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# The Biology and Evolution of Mammalian Y Chromosomes

Jennifer F. Hughes and David C. Page

Whitehead Institute, Howard Hughes Medical Institute, and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142; email: [jhughes@wi.mit.edu](mailto:jhughes@wi.mit.edu)

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## Keywords

MSY, sex chromosomes, sex determination, spermatogenesis, Turner syndrome, sexual dimorphism

## Abstract

Mammals have the oldest sex chromosome system known: the mammalian X and Y chromosomes evolved from ordinary autosomes beginning at least 180 million years ago. Despite their shared ancestry, mammalian Y chromosomes display enormous variation among species in size, gene content, and structural complexity. Several unique features of the Y chromosome—its lack of a homologous partner for crossing over, its functional specialization for spermatogenesis, and its high degree of sequence amplification—contribute to this extreme variation. However, amid this evolutionary turmoil many commonalities have been revealed that have contributed to our understanding of the selective pressures driving the evolution and biology of the Y chromosome. Two biological themes have defined Y-chromosome research over the past six decades: testis determination and spermatogenesis. A third biological theme begins to emerge from recent insights into the Y chromosome's roles beyond the reproductive tract—a theme that promises to broaden the reach of Y-chromosome research by shedding light on fundamental sex differences in human health and disease.

**Male-specific region of the Y chromosome (MSY):** region of the Y chromosome that does not ordinarily engage in crossing over with the X chromosome during male meiosis; excludes pseudoautosomal regions

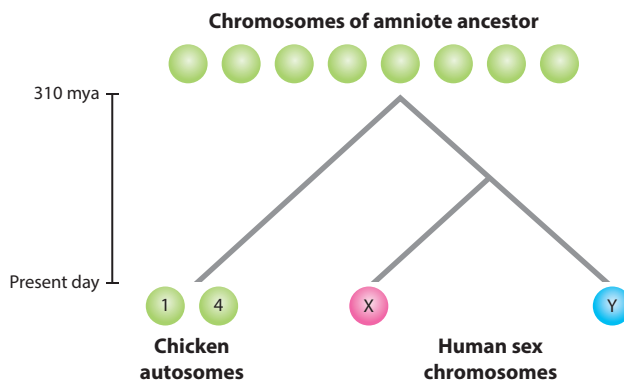
## INTRODUCTION

### The Evolutionary History of the Y Chromosome

Mammalian sex chromosomes evolved from an ordinary pair of autosomes (85). The X and Y chromosomes began to differentiate at least 180 million years ago, before the divergence of the marsupial and placental mammalian lineages. The autosomes that gave rise to the mammalian sex chromosomes still exist as autosomes in birds today (**Figure 1**). The first step in X-Y differentiation was the acquisition of a testis-determining gene by the proto-Y chromosome. Next, a series of large-scale inversions, most likely on the Y chromosome, suppressed recombination between the X and Y chromosomes in a stepwise fashion, creating at least four evolutionary strata (4, 48, 63, 89). Throughout this process, the X chromosome retained a partner for crossing over in females, but the Y chromosome's opportunities for crossing over became increasingly restricted as stratification progressed. Outside the pseudoautosomal regions (PARs), highly differentiated mammalian sex chromosomes do not ordinarily engage in crossing over with each other. In the absence of crossing over, the male-specific region of the Y chromosome (MSY) (which excludes the PARs) was subject to genetic decay, resulting in deletions and gene losses that collectively decimated it (15, 82, 99). In humans, the euchromatin of the present-day MSY is less than one-sixth the size of the X chromosome (23 Mb compared to 155 Mb) and has retained only 3% of the genes that were present on the ancestral autosome pair (17 of ~640) (4, 100, 107). The dearth of molecularly defined genes in the human MSY confirmed, in the minds of many biologists, its earlier characterization as a genetic wasteland and even fueled jovial speculation that the demise of the Y chromosome was imminent (1, 38, 39).

### Ignorance of Y Chromosomes

In diverse species with XX/XY sex chromosomes, biological understanding of Y chromosomes has consistently and dramatically lagged behind that of autosomes and X chromosomes. This is true both in human genetics and in experimental genetic systems like *Drosophila* and mouse. Consider the human Y chromosome. In the first half of the twentieth century, dozens of scientific publications claimed to provide evidence that one trait or another was Y-linked. By the 1950s,



**Figure 1**

Human sex chromosomes evolved from autosomes. Schematic representation of the evolution of human sex chromosomes, derived from an autosome pair present in the mammalian-avian ancestor. The same autosome pair contributed to present-day chicken autosomes 1 and 4. Bird sex chromosomes arose from a distinct pair of autosomes that contributed to present-day human chromosomes 5, 9, and 18. Timescale at left indicates approximate branching times. Abbreviation: mya, million years ago.

## Y-LINKED HEARING IMPAIRMENT: AN EXCEPTIONAL CASE

Only once has a Mendelian trait (apart from testis determination) been definitively linked to the Y chromosome: an unusual case of hearing impairment. In 2004, Y-linked hearing loss in a large Chinese family was reported, and in 2012 the molecular underpinnings of this curious phenomenon came to light (123, 124). Analysis of the family's nine-generation pedigree revealed that hearing loss appeared spontaneously and then displayed Y-linked dominant inheritance, appearing in all male descendants of the original affected male. A combination of Y-chromosome resequencing, read-depth mapping, and fluorescence in situ hybridization (FISH) in affected and unaffected males revealed that hearing impairment was associated with a complex Y-chromosome rearrangement that included an ~160-kb transposition from chromosome 1. This chromosome-1 segment is located within a genetic interval previously implicated in hearing impairment, implying that an extra copy of a dosage-sensitive gene or genes contained within this chromosome-1 region is the causal factor in this special case of Y-linked hearing impairment.

however, all such pedigree-based evidence of Y-linked genes had been discredited by the noted geneticist Curt Stern (109). In no case has the existence of an MSY gene been correctly inferred from the transmission of phenotypes in human pedigrees (but see sidebar, Y-Linked Hearing Impairment: An Exceptional Case). This methodological difficulty was misinterpreted as evidence that there are few or no genes in the human MSY. Consequently, the human Y chromosome came to be viewed as having little or no function—and little or no medical relevance.

