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ROLE OF X-LINKED GENES ON SEX DIFFERENCES IN NEUROGENIN 3 EXPRESSION IN DEVELOPING HYPOTHALAMIC NEURONS

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INTRODUCTION

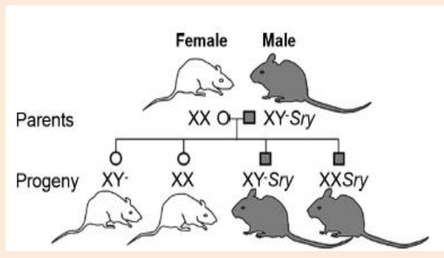
Our previous findings indicate that sex chromosome complement regulates the generation of sex differences in mouse hypothalamic neuronal development. Higher expression of neurogenin 3 (Ngn3) in XX neurons mediates sex differences in the rate of neuronal differentiation. Since Ngn3 is located in chromosome 10, these sex differences should be consequence of differences in the expression of X or Y chromosome genes that result from the inherent sex difference in the number (two copies of X) and/or type (presence or absence of Y) of sex chromosomes.
We tested the hypothesis that X genes that escape X-inactivation are involved in regulation of sex differences in autosomal expression of Ngn3 and axonal length of hypothalamic neurons. To deal with this aim we evaluated the expression of Ddx3x, Eif2s3x, Kdm5c, Kdm6a, Mid1 and Usp9x in primary neuronal cultures from E14 male and female mice.

Table 1: X-linked genes that escape from X-Inactivation in mouse brain

Gene Symbol	Gene name	Gene function
Ddx3x	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked	Effector of TBK1 necessary for type I IFN induction
Eif2s3x	Eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	Role in translation initiation
Kdm5c	Lysine (K)-specific demethylase 5C	Removes tri-methylation of histone H3 lysine 4
Kdm6a	Lysine (K)-specific demethylase 6[a]	Removes tri- and di-methylation of histone H3 lysine 27
Mid1	Midline 1	Microtubule binding
Usp9x	Ubiquitin specific peptidase 9	Deubiquitinating enzyme with role in hippocampus development and axon extension

MATERIALS AND METHODS

- The embryos used for this study were obtained from CD1 mice raised in the Cajal Institute (Madrid, Spain) and MF1 four core genotypes (FCG) mice born and reared in the Ferreyra Institute (Córdoba, Argentina).
- Neuronal hypothalamic cultures of E14 embryos were performed segregated by sex and genotype. For morphometric analyses cells were treated either with 17 B-Estradiol (E2, 10^{-7}M) or vehicle. For gene expression studies some cultures were treated either with E2 (10^{-10}M) or WP1130 (5uM) for 6 or 24 h.
- RNA were extracted and cDNA obtained by reverse transcription. Real-time PCRs were performed using the TaqMan and Sybr Green PCR Master Mix. Relative mRNA expression level was calculated using the ΔΔCT method.



XYF XXF XYM XXM

RESULTS

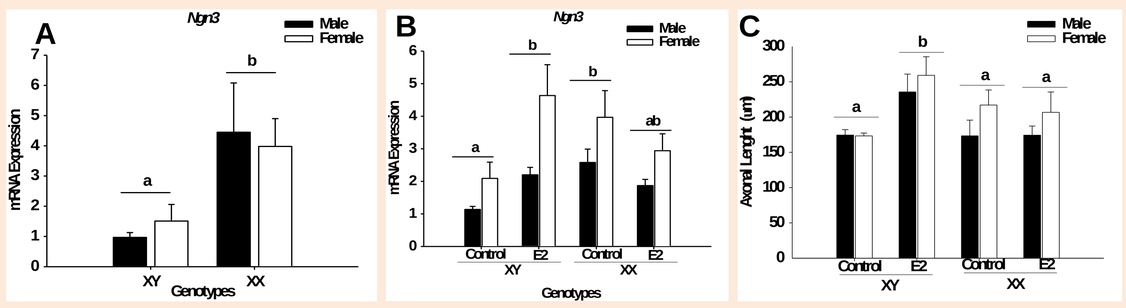


Figure 1: Sex differences in Ngn3 expression and axonal length in primary hypothalamic neurons were determined by sex chromosomes.
A) Ngn3 mRNA levels in FCG model. Two-way ANOVA revealed no effect of gonadal sex but a significant effect of sex chromosome complement. Sex chromosome complement regulates Estradiol effects on mRNA expression of Ngn3 (B) and axonal length of hypothalamic neurons (C). Different letters indicate significant differences with $p < 0.05$ (LSD Fisher). Data are mean ± SEM. n = 4 - 6 independent cultures for each genotype and treatment.

