Over-expression of XIST, the Master Gene for X Chromosome Inactivation, in Females With Major Affective Disorders

Baohu Ji, Kerin K. Higa, John R. Kelsoe, Xianjin Zhou *

Department of Psychiatry, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

ARTICLE INFO

Article history: Received 30 April 2015
Received in revised form 5 June 2015
Accepted 11 June 2015
Available online 14 June 2015

Keywords:
XIST
X chromosome inactivation
X-linked escapee genes
KDM5C
Major affective disorders

ABSTRACT

Background: Psychiatric disorders are common mental disorders without a pathological biomarker. Classic genetic studies found that an extra X chromosome frequently causes psychiatric symptoms in patients with either Klinefelter syndrome (XXY) or Triple X syndrome (XXX). Over-dosage of some X-linked escapee genes was suggested to cause psychiatric disorders. However, relevance of these rare genetic diseases to the pathogenesis of psychiatric disorders in the general population of psychiatric patients is unknown.

Methods: XIST and several X-linked genes were studied in 36 lymphoblastoid cell lines from healthy females and 60 lymphoblastoid cell lines from female patients with either bipolar disorder or recurrent major depression. XIST and KDM5C expression was also quantified in 48 RNA samples from postmortem human brains of healthy female controls and female psychiatric patients.

Findings: We found that the XIST gene, a master in control of X chromosome inactivation (XCI), is significantly over-expressed \( (p = 1 \times 10^{-7}, \text{corrected after multiple comparisons}) \) in the lymphoblastoid cells of female patients with either bipolar disorder or major depression. The X-linked escape gene KDM5C also displays significant up-regulation \( (p = 5.3 \times 10^{-7}, \text{corrected after multiple comparisons}) \) in the patients' cells. Expression of XIST and KDM5C is highly correlated \( (\text{Pearson's coefficient}, r = 0.78, p = 1.3 \times 10^{-13}) \). Studies on human postmortem brains supported over-expression of the XIST gene in female psychiatric patients.

Interpretations: We propose that over-expression of XIST may cause or result from subtle alteration of XCI, which up-regulates the expression of some X-linked escapee genes including KDM5C. Over-expression of X-linked genes could be a common mechanism for the development of psychiatric disorders between patients with those rare genetic diseases and the general population of female psychiatric patients with XIST over-expression. Our studies suggest that XIST and KDM5C expression could be used as a biological marker for diagnosis of psychiatric disorders in a significantly large subset of female patients.

Research in context: Due to lack of biological markers, diagnosis and treatment of psychiatric disorders are subjective. There is utmost urgency to identify biomarkers for clinics, research, and drug development. We found that XIST and KDM5C gene expression may be used as a biological marker for diagnosis of major affective disorders in a significantly large subset of female patients. Our studies show that over-expression of XIST and some X-linked escape genes may be a common mechanism for development of psychiatric disorders between the patients with rare genetic diseases (XXY or XXX) and the general population of female psychiatric patients.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The presence of an extra X chromosome causes Klinefelter syndrome (XXY) and Triple X syndrome (XXX). In the general population, the frequency of Klinefelter syndrome in men or Triple X syndrome in women is about 0.1%. However, there is a 4 to 5 fold increase of the prevalence of Klinefelter syndrome or Triple X syndrome in inpatients with psychiatric disorders (DeLisi et al., 1994). The increased prevalence in the inpatient psychiatric populations may underestimate the frequency of psychiatric symptoms in subjects who carry these rare genetic syndromes but are not admitted as psychiatric patients. In the non-psychiatric patients with Klinefelter syndrome, psychosis was reported in up to 50–65% of patients in some studies in addition to other psychiatric symptoms (DeLisi et al., 1994; Otter et al. (2010)) summarized psychological impairments of women with Triple X syndrome. Mood disorders and psychosocial impairments in Triple X syndrome ranged from 25% to 70% in different studies (Otter et al., 2010; Bender et al., 2001). Large variations in the frequency of psychiatric symptoms in different studies can partially be attributed to patients’ ages. About 30% to 50% of children with either XXY or XXX display attention-deficit hyperactivity disorder (ADHD) symptoms (Tartaglia et al., 2012). Supernumerary X chromosomes cause over-expression of

http://dx.doi.org/10.1016/j.ebiom.2015.06.012
2352-3964/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
X-linked escapee genes (Sudbrak et al., 2001). It was suggested that over-dosage of X-linked escapee genes may contribute to the pathogenesis of psychiatric phenotypes in these rare patients carrying aneuploid X chromosomes (DeLisi et al., 2005). Whether over-dosage of X-linked genes may be present in the general population of psychiatric patients with a normal karyotype remains unknown.

X chromosome inactivation (XCI) is a fundamental biological process to prevent over-expression of X-linked genes in females to ensure the same dosage between men and women (Lee and Bartolomei, 2013). In mice, Xist is the master gene in the initiation of XCI (Kay et al., 1993; Penny et al., 1996; Plath et al., 2002). Tsix, Ftx, and Jpx genes, localized in X chromosome inactivation center (XIC), encode non-coding RNAs to regulate expression of Xist (Lee et al., 1999; Chureau et al., 2011; Tian et al., 2010). Most genes on the inactive X chromosome are silenced in somatic cells, but about 10 to 15% of X-linked genes escape XCI (Johnston et al., 2008). XCI is stable and well preserved in human lymphoblastoid cells (Johnston et al., 2008; Zhang et al., 2013) that provide convenient resources to study XCI effects in human diseases (Amir et al., 2000).

We came across XCI from a very different research journey. Our previous studies found that inhibition of protein translation may contribute to pathogenesis of major psychiatric disorders in a rare Scottish family (Ji et al., 2014). Given that excessive protein translation was suggested in fragile X syndrome and autism, we speculated that abnormal protein translation might contribute to a wide range of mental disorders not only in rare families, but also in the general population. In this study, we analyzed protein translation activity in psychiatric patients’ lymphoblastoid cells. The lymphoblastoid cells from patients exhibited a significantly larger variation in protein translation activity than that from the healthy controls. Surprisingly, all variation in protein translation activity came from the female patients. These findings prompted us to investigate functions of the X chromosome in the female patients’ lymphoblastoid cells. We examined expression of XIST and several other X-linked genes in the lymphoblastoid cells of patients with different psychiatric disorders. XIST expression was also investigated in the post-mortem brains of patients with bipolar disorder, major depression, and schizophrenia.

