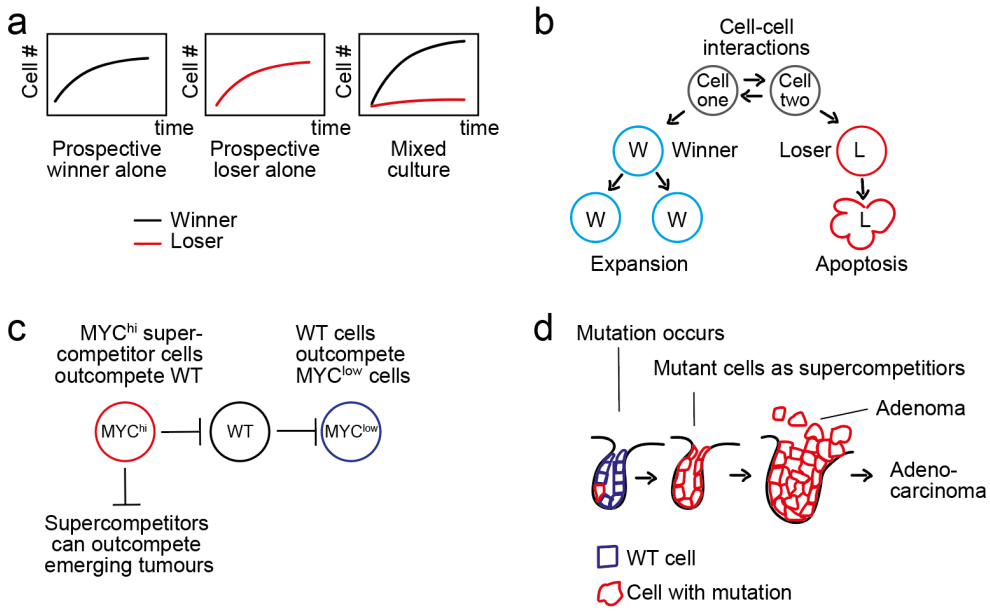


Figure 3. Cell competition. **a,** Cell competition amplifies minor fitness differences between prospective winner and loser cells. On their own, loser cells are capable of proliferation but

fail to expand in the presence of winner cells [35,36]. **b,** Cell competition designates winner and loser cells. Loser cells are eliminated by apoptosis only in the presence of winner cells. **c,** Somatic changes, including changes that drive overexpression of MYC, can turn cells into super-competitors that displace cells without mutations and can eliminate nascent tumours [44] **d,** Cell competition can be hijacked by super-competitors that eliminate their neighbours. This creates space for the mutant clones and facilitates the emergence of adenomas and potentially adenocarcinomas [53,55].

Cell competition — a protective mechanism?

Cell competition can be thought of as a protective mechanism that removes less-fit cells from the embryo to ensure normal development [31,39-41]. The power of cell competition is illustrated by its ability to protect the host from tumours. Human epithelial tissues accumulate cancer driver mutations with age, and normal adult tissues resemble a patchwork of mutant clones. This accumulation of mutant clones involves positive selection of somatic mutations that alter cellular fitness. Clones compete for space and survival, and the fittest clones expand by eliminating their less-competitive neighbours [42]. Epithelia may recognize and eliminate potentially transformed single cells through cell competition in a process dubbed EDAC, for epithelial defence against cancer [43]. In a mouse model of oesophageal cancer, many emerging tumours were eliminated by competition from surrounding cells, which had accumulated mutations but remained untransformed [44] (Fig. 3c).

Cell competition can also reduce the incidence of tumour formation by driving clonal dynamics in ageing [45], DNA damage [46] and cancer [47-49]. For example, ageing T cell progenitors in the thymus are eliminated by competition from genetically identical but more recently born (younger) progenitors [45]. Elimination of aged T cell progenitors is driven at least in part by a reduction in the ability to use the survival factor IL-7 and, crucially, is required for preventing the emergence of T-cell acute lymphoblastic leukaemia [45].

