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Sex differences in brain expression of X- and Y-linked genes

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ABSTRACT

The X chromosome plays an important role in brain development and function, as evidenced by its disproportionately high content of genes whose mutations cause mental retardation. These X-linked brain genes may play a role in sexual differentiation if they are expressed at a higher level in XX females than in XY males, due to incomplete X inactivation in females. The expression of several X escapee genes is indeed higher in brain tissues from females when compared to males. In mouse, some of the sex differences are only found in adult brains but not in other tissues. Determining the brain expression pattern of these X escapee genes is important for a better understanding of their role in the neurological phenotypes of XO Turner syndrome.

In a seminal result, Raisman and Field (1973) found more than 30 years ago that the pattern of synaptic connection in the medial preoptic area is different between male and female rats; since then, hundreds of sex differences have been identified in the brain, ranging from behaviors to molecules in humans and animals. Men and women, for instance, respond differently to nociceptive pain or to emotional faces (Craft et al., 2004; Cahill, 2006). The functional sex differences might be related to differences in brain structure between men and women—postmortem histologic examination revealed that men have more neurons in the neocortex where women have more synapses (de Courten-Myers, 1999; Rabinowicz et al., 1999); in agreement, several synaptic genes are found to be expressed differently between the two sexes (Amateau and McCarthy, 2004; Xu et al., 2005b). A recent comprehensive microarray analysis of gene expression in mouse brains detected 4508 genes to be actively transcribed in the brain, among which 355 genes are expressed more highly in females and 257 genes more highly in males (Yang et al., 2006). Many of these sexual dimorphisms in brain structure and gene expression have been proven to be due to testosterone and its metabolites, acting in the developing brain and permanently wiring the brain in a sex specific fashion (McCarthy and Konkle, 2005; Becker et al., 2005; Morris et al., 2004).

Recent evidence, however, indicates that certain sexual dimorphisms arise under the influence of the sex chromosome complement – in mammals, XY in males and XX in females – independent of steroid hormone levels (Arnold, 2004). This influence has been unequivocally established through study of transgenic mice. A mouse model was

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developed to specifically examine the role of genes on the sex chromosomes, independent from the effects of hormonal differences. In one set of mice, the Y chromosome is deleted for the testis determining gene *Sry* (thereafter termed Y^-), which results in XY^- female mice with ovaries similar to XX females. In a second set of mice, an *Sry* transgene is inserted into an autosome, which results in XX^{Sry} male mice with testes similar to XY males. This elegant approach allows for the separation of sex chromosome-linked gene effects from hormonal effects (Lovell-Badge and Robertson, 1990; Mahadevaiah et al., 1998). For example, XY mice, whether with testes or ovaries, contain a higher level of the neurotransmitter vasopressin in the nerve fibers innervating the lateral septum than XX mice (De Vries et al., 2002). When encountering an intruder, XY female mice initiate offensive attack faster than XX females, while the same XY females spend less time retrieving pups than their XX littermates (Gatewood et al., 2006). These differences point to an important role for the sex chromosomes in sex differences in behavior. Sex chromosome complement probably affects brain structure and behaviors because X- and Y-linked genes are transcribed differentially between the sexes: Y-linked genes are expressed in males only, while X-linked genes are transcribed more highly in females, at least for some X-linked genes (Xu et al., 2002). For instance, the male specific *Sry* gene is transcribed in the substantia nigra of adult male rat brains and regional specific down-regulation of *Sry* expression causes motor deficits in male rats (Dewing et al., 2006). The sex chromosomes could directly affect brain sex differentiation, as suggested by *in vitro* studies in which more XY than XX primary cultured neurons were differentiated into dopaminergic cells (Carruth et al., 2002); alternatively, the sex chromosomes could promote sexual differentiation of another non-gonadal tissue and affect the brain indirectly.

The X and Y chromosomes are hypothesized to have evolved from a pair of autosomal chromosomes in ancestral vertebrates (Graves et al., 2006). Identical initially, the sex chromosomes diverged when mutations occurred at the Y-linked *Sry* gene, which became the testis determinant. Following the development of genetic sex determination, recombination between the ancestral X and Y chromosomes was suppressed due to large Y inversions. Once recombination stopped, mutations started to accumulate on the Y, leading to loss of Y genes and evolving of genes advantageous to males, such as testis-specific genes. The good-for-male genes could have been on the Y chromosome prior to the divergence of the proto-X and proto-Y; their linkage to the sex-determining region was favored during evolution. It is also possible that an effect in male reproduction for a Y gene was acquired once the gene became transmitted only in the male lineage and was therefore released from any need to function in females. Presently, one region (or two in humans), called the pseudoautosomal region or PAR, retains similar DNA sequences where pairing and recombination between the sex chromosomes takes place in male meiosis. The non-recombining Y region (also called male-specific region) contains about 27 protein-coding genes (Skaletsky et al., 2003), which have been divided into two groups—testis-specific Y genes and ubiquitously expressed Y genes (the division has become less clear; some "testis-specific" genes are expressed in other tissues such as brain, as shown by Xu et al., 2002). Additionally, 29 genes with diverse functions reside in the PAR (Ross et al., 2005). Despite gene loss from the Y, some X/Y gene pairs have been retained on present-day sex chromosomes and are called X–Y paralogues.

The mammalian X chromosome is estimated to contain about 1400 genes (including non-coding RNA), among which genes involved in brain function and reproduction are disproportionately represented. It is suggested that about 40% of X-linked genes are expressed in the brain (Ropers and Hamel, 2005). Furthermore, recent evidence indicates that X-linked genes are expressed at a higher level in brain than in other tissues (Nguyen and Disteche, 2006; this issue). Zechner et al. (2001) analyzed all the genetic diseases compiled in the Online Mendelian Inheritance in Man (OMIM) database and found that mental retardation is associated more with the X chromosome than with autosomes. This uneven distribution of mental retardation genes in the genome is only partly due to an ascertainment bias (an X-linked mental disorder is about 2 times more likely to be identified, due to its apparent transmission pattern, than an autosome-linked disorder). After having corrected for this bias, mental retardation-related genes are still three times more abundant on the X chromosome than anywhere else in the genome. A conservative estimate is that 10% of X-linked genes cause mental retardation when mutated. For example, *FMR1* mutations lead to fragile X syndrome, the second most common mental retardation syndrome due to a genetic disorder (next to Down syndrome caused by trisomy 21). Most X-linked disorders affect boys more often than girls. One exception is Rett syndrome, caused by mutations in *MECP2*, which is usually embryonic lethal in male fetuses and affects carrier girls by impairing their language and motor development (Amir et al., 1999). Many X-linked mental disorders display additional symptoms besides cognitive disability and are thus classified as syndromic X-linked mental retardation (S-XLMR). Other X-linked disorders are known as non-syndromic X-linked mental retardation (NS-XLMR) where mental retardation is the only diagnostic symptom. The X-linked genes involved in NS-XLMR have been difficult to identify using traditional mapping methods, because patients who share similar phenotypes of mental retardation are highly heterogeneous in the gene involved. The situation has been improved with the sequencing of the human X chromosome and with international collaboration, resulting in a fast growing list of NS-XLMR genes (Ropers and Hamel, 2005; Ropers, 2006).

X-linked genes are not traditionally considered to play a role in sexual differentiation, because their expression is thought to be balanced between males and females due to X-inactivation, which silences gene transcription of one of the two X chromosomes in females (Lyon, 1961). X inactivation-specific transcript (Xist), a non-coding RNA gene on the X chromosome, is expressed only from the inactivated X chromosome and coats this chromosome at discrete foci (Brown et al., 1991). In mouse, at the 2-cell stage, the paternal X chromosome is inactivated in an imprinted fashion (Huynh and Lee, 2003; Okamoto et al., 2004). At implantation, the imprinted inactivation on the paternal X is erased; both X chromosomes become active briefly, after which random X