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Original Article

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

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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 ($p = 1 \times 10^{-7}$, corrected after multiple comparisons) in the lymphoblastoid cells of female patients with either bipolar disorder or major depression. The X-linked escapee gene *KDM5C* also displays significant up-regulation ($p = 5.3 \times 10^{-7}$, corrected after multiple comparisons) in the patients' cells. Expression of *XIST* and *KDM5C* is highly correlated (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 from the general population. Our studies show that over-expression of *XIST* and some X-linked escapee 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.

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

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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% CO_2. 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 $R^2 > 0.99$. 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
Lymphoblastoid cell lines from patients and controls.

	Gender	Age[a] (mean +/− SD)	European Caucasians	Family history	Mania	Psychosis	Depression	Severity
CTRL	Female (13)	39.9 +/− 13.7	Y					
	Male (13)	44.6 +/− 11	Y					
BP	Female (13)	41.7 +/− 13	Y	Y	Y	Y		Severe
	Male (14)	44.7 +/− 10.5	Y	Y (13), N (1)	Y	Y		Severe
MDR	Female (10)	44.3 +/− 11.5	Y	Y (9), N (1)			Y	Moderate
CTRL (mixed)	Female (23)	35.8 +/− 11.6	Mixed					
BP (mixed)	Female (23)	42.4 +/− 15.6	Mixed	Y	Y	Y		
MDR (mixed)	Female (14)	41.9 +/− 11.3	Mixed	Y			Y	Moderate

[a] At age when blood was taken.