Gender-Specific Reduction of Estrogen-Sensitive Small RNA, miR-30b, in Subjects With Schizophrenia

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Estrogen signaling pathways affect cortical function and metabolism, are thought to play a role in the pathophysiology of schizophrenia, and exert neuroprotective effects in female subjects at risk. However, the molecular signatures of estrogen signaling in normal and diseased cerebral cortex remain largely unexplored. Expression of the estrogen-sensitive small RNA, microRNA-30b (miR-30b), was studied in 30 controls and 30 matched samples from subjects diagnosed with schizophrenia from prefrontal cortex (PFC), as well as in 23 samples from parietal cortex (12 controls and 11 schizophrenia cases). The majority of case and control samples were genotyped for an estrogen receptor α (Esr1) sequence variant (rs2234693) previously associated with genetic risk, and a subset of them were subjected to further analysis to determine expression of mature and precursor forms of miR-30b (pre/pri-miR-30b). Gender-dimorphic expression was also explored in mouse frontal cortex and hippocampus. A significant interaction between gender and diagnosis was discovered for changes in mature miR-30b levels, so that miR-30b expression was significantly reduced in the cerebral cortex of female but not male subjects with schizophrenia. In addition, disease-related changes in miR-30b expression in a subset of female subjects were further modulated by Esr1 genotype. Changes after antipsychotic drug exposure remained insignificant. These preliminary findings point to the possibility that disease-related changes in the expression of small noncoding RNAs such as miR-30b in schizophrenia could be influenced by gender and potentially regulated by estrogen signaling.

Key words: microRNAs/estrogen/schizophrenia/miR-30b/prefrontal cortex

Introduction

Numerous studies have provided a link between estrogen and psychiatric disease. For example, age of onset of schizophrenia, its progression, and patients’ response to antipsychotic medication are different between males and females.1–7 Notably, periods of reduced estrogen activity (menopause, postpartum, etc.) are associated with a significantly increased risk for psychotic relapse or emergence of a first episode of the illness.5,6 Indeed, there is also evidence that estrogen supplementation exerts therapeutic benefits in certain patients with schizophrenia.4 Furthermore, metabolic activity and function of prefrontal circuitry, particularly in females of reproductive age, are highly regulated by estrogen signaling.2 These mechanisms could be involved in the pathogenesis of schizophrenia because sequence variants and truncating mutations in estrogen receptor alpha (ERα) (Esr1) confer an increased risk for schizophrenia.7 However, molecular targets that operate downstream of estrogen signaling pathways in human cerebral cortex remain largely unexplored, including those of potential relevance to the pathophysiology of schizophrenia.

MicroRNAs (miRNAs) are evolutionary conserved small noncoding RNAs that have been shown to mediate the posttranscriptional regulation of at least one-third of protein-coding genes.8,9 They are derived from longer precursor molecules (pri- and subsequently pre-miRNAs), which are cleaved to generate mature miRNA forms of approximately 20 nt in length.8,9 miRNAs are abundantly expressed in mammalian nervous system10 and have been shown to be important for brain development, neuronal differentiation, and synaptic plasticity.11–16 Interestingly, gender-dimorphic expression of a subset of miRNAs has been uncovered in mouse
Given the link between estrogen signaling and schizophrenia, we hypothesized that a subset of miRNAs involved in schizophrenia might be among those shown to be estrogen regulated in cell lines and estrogen-responsive tissues. The results from the comparison between related studies revealed that many of the miRNAs expressed at altered levels in schizophrenia postmortem brain and 2 additional miRNAs reported to contain sequence polymorphisms associated with the disease (miR-198 and miR-206) are regulated by estrogen signaling (table 1). Interestingly, miRNAs shown to be increased in schizophrenia were more likely to be reported as estrogen inhibited, whereas estrogen-responsive miRNAs were more likely to be among those reduced in schizophrenia ($P = .019$, chi-square test; $P = .035$, Fisher’s exact test) (table 1). The list of estrogen-sensitive miRNAs included miR-30b, a member of the miR-30 family of miRNA molecules for which we recently described the developmental and lamina-specific patterns of expression in the normal human prefrontal cortex (PFC). miR-30b, previously reported to be reduced in schizophrenia, is positively regulated by both estradiol and ER$\alpha$ signaling. Here, we provide evidence that miR-30b is regulated by estrogen signaling in neuronal tissues and is present at higher levels in female as compared with male mouse brain. Furthermore, we show that miR-30b is selectively downregulated in PFC of female but not male subjects with schizophrenia and this is associated with sequence variations in the Esr1 estrogen receptor. These findings identify miR-30b as an estrogen-responsive miRNA that is differentially expressed between genders in mouse brain and is reduced in a gender-specific and potentially ER$\alpha$ status-specific manner in schizophrenia.

