Received: 10 March 2020 | Revised: 1 May 2020 | Accepted: 3 May 2020

DOI: 10.1002/ajmg.c.31800

RESEARCH REVIEW

AMERICAN JOURNAL OF medical genetics PART C Seminars in Medical Genetics

WILEY

Genes that escape from X-chromosome inactivation: Potential contributors to Klinefelter syndrome

Maria Jose Navarro-Cobos | Bradley P. Balaton | Carolyn J. Brown

Department of Medical Genetics, Molecular Epigenetics Group, Life Sciences Institute, Vancouver, British Columbia, Canada

Correspondence
Carolyn J. Brown, Department of Medical Genetics, Molecular Epigenetics Group, Life Sciences Institute, 2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada.
Email: carolyn.brown@ubc.ca

Funding information
Canadian Institutes of Health Research, Grant/Award Number: PJT-162120

Abstract

One of the two X chromosomes in females is epigenetically inactivated, thereby compensating for the dosage difference in X-linked genes between XX females and XY males. Not all X-linked genes are completely inactivated, however, with 12% of genes escaping X chromosome inactivation and another 15% of genes varying in their X chromosome inactivation status across individuals, tissues or cells. Expression of these genes from the second and otherwise inactive X chromosome may underlie sex differences between males and females, and feature in many of the symptoms of XXY Klinefelter males, who have both an inactive X and a Y chromosome. We review the approaches used to identify genes that escape from X-chromosome inactivation and discuss the nature of their sex-biased expression. These genes are enriched on the short arm of the X chromosome, and, in addition to genes in the pseudoautosomal regions, include genes with and without Y-chromosomal counterparts. We highlight candidate escape genes for some of the features of Klinefelter syndrome and discuss our current understanding of the mechanisms underlying silencing and escape on the X chromosome as well as additional differences between the X in males and females that may contribute to Klinefelter syndrome.

KEYWORDS

dosage compensation, escape from XCI, Klinefelter syndrome, sex chromosome aneuploidy, X-chromosome inactivation

1 | INTRODUCTION

The human sex chromosomes are derived from ancestral autosomes that diverged 166–190 million years ago (mYa) when the Y chromosome acquired sex-determining function (reviewed in Marshall Graves (2016)). The once homologous X and Y chromosomes then diverged as the Y chromosome specialized, favoring loss of recombination between the emergent gametologs, and leading to degradation of much of the ancestral Y chromosome (Marshall Graves, 2016). The loss of Y genes led to the need for dosage compensation—both between the now single X-linked copy of genes and the ancestral autosomes; as well as between the single copy of X-linked genes in males and the two copies in females. How (and indeed, whether) genes became upregulated on the X chromosome to maintain ancestral levels of expression has been debated (reviewed in Disteche (2016) and Veitia, Veyrunes, Bottani, and Birchler (2015)). Equivalent X-linked gene expression between XX females and XY

Maria J. Navarro-Cobos and Bradley P. Balaton authors contributed equally to the manuscript.

males is generally achieved by X-chromosome inactivation (XCI), the transcriptional silencing of one X chromosome in females (Lyon, 1961). Loss of Y genes occurred in a stepwise fashion as recombination with the X chromosome was lost, and thus the pressure for XCI has existed for different lengths of time across the evolutionary strata of the X chromosome (Lahn & Page, 1999).

The inactive X chromosome (Xi) is silenced by the recruitment of multiple heterochromatin pathways, initiated in eutheria (placental mammals) by transcription of a long noncoding RNA named XIST. XIST expression from the Xi recruits a series of RNA binding proteins (Chu et al., 2015; McHugh et al., 2015; Minajigi et al., 2015) including SPEN that recruits histone deacetylase (Dossin et al., 2020); HNRNPK that recruits the polycomb complexes PRC1 and PRC2 (Pintacuda et al., 2017); and matrix-associated proteins (such as CIZ1 (Ridings-Figueroa et al., 2017) and HNRNPU (Sakaguchi et al., 2016)) that may contribute to phase-separation (Cerase et al., 2019). The Xi acquires additional features of silent heterochromatin, including late replication (Koren & McCarroll, 2014) and promoter DNA methylation (Cotton et al., 2015). Despite these multiple pathways to ensure silencing, some genes on the Xi continue to be expressed. While the silencing of all but one X chromosome is sufficient to allow viability of some individuals with X chromosome aneuploidies, the incomplete silencing of the Xi could be a significant contributor to the phenotypes associated with such aneuploidies. We will review the evidence for genes that escape from XCI; which genes escape from XCI and how they might contribute to Klinefelter syndrome (KS); and what we understand about how genes "escape" silencing and contribute to sex differences.

2 | EVIDENCE THAT GENES ESCAPE FROM XCI

Advances in genomic techniques have permitted the analysis of transcriptional activity from the Xi for over 600 of the over 1000 X-linked genes. Underlying limitations to determining the inactivation status of genes include restricted expression of the gene (e.g., over 100 cancer-testes antigen genes account for 25% of the unclassified genes), low levels of expression of some genes (particularly in accessible human tissues), or limited informative polymorphisms within genes (Balaton, Cotton, & Brown, 2015). Overall, the level of expression from the Xi is not as high as from the active X chromosome (Xa), with a general threshold for calling escape from XCI being Xi expression greater than 10% of the level of expression from the Xa (Carrel & Willard, 2005), although some methods of detecting Xi expression are robust to detecting even lower levels of relative expression. While some genes appear to be consistent in escape from XCI in all individuals and all tissues, many genes are variable in their inactivation, escaping from XCI in some individuals, tissues or cells (see Figure 1; Balaton et al., 2015). No consistent threshold for proportion of cells/individuals/tissues has been established, leading to some studies describing very high proportions of genes classified as (variably) escaping from XCI. Below we discuss many of the approaches that have been used for identifying genes that escape or variably escape from XCI.

2.1 | Biallelic expression

The most definitive evidence that genes escape from XCI is observing expression from both alleles of the gene (biallelic expression). However, as females are mosaic for populations of cells with each parental X being active, such an approach requires examining a sample which is significantly skewed for which X chromosome is inactivated. There are multiple causes for skewing (reviewed in Vacca, Della Ragione, Scalabrì, and D'Esposito (2016)), with skewing being more common in blood (Fey et al., 1992). A further limitation is the need for an informative expressed polymorphism in such a skewed population. A study examining XCI status in lymphoblast cell lines from the HapMap Project as well as fibroblasts found over 30% of genes were variable between informative individuals (see Figure 1a; Cotton et al., 2013). A single individual with skewed XCI was identified in the extensive GTEx project, allowing detailed analysis of tissue-specificity, finding that 10.6% of genes variably escaped across the 16 tissues examined, with 5.8% of genes only escaping in a single tissue (see Figure 1b; Tukiainen et al., 2017).

Biallelic expression can also be used to find genes that escape from XCI in single cell RNA-seq of samples without skewing of XCI (Tukiainen et al., 2017; Wainer Katsir & Linial, 2019); reviewed in Keniry and Blewitt (2018)). The GTEx study saw variation in the XCI status of genes within an individual depending on which allele was on the Xi (e.g., *MSL3*, see Figure 1c), or, as for the gene *TIMP1*, this variation was random and did not depend on which allele was on the Xi. Additionally, another single cell RNA-seq study found heterogeneity in XCI status between cells, with some cells having more escape genes than others depending on cell cycle and XIST expression level, a finding that had not been seen by other approaches (Balaton et al., 2015; Garieri et al., 2018). Single cell RNA-seq is most robust for well-expressed genes, and approaches that analyze only the 3′ end of the gene restrict the frequency of polymorphisms. In the absence of a polymorphism, fluorescence in situ hybridization of RNA (RNA-FISH) can be used to see expression from the Xa and Xi in single cells and visualize the biallelic expression of genes escaping from XCI (Al Nadaf et al., 2012), an approach that also detects heterogeneity between cells.

2.2 | Somatic cell hybrids

Expression from the Xi and Xa can be separated using rodent-human hybrids where a human cell is fused to a rodent cell and then selected to retain either the Xa or Xi. Active and inactive X-containing hybrid cells can then be examined for the expression levels of the human genes in comparison to housekeeping genes in the rodent. Such an approach obviates the need for polymorphisms to distinguish the X chromosomes, but relies on retention of expression patterns in the hybrid cells. An extensive survey of escapees demonstrated remarkable consistency between allelic calls and hybrids (Carrel & Willard, 2005). Nine different Xi-containing hybrids were used, and heterogeneous expression between hybrids (defined as genes

