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Four Core Genotypes and XY* mouse models: update on impact on SABV research

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Abstract

The impact of two mouse models is reviewed, the Four Core Genotypes and XY* models. The models are useful for determining if the causes of sex differences in phenotypes are either hormonal or sex chromosomal, or both. Used together, the models also can distinguish between the effects of X or Y chromosome genes that contribute to sex differences in phenotypes. To date, the models have been used to uncover sex chromosome contributions to sex differences in a wide variety of phenotypes, including brain and behavior, autoimmunity and immunity, cardiovascular disease, metabolism, and survival. In some cases, use of the models has been a strategy leading to discovery of specific X or Y genes that protect from or exacerbate disease. Sex chromosome and hormonal factors interact, in some cases to reduce the effects of each other. Future progress will come from more extensive application of these models, and development of similar models in other species.

Keywords

Sex differences; SABV; sex chromosomes; X chromosome; Y chromosome; Eif2s3x; Paul

The study of sex as a biological factor (SABV) in disease (Tannenbaum et al., 2016) requires knowledge of factors inherent in the two sexes that cause sex differences in structure and function.† Theories of sexual differentiation of the brain (or other tissues) have changed in the last 25 years (Arnold, 2012, 2017a; McCarthy and Arnold, 2008). By the end of the 20th century, sexual differentiation was presumed to be virtually entirely caused by differential action of gonadal hormones (Cooke et al., 1998). Since then, strong evidence has emerged

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†This manuscript is dedicated to the memory of Paul Burgoyne, who passed away in August 2020. Paul was a true pioneer in the study of sex chromosomes. He was co-creator of the Four Core Genotypes mouse model. His research also advanced understanding of the XY* model. He originated the analytical pipeline involving coordinated use of the FCG and XY* models, discussed at length here, as a method to determine if sex differences in any mouse phenotype were caused in part by sex chromosome effects, and if the effects were due to X or Y genes. Thus, the information in this manuscript derives directly from Paul's insightful use of mouse models, and demonstrates one aspect of his transformative influence on the study of sexual differentiation. Importantly, Paul shared his mice and knowledge generously with many colleagues, including the author, thus enabling most of the mouse research reviewed here.

for a second major pathway contributing to sex differences (Burgoyne et al., 1995). The inherent sexual inequality X and Y genes or chromatin within non-gonadal cells is now known to produce sex differences in tissue functions, not because of effects on gonadal hormones. This progress in understanding has been possible because of several advances in biomedical research. On the one hand, the explosion of information from sequencing of the sex chromosomes, and studies of X and Y chromosome evolution and function, have greatly improved our concepts of how the two sex chromosomes differ in gene content and function (Birchler et al., 2006; Burgoyne and Mitchell, 2007; Charlesworth, 1996; Disteche, 2016; Graves, 2006; Hughes and Page, 2015). Secondly, the discovery of *Sry* as the testis-determining gene in 1989–1991 (Goodfellow and Lovell-Badge, 1993) led to production of mice in which the action of *Sry* could be dissociated from the effects of other Y chromosome genes. A major side effect of *Sry* and sex chromosome research has been the availability of several mouse lines that are broadly useful for studying sexual differentiation of any tissue. The two most useful lines are the Four Core Genotypes (FCG) model, and the XY* model (Burgoyne and Arnold, 2016; Burgoyne et al., 1998; De Vries et al., 2002; Eicher et al., 1991; Mahadevaiah et al., 1998). These two models provide tools for any investigator who wishes to ask if a phenotypic sex difference in mice is caused by differential action of sex chromosome genes, or by gonadal hormones (Burgoyne and Arnold, 2016). Here, I briefly discuss the two models, and summarize selected recent published research to illustrate the utility of the models. I also discuss a few limitations of the models, and unanswered questions that can be studied in the near future.

Where FCG and XY* mice came from

Lovell Badge and Robertson (1990) (Gubbay et al., 1992) discovered an XY mouse with ovaries, which was subsequently found to have an 11kb deletion of the testis-determining gene *Sry* (Vernet et al., 2011), producing the “Y minus” chromosome, Y^{-} . The same lab produced a DNA construct encoding mouse *Sry*, which was inserted as a transgene onto mouse chromosome 3 by Washburn and Eicher (Itoh et al., 2015; Mahadevaiah et al., 1998). Burgoyne used a creative breeding scheme to move the *Sry* transgene to a mouse with the Y^{-} chromosome (Mahadevaiah et al., 1998), making fertile XY^{-}(*Sry*+) males (see Jackson Lab website entry for development of strain 010905). When *Sry* is autosomal, gonadal differentiation is not controlled by sex chromosome complement (XX vs. XY). Mating an XY^{-}(*Sry*+) male to an XX female produces XX(*Sry+*) and XY^{-}(*Sry*+) males with *Sry* and testes, and XX and XY^{-} females with ovaries, lacking *Sry* (Figure 1). Differential effects of XX vs. XY sex chromosomes can be measured by comparing XX and XY mice with the same type of gonad, either with testes or ovaries (Arnold, 2014; Burgoyne and Arnold, 2016). Differential effects of gonadal secretions can be measured by comparing mice with ovaries vs. testes (keeping sex chromosome complement constant, XX or XY). The FCG model tests for the effects of sex chromosome complement (XX vs. XY), or of gonadal hormones, or their interaction, on virtually any mouse trait (De Vries et al., 2002).