**Methods**

**Postmortem Brains**

A total of 83 postmortem brain samples were used in our study (30 PFC samples from subjects diagnosed with schizophrenia and 30 PFC samples from controls, 11 parietal cortex samples from schizophrenia cases, and 12 parietal cortex samples from controls [table 2 and supplementary file 1, tables S1–S2]. A subset of these cases and controls were used in 2 recent miRNA expression studies. All procedures were approved by the Institutional Review Board of the University of Massachusetts Medical School. For the PFC samples, each sample from a disease case was matched to a control according to gender, age, postmortem interval (PMI), and brain hemisphere. Demographics, medication status, and postmortem confounds, including RNA Integrity Number (RIN) are provided in table 2 and supplementary file 1, table S1. Matched pairs 1–7 and 13–25 of the PFC samples were from the pole of the frontal cortex (rostral portion of Brodmann area 10 [BA10] cut through the full vertical thickness of the cortex) collected from a brain bank at the University of California at Davis (Dr Edward G. Jones, Center for Neuroscience, University of California at Davis), and matched pairs 8–12 and 26–30 were from the lateral/dorsal portion of rostral PFC (corresponding to BA10 and/or rostral most of BA9) obtained from a brain collection at the Maryland Psychiatric Research Center (Dr R. Roberts), University of Maryland, Baltimore (table 2 and supplementary file 1, table S1). The matching process had been completed prior to the experiments. Diagnosis of schizophrenia was based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Revised, and control brains had no history of psychiatric or neurological disease. All parietal cortex RNA samples were isolated from BA7 (12 normal individuals and 11 cases with schizophrenia from the Stanley Brain Bank—see also table 2 and supplementary file 1, table S2). All samples included in this study had an RIN $\geq 4.0$ as determined by Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA), which has been proposed as a minimum standard for postmortem RNA quality.

**Animal Studies**

All procedures were approved by the Institutional Animal Care and Use Committee of the participating institutions. C57BL/6 mice were used for all animal experiments. For antipsychotic drug studies, adult male mice, 10 to 15 weeks of age, were treated for 21 days with once daily intraperitoneal injections of vehicle, haloperidol (0.5 mg/kg), or clozapine (5 mg/kg) (Sigma, St Louis, Missouri) and then killed 1 h after the last treatment. To dissect mouse frontal cortex, the brain was positioned in a coronal brain matrix, and a block between 4 and 2 mm (anterior/posterior, all coordinates relative to Bregma$^3$) was removed. The block was then placed on a flat surface on ice, posterior side up. The dorsal portion (−1 mm wide), containing both frontal association cortex and prelimbic cortex, was collected. The rest of the brain was transferred from the matrix into a beaker containing dry ice in isobutane. Once frozen, cortical samples were stored at $-80^\circ$C until processing. Tissue from dorsal hippocampus was isolated on a cryostat with a 0.5-mm micropuncher based on the following coordinates:
(-1.2 anterior/posterior, ±1.0 medial/lateral, and 1.2 dorsal/ventral). Estrus cycle was determined after microscopic examination of vaginal smear.

