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RESEARCH REVIEW

A strategic research alliance: Turner syndrome and sex differences

Adrianna K. San Roman[1] | David C. Page[1,2,3]

[1]Whitehead Institute, Cambridge, Massachusetts

[2]Howard Hughes Medical Institute, Whitehead Institute, Cambridge, Massachusetts

[3]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Correspondence
David C. Page, Whitehead Institute, 455 Main St., Cambridge, MA 02142.
Email: dcpage@wi.mit.edu

Sex chromosome constitution varies in the human population, both between the sexes (46,XX females and 46,XY males), and within the sexes (e.g., 45,X and 46,XX females, and 47,XXY and 46,XY males). Coincident with this genetic variation are numerous phenotypic differences between males and females, and individuals with sex chromosome aneuploidy. However, the molecular mechanisms by which sex chromosome constitution impacts phenotypes at the cellular, tissue, and organismal levels remain largely unexplored. Thus, emerges a fundamental question connecting the study of sex differences and sex chromosome aneuploidy syndromes: How does sex chromosome constitution influence phenotype? Here, we focus on Turner syndrome (TS), associated with the 45,X karyotype, and its synergies with the study of sex differences. We review findings from evolutionary studies of the sex chromosomes, which identified genes that are most likely to contribute to phenotypes as a result of variation in sex chromosome constitution. We then explore strategies for investigating the direct effects of the sex chromosomes, and the evidence for specific sex chromosome genes impacting phenotypes. In sum, we argue that integrating the study of TS with sex differences offers a mutually beneficial alliance to identify contributions of the sex chromosomes to human development, health, and disease.

KEYWORDS

sex chromosome aneuploidy, sex chromosome evolution, sex differences, Turner syndrome

1 | INTRODUCTION

The clinical features that define Turner syndrome (TS), including short stature, hypogonadism, and webbed neck, were first described in 1938 and TS was associated with the monosomy X karyotype (45,X) in 1959 (Ford, Jones, Polani, Dealmeida, & Briggs, 1959; Turner, 1938). In the 60 years since this association, questions regarding the causal role of the 45,X karyotype in TS phenotypes have accumulated. In parallel, another field has been considering the contributions of the sex chromosomes: the biology of sex differences. It is well-appreciated that males and females differ in sex chromosome constitution—most males are 46,XY, while most females are 46,XX—and that there are extensive phenotypic differences between males and females in health and disease (Wizemann and Pardue, 2001). For example, a variety of autoimmune diseases, including lupus, multiple sclerosis, and rheumatoid arthritis are more prevalent in females (Ngo, Steyn, & McCombe, 2014). In contrast, certain neurodevelopmental disorders, such as autism, attention-deficit hyperactivity disorder, and intellectual disability are male-biased (Boyle et al., 2011; Werling & Geschwind, 2013).

Nonetheless, we know little about how the sex chromosomes contribute to many sex-biased phenotypes. Fundamentally, the TS and sex differences fields are united by the pursuit of connections between variation in sex chromosome constitution and phenotype. Thus, as we have previously argued (Page & Miller, 2016), it is time for a strategic alliance between TS and sex differences researchers.

A primary goal of this alliance should be to decipher the complex landscape of variables that contribute to TS and sex differences. These include sex chromosome constitution, gonadal hormone profiles, and disparate environmental exposures. In fact, the impact of sex chromosome constitution has long been minimized compared with the effects of gonadal hormones. In this regard, it was commonly thought that sex chromosomes modulate phenotypes throughout the body *indirectly* through their primary role in sex determination and the subsequent organizing effects of gonadal hormones. Progress in understanding the biology of the sex chromosomes has led to a significant conceptual departure from this idea: that the sex chromosomes have *direct* effects on cells and tissues outside of the reproductive tract, which are not mediated through gonadal hormones (Skaletsky et al., 2003). Indeed,

many genes on the sex chromosomes are expressed throughout the body and participate in fundamental cellular processes such as transcription and translation (Bellott et al., 2014; Tukiainen et al., 2017). However, proving that sex chromosome constitution independently contributes to a given phenotype requires experimental models and strategies that can distinguish between effects of these co-occurring variables.

Here, we will review the evidence that sex chromosome constitution directly affects phenotypes in both TS and sex differences. We begin with a discussion of sex chromosome evolution, which can be considered a natural experiment that reveals promising candidate genes for mediating phenotypes throughout the body. Second, we consider experimental models that allow researchers to disentangle the effects of the sex chromosomes from other co-occurring variables. Third, we highlight two case studies of sex chromosome genes that have been convincingly implicated in mediating specific phenotypes in TS and may also contribute to sex differences. Finally, we suggest future approaches for increasing synergy between research in TS and sex differences.