When a sex chromosome effect (XX not equal to XY) is detected in FCG mice, it could be caused by either the number of X chromosomes (including X dose, X imprint or indirect effects of X inactivation), or the presence / absence of the Y chromosome (Arnold, 2017a; Burgoyne and Arnold, 2016). The XY* model is then useful to discriminate between these

possibilities. Discovered by Eicher et al. (Eicher et al., 1991), XY* mice have an aberrant pseudoautosomal region on the Y chromosome, which recombines abnormally with the X chromosome (Burgoyne and Arnold, 2016; Burgoyne et al., 1998). XY* fathers, mated to XX females, produce mice that are very similar to XX and XO gonadal females, and XY and XXY gonadal males (Burgoyne and Arnold, 2016)(Figure 1). The effects of one vs. two X chromosomes is measured by comparing XO vs. XX females, or XY vs. XXY males. The effects of one vs. no Y chromosome is measured by comparing XY vs. XO, and XXY vs. XX. In the XY* model, mice with a Y chromosome are gonadal males.

The study of FCG and XY* mice is not an end in itself, but the beginning of an investigation of the downstream effects and molecular pathways controlled by specific sex-biasing factors, hormonal and/or sex chromosomal. Once a sex chromosome effect is discovered in FCG mice, and attributed to the X or Y chromosome using XY* mice, specific X or Y candidate genes can be identified to assess if they are responsible for the sex chromosome effects. The top candidates are X genes that escape X-inactivation, and which are therefore expressed inherently higher in XX than XY cells (Disteche, 2016), or X genes that have a differential parental imprint in XX and XY cells because XY cells received only a maternal imprint (Burgoyne and Arnold, 2016). Top candidates on the Y chromosome are any of the genes not found on the pseudoautosomal region. The discovery of specific genes then leads to further investigation of the molecular mechanisms of X or Y gene action in tissues of interest.

In the FCG model, if gonadal males and females show no difference in a trait, one concludes that under conditions of the experiment, gonadal hormones did not cause a difference among groups. One cannot conclude that gonadal hormones have no impact on the trait, because the effects of testicular and ovarian hormones might be the same, and thus do not cause a group difference based on gonad type. Also, variations in the effect of one type of gonadal hormone (e.g., reduction in ovarian estrogens during reproductive senescence, or reduction in androgen levels during stress) might give rise to male-female differences under different testing conditions. Similarly, equivalence of XX and XY groups means that the XX and XY cells are not different under conditions of the experiment, not that the sex chromosome genes do not influence the trait. All of the sex-biasing factors, hormonal and sex chromosomal, can have compensatory effects that reduce the role of other factors (De Vries, 2004).

FCG mice are available from Jackson Laboratory, on a C57BL/6J genetic background (strain 10905). XY* mice on the same background are available from the Mutant Mouse Resource & Research Centers supported by the NIH (MMRRC, strain 43694-UCD). Transgenic and knockout mice for specific X and Y genes that are candidates for causing sex chromosome effects are also available from the MMRRC, and from international mouse gene knockout consortia.

Overview of results using FCG and XY* models

Table 1 is an updated list of published papers that use FCG and/or XY* mice to search for sex chromosome effects on diverse phenotypes. Because both models are informative and

easy to breed in the laboratory, their use has expanded. The diversity of sex chromosome effects is impressive because of the many tissues and functions that are influenced by sex chromosome complement. Sex chromosome effects have been discovered that contribute to sex differences in behavior (addiction, pain, learning, feeding, parental, sleep, social), in brain phenotypes and diseases, and in mouse models of various diseases including autoimmune, aging, neural tube closure defects, cardiovascular diseases (hypertension, cardiac ischemia / reperfusion injury, stroke, hypertension, atherosclerosis, and abdominal aortic aneurysms), immunity, metabolic disease, etc. (Table 1)(Cox et al., 2014). This diversity likely reflects sexual inequality of sex chromosome action that is widespread across cell types, reflecting the involvement of numerous X and Y genes in many fundamental cellular processes (Arnold, 2019; Arnold et al., 2017). Table 1 emphasizes the effects of sex chromosome complement, but the investigations referenced also have uncovered numerous cases in which gonadal hormones contribute to sex difference in phenotype. Often, a specific physiological or disease phenotype is influenced by both sex chromosome and gonadal effects.