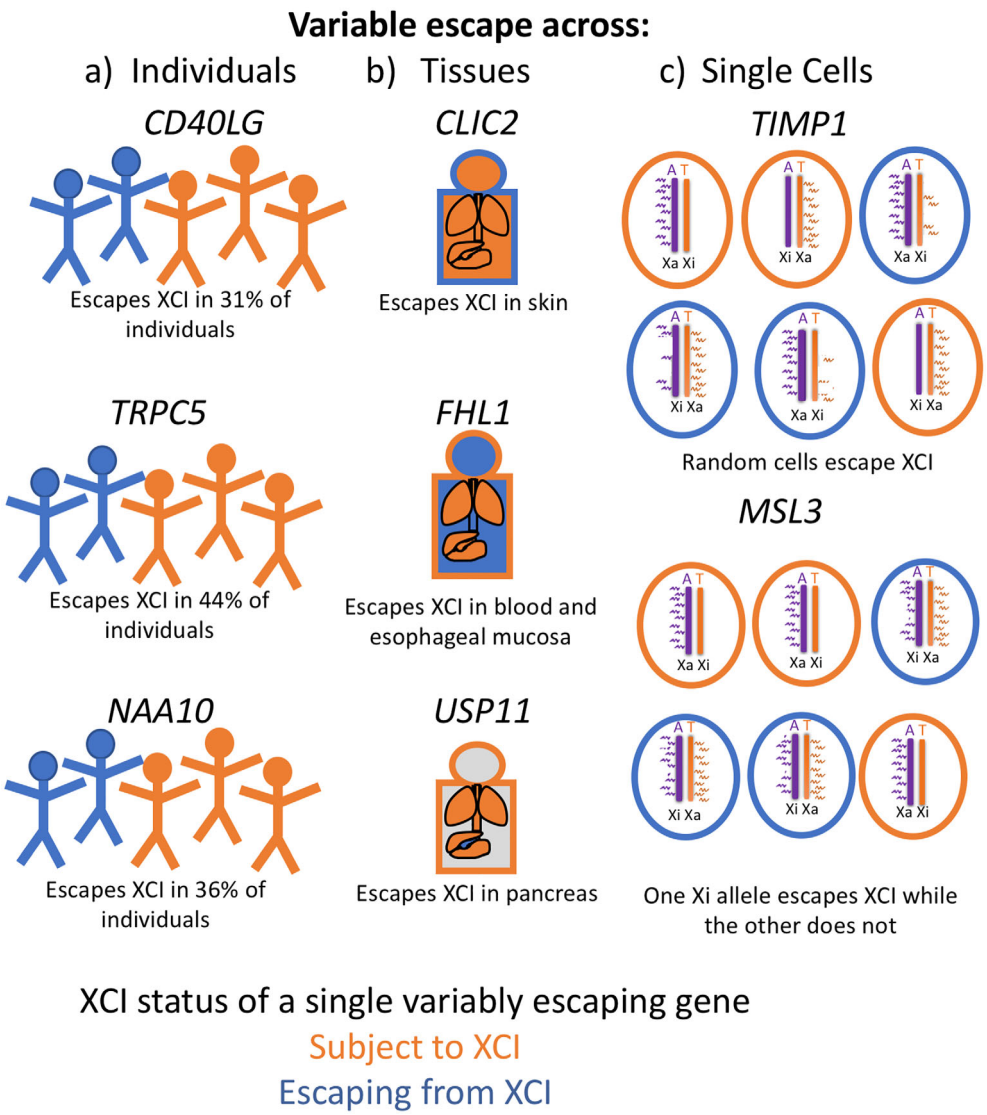

FIGURE 1 Different scales of variable escape from XCI. (a) Genes variably escape from XCI such that the gene escapes XCI in some individuals and is subject to XCI in other individuals (Cotton et al., 2013). (b) Tissue-specific variable escape from XCI is observed for some genes, which have a consistent XCI status in some tissues, but the opposite XCI status in other tissues (Tukiainen et al., 2017). (c) Heterogeneous escape from XCI occurs at the level of single cells, with some cells escaping from XCI while other cells are subject to XCI. *TIMP1* shows a random pattern while for *MSL3* one allele is subject to XCI while another allele escapes from XCI (discussed in Tukiainen et al., 2017)

expressed in three to six lines) was seen for 10% of genes examined. Overall, however, the various hybrid lines had similar numbers of escaping genes, suggesting that the variation in escape was due to a feature intrinsic to the genes and not the hybrid environment or the chromosome.

2.3 | Expression level comparisons

Another approach to differentiate expression between the Xi and Xa is to compare expression between males and females, and this was extensively analyzed with the GTEx samples (Tukiainen et al., 2017). While escape from XCI would mathematically result in more expression in females (Xa + Xi) than males (Xa), mechanisms such as feedback regulation that limit total aggregate expression would biologically limit such differences. Additionally, other factors that differ between the sexes, notably hormones, will confound the ability to attribute expression to genes that escape from XCI. Extending the analysis to include sex chromosome aneuploids by comparing XY to XXY or X to XX can assess the impact of an Xi within a sex, but can be confounded by secondary effects associated with aneuploids (discussed below). Comparison of expression levels in X, XX, XY, XXY, XYY, and XXYY individuals showed clear effects of X chromosome ploidy on the escape genes (see Figure 2; Raznahan et al., 2018). There were also 6 Y genes with statistically significant expression changes with increasing number of Y chromosomes, all of which have conserved X homologs. Additional regulatory influences are apparent however, as increasing X or Y count did not increase expression linearly, there were autosomal effects from increasing X or Y ploidy, and there was a clear impact of the Y chromosome on X-linked gene expression. Figure 2 shows the most consistently described genes that escape from XCI (Balaton et al., 2015), along with their expression patterns and female: male expression ratios from GTEx (https://gtexportal.org/home/). Clearly, there is substantial variation in the extent of sex-biased expression across tissues. We summarize the impact on escape gene expression in KS (Belling et al., 2017; Raznahan et al., 2018; Skakkebæk et al., 2018; Zhang et al., 2020; Zitzmann et al., 2015) in Figure 2. Within PAR1, relative expression is

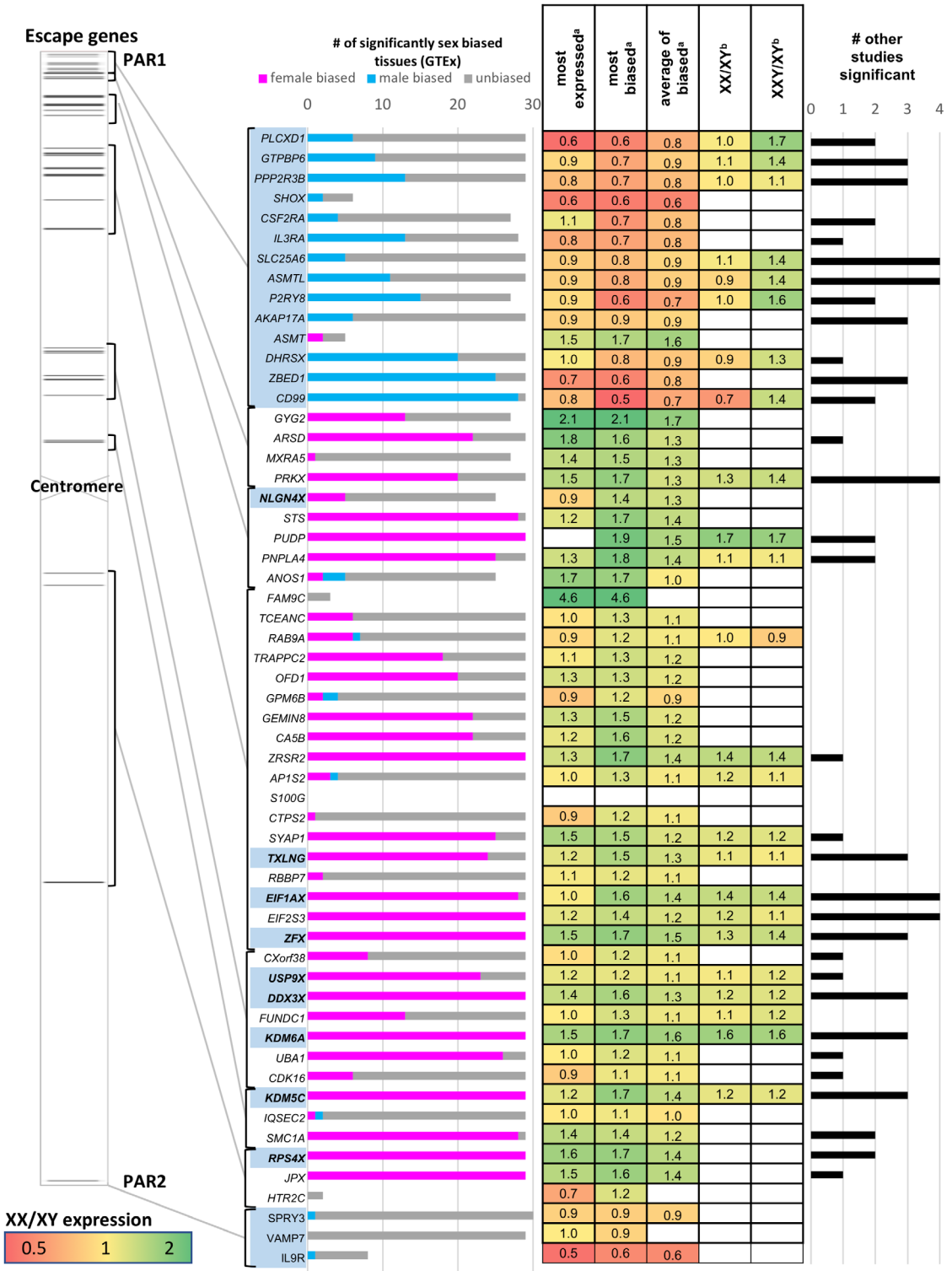

FIGURE 2 Sex differences in expression for genes in the PARs or that escape from XCI. The genes shown are ones which have been shown to escape from XCI in multiple studies. At the left, a schematic shows the location of the PARs and genes escaping XCI. The number of tissues in GTEx (out of 29) with expression and sex bias per gene are shown (center left). The XX/XY expression ratio per gene for the tissues in GTEx ([a]) with the most expression, the most biased expression, or averaged for the significantly sex-biased tissues. The final two columns show matched XX/XY and XXY/XY ratios for lymphoblastoid cell lines ([b]) from Raznahan et al. (2018) (center right). The number of other studies with significant XXY/XY expression per gene (out of four studies: Belling et al., 2017; Skakkebæk et al., 2018; Zitzmann et al., 2015; Zhang et al., 2020) is shown to the right. Genes with Y homology are shown in blue, with those outside the PARs in bold font

higher in KS, while for escape genes outside of the PAR, relative expression of KS to male is similar to female to male ratios.

2.4 | Epigenetic differences

DNA methylation is also used to identify genes escaping XCI, as the Xa and Xi differ greatly in their methylation (Cotton et al., 2015). In females, genes subject to XCI appear hemimethylated, with one X being hypermethylated and one X being hypomethylated, while genes escaping XCI are hypomethylated on both chromosomes. Genes escaping XCI also differ from genes subject to XCI in their gene body methylation (Cotton et al., 2015), as transcription-associated proteins and histone marks lead to DNA methylation (Teissandier & Bourc'his, 2017), although this difference is much less dramatic than that seen at promoters. Differences in non-CG dinucleotides in gene bodies have also been seen between genes subject to or escaping from XCI, particularly in the brain (Keown et al., 2017; Lister et al., 2013) but also in other tissues (Schultz et al., 2015). While DNA methylation-predicted XCI status was seen to be constant across tissues (finding only three genes that have one tissue consistently subject to XCI and another tissue consistently escaping); DNA methylation suggests 29% of genes are variable between individuals in escape from XCI (in at least one tissue, see Figure 1; Cotton et al., 2015).