2. Materials and methods

2.1. Lymphoblastoid cell culture

Studies on human lymphoblastoid cells were conducted under a University of California San Diego IRB-approved protocol. All lymphoblastoid cell lines were kindly provided by Dr. John R. Kelsoe at UCSD. The cells were in their early passages (~20 passages) (Oh et al., 2013). Demographic information of the subjects is summarized in Table 1. All female patients except one have a family history with one or more relatives suffering from psychiatric disorders. There is no significant difference in age between healthy controls and patients when blood was taken to generate lymphoblastoid cell lines by transformation with Epstein–Barr virus (EBV). Samples with mixed ethnic backgrounds were collections of subjects with various and mixed ethnicity. Lymphoblastoid cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (Life Technologies, CA) with penicillin and streptomycin (Life Technologies, CA) at 37 °C in a humidified atmosphere of 5% CO2. Fresh medium was added or cells were sub-cultured every 4 days. Cells were harvested for analysis 24 h after addition of fresh medium. The group of lymphoblastoid cell lines from the healthy European Caucasian female controls was co-cultured with the cell lines from each of the other groups. It served as the common control that was used to calibrate gene expression across all samples (Supplemental method I).

2.2. Quantitative real-time RT-PCR

Total RNA was extracted from lymphoblastoid cells with TRIzol reagent (Invitrogen, Carlsbad, CA). Primers were selected to amplify exons without alternative splicing in order to quantify all RNA isoforms. All PCR primers were first confirmed to specifically amplify the target cDNA genes without background before being used for qRT-PCR. cDNA was synthesized from 5 μg of total RNA using Superscript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) with random hexamers. SYBR Green real-time PCR was used to quantify relative expression of all genes with a comparative Ct method. The standard curve of PCR amplification was examined. All amplifications had R² > 0.99. Amplification efficiency for each pair of primers was determined using known molecular concentration of template DNA. Each sample had 4 amplification replica wells. After amplification, Ct was set in the exponential amplification phase of the curve as recommended by the manufacturer’s protocol (Bio-Rad CFX384). Variation of cycles between amplification replica wells was smaller than 0.5 cycles. β-Actin was used as a reference control for normalization. The primers used in real-time qRT-PCR are in Supplemental method II.

2.3. Western blot

Lymphoblastoid cells were collected by centrifugation and sonicated in passive lysis buffer (Promega, WI) with 1× protease inhibitor cocktail (P8340, Sigma). Protein concentration was measured using the Bradford (Abs 595 nm) method with Coomassie Plus Protein Assay (Thermo Scientific, IL). 50 μg of proteins were loaded for Western blot analysis (Ji et al., 2014). Rabbit anti-KDM5C (cat. 39230, dilution at 1:7,500, Active Motif, Carlsbad) and mouse anti-β-actin (sc-47778, dilution at 1:10,000, Santa Cruz) were used as primary antibodies. Horseradish peroxidase (HRP)-conjugated anti-mouse IgG (dilution at 1:10,000, Cell signaling, MA) or HRP-conjugated anti-rabbit IgG (dilution at 1:10,000, Santa Cruz, CA) were used as secondary antibodies. Pierce ECL Western blot substrate (Pierce, IL) was used for chemiluminescence visualization. Quantification of Western blot bands was conducted using Image J.

Table 1

| Gender | Age (mean +/− SD) | European Caucasians | Family history | Mania | Psychosis | Depression | Severity |
|--------|-----------------|----------------------|----------------|-------|-----------|------------|----------|
| CTRL (Female) | 39.9 +/− 13.7 | Y | Y | Y | Y | Moderate |
| BP (Female) | 44.6 +/− 11 | Y | Y | Y | Y | Moderate |
| MDR (Female) | 44.7 +/− 10.5 | Y | Y (13), N (1) | Y | Y | Moderate |
| CTRL (Male) | 35.8 +/− 11.6 | Mixed | Y | Y | Y | Moderate |
| BP (Male) | 42.4 +/− 15.6 | Mixed | Y | Y | Y | Moderate |

* At age when blood was taken.
2.4. Chromatin immunoprecipitation (ChIP)

Lymphoblastoid cell lines from 4 female controls and 4 female patients with high XIST expression were selected for examination of H3K27me3 on the inactive X chromosome. 1.5 × 10⁷ cells were harvested from each cell line. Cells were fixed, and chromatin was sheared. Chromatin immunoprecipitation was performed using ChIP-IT High Sensitivity Kit (Active Motif) according to its manual. Mouse monoclonal antibody against H3K27me3 (cat. 61017) was purchased from Active Motif. 6 pairs of primers were designed to amplify immunoprecipitated genomic DNA (each about 150 bp) around the transcription start site of the XIST gene. 2 pairs of primers were selected to amplify immunoprecipitated genomic DNA around the transcription start site of the KDM5C gene. Amplification of immunoprecipitated β-actin genomic DNA was used as a reference control to quantify enrichment of H3K27me3 at the promoters of XIST and KDM5C genes. All primers were first tested for specific amplification of their target DNA without non-specific background before being used for ChIP-QPCR. SYBR Green real-time PCR was used to quantify the amount of DNA immunoprecipitated by anti-H3K27me3 with a comparative Ct method. The primers used in ChIP-QPCR are in Supplemental method III.

2.5. RNA from postmortem human brains

We received 48 RNA samples from cingulate cortex of female subjects from both Array Collection and Depression Collection (Stanley Medical Research Institute, SMRI). Expression of XIST and KDM5C was quantified blind to diagnosis. After submitting the quantification data to SMRI, we received the diagnostic code for each subject. All subjects are European female Caucasians. Detailed information about the patients and controls can be found online (http://www2.stanleyresearch.org/brain-research/).

2.6. Outcomes

Our primary objective was to find whether expression of X-linked genes including XIST is altered in the general population of female patients with psychiatric disorders. Our secondary objective was to find whether alteration of these genes’ expression in lymphoblastoid cells may potentially serve as a biological marker for the diagnosis of psychiatric disorders in a subset of female patients.

2.7. Statistical analyses

All data were first tested for normal distribution using the Kolmogorov–Smirnov test. F-tests were used to assess the equality of variances between two samples. Student’s t-test was used for statistical analyses between two samples. Correction of multiple comparisons was conducted by using false discovery rate (FDR) (http://www.sdmproject.com/utilities/?show=FDR).

2.8. Role of the funding sources

Not applicable.