In cases when a sex chromosome effect is detected and attributed to the effects of one of the sex chromosomes, that chromosome is more often the X chromosome than the Y chromosome (Arnold et al., 2016). Table 1 lists 22 studies reporting an effect of the X chromosome, and two studies reporting an effect of the Y chromosome. Other mouse models also implicate Y genetic material in variation in disease (Case and Teuscher, 2015). The Y chromosome has been refractory to study because of the difficulty of linking specific genes to specific traits (Arnold, 2017b). Moreover, the higher number of effects of X genes is partly because the XY* model is more often used to find X chromosome effects than Y chromosome effects. Nevertheless, the disproportionate involvement of the X chromosome is also likely because of the larger number of X chromosome genes, and suggests that escape from X inactivation is a major source of sex bias in the genome. The degree of sex bias stemming from the X chromosome may be greater in humans than in mice, because about 25% of X genes in humans escape X inactivation and are expressed higher in females than males, in many tissues of the body (Carrel et al., 1999; Tukiainen et al., 2017). In contrast, 3–8% of X genes escape inactivation in mouse tissues (Berletch et al., 2015).

To date, a few research programs using FCG and/or XY* mice have progressed far enough to have identified specific genes responsible for sex chromosome effects. Two X-linked histone demethylases, *Kdm6a* and *Kdm5c*, escape X-inactivation and are expressed higher in XX cells than XY cells. *Kdm6a* has been reported to contribute to sex differences in mouse models of multiple sclerosis and Alzheimer's Disease, and *Kdm5c* in sex differences in metabolism, discussed below (Davis et al., 2020; Itoh et al., 2019; Link et al., 2020). In the bladder cancer studies, *Kdm6a* is implicated in female-biased protection leading to greater survival when two X chromosomes are present, relative to one X chromosome (Kaneko and Li, 2018).

The use of sex chromosome mouse models is part of a novel strategy to detect factors that modify disease. It represents a new tool in the armamentarium for investigators studying disease mechanisms. The models lead to discovery of genes that might well have gone undetected using traditional methods. The mouse models test, for example, whether the copy

number of specific X genes modulates disease mechanisms. Traditional methods such as Genome-wide Association Studies ask whether variations in the genetic sequence of a gene correlates with disease, not its copy number. Although variation in genetic sequence might mimic the effects of copy number (*e.g,* because both might influence level of expression), it might not. Because copy number of X genes is confounded in human populations with numerous other variables that vary by sex and gender, it might be difficult to recognize an association between X copy number and disease incidence or progression. Studies of the FCG and XY* models have and will lead to discovery of effects of specific X and Y genes. Translating this information for better understanding of human disease is probably best done once a specific gene is implicated. For example, discoveries that *Kdm6a* modulates bladder cancer or autoimmune disease or Alzheimer's Disease, or *Kdm5c* modulates fat metabolism, now rationalize further study of the role of these genes in disease mechanisms in humans.

Salient examples of recent research on FCG and XY* mice

1. Unusually high incidence of sex chromosome effects uncovered by magnetic resonance imaging of sex differences across the entire brain.

High-resolution MRI studies of mouse brain have uncovered sex differences in many brain regions (Spring et al., 2007), not just the limbic regions that have been used as dominant models of brain sexual differentiation. In MRI studies of gonad-intact FCG mice, 62 brain regions were segmented and compared across groups (Corre et al., 2016). Sixteen of the regions showed differences dependent on gonadal sex, and 11 showed differences attributed to sex chromosome complement. Three brain regions showed effects of both variables that were additive rather than interactive. For example, XY>XX difference in brain region volumes were found in the superior colliculus, several regions of the medulla, basal forebrain, and parietal-temporal lobe, and XX>XY differences were found in the cerebellar cortex, occipital cerebral cortex, corpus callosum, fimbria and septum. The number of brain regions showing sex chromosome effects is larger than expected, based on established models of hormone-dependent sexual differentiation. To test if the XX-XY differences are potentially caused by group differences in gonadal hormone levels, FCG mice were gonadectomized before puberty and their brains measured by MRI in adulthood (Vousden et al., 2018). Most of the sex chromosome effects were also found in gonadectomized mice, although the size of the differences were sometimes reduced modestly. Nevertheless, the authors detected instances in which hormonal and sex chromosomal effects were cooperative or compensatory.