DNA methylation levels on the X chromosomes of Klinefelter patients were distinct from both XX and XY controls (Skakkebæk et al., 2018) and also between KS and Turner syndrome (TS) patients, with hypomethylation in TS and hypermethylation in KS (Zhang et al., 2020). A large difference from XY controls would be expected given the presence of an Xi; but the difference from females with a similar Xa/Xi X chromosome complement further supports an impact of the Y chromosome upon XCI. DNA methylation changes were distinct from those genes with methylation changes in TS (Sharma et al., 2015). As with expression analyses, not all of the DNA methylation changes were on the X chromosome, the autosomes also differed from controls (Sharma et al., 2015; Skakkebæk et al., 2018). DNA methylation at *XIST* (an Xi-specific gene) and *FMR1* (an Xi inactivated gene) was enough to differentiate XY from XXY (Mehta et al., 2012).

Other epigenetic and regulatory differences can be seen between genes subject to or escaping from XCI. Histone modifications associated with inactive genes are enriched at genes subject to XCI and depleted on genes escaping XCI while those associated with active marks are enriched at genes escaping XCI and depleted on genes subject to XCI (reviewed in Balaton and Brown (2016)). RNA polymerase II occupancy (Kucera et al., 2011) and transposase accessibility (Qu et al., 2015) are both active marks that are noticeably enriched at genes escaping XCI.

Combining results from many studies using a variety of the aforementioned methods, we previously attained an XCI status call for 639 genes, with 12% of genes escaping from XCI (Balaton et al., 2015). In addition, another 8% of genes vary in their XCI status between populations, tissues or individuals within studies and 7% of genes were found consistent within studies, but discordant across studies (Balaton et al., 2015). As the number of studies, and breadth of tissues increase, the number of genes identified to variably escape from XCI is anticipated to rise. In Figure 1 we distinguish variable escape genes (which differ between individuals) from tissue-specific variable escape (wherein escape occurs in one tissue rather than another) and heterogeneous escape in which there is cell to cell variability. The timing of XCI has not been well-established in humans (Petropoulos et al., 2016), but in mice the presence of two Xa prior to XCI establishment is suggested to prime the epigenome for later gene expression differences (Deegan, Karbalaei, Madzo, Kulathinal, & Engel, 2019; Engel, 2018).

3 | THE GENES THAT ESCAPE FROM XCI

Those genes that retain Y homology were predicted by Lyon to escape from XCI (Lyon, 1962), thus keeping two active copies in both males and females. Pseudoautosomal regions (PARs) are homologous segments present on both the X and Y chromosomes. PAR1 is located at the end of the short arm of both sex chromosomes and has an essential role in recombination during male meiosis. The region spans 2.7 Mb, contains at least 25 genes, and all genes examined escape from XCI (Balaton et al., 2015). The PAR2 in humans is at the end of the long arm of the X and Y chromosomes, having a length of 320 kb and, contrary to PAR1, in this region recombination is not a common event (Raudsepp & Chowdhary, 2015). The PAR genes with known X inactivation status are shown in Figure 2. Genes in PAR1 tend toward a significant male bias in expression, but not in all tissues in which they are expressed, and *ASMT* often shows a female bias in expression. In the most sex-biased tissues, expression in males is almost 40% higher than in females; whereas for the tissues with the most expression, the differential is less than 15% on average. This suggests that the Xi is not as well expressed as the Y chromosome, but that either Y expression or X repression varies between tissues. Within PAR2, there is limited sex-differential expression for *SPRY3* and *VAMP7*, while *IL9R* is expressed in limited tissues, but shows a strong male bias in expression. *SPRY3* and *VAMP7* are subject to XCI (De Bonis et al., 2006), as well as being silenced on the Y chromosome, while the more recently acquired terminal *IL9R* gene escapes from XCI (Ciccodicola et al., 2000). Regulation of this region is influenced by more than DNA sequence, as the Xi appears more condensed than the Y and is more sensitive to loss of DNA methylation. Yet the *SPRY3* and *VAMP7* genes are associated with repressive histone marks and located within or at the edge of the chromosome territory when silenced on both the Xi and Y, while the Xa allele is less frequently inside the chromosome territory (Matarazzo, Boyle, D'Esposito, & Bickmore, 2007).

Another X-Y homologous region in the long arm of the X chromosome (Xq21.3/Yp11.2) has almost 4 Mb of shared DNA generated approximately 6 mYa by duplication onto the Y chromosome and subsequent inversion; resulting in deletion of *PABPC5* and truncation of *TGIF2LY*, leaving *PCDH11X/Y* as gametologs in the region (Priddle &

Crow, 2013). The *PCDH11X* promoter is unmethylated suggesting that it escapes from XCI (Lopes et al., 2006); however, replication asynchrony, a feature generally associated with monoallelic expression, was observed for the region (Wilson et al., 2007).

While Y homology is tightly associated with the X-linked gametologs that escape from XCI; many of the genes that escape lack a functional Y homolog (Balaton et al., 2015). There is an unequal distribution of the genes that escape from XCI along the X chromosome, with substantially more escape genes being found in regions which lost recombination with the Y chromosome more recently (Carrel & Willard, 2005). Figure 2 details the genes that consistently escape from XCI across multiple studies as described above, and of these only *RPS4X*, *JPX*, and *HTR2C* are from the long arm of the X chromosome. As seen in Figure 2, the non-PAR genes with Y homology show a female-expression bias in the majority of tissues, while the other genes that escape from XCI show expression bias (again, higher in females) in only a subset of expressed tissues. The extent of sex bias is similar (over 50% in the most sex-biased tissue, but 25–30% in the most expressed tissue) between genes that escape from XCI, regardless of whether there is a Y gametolog; however, for those genes with a gametolog, the Y version may compensate for the Xi expression or may have evolved distinct functions. Not shown in Figure 2 are genes that are variable between or within studies, and these generally show less dramatic, but still potentially biologically significant differences in expression between the sexes. Overall, escape genes do not show significant ontologies (PANTHER.db.org); however, 12 of the non-PAR escape genes are members of a set of ancestral Y genes that are functionally conserved across mammals due to dosage-sensitivity likely reflecting their important roles as transcription factors or chromatin regulators (Bellott et al., 2014; Naqvi, Bellott, Lin, & Page, 2018).

4 | ROLE FOR GENES THAT ESCAPE FROM XCI IN KS

The role of a supernumerary X chromosome in KS was discovered in 1959. 80–90% of the KS patients have the classical karyotype 47,XXY, while the rest show multiple aneuploidies such as 48,XXXY; 48,XXYY; 49,XXXXY. With an incidence of 1 in 600 births, KS is the most common sex chromosomal aneuploidy. The actual number of KS men could be higher as a substantial proportion remain undiagnosed (Bird & Hurren, 2016; O'Connor, Snyder, & Hayes, 2019; Samango-Sprouse et al., 2019; Shiraishi & Matsuyama, 2019). As the supernumerary X becomes inactivated, it is natural to question whether it is the genes that escape from XCI that contribute to the KS phenotype. Yet, surprisingly, the only well-established genotype/phenotype correlation with escape genes and KS is for stature (discussed below), thus the story is clearly more complex than single gene—single phenotype. To find candidate genes underlying KS, expression analyses have been conducted to find differentially expressed genes (DEGs) or differentially methylated regions (DMRs) in aneuploid patient samples compared with healthy controls. We have included results for several expression studies in Figure 2. An important caveat to these studies is that overexpression of escape genes need not result in overexpression of the protein product. Post-translational regulation has been implicated for proteins as part of multiprotein complexes (Veitia, 2010).

The unique evolutionary history of the X chromosome impacts the genes that are present on the chromosome as it spends more time in females, yet mutations are immediately hemizygous and exposed to selection in males (reviewed in Wilson Sayres (2018)). It has been argued this results in a "smart and sexy" chromosome (Graves, Gécz, & Hameister, 2002), which may be relevant to many of the characteristics of KS. In the following sections, we highlight the potential involvement of particular genes in not only stature, but also the immune response, cancer, gonadal development, and neurodevelopment.

4.1 | Escape genes and stature

Tall stature in KS patients can be detected at an early age, and in adulthood it is observed as an increase in leg length, decreased body ratio and a mean height greater than the 95th percentile. Increased height is generally attributed to an extra copy of the PAR1 gene *SHOX*, but testosterone deficiency can also contribute via delayed epiphyseal fusion (Samango-Sprouse et al., 2019). Beyond the widely known influence of *SHOX*, there are no reports of other genes contributing with tall stature in KS. Loss of *SHOX* expression is also causative for the short stature and skeletal features in TS, while an excess of *SHOX* expression in females with supernumerary X chromosome has also been reported (Fukami, Seki, & Ogata, 2016; Skakkebæk et al., 2018; Tuttelmann & Gromoll, 2010). The *SHOX* gene (short stature homeobox), encodes a transcription factor that controls chondrocyte proliferation and differentiation in the growth plate. Haploinsufficiency is implicated in idiopathic short stature (ISS) and Leri-Weill dyschondrosteosis, and can be treated with recombinant growth hormone (Genoni et al., 2018). A role for *SHOX* in microdeletions that cause infertility was excluded (Chianese et al., 2013), thus so far no other effects besides stature have been correlated with this gene.