**Table 1. Link Between Estrogen and Schizophrenia-Related miRNAs**

<table>
<thead>
<tr>
<th>No.</th>
<th>miRNA</th>
<th>Reported Changes in Schizophrenia</th>
<th>Estrogen Pathway Effect on miRNA Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>miR-30b</td>
<td></td>
<td>ERα induced, ERα induced, E2 inhibited</td>
</tr>
<tr>
<td>2</td>
<td>miR-346</td>
<td></td>
<td>Xenoestrogen induced</td>
</tr>
<tr>
<td>3</td>
<td>miR-30a-5p</td>
<td></td>
<td>ERα induced, ERα induced, E2 inhibited</td>
</tr>
<tr>
<td>4</td>
<td>miR-30c</td>
<td></td>
<td>ERα induced, E2 induced, E2 inhibited</td>
</tr>
<tr>
<td>5</td>
<td>miR-30d</td>
<td></td>
<td>ERα induced, E2 induced, E2 inhibited</td>
</tr>
<tr>
<td>6</td>
<td>miR-30e</td>
<td></td>
<td>ERα induced, E2/ERα induced, xenoestrogen inhibited</td>
</tr>
<tr>
<td>7</td>
<td>miR-92</td>
<td></td>
<td>ERα induced, E2/ERα induced</td>
</tr>
<tr>
<td>8</td>
<td>miR-29b</td>
<td></td>
<td>E2/ERα induced, xenoestrogen inhibited</td>
</tr>
<tr>
<td>9</td>
<td>miR-20b</td>
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<td>miR-24</td>
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<tr>
<td>13</td>
<td>miR-9-3p</td>
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<td>miR-7</td>
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<td>E2 induced</td>
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<td>miR-26b</td>
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<td>miR-195</td>
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</tr>
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</tr>
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<td>31</td>
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</tr>
<tr>
<td>32</td>
<td>miR-206</td>
<td>(SNP—rs17578796)</td>
<td>ERα inhibited, E2 inhibited</td>
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</tbody>
</table>

*Note:* miRNA, microRNAs; ERα, estrogen receptor alpha; SNP, single-nucleotide polymorphism.

**Estradiol Treatment of Glioblastoma Cell Line**

U-87 MG glioblastoma cell lines were cultured in 6-well plates in high-glucose Dulbecco’s Modified Eagle Medium with 10% Fetal Bovine Serum and 1% penicillin/streptomycin and treated with 10^-8 M synthetic estradiol diluted in ethanol or with ethanol-containing vehicle. Cells were harvested 4 h after treatment.

**RNA Isolation**

Total and small RNAs (<200 nt) were isolated by using the mirVANA PARIS Kit (Ambion, Austin, TX), or mirVANA small RNA isolation kit (Ambion), according...
to the manufacturer’s instructions and as described before.\textsuperscript{34} For isolation of <40-nt RNA, the flashPAGE Fractionator System (Ambion) was used according to the manufacturer’s instructions. Briefly, 5 μl of total RNA was run for 12 min at 75 mV, and RNA from the lower running buffer was purified using flashPAGE Reaction Clean-Up Kit (Ambion). In this case, total RNA was isolated by using RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, California) and then treated with DNase I (Ambion).

RNA Quantification and Normalization

The mirVANA quantitative real-time polymerase chain reaction (qRT-PCR) miRNA Detection Kit (Ambion) was used for measuring human miR-30b in PFC samples of <200- and <40-nt RNA. For each sample and amplicon, cycle thresholds (Ct) were averaged from triplicate reactions and normalized to either 5S ribosomal RNA (rRNA) (for <200-nt RNA) or miR-191 (for <40-nt RNA; see also Peltier and Latham\textsuperscript{38}) as described before.\textsuperscript{34} For human parietal samples, independent measurements were undertaken using TaqMan MicroRNA Assays (Applied Biosystems, Foster City, CA). Stanley Foundation brain collection specimens were used for comparative analysis in replications and normalization as recommended in manufacturer’s protocols. The average mean of PCR replication of each specimen was used for comparison. Normalization was performed using expression of let-7a, which was shown to be abundant in parietal cortex and not affected by diagnosis or gender (data not shown). TaqMan miRNA assays (Applied Biosystems) were also used according to the manufacturer’s instructions for determining miR-30b levels in mouse frontal cortex and dorsal hippocampus (normalized to snoRNA202) and for determining miR-30b levels in U-87 MG human glioblastoma cell lines (normalized to U6 small nuclear RNA). Human pri-miR-30b and pre/pri-miR-30b were detected as shown before\textsuperscript{39} and with the following primers: 5’-GTGAATGCTGTGCCTGTTC-3’ and 5’-GCCTCTGTATACTATTCTTGCCA-3’ for pri-miR-30b and 5’-CATGTAAACATCCTACACTCAGCT-3’ and 5’-ATCCACCTCCCAGCCAAT-3’ for pre/pri-miR-30b. TaqMan One-Step RT-PCR (Applied Biosystems) was used according to the manufacturer’s instructions for determining miR-30b levels with primers as shown before.\textsuperscript{40} In the latter precursor and 18S rRNA qRT-PCRs, total RNA was extracted with mirVANA small RNA isolation kit (Ambion). Quantifications were based on equation 2\textsuperscript{-ΔΔCt} or in case of mirVANA qRT-PCR as shown before.\textsuperscript{34}