3. Results

To measure protein translation activity, lymphoblastoid cells were first serum-starved for 8 h to synchronize cell growth before re-stimulated with a normal concentration of serum. Protein translation activity was measured 8 h after serum re-stimulation using the SunSet (surface sensing of translation) method (Ji et al., 2014; Schmidt et al., 2009). After analyzing 26 healthy controls and 28 bipolar patients with severe mania and psychosis, we found a significantly larger variation of protein translation activity in the patients’ lymphoblastoid cell lines (Fig. S1A and B). The different SunSet intensities between cell lines came from differential activities of protein synthesis rather than differential uptake of puromycin (Fig. S1C). A high correlation of SunSet intensities between the synchronized cells and asynchronized proliferating cells was observed (data not shown). No correlation was found between individuals’ age and protein translation activity (Fig. S1D). When the SunSet data were sub-grouped according to gender, however, we found that all variations in protein translation activity came from the female patients (Fig. S1E).

Restriction of abnormal protein translation activity to the female patients’ lymphoblastoid cells prompted us to investigate potential dysregulation of X chromosome functions. We examined XCI in female lymphoblastoid cells by quantifying expression of XIST, the master gene for XCI (Brown et al., 1992), and other noncoding RNA genes that are involved in the regulation of XIST expression such as TSIX (Migeon et al., 2002), FTX (Chureau et al., 2002), and JPX (Chureau et al., 2002). We found that expression of XIST is significantly higher (p = 0.001) in the female patients than in the female controls (Fig. 1A). Contrary to high XIST expression, TSIX expression is significantly lower (p < 0.01) in the patients’ cells (Fig. 1B). We also observed a trend of high expression of FTX (p < 0.1) in the patients (Fig. 1C), but no difference was found in JPX expression (Fig. 1D). Altered expression of XIST and its negative (TSX) and positive (FTX) regulator genes within XIC indicates a potential alteration in XCI in the lymphoblastoid cells of the female patients.

To investigate whether other X-linked genes beyond XIC are affected, we randomly selected a few genes that are either completely inactivated by or escapes from XCI. PKG1, G6PD and HPRT1 genes are known to be inactivated by XCI, and KDM5C, KDM6A and RPS4X genes are the well-established escapees from XCI in human lymphoblastoid cells (Johnston et al., 2008). We found that expression of KDM5C is significantly higher (p < 0.05) in the female patients than in the female controls (Fig. 1E). A trend of high expression (p < 0.1) of KDM6A (Fig. 1F), PKG1 and G6PD genes (Fig. S2A and B) was observed in the patients’ cells before correction of multiple comparisons. No difference was found in expression of either HPRT1 or RPS4X gene between the patients and the controls (Fig. S2C and D). Expression of all these genes was quantified with β-actin as the reference control. Using GAPDH as a different reference control, we confirmed over-expression of XIST in the patients’ cells (Fig. S2E). Since GAPDH expression is likely more sensitive to cell culture conditions, we continued to use β-actin expression as the reference control for all subsequent studies.

Altered expression of XIST, TSIX, KDM5C and KDM6A genes in patients’ lymphoblastoid cells is unlikely caused by any medication history of the patients because prior treatment of lymphoblastoid cells with both antipsychotics clozapine (CLZ) and mood stabilizer valproic acid (VPA) has no effect on expression of any of these genes (Fig. S3A, B, C, D). Additionally, expression of KDM5C and KDM6A genes was not different in the lymphoblastoid cells of male patients from the healthy male controls (Fig. S4), supporting that patients’ medication history has no effect on expression of X-linked genes in their lymphoblastoid cells. Neither XIST nor KDM5C expression is affected by age (Fig. S5A, B). There is no correlation between XIST expression and protein translation activity (SunSet) (Fig. S5C). A weak correlation between KDM5C expression and SunSet intensity was observed, but statistically insignificant (Fig. S5D). It is possible that other autosomal genes that vary between individuals may also be involved in the regulation of protein translation activity.

To investigate whether abnormal expression of XIST and other X-linked genes also presents in females with a different psychiatric disorder, we examined the expression of XIST, TSIX, KDM5C, KDM6A, PKG1, and G6PD genes in the lymphoblastoid cells of female patients with recurrent major depression. These genes were chosen because they displayed significant or trend increases of expression in the female bipolar patients with mania and psychosis. Expression of XIST was significantly higher (p < 0.01) in the female patients with recurrent major depression than in the female healthy controls (Fig. 2A). Down-regulation of TSIX expression was, however, not statistically significant.
Expression of KDM5C was also significantly higher \((p < 0.001)\) in the patients with recurrent major depression (Fig. 2B). Consistent with a trend of high KDM6A expression in the female bipolar patients, we observed a significantly higher expression of KDM6A \((p < 0.05)\) in the lymphoblastoid cells of female patients with recurrent major depression (Fig. 2D). No difference was found in either PGK1 or G6PD expression between the female patients and the healthy female controls (Fig. S6A and B). To enlarge the sample sizes of the healthy female controls, we analyzed expression of XIST, KDM5C and KDM6A genes in a group of healthy females who have various ethnic genetic backgrounds (“mixed”). No difference was observed in either XIST or KDM5C expression between the European Caucasian female controls and the female controls with various ethnic genetic backgrounds (Fig. 2E and F). A slightly higher level of KDM6A expression was observed in the controls with various ethnic backgrounds (Fig. S6C). Given that KDM6A expression varies between different cell cultures of the control samples (Fig. 1F vs Fig. 2D), it is not surprising that its expression was also affected by different ethnic backgrounds. We concluded that ethnic background has little effect on XIST and KDM5C expression.

in the female patients with recurrent major depression (Fig. 2B). Expression of KDM5C was also significantly higher \((p < 0.001)\) in the patients with recurrent major depression (Fig. 2C). Consistent with a trend of high KDM6A expression in the female bipolar patients, we observed a significantly higher expression of KDM6A \((p < 0.05)\) in the lymphoblastoid cells of female patients with recurrent major depression (Fig. 2D). No difference was found in either PKC1 or G6PD expression between the female patients and the healthy female controls (Fig. S6A and B). To enlarge the sample size of the healthy female controls, we analyzed expression of XIST, KDM5C and KDM6A genes in a group of healthy females who have various ethnic genetic backgrounds (“mixed”). No difference was observed in either XIST or KDM5C expression between the European Caucasian female controls and the female controls with various ethnic genetic backgrounds (Fig. 2E and F). A slightly higher level of KDM6A expression was observed in the controls with various ethnic backgrounds (Fig. S6C). Given that KDM6A expression varies between different cell cultures of the control samples (Fig. 1F vs Fig. 2D), it is not surprising that its expression was also affected by different ethnic backgrounds. We concluded that ethnic background has little effect on XIST and KDM5C expression.

