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The impact of chromosomal sex in cardiometabolic health and disease

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Abstract

Many aspects of metabolism are sex-biased, from gene expression in metabolic tissues to the prevalence and presentation of cardiometabolic diseases. The influence of hormones produced by male and female gonads have been widely documented, but recent studies have begun to elucidate the impact of genetic sex (XX or XY chromosomes) on cellular and organismal metabolism. XX and XY cells have differential gene dosage conferred by specific genes that escape X chromosome inactivation or presence of Y chromosome genes that are absent from XX cells. Studies in mouse models that dissociate chromosomal and gonadal sex have uncovered mechanisms for sex-biased epigenetic, transcriptional, and post-transcriptional regulation of gene expression in conditions such as obesity, atherosclerosis, pulmonary hypertension, autoimmune disease, and Alzheimer's disease.

Keywords

Sex broadly affects metabolism across the lifespan

Thousands of published studies document sex differences in cardiometabolic traits including obesity, plasma lipid profile, insulin sensitivity, and atherosclerosis. Nevertheless, in studies of both humans and experimental models, females have been underrepresented, and many studies that included both sexes failed to stratify data by sex. At present, much remains to be learned regarding the mechanisms that contribute to sex differences in metabolism. A more complete understanding of the physiological, molecular, and genetic sex differences in metabolism is critical for the development of 'personalized medicine' approaches to prevent and treat disease.

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The pervasive effect of sex on metabolism has been demonstrated by comparing the levels of metabolites in the blood and urine of healthy men and women using unbiased approaches such as mass spectrometry and nuclear magnetic resonance [1,2]. For example, in a targeted metabolomic study of a population-based sample of more than 3000 individuals, 102 out of 131 metabolites assessed exhibited significant concentration differences between men and women [3]. This was true even when adjusted for covariates that could differ due to sex or gender including smoking, alcohol consumption, and waist-to-hip ratio. Although individual studies differ in methodologies and findings, consistent sex differences in serum metabolite concentrations across studies include higher levels of most amino acids (including branched-chain amino acids) and acylcarnitines in men, and higher levels of lipid species such as phosphatidylcholines and sphingomyelins in women [2,3]. A better understanding of the 'baseline' levels of metabolites and other clinical parameters in healthy women and men may enhance the accuracy of predicting risk and tracking the progression of common metabolic diseases. This concept is illustrated by the recent finding that using the same risk threshold for systolic blood pressure values in men and women is inadequate. In an analysis of longitudinal data from more than 27,000 individuals with blood pressure measurements at baseline who were followed for the ensuing decades, women were found to experience cardiovascular disease outcomes (including myocardial infarction, heart failure, or stroke) after lower baseline systolic blood pressure readings than men [4]. Findings of this type emphasize the necessity to establish sex-specific clinical ranges for diagnostic measurements of cardiometabolic disease.

Sex differences are also evident in metabolism in fetuses and infants. At one year of age, baby girls had higher levels than baby boys of numerous lipid and lipoprotein species and serum albumin, but did not exhibit the sex differences in amino acid levels that occur in adults [5]. A separate study with longitudinal metabolite measurements in the same individuals demonstrated higher levels of triglyceride-rich lipoproteins in females at 8 years of age, but by 16 years (*i.e.*, following adolescence) these levels were higher in males, and higher still in adult males [1]. Furthermore, studies during human fetal development have shown that male and female fetuses differ in body size and body proportions. Beginning in the first trimester, male fetuses are longer than female fetuses (crown-to-rump length), and by the second and third trimesters, males have greater abdominal and head circumference, while females have longer femurs [6,7].

To decipher the mechanisms that underlie sex differences in metabolism, it is important to consider the components of biological sex, which encompass both **chromosomal sex** (see Glossary) and **gonadal sex** (Box 1). A traditional view of the sex chromosomes is that they serve a role primarily to specify the development of male or female gonads, and that the hormones produced by ovaries and testes are responsible for subsequent sex differences in physiology (Figure 1A, *left*). There is substantial evidence now to revise this view to encompass an independent role for sex chromosome complement in every cell of the body as a determinant of gene expression and cellular metabolism throughout tissues of the body (Figure 1A, *right*). Prior to gonadal hormone exposure, male and female cells exhibit substantial differences in gene expression. For example, sex-biased expression of hundreds of genes is detectable in male vs. female mouse embryonic stem cells and pre-implantation

embryos, and in human induced pluripotent stem cells [8–10]. Furthermore, comparisons of XX and XY embryonic stem cells have revealed that sex chromosome complement influences gene regulatory networks during complex events relevant to cardiovascular health, such as cardiac development and susceptibility to hypertrophic stimuli [10,11]. Thus, chromosomal sex is expected to have an impact throughout the entire lifespan, from early embryonic development through old age, including at times when gonadal hormone levels are low (Figure 1B). These findings raise the possibility of modeling sex differences in development and disease in various cell types using induced pluripotent stem cells that are engineered to have different sex chromosome complements on the same genetic background [12].

Sex chromosomes and gonadal hormones promote sex-specific cellular phenotypes

Gonadal hormones and sex chromosomes both influence metabolism through effects on gene expression, leading to sex-specific cellular phenotypes (Figure 2). During early development, one of the X chromosomes in XX cells is inactivated through the action of the long non-coding RNA, *Xist*, followed by epigenetic modifications and condensation of the DNA into heterochromatin [13,14]. Although the general process of **X chromosome inactivation** during embryogenesis is conserved from rodents to primates, it should be noted that the timing and maintenance of X inactivation may differ, and the process appears to be more complex in human compared to mouse cells [15]. As a result of X inactivation, the expression levels of ~80% of the >1000 protein coding genes on the X chromosome are similar between XX and XY cells. However, a subset of X chromosome genes escapes inactivation and is expressed from both X chromosomes in XX cells, leading to higher expression levels than in XY cells [13,14,16,17] (Figure 2A,B). These genes, referred to herein as '**X escape genes'**, encode proteins that have the potential to promote sex differences in gene expression throughout the genome, including histone modifying enzymes, translation initiation factors, transcription factors, and RNA helicases.