2 | STUDIES OF SEX CHROMOSOME EVOLUTION REVEAL CANDIDATE GENES FOR TS AND SEX DIFFERENCES

The investigation of sex chromosome evolution has been a successful strategy to identify genes that potentially contribute to TS and sex differences. Although research in TS typically focuses on the X chromosome, much of our understanding of sex chromosome evolution comes from the complementary study of the Y chromosome, as X and Y evolution occurred in parallel. The human sex chromosomes evolved from a pair of ordinary autosomes present in the common ancestor of mammals and birds approximately 300 million years ago (Lahn & Page, 1999; Ohno, 1967). X and Y chromosome differentiation began with the emergence of SRY, the male sex-determining gene, on the proto-Y chromosome before the evolutionary divergence of marsupials and placental mammals. Subsequently, a series of chromosomal inversions on the Y chromosome suppressed crossing-over with the X chromosome. In this region of suppressed X–Y crossing-over, the X and Y chromosomes diverged, resulting in a male-specific region on the Y (MSY; Figure 1a). The X and Y chromosomes are identical in two pseudoautosomal regions (PARs) at the tips of the chromosomes, where X–Y crossing-over occurs and is required for proper pairing and chromosome segregation in male meiosis (Cooke, Brown, & Rappold, 1985; Freije, Helms, Watson, & Donis-Keller, 1992). The loss of crossing-over with the X chromosome across the MSY led to genetic decay; only 3% of genes from the ancestral chromosome (17 of ~640 ancestral genes) survive on the human Y chromosome (Bellott et al., 2014; Skaletsky et al., 2003). In contrast, the X chromosome retained 98% of the ancestral genes (Bellott et al., 2014).

Although the X chromosome retained almost all of the ancestral genes, they did not follow a unified evolutionary path. Recent evidence shows that prior to sex chromosome differentiation, genes on the ancestral autosomes had inherent differences in sensitivity to gene dosage that resulted in four classes of divergent evolutionary trajectories on the X chromosome in the context of Y chromosome decay (Bellott et al., 2014; Bellott et al., 2017; Naqvi, Bellott, Lin, & Page, 2018). First are the PAR genes, which are distinguishable from the rest of the classes because this region never stopped crossing-over with the Y chromosome (Figure 1b). Here, we specifically refer to the PAR1 genes (on the tip of the short arm), since PAR2 (on the end of the long arm) was not part of the ancestral chromosomes (Ciccodicola et al., 2000). The PAR genes remain expressed from both copies of the X chromosome in 46,XX females, and on the X and Y chromosomes in 46,XY males. TS and sex-biased phenotypes may be impacted by PAR genes that are dosage sensitive.

The second class is comprised of genes on the ancestral autosomes with a high inherent sensitivity to gene dosage. As a result, they could not tolerate transient dosage changes and resisted decay on the Y chromosome. These 14 genes remain as homologous pairs with a single copy on the X and Y chromosomes and continue to be expressed from both X chromosomes in 46,XX females, and from the X and Y chromosomes in 46,XY males (Lahn & Page, 1997) (Figure 1b). As the X–Y pair genes do not crossover during meiosis, they have diverged and encode distinct protein isoforms; the X and Y copies retain 73–98% DNA and 71–99% protein identity (Skaletsky et al., 2003). Molecular functions of the X–Y pair genes have not been extensively compared; however, there is some evidence from mouse studies and in vitro experiments demonstrating either functional interchangeability or divergence, depending on the experimental context. For example, the histone lysine demethylase Uty is redundant with Kdm6a (Utx) in mouse development (Lee, Lee, & Lee, 2012; Shpargel, Sengoku, Yokoyama, & Magnuson, 2012), but the human UTY protein shows reduced demethylase activity in vitro compared to UTX (Hong et al., 2007; Lan et al., 2007).

Studies of independent sex chromosome systems reinforce the idea that survival of ancestral genes on sex-specific chromosomes is an important indicator of dosage sensitivity. Indeed, the same principle holds for the chicken ZW system, in which the W chromosome is present exclusively in females. Like the Y, the W chromosome has extensively degenerated over time, yet retains a set of genes that are highly dosage sensitive (Bellott et al., 2017). In the case of both the Y and W, the remaining ancestral genes are broadly expressed throughout the body and involved in fundamental cellular processes including transcription, epigenetic regulation, and translation (Figure 1c) (Bellott et al., 2014; Bellott et al., 2017). As we discuss below, these genes are some of the top candidates for contributing to TS and sex-biased phenotypes.