To enlarge the sample sizes of the psychiatric patients, we included more patients who have either bipolar disorder (mania and psychosis) or recurrent major depression with various ethnic backgrounds. Significant difference in either XIST or KDM5C expression was observed between the control group and each of the two patient groups (Fig. 3A and B). Since the same healthy European female Caucasians were used as a calibrator and control for analyses of all other control and patient groups, gene expression in each subject was therefore normalized against the group mean of the healthy European female Caucasians whose cells were co-cultured for quantification. There was a significantly larger variation of XIST expression between the combined control and the combined groups of either bipolar disorder \((p = 8 \times 10^{-5}, \text{F-test})\) or major depression \((p = 1 \times 10^{-10}, \text{F-test})\) (Fig. 3C). When both bipolar and major depression were combined \((\text{BP + MDR})\), a significantly larger variation was observed \((p = 1 \times 10^{-8}, \text{F-test})\). Additionally, significant increase of XIST expression was also detected in bipolar \((p = 1 \times 10^{-7})\), major depression \((p = 0.004)\), and the combined \((\text{BP + MDR})\) group \((p = 1 \times 10^{-7})\). Similarly, a larger variation of KDM5C expression was found in bipolar \((p = 0.002, \text{F-test})\), major depression \((p = 0.004, \text{F-test})\), and the combined \((\text{BP + MDR})\) group \((p = 0.001, \text{F-test})\) (Fig. 3D). Significant increase of KDM5C expression was also observed in bipolar disorder \((p =

---

Fig. 1. Abnormal expression of XIST and its regulator genes involved in XCI. Each dot represents a human subject. Black = healthy European Caucasian female controls (CTRL); red = European Caucasian female bipolar (BP) patients with mania and psychosis. Multiple comparisons of Student’s t-tests were corrected with FDR. (A) A significantly higher level of XIST RNA expression was observed in the patients than in the controls \((t(23) = -4.23, p = 0.001)\). (B) Consistent with its negative role, there was a significantly lower level of TSIX expression in the patients \((t(23) = 3.43, p < 0.01)\). (C) Consistent with its positive role, a trend of high expression of FTX was detected in the patients \((t(23) = -2.00, p < 0.1)\). (D) No difference was observed in JPX expression between the controls and patients \((t(23) = -0.24, \text{ns})\). (E) A significantly higher level of KDM5C expression \((t(23) = -2.89, p < 0.05)\) was observed in the patients. (F) There was a trend of high expression in KDM6A \((t(23) = -2.00, p < 0.1)\) in the patients before correction of multiple comparisons. (**p < 0.001, *p < 0.01, *p < 0.05, #p < 0.1).
and/or above the mean (dashed line, Fig. 4B). Out of 60 patients, 36 have expression above their reference ranges. Among them, a significantly larger variation was observed in XIST expression in the patients group ($p = 0.001, F$-test). There was also a significantly higher level of XIST expression ($t(11) = -4.16, p = 0.01, \text{unequal variances}$) in the patients than in the controls. (B) Down-regulation of TSIX expression was not statistically significant ($t(21) = 1.35, \text{ns}$). Larger variation was observed in either KDM5C ($p = 0.027, F$-test) or KDM6A ($p = 5.9 \times 10^{-8}, F$-test) gene expression in the patients. There was also significantly higher expression of KDM5C ($F(t(13)) = -4.45, p < 0.001, \text{unequal variances}$) and KDM6A ($F(t(10)) = -2.23, p = 0.05, \text{unequal variances}$) in the patients lymphoblastoid cells. There is no difference in XIST (E) or KDM5C (F) expression between healthy European Caucasian female controls and healthy female controls with various ethnic genetic backgrounds. (*$p < 0.01, ^*p < 0.05$).

5.4 $\times 10^{-6}$), major depression ($p = 0.001$), and the combined (BP + MDR) group ($p = 5.3 \times 10^{-7}$). A high correlation was observed between $XIST$ and $KDM5C$ expression in all patients’ cells (Pearson’s coefficient, $r = 0.78, p = 1.3 \times 10^{-13}$) (Fig. 4A). We explored whether a combination of $XIST$ and $KDM5C$ expression can be used as a potential diagnostic marker to separate the patients from the controls. According to calculation of reference range in clinical blood tests (William et al., 2008), we calculated the reference ranges of $XIST$ and $KDM5C$ expression in the control females to define a “normal” distribution. The upper limit of the reference range is defined as 2 standard deviations above the mean (dashed line, Fig. 4B). Out of 60 patients, 36 have $XIST$ and/or $KDM5C$ expression above their reference ranges. Among them, 20 patients have both $XIST$ and $KDM5C$ expressions above their reference ranges. None of the 36 controls display abnormally high $XIST$ and/or $KDM5C$ expression. Since it would be rare to have a control sample expressing both $XIST$ and $KDM5C$ above their reference ranges, we propose that a combination of $XIST$ and $KDM5C$ gene expressions may serve as a reliable biological marker for diagnosis of bipolar disorder or major depression in a significantly large subset of female patients.

We investigated whether cell passages may alter expression of $XIST$, $KDM5C$, and $KDM6A$ genes. Batches of cells from different cell passages were collected and analyzed. The level of $XIST$ expression is stable between different cell passages (Fig. 5A). A high correlation of $XIST$ expression (Pearson’s coefficient, $r = 0.88, p = 2.6 \times 10^{-8}$) was observed between the two different passages of individual cell lines. A strong correlation in $KDM5C$ expression was also observed between the two different cell passages (Pearson’s coefficient, $r = 0.5, p = 0.0028$) (Fig. 5B). As expected, $KDM6A$ expression appeared to be more susceptible to variations between different cell cultures (Fig. 5D). We did not detect an increase of X chromosome DNA copies in the patients with high $XIST$ expression, ruling out the presence of patients with Triple X syndrome in our samples (Fig. 5E).