2. Sex chromosomes regulate sensitivity to effects of sex steroid hormones in developing limbic system.

The decades-old model of sexual differentiation of hypothalamic and limbic brain regions is that permanent male-female differences are caused by effects of testosterone secreted from the male's testes (Arnold and Gorski, 1984). Once testosterone enters the brain, it is converted to estradiol by the aromatase enzyme, and acts on estrogen receptors to cause differential development. In this model, XX and XY cells were seen as equally responsive to hormonal effects, but the sex differences were simply induced by different levels of testosterone as a result of testicular secretions. New evidence suggests, however, that XX

and XY cells are not equally responsive. In cultures of embryonic day 16 neurons from amygdala and stria terminalis of FCG mice, XY neurons had higher expression of aromatase than XX neurons (Cisternas et al., 2015). Moreover, treatment of cultures with either estradiol or DHT increased aromatase expression in XX but not XY neurons. Expression of ERβ is also regulated by sex chromosomes (XY>XX) (Cisternas et al., 2017). Thus, sex chromosome complement potentially regulates estrogen levels and signaling mechanisms. In cultures from hypothalamus, expression of neurogenin 3 (*Ngn3*), which is required for sex differences in neuritic outgrowth *in vivo*, is higher in XX than XY cells, accounting for the inherent sex difference in *Ngn3* expression (Scerbo et al., 2014). These studies are particularly interesting because they are among the first to address the pathways by which two major sources of sex bias, the sex chromosomes and sex steroids, intersect and modulate each other to cause sex differences in the patterns of development (Cisternas et al., 2018). The results so far are quite tantalizing, and rationalize a great deal of further research, both *in vivo* and *in vitro*, to clarify the degree to which sex bias in one factor (e.g., sex chromosomes) limits or increases the effects of other factors (e.g., estrogens). The final result of these interactions is not yet clear. One hypothesis is that the greater effect of estradiol in XX cells, to increase aromatase, may have the result of increasing estrogen signaling by locally synthesized estradiol, to compensate for the estrogen action derived from testosterone secretion from the testes of males. Thus, the development of these brain regions may be more similar in the two sexes because of offsetting and intersecting effects of sex chromosome genes and gonadal hormones (Cambiasso et al., 2017; Cisternas et al., 2018; Rulli et al., 2018). It will be exciting to see how further studies unravel these interactions.

3. X chromosome mechanisms contributing to sex differences in autoimmunity

In EAE, a mouse model of multiple sclerosis, females are more affected than males, mirroring the higher incidence of MS in women than in men. Studies of EAE demonstrate that androgens reduce the severity of disease, as does estriol, an estrogen that is elevated in late pregnancy when women experience remission of MS symptoms (Gold and Voskuhl, 2016). The sites of action of estrogens mediating these effects have been studied extensively in mice (Golden and Voskuhl, 2017; Itoh et al., 2017; Spence and Voskuhl, 2012). In FCG mice, XX mice show worse disease and more neurodegeneration than XY mice (Smith-Bouvier et al., 2008). This direction of the sex chromosome effect likely reflects the action of sex chromosomes in the immune system, because a reverse effect is found in the brain. An XY brain shows worse disease than an XX brain (Du et al., 2014). Thus, the sex chromosomes likely have different effects in different cellular components involved with induction and effector phases of the disease. As indicated above, in CD4+ T cells, expression of the X gene *Kdm6a*, which escapes inactivation, is higher in XX than XY, and likely exacerbates EAE, because deletion of *Kdm6a* in those cells protects against clinical disease and neurodegeneration (Itoh et al., 2019). These results implicate a specific X gene as one contributor to sex differences in EAE. However, the different imprint on the X chromosome in males and females may also play a role. In CD4+ T lymphocytes, the paternal X chromosome is more highly methylated than the maternal X, which leads to higher expression of X genes in XY cells relative to XX cells because they lack the paternal X chromosome (Golden et al., 2019). Moreover, XY mice with different types of Y

chromosomes show different severity of EAE, indicating that Y genes or non-genic regions may also contribute to the sex difference in EAE (Arnold, 2017b; Case and Teuscher, 2015; Case et al., 2013). EAE is a case in which numerous independent sex-biasing factors act on different cell types to influence the course of disease, in a complex sex-biased pattern. Because some factors are protective and others harmful, the effects of the different factors can amplify or undermine the effects of other factors.