The third, and largest, class of X chromosome genes includes genes on the ancestral autosomes with intermediate dosage sensitivity. These genes went through a complex, three-step evolutionary process: (a) decay of the Y-linked homolog; (b) X chromosome upregulation; and (c) X chromosome inactivation (Jegalian & Page, 1998; Ohno, 1967). Following loss of the Y homologs, the genes were transcriptionally upregulated on the X chromosome in both sexes to maintain the ancestral gene dosage in males. The increased expression would have been deleterious to females; therefore, a mechanism of transcriptional dosage compensation, X chromosome inactivation (XCI), arose to silence one copy (Figure 1b). As these "X-inactivated" genes have equivalent expression in individuals with one or more
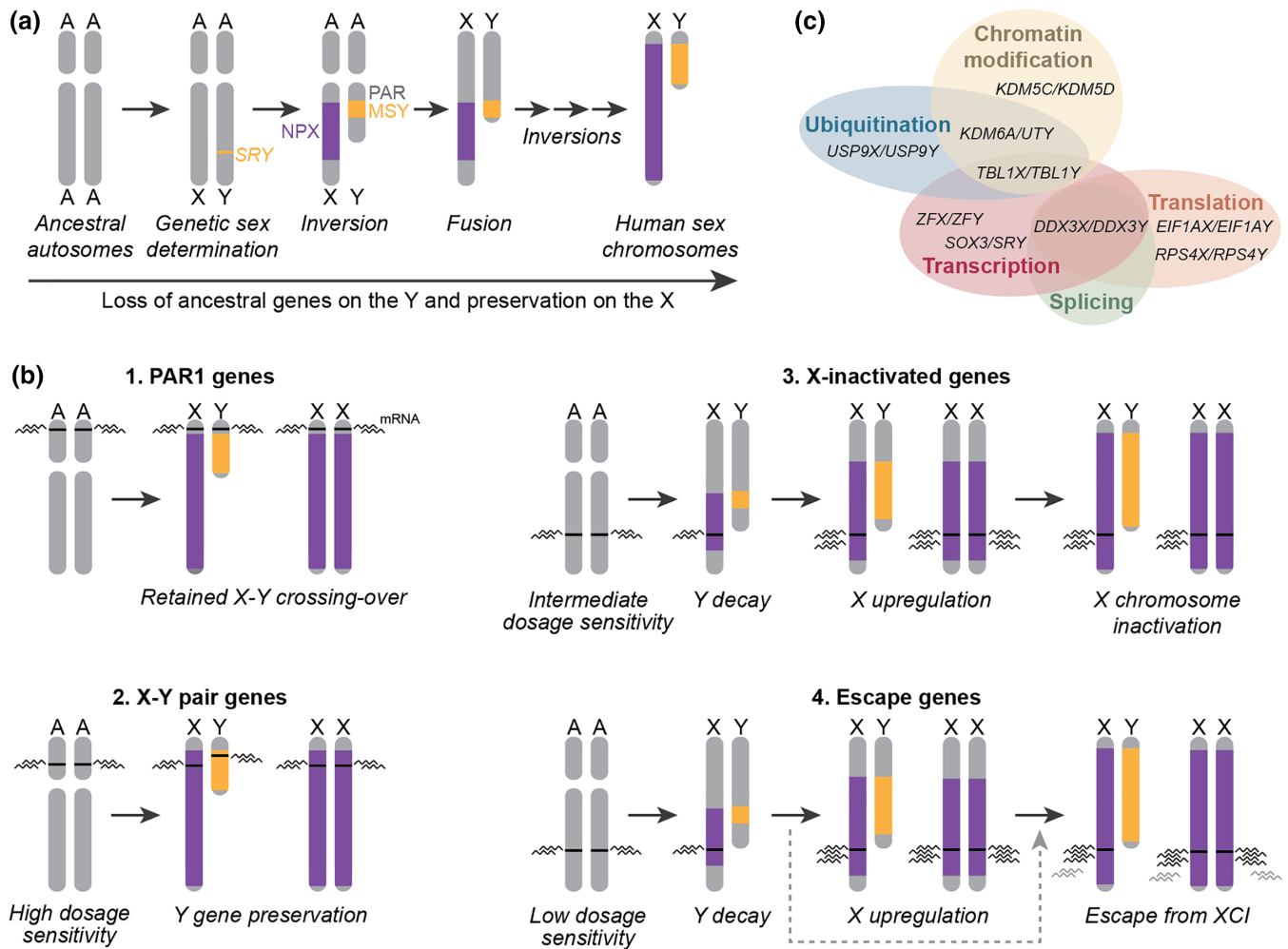
FIGURE 1 Evolution of the human sex chromosomes resulted in four gene classes on the X chromosome. (a) Sex chromosome evolution began with ordinary autosomes. SRY emergence began Y chromosome differentiation, followed by a series of inversions that resulted in loss of crossing-over between the male-specific region of the Y (MSY) and the non-pseudoautosomal region of the X (NPX). The pseudoautosomal region (PAR) on the short arm retained X–Y crossing-over. (b) Four classes of genes on the X chromosome underwent different evolutionary trajectories as a result of Y chromosome decay. The black wavy lines indicate the level of mRNA expressed from each gene. PAR1 genes (1) retained X-Y crossing-over and remain expressed from both the X and Y chromosomes. X-Y pair genes (2) reside in the NPX and MSY, and do not cross-over, but due to high dosage sensitivity the Y gene was preserved, and expression retained on both X chromosomes in 46,XX females. X-inactivated genes (3) had intermediate dosage sensitivity, which allowed for Y chromosome decay. This was followed by upregulation on the X chromosome to retain ancestral dosage in males, and subsequent inactivation of one allele in females. Escape genes (4) followed the same initial path as the X-inactivated genes, but did not become inactivated. Some escape genes may have avoided the step of X upregulation, indicated by the gray dashed arrow bypassing this step. These genes would have lower levels of mRNA expression (gray wavy lines) compared to genes that underwent X upregulation. (c) Functions of select human X–Y pair genes, adapted from "Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators," by Bellott et al., 2014, *Nature*, 508, p. 494