The dark side of cell competition

The protective function of cell competition is easily undermined. Mutations that increase fitness, for example, through the activation of oncogenes or loss of tumour suppressor genes, can enable cells to hijack cell competition to their advantage [50-54]. Just as variation in the expression of the oncogene *MYC* expression triggers the elimination of cells with low MYC levels[34,41,55], somatic mutations that increase MYC expression turn cells into super-competitors that readily eliminate their neighbours (Fig. 3c). Similarly, loss of the tumour suppressor gene *APC* can confer super-competitor status. Mechanistically, *Apc*-deficient stem cells in the intestinal crypt secrete the WNT inhibitor NOTUM, which drives adjacent wild-type stem cells to differentiate and leave the crypt. This creates space for mutant clones to expand and to ultimately form adenomas and adenocarcinomas [53,54] (Fig. 3d). Similarly, leukaemic cells outcompete normal haematopoietic stem cells for access to niches and associated resources that are required to maintain stem cell self-renewal and differentiation [56]. Even the 'guardian angel' function of cell competition described above — where non-transformed cells eliminate nascent tumours — requires that the 'protective' cells have already acquired mutations that turn them into super-competitors. As a result, they outcompete not only tumours but also normal cells that lack fitness-enhancing mutations [44].

In conclusion, cell competition is a double-edged sword: although competition can be protective, 'selfish' behaviour of rogue clones may harm the host. The ease with which cell competition is undermined may be rooted in the fact that competition operates at the level of the survival of cells, not survival of the organism.

Comparing X-linked and classic cell competition

Both X-linked and classic cell competition alter the behaviour of cells based on differences with their neighbours. However, there are apparent differences between the two forms of competition that warrant further analysis. X-linked competition is unique to XX individuals. Although both classic cell competition and X-linked competition are based on clonal differences, in X-linked competition these consist of genetic variants between the maternal and the paternal X-chromosomes. XX cells are programmed to selectively express one of their two X chromosomes, which results in two distinct families of clones that express either the maternally or paternally inherited X-chromosome. The potential for X-linked competition is therefore 'hard-wired' into the developmental programme of XX individuals. Competitive interactions between epigenetically distinct clones within XX individuals can restrict cell fate choices and thereby alter the course of organismal development by skewing the representation of X-linked sequence diversity across cell types and tissues [13].

Competition can be cell type-specific [13,31,45]. In X-linked competition, variants deemed unfit to contribute to the lymphoid compartment can nevertheless contribute to other cell types and tissues such as the brain to the same extent as wild-type [13]. In classic cell competition paradigms based on cell-autonomous differences in growth rates [31], variant clones remain smaller and therefore contribute less than wild-type even in tissues where there is no active cell competition.

Loser status depends on the relative fitness of competitor cells, and the prospect of loser cells is therefore improved by imposing genetic 'handicaps' on their competitors [57]. *Stag2* variant cells can be rescued from X-linked competition even after prolonged exposure to *Stag2* wild-type competitors throughout early development: conditional deletion of *Stag2* from designated winner cells at the later haematopoietic stem cell stage restores the ability of wild type-exposed *Stag2* variant cells to form lymphocytes [13]. Since the classic experiments that founded the field, views on what constitutes cell competition have considerably expanded [32], and mechanistic studies on X-linked competition are likely to continue this trend.

X-chromosome usage and X-linked disease

Differences in the disease manifestation of X-linked versus autosomal traits

Disease-associated variants are known for many of the ~600 protein-coding genes on the human X-chromosome [58]. The X-chromosome has a unique biology compared to autosomes, providing an interesting contrast in the genetics of X-linked versus autosomal traits. The main reason for this is the difference in the number of X-chromosomes in female and male individuals, and the resulting requirement for dosage compensation by X-chromosome inactivation in XX individuals. Knowing whether a disease gene is X-linked is thus important in clinical genetics, in particular for Mendelian conditions that can be caused by mutations in more than one gene, some of which may be X-linked and others autosomal (for example, urea cycle disorders or Cornelia de Lange syndrome).

