Early sex differences in histone methyltransferase *Ezh2* expression in developing hypothalamus of the mouse brain

Villarreal M.[1], Bigarani R. [1], Sosa C. [1], Cabrera Zapata L.E. [1], Cisternas C.D. [1,2]✉, Cambiasso M. J. [1,3]

[1] Instituto de Investigación Médica M y M Ferreyra, INIMEC-CONICET-UNC, Córdoba, Argentina.

[2]Cátedra de Fisiología Animal, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina.

[3]Cátedra de Biología Celular, Facultad de Odontología, Universidad Nacional de Córdoba, Argentina. ✉ ccisternas@immf.uncor.edu

Introduction

- Independent to gonadal hormones effects, several studies have shown that sex chromosomes have effects on sexually dimorphic gene expression in the mouse brain.[1,3,5,8] These sex differences were observed before the critical period of perinatal masculinization (embryonic day (E) 17 to postnatal day 10).[6] Many of X- and Y-linked genes are epigenetic modifiers and pivotal evidence in past 7 years implicates epigenetic mechanisms as mediators of brain sexual differentiation.[1]
- Neurogenin 3 (Ngn3) is an autosomal gene located in chromosome 10, it is required for specification of neuronal subtypes and regulates sex differences in neuritogenesis in the hypothalamus.[7,8] Recent results from our lab demonstrate that *Ngn3* expression is higher in XX than XY hypothalamic neurons before the critical period[8], and this sex difference is regulated by the X chromosome-linked H3K27 demethylase, Kdm6a.[3,4]
- Kdm6a interacts with numerous epigenetic modifiers, such as histone lysine methyltransferases (KMTs), implying that they could act together, influencing each other in a context-dependent manner, writing a histone crosstalk language.

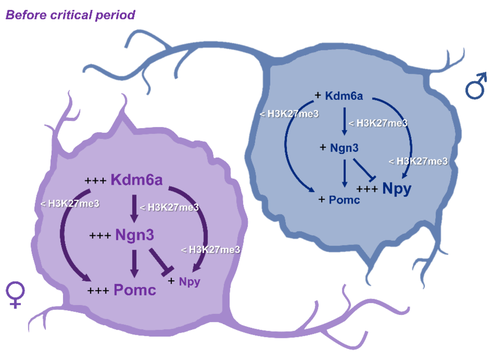

Figure 1: Hypothesis proposed to explain the sexually dimorphic mechanisms by which Kdm6a regulates the expression of Ngn3, Pomc and Npy in the developing hypothalamus. Before the critical period of hormonal organization of the brain, female hypothalamic neurons show higher expression of the X-linked Kdm6a than male neurons.[3] Kdm6a promotes Ngn3, Pomc and Npy transcription by removing repressive H3K27me3, with a stronger effect in females than in males. Adapted from Cabrera Zapata *et al*., 2022.

Aim

- Analyse the role of gonadal sex and sex chromosome complement on gene expressions of histone lysine methyltransferase *Ezh2* and *Ehmt2* in ventromedial hypothalamus at embryonic day 15.

Methods and Materials

We used MF1 wild-type mice and the "Four Core Genotypes" mouse model, which allows the evaluation of gonadal sex, sex chromosome complement, and their interaction (Figure 2).

Hypothalamic tissue was obtained from E15 mice. Embryos age was selected to avoid in utero exposure to the peak of testosterone secretion. The ventromedial hypothalamic region was dissected out and stripped off the meninges.

RNA was extracted from hypothalamic tissues. cDNA was obtained by reverse transcription and Real-Time PCR was performed using SYBR® Green PCR Master Mix for *Ezh2* and *Ehmt2*. Relative mRNA expression levels was calculated using *18S* as housekeeping gene with the ΔΔCt method.