### Three Biological Themes in Y-Chromosome Research

The advent of molecular genetics and genomics in the closing decades of the twentieth century helped bring to light the biological and medical relevance of the Y chromosome. With new molecular tools in hand, such as DNA cloning and nucleic acid hybridization, studies of Y-chromosome gene function came to rely not on classical transmission genetics but on DNA-based characterization of spontaneously arising sex chromosome anomalies. Three biological themes—one of which is just now beginning to unfold—have come to define Y-chromosome research in the age of molecular genetics and genomics.

The first biological theme, which defined the field of Y-chromosome research for more than three decades, involved the pursuit of the Y-linked testis-determining factor, which was the one and only genetic function attributed to the Y chromosome well into the 1970s and which offered the only obvious avenue for study. Studies of humans lacking portions of the Y chromosome, combined with functional studies in mice, culminated in 1991 in the establishment of *SRY* (sex-determining region of the Y chromosome) as the testis-determining gene on the mammalian Y chromosome (5, 42, 54, 59).

The second biological theme, which the field has pursued in earnest since the 1990s, concerns the roles of the Y chromosome in sperm production and fertility. Research accelerated with the publication of the DNA sequence of the human MSY in 2003. Over the ensuing decade, and following the sequencing of two additional primate MSYs (49, 50), the first MSY from a model organism—the mouse—was sequenced to completion (108). The mouse MSY sequence dramatically expanded the known range of diversity in mammalian Y-chromosome structure and gene content, enabling investigators to understand more deeply the selective forces driving Y-chromosome evolution that operate at the level of sperm production and fertility.

Although the roles of the Y chromosome in testis determination and spermatogenesis are firmly established, a third biological theme is just emerging: the quest to comprehend the Y chromosome's

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**Sex-determining region of the Y chromosome (*SRY*):** testis-determining gene found on all therian mammalian Y chromosomes; its expression in gonadal supporting cells causes the embryonic gonad to develop as a testis

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influence beyond the reproductive tract. Recent comparative genomic analyses, combined with earlier associations made between the Y chromosome and the multifaceted phenotypes found in females with Turner Syndrome [XO (or more formally, 45,X)] (29), provide intriguing clues regarding the roles that many Y-linked genes play throughout the body. The presence or absence of a Y chromosome (i.e., XY or XX sex chromosome constitution) may influence development and physiology on a multitude of levels, contributing to sex differences in health and disease both within and beyond the reproductive tract.

## BIOLOGICAL THEME #1: TESTIS DETERMINATION

### Sex Chromosomes and Sex Reversal

The first era in molecularly grounded Y-chromosome research focused on one biological role of the Y chromosome: testis determination. In 1959, reports of XO females (Turner syndrome, with oocyte-depleted ovaries) and XXY males (Klinefelter syndrome, with germ-cell-depleted testes) established the existence of a testis-determining gene on the human Y chromosome (32, 53), and the ensuing three decades of Y-chromosome research focused on the pursuit of this gene. In therian mammals (marsupials and placentals), the presence of a Y chromosome is sufficient to trigger testis formation during fetal development, setting in motion a cascade of events required for anatomic masculinization more broadly, including the production of testosterone (by testicular somatic cells known as Leydig cells). Although the Y chromosome triggers or activates the pathway of testis development, many other genes—most of which are located in autosomes or the X chromosome—are also involved in testicular and subsequent male development (80). For example, XY individuals with complete androgen insensitivity syndrome carry intact Y chromosomes and form testes rather than ovaries, but they develop, externally, as females because their cells cannot respond to testosterone or other androgens (47). In human and mouse, mutations in at least 17 autosomal or X-linked genes cause male-to-female gonadal sex reversal and/or abnormal gonadal development (90).

The first studies investigating the Y-chromosome's role in testis determination focused on individuals with Y-chromosome deletions and translocations that presented with sex reversal phenotypes (23, 26, 43, 76). It became evident that these Y-chromosome rearrangements frequently arose from aberrant crossing over between the X and Y chromosomes (132). The human X and Y chromosomes normally cross over only within the small PARs located at their termini. However, several loci on the short arm of the MSY are sites of occasional ectopic crossing over with the X chromosome (122, 127). Depending on which product of this ectopic crossing over is transmitted by the father, two very different phenotypes are observed in resultant offspring (**Figure 2**): (a) XX males (with germ-cell-depleted testes) in whom a terminal portion of the short arm of the Y chromosome (Yp) has been translocated to an almost complete paternal X chromosome and (b) XY females (with ovaries lacking normal germ cells) in whom a terminal portion of Yp has been replaced by a terminal portion of Xp. These observations led to the conclusion that the testis-determining gene is located on the terminal portion of Yp.

### Searching for the Testis-Determining Gene

Fine mapping of X-Y crossover products in XX males and XY females eventually narrowed the region on the human Y chromosome carrying the testis-determining gene to a 300-kb segment of Yp (86). The first candidate gene identified within this region was *ZFY*, encoding a zinc-finger protein that has two homologs on the mouse Y chromosome (87). Subsequent studies excluded



**Figure 2**

Ectopic X-Y recombination and the origins of human XX males and XY females. (a) The short arm of the X chromosome and the entire Y chromosome are shown. Euchromatic X chromosome sequence is shown in pink; all euchromatic MSY (male-specific region of the Y chromosome) sequence classes are shown in blue for simplicity; pseudoautosomal regions, green; heterochromatin, gray. (b) One of many possible sites of ectopic X-Y recombination is shown here. The crossover products are depicted below and the phenotypic consequences are indicated. Abbreviations: cen, centromere; Mb, megabase.

*ZFY* based on refined mapping data in human and expression analysis in mouse (58, 88). The second gene cloned from the critical region of Yp was the transcription factor *SRY*, which was found to be conserved on the Y chromosome of multiple mammals and expressed in mouse gonads during the appropriate stage of fetal development (42, 60, 106). After *SRY* was cloned, evidence unequivocally confirming its pivotal role in testis determination soon followed. First, two separate studies identified human XY females (with ovaries lacking normal germ cells) bearing de novo point mutations in *SRY* (5, 54). Second, XX mice bearing an autosomal transgene of the mouse *Sry* genomic locus were shown to develop as anatomic males, with testes and male internal and external genitalia (59).