X chromosomes, they are not likely to underlie phenotypes related to variation in sex chromosome constitution.

Finally, genes with the lowest dosage sensitivity followed a fourth path: they were lost from the Y chromosome but are not subject to XCI today. Instead, they "escaped" XCI and are expressed from both X chromosomes in 46,XX females. The exact evolutionary trajectory they took to get there is not clear; they either were not upregulated following Y decay and did not require XCI, or they were upregulated but did not become inactivated (Figure 1b). Escape genes are expressed at higher levels in 46,XX females compared to 46,XY males across tissues (Tukiainen et al., 2017), and are plausible candidates for contributing to sex-biased phenotypes.

Since the phenotypes in TS are related to the loss of all or part of a sex chromosome, candidate genes should be sensitive to gene dosage and require expression from two alleles on the sex chromosomes (Ferguson-Smith, 1965; Held et al., 1992; Hook & Warburton, 1983). Given this, and because 46,XY males do not display TS phenotypes despite only having a single X chromosome, causal TS genes must be common to X and Y, and functionally interchangeable (Watanabe, Zinn, Page, & Nishimoto, 1993; Zinn, Page, & Fisher, 1993). There are two groups of genes that fit these criteria: (a) PAR genes and (b) X–Y pair genes (if they prove to be functionally interchangeable in specific phenotypic contexts relevant to TS; Figures 2 and 3).
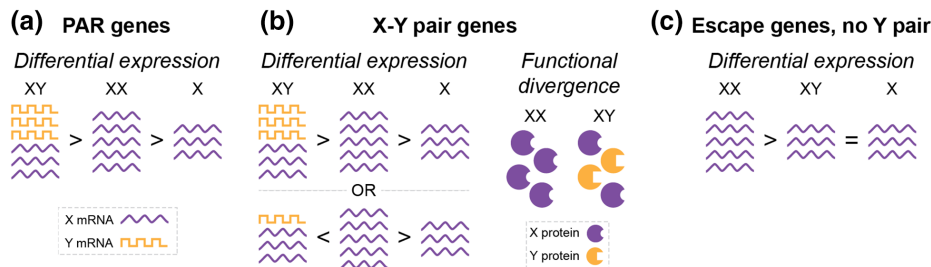
FIGURE 2 Mechanisms of sex chromosome contributions to TS and sex differences. (a) PAR genes are typically more highly expressed in 46,XY males compared with 46,XX females, due to spreading of XCI, and expressed at the lowest levels 45,X individuals. (b) Left, X-Y pair genes may have differential mRNA expression levels from the X and Y homologs such that 46,XY or 46,XX individuals have unequal total expression. These genes escape X chromosome inactivation in 46,XX females, so expression is lower in 45,X females. Right, X-Y pair genes may encode functionally divergent proteins that lead to molecular sex differences between 46,XX and 46,XY individuals. A combination of both mechanisms may also exist. A combination of both mechanisms may also exist. (c) Genes that escape XCI without a Y homolog may contribute to sex differences due to differences in mRNA expression. Expression of genes that escape XCI is higher compared to 46,XY and 45,X individuals, in which these genes are equally expressed. Since 46,XY individuals do not have TS, we do not anticipate that these genes will underlie phenotypes in TS