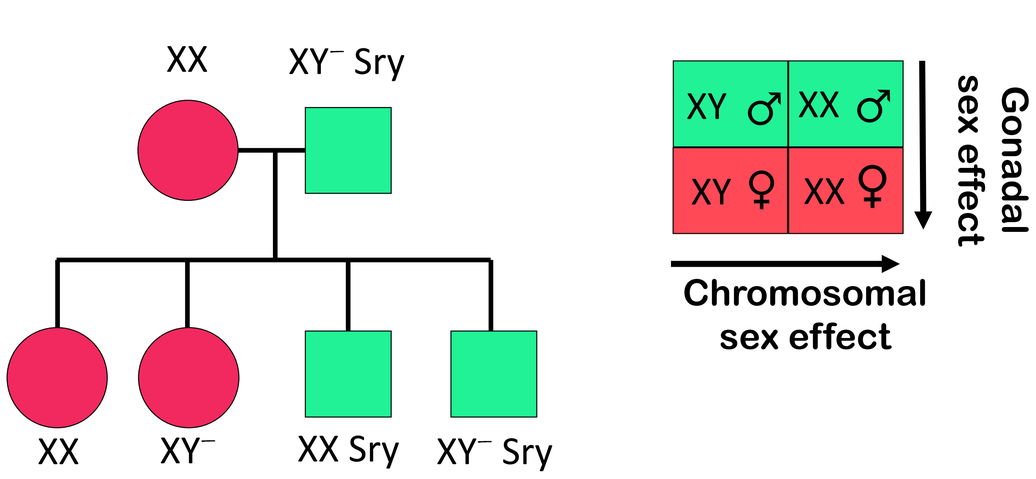

Figure 2. Four Core Genotypes mouse model. The testis-determining *Sry* gene is deleted from the Y chromosome (Y^-) and is inserted onto an autosome, in this way the gonadal sex of the mouse becomes independent of the sex chromosomes. This model makes it possible to study the differences caused by gonadal secretions, or by the complement of sex chromosomes, as well as their interaction. Adapted from Arnold *et al*., 2004.

Acknowledgements

CONICET · INSTITUTO FERREYRA · UNC Universidad Nacional de Córdoba · I N I M E C · AGENCIA · Ministerio de CIENCIA Y TECNOLOGÍA

Results

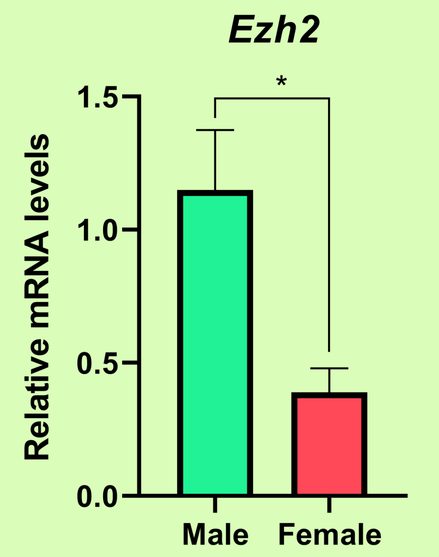

Figure 3. Relative *Ezh2* mRNA levels in ventromedial hypothalamic tissue of E15 mice. Data are mean ± SEM. n=6 individuals for each sex. *p < 0.05

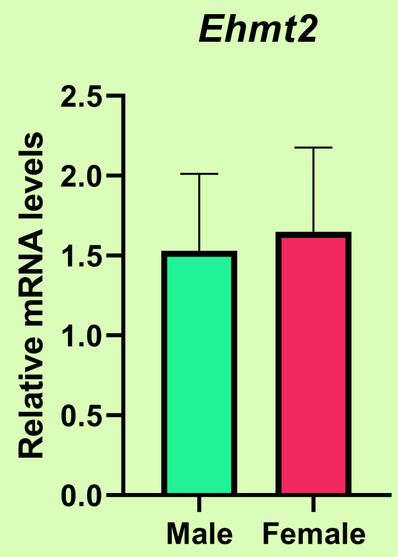

Figure 4. Relative *Ehmt2* mRNA levels in ventromedial hypothalamic tissue of E15 mice. Data are mean ± SEM. n= 8-9 individuals for each sex.