## BIOLOGICAL THEME #2: SPERMATOGENESIS

The existence of a testis-determining gene on the MSY, and the molecular identification of that gene as *SRY*, was widely viewed as an isolated, exceptional counterpoint on an otherwise barren and desolate chromosome. The genetic wasteland model of the MSY dominated thinking among biologists into the 1990s, when advances in Y-chromosome genomics ushered in a second era of intensive molecular genetics research. Direct genomic analysis of the human MSY, coupled with molecular characterizations of recurrent MSY deletions and their phenotypic consequences, led to an appreciation of the MSY's central role in spermatogenesis.

### Sequence of the Human MSY: A Fragile Hall of Mirrors

In 1992, a nearly complete physical map of the human MSY was produced (31), representing one of the first two human chromosomes to be mapped and cloned in its entirety [the other being chromosome 21 (16)]. The YAC (yeast artificial chromosome)-based physical map of the human MSY was complemented by a second map, published concurrently and based on naturally occurring deletions as found in children or adults (120). However, certain regions of the human MSY proved to be extremely difficult to map; YACs could not be assembled into large contigs, and markers

## TRADITIONAL SEQUENCING APPROACHES DO NOT WORK FOR Y CHROMOSOMES

All standard approaches to genome sequencing, including both BAC-based and whole-genome-shotgun (WGS), fall short in ampliconic regions. Take the human reference sequence as an example: It was originally assembled from a patchwork of BAC clones derived from 13 different individuals representing 26 haplotypes. Ampliconic sequences consist of repeats that may differ from one another by less than 1 bp in 10,000, which is an order of magnitude lower than the nearly 1 bp in 1,000 difference typically observed between alleles or haplotypes. In assembling the sequence of ampliconic regions, only occasional nucleotide differences distinguish BACs that derive from different copies of an amplicon as opposed to those that truly overlap. If an investigator employs BACs derived from multiple haplotypes, then the relatively frequent differences between haplotypes mask the rare differences between amplicon copies. In the multihaplotype assembly of the human genome, amplicons were thus frequently misassembled or mistakenly abandoned as redundant.

The WGS approach, which is routinely employed in both human medical resequencing and nonhuman *de novo* genome sequencing, has an even more profoundly negative effect on the representation of ampliconic sequences. WGS assemblies rely solely on either capillary-based or next-generation sequencing reads. Ampliconic regions are completely inaccessible by this strategy because of the disparity between the length of WGS reads (100 to 500 bp) and the length of amplicon-repeat units (10 kb to >1 Mb). Thus, reads deriving from different repeat units are invariably collapsed into single contigs during assembly because WGS methods do not incorporate mapping information, which is necessary to identify and disentangle large amplicons.

could not be ordered with confidence. The most problematic region was *AZF<sub>c</sub>* (azoospermia factor c), which came to be recognized as the most frequently deleted portion of the Y chromosome in men with spermatogenic failure (37, 57, 83, 94, 105, 119). Several independent efforts to map the *AZF<sub>c</sub>* region encountered similar difficulties (56, 110, 131). Such difficulties are frequently associated with highly repetitive genomic sequences, of which the Y chromosome in general and the *AZF<sub>c</sub>* region in particular would eventually prove to be unmatched exemplars.

Although the technical challenges of these mapping studies hinted at the repetitiveness and structural complexity of the *AZF<sub>c</sub>* region, the region remained an enigma until it was mapped and sequenced to completion in 2001. Because of the technical challenges that the *AZF<sub>c</sub>* region posed, its mapping and sequencing required the conception and implementation of a new method known as SHIMS (single-haplotype iterative mapping and sequencing) to disentangle extremely repetitive genomic regions (48, 61) (see sidebar, Traditional Sequencing Approaches Do Not Work for Y Chromosomes). First, bacterial artificial chromosomes (BACs) or fosmids are used as sequencing templates because they are less prone to chimerism than the larger YAC clones. Second, clones derived from the Y chromosome of one individual, bearing a single haplotype, are used for sequencing to eliminate polymorphisms, which could otherwise be confused with paralogous duplications. Third, tiling paths are constructed with a high degree of overlap between adjacent clones so that rare, single-nucleotide differences between paralogous repeat copies can be identified. Last, the map is refined through an iterative process, sorting repeat copies into correct tiling paths based on sequence information. The finished *AZF<sub>c</sub>* sequence, assembled using SHIMS, spanned 4.5 Mb and contained three massive palindromes, three other large inverted repeats, and three direct repeats (**Figure 3**) (61). This region is gene-rich, harboring a total of 15 genes in five distinct gene families, all expressed predominantly, if not exclusively, in testes.

In 2003, the SHIMS-produced sequence of the entire human MSY was completed (107), and the MSY still stands as the only chromosome in the human reference sequence that was

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### *AZF<sub>c</sub>* (azoospermia factor c):

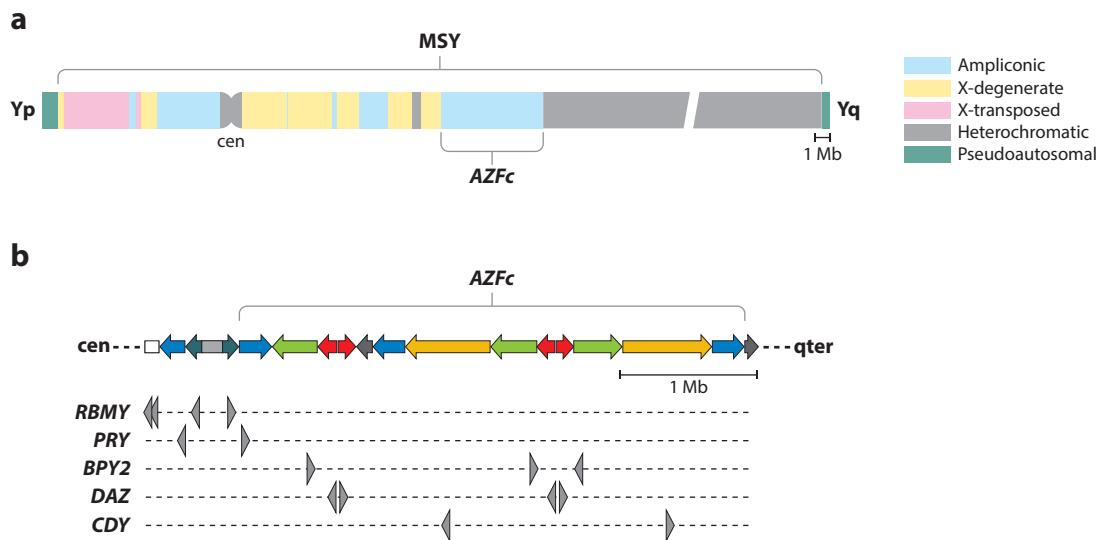
region of the human Y chromosome consisting of amplicons that provides substrates for nonallelic homologous recombination, generating recurrent deletions and rearrangements that cause spermatogenic failure