Sex-biased phenotypes must be mediated by genes that are (a) expressed at different levels based on sex chromosome dosage, and/or (b) encode Y chromosome proteins with unique functions. Escape genes and PAR genes fit the first criterion, while X–Y pairs fit both criteria. Genes that escape XCI are expressed higher in females (Carrel, Cottle, Goglin, & Willard, 1999); conversely, PAR genes tend to be more highly expressed in males due to spreading of XCI into the PAR in 46,XX females (Tukiainen et al., 2017). X-Y pair genes may have differences in the cumulative expression from both of the X chromosomes in 46,XX females or summed expression from the X and Y chromosomes in 46,XY males. Additionally, the X–Y pair genes may exhibit functional differences because they encode distinct protein isoforms (Skaletsky et al., 2003). This divergence allows for the intriguing possibility that the X and Y protein isoforms lead to distinct biochemical activities in males and females (Figures 2 and 3).

3 | EXPERIMENTAL MODELS FOR IDENTIFYING PHENOTYPES MEDIATED BY SEX CHROMOSOME CONSTITUTION

At this point, we have a sufficient understanding of the types of genes that likely contribute to phenotypes due to variation in sex chromosome constitution. However, obtaining experimental evidence to prove the involvement of any specific gene or the sex chromosomes in general is not trivial. This is complicated by the co-occurrence of other variables with sex chromosome constitution, including gonadal hormone profiles and environmental exposures. For example, typical 46,XX females and 46,XY males differ in the levels of circulating estrogens and androgens, while women with TS have reduced estrogen levels compared to 46,XX females (Turner, 1938). There have been many strategies applied to this challenge, each with its pros and cons; we will evaluate some of them here and provide suggestions for moving forward.

One common strategy for deciphering genotype–phenotype relationships is to develop a mouse model, which has been applied in both TS and sex differences. For TS, there is a monosomy X mouse model (39,X karyotype); however, it does not recapitulate the infertility, embryonic lethality, or the severity of phenotypes present in TS (Welshons & Russell, 1959). This is likely because the mouse and human sex chromosomes have significantly diverged in gene content and regulation. Seven of the 14 human X–Y pair genes are not present on the mouse Y chromosome: five were lost and two were retrotransposed onto autosomes (Hughes, Skaletsky, Koutseva, Pyntikova, & Page, 2015; Soh et al., 2014). Additionally, almost all of the orthologs of the human PAR genes are found on mouse autosomes or are missing in the mouse genome (Table 1) (Raudsepp & Chowdhary, 2015). Moreover, sex chromosome gene dosage regulation differs between mice and humans; only 3–7% of X-linked genes are expressed from both X alleles in the mouse, compared to at least 23% in humans (Berletch et al., 2015; Tukiainen et al., 2017).
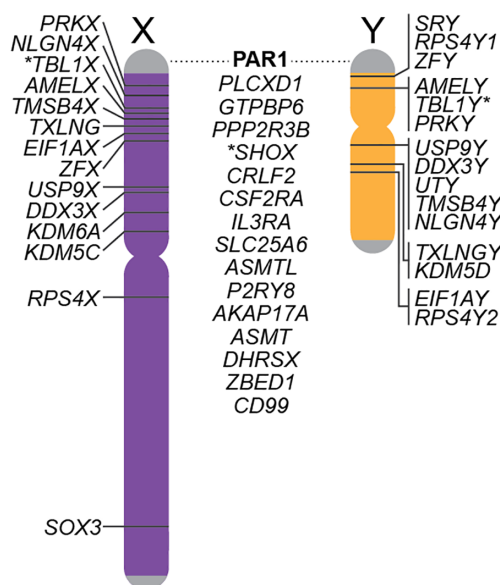

FIGURE 3 X and Y genes that may contribute to Turner syndrome and sex differences. This schematic includes two categories of genes that contribute to TS and sex differences phenotypes: X–Y pair genes and PAR1 genes (not shown are X escape genes without a Y homolog; these may also contribute to sex differences). PAR1 genes are listed in order from distal to proximal. Genes discussed in case studies are marked with an asterisk