4.2 | Escape genes and the immune response

The X chromosome is often considered to be overrepresented for immune-related genes, although the observation has recently been questioned (Meester et al., 2020). Autoimmune diseases are diagnosed at strikingly elevated incidence in KS, approaching a predisposition similar to normal females (Syrett & Anguera, 2019). In a cohort of 2,208 men with KS compared with a control cohort of 5 million men in the UK a significantly increased risk for seven diseases was detected: Addison's disease (relative risk - RR: 11.7); diabetes mellitus type 1 (RR: 6.1); multiple sclerosis (RR: 4.3); acquired hypothyroidism (RR: 2.7); rheumatoid arthritis (RR: 3.3); Sjögren syndrome (RR: 19.3); and systemic lupus erythematosus (SLE) (RR: 18.1) (Seminog, Seminog, Yeates, & Goldacre, 2015). SLE is not only widely described in patients

with KS, it is underrepresented in TS (Tuttelmann & Gromoll, 2010). There are further reports of KS patients with other rheumatic diseases, for instance, juvenile idiopathic arthritis, psoriatic arthritis, polymyositis/dermatomyositis, systemic sclerosis and mixed connective tissue disease (Rovenský, Imrich, Lazúrová, & Payer, 2010).

Candidate genes for the female (and KS) excess in SLE include the *TLR7* and *CD40LG* genes found overexpressed in females and KS males but underexpressed in TS (Sarmiento, Svensson, Barchetta, Giwercman, & Cilio, 2019). *TLR7* is part of the toll-like receptor (TLR) family with a role in viral response and activating innate immunity. A significant association of *TLR7* and SLE has been detected in genome-wide association studies (Laffont & Guéry, 2019; Souyris et al., 2018) and *TLR7* biallelic expression has been observed in immune cells from both women and KS patients (Souyris et al., 2018). Interestingly, the biallelic expression of *TLR7* and *CD40LG* was reported to occur concomitantly with loss of XIST and heterochromatic mark localization during T cell development (Syrett & Anguera, 2019). The variable escape gene *CXorf21* is highly expressed in transformed lymphocytes and its protein colocalizes with *TLR7* (Odhams et al., 2019). Additionally, when *Tlr7* is duplicated in Yaa mice they are prone to autoimmunity (Syrett & Anguera, 2019) and *Tlr7* can be activated in murine models of Sjögren syndrome, another autoimmune disease with female and KS male bias (Kiripolsky & Kramer, 2018). *CD40LG* participates in the regulation of B cell activation, and overexpression causes autoimmunity in mice and a microduplication including the gene in humans resulted in various autoimmune diseases (Syrett & Anguera, 2019).

KS overexpression of PAR genes (Figure 2; average 1.4 relative to XY) is greater than of other genes that escape from XCI (Figure 2; average 1.25 (Raznahan et al., 2018)), and multiple genes in the PAR have functions relevant to immunity. Gene set enrichment analysis revealed that the "cytokine-cytokine receptor interaction" pathway involved two PAR1 genes (*IL3RA* and *CSF2RA*), which were upregulated in KS (Belling et al., 2017). *CSF2RA* (colony-stimulating factor 2 receptor, alpha) is a cytokine subunit that has an important role in the production, differentiation, and function of granulocytes and macrophages (Mangs & Morris, 2007). *IL3RA* (interleukin 3 receptor, alpha) encodes a subunit of the receptor for interleukin 3 (Mangs & Morris, 2007) and its aberrant expression is associated with poor prognosis in acute myeloid leukemia (Arai et al., 2019). Another PAR1 gene, *CRLF2* (cytokine receptor-like factor 2), encodes for the thymic stromal lymphopoietin receptor that promotes the proliferation of CD4+ T cell (Mangs & Morris, 2007) and is overexpressed (and often rearranged) in acute lymphoblastic leukemia (ALL; Potter et al., 2019). The protein encoded by *CD99* is a cell surface glycoprotein involved in leukocyte migration, T-cell adhesion and activation of caspase-independent death pathway in T-cells (Mangs & Morris, 2007), with abnormal expression associated with solid and hematologic malignancies (Cardoso et al., 2019; Chen et al., 2019; Koufopoulos et al., 2019). *IL9R* (interleukin 9 receptor), an escape gene in PAR2, is reported to be contributing to psoriatic arthritis and rheumatoid arthritis (Raychaudhuri, Abria, Maverakis, & Raychaudhuri, 2018). Notably, many of these immune-related genes are linked to cancers.

4.3 | Escape genes and the EXiTS hypothesis of cancer

There is a well-established male bias to many cancers, and six X-chromosome genes that escape or variably escape from XCI (*ATRX*, *CNKSR2*, *DDX3X*, *KDM5C*, *KDM6A*, and *MAGEC3*) were found to have loss-of-function mutations more frequently in males than females, validating the "EXiTS" hypothesis wherein genes that escape from X inactivation are tumor suppressors, with the expression from the Xi protecting females from cancer (Dunford et al., 2017). Thus, a second X chromosome could be protective from cancer for KS individuals. Several other genes that escape from XCI have been suggested to be tumor suppressors (including the PAR gene *PPP2R3B*), and loss of the Xi is often associated with poor prognosis and survival in cancer (van Kempen et al., 2016). Notable cancer risks for KS patients are the well-known elevation for breast cancer (being as high as 19-fold) and a high prevalence of extragonadal germ cell tumors, especially teratomas (Kanakis & Nieschlag, 2018).

4.4 | Escape genes and spermatogenesis

Most KS adults display signs and symptoms of hypogonadism, such as poor muscle development, short penis, sparse pubic, axillary, and facial hair, which can also be present at puberty. Gynecomastia is usually the consequence of high levels of estradiol (Shiraishi & Matsuyama, 2019). KS patients comprise 10–12% of males with non-obstructive azoospermia (NOA), and while reduction in testicular size has been reported in some studies prior to puberty, after this period the degeneration of testicular structures is noticeable, and the seminiferous tubules exhibit hyalinization, fibrosis and degeneration of Sertoli cells (Bird & Hurren, 2016; Samango-Sprouse et al., 2019; Shiraishi & Matsuyama, 2019). The X chromosome is enriched in genes involved in both oogenesis and spermatogenesis, and resetting of chromatin marks results in germ cells having X dosage that differs between the sexes, and also differs from somatic cells (reviewed in Sangrithi and Turner (2018)). Less than 9% of KS patients have sperm in the ejaculate, which can be used for intracytoplasmic sperm injection. There are two hypotheses of how spermatogenesis occurs in those patients. The first one postulates that 47,XXY spermatogonia complete meiosis, which additionally explains the increase in sex chromosomal aneuploidy rate and haploid spermatozoa. The second one suggests that the spermatozoa arise from 46,XY spermatogonial stem cell patches (Maiburg, Repping, & Giltay, 2012).

4.5 | Escape genes and neurodevelopment

The X chromosome is over-represented for genes with a role in brain. While the X chromosome is only 5% of the genome (and also gene-poor) it has approximately 15% of the genes identified to underlie intellectual disability, with 141 known genes (Neri, Schwartz, Lubs, & Stevenson, 2018). During childhood, KS boys can struggle to reach

developmental milestones, having developmental speech and motor delays, cognition, and adaptive problems and an increased incidence of attention-deficit/hyperactivity disorder and psychiatric disorders including schizophrenia (van Rijn, Aleman, Swaab, & Kahn, 2006). Neuroimaging studies reveal reductions in brain volume in the caudate, frontal and temporal regions. As adults, anomalies in socialization and behavior and executive dysfunction have been described (Bird & Hurren, 2016; Bruining et al., 2010; Samango-Sprouse et al., 2019; Shiraishi & Matsuyama, 2019). In considering the impact of sex chromosome aneuploidy on the frequency of psychiatric disorders, an "extra X" effect was most notable for attention-deficit/hyperactivity disorder (Green, Flash, & Reiss, 2019). The PAR1 gene *GTPBP6* (putative binding protein 6), might be affecting language development (Vawter, Harvey, & DeLisi, 2007), and pathogenic variants in this gene have also been reported in patients with X-linked mental retardation (Tarpey et al., 2009). *CXorf21* encodes a protein with unknown functions that is variable in its inactivation status (Balaton & Brown, 2016), which is also expressed in fetal and adult brain and correlated with cognitive functions (Vawter et al., 2007).

In another approach, human induced pluripotent stem cells from two azoospermic KS patients were used for transcriptomic analysis, revealing 23 up-regulated X-linked genes, including PAR1 genes and high confidence escape genes such as: *AP1S2*, *CTPS2*, *GYG2*, *NLGN4X*, *PUDP*, *SYAP1*, *PLCXD1*, and *SLC25A6* related to nervous system development, synaptic transmission and metabolic processes (Panula et al., 2019). Using in situ hybridization it was shown that the X and Y gametologs *PCDH11X/Y* and *NLGN4X/Y* are expressed in specific and heterogeneous cell populations in the brain (Johansson et al., 2016). The *XG* blood group gene, which shows a female-bias in expression spans the pseudoautosomal boundary and generates a cell-surface antigen (Mangs & Morris, 2007). As this region of the X chromosome has shown an association with autism spectrum disorders (ASD; Chang, Pauls, Lange, Sasanfar, & Santangelo, 2013), this may reflect a potential immune/neural development connection also reported for genes such as *CXorf21* (Odhams et al., 2019).

4.6 | Escape genes in mouse

Studying XCI in mice has many advantages, including access to early development (*in vivo* and *in vitro* using differentiating embryonic stem cells), highly informative genetic crosses providing many polymorphisms, and the ability to control XCI skewing through *Xist* mutations (e.g., Berletch et al., 2015), studying imprinted trophoblast cells (Calabrese et al., 2012) or the use of fluorescent markers to tag both X chromosomes (Wu et al., 2014; Kobayashi et al., 2016). However, mice have only 3–7% of their genes escaping from XCI, depending on tissue examined (Berletch et al., 2015), and additional differences in XCI between mouse and humans are reviewed in (Carrel & Brown, 2017). Even though mice have less genes that escape from XCI, mouse models are useful for studying KS for the ease of manipulating their genetics and controlling for nongenetic factors. There are two main mouse models to examine the effect of sex aneuploidies. The four core genotype model involves a translocation of the *SRY* gene onto an autosome, allowing for the reliable breeding and comparison of XX and XY males and XX and XY females (Arnold & Chen, 2009). Much of the work using this model has been in the fields of neuroscience and behavior (Arnold & Chen, 2009), but other sex differences have also been investigated (Itoh et al., 2015). The strength of the four core genotypes model is differentiating the effects of sex chromosomes from the effects of sex hormones; a limitation of the four core genotypes model is the inability to differentiate the addition of an Xi from the loss of a Y chromosome (Arnold & Chen, 2009). Another mouse model to study sex aneuploidies is the Y* model (Hunt & Eicher, 1991). The Y* model uses mice with an additional centromere on the Y, which when recombined with the X, forms 2 new chromosomes, XY* that consists- of the whole X and most of the Y chromosome and Y^{*X} that contains the Y* centromere and PARs. Breeding these mice for 4 generations will consistently produce XX, XY, and XXY mice with normal chromosomes, along with XY^{*X}, XXY^{*X}, and XYY^{*X} (Hunt & Eicher, 1991), which have various numbers of X, Y and PAR genes and may also be interesting to study. The XXY mice produced in this model have been shown to have similar testicular changes and learning deficits as humans with KS (Lue et al., 2005).