### Single-haplotype iterative mapping and sequencing (SHIMS):

a super-resolution sequencing method useful in accurately and completely assembling ampliconic regions of genomes

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**Figure 3**

The human MSY (male-specific region of the Y chromosome) and *AZFc* (azoospermia factor c). (*a*) Schematic representation of the entire human Y chromosome, including a large heterochromatic region on Yq; color-coding indicates sequence class. The X-transposed region arose from an X-to-Y transposition event that occurred three to four million years ago. (*b*) Repeat structure and gene locations within the reference *AZFc* region. Different families of repeats are color-coded and arrows indicate repeat orientation. Each of the five gene families found in this region is present in multiple copies within *AZFc*; the location and orientation of each gene copy are indicated. Abbreviations: cen, centromere; Mb, megabase; qter, long-arm terminus of Y chromosome.

assembled from a single haplotype. Most euchromatic sequences in the human MSY fall into two classes based on patterns of similarity to other sequences on the X or Y chromosomes (**Figure 3**) (107). X-degenerate regions are strewn with single-copy genes that have homologs on the X chromosome. These X-degenerate genes on the Y chromosome are living fossils that attest to the X and Y chromosomes' shared evolutionary origins as an ordinary pair of autosomes. Ampliconic regions of the MSY are composed of sequences that exhibit striking similarity—as much as 99.99% identity over tens or hundreds of kilobases—to other MSY sequences. Some ampliconic sequences are derived from autosomal transpositions and others are derived from the X-Y common ancestor. The ampliconic regions of the human MSY are dominated by large palindromes, or mirror-image repeat structures. The X-degenerate and ampliconic sequence classes are also distinct in their functional characteristics, including their gene content (64, 107). The X-degenerate genes are single copy, and most are expressed in multiple tissues. The ampliconic genes are multicopy and expressed predominantly, if not exclusively, in the testis, suggestive of specialized functions related to spermatogenesis.

The availability of the human MSY sequence enabled studies of the molecular mechanisms underlying recurrent MSY deletions and other types of rearrangements, and this in turn led to a deeper appreciation of the MSY's central role in spermatogenesis. MSY deletions were known to be the most common genetic cause of spermatogenic failure (reduced or absent sperm production, with few or no sperm in semen) in human populations (91, 93, 94, 116, 119). With the human MSY sequence in hand, it became evident that nonallelic (ectopic) homologous recombination mediated by MSY amplicons was the underlying cause of five major, recurrent, and precisely defined classes of interstitial deletions that cause or increase the risk of spermatogenic failure:

**Bacterial artificial chromosomes (BACs):** a bacterially propagated recombinant DNA construct carrying up to 200 kb of eukaryotic DNA; essential sequencing template for SHIMS approach

**Ampliconic sequence:** genomic sequence comprising repeat units that display >99% identity over >10 kb. The term amplicon refers to a single repeat unit

*AZFa*, *AZFc* (or b2/b4), P5/distal-P1, P5/proximal-P1, and gr/gr deletions (61, 95–97, 113). Most of these deletions remove some or all copies of several multicopy, testis-specific gene families. Knowledge of the repeat structure of the MSY also enabled the design of high-throughput assays to investigate the frequency of deletions within the *AZFc* region in the general population. In >20,000 men unselected for spermatogenesis phenotype, 775 men were found to have a deletion involving the *AZFc* region, yielding an estimated prevalence of 1 in 27 (101). Much ongoing work in this field focuses on possible associations between spermatogenesis phenotypes and copy number variation in specific testis-expressed gene families (35, 36, 74, 75, 84).

### Sequence of the Mouse MSY: Fallout of X Versus Y Battle

The mouse MSY's critical role in sperm production has been studied intensely in recent decades, aided by the identification of spontaneously arising MSY deletions and rearrangements in laboratory colonies. These studies have uncovered connections between specific gene deficits and distinct spermatogenesis defects, providing insights into gene function (8).

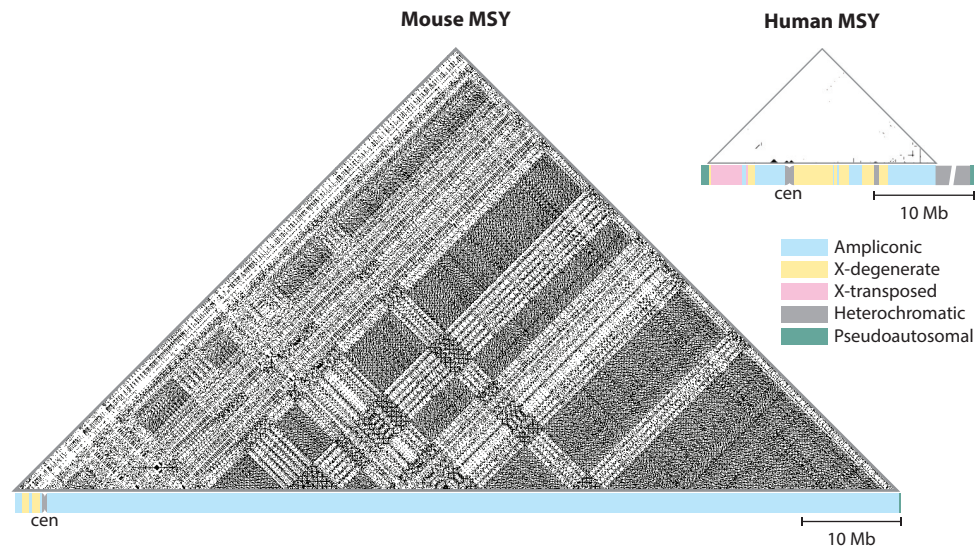
The short arm of the mouse MSY was shown to contain genes required early in spermatogenesis. The *Sxr* (sex-reversed) strain, first identified in 1971 (12), carries most of the MSY short arm, including *Sry* and 10 additional MSY genes, translocated to an X chromosome (78). Mice carrying this X-Y fusion develop as males, with testes and germ (spermatogenic) cells. However, sperm produced by *Sxr* mice exhibit morphological defects. An *Sxr*-deletion variant was later discovered that retains *Sry* but is missing six of the additional genes (79). These *Sxr*-deleted mice have a more severe spermatogenesis phenotype compared to the original *Sxr* mice, with meiotic arrest and defects in spermatogonial proliferation (114). Through transgenic analyses, a single gene within the *Sxr*-deletion interval, *Eif2s3y*, was shown to be required for normal spermatogonial proliferation (77). A more recent study indicated that *Eif2s3y* and *Sry* are the only two Y-linked genes required for spermatogenesis to progress through the first meiotic division (130).