Approximately 5% of X-linked genes escape inactivation in the mouse and ~25% escape to some degree in humans, although these estimates vary depending on the method used to identify expression from the inactivated X chromosome. Importantly, a core set of X-linked genes escape inactivation across mammals and in most tissues [14]. Other X escape genes are expressed from the inactivated X chromosome in a tissue-specific manner, and degree of expression may vary among individuals [18–20]. Recent work indicates that genes on the inactive X chromosome may also influence expression levels of specific genes on the active X chromosome [21]. The effects of sex chromosome complement on autosomal gene expression have been cataloged in RNA-sequencing studies of individuals with sex chromosome aneuploidies such as XXX, XYY, and XXYY [22,23]. Furthermore, gene expression analyses in mouse models with unusual sex genotypes such as the **Four Core Genotypes mouse model** (described below) have identified a sex chromosome effect on autosomal gene expression in white adipose tissue and liver that is independent of gonadal type [24,25].

The majority of the ~50 protein coding genes that are unique to the Y chromosome have roles in male reproduction. However, the Y chromosome also includes homologs to some of the X escape genes [26]. Since these X-Y gene pairs do not undergo genetic recombination, the members of these pairs may have diverged in their regulation and/or protein function [27–29] and could contribute to differences between males and females in normo- and pathophysiology.

Differential levels of female and male gonadal hormones (and potentially hormone receptors) also lead to distinct gene expression profiles in males and females (Figure 2C,D). Estrogens and androgens act through estrogen receptor alpha (ERα) or beta (ERβ), and androgen receptor (AR), respectively [30,31]. When activated, these receptors translocate to the nucleus and bind site-specific DNA elements to activate or repress target genes throughout the genome. Estrogens also exert non-genomic, rapid action through membrane-bound G protein-coupled receptors (G protein-coupled estrogen receptor 1, GPER1, and G_q-coupled membrane estrogen receptor, G_q-mER), which activate signaling cascades that modulate gene expression.

Sex chromosome dosage is critical for normal physiology

The critical role of sex chromosome dosage in physiology and disease is exemplified by **Turner syndrome** (TS), which results from a complete or partial loss of one of the X chromosomes in biological females (denoted 45,X) [32]. An estimated 1% of 45,X human fetuses survive gestation, and account for about 1 in 2500 live female births, although diagnosis typically does not occur until the teen years [33]. 45,X individuals exhibit a complex phenotype that often includes short stature, delayed puberty, gonadal dysfunction, congenital heart malformations, and metabolic disorders such as type 2 diabetes mellitus [32,33]. Phenotypic variation among TS individuals is associated with genetic mosaicism (in which some cells within the same individual carry a normal 46,XX chromosome complement while other cells are 45,X), or retention of portions of the second X chromosome rather than its complete absence. Studies of 45,X individuals have affirmed the importance of X escape genes in physiology [34].

The most common human sex chromosome aneuploidy is XXY (**Klinefelter syndrome**, KS), which occurs in about 1 of 650 newborn boys. KS individuals present with hypogonadism, infertility, and increased risk for the Metabolic Syndrome, including abdominal obesity, hyperlipidemia, and insulin resistance/type 2 diabetes [35,36]. Metabolic disease in KS patients is impacted by decreased male gonadal hormones; however, testosterone restoration does not fully mitigate KS metabolic symptoms [37]. One interpretation is that the higher dosage of X chromosome escape genes in XXY compared to XY individuals contributes to altered cellular metabolism. This hypothesis is supported by increased abdominal obesity and insulin resistance in XXY boys before the onset of puberty [35,38,39]. A reduced postpartum testosterone surge in some XXY individuals may also contribute to altered adipose tissue expansion [40–42]. Overall, the disrupted metabolic physiology in KS individuals supports an important role for X chromosome dosage in metabolism.

Detecting chromosomal and gonadal contributions to metabolic traits

Distinguishing the metabolic effects of gonadal hormones from those of sex chromosome gene dosage is challenging in humans, since XX chromosomes are generally coupled with ovaries, and XY chromosomes with testes. While sex chromosome aneuploidies described above have demonstrated that sex chromosome complement influences metabolic disease phenotypes such as obesity, type 2 diabetes, and metabolic syndrome, the interpretation of genotype–phenotype relationships in human sex chromosome aneuploidies is confounded by small patient populations, genetic heterogeneity, and mosaicism. This has led to the use of engineered mouse models to interrogate the independent effects of chromosomal and gonadal sex in physiology.

The most widely used model for analysis of sex components is The Four Core Genotypes (FCG) mouse model. This model was made possible with contributions of mouse strains from several investigators, which were bred by Burgoyne and colleagues to generate the four genotypes in a single litter [43]. This model has been championed by Arnold and colleagues for investigation of sex components in several fields [44,45]. The FCG model allows the disentanglement of XX or XY chromosomes from ovaries and testes through the generation of four genotypes within a single litter of C57BL/6 mice: XX or XY mice with ovaries, and XX or XY mice with testes (Figure 3). In the FCG model, the testis-determining *Sry* gene is deleted from the tip of the Y chromosome (which occurred through spontaneous mutation) and its function is provided by an *Sry* transgene that integrated on chromosome 3. The comparison of the four genotypes for a trait of interest allows identification of effects that are determined by gonadal sex, sex chromosome complement, or interactions between chromosomal and gonadal sex (Figure 3; Box 2).

Sex chromosome dosage impacts obesity, hyperlipidemia, atherosclerosis, Alzheimer's disease, autoimmunity, and pulmonary hypertension

Given that the effects of gonadal hormones on cardiometabolism have been extensively reviewed previously [30,46], here we focus on examples of metabolic traits that are influenced by chromosomal sex, most of which have been revealed through initial studies with the FCG mouse model.

Obesity is a key risk factor in cardiometabolic disease. Fat comprises a greater proportion of body weight in women compared to men, and sex differences occur in adipose tissue cellularity, inflammation, adipokine secretion, efficiency of lipolysis, lipid oxidation, adipose tissue expansion in response to nutrient excess, and anatomical fat distribution [47–50]. As in many mammalian species, young adult C57BL/6 males fed a chow diet weigh more than females by about 25%. Consistent with this, studies with FCG mice detected greater body weight in mice with testes compared to ovaries, but also greater body weight and fat mass in XX compared to XY mice within each gonad type [51,52]. The effect of the XX chromosome complement on body fat was enhanced in mice fed a high-fat diet or following removal of the activational effects of gonadal hormones in adult mice [51,52]. The enhanced weight gain in XX compared to XY mice was associated with increased

food intake specifically during the inactive phase of the diurnal cycle, suggesting a potential effect of sex chromosomes on circadian regulation [51–53].