4. Identification of an X gene escaping inactivation that contributes to sex differences in fat metabolism

In the FCG model, adult mice with testes weigh about 25% more than mice with ovaries, indicating that gonadal hormones have a large effect on body weight. When FCG mice are gonadectomized as adults, XX mice develop much greater body weight and body fat than XY mice (Chen et al., 2012; Chen et al., 2013). Thus, sex chromosome complement also contributes significantly to sex differences in body weight and fat. If fed a high fat diet, the XX mice eat more than XY mice, develop much higher levels of liver triglycerides, and higher plasma levels of high density lipoproteins (Chen et al., 2012; Link et al., 2015; Link et al., 2020). Studies of XY* mice show that the sex chromosome effect is caused by the number of X chromosomes. The X chromosome effect is largely attributed to the dose of *Kdm5c*, a histone demethylase that escapes X inactivation and is expressed higher in XX than XY cells (Link et al., 2020). KDM5C regulates chromatin accessibility, gene expression, and adipocyte differentiation. These studies rationalize a focus on the same gene in humans, in whom adipose tissue KDM5C mRNA levels and KDM5C genetic variants are associated with variations in body mass (Link et al., 2020).

5. *X-linked* Kdm6a *contributes to sex differences in Alzheimer's disease and longevity*

One mouse model of Alzheimer's disease involves introduction of a mutated form of human amyloid precursor protein (hAPP) in mice. In hAPP-FCG mice, XY mice have more memory deficits and shorter life span than XX mice, indicating a sex chromosome effect (Davis et al., 2020). Using XY* mice, the sex chromosome effect was attributed to the dose of X chromosomes, because mice with one X chromosome (XO, XY) had more memory deficits and shorter life span than mice with two X chromosomes (XX, XXY). In addition to the X chromosome effects, the studies of FCG-hAPP and XY*-hAPP mice showed a probable interaction of sex chromosome effects and gonadal hormones. For example, the X chromosome difference in longevity in FCG and XY* mice was greater in mice with ovaries than testes. These authors focused further on *Kdm6a*, an X-linked gene that escapes inactivation and is expressed higher in XX than XY cells of the brain and other tissues. A minor variant of *Kdm6a* sequence, found in humans, was associated with increased expression of *Kdm6a* and lower cognitive decline among patients with mild cognitive impairment. When Kdm6a levels were increased or decreased in the dentate gyrus of mice, increasing or decreasing performance on the Morris water maze learning task, respectively. *In vitro*, higher expression of *Kdm6a* prevented toxicity of amyloid beta peptide in neuronal cultures. The results suggest a combined effect of *Kdm6a* and gonadal hormones in regulating sex differences in this model of Alzheimer's Disease. The neurotoxic effects of amyloid protein β *in vitro*, and learning deficits *in vivo*, were lower in mice with two copies

of *Kdm6a*, relative to one copy, suggesting that some of the protective effects of a second X chromosome derive from the second dose of *Kdm6a* (Davis et al., 2020).

Why answering the sex difference question is important

The study of both sexes is rationalized, first and foremost, by the realization that the two sexes show different patterns of disease. Studying one sex does not necessarily reveal disease mechanisms of the other sex. A second reason is that greater disease incidence or progression in one sex means that the other sex is protected by some sex-biased factors (gonadal hormones or sex chromosome genes), or the afflicted sex harbors harmful factors. Research to understand the effects of sex-biasing factors may uncover novel mechanisms of protection, which could be targets for novel therapies. Of course, sex bias in animal systems will not always be identical to sex bias found in humans. The importance of animal research is not just that animal physiology has some similarities to that of humans. Perhaps more importantly, animal research has a major role in framing the questions that are asked about human disease. The conceptual framework for understanding human physiology and disease is fundamentally dependent on animal research. Both differences and similarities between animals and humans can be informative.

Limitations of FCG and XY* models

Potential caveats for use of FCG and XY* models have been reviewed in detail (Arnold, 2014; Burgoyne and Arnold, 2016). Among the issues is whether XX and XY mice differ in any inherent non-sex-chromosomal factors that could explain differences between groups in the two models, and if effects of those other factors might be mistaken for effects of sex chromosome complement. Although there are some potentially confounding factors, the models can nevertheless be used fruitfully to detect *bone fide* sex chromosome effects, especially when the same XX-XY difference is detected under different conditions, for example in both gonadal males and females, in the presence or absence of an *Sry* transgene, and/or in both FCG and XY* models.

