Brief report

The placenta and neurodevelopment: sex differences in prenatal vulnerability

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Prenatal insults, such as maternal stress, are associated with an increased neurodevelopmental disease risk and impact males significantly more than females, including increased rates of autism, mental retardation, stuttering, dyslexia, and attention deficit/hyperactivity disorder (ADHD). Sex differences in the placenta, which begin with sex chromosomes, are likely to produce sex-specific transplacental signals to the developing brain. Our studies and others have identified X-linked genes that are expressed at higher levels in the female placenta. Through a genome-wide screen after maternal stress in mice, we identified the X-linked gene O-linked N-acetylglucosamine transferase (OGT) and demonstrated its causality in neurodevelopmental programming producing a male-specific stress phenotype. Elucidating the sex-specific molecular mechanisms involved in transplacental signals that impact brain development is key to understanding the sex bias in neurodevelopmental disorders and is expected to yield novel insight into disease risk and resilience.

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Introduction

Males are four to eight times likelier than females to be affected by a neurodevelopmental disorder; this complex statistic is dependent on a number of variables, including comorbidities and genetic variants.[1-10] It is clear that prenatal insults, such as maternal stress, are associated with increased neurodevelopmental disease risk and impact males significantly more than females, including increased rates of autism, mental retardation, stuttering, dyslexia, and attention deficit/hyperactivity disorder (ADHD).[11-15] Suggested explanations as to why males present with higher rates of these disorders range from the male brain developing more slowly than the female—placing the male brain at increased vulnerability over a greater period of time—to the perception that males are less able to compensate when unilateral brain damage occurs developmentally.[16-19] A more recent and exciting hypothesis focuses on the critical involvement of the placenta in relaying sex-specific transplacental signals to the developing brain regarding changes or perturbations in the maternal milieu.[20-26] Furthermore, such maternal insults during pregnancy

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produce robust sex differences in transcriptional responses in the placenta.[22-24,27] This Brief Report focuses on the mechanistic evidence that the known sex bias in neurodevelopmental disorder risk begins with placental sex differences.

Surprisingly, placental sex (XX vs XY) is a major determinant in the magnitude and functional responses of the placenta to maternal perturbations during pregnancy, where the female placenta appears to be protective.[7,20,25,28-43] Placental cellular mechanisms give rise to sex-specific neurodevelopmental changes and are expected to provide novel insight into disease risk and resilience. The placenta is a highly dynamic endocrine tissue, producing nutrients and growth factors and maintaining a protective barrier, all critical for appropriate fetal development.[22,27,44-47] Sex differences in the placenta begin as early as the trophectoderm stage and remain robust throughout trophoblast cell differentiation, all of which occurs in the first third of gestation in humans and rodents. Sex-chromosome genes are differentially expressed early on, suggesting a mechanism whereby sex differences could be driven by the ability of the placenta to respond to changes in the maternal milieu and ultimately by the transplacental signals received by the developing fetus. We and others have reported significant differences in male and female placental gene expression patterns across gestation in rodent models; these differences were similarly found in human placenta.[11,20,23,24,39,48] Despite differences in placental structure between rodents and humans, such studies suggest that key functional similarities exist and that mechanisms uncovered in animal models are translatable.[24]

Placental mechanisms

Why male, but not female, placentas are consistently so responsive to changes in the maternal environment (eg, in response to maternal stress, infection, and diet) remains a key question in defining potential sex-specific disease mechanisms.[26,49-55] Addressing the possible mechanisms underlying these sex differences, animal models have demonstrated (through functional and causal studies) the importance of specific sex-chromosome genes. As an example, in our established mouse model of early prenatal stress (EPS), male, but not female, offspring present with increased stress sensitivity as adults, including increased hypothalamic-pituitary-adrenal (HPA) stress axis activity, reduced growth after weaning, and hypothalamic mitochondrial dysfunction.[34,35,39,56] In a genome-wide screen for sex-specific changes in the placenta after EPS, we identified the X-linked gene *O*-linked N-acetylglucosamine transferase (*OGT*) as a top candidate for having an important role in the placenta in guiding broad chromatin changes that are probably important in placental function because of its sex specificity in expression and response to maternal stress. In the placenta, *OGT* escapes X inactivation; consequently, levels are twice as high in the female placenta than the male placenta both in rodents and humans.[23,24] In our EPS mouse model, placental OGT is further reduced by stress in both sexes, resulting in significant and lasting reductions throughout gestation in OGT levels in male placentas owing to the lower starting point of OGT in males.[34,35]