Human genetic diseases are traditionally grouped into recessive, where two mutant versions of a gene are required for disease to occur, versus dominant, where a single mutated copy is sufficient to elicit disease. Due to the presence of only one X chromosome in male individuals, a single copy of a mutated X-linked gene will drive X-linked disease. For women, the expectation is that X-linked dominant but not X-linked recessive disease should manifest in heterozygous individuals. In practice, the phenotypic presentation of X-linked conditions can be more complex [59], with a spectrum ranging from no (or mild) symptoms to full-blown disease in heterozygous individuals (Fig. 4). Diseases that manifest in heterozygous female individuals often have very severe phenotypes in hemizygous male individuals, including embryonic lethality[60]. Of note, aberrations in sex chromosome numbers [61-63] (sex chromosome

aneuploidies), detrimental effects of mosaic expression [64,65] (cellular interference) and escape from X-chromosome inactivation [66-75] can all modulate the manifestation of X-linked disease (Box 1). The term 'X-linked' is favoured over the strict distinction between X-linked recessive and X-linked dominant, whereas the distinction between recessive and dominant manifestation applies more consistently for autosomal variants [59].

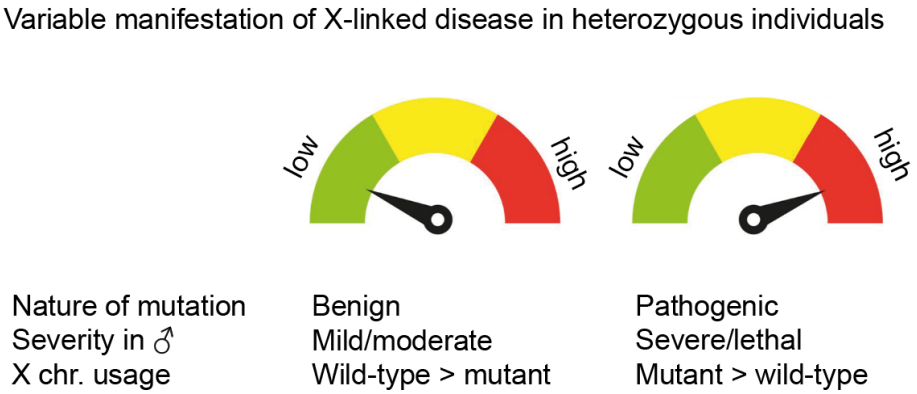


Figure 4. Variable manifestation of X-linked disease in heterozygous individuals. The expressivity of X-linked mutations and the manifestation of X-linked disease in heterozygous individuals can be affected by the nature of the mutation and the disease and is often correlated with the fraction of cells that use the X chromosome harbouring the mutant allele in disease-relevant cell types and tissues.

X-chromosome usage can tune the impact of X-linked disease mutations

Although X-chromosome inactivation is fundamentally random, significant deviations from the expected 50:50 pattern can occur, either by chance or by genetic mechanisms [69]. It therefore seems intuitive that the manifestation of X-linked mutations in heterozygous individuals is often [76-80] (but not always [81,82]) correlated with the fraction of cells that express the X chromosome harbouring the mutant allele in disease-relevant cell types and tissues: skewed usage of the wild-type X chromosome may explain heterozygous females that are healthy or more mildly affected. By contrast, skewing in favour of the mutant over the wild-type allele may explain heterozygous females that present with X-linked disease [80,83] (Fig. 4). Although most clinical studies agree with this prediction, a note of caution is that these usually involve small numbers of patients, and that most disease-relevant tissues are not accessible for analysis of X-chromosome usage. As a case in point, X-linked cerebral adrenoleukodystrophy is caused by mutations in *ABCD1* and principally affects the nervous system. Heterozygous female individuals experience later disease onset and milder symptoms than male individuals and, interestingly, show dominance of *ABCD1*^{mut} cells in blood. However, strikingly, X chromosome usage in blood does not predict the likelihood of symptoms [84], even though transplantation of wild-type (or gene-corrected) haematopoietic stem cells treats the disease. This is because the critical cells in X-linked cerebral adrenoleukodystrophy are not blood cells but microglia, brain support cells of haematopoietic origin, and even partial repopulation of the brain by *ABCD1*$^{wild\text{-}type}$ microglia alleviates the disease [85].