Another issue is that to date, the mouse is the only species in which these models are available. Thus, when a sex difference is discovered in physiological, behavioral, or disease processes in a species other than the mouse, the investigator does not have comparable models to discover whether the sex difference is caused by gonadal hormones vs. sex chromosome genes. This is a significant limitation, for at least two reasons. Firstly, many disease processes cannot be studied well in mice. Some phenotypes and diseases are much easier to study in other species, because of their size, physiology, and tractability for specific experimental manipulations. Secondly, the peculiar physiology and genome of the mouse will shape the kinds of answers that one gets from studying sex chromosome effects. The interplay of sex chromosome and hormonal effects is almost certainly different across species, and the answer from mouse studies does not necessarily establish general principles that are valid across species. As Frank Beach pointed out long ago, concentration of studies to a small number of species is highly disadvantageous (Beach, 1950). Broad principles of biology are established by comparative studies of numerous species (Capel, 2017; Hughes and Page, 2015). With the recent advent of powerful gene knockout and transgenic strategies

that can be applied across species (Aitman et al., 2016; Shimoyama et al., 2017) , we hope that more models can be created in diverse species to test for sex chromosome effects.

Prognosis for the future

Although Table 1 shows that dozens of studies have used FCG and/or XY* models to date, we expect the use of these models to expand more rapidly in the coming years. This is partly because of the mandate by the NIH that investigators consider sex as a biological variable, but also because of the increasing investment in SABV research by investigators who are discovering that sex is a critical variable in modulation of disease processes. The availability and tractability of these models is attractive. Moreover, knockouts and floxed alleles of important X and Y genes are becoming more available, at public mouse repositories, and in individual labs. Thus, the pipeline for analysis of sex chromosome effects (Burgoyne and Arnold, 2016) is being utilized with increasing frequency, and provides increasingly diverse examples of how the mouse resources can be utilized fruitfully.

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Highlights

- + The Four Core Genotypes and XY* mouse models uncover sex chromosome effects in physiology and disease
- + XX vs. XY sex chromosome complement contributes to sex differences in a wide variety of tissues
- + Sex chromosomes cause sex difference in fundamental cellular processes.
- + The mouse models anchor a novel strategy for uncovering factors that protect from disease.
- + Specific X and Y genes have been discovered that contribute to sex differences in traits.

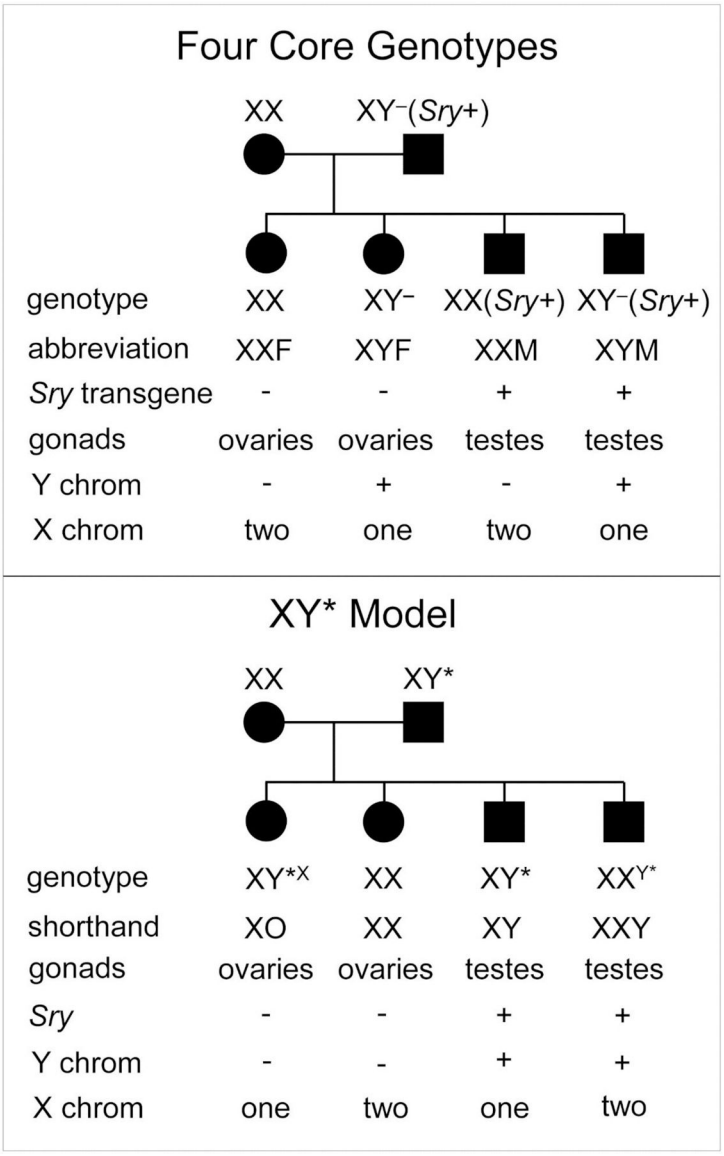

Figure 1.