By contrast, genes on the long arm of the mouse MSY (Yq) appear to be required for the later stages of spermatogenesis, after meiosis has been completed. Yq-deleted mice have abnormal sperm morphology and impairment of sperm function (111, 112); the size of the deletion correlates with the severity of the defects (20, 117, 125). Yq deletions are also associated with a skewed sex ratio in offspring in favor of females (20).

The sequence of the mouse MSY, the largest mammalian MSY known, was reported in 2014 (108), and the sequencing data have been and continue to be instrumental to understanding the mouse MSY's striking biology. The euchromatic sequence of the mouse MSY is roughly 90 Mb in size, which is larger than the euchromatic portions of all three sequenced primate MSYs (human, chimpanzee, and rhesus macaque) combined. Less than 2% of the mouse MSY is demonstrably homologous to the primate MSYs or the ancestral autosomes from which the mammalian X and Y are derived. This ancestral sequence is confined to the short arm of the mouse MSY. The long arm of the MSY is composed of a mouse-specific ampliconic sequence (**Figure 4**). The basic element of the ampliconic sequence is a 0.5-Mb repeat unit, present in ~180 copies, and in aggregate, these repeats account for an impressive 3% of the mouse genome. This is the most ampliconic mammalian MSY known. Each repeat unit contains members of four distinct gene families that are relatively recent additions to the mouse Y chromosome. Consequently, the mouse MSY is gene rich, with a total of ~700 genes compared to 78, 37, and 31 in human, chimpanzee, and rhesus macaque, respectively.

The amplified mouse Y gene families have nonallelic X homologs, which were also acquired and amplified in the rodent lineage (81, 98, 108). Both the X-linked and Y-linked gene families





**Figure 4**

Comparison of ampliconic sequence content of mouse and human MSYs (male-specific regions of the Y chromosome). Triangular dot-plots (*drawn to scale*) illustrate repeat structures of sequences. Each dot within the plot represents 100% nucleotide sequence identity within a 200-bp window. Direct repeats appear as horizontal lines, inverted repeats as vertical lines. Schematic representations of chromosomes are shown below plots; color-coding indicates sequence class. Abbreviations: cen, centromere; Mb, megabase.

are expressed specifically in testicular germ cells (108), raising the possibility that X versus Y antagonism in the male germline may have driven the acquisition and massive amplification of these genes on the sex chromosomes. Gene knockdowns of the X- and Y-linked homologs of one multicopy gene family, *Sly/Slx*, have been shown to distort the sex ratio in opposite directions. Sex ratio favors females when *Sly* expression is reduced, but it favors males when *Slx* expression is reduced, providing support for sex-chromosome-driven intragenomic conflict (18, 19).

It is not known whether parallel amplification of male germ-cell-expressed gene families on X and Y chromosomes is a peculiarity of the mouse lineage or whether this phenomenon might be a widespread feature of mammalian sex chromosome evolution. One other notable example of X-Y coamplification, albeit less dramatic, can be found in humans. The male germ-cell-expressed genes *VCX* and *VCY* were acquired and amplified on the sex chromosomes of the human-chimpanzee ancestor (62). The functions of these gene families and the consequences, if any, of their disruption in human are unknown.

### **BIOLOGICAL THEME #3: BEYOND THE REPRODUCTIVE TRACT**

The roles of the MSY in testis determination and sperm production are firmly established, and the genes responsible for these phenotypes have been the focus of intense study. Far less is known about the functions of the remaining single-copy, broadly expressed genes in the MSY. These genes were once considered lucky survivors of the degeneration process that wiped out more than 600 genes on the MSY during the process of X-Y evolution and differentiation, which spanned

hundreds of millions of years. However, some intriguing studies point to the biological and medical importance of these single-copy genes.

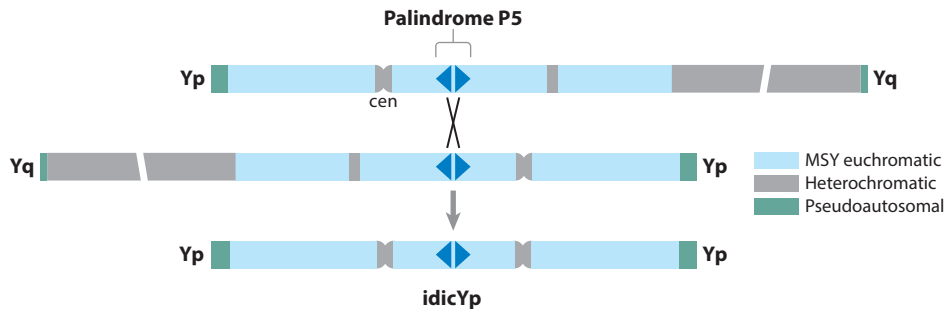
### MSY Genes and Turner Syndrome

Turner syndrome occurs in approximately 1 in 2,000 live female births, making it one of the most frequent chromosomal abnormalities in females. Turner phenotypes include short stature, ovarian insufficiency (oocyte depletion), congenital lymphedema, cardiovascular malformations, and specific neurocognitive deficits (40). Affected individuals are missing all or part of one sex chromosome, the most widely recognized form being monosomy X (XO). It is estimated that >99% of XO conceptuses die in utero (17) and that most individuals with Turner syndrome are mosaic for all or part of a second sex chromosome (44–46).