Further investigation of the role of X and Y chromosomes in body weight and adiposity in XY* mice (see Box 2) revealed accelerated weight gain in XX and XXY mice compared to XY and XO mice, indicating an association with two vs. one X chromosome [51]. This led to the hypothesis that a gene(s) that escapes X inactivation may promote adiposity. Indeed, manipulating the dosage of the X escape gene, *Kdm5c*, recapitulated many effects of the X chromosome dosage on adiposity in the mouse, and in humans, *KDM5C* expression levels in adipose tissue were correlated with body mass index [52]. *Kdm5c/KDM5C* encodes a histone 3 lysine 4 (H3K4) demethylase, and its gene dosage was found to regulate chromatin accessibility, gene expression, and differentiation of cultured preadipocytes [52]. Thus, the higher KDM5C levels in XX compared to XY mice (due to escape from X inactivation) contribute to sex differences in adipose tissue gene regulation, body fat accumulation, and diet-induced weight gain.

XX chromosome dosage also influences plasma lipid levels and development of atherosclerosis. In mice fed an atherogenic diet or made hypercholesterolemic by genetic modification, mice with two X chromosomes had higher cholesterol levels than mice with a single X chromosome [54,55]. Gonadal sex also influenced cholesterol levels in mice fed a chow diet, but the sex chromosomes were the key determinant in mice with hypercholesterolemia. Furthermore, in hypercholesterolemic mice, XX animals developed larger atherosclerotic lesions in the aortic arch than XY mice [55]. Increased lipid and lesion levels in XX mice correlated with enhanced intestinal expression levels of genes involved in dietary fat absorption and assembly of intestinal lipoproteins, and there was a trend to increased fat absorption in intestine of XX compared to XY mice [55]. Further studies are necessary to determine whether genes on the X chromosome promote atherogenesis, or genes on the Y chromosome have a protective effect. In humans, Y chromosome loss in leukocytes is a strong risk factor for cardiovascular and fibrotic diseases, and occurs spontaneously with age (40% of peripheral white blood cells have lost the Y chromosome in men over 70 years of age) [56,57]. Studies from the UK Biobank have implicated a specific haplogroup of the Y chromosome, and the *UTY* gene, as a determinant of coronary artery disease risk [58]. Further studies are required to better define the role of chromosomal sex in cardiovascular disease.

Another vascular disease that is influenced by sex chromosome complement is pulmonary arterial hypertension (PAH). PAH is more prevalent in women than men by a factor of four and is characterized by inflammation and vascular remodeling in the lungs. In FCG mice lacking circulating gonadal hormones, the Y chromosome was protective against experimental PAH [59]. An evaluation of four Y chromosome genes expressed in lung tissue identified *Uty* (the Y chromosome homolog of X escape gene, *Kdm6a*) as the protective Y chromosome gene [60]. *Uty* expression was inversely correlated with *Cxcl9* and *Cxcl10* chemokine gene expression, which promote the development of experimental PAH.

Studies of sex differences in Alzheimer's disease and autoimmune diseases have implicated XX chromosome dosage as a determinant of disease risk. Specifically, the dosage of

Kdm6a, an X escape gene that encodes histone demethylase activity that regulates H3K27 methylation, may be a key determinant. Men with Alzheimer's disease show greater cognitive defects than women, and are more likely to die earlier [61]; this sex difference is recapitulated in a mouse model of Alzheimer's disease produced by expressing the human amyloid precursor protein (hAPP) to promote amyloid plaque development in the brain [62]. Sex chromosomes are a major determinant of this sex difference. In FCG mice expressing hAPP, XX mice were more resilient than XY mice, and adding a second X chromosome to XY mice or XO mice increased their resilience. The X escape gene, *KDM6A/Kdm6a,* has a known role in cognition in humans and mice, which led to testing its potential role in Alzheimer's disease. Enhancing *Kdm6a* expression in the dentate gyrus (a center for memory and spatial learning) of XY-hAPP mice attenuated cognitive impairment. A recent study suggests that the protective function of KDM6A is independent of its histone demethylase activity as the cognitive ability in aged male mice was improved with acute overexpression of KDM6A lacking enzyme activity [63]. Future identification of the gene targets regulated by KDM6A H3K27 histone demethylase activity, as well as identification of enzyme-independent activity, may provide insight into the molecular mechanisms that underlie greater Alzheimer's disease resilience in females. In humans, transcriptome analysis in the prefrontal cortex of men and women with Alzheimer's disease showed that increased expression of 19 X chromosome genes was associated with slower cognitive decline in women [64].

Autoimmune diseases are among the most sex-biased of all diseases, with women accounting for 70–80% of those affected [65]. The bias toward greater autoimmune disease prevalence in females is correlated with greater immune response to infection and vaccines than seen in males [66]. This, in turn, places women at greater risk for diseases such as systemic lupus erythematosus, scleroderma, rheumatoid arthritis, multiple sclerosis, and even AIDs [65]. Gonadal hormones have immunomodulatory effects, and clinical trials demonstrate that estriol reduces relapse rate in women with multiple sclerosis [67,68]. But studies in an FCG model of multiple sclerosis (experimental autoimmune encephalomyelitis) revealed that XX chromosome complement is also critical in modulating the inflammatory component of the disease in CD4+ T cells [69]. It may be that gonadal and chromosomal sex components influence distinct mechanisms of the disease, or that they converge at specific points of the inflammatory network. Reduction in dosage of the X escape gene, *Kdm6a*, in T cells was protective in experimentally induced autoimmune disease [69]; however, Y chromosome genes may also influence disease severity [70,71]. It is likely that multiple sex-biasing factors interact in immunometabolism to influence the sex bias observed with many autoimmune diseases.

Molecular mechanisms that promote sex-biased gene expression

As described in the preceding sections, studies with mouse models and human chromosome aneuploidies have established that sex chromosome complement acts alongside gonadal hormones to confer sex biases in gene expression that influence metabolic traits. Here we highlight multiple levels at which sex influences tissue metabolism, from the cellular composition of metabolic tissues to transcriptional and post-transcriptional regulation of gene expression (Figure 4).