TABLE 1 Mouse orthologs of human X–Y pair and PAR1 genes

		Chromosomal location	
	Human gene	Human	Mouse[c]
Ancestral X–Y pair genes	SOX3/SRY	X/Y	X/Y
	ZFX/ZFY	X/Y	X/Y
	DDX3X/Y	X/Y	X/Y[a]
	KDM6A/UTY	X/Y	X/Y[a]
	KDM5C/D	X/Y	X/Y[a]
	USP9X/Y	X/Y	X/Y
	EIF1AX/Y	X/Y	X/18[g]
	RPS4X/Y	X/Y	X/6[g]
	AMELX/Y	X/Y	X/−
	TXLNG/Y	X/Y	X/−
	TMSB4X/Y	X/Y	X/−
	NLGN4X/Y	X/Y	X/Y (PAR)
	TBL1X/Y	X/Y	X/−
	PRKX/Y	X/Y	X/−
PAR genes	PLCXD1	X/Y (PAR1)	5
	GTPBP6	X/Y (PAR1)	5[d]
	PPP2R3B	X/Y (PAR1)	9
	SHOX	X/Y (PAR1)	−[b]
	CRLF2	X/Y (PAR1)	5
	CSF2RA	X/Y (PAR1)	19[e]
	IL3RA	X/Y (PAR1)	14[f]
	SLC25A6	X/Y (PAR1)	−[f]
	ASMTL	X/Y (PAR1)	−
	P2RY8	X/Y (PAR1)	−
	AKAP17A	X/Y (PAR1)	X/Y (PAR)
	ASMT	X/Y (PAR1)	X/Y (PAR)
	DHRSX	X/Y (PAR1)	4[d]
	ZBED1	X/Y (PAR1)	−[d]
	CD99	X/Y (PAR1)	4[h]

PAR = pseudoautosomal region.
[a] X homolog expressed from both X chromosomes in human and mouse (Berletch et al., 2015; Yang, Babak, Shendure, & Disteche, 2010).
[b] Human and mouse have a paralagous gene, SHOX2, on chr3 (Rao et al., 1997).
[c] For X-Y pairs, presence or absence of the Y copy in mouse is confirmed in (Soh et al., 2014).
[d] (Gianfrancesco et al., 2001).
[e] (Disteche et al., 1992).
[f] (Ellison, Li, Francke, & Shapiro, 1996).
[g] (Hughes et al., 2015).
[h] (Park et al., 2005).

To study sex differences, a sex-reversed mouse model was developed to uncouple the effects of sex chromosome constitution and gonadal hormones. In this model, Sry, the Y-linked testis-determining gene, is deleted from the Y chromosome and relocated to an autosome as a transgene, resulting in the ability to generate gonadal males with either XY or XX chromosomes, and gonadal females with either XX or XY chromosomes. Therefore, this model allows for the identification of phenotypes independently associated with either gonadal sex or sex chromosome constitution. To date, several phenotypes dependent on sex chromosome constitution have been identified with this model, such as pathogenesis of autoimmune disease, addiction behaviors, and fat metabolism (Chen, McClusky, Itoh, Reue, & Arnold, 2013; Quinn,

Hitchcott, Umeda, Arnold, & Taylor, 2007; Smith-Bouvier et al., 2008). Due to the divergence of the sex chromosomes, it will be useful to determine whether the genes mediating these phenotypes are conserved between mouse and human. The most informative genes in this regard are Kdm6a/Uty, Kdm5c/Kdm5d, and Ddx3x/Ddx3y, since, similar to humans, they remain as X–Y pairs and the X homologs are expressed from both X chromosomes in 40,XX female mice (Table 1).

Other strategies have been developed to study the effects of sex chromosome constitution directly in humans. The preimplantation embryo is a promising place to look at phenotypes mediated by the sex chromosomes prior to the initiation of gonadal development and hormone production. A single-cell study of human embryos from the eight-cell stage to embryonic day 7 found sex-biased expression of 58 autosomal genes, 13 Y-linked genes, and 105 X-linked genes (Petropoulos et al., 2016). It is likely that there were so many sex-biased X-linked genes observed because X chromosome inactivation is not yet complete during this developmental window. These results indicate that the sex chromosomes significantly shape the early transcriptome and may contribute to sex differences that include rate of growth to the blastocyst stage and glucose metabolism, which differ markedly between XX and XY human preimplantation embryos (Ray, Conaghan, Winston, & Handyside, 1995). Other groups have used human pluripotent stem cells (both embryonic and induced pluripotent stem cells) to model TS and sex differences in the preimplantation period (Ronen & Benvenisty, 2014; Syrett, Sierra, Berry, Beiting, & Anguera, 2018; Urbach & Benvenisty, 2009). These are promising model systems because they enable genetic manipulation and the potential to recapitulate tissue differentiation in vitro. However, many pluripotent cell lines have perturbed XCI status and must be carefully evaluated on a case-by-case basis prior to being used to address questions related to sex chromosome constitution.

