Review

Mouse model systems to study sex chromosome genes and behavior: Relevance to humans

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ABSTRACT

Sex chromosome genes directly influence sex differences in behavior. The discovery of the Sry gene on the Y chromosome (Gubbay et al., 1990; Koopman et al., 1990) substantiated the sex chromosome mechanistic link to sex differences. Moreover, the pronounced connection between X chromosome gene mutations and mental illness produces a strong sex bias in these diseases. Yet, the dominant explanation for sex differences continues to be the gonadal hormones. Here we review progress made on behavioral differences in mouse models that uncouple sex chromosome complement from gonadal sex. We conclude that many social and cognitive behaviors are modified by sex chromosome complement, and discuss the implications for human research. Future directions need to include identification of the genes involved and interactions with these genes and gonadal hormones.

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1. Introduction

In species with sexual reproduction males and females must accommodate the demands of both shared and divergent evolutionary pressures. The responses to different demands are reflected in sex differences in morphology and behaviors. Primary sex differences, such as male versus female gonads, are distinctly different between the two sexes, while secondary sexual characteristics like antlers, body hair, and musculature of the larynxes can be completely sexually dimorphic or can vary by degrees. Importantly, sexually dimorphic structures are also present in the central nervous system, and are necessary for the regulation of behavioral sex differences.

Morphological sex differences are primarily orchestrated by developmental gene expression, and, in many vertebrates, sex chromosome genes initiate sex-specific gene expression during early development. These genes, and the proteins they encode, propagate a cascade of signaling events within gene-networks to drive sexual differentiation programs. The best-documented example of this in mammals is the gene of the sex-determining region of the Y chromosome, *Sry*. When *Sry* is expressed at the correct time and place it causes the undifferentiated gonads to become testes. In turn, androgens, produced by the male testes

during development, organize the brain to respond to male-typical steroid hormones in adulthood, thereby activating sexually dimorphic masculine behaviors.

In addition to steroid hormones, the substantial genetic difference between males (bearing a single X and a Y chromosome) and females (bearing two X chromosomes) plays a significant role in brain development and may lead to neurological disorders (Lyon, 1961; Qureshi and Mehler, 2010; Raymond, 2006; Savic, 2012). Notably, the proportion of genes on the X chromosome that are expressed in brain is higher than any other single chromosome (Xu and Andreassi, 2011), and it is well known that some of these genes play a role in cognitive behavior. Human males with functional mutations in their only allele of these X-genes (i.e. *FMR1*, *JARID1C*, *ATRX*, etc.) exhibit mental illnesses (Raymond, 2006). In addition, males with three sex chromosomes (XXY or XYY) can have behavioral problems and low IQs (van Rijn et al., 2006), and XXY (Klinefelter syndrome) boys are diagnosed with autism spectrum disorders, schizophrenia, affective disorders, and language disabilities more often than XY boys (Savic, 2012). Likewise, XO females (Turner syndrome) have variable behavioral phenotypes but are often described as socially immature or fearful (Elsheikh et al., 2002; Lesniak-Karpiak et al., 2003). These are very common diseases, ranging in incidence from 1:2500 (Turner) to 1:600–1000 (Klinefelter), which highlights the potential clinical applications of research using mouse models of sex chromosome aneuploidy, as well as models that isolate the behavioral actions of sex chromosome genes from actions of hormones.

Over the past ten years, a surge in interest in sex chromosome genes and behavior has been driven by the hypothesis that these genes play a role in sexual differentiation of the brain (Arnold,

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2009; Arnold et al., 2003; De Vries et al., 2002). Because the sex chromosomes are intrinsically linked to gonadal development, and thereby hormonal differences, genetically manipulated mice along with spontaneous mutants, have been used as tools for examining direct, genetically derived sex differences that arise independently of hormones. While many non-behavioral phenotypes have been described from these models, this review will focus on and summarize sex chromosome gene effects on sexually dimorphic behaviors. We aim to broaden the readers' interests in the roles that sex chromosome genes play in sexually dimorphic behavior as well as other behaviors that may relate to human neurodevelopmental disorders with sex-biased incidences.

2. The sex chromosomes

The mammalian sex chromosomes, X and Y, are structurally heteromorphic, and unequally represented in the cells of XX females and XY males. It is hypothesized that the mammalian sex chromosomes started as identical autosomes that diverged once the precursor to the modern day Y chromosome acquired the gene(s) and mutations necessary for genetic inheritance of testes determination (Graves, 1995; Graves et al., 2006). After the Y chromosome captured the testis determining gene(s), three major genetic domains among the two sex chromosomes of mammals evolved (Fig. 1; Ellegren, 2011). The two chromosomes share a region of identical sequence homology, called the pseudoautosomal region (PAR; Ellis and Goodfellow, 1989) that, in humans, contains about 29 genes (Ross et al., 2005). Recombination of the sex chromosome PAR region, like recombination in autosomes, is essential for meiosis. In addition to the PAR genes shared with X, the human Y chromosome has approximately 27 known unique genes (429 predicted) in the non-recombining region of Y (NRY; (Skaletsky et al., 2003); NCBI human genome assembly), while the X chromosome contains approximately 1400 known unique genes (1672 predicted) in the non-pseudoautosomal region of X (NPX; Xu and Disteche, 2006; NCBI human genome assembly). In contrast to humans, the single PAR region in mouse sex chromosomes contains only two known genes (Dal Zotto et al., 1998; Perry et al.,