Sex differences in the cell type composition of metabolic tissues have been uncovered by the recent surge of single-cell RNA-sequencing and proteomics studies (Figure 4A). For example, in white adipose tissue, the cellular composition of the stromal vascular fraction (which includes preadipocytes, stem cells, immune cells, and endothelial cells) differs between males and females in a depot-specific manner, and also in changes that occur in response to a high-fat diet [50,72,73]. It is unclear how gonadal and chromosomal sex influence tissue cell-type composition. To assess the effects of circulating gonadal hormones and chromosome complement on cellular composition of metabolic tissues, a recent study performed deconvolution of bulk RNA-sequence data of adipose tissue and liver from FCG mice [24]. In this analysis, circulating gonadal hormones had the largest impact on cell type composition, but the level of impact varied based on the presence of XX or XY sex chromosomes. This indicates that both gonadal hormones and sex chromosome complement impact the cellular composition of adipose tissue and liver. Further studies (such as single-cell –omics in FCG mice) are warranted to delineate the influence of both chromosomal and gonadal sex on metabolic tissue cell type composition.

Given that sex differences exist in cell type composition of metabolic tissues, some RNA-sequencing datasets that compared sexes without taking this difference into account may have provided incomplete information regarding the underlying basis for sex differences in tissue gene expression. Fortunately, a recent analysis of sex differences in human gene expression across 44 tissues did account for sex differences in cell type abundance in the interpretation of their data [74]. This study reported that 37% of the human genome (~13,300 genes) had sex-biased expression in at least one tissue type. Importantly, 96% of the genes with sex-biased expression are encoded on autosomal chromosomes [74]. The majority of sex-biased expression was tissue-specific, with 18% of the sex-biased genes (>2400 genes) being unique to a single tissue type and only 30 genes shared across all tissue types. Of the 30 genes with tissue-wide sex-biased expression, 22 are X escape genes. Sex-biased genes were enriched for functions in transcriptional regulation and metabolic pathways, including genes with roles in modulating epigenetic marks, fat metabolism, xenobiotic metabolism, mitochondrial genes, and fatty acid oxidation. These data demonstrate that sex impacts gene expression across the human genome in a tissue-specific manner that may influence metabolic homeostasis.

There is evidence that multiple gene regulatory processes contribute to sex-biased gene expression, including epigenetic modification of histones and CpG sequences, the impact of DNA variants, and post-transcriptional regulation (Figure 4B–D). As described in preceding sections, at least two X escape genes (*KDM5C* and *KDM6A*) have direct roles in modulating chromatin structure, by regulating histone marks (H3K4 and H3K27 methylation, respectively) that determine the accessibility of promoter and enhancer sequences to transcription factor binding. KDM5C modulates chromatin landscape and corresponding gene expression in mouse preadipocytes that contribute to sex differences in adipocyte differentiation (Figure 4B) [52]. KDM6A modulates histone modifications at key gene promoters during hypothalamic neuron differentiation [75], and in embryonic stem cells [76]. Further analysis of genomic targets of the X escape histone demethylases (and potentially their Y chromosome homologs, KDM5D and UTY) using techniques such as ChIP-seq and CUT&RUN across cell types may identify specific genes with sex-biased

expression due to sex chromosome-encoded chromatin modifiers. Chromatin structure is also regulated by sex-biased hormone action. For example, sex differences in growth hormone regulation have been associated with modulation of hepatic gene expression through sex-biased repressive histone marks [77].

Male/female differences have been identified in DNA methylation patterns across multiple tissues [78,79]. Differential DNA methylation patterns in peripheral blood leukocytes from Turner syndrome and Klinefelter syndrome patients compared to their XX and XY counterparts support a role for sex chromosome dosage in the regulation of autosomal DNA methylation patterns. The genomes of 45,X females are hypomethylated compared to XX females, and XXY individuals have differential DNA methylation patterns compared to either XY males or XX females, including differences in both hypermethylated and hypomethylated regions [80–82]. It would be informative to assess DNA methylation patterns in FCG mice, where the confounding effects of heterogeneous human genetic background are not present.

Sex differences also occur in the impact of genomic regulatory variants on gene expression levels. This is assessed by genome-wide analysis of genetic variants that influence gene expression levels, and these variants are known as expression quantitative trait loci (eQTL). eQTLs can exhibit an effect exclusively in one sex, or may exhibit a sex-bias in the direction or magnitude of their effect on gene expression (Figure 4C) [74,83]. In human subcutaneous adipose tissue, >2400 eQTLs with a sex-by-SNP (single nucleotide polymorphism) interaction were identified [83]. A sex-specific effect was observed for 33% of the eQTLs and 66% of the eQTLs exhibited a sex difference in the direction of their effect (increase or decrease) on gene expression levels. These sex-biased eQTLs may represent genetic variants located in regulatory regions that interact differentially with gene regulatory enzymes in males compared to females.

Finally, sex-bias exists in post-transcriptional regulation, including mRNA splicing, mRNA covalent modifications, and microRNA action (Figure 4D). A study of three primate species (humans, chimpanzees, and rhesus macaques) that were sequenced with high coverage revealed sex-bias in alternative mRNA splicing, with a subset of sex-biased splice variants conserved across species [84]. RNA modifications such as N^6-methyladenosine (m^6A), which influences mRNA degradation, also exhibit sex-bias. Lipogenic mRNAs in mouse liver exhibited sex differences in m^6A modification, which was correlated with hepatic lipid accumulation [85]. The *Mettl4* methyltransferase that catalyzes m^6A modification was found to be regulated by both chromosomal and gonadal sex in FCG mice [85]. Another important post-transcriptional mechanism is translational modulation by small non-coding microRNAs. RNA-sequencing of microRNAs in gonadal fat of FCG mice identified specific microRNAs that are regulated by circulating gonadal hormones and others that are regulated by sex chromosome complement [86].

Cumulatively, sex influences gene regulation at many levels, with a composite effect of sex chromosome complement and gonadal hormones. A better understanding of how each biological sex component contributes to these regulatory mechanisms may provide additional insight into their contribution to sex-based physiology and disease processes.

Concluding remarks and future perspectives

Sex is an important determinant of both normo- and pathophysiology, and we propose that working to better understand the mechanisms driving sex differences is necessary to design optimal strategies for the diagnosis and treatment of cardiometabolic and immunometabolic diseases (see Outstanding Questions). At the level of basic research, this involves rejection of the widely held—but disproven—belief that female animals show greater variability in physiological and behavioral traits than males [87,88]. Fortunately, it appears that over the past dozen years, a greater proportion of studies published across biological disciplines include both sexes; however, in eight of the nine disciplines assessed, there has been no increase in the proportion of studies that analyzed data individually for each sex [89]. At the level of clinical research, there has been substantial progress in the inclusion of women in clinical trials since 1993, when the Food and Drug Administration rescinded a 1977 policy that banned women from participating in clinical trials [90,91]. Despite this, there remain large gaps in understanding of how sex influences the parameters that are routinely used to monitor health. This is illustrated by recent data showing that thresholds for blood pressure measurements as predictors for cardiovascular disease risk may need to be recalibrated by sex [4]. It is likely that baseline clinical values for many widely assessed metabolites (lipids, glucose, amino acids, etc.) differ by sex and should be evaluated against sex-specific standards.