Mechanisms that skew X-chromosome usage in human disease

Chance is likely the biggest factor in skewed X-chromosome usage in X-linked disease; however, deterministic forces are also at play. Rarely, biased X-chromosome choice is programmed from an early stage, for example, by mutations that affect the X-inactivation machinery itself [6] or by X-autosome translocations where clones that inactivate the normal X chromosome may be selected, preventing severe dosage imbalances [86]. In most cases of skewed X-chromosome usage due to disease-causing variants, skewing occurs subsequent

to the initial choice which X chromosome to inactivate [8,77,7987-93]. Various terms have been coined to describe this form of skewed X-chromosome usage, including “hemizygous selection” [94], “somatic selection after X inactivation” [95] and “allelic selection” [10].

Skewed X-chromosome usage can be mechanistically explained for a subset of X-linked mutations that exclude clones expressing X-linked disease mutations from specific cell types or tissues. For example, mutations in *IL2RG* ([96]) or *BTK* ([97,98]) result in cell-intrinsic defects, and consequently an inability of lymphoid progenitors to complete their maturation. The *IL2RG* gene encodes an essential component of a cytokine receptor and is cell-intrinsically required for the survival and differentiation of B-cell as well as T-cell progenitors. Male individuals with *IL2RG* mutations lack B cells and T cells and suffer from X-linked severe combined immunodeficiency (X-SCID). Because *IL2RG* mutations have a severe impact on the fitness of B-cell and T-cell progenitors, selection explains why only those clones that express wild-type *IL2RG* form B and T cells in heterozygous female individuals [96].

Similarly, the *BTK* gene encodes Bruton's Tyrosine Kinase, which mediates pre-B cell receptor signalling and is therefore cell-intrinsically required for the differentiation of B-cell (but not T-cell) progenitors. Male individuals with *BTK* mutations lack B cells and suffer from X-linked agammaglobulinemia (XLA), indicating that *BTK* mutations severely impact the fitness of B-cell progenitors. In female carriers of *BTK* mutations, only clones with an active X chromosome that harbours the wild-type *BTK* allele can differentiate into B cells, explaining skewed X-chromosome usage [97,98].

Mutations in the Wiscott–Aldrich Syndrome gene *WAS* ([99,100]) and the incontinentia pigmenti gene *NEMO* (*IKBKG*) also seem to directly impair the fitness of blood cells and thereby drive the selection of clones expressing the wild-type X chromosome. WAS protein (WASP), which has cell-intrinsic functions in platelets and lymphocytes, including cell proliferation, differentiation, homeostasis, and survival. *WAS* mutant males have dysfunctional lymphocytes and X-linked primary immune deficiency[99,100]. In clinically normal heterozygous females, all platelets and T cells use the X chromosome that harbours the wild-type allele [89,101]. Males with *NEMO* mutations show X-linked immune deficiency and other immunological abnormalities [102,103], indicating a cell-autonomous requirement for NEMO, and in *NEMO* heterozygous females ~98% of blood leukocytes use the X chromosome that harbours the wild-type allele [104].

