Transgenerational Epigenetic Inheritance and Autism Spectrum Disorder: Linking Meiotic Dysfunction and Cognitive Impairment

Katelyn R. DeNegre[1,2,3]; Michael J. O'Neill[1,2,3]

1. Department of Molecular and Cell Biology, University of Connecticut, Storrs, United States
2. Institute for Systems Genomics, University of Connecticut, Storrs, United States
3. Institute for the Brain and Cognitive Sciences, University of Connecticut, Storrs, United States

We identified the first imprinted genes on the mouse X chromosome, *Xlr3b/4b/4c*. They are imprinted in the brain in a pattern of paternal repression and are highly expressed in testis. Given these observations, we have been interested in the potential link between the events of meiosis and those of neurodevelopment. We have generated transgenic mice using siRNA to target the functional copies of *Xlr3* in germ cells. Functional analyses of our transgenic and wild-type animals have revealed that Xlr3 protein localizes to the sex chromosomes during Meiotic prophase I. Since Xlr3 is present on the sex chromosomes during prophase I, including the period encompassing Meiotic Sex Chromosome Inactivation (MSCI), we next assessed the expression of several X and Y linked genes, which are normally epigenetically silenced during MSCI. We found that Xlr3 knockdown results in disruption of MSCI, leading to inappropriate expression of X and Y genes. These observations led to the hypothesis that Xlr3 knockdown begets the establishment of epimutations on the X chromosome during male meiosis and are then passed across generations via the sperm, an example of transgenerational epigenetic inheritance. The transgenerational effect of this epimutated X is manifest in daughters of transgenic knockdown males by disruption of the imprinting of *Xlr3b/4b/4c* in the brain. In other words, we observed reactivation of the normally silent paternal allele of these genes. To explore the persistence of this epimutation across multiple generations, we devised a novel breeding scheme using a full-length inverted X opposite the epimutated paternal progenitor X to prevent recombination in F1 females. Thus, they pass the putatively epimutated X intact to sons which allowed us to generate a cohort of 25 F2 male mice that only possessed the putatively epimutated X. These F2 males were subjected to a battery of behavioral tests for social interaction, spatial learning, and repetitive behaviors. F2 males with epimutated X chromosomes were behaviorally distinct from WT controls in the novel object recognition, Morris Water Maze, and open field tests. After behavioral testing was completed, testes and brains of the F2 males were collected and RNA sequencing will be performed to assess differential gene expression. Finally, we explored the molecular nature of the X chromosome epimutation by examining DNA methylation patterns in the sperm of knockdown males compared to controls. Transgenic knockdowns have methylation patterns that are distinct from controls, and we have identified differentially methylated regions (DMRs), with many loci of interest on the X chromosome. Intriguingly, the DMRs identified on the X chromosome are restricted to the pseudoautosomal region (PAR), and *Gcna* which has necessary functions in spermatogenesis. We hypothesize that knockdown of Xlr3 causes epigenetic changes that destabilize the PAR which may lead to X-Y pairing defects and aneuploidy. In summary, *Xlr3* function is critical to MSCI and the establishment of epigenetic signatures on the X chromosome during male meiosis. Disruption of Xlr3 function leads to the production of sperm carrying a broadly epimutated X with

multigenerational consequences to gene regulation and neurodevelopment. This research has implications for male biased disorders such as Autism Spectrum Disorder. We hypothesize a female protective effect in Autism susceptibility may rely on proper establishment and maintenance of epigenetic programming of the X chromosome involving Meiotic Sex Chromosome Inactivation in males.