Beyond ensuring the inclusion of males and females in research studies and clinical trials, it is critical to delineate the molecular mechanisms that determine sex differences in metabolism. A key aspect is to define the genetic (sex chromosome) and hormonal (gonadal sex) components of biological sex that influence metabolic processes. The important role of gonadal hormones in metabolism has been studied for decades, and has provided critical information about why sex differences in some cardiometabolic traits are diminished after levels of circulating hormones wane with age [92]. But there remains a large gap in our appreciation of how genetic sex—the presence of XX or XY chromosomes in every cell of an individual—impacts metabolic homeostasis and disease. One area for improvement is in the analysis of genome-wide association (GWAS) studies to identify cardiometabolic disease genes. At present, the X chromosome remains excluded in the majority of published GWAS data due to perceived analytical hurdles [93]. The genetic content of the X chromosome is 5% of the genome, and nearly 10% of Mendelian disorders are linked to X chromosome genes. Thus, inclusion of the X chromosome in large-scale genetic studies offers an opportunity to uncover new biology, and tools for GWAS analyses including the X chromosome are now widely available [94–97].

Finally, beyond the role of biological sex, the intersection of sex with gender is a critical consideration in both health and disease states. As discussed earlier, sex and gender are distinct but interrelated facets of each individual, but the data reviewed here has referred specifically to the biological attributes that define sex. Although animal models are valuable for the investigation of biological sex and metabolism, they are not useful to study the role of gender, which refers to a set of social attributes including the way in which individuals perceive themselves and how they interact with others. Gender influences diet, lifestyle, stress, and attitudes towards medical care, which impacts the diagnosis and treatment of

disease conditions [46]. As the inclusion of biological sex variables becomes the norm in biology and medicine, efforts must also be made to design human studies and clinical trials to consider the impact of gender.

Acknowledgements

Glossary

Activational effects of gonadal hormones

the acute effect of transient hormone action

Chromosomal sex

biological sex determined by sex chromosome complement, such as the presence of XX or XY sex chromosomes

Four Core Genotypes mouse model

mouse model that allows the detection of chromosomal sex and gonadal sex effects on a trait of interest through the analysis of four sex chromosome/gonad combinations—XX with ovaries, XX with testes, XY with ovaries, and XY with testes

Gonadal sex

biological sex determined by gonadal type and corresponding hormone secretions

Klinefelter syndrome

a chromosome aneuploidy syndrome (47,XXY) that influences fertility and metabolic traits such as adiposity and risk for type 2 diabetes

Organizational effects of gonadal hormones

Permanent effects of gonadal hormones that occur during development of an organism

Turner syndrome

a chromosome aneuploidy syndrome (45,X) with a complete or partial absence of a second X chromosome, which results in impaired ovarian development and heart defects, among other symptoms

X chromosome inactivation

The suppression of gene expression from one X chromosome when two X chromosomes are present within a cell

X escape genes

X chromosome genes that escape X inactivation, leading to higher expression levels in XX cells than XY cells

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Box 1.

Determinants of biological sex

In mammals, every cell in a female body carries two X chromosomes while male cells carry an X and a Y chromosome. The *Sry* gene encoded on the Y chromosome specifies the development of testes, and the absence of *Sry* in XX individuals leads to the development of ovaries [98]. The main hormone secreted by testes is testosterone, and the main ovarian hormones are 17β-estradiol and progesterone. The hormones secreted by ovaries and testes have both permanent effects (such as the effect of prenatal testosterone surges on 'masculinization' of brain regions, including the hypothalamus), and reversible, acute effects (such as the binding of circulating estrogen to cellular receptors to regulate gene expression in tissues throughout the body) [45]. The effects of gonadal hormones on metabolism (particularly estrogens) have been reviewed extensively [30,99,100]. In humans, a protective role for estrogen on cardiometabolic risk factors such as obesity, hypertension, hyperlipidemia, and insulin resistance have been inferred by comparing these traits in groups of pre-menopausal and post-menopausal women. It has been shown that vasomotor symptoms during the menopausal transition are associated with a more unfavorable cardiometabolic profile [101], suggesting potential determinants beyond loss of ovarian function, which may vary between individuals. Comparison of metabolic traits in pre- and post-menopausal women is also confounded by age differences between these groups and the associated changes in physical activity, basal metabolic rate, and lifestyle.

Box 2.

Deciphering chromosomal versus gonadal effects on sex-biased traits in mouse models

The Four Core Genotypes mouse model is useful to begin an investigation of the molecular mechanisms that underlie a sex-biased metabolic trait. Analysis of the four genotypes for a trait of interest may reveal roles for sex chromosome complement and/or gonadal sex. If analysis of FCG mice reveals that a trait is influenced by sex chromosome complement (that is, XX mice with ovaries and XX mice with testes differ from XY mice with ovaries and XY mice with testes), it could be caused by the presence of two vs. one X chromosome or the presence vs. absence of the Y chromosome. Subsequent distinction of the involvement of the X vs. Y chromosome can be made with the XY* model, which compares XO, XX, XY, and XXY genotypes [98,102]. Results that show X chromosome number as a determinant of a trait turns attention to candidate genes that escape X chromosome inactivation, that exhibit parent-of-origin imprinting, or (as yet ill-defined) indirect effects of X chromosome inactivation. Results that implicate presence of the Y chromosome may focus attention on Y-specific genes, including genes that are homologs of X escape genes (described in the text). An additional model for interrogation of sex chromosome dosage effects is the Sex Chromosome Trisomy model (XX, XY, XXY and XYY) [102,103].

If analysis of FCG mice indicates that a trait is influenced by gonadal type (that is, XX and XY mice with ovaries differ from XX and XY mice with testes), further investigation can distinguish between the permanent, **organizational effects** of gonadal hormones that occur during development and acute, **activational effects** that occur during transient hormone action. A useful experimental design to distinguish organizational from activational hormone effects is to allow FCG mice to develop to adulthood (such that the organizational effects of gonadal hormones occur) and then remove gonads from adult mice to abolish acute gonadal hormone action, as well as interactions between sex chromosome complement and acute gonadal hormone action.