Consistent with over-expression of $KDM5C$ mRNA in the patients’ cells, Western blot analyses found a significantly higher level of $KDM5C$ protein ($p < 0.01$) in the patients’ cells than in the controls’ cells (Fig. 5C and D). Epigenetic modification of $XIST$ and $KDM5C$ genes was also compared between the controls and the patients with increased $XIST$ and $KDM5C$ expression. H3K27me3 is particularly enriched on the inactive X chromosome because of Xist-dependent recruitment of Polycomb repressor complex 2 (PRC2) that methylates lysine 27 on histone 3 (Plath et al., 2003; Maenner et al., 2010; Zhao et al., 2008). H3K27me3 was therefore examined around the promoters of $XIST$ and $KDM5C$ genes using chromatin immunoprecipitation. $XIST$ (1) and $XIST$ (3) sites have significantly more abundant H3K27me3 in the patients’ lymphoblastoid cells than in the controls’ lymphoblastoid cells (Fig. 5E). $KDM5C$ gene also displayed more abundant H3K27me3 at...
**KDM5C** (1) in the patients’ lymphoblastoid cells (Fig. 5F). Altogether, different lines of evidence support that aberrant regulation and expression of **XIST** and **KDM5C** genes present in the lymphoblastoid cells of female patients with psychiatric disorders.

With a limited number of available postmortem human brains from female patients with major psychiatric disorders, we double-blindly examined expression of **XIST** and **KDM5C** genes in the RNA of cingulate cortex from both Array Collection and Depression Collection from Stanley Medical Research Institute (SMRI). One of many confounding factors in gene expression analysis is the potential cell heterogeneity of the brain samples. If some brain tissues have significantly more glial cells, they may particularly distort quantification of relative **XIST** expression, presumably because **XIST** expression is more dependent on the number of individual cells than the β-actin reference control. Therefore, we first conducted analysis of **GFAP** mRNA expression to evaluate relative abundance of glial cells in all 48 female samples received from SMRI. Two brain samples exhibited very high levels of **GFAP** mRNA expression that was over 3 standard deviations above the group mean (Fig. 6A). Both samples were determined as extreme outliers because they lie outside of 3 interquartile ranges (IQR) above the upper quartile using boxplot analysis. They were therefore excluded for further analysis. Both **XIST** and **KDM5C** expression were quantified in the cingulate cortex. One control sample displayed a very high level of **XIST** expression. Fig. 3. **XIST** and **KDM5C** over-expression in additional groups of patients with bipolar disorder or recurrent major depression. Each dot represents a human subject. Black = healthy European Caucasian female controls (CTRL); red = patients with various ethnic backgrounds with either bipolar (mixed BP) or recurrent major depression (mixed MDR). Multiple comparisons of Student’s t-tests were corrected with FDR. (A) Significantly larger variations were observed in **XIST** expression in either bipolar (p = 0.0038, F-test) or major depression (p = 0.0009, F-test). There was significantly higher **XIST** expression (t(33) = −5.1, p < 0.00001, unequal variances) in the bipolar patients than in the controls. The mean of **XIST** expression of major depression group was not significantly different from the mean of the control group. [B] There is no difference in the variation of **KDM5C** expression between groups. Significantly higher expression of **KDM5C** was observed in both bipolar (t(58) = −2.71, p < 0.01) and major depression (t(24) = −2.84, p < 0.01) than in the controls. (C) Expression of **XIST** in all individuals was normalized against the mean of the European female controls that were co-cultured. After combining all data, significantly larger variation was observed in **XIST** expression in bipolar (p = 8 × 10⁻⁵, F-test), major depression (p = 1 × 10⁻⁷, F-test), and their combined group (p = 1 × 10⁻⁷, F-test). In addition to the difference in variation, there was significantly higher **XIST** expression in bipolar (t(52) = −6.5, p = 1 × 10⁻⁷, unequal variances), major depression (t(26) = −2.86, p = 0.004, unequal variances), and their combined group (t(83) = −5.63, p = 1 × 10⁻⁷, unequal variances). (D) Significantly larger variation was observed in **KDM5C** expression in bipolar (p = 0.002, F-test), major depression (p = 0.004, F-test), and their combined group (p = 0.001, F-test). Significantly higher **KDM5C** expression was observed in bipolar (t(58) = −4.9, p = 5.4 × 10⁻⁶, unequal variances), major depression (t(34) = −3.3, p = 0.001, unequal variances), and their combined group (t(94) = −5.4, p = 5.3 × 10⁻⁷, unequal variances). (**p < 0.01, ***p < 0.001).
A significant difference was observed in KDM5C expression in the cingulate cortex between the control group and each of the patient groups (Fig. 6C). Neither a correlation was found between XIST and KDM5C expression in the cingulate cortex of postmortem human brains (Fig. 6D). In summary, the postmortem brain data supported that over-expression of XIST occurs in both lymphoblastoid cells and the postmortem brains of female patients with bipolar disorder or major depression.

4. Discussion

Our studies suggest that over-expression of both XIST and KDM5C genes may serve as a biological marker for diagnosis of bipolar disorder with mania and psychosis or recurrent major depression in females. In 60 lymphoblastoid cell lines established from randomly collected female patients from the general population, about 30% to 60% of the patients can be diagnosed by this markers using different stringency. Not all female patients displayed over-expression of XIST and/or KDM5C genes, suggesting heterogeneity of genetic etiologies in the general population of female patients. Most of the female patients used in our studies have a family history of mental disorders and display severe psychiatric symptoms. Therefore, our studies may over-estimate the prevalence of abnormal XIST and KDM5C expression in the general population of female psychiatric patients without family history and/or with milder psychiatric symptoms.

The levels of XIST and KDM5C expression are stable between different passages of lymphoblastoid cells. Their expression is unaffected by patients' ages when the blood was drawn for establishment of lymphoblastoid cell lines. Consistent with our studies on humans, mouse Xist expression maintains the same high level of expression across development (Buzin et al., 1994) and between different adult tissues (Kay et al., 1993; Ma and Strauss, 2005). We propose that XIST expression could be a stable trait that associates with the state of X chromosomes in female somatic cells since establishment of XCI. Given that psychiatric symptoms mostly develop in late adolescence, it may be feasible to measure XIST and KDM5C expression in the lymphocytes from girls at childhood or early adolescence to predict the risk of developing major psychiatric disorders in young adulthood. Such studies merit further investigation in the future, as early diagnosis can greatly help early intervention as demonstrated in autism (Rogers et al., 2014).