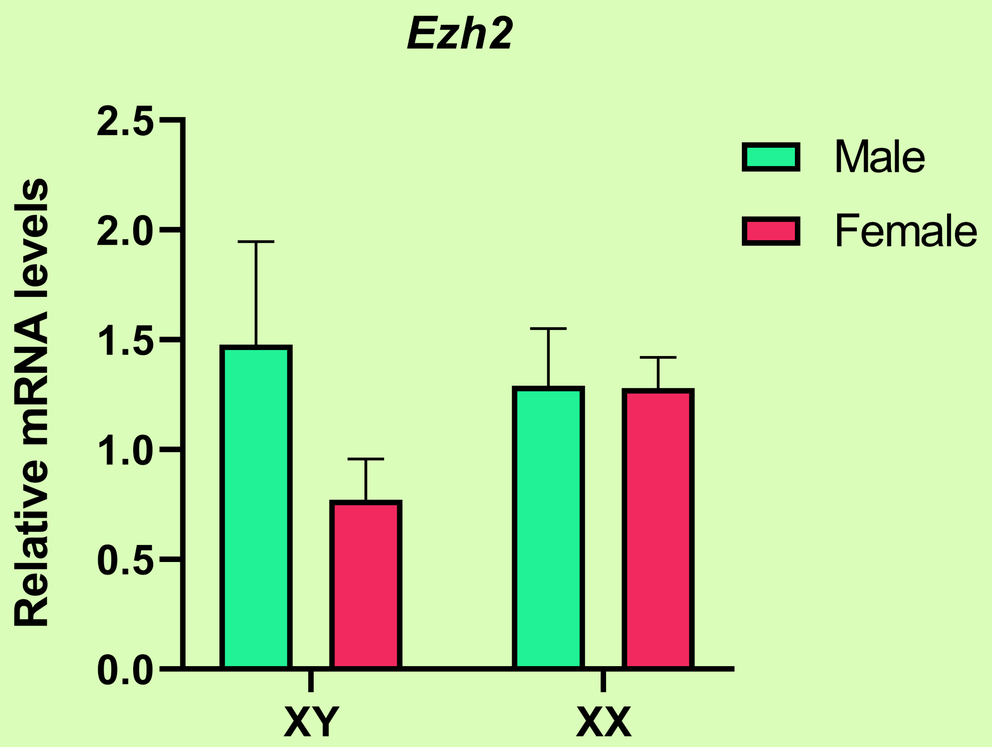

Figure 5. Relative *Ezh2* mRNA levels in ventromedial hypothalamic tissue of E15 mice. Data are mean ± SEM. n= 5-6 individuals for each genotype.

Conclusions

- *Ezh2* showed sex differences in expression. Male ventromedial hypothalamus (VMH) showed higher mRNA levels than female VMH. These sex differences were not due to sex chromosomes since no differences in expression were found among genotypes.
- *Ezh1* expression was not detected in this region at E15 (results not shown).
- *Ehmt2* expression did not show sex differences.
- These results suggest that gonadal sex determines sexually dimorphic expression of *Ezh2* in ventromedial hypothalamus.
- Further experiments are required to determine if higher levels of *Ezh2* in males are responsible for the sexually dimorphic regulation of H3K27me3 on Ngn3 promoter region.

References

1. Arnold A. P. (2022). X chromosome agents of sexual differentiation. *Nature reviews. Endocrinology*, *18*(9), 574–583.
2. Arnold, A. P., & Burgoyne, P. S. (2004). Are XX and XY brain cells intrinsically different?. *Trends in endocrinology and metabolism: TEM*, *15*(1), 6–11.
3. Cabrera Zapata L.E., Cisternas C.D., Sosa C., Garcia-Segura L.M., Arevalo M.A. & Cambiasso M.J. (2021). X-linked histone H3K27 demethylase Kdm6a regulates sexually dimorphic differentiation of hypothalamic neurons. *Cell Mol Life Sci*. 78(21-22): 7043-7060.
4. Cabrera Zapata L. E, Cambiasso M. J. & Arevalo M.A. (2022). Epigenetic Modifier Kdm6a/Utx Controls the Specification of Hypothalamic Neuronal Subtypes in a Sex-Dependent Manner. *Frontiers Cell and Developmental Biology*, in press.
5. Cisternas, C. D., Tome, K., Caeiro, X. E., Dadam, F. M., Garcia-Segura, L. M., & Cambiasso, M. J. (2015). Sex chromosome complement determines sex differences in aromatase expression and regulation in the stria terminalis and anterior amygdala of the developing mouse brain. *Molecular and cellular endocrinology*, *414*, 99–110.
6. Arnold A. P. (2009). The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Hormones and Behavior* 55: 570–578.
7. Pelling, M., N. Anthwal, D. Mcnay, G. Gradwohl, A. B. Leiter, F. Guillemot & S. L. Ang. (2011). Differential requirements for neurogenin 3 in the development of POMC and NPY neurons in the hypothalamus. *Developmental Biology* 349: 406–16.
8. Scerbo, M. J., A. Freire-Regatillo, C. D. Cisternas, M. Brunotto, M. A. Arevalo, L. M. Garcia-Segura & Cambiasso M. J. (2014). Neurogenin 3 mediates sex chromosome effects on the generation of sex differences in hypothalamic neuronal development. *Frontiers in Cellular Neuroscience* 8: 188.