Highlights

- Both sex chromosomes and gonadal hormones impact cellular metabolism to generate sex-biases in cardiometabolic traits.

- Males and females exhibit widespread differences in gene expression across the autosomes as well as the sex chromosomes.

- Sex biases exist at several levels of gene regulation including epigenetic modifications and pre- and post-translational processes.

- X chromosome dosage has direct effects on gene expression levels through enhanced expression of specific genes that escape X chromosome inactivation.

- Genes that escape X chromosome inactivation include histone demethylases, which regulate autosomal gene expression.

- Using mouse models that distinguish chromosomal and gonadal sex effects, sex chromosomes have been shown to impact sex-biased diseases such as obesity, hyperlipidemia, atherosclerosis, Alzheimer's disease, autoimmunity, and pulmonary hypertension.

Outstanding Questions

- How do gonadal hormones and sex chromosomes interact to influence key metabolic phenotypes?

- How can research in animal models contribute to understanding the interaction between sex-biasing biological factors and environmental factors that impact cardiometabolic disease, including diet and physical activity?

- How does the sex chromosome complement in widely used cell lines (which are often characterized by aneuploidy) impact cellular phenotypes?

- Is the impact of sex chromosome complement on metabolism amplified during periods of low gonadal hormones, such as prior to puberty and after menopause/andropause?

- Do values for commonly used clinical measurements that are used to predict cardiometabolic disease risk (such as glucose and lipid levels) need to be defined separately for males and females?

- What are the genomic targets of X and Y chromosome genes that encode histone modifying enzymes (KDM5C, KDM6A, KDM5D, UTY) in metabolic tissues such as adipose tissue, liver, and muscle?

- What additional sex differences will be revealed in metabolic tissues by single cell -omics techniques?

- How do the sex chromosomes and gonadal hormones interact to influence metabolic health and disease, and how are these interactions influenced in transgender individuals?

- How do sex-biasing factors influence the efficacy and adverse effects of drugs that are used to prevent or treat cardiometabolic disease?

- What are the best experimental designs to elucidate the impact of both biological sex and gender on cardiometabolic health and disease?
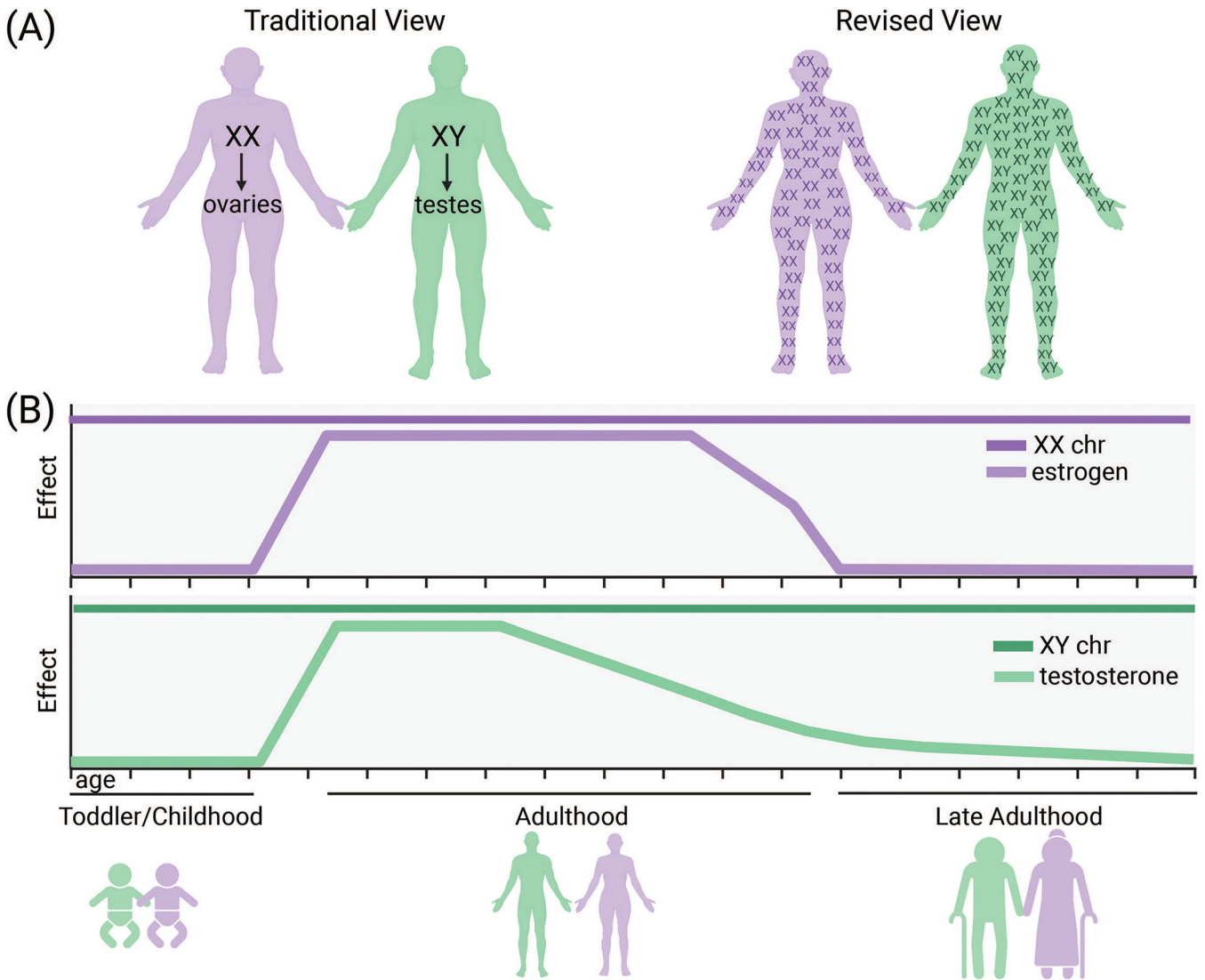
Figure 1. Sex chromosome and gonadal hormone effects over the lifespan.
(A) *Left*, A traditional view has been that sex chromosomes are primarily important during fetal development, and that gonadal hormones are responsible for sex differences postnatally. *Right*, Data support the ongoing influence of sex chromosome complement in each cell across the lifespan. **(B)** Sex chromosomes act throughout the life span from early development through old age, whereas gonadal hormone effects increase during puberty and wane with advancing age.
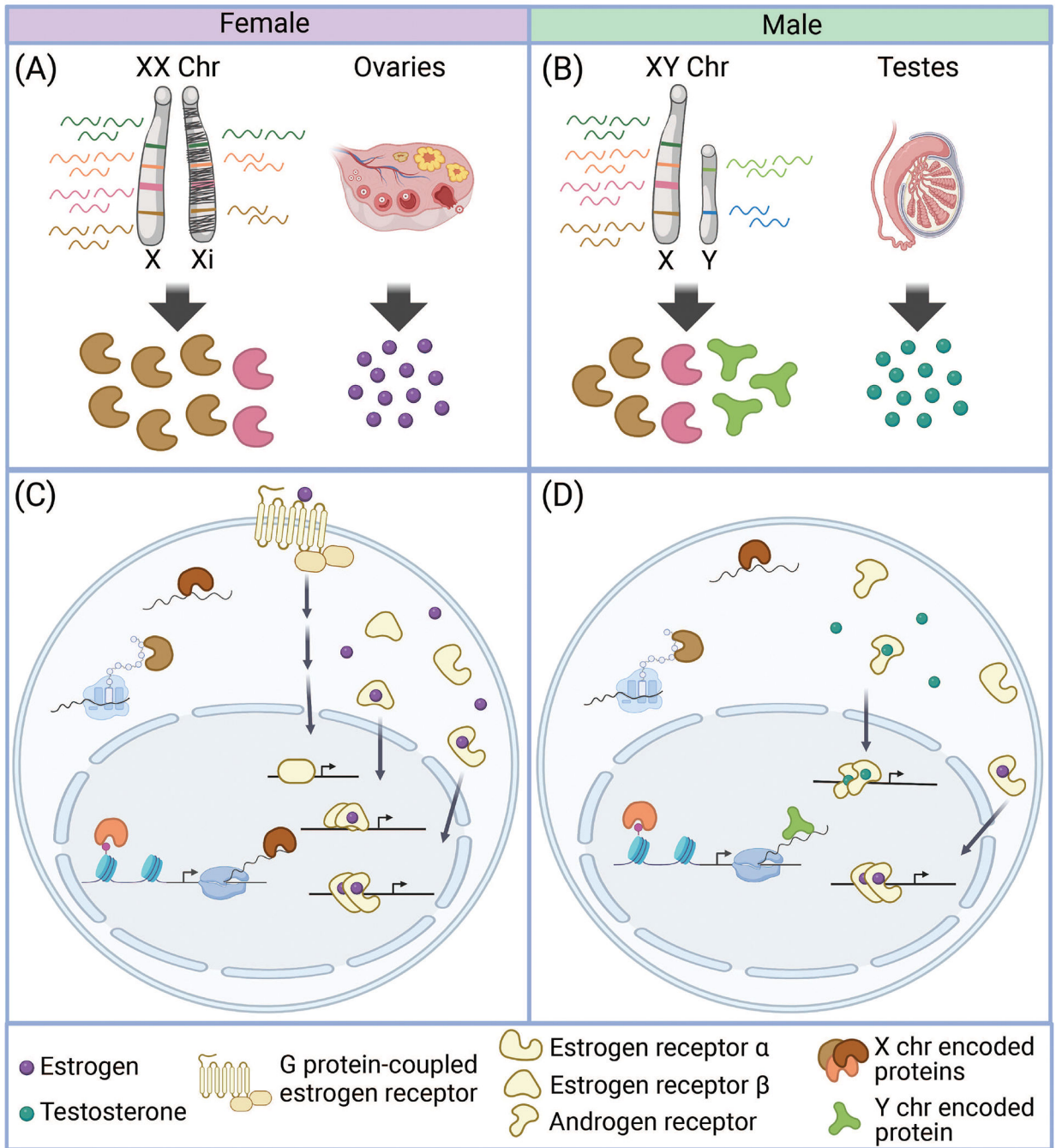
Figure 2. A combination of chromosomal and gonadal sex determine cellular gene expression phenotype.