5 | MECHANISM OF ESCAPE

The genes that escape XCI generally show epigenetic features similar to their Xa copies (see Balaton and Brown (2016) for review), somehow avoiding the sweep of XIST-induced heterochromatin that extends across the Xi. When autosomal material is translocated to the X chromosome, it is able to be inactivated, although less robust "spread" of silencing into the autosome led to the hypothesis of waystations that would amplify silencing and were enriched on the X chromosome (Balaton & Brown, 2016; Gartler & Riggs, 1983). The LINE retrotransposons are considerably enriched on the X chromosome (Bailey, Carrel, Chakravarti, & Eichler, 2000), leading Lyon to propose they could be the waystations that promote silencing (Lyon, 1998). LINE density correlates with silencing in X/autosome translocations (Tannan et al., 2014) as does pre-existing PRC2 occupancy (Cotton et al., 2014), also seen in mouse models (Loda et al., 2017).

The conservation of escape from XCI, particularly for the Y-conserved ancestral genes, suggests the presence of elements that favor expression. With a series of X-linked ~150 kb transgenes of the mouse *Kdm5c* gene, Li and Carrel demonstrated that escape from XCI was an intrinsic property of the locus (Li & Carrel, 2008). We have observed a similar recognition of an escape-favoring element in the human *RPS4X* region, and in this case, the element(s) could be recognized by mouse, despite the mouse ortholog being subject to XCI (Peeters, Korecki, Simpson, & Brown, 2018). Enrichment of sequence motifs and the transcription factors CTCF and YY1 near escape genes has been identified by bioinformatic assessment of X-linked genes or transgenes that are either subject to, or escape from, XCI (Chen et al., 2016; Loda et al., 2017). ALU element density correlates with

escape from XCI in X/autosome translocations (Cotton et al., 2014) which might reflect the general anti-correlation of LINE and ALU elements in the genome, or could also reflect the role of ALU elements in transcriptional control and CTCF binding (Ferrari et al., 2020). Between the euchromatin of genes that escape from XCI and the heterochromatin of genes subject to XCI, there are likely to be boundary elements. Deletions of the integrated mouse *Kdm5c* region resulted in the spread of escape from XCI (Horvath, Li, & Carrel, 2013). Such boundaries presumably differ between humans and mice, resulting in larger domains of escape genes in humans than mice, with CTCF being the most likely candidate (Filippova et al., 2005).

Recent chromosome-wide (Borensztein et al., 2017) and gene-specific (Peeters et al., 2019) studies demonstrate that escape is generally an avoidance of silencing rather than a failure to maintain an initial silencing. For those genes that escape from XCI in only a subset of tissues, it seems that there must be some instability to the silencing; despite XCI of most genes being stably maintained—such that the billions of clonally derived cells in a cancer retain their XCI status (Linder & Gartler, 1965). Reactivation of individual genes can be achieved by targeting both XIST expression and DNA methylation (Carrette et al., 2018). Recently, CRISPR was used to specifically demethylate and activate the *CDKL5* gene from the Xi (Halmai et al., 2020). As mentioned above, during lymphocyte development, loss of XIST localization has been correlated with reactivation of genes including *TLR7* (Syrett et al., 2017). However, loss of *Xist* in mouse somatic tissues is seen to have a limited effect, except under stress in particular tissues (Halmai et al., 2020; Yang, Yildirim, Kirby, Press, & Lee, 2020). Genes that reactivate in specific tissues could contribute to the phenotype of KS; however, the understanding of such events is currently limited.

6 | ADDITIONAL ROLES FOR THE X CHROMOSOMES BETWEEN THE SEXES AND QUESTIONS REMAINING

We have reviewed the genes that escape from XCI and their potential contribution to sex differences and phenotypes seen in KS. To date, the only strong correlation of phenotypic outcome and Xi gene expression is the role of *SHOX* in stature, and thus there is a need for more study to understand both the genes that escape XCI and the transcriptomic and phenotypic variability in KS. The picture of genes that escape from XCI is growing more complex as more individuals, more tissues and more single-cell analyses reveal the extent of variability in escape (see Figure 1). Additionally, there are many X-linked genes for which an inactivation status has yet to be determined. The long and short noncoding RNAs are a substantial portion of these. Long noncoding RNAs in addition to XIST have been shown to be involved in the ultrastructure of the Xi (reviewed in Fang, Disteche, and Berletch (2019)). miRNAs are over-represented on the X, but have rarely been examined for XCI status (Peeters et al., 2019).

There are multiple additional ways in which having an Xi may impact biology. The random nature of XCI means that females are mosaic for two populations of cells which differ in which X is inactivated. XCI is also generally seen to be random in Klinefelter individuals (Kinjo et al., 2020). Skewing of inactivation can occur by chance, but also due to selection—often to minimize impact of a deleterious allele. Thus, similar to females, males with a second X may be protected from X-linked disease, including cancer through the EXiTS genes (Dunford et al., 2017). Maladaptively, skewed inactivation is reported in some autoimmune disease, leading to the speculation that limited expression of an antigen might predispose to autoimmunity (Lambert, 2009), although loss of mosaicism may also reflect chronic inflammation (Kanaan et al., 2016; Lambert, 2009). Mosaicism is also a contributor to rare X-linked dominant disorders only present in females (Twigg et al., 2013) due to cellular interference.

We have excluded the *XIST* gene from our tables of genes that escape from XCI as it is expressed solely from the Xi. Thus, the difference in expression between individuals with and without an Xi is immense. While the primary function of XIST is to silence the X in *cis*, secondary impacts are possible. Many articles propose an impact through "miRNA sponging" whereby the lncRNA can act as a decoy for miRNAs, reducing their abundance. Such a role is challenging to demonstrate, but given the XIST localization to the nucleus, should only be effective on nuclear miRNA populations (Marshall, Stewart, Sage, Lam, & Brown, 2019). The presence of the heterochromatic Xi could also impact the cell - potentially by either depleting the availability of chromatin regulators or serving as a stockpile of such elements (see Déjardin (2015)).

Males always inherit their single X chromosome from their mothers, as they inherit the Y chromosome from their fathers. Thus inheritance of a paternal X is unique to females, but can also be found in 47,XXY individuals. 47,XXY happens as a consequence of nondisjunction errors during meiotic division, and the origin of the extra X chromosome is maternal in approximately 50% of the cases (Samango-Sprouse et al., 2019; Tuttelmann & Gromoll, 2010). Developmental impairment in speech or motor areas, incidence of schizotypal traits and later onset of puberty have been variably reported as influenced by the parental origin of the extra X chromosome (Bruining et al., 2010; Stemkens et al., 2006; Wikström, Painter, Raivio, Aittomäki, & Dunkel, 2006).

Separating the role of the Xi and Y chromosome from hormonal differences in sex differences is challenging; however, there are clear differences in embryonic growth of XX and XY individuals before gonadal differentiation (reviewed in Engel (2018)), arguing that the sex chromosome complement is an important contributor. Further transcriptomic studies of KS individuals can contribute to our understanding of the contribution of the Xi to sex differences, as well as the role of the Xi in the clinical manifestations of sex chromosome aneuploids. The analysis of iPS cells (Panula et al., 2019) can be extended by differentiation into multiple tissue types, and through nondisjunction could also generate isogenic nonaneuploid lines for comparison, which may inform how much variability is attributable to underlying genetic variation (discussed in Sangrithi and Turner (2018)). Understanding of the mechanism(s) by which genes are able to escape from XCI, and whether reactivation of the Xi, or some genes of the Xi is

occurring in some tissues will substantially further our ability to correlate KS phenotypic variation with genes that escape from XCI.

ACKNOWLEDGMENTS

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the tables presented in this manuscript were obtained from: the GTEx Portal on February, 2020. This study was supported by Canadian Institutes of Health Research Project Grant PJT-162120.

CONFLICT OF INTEREST

None.

ORCID

Carolyn J. Brown https://orcid.org/0000-0002-8959-0101

REFERENCES

Al Nadaf, S., Deakin, J. E., Gilbert, C., Robinson, T. J., Graves, J. A. M., & Waters, P. D. (2012). A cross-species comparison of escape from X inactivation in Eutheria: Implications for evolution of X chromosome inactivation. *Chromosoma*, *121*, 71–78.

Arai, N., Homma, M., Abe, M., Baba, Y., Murai, S., Watanuki, M., … Takimoto, M. (2019). Impact of CD123 expression, analyzed by immunohistochemistry, on clinical outcomes in patients with acute myeloid leukemia. *International Journal of Hematology*, *109*, 539–544.

Arnold, A. P., & Chen, X. (2009). What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? *Frontiers in Neuroendocrinology*, *30*, 1–9.

Bailey, J. A., Carrel, L., Chakravarti, A., & Eichler, E. E. (2000). Molecular evidence for a relationship between LINE-1 elements and X chromosome inactivation: The Lyon repeat hypothesis. *Proceedings of the National Academy of Sciences*, *97*, 6634–6639.

Balaton, B. P., & Brown, C. J. (2016). Escape artists of the X chromosome. *Trends in Genetics*, *32*, 348–359.