Another human-centric approach is to study individuals with chromosomal mosaicism, in which cells with different sex chromosome constitutions are exposed to the same levels of gonadal hormones and environmental stimuli. Individuals with typical 46,XX or 46,XY karyotypes lose sex chromosomes in blood cells with increased age, which has stimulated interest in understanding the contributions of this phenomenon to disease phenotypes (Guttenbach, Koschorz, Bernthaler, Grimm, & Schmid, 1995; Jacobs, Doll, Goldstein, Brunton, & Courtbrown, 1963). Indeed, researchers have found that loss of the Y chromosome in men correlates with increased risk of Alzheimer's disease and cancer (Dumanski et al., 2016; Forsberg et al., 2014). It is worth noting that general chromosomal instability predisposing to cancer may result in Y loss simply because this chromosome is easier to lose, so more research must be done to prove causality (Wright et al., 2017). Mosaicism is also a common phenomenon in women with TS; in fact, it is thought that all women with TS must be mosaic for cells of another karyotype, such as 46,XX, because ~99% of fetuses with a complete 45,X karyotype do not survive fetal development (Hook & Warburton, 1983, 2014). Mosaicism is associated with reduced severity of many phenotypes in TS, for example 45,X/46,XX mosaicism is associated with enhanced follicle development and fertility (Borgstrom et al., 2009). To most effectively harness mosaicism as a model in the future, we must learn more about its

embryonic origins, dynamics throughout the lifespan, distribution across the body, and variability between individuals.

These experimental strategies have begun to help us understand the effects of the sex chromosomes in development, health, and disease, each with their strengths and weaknesses. Many of the approaches are used to investigate sex chromosome constitution as a whole, and not to study the influence of specific genes on the sex chromosomes on a given phenotype. We will now present two case studies that implicate one PAR gene and one X–Y pair in specific TS phenotypes. In addition, we speculate about how these genes may be relevant to sex differences.

4 | CASE STUDIES: DIRECT EFFECTS OF SEX CHROMOSOME GENES ON PHENOTYPES IN TS AND SEX DIFFERENCES

The best characterized genetic explanation for a phenotype in TS is for the PAR gene *short stature homeobox* (*SHOX*), which, as the name describes, is associated with short stature. The *SHOX* gene was initially identified through studies of short-statured individuals with deletions on the tip of the short arm of the X chromosome (Rao et al., 1997). Since then, the molecular functions of SHOX have been comprehensively investigated (see (Marchini, Ogata, & Rappold, 2016) for an extensive review). Human studies show that *SHOX* is expressed in the developing long bones of the forearms and lower limbs, as well as the first and second pharyngeal arches, which contribute to the bones of the face (Clement-Jones et al., 2000). Thus, *SHOX* is also thought to be responsible for the skeletal phenotypes in TS, including cubitus valgus, short metacarpals, and a high arched palate. As a testament to the dosage sensitivity of *SHOX* in bone growth, individuals with an extra sex chromosome, and therefore an extra copy of *SHOX*, are taller than average (Ogata & Matsuo, 1993). It is also interesting to speculate about the possibility that *SHOX* may contribute to sex differences in height. Similar to other PAR genes, there is evidence that *SHOX* is more highly expressed in 46,XY males, compared to 46,XX females. This result comes from a gene expression survey of adult human tissues in which male bias was observed in all six tissues where *SHOX* expression could be detected, but it remains to be seen whether this is also true in the context of the developing long bones (Tukiainen et al., 2017). Given the dosage sensitivity of *SHOX* demonstrated by studies of individuals with sex chromosome aneuploidies, a small difference in the expression of this gene may lead to nontrivial sex differences in height.

Recently, the X–Y pair transducin beta like 1 X and Y (TBL1X/Y) *TBL1X/Y* emerged as a compelling genetic explanation for the high prevalence of sensorineural deafness in TS (Elsheikh, Dunger, Conway, & Wass, 2002; Gravholt, 2004). Genetic sequencing of a family with X-linked inheritance of late-onset sensorineural deafness identified a causal loss-of-function mutation in *TBL1X* (Bassi et al., 1999). Another family, this time with a Y-linked inheritance pattern of hearing loss, was found to have a loss-of-function mutation in *TBL1Y*, the Y homolog of *TBL1X* (Di Stazio et al., 2018). This is in contrast to a different family in which Y-linked hearing loss was associated with transposition of a region of Chromosome 1 previously implicated in hearing