(A,B) Female sex is associated with the presence of XX sex chromosomes, which leads to the development of ovaries. Male sex is determined by the presence of a Y chromosome, which leads to the development of testes. Sex chromosome complement directly influences the protein repertoire in a cell: in XX cells, a subset of X chromosome genes escapes inactivation leading to higher mRNA and protein levels of these genes compared to XY cells, whereas proteins encoded by the Y chromosome genes are exclusively present

in cells carrying this chromosome. **(C,D)** Sex-specific cellular phenotypes arise through the interaction of sex chromosome complement and gonadal hormones. These include differential expression X and Y chromosome genes (as described above), differential gonadal hormone levels, and levels and action of cellular receptors that respond to gonadal hormones.
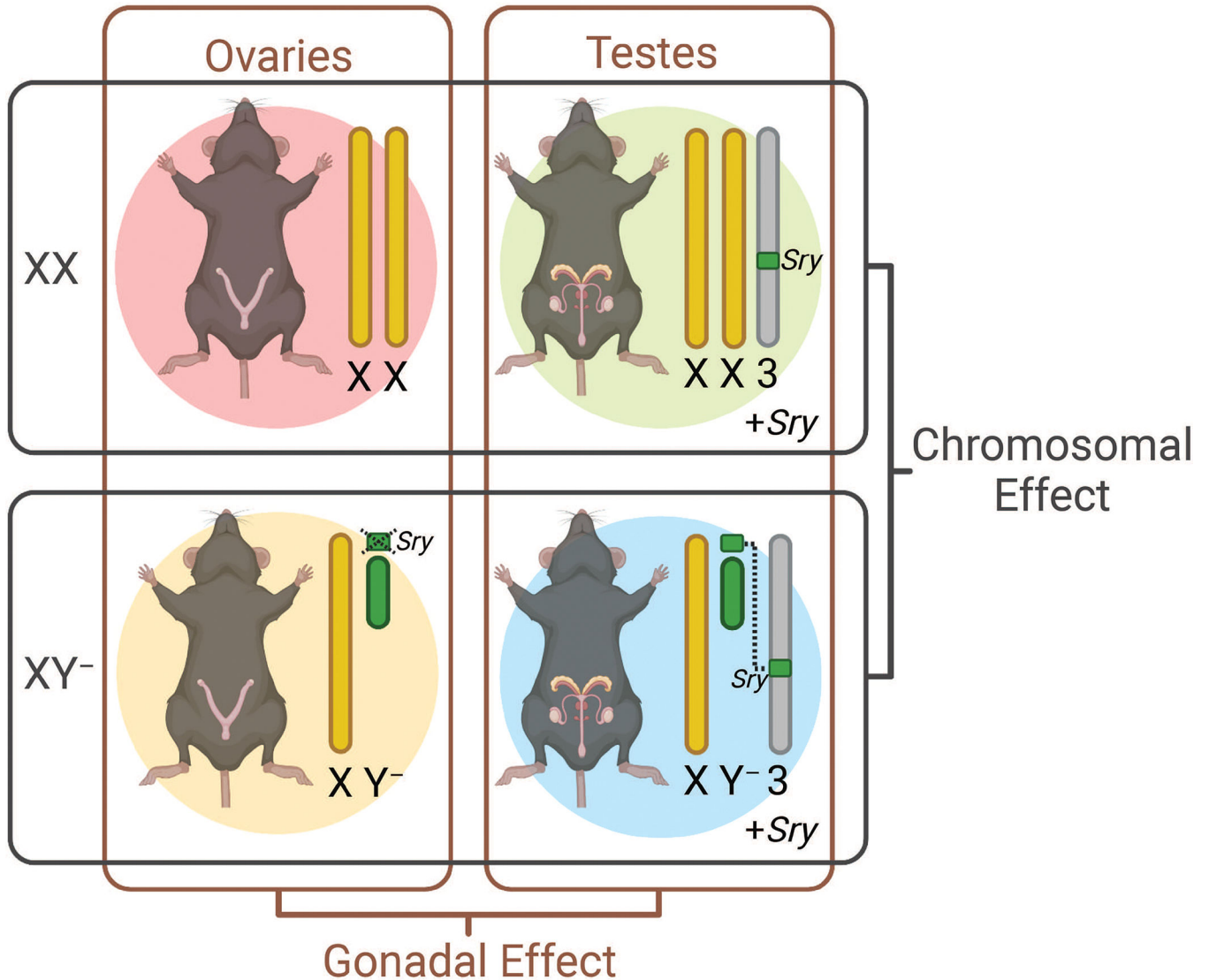
Four Core Genotypes