For other X-linked mutations, the mechanisms that favour X chromosomes harbouring wild-type or mutant alleles remain less well understood. For example, mutations in the *ABCD1* gene associated with X-linked adrenoleukodystrophy drive skewing in blood cells in favour of the X chromosome that harbours the mutant allele [85,90,105], whereas *HPRT1* mutations associated with Lesch–Nyhan syndrome usually skew in favour of the wild-type X chromosome [79,87]. Skewing in favour of the wild-type X chromosome has been attributed to selection against HPRT-deficient cells at the level of haematopoietic stem cells [91], which may depend on an intact purine salvage pathway and therefore on HPRT activity [106]. Interestingly, some *HPRT1* heterozygous females show characteristic manifestations of Lesch–Nyhan syndrome, and these individuals preferentially express mutant *HPRT1* ([107,108]).

X-chromosome usage has been suggested as an indicator of pathogenic X-linked mutations [109,110]. However, X-chromosome usage is clearly not a failsafe mechanism against X-linked disease, as it does not necessarily favour the expression of X-linked alleles that are beneficial for the host [84,90,105]. Skewing of X-chromosome usage in blood increases sharply with age and reflects the accumulation of somatic mutations that confer a competitive clonal advantage in age-related clonal haematopoiesis [111-116]. However, driver mutations in this scenario are mostly autosomal, in which case X-chromosome usage is merely a marker for the expansion of super-competitor clones that happen to express one or the other X chromosome.

Moreover, even if X-chromosome usage is skewed away from the X chromosome that harbours the mutant allele in blood, skewed X chromosome usage in blood alone may not protect against the manifestation of X-linked diseases for which blood is not the disease-relevant tissue. Indeed, blood is often skewed in favour of the wild-type X chromosome in female patients with X-linked intellectual disability [77] including patients with cohesinopathies, holoprosencephaly and other clinical syndromes associated with heterozygous mutations in the X-linked cohesin gene *STAG2* ([22-25]) and the X-linked cohesin regulator *HDAC8* ([19-21]). X chromosome usage in the brain of these patients has not been assessed, but it is notable that in mouse models, X-linked competition can strongly favour the wild-type X chromosome in blood while allowing both X chromosomes to contribute equally to brain and other organs [13] (Fig. 2).

Conclusions and future directions

Human populations comprise a rich spectrum of phenotypic diversity, driven in part by germline variation between, as well as somatic mutation within, human individuals. Genetic variation on the X chromosome behaves differently from genetic variation on autosomes [117]: X-linked germline variants are heritable but active only in a subset of cells in each XX individual due to X-chromosome inactivation and its epigenetic propagation. X-linked variants therefore contribute to intra-individual (epi)genetic diversity, and clones expressing distinct genetic variants may interact with each other to shape the contribution of X-linked clonal diversity across cell types and tissues in XX individuals [13]. It will be key to investigate the spectrum of X-linked variants that trigger X-linked competition, and how frequently this process occurs in XX individuals.

As previously demonstrated for classic cell competition [118], X-linked competition curtails clonal diversity in affected tissues. In this way, X-linked competition effectively sacrifices the complement of X-linked genetic variation present on one X chromosome. Future work will pursue potential consequences of skewed X-chromosome usage. For example, skewed X-chromosome usage has been preliminarily associated with both risk [113] and protection [119] from disease. Of note, it remains to be determined whether the exclusion of *STAG2* variant-expressing clones from lymphoid specification and differentiation has adaptive value, for example, by safeguarding against potentially deleterious consequences of *Stag2* variant expression. Of note, somatically acquired *STAG2* mutations are associated with haematopoietic malignancies [120,121] where they disrupt normal haematopoiesis and contribute to myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML).

This Perspective featured many examples of blood cell phenotypes. High cell turnover over the lifetime may render the blood particularly prone to X-linked competition, and it will be important to explore the potential link between turnover and competition in other tissues with high turnover such as the skin or the intestine. Differential fitness or developmental plasticity of cells could potentially be acted upon by any of multiple cellular differentiation waypoints across one or more tissues.

