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Number of X-chromosome genes influences social behavior and vasopressin gene expression in mice

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Summary Sex differences in behavior are widespread and often caused by hormonal differences between the sexes. In addition to hormones, the composition and numbers of the sex chromosomes also affect a variety of sex differences. In humans, X-chromosome genes are implicated in neurobehavioral disorders (i.e. fragile-X, autism). To investigate the role of X-chromosome genes in social behavior, we used a mouse model that has atypical sex chromosome configurations resembling Turner (45, XO) and Klinefelter syndromes (47, XXY). We examined a number of behaviors in juvenile mice. Mice with only one copy of most X-chromosome genes, regardless of gonadal sex, were less social in dyadic interaction and social preference tasks. In the elevated plus maze, mice with one X-chromosome spent less time in the distal ends of the open arms as compared to mice with two copies of X-chromosome genes. Using qRTPCR, we noted that amygdala from female mice with one X-chromosome had higher expression levels of vasopressin (*Avp*) as compared to mice in the other groups. Finally, in plasma from girls with Turner syndrome we detected reduced vasopressin (AVP) concentrations as compared to control patients. These novel findings link sex chromosome genes with social behavior via concentrations of AVP in brain, adding to our understanding of sex differences in neurobehavioral disorders.

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1. Introduction

Sex differences in behavior have been attributed to differences in hormone levels between males and females (Arnold, 2009). In addition, sex chromosome complement correlates with differences in the brain and behavior in mice (reviewed in Cox et al., 2014). The basic mechanisms that cause sex differences are important to study and, more broadly, can also help us understand neurobehavioral diseases, many of which are sexually dimorphic in their incidence, severity, and/or timing of onset. Autism spectrum disorders (ASD) are one example of such gender dimorphism in human disease, as ASD is diagnosed five times more commonly in males than in females (Giarelli et al., 2010). Several sex chromosome genes, particularly on the X-chromosome, are implicated in neurobehavioral disorders (Raymond, 2006) and patients with Turner (45, XO) and Klinefelter syndrome (47, XXY), two of the most common sex chromosome aneuploidies, have a higher incidence of psychological dysfunction and autism than the general population (Lesniak-Karpiak et al., 2003; Russell et al., 2006; van Rijn et al., 2006).

Here we used a mouse model with atypical sex chromosome configurations to assess the contribution of X-chromosome gene copy number to juvenile social and anxiety behaviors. These mice (Eicher et al., 1991; Burgoyne et al., 1998; Cox et al., 2014) produce offspring with four sex chromosome genotypes: XY males, XXY males, XO females, and XX females (hereafter referred to as 1X males, 2X males, 1X females, and 2X females; Table 1). In order to control for the hormonal differences seen in adult mice, prepubertal mice were tested for social and anxiety behavior. The mRNA of candidate genes, including several that escape X-inactivation and which are typically expressed 1.5-fold higher in XX females as compare with XY males (Berletch et al., 2011), was quantified in the amygdala, a brain region involved in emotional, social and anxiety behaviors in rodents (Veenema and Neumann, 2008) and humans (Adolphs, 2010). Finally, to check for correlations between vasopressin and the XO genotype in young girls, we measured vasopressin (AVP) in plasma from Turner syndrome patients and controls.

2. Materials and methods

2.1. Animals

The Y* strain used for all studies originated from males with a mutation in the pseudoautosomal region of the Y-chromosome that resulted in a neocentromere and misalignment with the X-chromosome during meiosis. Mating these males with wild-type females produces offspring of both sexes with either 1 or 2 copies of X-chromosomes (Table 1) (Eicher et al., 1991; Burgoyne et al., 1998; Cox et al., 2014). Our Y* colony was established with XY* males (in the 6JEiJ substrain) and C57BL/6J (B6) females purchased from The Jackson Laboratory (Bar Harbor, ME; stock numbers 002021 and 000924), and maintained in the B6 sub-strain. To genotype the mice, RNA was isolated from tail clippings as previously described (Wolstenholme et al., 2013b) and reverse transcribed into cDNA. *Xist* mRNA was measured and normalized to *Gapdh* as an indication for the number of X chromosomes present for mice of each sex. The primers used for *Xist* genotyping were 5′-TAAGGACTACTTAACGGGCT-3′ (forward) and 5′-TACTCAGACATTCCCTGGCA-3′ (reverse), while the primers for *Gapdh* were 5′-ACCACAGTCCATGCCATCAC-3′ (forward) and 5′-TCCACCACCCTGTTGCTGTA-3′ (reverse).

Mice were bred and maintained at the University of Virginia School of Medicine, Jordan Hall Animal Facility, and all procedures were conducted in compliance with the University of Virginia Animal Use and Care Committee. Mice were maintained on a 12:12 light/dark cycle (lights off at 1800 EST) and food (Harlan Teklad no. 7912) and water were provided ad libitum. Mice were reared with both parents and left largely unhandled (excluding routine cage changes) until postnatal day (PN) 20. We chose to begin testing animals on PN21 to assure that the mice remained prepubertal throughout the testing series, thus avoiding the potential confounds of hormone differences. One cohort was used for the dyadic social interaction and preference tests, but separate cohorts were used for each of the other behavior tests, the physiological measurements, and the gene expression analysis. In total, 51 litters were used, with animals from at least 5 different litters used in each study to reduce litter effects.

2.2. General behavior testing procedures

All behavior tests, except social recognition, were conducted in the dark, approximately 1 h after lights off (1900 EST), under red-light illumination, videotaped, and scored using Noldus Observer (5.0) software (Noldus, Leesburg, VA, USA). The social recognition task was scored live in the light (between 1000 and 1400 EST). All tests were scored by an observer (KHC), blind to both the sex and genotype of the test subjects.

Table 1 Offspring produced and tested.

Genotype	Model for	Gonadal sex	Copy of non-PAR X Genes	Copy of non-PAR Y Genes	Nomenclature
XY^{*}	XY	Male	1	1	1X male
XX^{Y*}	XXY (Klinefelter syndrome)	Male	2	1	2X male
XY^{*X}	XO (Turner syndrome)	Female	1	0	1X female
XX	XX	Female	2	0	2X female

Details on the genotypes produced and tested in these studies. In the genotypes column, the first X-chromosome listed, by convention, is maternally inherited, while the second is inherited from the father. The offspring have either 1 or 2 copies of non-pseudoautosomal (non-PAR) X-chromosome genes, with gonadal males possessing a Y* or X^{Y*} chromosome.