Fig. 1. Structural domains of mouse X and Y sex chromosomes. Schematic of the sex chromosomes in mice. PAR (in green), the pseudoautosomal region has sequence homology between X and Y with identical genes that recombine during male meiosis. *Sts* (in white), the steroid sulfatase gene. NPX (in pink), the non-pseudoautosomal region of X-chromosome genes unique to X. NRY (in yellow), the non-recombining region of Y-chromosome genes unique to Y. *Sry* (in blue), the sex determining region of Y encoding the testis-determining transcription factor. The centromere is represented as a white circle.

2001; Raudsepp and Chowdhary, 2008), the NRY contains 54 predicted Y-unique genes, and the NPX contains 2025 predicted X-unique genes (NCBI mouse genome assembly).

In order to deal with the stoichiometric problem of different doses of sex chromosome genes between the sexes, mammals developed X-inactivation dosage compensation, whereby one of the two X chromosomes is transcriptionally silenced at random in every female cell to match the single X expression levels in XY males (Lyon, 1961; Nguyen and Disteche, 2006; Wutz, 2011). However, in humans, 15% (Carrel and Willard, 2005), and in mice 3% (Berletch et al., 2011; Disteche et al., 2002; Yang et al., 2010), of genes on the silenced X chromosome are capable of escaping inactivation. This can result in a genetic imbalance between males and females such that, besides the unique genes on the Y chromosome that are only expressed in males, some of the genes unique to the X chromosome which escape X-inactivation may be more highly expressed in females. While the number of differentially expressed X chromosome genes is small (Itoh et al., 2007; Johnston et al., 2008), some of these genes are important regulators of downstream gene expression (Xu and Andreassi, 2011). In addition, females, not males, inherit a paternal X chromosome; therefore, any paternally imprinted X genes will be expressed in females only. Dimorphic sex chromosome gene expression is apparent in brain tissue and thus may cause sex differences in behavior (Xu and Andreassi, 2011; Xu et al., 2002, 2008a,b, 2005; Xu and Disteche, 2006). In the case of human sex chromosome aneuploidies, which have more or fewer than two sex chromosomes, variation in expression of sex chromosome genes within the brain may also affect behavior (Knickmeyer, 2012; Savic, 2012). For example, when compared with XX females, females with Turner syndrome (XO) would have decreased expression of any X chromosome genes that escape X-inactivation, while individuals with Klinefelter syndrome (XXY) would have over expression of these same genes when compared to XY males.

3. The mice

Before reviewing the behavioral data, we provide detailed descriptions of many of the mouse models that have been used for evaluating sex chromosome effects on behavior. A simplified summary of these models is found in Table 1. A critical consideration when interpreting results in mice is that the background strain influences most behaviors; therefore, we make note of model background strains, and, where appropriate, the Y chromosome strain of origin.

3.1. Four core genotypes

A mouse model now known as the "Four Core Genotypes" (FCG) has been used to directly test the contributions of sex chromosome complement versus gonadal sex (De Vries et al., 2002). This model takes advantage of a spontaneous mutation that resulted in a deletion of the testis determining gene, *Sry*, on the mouse Y chromosome (Lovell-Badge and Robertson, 1990), which is rescued by a transgenic insertion of *Sry* within an autosome (Mahadevaiah et al., 1998). An animal with an *Sry*-deleted Y chromosome (henceforth called 'Y⁻') and an autosomal *Sry* transgene (XY⁻*Sry* male) develops normal testes and is a fertile male. Mating a XY⁻*Sry* male with a normal XX female produces offspring of four genotypes (Fig. 2): females with two X chromosomes (XXF), females with an X and a Y⁻ chromosome (XYF), males with two X chromosomes and no Y chromosome (XXM), and males with an X and a Y chromosome (XYM). The FCG unlinks gonadal determination from the inheritance of the sex chromosomes, and allows for indepen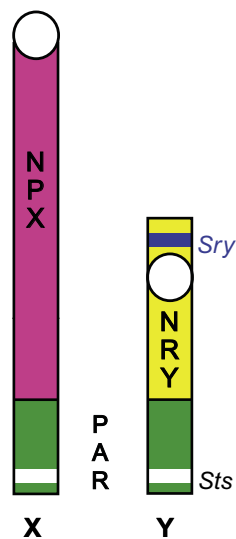dent analysis of the two factors.