XIST is a unique gene. It encodes a 19 kb long noncoding RNA and serves as the master gene in both initiation (Kay et al., 1993; Penny et al., 1996; Plath et al., 2002) and maintenance (Yildirim et al., 2013) of X chromosome inactivation (XCI). Mouse Tsix, Ftx, and Jpx genes, localized in X chromosome inactivation center (XIC), have been demonstrated to regulate expression of Xist during XCI initiation (Lee et al., 1999; Chureau et al., 2011; Tian et al., 2010). In the lymphoblastoid cell lines, significant down-regulation of Tsix (a negative regulator of XIST expression) and a trend of high level of FTX (a positive regulator of XIST expression) were observed in the patients with mania and psychosis. However, we failed to detect significant differences in their expression in the patients with recurrent major depression. It is unclear what roles these negative and positive noncoding RNAs play in regulation of XIST expression in the lymphoblastoid cells. In addition, epigenetic modifications of XIST and KDM5C loci are also altered, which accompany over-expression of both genes in patients' lymphoblastoid cell lines. Taken together, all of these data support aberant regulation and expression of XIST and some other X-linked genes in the patients' lymphoblastoid cells. We suggest that subtle XCI deficiency may occur in the patients' lymphoblastoid cells to cause over-expression of X-linked escapee genes KDM5C and KDM6A. Over-expression of XIST may be a compensatory response to XCI deficiency. Such a subtle deficiency appears to be insufficient to cause over-expression of most X-linked genes since we did not observe consistent up-regulation of PGK1, G6PD, and HPRT1 genes in patients' lymphoblastoid cell lines. However, over-expression of XIST may alter epigenetic state of inactive X chromosome via recruiting excessive XIST-dependent protein complexes. Therefore, we cannot rule out potential active roles of XIST over-expression in regulation of XCI, although the exact mechanisms remain to be investigated. There is a robust correlation between XIST and KDM5C expression in patients' lymphoblastoid cell lines. However, we did not observe such a correlation in the cingulate cortex of postmortem brains. Given that X-linked escapee genes display tissue-specific variation in degree of escape (Berleth et al., 2015; Sheardown et al., 1996), the correlation between XIST and KDM5C expression may be cell-specific. Additionally, other confounding factors such as tissue heterogeneity in the postmortem brains may disrupt such a correlation (if there is any). Due to lack of allele-specific polymorphisms of XIST RNA in our samples, we do not know whether XIST over-expression comes exclusively from the inactive X chromosome in patients' lymphoblastoid cell lines. RNA fluorescent in situ hybridization (FISH) experiments on patients' lymphoblastoid cells may be conducted in the future to clarify the localization of XIST over-expression.

X chromosome plays an important role in the development of psychiatric disorders (Goldstein et al., 2013; Perrin et al., 2010; Crow, 2013; Ross et al., 2001, 2006). Over-dosage of X-linked escapee genes...
due to an extra X chromosome was suggested to contribute to the development of psychiatric symptoms in both Klinefelter syndrome (XXY) and Triple X syndrome (XXX) (DeLisi et al., 2005). In lymphoblastoid cells from the female psychiatric patients, not all studied X-linked escapee genes displayed concomitant up-regulation with XIST over-expression. For example, escapee gene RPS4X has normal expression across passages and alteration of epigenetic modifications in the patients. Each dot represents a human subject. Black = healthy European Caucasian female controls (CTRL); red = European Caucasian female patients with recurrent major depression (MDR). XIST (A) and KDM5C (B) gene expression was examined in the first batch of lymphoblastoid cells (XIST-1 and KDM5C-1). A high correlation of XIST (Pearson’s coefficient, r = 0.88, p = 2.6 × 10⁻⁸) and KDM5C (Pearson’s coefficient, r = 0.6, p = 0.0028) expression was observed between the two batches of cells with different passages (XIST-2 and KDM5C-2). After more than a month of continuous cell culture, the same set of genes was examined again in the second batch of the same cell lines with different cell passages (XIST-2 and KDM5C-2). A high correlation of XIST (Pearson’s coefficient, r = 0.88, p = 2.6 × 10⁻⁸) and KDM5C (Pearson’s coefficient, r = 0.6, p = 0.0028) expression was observed between the two batches of cells with different passages. (C) Western blot analyses of KDM5C protein expression in the lymphoblastoid cells of female patients with recurrent major depression. A single band at 180 kD, the calculated size of human KDM5C protein, was detected. C: controls; D: recurrent major depression. β-actin was used as an internal control for normalization. (D) Consistent with increased mRNA expression, significantly higher KDM5C protein expression was found in the patients (t(22) = −2.85, p < 0.01). (E) Lymphoblastoid cell lines from 4 female controls (black) and 4 female patients (red) with either recurrent major depression or mania and psychosis were selected for chromatin immunoprecipitation experiments. Six sites, separated by ~1 kb (except a 2 kb between 1 and 2) around the promoter of XIST gene, were examined by ChIP-QPCR. Multiple comparisons of Student’s t-tests were corrected with FDR. Significantly more H3K27me3 was observed in the patients’ lymphoblastoid cells at XIST (1) (t(6) = −3.83, p < 0.05) and XIST (3) (t(6) = −3.2, p < 0.05). A trend of more H3K27me3 at XIST (4) (t(6) = −2.7, p < 0.1) was also observed. (F) Two sites around the promoter of KDM5C gene were also examined by ChIP-QPCR. H3K27me3 is significantly more enriched at KDM5C (1) (t(6) = −3.39, p < 0.05) in female patients’ lymphoblastoid cells.