Even taking mosaicism into account, it is of note that Turner syndrome is the only human monosomy that even occasionally survives to birth, and this offers valuable clues regarding the etiology of Turner syndrome (134). The unique tolerance for monosomy of the X chromosome, compared to any of the autosomes, stems from the fact that one X chromosome is largely transcriptionally silenced, or inactivated, in the cells of XX females. This mechanism of X inactivation evolved to compensate for different dosages of X-linked genes in males and females. Why then is partial or complete sex chromosome monosomy associated with any phenotype at all if just one X chromosome is normally active in the cells of XX females? Part of the answer lies in the finding that X-chromosome inactivation is far from complete (25). At least 15% of genes on the inactive X chromosome are actually transcribed in human (11). These genes that escape X inactivation may be required in two active copies for normal development, and the absence of the second copy could contribute to the phenotypic features of Turner syndrome. However, these arguments alone do not explain why XY males do not exhibit Turner features. The most important group of candidate Turner genes on the X chromosome may be those that also have single-copy, broadly expressed homologs on the Y chromosome (27, 55). These Y-linked genes are generally well conserved along with their X-linked homologs, consistent with their having shared functions. The PARs of the X and Y chromosomes may also contain candidate Turner genes, as XO individuals retain only one copy of each PAR. Indeed, the absence of a second copy of the pseudoautosomal gene *SHOX* contributes to the short stature seen in girls and women with Turner syndrome (92), but no other pseudoautosomal genes have been implicated thus far.

Further evidence of the involvement of Y-linked genes in Turner syndrome comes from studies of patients with X-Y translocations generated by ectopic X-Y crossovers (**Figure 2**). Several XY females arising from such translocations were reported to have certain phenotypic features of Turner syndrome, with lymphedema being the most commonly observed feature (6, 10, 26, 71). Notably, these XY Turner females retain two intact copies of the PAR. Therefore, the missing portion of the MSY seems likely to contain one or more genes whose deficiency contributes to the Turner phenotype. Molecular mapping studies narrowed down the Y-linked interval associated with Turner phenotypes in XY sex reversal cases to a 90-kb region that contains a single gene, *RPS4Y* (30). *RPS4Y* is a strong candidate because its X-linked homolog, *RPS4X*, escapes X inactivation, and *RPS4Y* and *RPS4X* have been shown to be functionally interchangeable in vitro (126).

The case for the involvement of MSY genes in Turner phenotypes is a strong one, and other disorders involving sex chromosome anomalies provide additional clues regarding nonreproductive phenotypes associated with the human Y chromosome. Although the evidence is far from definitive, phenotypes as diverse as height, tooth size, and brain development have all been linked to the Y chromosome through studies involving individuals with sex chromosome aneuploidies



**Figure 5**

Isodicentric Yp (*idicYp*) formation. Ectopic recombination and crossing over between sister chromatids within human MSY (male-specific region of the Y chromosome) palindrome P5 (shown as a pair of blue triangles) generates an *idicYp* chromosome (bottom). Most other Yq palindromes and inverted repeats have been found to mediate ectopic recombination in *idicYp* cases. Abbreviation: cen, centromere.

[XXY (Klinefelter syndrome) and XYY males] and individuals with sex reversal (XY females and XX males) (2, 3, 7, 22, 80, 118).

### Palindromes, Isodicentric Y Chromosomes, and Turner Syndrome

One property of Turner syndrome that distinguishes it from other human aneuploidies is that most cases are not due to chromosome segregation errors during maternal meiosis. Instead, in approximately 75% of XO individuals, the single X chromosome is of maternal origin, implying that a paternally derived sex chromosome has gone missing (52). The rearrangement-prone architecture of the human MSY may be responsible in part for this phenomenon (66). The large palindromes of the MSY undergo frequent gene conversion, a manifestation of intrachromatid exchange, which maintains the high degree of identity between palindrome arms. The MSY palindromes can also engage in interchromatid exchange, sometimes resulting in the generation of isodicentric Y chromosomes (Figure 5). The most common such rearrangement results in a symmetrical chromosome with two short arms (and, therefore, two copies of *SRY*), two centromeres, and two truncated long arms fused together. This configuration is referred to as an isodicentric Yp (*idicYp*) chromosome.

The clinical consequences of *idicYp* formation can be diverse (66). One such consequence is spermatogenic failure: Most *idicYp* chromosomes are missing a number of the Y chromosome's ampliconic genes, including those within the critical *AZFc* region. Some of these individuals are missing several single-copy genes as well, but phenotypes beyond the reproductive tract have not been well documented in these cases. A second, and less intuitive, consequence of *idicYp* formation is sex reversal and Turner syndrome. The presence of two centromeric regions makes *idicYp* chromosomes mitotically unstable, and they are frequently lost during cell division, leading to mosaicism for XO and X;*idicYp* cells. If a large proportion of a fetus's cells (or a particularly critical subset of fetal cells) are XO, the fetus develops as an anatomic female (because the *SRY* gene was not present in the fetal gonad to trigger testis differentiation) with phenotypic features of Turner syndrome (because the second sex chromosome was missing in certain somatic tissues during development). Therefore, what appears to be a paternal transmission error in Turner syndrome (41, 69) may actually be due, in some cases, to transmission of an *idicYp* chromosome and its subsequent loss during embryonic or fetal development.