Schematic drawing showing the breeding scheme and genotypes in the Four Core Genotypes (FCG) and XY* mouse models. Above, the FCG model produces four genotypes that are XX or XY, each genotype with either testes or ovaries. Comparison of the XX and XY mice with the same type of gonad leads to discovery of phenotypes in which the complement of sex chromosomes causes sex differences. Comparison of mice with testes vs. ovaries, with the same sex chromosomes, leads to discovery of phenotypes in which the presence or absence of *Sry* causes sex differences, including the effects of testicular vs. ovarian secretions. Below, the XY* model is especially good for detecting effects of X chromosome number, in comparisons of XO and XX gonadal females, or XY vs. XXY gonadal males. Comparisons of mice with a Y chromosome or not (XO vs. XY, XX vs. XXY) leads to detection of phenotypes in which the Y chromosome, or gonadal hormones, causes a sex difference. Figure modeled after (Arnold and Chen, 2009).

Table 1.

The table lists primary literature articles using Four Core Genotypes (FCG) and/or XY* mouse models for detecting sex chromosome effects on mouse phenotypes. ChrX, effect of chromosome X; ChrY, effect of chromosome Y; CVD, cardiovascular disease, Xm, X chromosome with maternal imprint; Xp, X chromosome with paternal imprint; SCE, sex chromosome effect.

Reference	Model	Effect	Phenotype
(Davis et al., 2019)	FCG	SCE	Aging, longevity
(Golden et al., 2019)	FCG	ChrX, Xm vs. Xp	Autoimmunity, EAE
(Itoh et al., 2019)	FCG	SCE, ChrX	Autoimmunity, EAE
(Palaszynski et al., 2005)	FCG	SCE	Autoimmunity, EAE
(Sasidhar et al., 2012)	FCG	SCE	Autoimmunity, EAE
(Smith-Bouvier et al., 2008)	FCG	SCE	Autoimmunity, EAE and lupus
(Du et al., 2014)	FCG	SCE	Autoimmunity, EAE, brain
(Barker et al., 2010)	FCG	SCE	Behavior, addiction
(Quinn et al., 2007)	FCG	SCE	Behavior, addiction
(Martini et al., 2020)	FCG	SCE	Behavior, addiction
(Gatewood et al., 2006)	FCG	SCE	Behavior, aggressive, parental
(Kuljis et al., 2013)	FCG	SCE	Behavior, circadian
(Aguayo et al., 2018)	FCG	no SCE	Behavior, circadian feeding
(Chen et al., 2015)	FCG	SCE	Behavior, feeding
(Kopsida et al., 2013)	FCG	SCE	Behavior, feeding, anxiety
(Seu et al., 2014)	FCG	SCE	Behavior, learning and motivation
(Aarde et al., 2020)	FCG	SCE	Behavior, learning
(Gioiosa et al., 2008a)	FCG	SCE	Behavior, pain
(Gioiosa et al., 2008b)	FCG	SCE	Behavior, pain
(Ehlen et al., 2013)	FCG	SCE	Behavior, sleep
(Cox and Rissman, 2011)	FCG	SCE	Behavior, social
(McPhie-Lalmansingh et al., 2008)	FCG	SCE	Behavior, social
(Tejada and Rissman, 2012)	FCG	no SCE	Behavior, social
(Cisternas et al., 2015)	FCG	SCE	Brain, aromatase expression
(Cisternas et al., 2017)	FCG	SCE	Brain, aromatase expression
(Moore et al., 2013)	FCG	SCE	Brain, callosal remyelination
(Markham et al., 2003)	FCG	no SCE	Brain, cortical thickness
(Abel et al., 2011)	FCG	SCE	Brain, gene expression
(Barko et al., 2019)	FCG	SCE	Brain, gene expression, stress
(Puralewski et al., 2016)	FCG	SCE	Brain, gene expression, stress
(Seney et al., 2013a)	FCG	SCE	Brain, gene expression, stress
(Seney et al., 2013b)	FCG	SCE	Brain, gene expression, stress
(Quinnies et al., 2015)	FCG	SCE	Brain, hypothalamus gene expression
(Kuo et al., 2010)	FCG	no SCE	Brain, hypothalamus in vitro