Fig. 5. XIST and KDM5C expression across passages and alteration of epigenetic modifications in the patients. Each dot represents a human subject. Black = healthy European Caucasian female controls (CTRL); red = European Caucasian female patients with recurrent major depression (MDR). XIST (A) and KDM5C (B) gene expression was examined in the first batch of lymphoblastoid cells (XIST-1 and KDM5C-1). A high correlation of XIST (Pearson’s coefficient, r = 0.88, p = 2.6 × 10⁻⁸) and KDM5C (Pearson’s coefficient, r = 0.6, p = 0.0028) expression was observed between the two batches of cells with different passages (XIST-2 and KDM5C-2). After more than a month of continuous cell culture, the same set of genes was examined again in the second batch of the same cell lines with different cell passages (XIST-2 and KDM5C-2). A high correlation of XIST (Pearson’s coefficient, r = 0.88, p = 2.6 × 10⁻⁸) and KDM5C (Pearson’s coefficient, r = 0.6, p = 0.0028) expression was observed between the two batches of cells with different passages. (C) Western blot analyses of KDM5C protein expression in the lymphoblastoid cells of female patients with recurrent major depression. A single band at 180 kD, the calculated size of human KDM5C protein, was detected. C: controls; D: recurrent major depression. β-actin was used as an internal control for normalization. (D) Consistent with increased mRNA expression, significantly higher KDM5C protein expression was found in the patients (t(22) = −2.85, p < 0.01). (E) Lymphoblastoid cell lines from 4 female controls (black) and 4 female patients (red) with either recurrent major depression or mania and psychosis were selected for chromatin immunoprecipitation experiments. Six sites, separated by ~1 kb (except a 2 kb between 1 and 2) around the promoter of XIST gene, were examined by ChIP-QPCR. Multiple comparisons of Student’s t-tests were corrected with FDR. Significantly more H3K27me3 was observed in the patients’ lymphoblastoid cells at XIST (1) (t(6) = −3.83, p < 0.05) and XIST (3) (t(6) = −3.2, p < 0.05). A trend of more H3K27me3 at XIST (4) (t(6) = −2.7, p < 0.1) was also observed. (F) Two sites around the promoter of KDM5C gene were also examined by ChIP-QPCR. H3K27me3 is significantly more enriched at KDM5C (1) (t(6) = −3.39, p < 0.05) in female patients’ lymphoblastoid cells.
expression in the patients’ lymphoblastoid cell lines. Investigation of more X-linked escapee genes is needed in the future. Nevertheless, it is plausible that over-expression of \textit{XIST} and some escapee genes may be a common mechanism underlying development of psychiatric disorders between the rare patients with aneuploid X chromosomes and a subset of the general population of female psychiatric patients with a normal karyotype. Among these escapee genes, \textit{KDM5C} is particularly interesting since its haploinsufficiency causes mental retardation (Ounap et al., 2012; Fieremans et al., 2015) or mood disorder (Jensen et al., 2005) in females. Mutations of \textit{KDM5C} generate much more severe mental retardation in males (Jensen et al., 2005; Rujirabanjerd et al., 2010). It will be interesting to know whether \textit{KDM5C} over-expression may contribute to psychiatric symptoms in the female patients. Our studies suggest that subtle alteration of XCI could be a unique genetic risk factor for psychiatric disorders in women. It could be argued that the risk factor is \textit{XIST} over-expression rather than subtle alteration of XCI. This alternative hypothesis, however, cannot readily explain the high correlation between expression of \textit{XIST} and the X-linked escapee gene \textit{KDM5C} in the patients. Why does over-expression of \textit{XIST} and \textit{KDM5C} occur in both bipolar disorder and major depression? One explanation is that psychiatric disorders are not distinct individual diseases, but a spectrum of mental disorders.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{Over-expression of \textit{XIST} in postmortem brains of female patients with major psychiatric disorders. All 48 female RNA samples from postmortem human brains were kindly provided by Stanley Medical Research Institute. Expression of \textit{XIST} and \textit{KDM5C} was double-blindedly quantified. Each dot represents a human subject. Gray = all subjects; black = healthy female controls (CTRL); red = female patients with bipolar disorder (BP), recurrent major depression (MDR), and schizophrenia (SCZ). (A) To assess cell heterogeneity of the samples, \textit{GFAP} expression was quantified to evaluate relative abundance of glial cells in the samples. 2 samples displayed high levels of \textit{GFAP} expression above 3 interquartile ranges (IQR) above the upper quartile using boxplot analysis. Therefore, both were determined as extreme outliers and excluded for further analyses. (B) There was one control sample with a very high level of \textit{XIST} expression that is qualified as an outlier. Due to small sample size, it was retained. There was significantly higher \textit{XIST} expression in bipolar disorder (t(24) = −2.07, p = 0.025), and a trend of high \textit{XIST} in major depression (t(20) = −1.35, p = 0.096). Significantly higher \textit{XIST} expression was also detected when bipolar disorder and major depression were combined or when all patients were combined. (C) There was no significant difference in \textit{KDM5C} expression between the control group and any patient group. (D) There was no correlation between \textit{XIST} and \textit{KDM5C} expression in the postmortem brains of the patients.}
\end{figure}
For example, some patients display both mania and depression. Another explanation is that there are other modifier genes on autosomes that interact with environment to result in different clinical symptoms. In fact, patients with either Klinefelter syndrome or Triple X syndrome display a variety of psychiatric symptoms that can be classified as bipolar disorder, major depression, schizophrenia, and other psychiatric disorders (DeLisi et al., 1994, 2005; Tartaglia et al., 2012). A thorough analysis to compare expression of X-linked genes between the patients with Triple X syndrome and female psychiatric patients with a normal karyotype will help understand the role of over-dosage of X-linked genes in the pathogenesis of psychiatric disorders. Reversing abnormal expression of the X-linked genes in affected females may potentially be a new strategy for future treatment of psychiatric disorders.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2015.06.012.

Contributions
X.Z. conceived of, analyzed, and wrote the studies. B.J. and K.H. performedExprimental and analyzed data. J.R.K. provided all lymphoblastoid cell lines for the studies.

Conflict of interest
The authors declare no conflict of interest.

Funding
There was no funding to directly support the studies.

Acknowledgments
We thank Tanya Shekhtman and Brian Chin for assistance with cell culture, and the Stanley Medical Research Institute for providing RNA samples from postmortem human brains.