#### Isodicentric Yp (*idicYp*):

mirror-image derivative of the Y chromosome with two copies of short arm (including *SRY* gene), centromere, and proximal long arm

## Evolutionary Analysis Reveals that Y-Chromosome Genes Are a Selected and Specialized Set

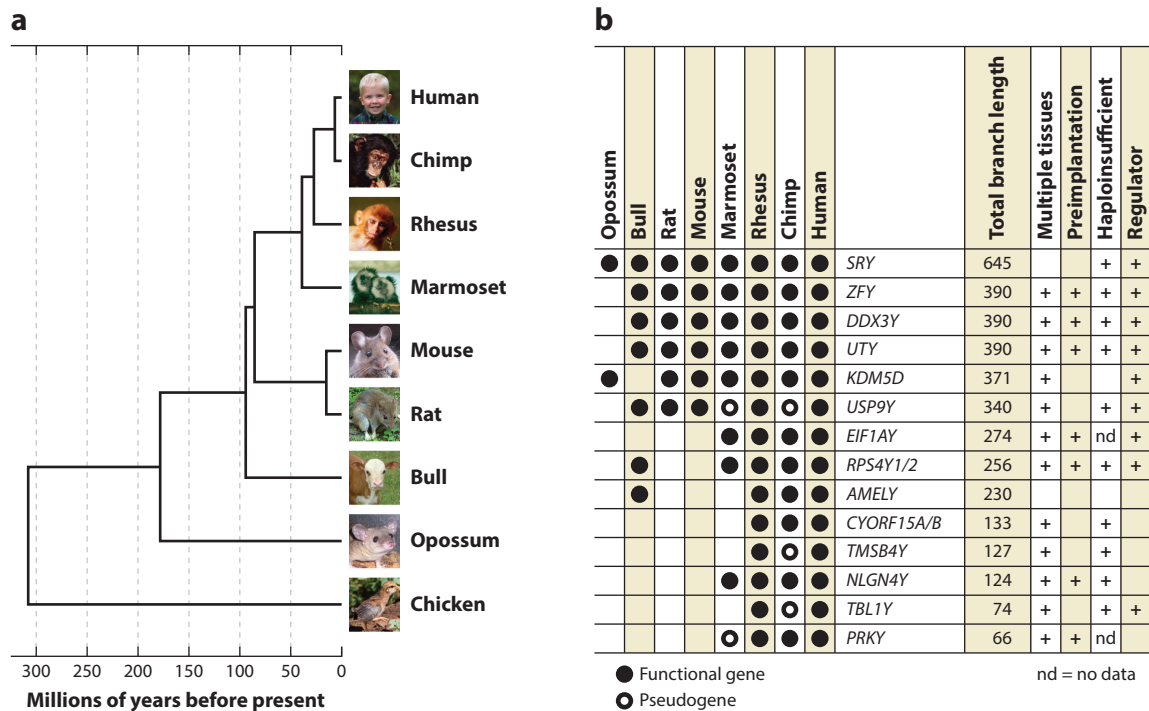
Clues regarding the functions of Y-chromosome genes beyond the reproductive tract have emerged from long-term studies of patients with sex chromosome anomalies. A second, complementary approach involves looking back tens or hundreds of millions of years in sex chromosome evolution. A comprehensive comparative genomic analysis of the Y chromosomes of multiple mammalian species has recently yielded novel insights into the extraordinary evolutionary longevity and fundamental biological functions of Y-linked genes (4).

Because of rampant genetic decay in the absence of recombination, the present-day human Y chromosome has lost all but 17 of the ~640 genes that it once shared with the X chromosome. Three of these ancestral Y-linked genes, *RBMY*, *TSPY*, and *HSFY*, ensured their survival by becoming ampliconic: They are now multicopy, are expressed exclusively in the testis, and likely play critical roles in spermatogenesis (107). From comparisons of three primate MSYs (human, chimpanzee, and rhesus macaque), it became evident that the remaining single-copy ancestral genes have been preserved quite effectively in the human lineage by natural selection (49–51). An expanded analysis including Y-chromosome sequences from five additional mammals—marmoset, mouse, rat, bull, and opossum—greatly increased our ability to reconstruct the evolutionary past, allowing us to look back at least 180 million years. This eight-species analysis not only strengthened previous findings that the single-copy ancestral genes of the MSY have impressive staying power but also revealed that they form a functionally coherent group enriched for genes that are dosage sensitive, are broadly expressed across the body, and encode general regulators of chromatin modification, transcription, translation, and protein stability (**Figure 6**) (4).

Multiple lines of evidence argue that the MSY's single-copy ancestral genes function beyond the reproductive tract (4). First, most of these genes are broadly expressed across adult tissues and across developmental time (**Figure 6**). Second, the X homologs of the MSY's broadly expressed genes appear to be highly dosage sensitive, in a manner that is consistent with their having vital roles in both sexes: The X homologs of all MSYs broadly expressed genes escape X inactivation, and they have a higher likelihood of being haploinsufficient (requiring a second copy on either the X or Y chromosome for normal organismal development or function) than other ancestral X-linked genes (**Figure 6**). X-linked intellectual disability syndromes in human provide further evidence for dosage sensitivity of these genes: Heterozygous mutations in *UTX*, *KDM5C*, and *NLGN4X*—all genes that have broadly expressed MSY homologs and escape X inactivation—cause intellectual disabilities of varying degrees, implying haploinsufficiency (4, 67, 72, 102).

Across the genome, haploinsufficient genes have been reported to be enriched for regulatory proteins and transcription factors (21), and this same bias is observed in the X homologs of broadly expressed MSY genes. Functional annotations for many of these genes suggest regulatory functions: nuclear organization, DNA binding, and RNA binding (**Figure 6**). For example, the X homologs of *UTY* and *KDM5D* are histone lysine demethylases (65, 115), the X homolog of *ZFY* is a transcriptional regulator of stem-cell self-renewal (34), and the X homologs of *EIF1AY* and *DDX3Y* are translation initiation factors (24, 68). *DDX3X* also appears to be a component of the spliceosome (133). The haploinsufficiency model predicts that the broadly expressed Y-linked genes share functions with their X-linked counterparts. Functional interchangeability of X and Y homologs has been demonstrated experimentally for a handful of genes: *RPS4X* and *RPS4Y* and *DDX3X* and *DDX3Y* are functionally interchangeable in vitro (103, 126), and *Utx* and *Uty* are functionally redundant during mouse embryonic development (70, 104, 128).