Establishing causality with placental-specific gene targeting of *OGT*, we recapitulated the EPS phenotype, confirming the importance of placental OGT in neurodevelopmental programming and metabolic regulation.[34] To do this, we utilized a trophoblast-specific transgenic mouse to express Cre-recombinase (only in the placenta) and crossed this mouse with a conditional *OGT* mouse, in which *loxP* insertion around the *OGT* gene allows for excision of *OGT* in the presence of the Cre-recombinase, in order to examine the role of placental OGT in neurodevelopment, according to gene dosage.[23,24] Hemizygous and homozygous deletion of *OGT* in trophoblast cells of the placenta completely recapitulated our EPS phenotype, producing offspring with significant hypothalamic programming changes, including dramatic and significant deficiency in mitochondrial function and transcriptional changes within the paraventricular nucleus of the hypothalamus (PVN), the energy and stress-regulating center of the brain.[23]

To delve even deeper into the cellular mechanisms of the placenta that may relate to brain development and explain these sex differences, we need to understand the molecular role of OGT in this tissue. One major function of OGT is to broadly control transcriptional repression via its stabilization of a protein, enhancer of zeste homolog 2 (EZH2)—a histone H3K27 methyltransferase—thereby increasing levels of methylation of this global histone repressive mark, H3K27me3 (histone H3 trimethylated at lysine 27) (*Figure 1*).[52,57,58] Therefore, from these data we can propose a hypothesis for a sex-specific mechanism that broadly controls pla-

cental transcription and function whereby the low levels of OGT in males result in low levels of the histone repressive mark, H3K27me3. This would position male chromatin to be in a much more "reactive" state, poised to produce robust transcriptional responses to maternal insults such as stress.[21,23-25,27]

Within the placenta, other sex-specific cellular processes have been examined, with the most common being related to maternal stress and glucocorticoids and the glucocorticoid receptor and/or inactivating enzyme levels within the placenta.[59-64] Within the placenta, maternal stress and glucocorticoids decrease placental glycogen levels in female, but not male, tissue suggesting important fetal sex differences in energy availability during maternal stress that may allow an adaptive response in females that is not found in males.[20,65] Although we are beginning to identify placental cellular processes that may contribute to the sex bias in neurodevelopmental disorder risk, we still lack a clear understanding of what the important sex-specific signals are that ultimately reach and program the brain during gestation.

Transplacental signals

Most of what is reported for changes in placental signals related to neurodevelopmental disorders or sex differences is limited to associational studies, as it is extremely difficult to demonstrate direct placental transmission

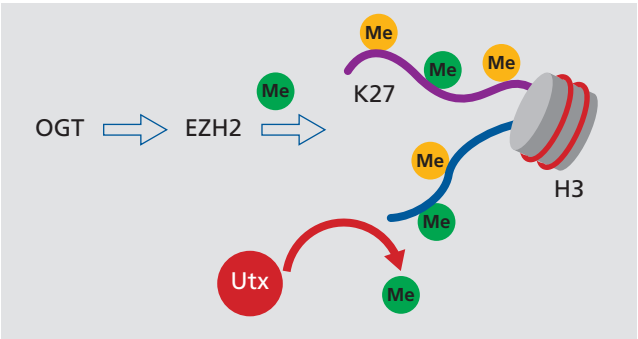

Figure 1. Schematic illustrating the chromatin regulation and transcriptional control by *O*-linked N-acetylgluosamine transferase (OGT). For transcriptional silencing, OGT controls protein stability of EZH2, the H3K27me3 methyltransferase. Utx is a H3K27me3 demethylase. Increased OGT positively correlates with levels of H3K27me3 and transcriptional repression. EZH2, enhancer of zeste homolog 2; H3, histone H3; K27, lysine 27; Me, methyl; OGT, *O*-linked N-acetylgluosamine transferase; Utx, a histone H3-lysine 27 demethylase

in animal models or in humans due to the complexity in determining the origination source of any given molecule.[20,24,32,55,66-74] However, one of the strongest stories we currently have centers on the production of, and changes in, fetal testosterone exposure and neurodevelopmental programming.[13,20,23,68-75] The sexually dimorphic brain is organized during a critical window of development via hormone exposure, with males experiencing elevated testosterone levels during the process of normal testes development, largely in utero, although the exact timing of this is species dependent (as reviewed in reference 76). Aromatization of this testosterone to estradiol in the brain drives masculinization, an active process affecting cell differentiation and connectivity in the brain in most mammals. In humans, a strong correlation exists between fetal testosterone levels and neurodevelopmental disease risk, as well as with adult cognitive and behavioral stress reactivity, supporting a potential importance of these processes involved in establishing the sexually dimorphic brain, which occurs very early in brain development.[68,69,73-75] Programming of important regulatory brain regions, including limbic regions such as the amygdala and hippocampus, and the neuroendocrine hypothalamus, via this developmental testosterone exposure and its effects on cell-migration patterns, probably also contributes to sex differences in how the individual responds to environmental challenges throughout life.[77]