References

Amir, R.E., Van den Veyver, I.B., Schultz, R., et al., 2000. Influence of mutation type and X chromosome inactivation on FRT syndrome phenotypes. Ann. Neurol. 47 (5), 670–679.
Bender, B.G., Linden, M.G., Harmon, R.J., 2001. Neuropsychological and functional cognitive skills of 35 unselected adults with sex chromosome abnormalities. Am. J. Med. Genet. 102 (4), 309–313.
Berlihet, J.B., Ma, W., Yang, F., et al., 2015. Escape from X inactivation varies in mouse tissues. PloS Genet. 11 (3), e1005075.
Brown, C.J., Hendrich, B.D., Rupert, J.L., et al., 1992. The human XIST gene: analysis of a non-coding RNA that contains conserved repeats and is highly localized within the X-inactivation center region in mouse, human, and bovine. Genome Res. 2 (12), 894–908.
Chureau, C., Prissette, M., Boudet, A., et al., 2002. Comparative sequence analysis of the X-inactivation center region in mouse, human, and bovine. Genome Res. 12 (6), 1360.
Goldstein, J.M., Cherkerzian, S., Tsuang, M.T., Petryshen, T.L., 2013. Sex differences in the genetic risk for schizophrenia: history of the evidence for sex-specific and sex-dependent effects. Am. J. Med. Genet. B Neuropsychiatr. Genet. 162B (7), 698–710.
Jensen, L.R., Amendt, A., Gurak, U., et al., 2005. Mutations in the JARIDIC gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am. J. Hum. Genet. 76 (2), 227–236.
Johnston, C.M., Lovell, F.L., Leongamornlert, D.A., Stranger, B.E., Dermitzakis, E.T., Ross, M.T., 2008. Large-scale population study of human cell lines indicates that dosage compensation is virtually complete. PLoS Genet. 4 (1), e9.
Johnston, C.M., Lovell, F.L., Leongamornlert, D.A., Stranger, B.E., Dermitzakis, E.T., Ross, M.T., 2008. Large-scale population study of human cell lines indicates that dosage compensation is virtually complete. PLoS Genet. 4 (1), e9.
Kuy, Y.F., Penny, G.D., Patel, D., Ashworth, A., Brockdorff, N., Rastan, S., 1993. Expression of Xist during mouse development suggests a role in the initiation of X chromosome inactivation. Cell 72 (2), 171–182.
Lee, J.T., Bartolomei, M.S., 2013. X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell 152 (6), 1308–1323.
Lee, J.T., Davidow, L.S., Warshawsky, D., 1995. Tsix, a gene antisense to Xist at the X-inactivation centre. Nat. Genet. 21 (4), 400–404.
Ma, M., Strauss, W.M., 2005. Analysis of the Xist RNA isoforms suggests two distinctly different forms of regulation. Mamm. Genome 16 (6), 391–404.
Maenner, S., Blaas, M., Fouillen, L., et al., 2010. 2-D structure of the A region of XIST RNA and its implication for lncRNA2 association. PloS Biol. 8 (1), e1000276.
Migeon, B.R., Lee, C.H., Chowdhury, A.K., Carpenter, H., 2002. Species differences in TSIX/TSix reveal the roles of these genes in X-chromosome inactivation. Am. J. Hum. Genet. 71 (2), 286–293.
Oh, J.H., Kim, Y.J., Moon, S., et al., 2013. Genotype instability during long-term subculture of lymphoblastoid cell lines. J. Hum. Genet. 58 (1), 16–20.
Otter, M., Schrandner-Stumpel, C.T., Curs, L.M., 2010. Triple X syndrome: a review of the literature. Eur. J. Hum. Genet. 18 (3), 265–271.
Ounap, K., Pausseg-Benazzouz, H., Peters, M., et al., 2012. A novel c-TET mutation of the KDM5C/JARID1C gene in one large family with X-linked intellectual disability. Eur. J. Med. Genet. 55 (3), 178–184.
Pei, D.G., Kay, G.F., Sheardown, S.A., Rastan, S., Brockdorff, N., 1996. Requirement for Xist in X chromosome inactivation. Nature 379 (6561), 131–137.
Perrin, M., Harlap, S., Kleinhaus, K., et al., 2010. Older paternal age strongly increases the morbidity for schizophrenia in sisters of affected females. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153B (7), 1329–1335.
Plath, K., Mlynarczyk-Evans, S., Nusinow, D.A., Panning, B., 2002. Xist RNA and the mechanism of X chromosome inactivation. Annu. Rev. Genet. 36, 233–278.
Plath, K., Fang, J., Mlynarczyk-Evans, S.K., et al., 2003. Role of histone H3 lysine 27 methylation in X inactivation. Science 300 (5616), 131–135.
Rogers, S.J., Vismara, L., Wagoner, A.L., McCormick, C., Young, G., Ozonoff, S., 2014. Autism symptoms in children and adolescents with sex chromosome aneuploidy: the XYY/(p22.33;p11.2) male with schizophrenia. J. Med. Genet. 38 (10), 710–719.
Ross, N.L., Wang, J., Sargent, C.A., et al., 2001. Triplication of several PAB1 genes and part of the Homo sapiens specific Ypl1.2/Xq21.3 region of homology in a 46, X, (X; Y)(p22.33;p11.2) male with schizophrenia. J. Med. Genet. 38 (10), 710–719.
Ross, N.L., Wadkar, R., Lopes, A., et al., 2006. Methylation of two Homo sapiens-specific X-Y homologous genes in Klinefelter’s syndrome (XYX). Am. J. Med. Genet. B Neuropsychiatr. Genet. 141B (5), 544–548.
Rujirabanjerd, S., Nelson, J., Tarpey, P.S., et al., 2010. Identification and characterization of two novel JARID1C mutations: suggestion of an emerging genotype–phenotype correlation. Eur. J. Hum. Genet. 18 (3), 330–335.
Schmidt, E.K., Clavaron, G., Ceppi, M., Pierre, P., 2009. SUnSET, a nonradioactive method to monitor protein synthesis. Nat. Methods 6 (4), 275–277.
Sheardown, S., Norris, D., Fisher, A., Brockdorff, N., 1996. The mouse Smcx gene exhibits developmental and tissue specific variation in degree of escape from X inactivation. Hum. Mol. Genet. 5 (9), 1355–1360.
Sudbrak, R., Wieczorek, G., Nuber, U.A., et al., 2001. 2-D structure of the A region of XIST RNA binding to XIST protein and chromatin remodeling, cause X-linked psychiatric disorders. Am. J. Med. Genet. 102 (4), 184.
Yildirim, E., Kirby, J.E., Brown, D.E., et al., 2013. Xist RNA is a potent suppressor of protein translation by the DISC1-Xist RNA interaction. Cell 152 (6), 1308–1323.
Zhang, Y., Castillo-Morales, A., Jiang, M., et al., 2013. Genes that escape X-inactivation in humans have high intraspecific variability in expression, are associated with mental impairment but are not slow evolving. Mol. Biol. Evol. 30 (12), 2588–2601.
Zhao, J., Sun, B.K., Erwin, J.A., Song, J.J., Lee, J.T., 2008. Polycyctome proteins targeted by a short repress RNA to the mouse X chromosome. Science 322 (5902), 750–756.