Balaton, B. P., Cotton, A. M., & Brown, C. J. (2015). Derivation of consensus inactivation status for X-linked genes from genome-wide studies. *Biology of Sex Differences*, *6*, 35.

Belling, K., Russo, F., Jensen, A. B., Dalgaard, M. D., Westergaard, D., Rajpert-De Meyts, E., … Brunak, S. (2017). Klinefelter syndrome comorbidities linked to increased X chromosome gene dosage and altered protein interactome activity. *Human Molecular Genetics*, *26*, 1219–1229.

Bellott, D. W., Hughes, J. F., Skaletsky, H., Brown, L. G., Pyntikova, T., Cho, T.-J., … Page, D. C. (2014). Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature*, *508*, 494–499.

Berletch, J. B., Ma, W., Yang, F., Shendure, J., Noble, W. S., Disteche, C. M., & Deng, X. (2015). Escape from X inactivation varies in mouse tissues. *PLoS Genetics*, *11*(3), 1–26.

Bird, R. J., & Hurren, B. J. (2016). Anatomical and clinical aspects of Klinefelter's syndrome. *Clinical Anatomy*, *29*, 606–619.

Borensztein, M., Syx, L., Ancelin, K., Diabangouaya, P., Picard, C., Liu, T., … Heard, E. (2017). Xist-dependent imprinted X inactivation and the early developmental consequences of its failure. *Nature Structural & Molecular Biology*, *24*, 226–233.

Bruining, H., van Rijn, S., Swaab, H., Giltay, J., Kates, W., Kas, M. J. H., … de Sonneville, L. (2010). The parent-of-origin of the extra X chromosome may differentially affect psychopathology in Klinefelter syndrome. *Biological Psychiatry*, *68*, 1156–1162.

Calabrese, J. M., Sun, W., Song, L., Mugford, J. W., Williams, L., Yee, D., … Magnuson, T. (2012). Site-specific silencing of regulatory elements as a mechanism of X inactivation. *Cell*, *151*(5), 951–963.

Cardoso, L. C., Laurentino, S. R. S., Lerario, T. D. S., Lerario, A. M., SKN, M., & Oba-Shinjo, S. M. (2019). CD99 expression in glioblastoma molecular subtypes and role in migration and invasion. *International Journal of Molecular Sciences*, *20*(5), 1137.

Carrel, L., & Brown, C. J. (2017). When the Lyon(ized chromosome) roars: Ongoing expression from an inactive X chromosome. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*, 20160355.

Carrel, L., & Willard, H. F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature*, *434*, 400–404.

Carrette, L. L. G., Wang, C.-Y., Wei, C., Press, W., Ma, W., Kelleher, R. J., & Lee, J. T. (2018). A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, E668–E675.

Cerase, A., Armaos, A., Neumayer, C., Avner, P., Guttman, M., & Tartaglia, G. G. (2019). Phase separation drives X-chromosome inactivation: A hypothesis. *Nature Structural & Molecular Biology*, *26*, 331–334.

Chang, S.-C., Pauls, D. L., Lange, C., Sasanfar, R., & Santangelo, S. L. (2013). Sex-specific association of a common variant of the XG gene with autism spectrum disorders. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *162B*, 742–750.

Chen, C.-Y., Shi, W., Balaton, B. P., Matthews, A. M., Li, Y., Arenillas, D. J., … Wasserman, W. W. (2016). YY1 binding association with sex-biased transcription revealed through X-linked transcript levels and allelic binding analyses. *Scientific Reports*, *6*, 37324.

Chen, D., Camponeschi, A., Wu, Q., Gerasimcik, N., Li, H., Shen, X., … Mårtensson, I.-L. (2019). CD99 expression is strongly associated with clinical outcome in children with B-cell precursor acute lymphoblastic leukaemia. *British Journal of Haematology*, *184*, 418–423.

Chianese, C., Lo Giacco, D., Tüttelmann, F., Ferlin, A., Ntostis, P., Vinci, S., … Krausz, C. (2013). Y-chromosome microdeletions are not associated with SHOX haploinsufficiency. *Human Reproduction*, *28*, 3155–3160.

Chu, C., Zhang, Q. C., da Rocha, S. T., Flynn, R. A., Bharadwaj, M., Calabrese, J. M., … Chang, H. Y. (2015). Systematic discovery of Xist RNA binding proteins. *Cell*, *161*, 404–416.

Ciccodicola, A., D'Esposito, M., Esposito, T., Gianfrancesco, F., Migliaccio, C., Miano, M. G., … D'Urso, M. (2000). Differentially regulated and evolved genes in the fully sequenced Xq/Yq pseudoautosomal region. *Human Molecular Genetics*, *9*, 395–401.

Cotton, A. M., Chen, C.-Y., Lam, L. L., Wasserman, W. W., Kobor, M. S., & Brown, C. J. (2014). Spread of X-chromosome inactivation into autosomal sequences: Role for DNA elements, chromatin features and chromosomal domains. *Human Molecular Genetics*, *23*, 1211–1223.

Cotton, A. M., Ge, B., Light, N., Adoue, V., Pastinen, T., & Brown, C. J. (2013). Analysis of expressed SNPs identifies variable extents of expression from the human inactive X chromosome. *Genome Biology*, *14*, R122.

Cotton, A. M., Price, E. M., Jones, M. J., Balaton, B. P., Kobor, M. S., & Brown, C. J. (2015). Landscape of DNA methylation on the X chromosome reflects CpG density, functional chromatin state and X-chromosome inactivation. *Human Molecular Genetics*, *24*, 1528–1539.

De Bonis, M. L., Cerase, A., Matarazzo, M. R., Ferraro, M., Strazzullo, M., Hansen, R. S., … D'Esposito, M. (2006). Maintenance of X- and Y-inactivation of the pseudoautosomal (PAR2) gene SPRY3 is independent from DNA methylation and associated to multiple layers of epigenetic modifications. *Human Molecular Genetics*, *15*, 1123–1132.

Deegan, D. F., Karbalaei, R., Madzo, J., Kulathinal, R. J., & Engel, N. (2019). The developmental origins of sex-biased expression in cardiac development. *Biology of Sex Differences*, *10*(1), 1–20.

Déjardin, J. (2015). Switching between epigenetic states at pericentromeric heterochromatin. *Trends in Genetics*, *31*, 661–672.

Disteche, C. M. (2016). Dosage compensation of the sex chromosomes and autosomes. *Seminars in Cell & Developmental Biology*, *56*, 9–18.

Dossin, F., Pinheiro, I., Żylicz, J. J., Roensch, J., Collombet, S., Le Saux, A., … Heard, E. (2020). SPEN integrates transcriptional and epigenetic control of X-inactivation. *Nature*, *578*, 455–460.

Dunford, A., Weinstock, D. M., Savova, V., Schumacher, S. E., Cleary, J. P., Yoda, A., … Lane, A. A. (2017). Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias. *Nature Genetics*, *49*, 10–16.

Engel, N. (2018). Sex differences in early embryogenesis: Interchromosomal regulation sets the stage for sex-biased gene networks: The dialogue between the sex chromosomes and autosomes imposes sexual identity soon after fertilization. *BioEssays*, *40*(9), 1–9.

Fang, H., Disteche, C. M., & Berletch, J. B. (2019). X inactivation and escape: Epigenetic and structural features. *Frontiers in Cell and Development Biology*, *7*, 219.

Ferrari, R., de Llobet Cucalon, L. I., Di Vona, C., Le Dilly, F., Vidal, E., Lioutas, A., … Beato, M. (2020). TFIIIC binding to Alu elements controls gene expression via chromatin looping and histone acetylation. *Molecular Cell*, *77*, 475–487.

Fey, M. F., Peter, H. J., Hinds, H. L., Zimmermann, A., Liechti-Gallati, S., Gerber, H., … Tobler, A. (1992). Clonal analysis of human tumors with M27 beta, a highly informative polymorphic X chromosomal probe. *The Journal of Clinical Investigation*, *89*, 1438–1444.

Filippova, G. N., Cheng, M. K., Moore, J. M., Truong, J.-P., Hu, Y. J., Nguyen, D. K., … Disteche, C. M. (2005). Boundaries between chromosomal domains of X inactivation and escape bind CTCF and lack CpG methylation during early development. *Developmental Cell*, *8*, 31–42.

Fukami, M., Seki, A., & Ogata, T. (2016). SHOX haploinsufficiency as a cause of syndromic and nonsyndromic short stature. *Molecular Syndromology*, *7*, 3–11.

Garieri, M., Stamoulis, G., Blanc, X., Falconnet, E., Ribaux, P., Borel, C., … Antonarakis, S. E. (2018). Extensive cellular heterogeneity of X inactivation revealed by single-cell allele-specific expression in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 13015–13020.

Gartler, S. M., & Riggs, A. D. (1983). Mammalian X-chromosome inactivation. *Annual Review of Genetics*, *17*, 155–190.

Genoni, G., Monzani, A., Castagno, M., Ricotti, R., Rapa, A., Petri, A., … Bellone, S. (2018). Improving clinical diagnosis in SHOX deficiency: The importance of growth velocity. *Pediatric Research*, *83*, 438–444.

Graves, J. A. M., Gécz, J., & Hameister, H. (2002). Evolution of the human X—A smart and sexy chromosome that controls speciation and development. *Cytogenetic and Genome Research*, *99*, 141–145.

Green, T., Flash, S., & Reiss, A. L. (2019). Sex differences in psychiatric disorders: What we can learn from sex chromosome aneuploidies. *Neuropsychopharmacology*, *44*, 9–21.

Halmai, J. A. N. M., Deng, P., Gonzalez, C. E., Coggins, N. B., Cameron, D., Carter, J. L., … Fink, K. D. (2020). Artificial escape from XCI by DNA methylation editing of the CDKL5 gene. *Nucleic Acids Research*, *48*, 2372–2387.

Horvath, L. M., Li, N., & Carrel, L. (2013). Deletion of an X-inactivation boundary disrupts adjacent gene silencing. *PLoS Genetics*, *9*, e1003952.