The current causal question then is how do sex differences within the placenta relate to disruptions in the sexually dimorphic brain? It is intriguing that whereas autism has been described as the "hypermasculinized brain," and many autism traits are directly correlated with high fetal testosterone levels,[68,75] schizophrenia, especially in men, has been described as the "hypomasculinized brain," and studies in male patients have reported low levels of testosterone[78,79] and reduced gestational programming by testosterone.[80,81] Therefore, one mechanism that may place placental function at the root of sex biases in neurodevelopmental disease risk is that of variations in steroid hormone production during key periods of brain development.[23,32,82] The placenta has robust and dynamic steroidogenic activity, and in many species, the male placenta produces high levels of testosterone during gestation.[23,83-85]

As an example of placental steroid hormones influencing the brain, in our EPS model, we identified a significant reduction in OGT association with the

17-β-hydroxysteroid dehydrogenase-3 (*Hsd17b3*) gene locus in male EPS placentas by ChIP-Seq analyses (chromatin immunoprecipitation combined with massively parallel DNA sequencing). As expected, this finding correlated with a significant reduction in Hsd17β3 expression in these placentas.[23] Hsd17β3 is a steroidogenic enzyme that converts androstenedione into testosterone. When we subsequently examined placental hormones in EPS male placentas, we found a concordant significant reduction in testosterone and increase in androstenedione levels, supporting a functional outcome resulting from EPS exposure.[23] Importantly, adult EPS males also demonstrated a dysmasculinized phenotype, where their stress behaviors, cognitive function, and spatial strategies were much more similar to the control females than to the control males.[39,56,86] We are certainly not the first to report such prenatal programming of dysmasculinized phenotypes due to maternal stress.[87-92]

Altogether, this Brief Report provides provocative discussion as to the potential critical involvement of the placenta in producing sex-specific responses to maternal insults in pregnancy that impact neurodevelopment and, ultimately, disease risk (*Figure 2*). Although steroid hormones may be involved as transplacental signals, these sex differences begin with genes from the sex chromosomes that drive sex-specific transcriptional regulation. A significant increase in the X-linked gene *OGT* and its control of the transcriptional repressive mark H3K27me3 in female (XX) compared with male (XY) trophoblast cells may position female placentas to be less responsive to a dynamic or changing gestational environment, providing a protective effect for the developing brain. Further studies are necessary to determine additional molecular mechanisms involved and their potential contribution to the known sex bias in neurodevelopmental disorders. ❑

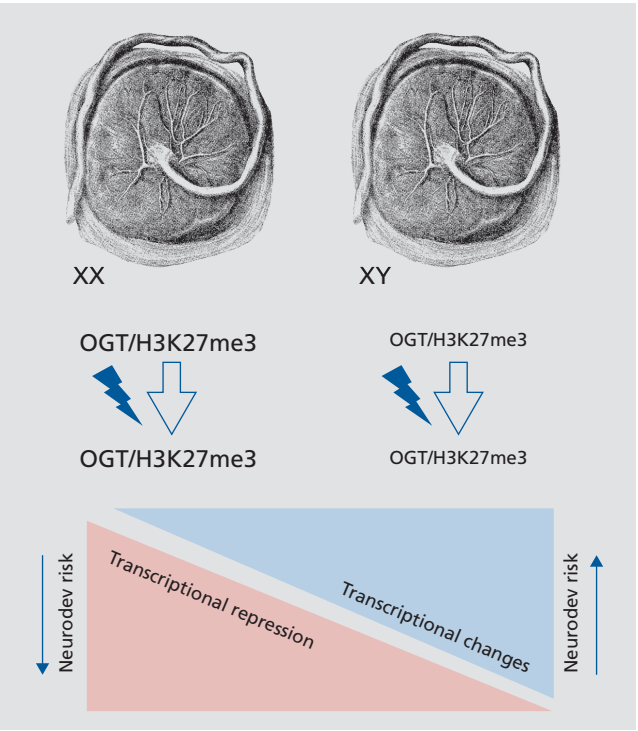

Figure 2. Summary illustration of the hypothesis regarding sex differences in chromatin regulation via *O*-linked N-acetylgluosamine transferase (OGT) and its regulation of the repressive histone mark H3K27me3 that may underlie the male bias for neurodevelopmental disorders. The increased expression of the X-linked gene OGT, in the female placenta provides them with a starting point from which a reduction due to a prenatal insult, such as maternal stress (orange lightning bolt), still affords significantly higher female OGT levels than found in males. Maternal insults can promote widespread transcriptional changes in male placentas (blue) but relatively little in female tissue due to differences in transcriptional repression (pink). The increased H3K27me3 levels in female placentas may then promote sex differences in transcriptional responses and, ultimately, placental function in response to early prenatal stress, placing males at an increased risk for neurodevelopmental disorders stemming from prenatal insults. EPS, early prenatal stress; H3K27me3, histone H3 trimethylated at lysine 27; Neurodev, neurodevopmental disorder; OGT, *O*-linked N-acetylgluosamine transferase; XX, female; XY, male.

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