Reference	Model	Effect	Phenotype
(Scerbo et al., 2014)	FCG	SCE	Brain, hypothalamus in vitro
(Carruth et al., 2002)	FCG	SCE	Brain, midbrain Pessoane expression in vitro
(Corre et al., 2016)	FCG	SCE	Brain, MRI morphology
(Vousden et al., 2018)	FCG	SCE	Brain, MRI morphology
(Wagner et al., 2004)	FCG	no SCE	Brain, neonatal hypothalamus
(De Vries et al., 2002)	FCG	SCE	Brain, septal anatomy
(Alsiraj et al., 2017)	FCG	SCE	CVD, abdominal aortic aneurysms
(Alsiraj et al., 2018)	FCG	SCE	CVD, aortic aneurysms
(AlSiraj et al., 2019)	FCG	SCE	CVD, atherosclerosis
(Manwani et al., 2015)	FCG	no SCE	CVD, brain, stroke
(McCullough et al., 2016)	FCG	SCE	CVD, brain, stroke
(Caeiro et al., 2011)	FCG	SCE	CVD, heart rate
(Ji et al., 2010)	FCG	SCE	CVD, Hypertension
(Pessoa et al., 2015)	FCG	SCE	CVD, hypertension
(Dadam et al., 2017)	FCG	SCE	CVD, hypertension, renal
(Liu et al., 2010)	FCG	no SCE	CVD, renal gene expression
(Dadam et al., 2014)	FCG	SCE	CVD, salt regulation
(Van Nas et al., 2009)	FCG	SCE	Gene expression
(Xu et al., 2002)	FCG	SCE	Gene expression, brain
(Xu et al., 2005a)	FCG	SCE	Gene expression, brain
(Xu et al., 2005b)	FCG	SCE	Gene expression, brain
(Xu et al., 2006)	FCG	SCE	Gene expression, brain
(Xu et al., 2008a)	FCG	SCE	Gene expression, brain
(Xu et al., 2008b)	FCG	SCE	Gene expression, brain
(Xu and Arnold, 2005)	FCG	no SCE	Gene expression, kidney
(Durcova-Hills et al., 2004)	FCG	SCE	Germline
(Sangrithi et al., 2017)	FCG	SCE	Germline
(Itoh et al., 2015)	FCG	no SCE	Growth, anogenital distance
(Holaskova et al., 2015)	FCG	SCE	Immunity, drug effect
(Dill-Garlow et al., 2019)	FCG	no SCE	Immunity, lymph node
(Robinson et al., 2011)	FCG	SCE	Immunity, viral infection
(Link et al., 2017)	FCG	SCE	Metabolism, adipose miRNA expression
(Link et al., 2020)	FCG	ChrX	Metabolism, adipocyte differentiation
(Wijchers et al., 2010)	FCG, XO	ChrX	Gene expression, autosomal
(Bonthuis et al., 2012)	FCG, XY*	ChrX	Behavior, reproductive
(Chen et al., 2009)	FCG, XY*	ChrX	Brain, striatum gene expression
(Davis et al., 2020)	FCG, XY*	Chr X	Alzheimer’s Disease, longevity
(Li et al., 2014)	FCG, XY*	ChrX	CVD, cardiac ischemia / reperfusion injury
(Umar et al., 2018)	FCG, XY*	ChrY	CVD, pulmonary hypertension

Reference	Model	Effect	Phenotype
(Chen et al., 2008)	FCG, XY*	ChrX	Developmental defect, neural tube closure
(Ishikawa et al., 2003)	FCG, XY*	SCE	Growth, placental
(Burgoyne et al., 2002)	FCG, XY*	SCE	Growth, postnatal
(Link et al., 2015)	FCG, XY*	SCE	Metabolism, adipose
(Chen et al., 2012)	FCG, XY*	ChrX	Metabolism, adiposity, body weight
(Chen et al., 2013)	FCG, XY*	ChrX, ChrY	Metabolism, adiposity, body weight
(Taylor et al., 2020)	FCG, XY*	Chr X	Behavior, pain
(Davies et al., 2007)	XY*	ChrX	Behavior, attention
(Davies et al., 2009)	XY*	ChrX	Behavior, attention
(Davies et al., 2005)	XY*	ChrX, Xm vs. Xp	Behavior, cognitive
(Isles et al., 2004)	XY*	ChrX	Behavior, fear
(Aarde et al., 2019)	XY*	SCE	Behavior, learning
(Lewejohann et al., 2009)	XY*	ChrX	Behavior, memory
(Wolstenholme et al., 2012)	XY*	ChrX	Brain, gene expression
(Bonthuis and Rissman, 2013)	XY*	ChrX	Brain, gene expression, body weight
(Cox et al., 2015)	XY*	ChrX	Brain; Behavior, social and anxiety
(Hinton et al., 2015)	XY*	ChrX, Xm vs. Xp	CVD, aortic morphology
(Werler et al., 2011)	XY*	ChrX	Gene expression, multiple tissues
(Werler et al., 2014)	XY*	ChrX	Testis function, germline
(Wistuba et al., 2010)	XY*	ChrX	Testis function, plasma hormones