Taken together, these medical and evolutionary analyses indicate that the MSY's broadly expressed genes have important biological functions throughout the body. Y-chromosome deletions



**Figure 6**

Comparative Y-chromosome sequencing reveals long life spans and functional coherence of human MSY (male-specific region of the Y chromosome) single-copy genes. (a) Species tree indicating evolutionary relationships between the eight mammals with SHIMS (single-haplotype iterative mapping and sequencing)-sequenced ancestral MSY sequences. Chicken is shown as an outgroup. Branch lengths are drawn to scale. (b) Species distribution and features (expression breadth across tissues, expression in preimplantation embryos, haploinsufficiency probability, and predicted regulatory function) of human MSY single-copy ancestral genes, which are ranked according to evolutionary longevity. Total branch length for a given gene is the sum of branch lengths for each species possessing an intact homolog of that gene.

and rearrangements that perturb these genes, or their copy number, will continue to be helpful in revealing the roles of these genes in development and physiology both within and beyond the reproductive tract.

## LOOKING FORWARD: THE Y CHROMOSOME AND SEXUAL DIMORPHISM

A distinct and largely unexplored hypothesis regarding the biological function of MSY genes is that the presence or absence of the Y chromosome directly influences an individual's disease risks. A myriad of human diseases exhibit striking sex biases in susceptibility, incidence, or severity, as highlighted in the Institute of Medicine's report probing the pervasive biological influence of sex (129). To name just a few examples: Lupus (6:1), rheumatoid arthritis (3:1), and unipolar depression (2:1) are strongly female biased, whereas autism (5:1), dilated cardiomyopathy (3:1), and ankylosing spondylitis (5:1) are strongly male biased. Historically, such biases have been attributed solely to hormonal, or extrinsic, factors. However, it is possible that intrinsic biochemical differences between XX and XY cells have biological consequences throughout the body and thus contribute



to sexual dimorphism in human health and disease. A spate of recent studies examining large populations has implicated the human Y chromosome in numerous somatic diseases, including cancer (9, 28, 33), coronary artery disease (14), autism (69), and primary biliary cirrhosis (73). However, the genetic architecture of these diseases is complex, and the Y-linked gene or genes contributing to these disease phenotypes have not been identified.

As discussed above, evolutionary analyses of ancestral regions of mammalian Y chromosomes yielded strong evidence that X-Y homologous genes serve ubiquitous gene regulatory functions (4). Therefore, slight differences in the biochemical properties of protein isoforms encoded by X-Y gene pairs—or differences in the levels or patterns of expression of the X- and Y-linked genes—could have significant phenotypic consequences. For example, two human X-Y gene pairs encode histone demethylases. If the X- and Y-encoded isoforms have different expression levels, substrate specificities, and/or enzyme affinities, this could contribute to genome-wide transcriptional regulatory differences between males and females. A complete understanding of the influence of the sex chromosome complement in male and female health and disease will create a better toolkit for scientists, clinicians, and developers of novel therapies.

Methods to explore this intriguing hypothesis will likely involve analysis of large-scale molecular data sets from a variety of mammals, as well as the generation of appropriate animal models, which is not a straightforward task when it comes to the Y chromosome. Access to the complete mouse Y-chromosome sequence and recent advances in genome-editing technologies have finally opened the door to directed mutagenesis of Y-linked genes in mice (108, 121). The mouse, however, may ultimately prove to be of limited use as a model for the human sex chromosomes because of the rapid evolution of the Y chromosome. Of the 12 broadly expressed, single-copy genes on the human MSY, only five have orthologs in the mouse MSY (**Figure 6**). By contrast, the rhesus macaque MSY carries an ortholog of each and every ancestral gene in the human MSY (49), and progress toward gene targeting in such nonhuman-primate models (13) may soon enable *in vivo* study of the function of all human MSY genes in the macaque or other primate models. New insights into MSY gene function can also guide systematic investigations of human organismal phenotypes associated with MSY gene deficits. Finally, thorough characterization of sex differences at the molecular and cellular level will provide a strong foundation for understanding the gene regulatory functions of X and Y homologs. Using this multifaceted approach, the field may come to understand the contribution of Y-linked genes to human biology and to medically consequential differences between the sexes at the molecular, cellular, and physiological levels. Ultimately, these insights will lead to a greater appreciation of the etiology of Turner syndrome and the underlying causes of sex biases in disease, enabling both medical research and practice to embrace and address the most fundamental polymorphism in our species, whether an individual has two X chromosomes, or one X plus one Y chromosome.

### SUMMARY POINTS

1. Three biological themes have defined Y-chromosome research in the age of molecular genetics and genomics: testis determination, spermatogenesis, and biological roles beyond the reproductive tract.
2. The Y chromosome's roles in testis determination and spermatogenesis are well defined and widely appreciated, especially in human and mouse, but the Y chromosome's roles beyond the reproductive tract are only beginning to be studied.



3. Complete MSY sequences from human, chimpanzee, rhesus macaque, and mouse have revealed that mammalian Y chromosomes vary widely in gene content, size, and structure.
4. The massive amplification of male germ-cell-specific gene families on the mouse MSY was accompanied by parallel amplification of homologous gene families on the X chromosome, providing insight into molecular mechanisms of the sex-linked meiotic drive in mammals.
5. Studies of patients with sex chromosome anomalies combined with comprehensive comparative genomic analysis of multiple mammalian MSYs indicate that many MSY genes have fundamental biological roles throughout the body.
6. The future of Y-chromosome research lies in understanding how differences in sex chromosome complement (XX versus XY) and the presence or absence of individual Y-chromosome genes influence sex differences in health and disease, both within and beyond the reproductive tract.

## FUTURE ISSUES

1. The X-Y coamplification observed in mouse may be widespread among mammals. In order to explore this possibility, SHIMS sequencing of additional mammalian X and Y chromosomes will be required. Complete and accurate X- and Y-chromosome sequences are essential for unraveling the ampliconic repeats that are intrinsic to this phenomenon. Functional studies in human may also be possible, taking advantage of predictable and recurrent structural variants on the X and Y chromosomes.
2. Exploring the contribution of Y-linked genes to health and disease beyond the reproductive tract will require a multifaceted approach involving targeting Y-linked genes in nonhuman primates, systematic clinical investigations of patients with sex chromosome anomalies, and large-scale molecular studies of male and female cells across tissues and species.

## DISCLOSURE STATEMENT

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