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Research Report

Identification of sexually dimorphic genes in the neonatal mouse cortex and hippocampus

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ABSTRACT

The cerebral cortex and hippocampus are important for the control of cognitive functions and social behaviors, many of which are sexually dimorphic and tightly regulated by gonadal steroid hormones via activation of their respective nuclear receptors. As different levels of sex steroid hormones are present between the sexes during early development and their receptors act as transcription factors to regulate gene expression, we hypothesize that sexually dimorphic gene expression in the developing mouse cortex and hippocampus might result in sex differences in brain structures and neural circuits governing distinct behaviors between the sexes as adults. To test our hypothesis, we used gene expression microarrays to identify 90 candidate genes differentially expressed in the neonatal cortex/hippocampus between male and female mice, including 55 male-biased and 35 female-biased genes. Among these genes, sexually dimorphic expression of eight sex chromosome genes was confirmed by reverse transcription with quantitative PCR (RT-qPCR), including three located on the X chromosome (*Xist*, *Eif2s3x*, and *Kdm6a*), three on the Y chromosome (*Ddx3y*, *Eif2s3y*, and *Kdm5d*), and two in the pseudoautosomal region of the X and Y chromosomes (*Erdr1* and *Mid1*). In addition, five autosomal genes (*Cd151*, *Dab2*, *Klk8*, *Meg3*, and *Prkdc*) were also validated for their sexually dimorphic expression in the neonatal mouse cortex/hippocampus. Gene Ontology annotation analysis suggests that many of these sexually dimorphic genes are involved in histone modifications, cell proliferation/death, androgen/estrogen signaling pathways, and synaptic organization, and these biological processes have been implicated in differential neural development, cognitive function, and neurological diseases between the sexes.

1. Introduction

Sex differences in normal brain function and behavior are substantial in many species, including humans and mice (Breedlove and Hampson, 2002; Jazin and Cahill, 2010; McCarthy and Arnold, 2011). Gender differences are also noted in the prevalence and symptomatology of many neurological diseases and mental illnesses such as multiple sclerosis, schizophrenia, and autism (Baron-Cohen et al., 2005; Deng et al., 2010; Greer and McCombe, 2011). Elucidation of the neural mechanisms that establish sexual

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dimorphism in brain structures and behaviors will help us understand the processes regulating sex-specific susceptibility to various diseases and assist in development of new treatments for sex-biased disorders.

In mammals, including humans and rodents, sexual differentiation is initiated by the combination of the two sex chromosomes, X and Y (chromosomal sex), generally XX in females and XY in males. The *Sry* gene located on the Y chromosome encodes a transcription factor that initiates differentiation of the testes in males. In mice, deletion of the *Sry* gene converts XY males to gonadal females while XX females receiving an *Sry* transgene inserted onto an autosome develop testes rather than ovaries (Gubbay et al., 1990; Koopman et al., 1990). The developing testes release testosterone (T) during late gestation and immediately after birth, causing a rise in circulating T that is essential for producing sex differences in many behaviors and in the neural structures and circuits underlying these behaviors (organizational effect) (Motelica-Heino et al., 1988; Phoenix et al., 1959). T acts directly on androgen receptor (AR) and/or indirectly on estrogen receptors (ERs) via locally synthesized estradiol (E2) from T by aromatase to masculinize brain structures and behaviors (Davis et al., 1996; Forger, 2009; Hines, 2006). AR and ERs are abundantly expressed in the developing mouse cortex and hippocampus (Ivanova and Beyer, 2000; Kerr et al., 1995). When activated, these receptors act as transcription factors to modulate gene expression, but their specific downstream target genes that are relevant to sex differences in the cortex and hippocampus remain unclear.