RESULTS

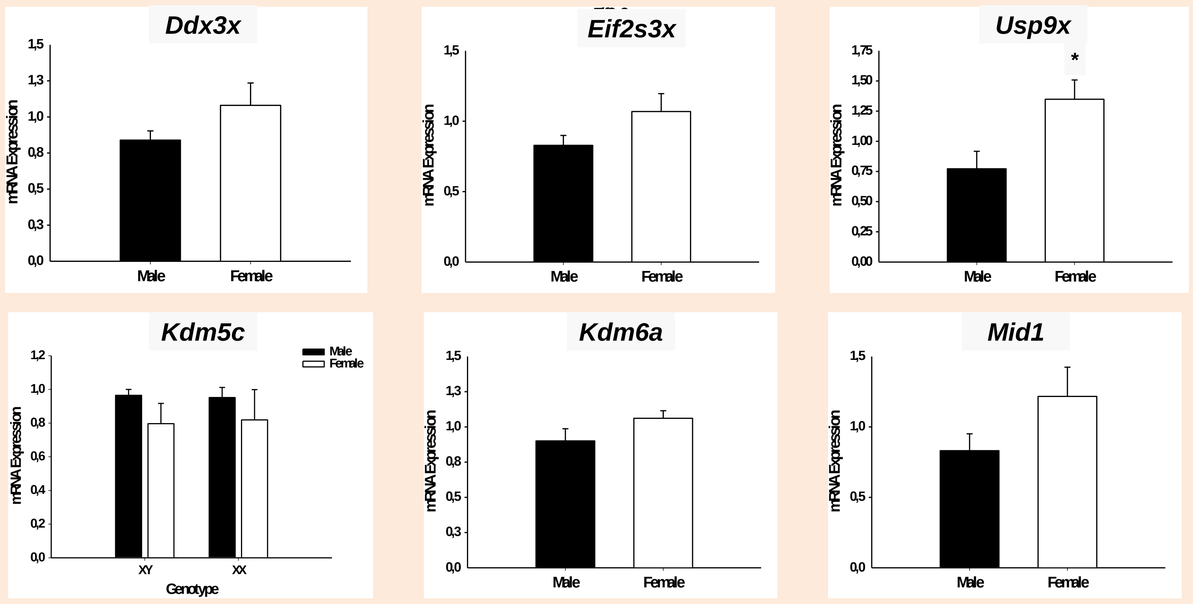
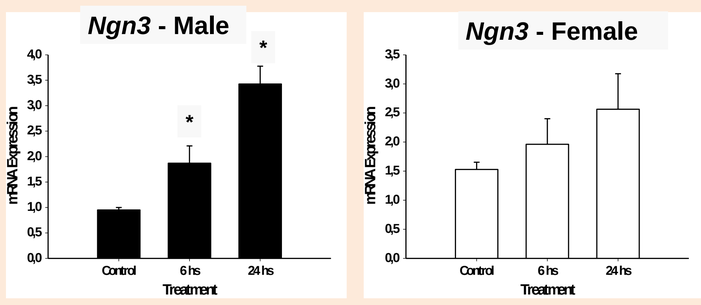


Figure 2: Sex differences of X-linked gene expression in hypothalamic neuronal cultures at E14.
Female neuronal cultures showed enhanced expression of the deubiquitinating enzyme *Usp9x* ($p<0.05$). Data are mean ± SEM. n = 4 - 6 independent cultures.

Figure 3: Role of *Usp9x* on Ngn3 expression in sexually segregated hypothalamic neuronal cultures at E14.
Treatment with WP1130 (DUB inhibitor) increases Ngn3 expression only in male neuronal cultures ($p<0.05$) abolishing sex differences.



CONCLUSIONS

These preliminary findings suggest that the X-linked gene *Usp9x* regulates the expression of the autosomal gene Ngn3 which is involved in sex differences in hypothalamic neuronal development. However, since WP1130 is a partly selective DUB inhibitor further experiments are required to determine the effect of specific down regulation of *Usp9x* expression on the observed sex differences in growth and differentiation of hypothalamic neurons.

Sexual dimorphisms are only partially explained through differences in sex hormones. Clearly, sex chromosomes have found other ways to promote sex differences in somatic tissues, most of which are probably mediated by sex differences in X/Y-linked gene expression. The contribution of X-linked transcriptional regulators that escape inactivation to sex differences will led us to elucidate the downstream pathways that differentially regulate neuronal differentiation in males and females.

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