Sex Differences in an *Fmr1* Knock-out Mouse Model of Fragile X Syndrome

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INTRODUCTION

Fragile X Syndrome (FXS) is the leading monogenetic cause of intellectual disabilities and autism spectrum disorder (ASD). Epilepsy, sensory hypersensitivities, and several other neuropsychiatric symptoms are also common in FXS and ASD. FXS is caused by a >200 CGG-repeat on the *FMR1* gene, located on the X-chromosome, that silences its transcription, preventing expression of the protein, FMRP. Possessing a single X-chromosome, FXS occurs more frequently in males than females. Males with FXS typically have more severe clinical symptoms than females with FXS. For example, males are more than twice as likely to have epilepsy. Genetic mosaicism (X-inactivation) in females with FXS is the presumed, exclusive reason for the lower symptom severity. We explored the hypothesis that other factors—sex hormones or sexually dimorphic brain systems—could be involved.

METHODS

Subjects. FVB.129P2-*Pde6b*⁺ *Tyr^{c-ch}* *Fmr1*^{tm1Cgr/J} (*Fmr1* KO mice) and FVB.129P2-*Pde6b*⁺ *Tyr^{c-ch}*/AntJ (WT mice) were purchased from Jackson Laboratory to develop a colony. Genotyping of litters was based on established protocols. KO males are hemizygous and females are homozygous for the knock-out *Fmr1* gene.

Audiogenic Seizure (AGS) Susceptibility. *Fmr1* KO male and female juvenile (P23-P25) mice were given IP injections 30 minutes prior to testing of vehicle (MilliQ water). Mice were exposed to a 120 dB alarm (Radioshack) for 5 min, and responses, including wild running and jumping (WRJ), tonic-clonic seizure, respiratory arrest and subsequent death, were recorded.

Radioligand Saturation Binding. The saturation binding assay was performed using cell membranes of brain tissue collected from adult (≥P60) male and female WT and *Fmr1* KO mice, as previously described (Canal et al., 2011; PMID: 20165943). [³H]5-CT (PerkinElmer) with cold ligands to block off-targets were used to label 5-HT_{1A}Rs.

RESULTS

<https://www.fraxa.org/fragile-x-syndrome/cause/>

Increased whole brain expression of 5-HT_{1A}Rs in adult *Fmr1* KO male mice

Left: Radioligand/[³H]5-CT saturation binding results showing the average expression level of 5-HT_{1A}Rs in WT (blue) and *Fmr1* KO (red) mice. Individual data points are shown, and males (left) are compared to females (right). Center: Representative saturation binding isotherm showing increased expression of brain 5-HT_{1A}Rs in an adult, male *Fmr1* KO mouse. Right: Representative data showing 5-HT_{1A}R expression differences between a WT and *Fmr1* KO mouse were blocked by the selective 5-HT_{1A}R antagonist, WAY-100635 (N=3)—thus, whole brain 5-HT_{1A}R expression is higher in adult, male *Fmr1* KO mice.

Increased incidence and severity of AGS in juvenile *Fmr1* KO male mice

CONCLUSIONS and DISCUSSION

During preclinical testing of *Fmr1* knock-out mice, we observed a statistically significant, higher prevalence of audiogenic seizures in juvenile, male *Fmr1* KO mice (100%) compared to juvenile, female *Fmr1* KO mice (50%). These observations closely parallel clinical data. Males with FXS have a higher incidence of epilepsy than females with FXS.² The reason for this clinical, sex difference is purportedly that females with FXS have one functioning *FMR1* allele, whereas males do not. Importantly, however, female *Fmr1* KO mice are homozygous for the *Fmr1* gene, and therefore, do not express FMRP, thus suggesting sex hormones or sexually dimorphic brain systems could be involved in protecting females from seizures. Our preclinical data challenge the presumption that reduced frequency of FXS and symptom severity in females may not be solely attributed to X-inactivation of the mutated *FMR1* allele. We also found that expression of the serotonin 5-HT_{1A} receptor was significantly elevated in whole brains of adult, male *Fmr1* KO mice, compared to WT mice; we did not observe this difference in female mice. We are currently investigating whether 5-HT_{1A}Rs contribute to the sex difference in AGS susceptibility. Notably, studies have shown that selective 5-HT_{1A}R agonists can prevent, reduce onset latency, and/or decrease the severity of seizures in multiple animal models.^{3,4} Our observations of significantly increased 5-HT_{1A}Rs in brains of adult *Fmr1* KO males suggests that targeting 5-HT_{1A}Rs might be therapeutic for FXS or ASD with epilepsy.

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