Hunt, P. A., & Eicher, E. M. (1991). Fertile male mice with three sex chromosomes: Evidence that infertility in XYY male mice is an effect of two Y chromosomes. *Chromosoma*, *100*, 293–299.

Itoh, Y., Mackie, R., Kampf, K., Domadia, S., Brown, J. D., O'Neill, R., & Arnold, A. P. (2015). Four core genotypes mouse model: Localization of the Sry transgene and bioassay for testicular hormone levels. *BMC Research Notes*, *8*, 69.

Johansson, M. M., Lundin, E., Qian, X., Mirzazadeh, M., Halvardson, J., Darj, E., … Jazin, E. (2016). Spatial sexual dimorphism of X and Y homolog gene expression in the human central nervous system during early male development. *Biology of Sex Differences*, *7*, 5.

Kanaan, S. B., Onat, O. E., Balandraud, N., Martin, G. V., Nelson, J. L., Azzouz, D. F., … Lambert, N. C. (2016). Evaluation of X chromosome inactivation with respect to HLA genetic susceptibility in rheumatoid arthritis and systemic sclerosis. *PLoS One*, *11*, e0158550.

Kanakis, G. A., & Nieschlag, E. (2018). Klinefelter syndrome: More than hypogonadism. *Metabolism, Clinical and Experimental*, *86*, 135–144.

Keniry, A., & Blewitt, M. E. (2018). Studying X chromosome inactivation in the single-cell genomic era. *Biochemical Society Transactions*, *46*, 577–586.

Keown, C. L., Berletch, J. B., Castanon, R., Nery, J. R., Disteche, C. M., Ecker, J. R., & Mukamel, E. A. (2017). Allele-specific non-CG DNA methylation marks domains of active chromatin in female mouse brain. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E2882–E2890.

Kinjo, K., Yoshida, T., Kobori, Y., Okada, H., Suzuki, E., Ogata, T., … Fukami, M. (2020). Random X chromosome inactivation in patients with Klinefelter syndrome. *Molecular and Cellular Pediatrics*, *7*, 1.

Kiripolsky, J., & Kramer, J. M. (2018). Current and emerging evidence for toll-like receptor activation in Sjögren's syndrome. *Journal of Immunology Research*, *2018*, 1246818.

Kobayashi, S., Hosoi, Y., Shiura, H., Yamagata, K., Takahashi, S., Fujihara, Y., … Ishino, F. (2016). Live imaging of X chromosome reactivation dynamics in early mouse development can discriminate naïve from primed pluripotent stem cells. *Development (Cambridge)*, *143*(16), 2958–2964.

Koren, A., & McCarroll, S. A. (2014). Random replication of the inactive X chromosome. *Genome Research*, *24*, 64–69.

Koufopoulos, N., Kokkali, S., Manatakis, D., Balalis, D., Nasi, D., Ardavanis, A., … Khaldi, L. (2019). Primary peripheral neuroectodermal tumor (PNET) of the adrenal gland: A rare entity. *Journal of BUON*, *24*, 770–778.

Kucera, K. S., Reddy, T. E., Pauli, F., Gertz, J., Logan, J. E., Myers, R. M., & Willard, H. F. (2011). Allele-specific distribution of RNA polymerase II on female X chromosomes. *Human Molecular Genetics*, *20*, 3964–3973.

Laffont, S., & Guéry, J.-C. (2019). Deconstructing the sex bias in allergy and autoimmunity: From sex hormones and beyond. *Advances in Immunology*, *142*, 35–64.

Lahn, B. T., & Page, D. C. (1999). Four evolutionary strata on the human X chromosome. *Science*, *286*, 964–967.

Lambert, N. C. (2009). The price of silence. *Arthritis and Rheumatism*, *60*, 3164–3167.

Li, N., & Carrel, L. (2008). Escape from X chromosome inactivation is an intrinsic property of the Jarid1c locus. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 17055–17060.

Linder, D., & Gartler, S. M. (1965). Glucose-6-phosphate dehydrogenase mosaicism: Utilization as a cell marker in the study of leiomyomas. *Science*, *150*, 67–69.

Lister, R., Mukamel, E. A., Nery, J. R., Urich, M., Puddifoot, C. A., Johnson, N. D., … Ecker, J. R. (2013). Global epigenomic reconfiguration during mammalian brain development. *Science*, *341*, 1237905.

Loda, A., Brandsma, J. H., Vassilev, I., Servant, N., Loos, F., Amirnasr, A., … Gribnau, J. (2017). Genetic and epigenetic features direct differential efficiency of Xist-mediated silencing at X-chromosomal and autosomal locations. *Nature Communications*, *8*, 690.

Lopes, A. M., Ross, N., Close, J., Dagnall, A., Amorim, A., & Crow, T. J. (2006). Inactivation status of PCDH11X: Sexual dimorphisms in gene expression levels in brain. *Human Genetics*, *119*, 267–275.

Lue, Y., Jentsch, J. D., Wang, C., Rao, P. N., Hikim, A. P. S., Salameh, W., & Swerdloff, R. S. (2005). XXY mice exhibit gonadal and behavioral phenotypes similar to Klinefelter syndrome. *Endocrinology*, *146*, 4148–4154.

Lyon, M. F. (1961). Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature*, *190*, 372–373.

Lyon, M. F. (1962). Sex chromatin and gene action in the mammalian X-chromosome. *American Journal of Human Genetics*, *14*, 135–148.

Lyon, M. F. (1998). X-chromosome inactivation: A repeat hypothesis. *Cytogenetic and Genome Research*, *80*, 133–137.

Maiburg, M., Repping, S., & Giltay, J. (2012). The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. *Fertility and Sterility*, *98*(2), 253–260.

Mangs, H. A., & Morris, B. J. (2007). The human pseudoautosomal region (PAR): Origin, function and future. *Current Genomics*, *8*, 129–136.

Marshall, E. A., Stewart, G. L., Sage, A. P., Lam, W. L., & Brown, C. J. (2019). Beyond sequence homology: Cellular biology limits the potential of XIST to act as a miRNA sponge. *PLoS One*, *14*, e0221371.

Marshall Graves, J. A. (2016). Evolution of vertebrate sex chromosomes and dosage compensation. *Nature Reviews. Genetics*, *17*, 33–46.

Matarazzo, M. R., Boyle, S., D'Esposito, M., & Bickmore, W. A. (2007). Chromosome territory reorganization in a human disease with altered DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 16546–16551.

McHugh, C. A., Chen, C.-K., Chow, A., Surka, C. F., Tran, C., McDonel, P., … Guttman, M. (2015). The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature*, *521*, 232–236.

Meester, I., Manilla-Muñoz, E., León-Cachón, R. B. R., Paniagua-Frausto, G. A., Carrión-Alvarez, D., Ruiz-Rodríguez, C. O., … García-Martínez, J. M. (2020). SeXY chromosomes and the immune system: Reflections after a comparative study. *Biology of Sex Differences*, *11*, 3.

Mehta, A., Malek-Jones, M., Bolyakov, A., Mielnik, A., Schlegel, P. N., & Paduch, D. A. (2012). Methylation-specific PCR allows for fast diagnosis of X chromosome disomy and reveals skewed inactivation of the X chromosome in men with Klinefelter syndrome. *Journal of Andrology*, *33*, 955–962.

Minajigi, A., Froberg, J., Wei, C., Sunwoo, H., Kesner, B., Colognori, D., … Lee, J. T. (2015). A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation. *Science*, *349*, aab2276.

Naqvi, S., Bellott, D. W., Lin, K. S., & Page, D. C. (2018). Conserved microRNA targeting reveals preexisting gene dosage sensitivities that shaped amniote sex chromosome evolution. *Genome Research*, *28*, 474–483.

Neri, G., Schwartz, C. E., Lubs, H. A., & Stevenson, R. E. (2018). X-linked intellectual disability update 2017. *American Journal of Medical Genetics. Part A*, *176*, 1375–1388.

O'Connor, M. J., Snyder, E. A., & Hayes, F. J. (2019). Klinefelter syndrome and diabetes. *Current Diabetes Reports*, *19*, 71.

Odhams, C. A., Roberts, A. L., Vester, S. K., Duarte, C. S. T., Beales, C. T., Clarke, A. J., … Vyse, T. J. (2019). Interferon inducible X-linked gene CXorf21 may contribute to sexual dimorphism in systemic lupus erythematosus. *Nature Communications*, *10*, 2164.

Panula, S., Kurek, M., Kumar, P., Albalushi, H., Padrell Sánchez, S., Damdimopoulou, P., … Stukenborg, J.-B. (2019). Human induced pluripotent stem cells from two azoospermic patients with Klinefelter syndrome show similar X chromosome inactivation behavior to female pluripotent stem cells. *Human Reproduction*, *34*, 2297–2310.

Peeters, S. B., Korecki, A. J., Baldry, S. E. L., Yang, C., Tosefsky, K., Balaton, B. P., … Brown, C. J. (2019). How do genes that escape from X-chromosome inactivation contribute to Turner syndrome? *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *181*, 28–35.

Peeters, S. B., Korecki, A. J., Simpson, E. M., & Brown, C. J. (2018). Human cis-acting elements regulating escape from X-chromosome inactivation function in mouse. *Human Molecular Genetics*, *27*, 1252–1262.

Petropoulos, S., Edsgärd, D., Reinius, B., Deng, Q., Panula, S. P., Codeluppi, S., … Lanner, F. (2016). Single-cell RNA-seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell*, *165*(4), 1,012–1,026.

Pintacuda, G., Wei, G., Roustan, C., Kirmizitas, B. A., Solcan, N., Cerase, A., … Brockdorff, N. (2017). hnRNPK recruits PCGF3/5-PRC1 to the Xist RNA B-repeat to establish Polycomb-mediated chromosomal silencing. *Molecular Cell*, *68*, 955–969.e10.

Potter, N., Jones, L., Blair, H., Strehl, S., Harrison, C. J., Greaves, M., … Russell, L. J. (2019). Single-cell analysis identifies CRLF2 rearrangements as both early and late events in Down syndrome and non-Down syndrome acute lymphoblastic leukaemia. *Leukemia*, *33*, 893–904.