Figure 3. Four Core Genotypes (FCG) Mouse Model.

The FCG mouse model allows the interrogation of independent effects of sex chromosome and gonad type. Four genotypes are generated through combinations of the *Sry* transgene and XX or XY chromosomes (depicted to the right of each mouse phenotype). A chromosomal effect is determined by comparing animals with XX sex chromosomes (XX ovaries + XX testes) to animals with XY sex chromosomes (XY ovaries + XY testes). A gonadal effect is determined by comparing animals with ovaries (XX ovaries + XY ovaries) to animals with testes (XX testes + XY testes).
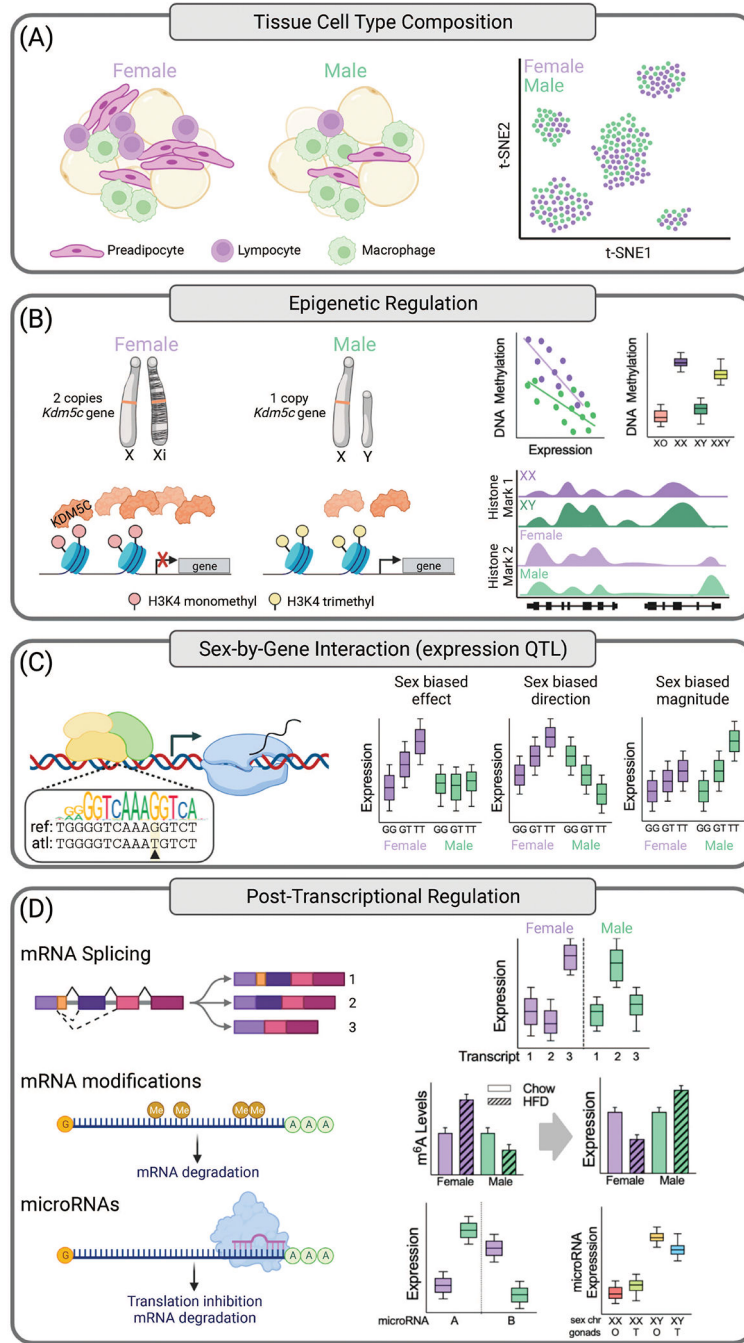
Figure 4. Mechanisms contributing to sex-biased gene expression.
(**A**) Tissue cell type composition differs between the sexes, which confounds bulk tissue gene expression analysis. (**B**) Epigenetic mechanisms such as histone modifications, DNA methylation, and chromatin structure are regulated by sex and impact autosomal gene expression. (**C**) Sex-by-gene interactions exhibit sex-biased effects, including differences in both the direction and magnitude of gene expression. (**D**) Biological sex impacts multiple post-transcriptional regulatory processes to influence gene expression levels, including

mRNA splicing, microRNA abundance, and covalent mRNA modifications that affect mRNA degradation.