Many cognitive behaviors served by the cerebral cortex and hippocampus, such as learning and memory, show sex differences, and gonadal steroids and their nuclear receptors are important for sexual differentiation of these behaviors (Frick and Gresack, 2003; Rizk et al., 2005; Sutcliffe et al., 2007). Associated with differential behavioral phenotypes between males and females, sex differences have been reported in the size and laterality of the mouse and rat hippocampus, which may be tied to a greater rate of neurogenesis in males during the first week after birth, as induced by T (Tabibnia et al., 1999; Zhang et al., 2008). Another reported difference in neuroanatomy is the cortical thickness, with adult male mice possessing a thicker cortex than females due to the effects of T (Markham et al., 2003). In humans, a thicker cortex is also seen in male patients suffering from autism compared to healthy male controls (Carper et al., 2002; Doyle-Thomas et al., 2013). Autism is a pervasive neurodevelopmental disorder characterized by deficits in social behavior and interpersonal communication that is four times more likely to occur in males than in females (Baron-Cohen et al., 2011). Auyeung et al. (2009) found that the more T levels in amniotic fluid of a pregnant woman, the higher her child scored on the tests of autistic traits although none of the children in that study were autistic (Auyeung et al., 2009). Along with the positive relationship between normal fetal T levels (ranging between 0.05 and 2.05 nM) and subclinical autistic traits, the cortical thickness influenced by T might be a potential mechanism underlying gender differences in cognitive functions and neuropsychiatric disorders, such as autism. If these sexual dimorphisms are caused by hormone receptors acting as transcription factors, then we should be able to identify specific gene expressions that create these structural and functional differences in the cortex and hippocampus between the sexes.

Besides gonadal hormones, emerging evidence has shown that brain sexual differentiation is also mediated by the action of genes located on the sex chromosomes. For example, the number of mesencephalic dopaminergic cells dissociated and cultured from the XY mouse embryos prior to gonadal differentiation is greater than that of XX mice (Carruth et al., 2002). In addition, a variety of behaviors are modulated by sex chromosome complement (Bonthuis et al., 2012; Cox and Rissman, 2011; Gatewood et al., 2006; Gioiosa et al., 2008; Grgurevic et al., 2012; Park et al., 2008; Quinn et al., 2007). Thus, we hypothesize that in the neonatal male cortex/hippocampus, differential expression of sexually dimorphic genes originating from both sex chromosomes and gonadal sex steroid hormones might lead to the development of distinct neural function and behaviors, as well as to underlying differences in brain structure, between the sexes. To test our hypothesis, we used gene expression microarrays to identify sexually dimorphic candidate genes expressed in the neonatal mouse cortex/hippocampus and confirmed the sex-biased expression of selected candidates with reverse transcription with quantitative PCR (RT-qPCR). Through this analysis, we discovered several sex chromosome and autosomal genes differentially expressed in the neonatal male and female mouse cortex/hippocampus.

Fig. 1 – Heatmap showing hierarchical clustering of the microarray probes differentially expressed in the neonatal cortex/hippocampus between male and female mice. The rows represent the sexually dimorphic probes with corresponding gene symbols on the left and right sides of the figure; male-biased probes are top, female-biased are bottom. The probes for the sexually dimorphic genes located on the X, Y and X/Y chromosomes are colored red, blue, and green, respectively. The expression level of each probe is displayed on the continuous color scale as shown in the Color Key; blue and red represents the expression level above or below the mean, respectively. The individual samples are shown as columns (1 sample per column). The sample IDs (M1–9 and F1–8) are listed on the top of the columns; the blue ones are from the first microarray assay and the rest are from the second. The dendrogram (clustering) to the left of the heatmap illustrates the similarities between sexually dimorphic probes in terms of their expression levels in different samples; of note are the close clustering of three Y chromosome genes (*Ddx3y*, *Eif2s3y*, and *Kdm5d*, in blue) up top and the close clustering of three X (*Xist*, *Eif2s3x*, and one of the two *Kdm6a* probes, in red) and two X/Y (*Erdr1* and *Mid1*, in green) chromosome genes below. Clustering displayed above the sample IDs illustrates the similarities between different samples in terms of how they express the sexually dimorphic genes, exemplified by samples of the same sex grouped together before joining samples of the opposite sex. * Indicates a gene that was examined by RT-qPCR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)