The cellular and molecular mechanisms that drive X-linked clonal dominance are currently unknown. Elucidating these mechanisms will be of interest for understanding clonal dynamics in organismal development, development and disease. The resulting insights may be clinically relevant since the severity of symptoms in female carriers of certain X-linked disease mutations depends at least in part on the clonal balance between clones expressing wild-type and mutant X chromosomes (Fig. 4). Harnessing the mechanisms that underlie competition may offer opportunities for redressing this balance. If one could direct X-chromosome usage away from disease-associated and in favour of wild-type alleles, this would potentially ameliorate manifestations of X-linked disease in females affected by X-linked disorders. Approaches to alter the representation of clones that express the wild-type versus

the mutant X chromosome in blood could have broad clinical applications in X-linked myelodysplastic, autoinflammatory or autoimmune syndromes, XLR immunodeficiencies, anaemias or thrombocytopenias. Outside of blood disorders, potential applications could include female OTC deficiency where it would potentially be curative if one could turn the minority of wildtype *OTC*-expressing liver cells into a majority. Urgent remaining questions therefore include what criteria are used by the X-linked competition process to distinguish winner and loser cells, and how X-linked competition prevents designated loser cells from realizing their developmental potential.

Although the focus of this Perspective has been on competition, clonal interactions may in principle also be cooperative, symbiotic, parasitic or commensal [122], and it will be of interest to explore these forms of interactions in the context of X-linked sequence diversity.

Box 1. Modifiers of X-linked disease

Sex chromosome aneuploidies. Some individuals have unusual sex chromosome numbers. Known as sex chromosome aneuploidies, these include X0 (Turner syndrome), XXY (Klinefelter syndrome, and XXX (triple X syndrome). This can alter the penetrance of X-linked conditions as illustrated by XO individuals who present with X-linked recessive conditions [61] or XXY individuals who present with attenuated X-linked disease phenotypes [62]. Acquired sex chromosome aneuploidies can occur by the loss of sex chromosomes from somatic cells [63].

Cellular interference. In a select few conditions, disease manifestation can be more severe in heterozygous females than in hemizygous males. Mutations in the X-linked genes *EFNB1* ([64]) and *PCDH19* ([65]) have more serious clinical manifestations in heterozygous females than in hemizygous males. This indicates that mosaic expression of *EFNB1* or *PCDH19* is worse than no expression at all, a phenomenon described as 'cellular interference'.

Escape from X chromosome inactivation. Not all genes located on the X chromosome are subject to X chromosome inactivation. Some 15% of human X-linked genes escape X chromosome inactivation, either partially or entirely, and this can contribute to sex-biased expression [69]. A subset of X-linked genes is therefore expressed from two active copies in XX individuals and may show an autosome-like pattern of inheritance, which can affect the manifestation of X-linked disease [66,67]. Interestingly, the extent of escape can vary from individual to individual [66]. The biallelic expression of X-linked immune genes may contribute to autoimmunity, which is markedly more common in XX individuals [70-73]. For example, genes that are normally silenced by XIST are overexpressed in patients with SLE compared to healthy donors [74], and their expression can be induced by inflammation in atypical B cells linked to autoimmunity [75].

The potential impact of escape from X chromosome inactivation X-linked competition is of interest but currently unknown: on the one hand, biallelic expression of escapee genes may buffer and thereby diminish phenotypic effects [80]. On the other hand, one may speculate that lymphocytes that overexpress X-linked immune genes as a result of escape from X inactivation expand preferentially and thereby gain an advantage.

A special case of “escape” involves genes in pseudoautosomal regions, short segments of homology between the mammalian X and Y chromosomes that recombine during meiosis. As an example, the PAR1-localized gene *SHOX*, a gene related to several short stature syndromes, is expressed from both X chromosomes in XX individuals and from both the X and Y (where it is called *SHOXY*) in XY individuals, and syndromes resulting from variants in this gene are inherited in a fashion that resembles autosomal rather than sex chromosome genes [68].

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Author contributions.

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Competing interests.

The authors declare no competing interests.