Priddle, T. H., & Crow, T. J. (2013). The protocadherin 11X/Y (PCDH11X/Y) gene pair as determinant of cerebral asymmetry in modern *Homo sapiens*. *Annals of the New York Academy of Sciences*, *1*(288), 36–47.

Qu, K., Zaba, L. C., Giresi, P. G., Li, R., Longmire, M., Kim, Y. H., … Chang, H. Y. (2015). Individuality and variation of personal regulomes in primary human T cells. *Cell Systems*, *1*, 51–61.

Raudsepp, T., & Chowdhary, B. P. (2015). The eutherian pseudoautosomal region. *Cytogenetic and Genome Research*, *147*, 81–94.

Raychaudhuri, S. K., Abria, C., Maverakis, E. M., & Raychaudhuri, S. P. (2018). IL-9 receptor: Regulatory role on FLS and pannus formation. *Cytokine*, *111*, 58–62.

Raznahan, A., Parikshak, N. N., Chandran, V., Blumenthal, J. D., Clasen, L. S., Alexander-Bloch, A. F., … Geschwind, D. H. (2018). Sex-chromosome dosage effects on gene expression in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 7398–7403.

Ridings-Figueroa, R., Stewart, E. R., Nesterova, T. B., Coker, H., Pintacuda, G., Godwin, J., … Coverley, D. (2017). The nuclear matrix protein CIZ1 facilitates localization of Xist RNA to the inactive X-chromosome territory. *Genes & Development*, *31*, 876–888.

Rovenský, J., Imrich, R., Lazúrová, I., & Payer, J. (2010). Rheumatic diseases and Klinefelter's syndrome. *Annals of the New York Academy of Sciences*, *1*(193), 1–9.

Sakaguchi, T., Hasegawa, Y., Brockdorff, N., Tsutsui, K., Tsutsui, K. M., Sado, T., & Nakagawa, S. (2016). Control of chromosomal localization of Xist by hnRNP U family molecules. *Developmental Cell*, *39*, 11–12.

Samango-Sprouse, C. A., Counts, D. R., Tran, S. L., Lasutschinkow, P. C., Porter, G. F., & Gropman, A. L. (2019). Update on the clinical perspectives and care of the child with 47,XXY (Klinefelter Syndrome). *The Application of Clinical Genetics*, *12*, 191–202.

Sangrithi, M. N., & Turner, J. M. A. (2018). Mammalian X chromosome dosage compensation: Perspectives from the germ line. *BioEssays*, *40*, e1800024.

Sarmiento, L., Svensson, J., Barchetta, I., Giwercman, A., & Cilio, C. M. (2019). Copy number of the X-linked genes TLR7 and CD40L influences innate and adaptive immune responses. *Scandinavian Journal of Immunology*, *90*, e12776.

Schultz, M. D., He, Y., Whitaker, J. W., Hariharan, M., Mukamel, E. A., Leung, D., … Ecker, J. R. (2015). Human body epigenome maps reveal noncanonical DNA methylation variation. *Nature*, *523*, 212–216.

Seminog, O. O., Seminog, A. B., Yeates, D., & Goldacre, M. J. (2015). Associations between Klinefelter's syndrome and autoimmune diseases: English national record linkage studies. *Autoimmunity*, *48*, 125–128.

Sharma, A., Jamil, M. A., Nuesgen, N., Schreiner, F., Priebe, L., Hoffmann, P., … El-Maarri, O. (2015). DNA methylation signature in peripheral blood reveals distinct characteristics of human X chromosome numerical aberrations. *Clinical Epigenetics*, *7*, 76.

Shiraishi, K., & Matsuyama, H. (2019). Klinefelter syndrome: From pediatrics to geriatrics. *Reproductive Medicine and Biology*, *18*, 140–150.

Skakkebæk, A., Nielsen, M. M., Trolle, C., Vang, S., Hornshøj, H., Hedegaard, J., … Gravholt, C. H. (2018). DNA hypermethylation and differential gene expression associated with Klinefelter syndrome. *Scientific Reports*, *8*, 13740.

Souyris, M., Cenac, C., Azar, P., Daviaud, D., Canivet, A., Grunenwald, S., … Guéry, J.-C. (2018). TLR7 escapes X chromosome inactivation in immune cells. *Science Immunology*, *3*, eaap8855.

Stemkens, D., Roza, T., Verrij, L., Swaab, H., van Werkhoven, M. K., Alizadeh, B. Z., … Giltay, J. C. (2006). Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. *Clinical Genetics*, *70*, 43–48.

Syrett, C. M., & Anguera, M. C. (2019). When the balance is broken: X-linked gene dosage from two X chromosomes and female-biased autoimmunity. *Journal of Leukocyte Biology*, *106*, 919–932.

Syrett, C. M., Sindhava, V., Hodawadekar, S., Myles, A., Liang, G., Zhang, Y., … Anguera, M. C. (2017). Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells. *PLoS Genetics*, *13*, e1007050.

Tannan, N. B., Brahmachary, M., Garg, P., Borel, C., Alnefaie, R., Watson, C. T., … Sharp, A. J. (2014). DNA methylation profiling in X; autosome translocations supports a role for L1 repeats in the spread of X chromosome inactivation. *Human Molecular Genetics*, *23*, 1224–1236.

Tarpey, P. S., Smith, R., Pleasance, E., Whibley, A., Edkins, S., Hardy, C., … Stratton, M. R. (2009). A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nature Genetics*, *41*, 535–543.

Teissandier, A., & Bourc'his, D. (2017). Gene body DNA methylation conspires with H3K36me3 to preclude aberrant transcription. *The EMBO Journal*, *36*, 1471–1473.

Tukiainen, T., Villani, A.-C., Yen, A., Rivas, M. A., Marshall, J. L., Satija, R., … DG, M. A. (2017). Landscape of X chromosome inactivation across human tissues. *Nature*, *550*, 244–248.

Tuttelmann, F., & Gromoll, J. (2010). Novel genetic aspects of Klinefelter's syndrome. *Planning Perspectives*, *386*, 395.

Twigg, S. R. F., Babbs, C., van den Elzen, M. E. P., Goriely, A., Taylor, S., McGowan, S. J., … Wilkie, A. O. M. (2013). Cellular interference in craniofrontonasal syndrome: Males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. *Human Molecular Genetics*, *22*, 1654–1662.

Vacca, M., Della Ragione, F., Scalabrì, F., & D'Esposito, M. (2016). X inactivation and reactivation in X-linked diseases. *Seminars in Cell & Developmental Biology*, *56*, 78–87.

van Kempen, L. C. L., Redpath, M., Elchebly, M., Klein, K. O., Papadakis, A. I., Wilmott, J. S., … Spatz, A. (2016). The protein phosphatase 2A regulatory subunit PR70 is a gonosomal melanoma tumor suppressor gene. *Science Translational Medicine*, *8*, 369ra177.

van Rijn, S., Aleman, A., Swaab, H., & Kahn, R. S. (2006). Klinefelter's syndrome (karyotype 47, XXY) and schizophrenia-spectrum pathology. *The British Journal of Psychiatry*, *189*, 459–460.

Vawter, M. P., Harvey, P. D., & DeLisi, L. E. (2007). Dysregulation of X-linked gene expression in Klinefelter's syndrome and association with verbal cognition. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *144B*, 728–734.

Veitia, R. A. (2010). A generalized model of gene dosage and dominant negative effects in macromolecular complexes. *The FASEB Journal*, *24* (4), 994–1002.

Veitia, R. A., Veyrunes, F., Bottani, S., & Birchler, J. A. (2015). X chromosome inactivation and active X upregulation in therian mammals: Facts, questions, and hypotheses. *Journal of Molecular Cell Biology*, *7*, 2–11.

Wainer Katsir, K., & Linial, M. (2019). Human genes escaping X-inactivation revealed by single cell expression data. *BMC Genomics*, *20*, 201.

Wikström, A. M., Painter, J. N., Raivio, T., Aittomäki, K., & Dunkel, L. (2006). Genetic features of the X chromosome affect pubertal development and testicular degeneration in adolescent boys with Klinefelter syndrome. *Clinical Endocrinology*, *65*, 92–97.

Wilson, N. D., Ross, L. J. N., Close, J., Mott, R., Crow, T. J., & Volpi, E. V. (2007). Replication profile of PCDH11X and PCDH11Y, a gene pair located in the non-pseudoautosomal homologous region Xq21.3/Yp11.2. *Chromosome Research*, *15*, 485–498.

Wilson Sayres, M. A. (2018). Genetic diversity on the sex chromosomes. *Genome Biology and Evolution*, *10*, 1064–1078.

Wu, H., Luo, J., Yu, H., Rattner, A., Mo, A., Wang, Y., … Nathans, J. (2014). Cellular resolution maps of X chromosome inactivation: Implications for neural development, function, and disease. *Neuron*, *81*(1), 103–119.

Yang, L., Yildirim, E., Kirby, J. E., Press, W., & Lee, J. T. (2020). Widespread organ tolerance to Xist loss and X reactivation except under chronic stress in the gut. *Proceedings of the National Academy of Sciences*, *117*, 4262–4272.

Zhang, X., Hong, D., Ma, S., Ward, T., Ho, M., Pattni, R., … Urban, A. E. (2020). Integrated functional genomic analyses of Klinefelter and Turner syndromes reveal global network effects of altered X chromosome dosage. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 4864–4873.

Zitzmann, M., Bongers, R., Werler, S., Bogdanova, N., Wistuba, J., Kliesch, S., … Tüttelmann, F. (2015). Gene expression patterns in relation to the clinical phenotype in Klinefelter syndrome. *The Journal of Clinical Endocrinology and Metabolism*, *100*, E518–E523.

How to cite this article: Navarro-Cobos MJ, Balaton BP, Brown CJ. Genes that escape from X-chromosome inactivation: Potential contributors to Klinefelter syndrome. *Am J Med Genet Part C*. 2020;184C:226–238. https://doi.org/10.1002/ajmg.c.31800