impairment to the Y chromosome (Wang et al., 2013). Available evidence points to the potential for functional redundancy of TBL1X and TBL1Y: both genes are expressed in the human cochlea, and in vitro studies show that they bind to the same transcriptional corepressor complex and have similar effects on gene expression (Di Stazio et al., 2018; Guenther et al., 2000). Previously, it was reported that a recurrent Y chromosome deletion encompassing *TBL1Y* and several other genes had "no major deleterious effects," which cast doubt on whether *TBL1X/TBL1Y* could contribute to phenotypes in TS (Jobling et al., 2007). However, the authors state that they had no phenotypic information about the subjects with the Y deletion, therefore the results are not inconsistent with *TBL1Y* contributing to sensorineural deafness. There are also clues that *TBL1X/Y* may be involved in sex differences in hearing loss. *TBL1Y* is expressed three times higher than *TBL1X* in the human cochlea, raising the possibility of a sex difference in TBL1 activity in the inner ear (Di Stazio et al., 2018). Interestingly, several large studies have found that hearing loss, especially at high frequencies, is more prevalent in men compared to women (Gates, Cooper Jr., Kannel, & Miller, 1990; Helzner et al., 2005; Moller, 1981). More research is required to definitively show that the *TBL1X/Y* pair is involved in hearing loss and to investigate its molecular functions in the inner ear. Nevertheless, these initial findings provide a promising avenue for a more thorough understanding of this prevalent phenotype in TS and may lead to novel therapeutic approaches.

5 | CONCLUSIONS AND FUTURE PROSPECTS

This is an exciting time to reinvigorate research into the sex chromosomes and apply our extensive evolutionary, structural, and functional understanding to new questions in the realms of TS and sex differences. The tandem approach of combining the study of sex chromosome aneuploidy and sex differences is already yielding promising results. It has been extensively applied toward understanding the contributions of the sex chromosomes to neurodevelopmental and psychiatric disorders (recently reviewed in Green, Flash, and Reiss (2019)). To make further progress, we issue the following recommendations. First, we must invest in resources such as large clinical datasets measuring phenotypes of interest in populations with different sex chromosome constitutions. We must also develop biorepositories to store cells and tissues from these individuals, as it is difficult for any single investigator to obtain enough samples for robust molecular studies of human tissues. Second, we must explore new methods for studying the sex chromosomes in the context of specific phenotypes, in a manner that is free of confounding variables. This includes improving our abilities to model sex differences and sex chromosome aneuploidies both in vivo, using engineered mouse models or exploring other model organisms, and in vitro through pluripotent stem cell cultures and directed differentiation into specific tissues of interest. Finally, we must engage multidisciplinary teams of clinicians and scientists, the sex chromosome aneuploidy communities, and representatives from the biotechnology and pharmaceutical industries to join in these pursuits. Ultimately, biology has linked TS and sex differences through the shared molecular phenomenon of differences in X and Y

chromosome dose. It is now up to researchers to achieve a broad understanding of the contributions of sex chromosome constitution to human development, health, and disease; and to use this knowledge to transform medical care for future generations.

ORCID

Adrianna K. San Roman https://orcid.org/0000-0003-4511-3052
David C. Page https://orcid.org/0000-0001-5489-6453

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AUTHOR BIOGRAPHIES

Adrianna K. San Roman, PhD, is a postdoctoral fellow in the laboratory of David Page at the Whitehead Institute for Biomedical Research. A graduate of Williams College, Dr. San Roman received her Ph.D. in Developmental and Regenerative Biology from Harvard University where she studied epigenetic regulation of stem cells in intestinal regeneration. In the Page lab she studies the molecular mechanisms underlying Turner syndrome phenotypes and how these are related to sex differences.

David C. Page, MD, is the Director of the Whitehead Institute for Biomedical Research, Professor of Biology at MIT, and an Investigator of the Howard Hughes Medical Institute. A graduate of Swarthmore College, Dr. Page earned his MD from Harvard Medical School and the Harvard-MIT Health Sciences and Technology program. He joined the Whitehead Institute, as the first Whitehead Fellow, in 1984. Dr. Page's laboratory explores fundamental differences between males and females in health and disease, both within and beyond the reproductive tract. The Page lab recently discovered that XY and XX sex chromosomes account for subtle differences in the molecular biology of male and female cells throughout the body. Dr. Page's honors include a MacArthur Prize Fellowship, Science magazine's Top Ten Scientific Advances of the Year (1992 and 2003), the Francis Amory Prize of the American Academy of Arts and Sciences, and the March of Dimes Prize in Developmental Biology. He is a Member of the National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences.