Behavioral/Systems/Cognitive

Sex-Specific Differences in Expression of Histone Demethylases *Utx* and *Uty* in Mouse Brain and Neurons

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Although X inactivation is thought to balance gene expression between the sexes, some genes escape inactivation, potentially contributing to differences between males and females. *Utx* (ubiquitously transcribed tetratricopeptide repeat gene on X chromosome) is an escapee gene that encodes a demethylase specific for lysine 27 of histone H3, a mark of repressed chromatin. We found *Utx* to be expressed higher in females than in males in developing and adult brains and in adult liver. XX mice had a higher level of *Utx* than XY mice, regardless of whether they had testes or ovaries, indicating that the sexually dimorphic gene expression was a consequence of the sex chromosome complement. Females had significantly higher levels of *Utx* than males in most brain regions except in the amygdala. The regional expression of the Y-linked paralogue *Uty* (ubiquitously transcribed tetratricopeptide repeat gene on Y chromosome) was somewhat distinct from that of *Utx*, specifically in the paraventricular nucleus of the hypothalamus (high *Uty*) and the amygdala (high *Utx*), implying that the two paralogues may be differentially regulated. Higher expression of *Utx* compared with *Uty* was detected in P19 pluripotent embryonic carcinoma cells as well as in P19-derived neurons. This transcriptional divergence between the two paralogues was associated with high levels of histone H3 lysine 4 dimethylation at the *Utx* promoter and of histone H4 lysine 16 acetylation throughout the gene body, which suggests that epigenetic mechanisms control differential expression of paralogous genes.

Key words: **neuron; cognition; Turner syndrome; Klinefelter's syndrome; estrogen; androgen**

Introduction

Males and females differ in physiology and behavior, as well as in brain structure. Such differences are mainly attributed to the action of steroid hormones (Becker et al., 2005). However, not all sexual dimorphisms can be explained as the result of gonadal steroid action (Arnold et al., 2004); nongonadal sources of sex-specific signals include genes on the X and Y chromosomes.

X-linked genes have been thought previously to be similarly expressed between the sexes, because X inactivation silences one of the two X chromosomes in females (Lyon, 1961). However, in humans, at least 15% of X-linked genes escape inactivation (Carrel and Willard, 2005), and presumably are expressed more highly in females. Some of the X escapees have paralogues on the Y chromosome (Xu and Disteche, 2006). For the Y paralogue to compensate for the female's second expressed X copy, the Y-linked gene should have the same function and be expressed in similar tissues. In the present study, we compared the expression patterns of *Utx* (ubiquitously transcribed tetratricopeptide repeat gene on X chromosome) and of *Uty* (ubiquitously transcribed tetratricopeptide repeat gene on Y chromosome) in adult mouse brain and in cell culture. In addition, we used a mouse model which allows for the separation of sex chromosome-linked gene effects from hormonal effects (Lovell-Badge and Robertson, 1990). 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, resulting in XX*Sry* males with testes similar to XY males.

The UTX protein contains a Jumonji-C domain, a structural motif shared by many histone demethylases (Klose and Zhang, 2007). UTX catalyzes the removal of methylation at lysine 27 of histone H3, a repressive chromatin modification (Agger et al., 2007; Hong et al., 2007; Lan et al., 2007; Lee et al., 2007); thus, UTX acts as a powerful activator of gene expression. The function of UTY, which has 84% amino acid sequence similarity with UTX, is unknown.

We found that although expression of *Utx* and *Uty* was broadly similar in male mouse brain, there were significant differences in the regional expression patterns. *Utx* was expressed preferentially in the amygdala, and *Uty* in the paraventricular nucleus (PVN) of the hypothalamus. These discordances be-

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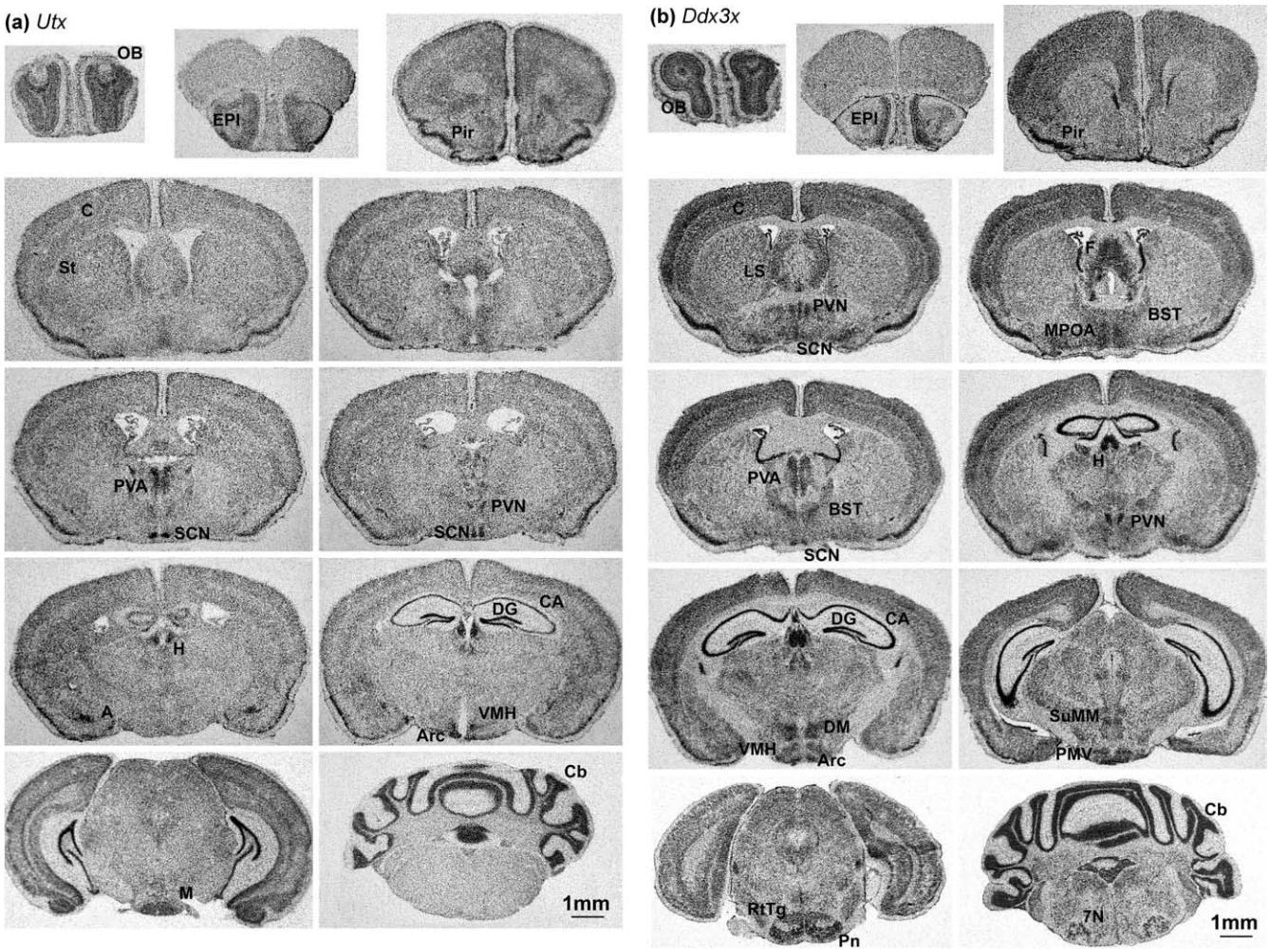

Figure 1. ***a***, ***b***, Expression of *Utx* (***a***) and *Ddx3x* (***b***) by *in situ* hybridization on sections of adult mouse brain. Specific brain regions, including the olfactory bulb (OB), piriform cortex (Pir), habenula (H), hypothalamus [SCN, ventromedial hypothalamic nucleus (VMH), arcuate nucleus (Arc), etc.], hippocampus (CA and DG), and cerebellum (Cb) had a relatively higher expression level. *Utx* was detected specifically in the amygdala (A). *Ddx3x* was detected in the fornix (F), whereas *Utx* was not. 7N, Facial nucleus; BST, bed nucleus of the stria terminalis; C, cortex; DM, dorsomedial hypothalamic nucleus; EPI, external plexiform layer of olfactory bulb; LS, lateral septal nucleus; M, mammillary nucleus; MPOA, medial preoptic area; PMV, premammillary nucleus; Pn, pontine nuclei; PVA, paraventricular thalamic nucleus; RtTg, reticulotegmental nucleus; SuMM, supramammillary nucleus; St, striatum.

tween X and Y paralogues in specific brain areas may lead to sex differences in brain function.

Discordant brain expression patterns between paralogous genes could arise because of differences in chromatin remodeling on the X and Y chromosomes. Both histone methylation and acetylation have been shown to activate or repress expression of certain genes in specific brain regions (Tsankova et al., 2007). Using P19 cells, an *in vitro* model of neuronal differentiation (McBurney, 1993), we demonstrated that the divergence in the expression between *Utx* and *Uty* was associated with differences in the levels of two histone modifications, H3 dimethylation at lysine 4 (H3K4me2) and H4 acetylation at lysine 16 (H4K16ac).

Materials and Methods

Animals. Procedures for mouse use were approved by the University of California, Los Angeles, Chancellor's Animal Research Committee. Mice were bred from stocks obtained from Jackson Laboratories (Bar Harbor, ME) (C57BL/6J) or as a gift (MF1 mice) from Dr. Paul Burgoyne [Medical Research Council (MRC), National Institute for Medical Research, London, UK]. Conditions of mouse husbandry, the breeding paradigm to generate sex-reversed and control mice (XY^- females, XY^- *Sry* males, XX females, and XX*Sry* males) as well as the modalities of tissue collection are described by Xu et al. (2006). Adult tissues were collected from 8- to 10-month-old mice, except for the four-core mouse brains, which were from 12- to 14-month-old animals.

Cell culture and neuronal induction. P19 embryonal carcinoma (EC) cells were cultured in DMEM medium containing 10% fetal bovine serum. Neural differentiation was initiated by plating P19 cells in medium containing 0.3 μM retinoic acid (RA; Sigma, St. Louis, MO) on nonadhesive Petri dishes to promote the formation of aggregates. After a 4 d exposure to RA, the aggregates were dispersed with trypsin (Invitrogen, Carlsbad, CA) and replated on cell culture dishes. Cytosine arabinoside (Ara-C; Sigma), which inhibits the proliferation of non-neuronal cells, was then added to the medium for neuronal selection. P19 neurons appeared to be fully differentiated by day 6. Cells were collected on day 10 for mRNA and histone modification analyses. Three independent samples were cultured and tested for each cell type.

In situ *hybridization. In situ* hybridization of brain sections was performed as described by Xu et al. (2006). The riboprobes were transcribed from linearized plasmids containing either a *Utx* or *Uty* cDNA insert. Both cDNA clones, originally generated by Dr. A. Greenfield (MRC Mammalian Genetics Unit, Oxfordshire, UK) (Greenfield et al., 1998), were rederived into pT7T3-Pac vectors. The *Utx* riboprobe corresponds to nucleotides 1284–2205 of GenBank sequence NM_009483 and the *Uty* riboprobe to nucleotides 1–2392 of sequence NM_009484. Concerned whether the differences in hybridization pattern between the two probes might be related to differences in probe length and location of the

Table 1. *Utx* expression in male and female brain measured by *in situ* hybridization

	Ratio (female/male)[a]	Paired *t* test
Cortex	1.17 ± 0.14	$t_{(7)} = 23.8, p < 0.0001$
Striatum	1.23 ± 0.19	$t_{(7)} = 18.0, p < 0.0001$
SCN	1.35 ± 0.14	$t_{(7)} = 7.3, p < 0.001$
Habenula	1.33 ± 0.11	$t_{(7)} = 9.7, p < 0.0001$
CA	1.21 ± 0.08	$t_{(7)} = 9.9, p < 0.0001$
Dentate gyrus	1.15 ± 0.07	$t_{(7)} = 6.5, p < 0.001$
PVN	1.29 ± 0.23	$t_{(5)} = 2.7, p < 0.05$
Amygdala	1.09 ± 0.08	$t_{(6)} = 1.1, p = 0.30$

[a] Data are mean ± SEM.

probes in the genes, we designed a shorter *Uty* probe (1300 bp) by linearizing the plasmid with *BsrGI*. This *Uty* probe was close in length to the *Utx* probe (922 bp) and located in a similar region of the gene. No differences in the level or regional distribution of hybridization signals were observed using either of the two *Uty* probes, suggesting that the differences we detected between *Utx* and *Uty* were independent of probe length or target gene region. To ensure specificity, the *Uty* riboprobe was applied to slides containing adjacent male and female brain sections. Only male sections hybridized, demonstrating specificity of the probe. Expression of *Ddx3x*, another X-linked escape gene, was also examined on brain sections. The *Ddx3x* riboprobe was transcribed from a linearized cDNA clone which contained a 624 bp PCR product (nucleotides 443–1067, GenBank sequence NM_010028) constructed with the pCRScript kit (Stratagene, La Jolla, CA).

Eight brain regions were selected for analysis including the striatum, cortex, suprachiasmatic nucleus (SCN), PVN, habenula, CA1 and CA3 subfields of the hippocampus (CA), dentate gyrus (DG), and amygdala. Cortex expression was quantified on the sections that contained striatum. Each region was compared between eight pairs of males and females except for the amygdala and PVN, for which only seven (amygdala) or six (PVN) pairs were compared. *Utx* and *Uty* expression was compared on adjacent sections of six male brains containing the amygdala, PVN, and habenula. The hybridization intensity of the *Utx* or *Uty* probe in the amygdala or PVN was averaged and normalized against that in the habenula (which was easily identifiable on the same brain sections). Paired *t* tests were applied to compare the relative expression of *Utx* versus *Uty*.

Northern blots. Northern blot hybridization was done as described previously (Xu et al., 2002). The template for synthesis of the *Utx* probe was the same 922 bp cDNA fragment used for *in situ* hybridization. To synthesize the control *Gapdh* probe, a 451 bp reverse transcriptase (RT)-PCR product (nucleotides 520–971, GenBank sequence XM_111622) was used as template. *Gapdh* is ubiquitously expressed across tissues and at different ages and there is no detectable sex difference in its expression (Xu et al., 2002). Quantification of band intensity was done as described previously (Xu et al., 2002). For comparison of gene expression in adult liver between wild-type males and females, four samples were tested in each sex and each sample was pooled from two mice. The neonatal brain blot contained four mRNA samples for each sex and each sample was derived from three to four day 1 pups. On the Northern blot of four core mouse brains, each genotype was represented by three samples, each sample being pooled from two adult mice.

Quantitative RT-PCR and expression arrays. Total RNA was isolated with an RNeasy kit (Qiagen, Valencia, CA) and was then reverse-transcribed using a first-strand synthesis kit (Invitrogen). The amount of *Utx* and *Uty* mRNA in P19 stem and neuron samples was determined on a LightCycler system (Roche, Indianapolis, IN). The forward and reverse primer sequences, obtained from the Primer Bank website (http://pga.mgh.harvard.edu/primerbank/), for *Utx* were 5′-AAG GCT GTT CGC TGC TAC G-3′ and 5′-GGA TCG ACA TAA AGC ACC TCC-3′; for *Uty*, 5′-GTT TTG TGG CAT GGG AGG ATA-3′ and 5′-GAT GGC ACT GTC TCA GGT GG-3′; and for *Gapdh*, 5′-AGA GAG AGG CCC TCA GTT GCT-3′ and 5′-TTG TGA GGG AGA TGC TCA GTG T-3′. Efficiencies of primer pairs judged by serial dilutions were similar. The level of *Gapdh* mRNA serves as a reference for sample loading. PCRs were repeated at least two times independently. Expression was compared between *Utx* and *Uty* using a paired *t* test in six P19 samples (three undifferentiated and three P19 neuron samples). Expression array data on the undifferentiated P19 cells and P19 neurons were obtained using two Agilent (Palo Alto, CA) mouse gene expression 44K arrays and two Affymetrix (Santa Clara, CA) GeneChip Mouse Genome 430 2.0 arrays following standard protocols. Analysis of arrays was done at the Microarray Center at the University of Washington.

ChIP assays. Chromatin was extracted from three P19 stem cell samples and three P19 neuron samples following the manufacturer's instruction (EZ-ChIP kit; Millipore, Billerica, MA). Briefly, 20 μg of fixed chromatin was incubated for 15 h at 4°C with 5 μg of antibody (antidimethylated H3 at lysine 4 and anti-acetylated H4 at lysine 16, Millipore). After a second incubation with protein A-Sepharose beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for 2 h and three rinses in wash buffer, bound DNA was eluted from the protein A beads by agitation in 1% SDS for 15 min. Chromatin immunoprecipitation (ChIP) DNA was purified, and then used as template in real-time PCR quantification of histone modifications using a Roche LightCycler. *Utx*- and *Uty*-specific PCR primers were designed to amplify regions spanning from nucleotides -328 through -148 on the *Utx* promoter and from nucleotides -364 through -231 on the *Uty* promoter. The sequences of the primers used are as follows: 5′-CTC CAT CTC TGC CTG TGT ATT T-3′ (*Utx* forward); 5′-CCA CTT AAC TTG CTA GCT GCT TT-3′ (*Utx* reverse); 5′-TTA GTG CTC TTT GTT CCT TGT GAG-3′ (*Uty* forward); and 5′-CTA AGA GCT CTA CCT ACC GAC CAC-3′ (*Uty* reverse). Efficiencies of primer pairs judged by serial dilutions were similar. The enrichment of H3K4me2 and H4K16ac was normalized against the input, which served as an internal control for possible differences in amplification efficiency between primer pairs. Histone modifications were compared between *Utx* and *Uty* using a paired *t* test in six P19 samples (three stem cell samples and three neuron samples). DNA from chromatin fractions obtained for histone H4 acetylation at lysine 16 was also submitted to Nimblegen (Madison, WI) for array analysis using the manufacturer conditions. Analysis was done using the manufacturer software.

Results

Expression of *Utx* and *Ddx3x* in mouse brain

Using *in situ* hybridization with specific antisense riboprobes, the expression patterns of *Utx* and *Ddx3x* were examined in adult mouse brain. In human, both *UTX* and *DDX3X* have been proposed as candidates for the facial emotion recognition deficiency found in Turner syndrome women (Good et al., 2003). These women have only one X chromosome and would therefore express X escapee genes at a reduced level relative to XX females. Although *Utx* and *Ddx3x* were found to be expressed throughout the brain, higher expression levels were apparent in specific regions (Fig. 1), including the olfactory bulb, piriform cortex, habenula, hypothalamus [SCN, ventromedial hypothalamic nucleus, arcuate nucleus, etc], hippocampus (DG and CA), and cerebellum. This is, in part, because of a higher cell density in areas such as hippocampus and cerebellum. *Utx* and *Ddx3x*, showed distinct expression patterns; for instance, *Ddx3x*, but not *Utx*, was expressed in the fornix (Fig. 1*a*,*b*).

The specificity of the *Utx* and *Ddx3x* riboprobes was verified by Northern blots on which specific hybridization bands were detected, distinct from those of their Y-linked paralogues *Uty* and *Ddx3y* (Xu et al., 2002). Furthermore, no hybridization signal was detected on brain sections when sense-strand riboprobes were used (data not shown).

Sex-specific differences in *Utx* expression in the cortex, striatum, SCN, habenula and hippocampus, but not in the amygdala

The amygdala-specific expression of *Utx* prompted a detailed analysis of its expression, which was quantified on *in situ* hybridization autoradiograms in individual brain regions including the

cortex, striatum, SCN, habenula, CA, and DG, as well as the amygdala. Females expressed *Utx* at a significantly higher level (ranging from 13 to 35% higher) than males in almost all of the regions tested (Table 1, Fig. 2). However, no significant sex-specific difference was found in the amygdala (Table 1, Fig. 2). Note that the expression levels shown in Figure 2 should not be directly compared across different brain regions, because the background hybridization intensity was measured in different brain areas and then subtracted from that of the region being quantified.

We have found previously, using Northern blot analyses, that *Utx* is expressed at a higher level in the brain of adult females compared with males (Xu et al., 2002). The female bias for *Utx* expression was confirmed in the present study both, in neonatal brain as well as in adult liver (Fig. 3) (neonatal brain, mean ± SEM]: 5.7 ± 0.6 (male) vs 8.2 ± 0.3 (female), $t_{(6)} = 4.0$, $p < 0.01$; adult liver: 3.6 ± 0.3 (male) vs 5.6 ± 0.7 (female), $t_{(6)} = 3.0$, $p < 0.05$), indicating that the sex-specific difference in *Utx* expression exists throughout development and in different tissues.

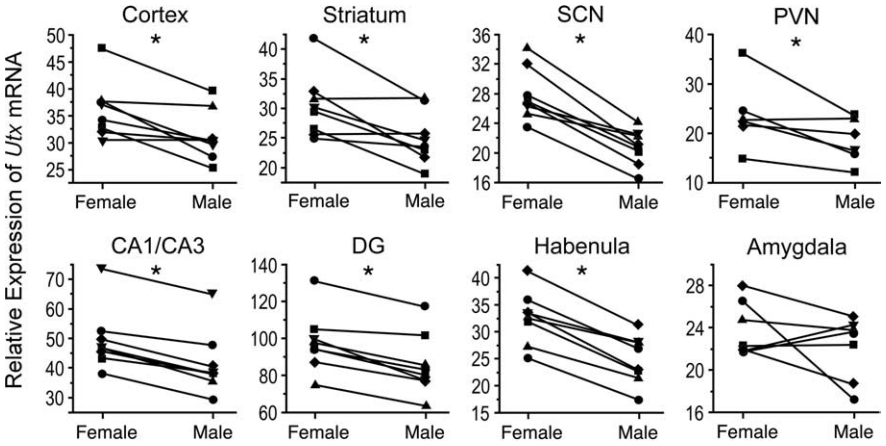

Figure 2. *Utx* expression quantified by *in situ* hybridization to sections of male and female adult mouse brains. Female mice had significantly higher *Utx* expression than males in the cortex, striatum, SCN, PVN, pyramidal layer of the hippocampus (CA1/CA3), DG, and habenula, but not in the amygdala. The *in situ* hybridization labeling intensity was measured as the mean pixel density minus background in six to eight pairs of females and males processed in parallel on the same slides. *$p < 0.05$.

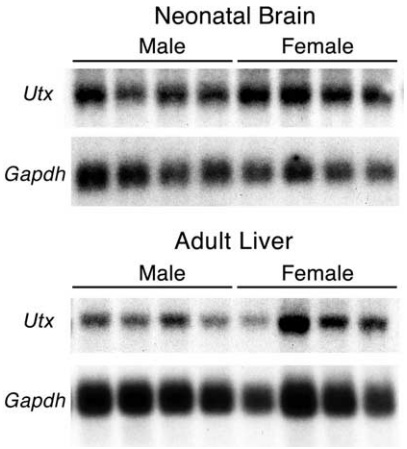

Figure 3. *Utx* expression in male and female neonatal mouse brains and in adult livers. Expression determined by Northern blot analysis was higher in females compared with males. *Gapdh* was used as a loading control.

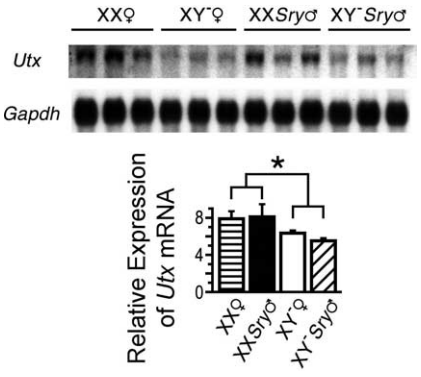

Figure 4. *Utx* expression in brains from XX females, XY$^-$ females, XX*Sry* males, and XY$^-$*Sry* males. Each of the three samples per genotype was derived from two adult brains. *Gapdh* was used as a loading control. Error bars indicate SEM. *$p < 0.05$.

Sex-specific differences in *Utx* expression depend on the complement of sex chromosomes

To confirm that the higher expression of *Utx* in females is indeed caused by the presence of two expressed copies of the gene, we compared *Utx* expression in gonadally intact adult mice between 12 and 14 months of age using the four core genotypes (i.e., XX females, XY$^-$ females, XX*Sry* males, and XY$^-$*Sry* males). Three independent samples, each from two adult brains, were tested by Northern blotting for each genotype. A two-way ANOVA revealed a main effect of the sex chromosome complement, i.e., XX vs XY, on *Utx* expression relative to *Gapdh* (Fig. 4) ($F_{(1,8)} = 7.04$; $p < 0.05$). Mice with two X chromosomes had higher levels of *Utx* mRNA than those with one X chromosome. There was no effect of gonadal sex (male vs female). However, expression in mice with two X chromosomes was not doubled, indicating that expression from the inactive X chromosome was significantly lower (15–35%) than that from the active X chromosome (Fig. 2, Table 1).

Differential expression of *Utx* and *Uty* in the amygdala and PVN in male mouse brain

We compared the expression pattern of *Uty* to that of *Utx* on adjacent sections from adult male mouse brains using *in situ* hybridization. In general, the expression patterns were similar for the two paralogues (Fig. 5), suggesting that *Uty* may compensate for the increased expression of *Utx* in females. In particular, *Uty* was well expressed in the hippocampus, habenula, hypothalamic nuclei, and cerebellum. However, there were notable differences between the paralogues: *Uty* was expressed more highly in the PVN, but less in the amygdala, relative to *Utx* (Fig. 5). Expression levels were quantified in six male brains by measuring the hybridization intensity of the riboprobes in the amygdala, PVN, and habenula on sections that contained all three regions. Using the habenula as a reference area, *Utx* was expressed at a significantly higher level in the amygdala than *Uty* (Table 2) ($t_{(5)} = 9.10$; $p < 0.001$), whereas in the PVN, *Uty* was expressed more highly than *Utx* (Table 2) ($t_{(5)} = -5.10$; $p < 0.01$).

Expression and histone modifications of *Utx* and *Uty* in the neural differentiation of P19 EC cells

P19 EC cells are pluripotent cells with a normal 40,XY male karyotype, which can be readily induced to differentiate into neurons. P19 cells were analyzed to determine whether the differ-

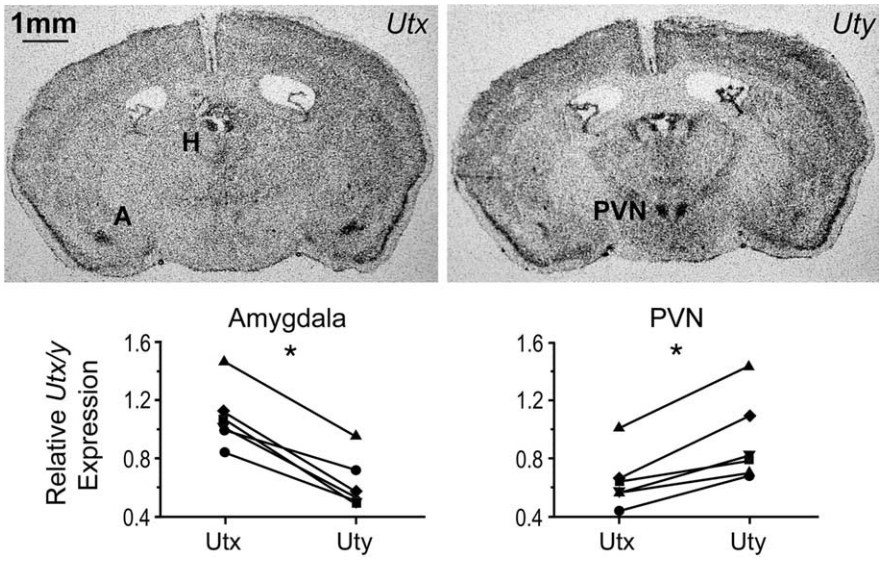

Figure 5. *Utx* and *Uty* differential expression in male mouse brain. Top, Expression patterns of *Uty* and *Utx* by *in situ* hybridization on adjacent adult male brain sections. *Uty* was expressed more highly in the PVN, but less in the amygdala (A) relative to *Utx*. The graphs below show the quantification of expression levels of *Uty* and *Utx* in the amygdala and PVN, using the habenula (H) as a reference area, based on sections that contained all three regions ($n = 6$). *$p < 0.05$.

Table 2. Expression of *Utx* and *Uty* in the amygdala and PVN, relative to the habenula, in adult male mice measured by *in situ* hybridization

	Utx (mean ± SEM)	*Uty* (mean ± SEM)	Paired *t* test
Amygdala	1.08 ± 0.09	0.63 ± 0.18	$t_{(5)} = 9.1, p < 0.0005$
PVN	0.64 ± 0.08	0.82 ± 0.12	$t_{(5)} = 5.1, p < 0.0005$

ences in brain expression patterns between *Utx* and *Uty* described above were associated with distinct epigenetic modifications of the paralogues. Using real-time quantitative PCR, we determined that *Utx* mRNA was much more abundant than *Uty* mRNA both in undifferentiated P19 cells and in P19 neurons (*Utx*, mean ± SEM, 292 ± 78; *Uty*, 0.08 ± 0.03; paired *t* test, $t_{(5)} = 3.7$; $p = 0.01$). Expression of *Utx* and *Uty* in P19 cells estimated on Agilent mouse 44K expression arrays and on Affymetrix mouse 430 2.0 GeneChips was consistent with the quantitative RT-PCR results, i.e., *Utx* was expressed more highly than *Uty* both in undifferentiated P19 cells (Agilent, *Utx*/*Uty* = 7.3 ± 0.9 and Affymetrix, *Utx*/*Uty* = 13.5) and in neurons (Agilent, *Utx*/*Uty* = 5.7 ± 0.1; Affymetrix, *Utx*/*Uty* = 10.9), although the difference between the paralogues was lower than when measured by real-time PCR. No significant difference in expression of *Utx* or *Uty* was detected between P19 neurons and P19 stem cells, either by RT-PCR (*Utx*, $t_{(4)} = 2.46, p = 0.06$; *Uty*, $t_{(4)} = 1.54, p = 0.20$), or by analysis of array data.

To determine whether the higher expression of *Utx* versus *Uty* was associated with chromatin differences, we examined two histone marks characteristic of transcription activation, H3K4me2 and H4K16ac, at the promoter regions of *Utx* and *Uty* using quantitative PCR analysis of ChIP fractions. For the six ChIP samples, three stem cell samples, and three P19 neuron samples, a pair *t* test between the paralogues indicated a trend of higher level of H3K4me2 on the *Utx* promoter relative to *Uty* ($t_{(5)} = 2.4$; $p = 0.06$). H3K4me2 was also found to be higher at the *Utx* promoter, but not the *Uty* promoter, in neurons versus stem cells (P19 stem, 0.42 ± 0.08; neuron, 1.27 ± 0.17; $t_{(4)} = 4.4$; $p = 0.01$ in the case of *Utx*). There were no apparent differences in H4K16ac at the 5′ end of the two genes. However, a ChIP-on-chip analysis on DNA tiling arrays, which represent the whole mouse X and Y chromosomes, showed a significantly higher accumulation of H4K16ac along the *Utx* gene body compared with *Uty*, the latter showing signals no higher than background (data not shown).

Discussion

Utx is one of a handful of X-linked genes that escape X-inactivation in both humans and mice (Disteche et al., 2002; Brown and Greally, 2003). In this study, we determined that *Utx* was expressed at a higher level in female than male mice in specific brain regions such as the cortex, hippocampus, and hypothalamus. A sex-specific difference in expression of *Utx* was also found in developing brains as well as in adult livers. The sex chromosome complement, not the gonadal sex, dictated the expression of *Utx* as demonstrated by higher expression in XX than XY mice, regardless of their gonadal type. We cannot, however, exclude the possibility of a gonadal steroid effect on *Utx* expression because we have not specifically tested the effect of steroids by manipulating their levels in gonadectomized mice, and did not record the estrous state of the females. Although the most likely explanation for the XX versus XY difference in expression is the difference in the genomic dose of *Utx*, it is also possible that a Y-linked factor, such as UTY, suppresses the expression of *Utx* in XY mice. The sex-specific differences in expression of *Utx* may not necessarily lead to differences at the protein level: we have shown previously that *Eif2s3x*, another X escapee gene, is more highly expressed in females than in males for mRNA only but not for the EIF2S3X protein (Xu et al., 2006). In addition, the effects of sex-linked genes and sex hormones may cancel each other out to yield similar physiology between the sexes. For instance, immune responses and cytokine production induced by autoantigens are enhanced in mice with an XY complement compared with mice with an XX complement, but such immune reactions are repressed by male sex hormones (Palaszynski et al., 2005).

The sex-specific differences in expression of an X escapee could conceivably be compensated for by the expression of a functionally similar Y-linked homolog in males (Arnold et al., 2004; Xu et al., 2005). However, biochemical analyses of UTX and UTY have shown that, whereas UTX demethylates H3K27, UTY does not appear to have this function (Hong et al., 2007; Lan et al., 2007). Our studies also show differences between *Utx* and *Uty* in terms of their expression patterns in brain. Although we found that *Utx* and *Uty* are expressed in a parallel manner for many brain regions, we also found brain regions where *Utx* and *Uty* had distinct expression patterns. In the amygdala, *Utx* had a higher expression than *Uty*, whereas in the PVN, *Uty* was expressed at a significantly higher level than *Utx*. The male-specific high expression of *Uty* in the PVN represents the first example of a Y-linked gene expressed in a male-typical manner and may play a role in sexual differentiation of the brain. The discordance in transcription between the X and Y paralogues suggests that they are not coregulated, which may be because of differences in gene struc-

ture (promoter, enhancer, 3′ end), epigenetic modifications, and/or tissue-specific transcriptional factors.

As a proof of principle, we tested two histone marks associated with active chromatin, H3K4me2 and H4K16ac, at the *Utx* and *Uty* genes in P19 stem cells and neurons. In both cell types, H3K4me2 exhibited a trend of higher enrichment at the 5′end of *Utx* compared with *Uty*, and H4K16ac was enriched over the *Utx* gene body, but not over *Uty*. This is consistent with the higher expression of *Utx* relative to *Uty*. Because P19 cells are derived from a day 7 mouse embryo (McBurney, 1993), the expression and epigenetic modifications of *Utx* and *Uty* seem to become divergent at very early stages of development. It is not clear whether similar epigenetic differences between the paralogues will also be found in specific brain regions in adult mice. ChIP assays in discrete brain regions will be needed to determine whether epigenetic modifications vary in a regional manner *in vivo*.

Interestingly, we detected *Utx* expression in the amygdala where other X-linked escapee genes, such as *Eif2s3x*, are not expressed (Xu et al., 2006). Studies have shown that the amygdala plays an important role in human emotion and social behavior, including recognition of other people's emotions from facial expressions (Adolphs, 2003). The amygdala is activated in functional brain imaging when photos of fearful faces are presented to subjects (Morris et al., 1996). When the amygdala is damaged bilaterally, one loses the ability to recognize emotions from other people's facial expressions (Anderson and Phelps, 2000). The molecular mechanism responsible for the role of the amygdala in social cognition is not clear. The X chromosome might play a role in normal development of the amygdala and, in turn, emotional facial recognition.

45,X Turner Syndrome women have been recognized for some time to have varying degrees of impairment in their ability to recognize emotional facial expressions (Ross et al., 2000), which was later correlated with a significant increase in amygdala volume (Good et al., 2003; Kesler et al., 2004). It is thus proposed that certain X escapee genes might influence the development of the amygdala, and therefore Turner women who are haploinsufficient for these X escapees are affected in their social cognitive ability (Skuse et al., 2003). By examining a group of Turner women with partial deletions on the X chromosome, Good et al. (2003) identified a 4.96 Mb critical interval that is specifically important for amygdala development and social cognition. Among 21 confirmed genes within this interval, *EFHC2* is a leading candidate for the social cognition phenotype (Weiss et al., 2007). Other candidates are *USP9X*, *DDX3X*, and *UTX*, which escape X inactivation in human. USP9X is a deubiquitinating enzyme known for its involvement in synaptic development. However, in a previous study we did not detect USP9X protein in mouse amygdala (Xu et al., 2005). In the present study, we examined the expression pattern of two other genes in the critical interval, *Ddx3x* and *Utx*. *Utx* was expressed in the amygdala at an impressive level, whereas *Ddx3x* was detected at a lower level. Thus, provided that its expression pattern is similar in human and mouse, *Utx* is an attractive candidate for the abnormalities in amygdala in Turner syndrome.

Social cognition, aggression, and parental behavior are among an increasing list of sexually dimorphic traits, the origin of which is not always clear (Skuse et al., 1997; Gatewood et al., 2006). Female mice with an XY karyotype are faster to attack an intruder in an aggression test and are slower to retrieve pups in a maternal task when compared with XX female mice (Gatewood et al., 2006). Because the amygdala is intrinsically involved in both aggressive and parental behavior (Byrnes et al., 2007; Nelson and Trainor, 2007), the specific expression of *Utx* we found suggests that this gene may play a role in these behavioral sexual dimorphisms.

The majority of sex-specific differences in the brain have been thought and in many cases proven to be mediated by the actions of steroid hormones (Arnold and Gorski, 1984; Morris et al., 2004). However, as shown in the present study, genes on the X and Y chromosomes may be an additional source of sex-specific signals (Arnold, 2004). The possibility of sexual dimorphisms induced by sex-linked genes is particularly relevant to brain function, because of the relatively high concentration of brain genes on the X chromosome (threefold higher than on autosomes) (Zechner et al., 2001) and the higher expression of X-linked genes in brain (Nguyen and Disteche, 2006). Trimethylation at histone H3K27, which is removed by UTX, is associated with repressed chromatin, in particular the inactive X chromosome (Heard and Disteche, 2006). Removal of this repressive mark would be important specifically in females to allow for X chromosome reactivation and for escape from X inactivation. This could explain why *Utx* itself escapes from X inactivation, leading to its higher expression in females compared with males. The specific role(s) of *Utx/Uty* in brain will need to be further studied. The retention of a Y-linked paralogue together with escape from X inactivation of the X paralogue may afford greater flexibility in gene expression level and possibly functional difference between the sexes.

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