Genotyping

Esr1 single-nucleotide polymorphism (SNP) genotyping was performed using direct sequencing and also matrix-assisted laser desorption/ionization mass spectrometry (Sequenom, San Diego, CA), in conjunction with SpecroDesign software (Sequenom) for PCR and MassEXTEND primers (Sequenom).

Data Analysis

The effects of gender and diagnosis were evaluated by fitting general linear mixed models\textsuperscript{41} with matching of samples as described above (see “Postmortem Brains” section), with matched sets as a random effect. Gender and diagnosis were modeled as a factorial design in the fixed effects portion of the mixed model. These data represented samples from 2 cohorts from the brain bank.
matched by the brain bank. A third cohort was added for which matching was not originally provided. Pairing was done ad hoc on the basis of gender and hemisphere. The analysis was originally performed with all cohorts together and cohort as a random effect. In this analysis, the covariance component for cohorts was more than 10 times that of any other component leading us to conclude that these cohorts were heterogeneous and led us to perform separate analyses on each cohort. The distributional characteristics of miR-30b were evaluated by analysis of model residuals using the Kolmogorov-Smirnov goodness-of-fit test for normality\textsuperscript{42,43} and by graphical inspection of frequency histograms of model residuals. The levels were transformed by natural logarithms and retested to confirm compliance with the distributional assumptions of the mixed model analysis.\textsuperscript{41} Analyses were performed using the SAS statistical software package\textsuperscript{44} using the mixed procedure.\textsuperscript{45,46} For the purpose of graph representation of relative miR-30b levels of all 3 cohorts, normalized miR-30b levels for each sample were divided to the cohort’s average.

For the calculation of S/C (Schizophrenia/Control) miR-30b ratios, normalized miR-30b levels for each case with schizophrenia were divided to each matched control for PFC samples and to the average of all controls of same gender and hemisphere for the nonmatched parietal cortex samples. Due to the small number ($N = 2$) of female cases with CC Esr1 genotype, we compared samples with TT genotype (absence of risk allele) to those with either CT or CC genotype (presence of risk allele) using 2-tailed $t$ test. To test if S/C miR-30b ratios for each of the above groups represented a significant reduction, 2-tailed 1-sample $t$ test was applied with 1.0 as the hypothetical value. In both cases, parametric statistical tests were selected after normality of distribution of data was verified as mentioned above.

**Results**

**miR-30b Levels Are Upregulated Upon Estradiol Treatment**

Because miR-30b has been shown to be both estradiol and ER\textsubscript{\alpha} responsive in peripheral tissues and cell lines\textsuperscript{19,23,26} and is expressed in high levels in human brain,\textsuperscript{31,34,47} we first tested whether estrogen-mediated upregulation of miR-30b is reproducible in central nervous system tissues. Toward that end, we treated human U-87 MG glioblastoma cells, which we first found to express high levels of ESR1 messenger RNA (mRNA) (supplementary file 1, figure S1A), with $10^{-8}$ M of synthetic estradiol (E2). Indeed, a significant 2-fold increase in miR-30b levels was observed 4 h after E2 treatment (supplementary file 1, figure S1B). This level of estradiol-induced upregulation of miR-30b expression is comparable with the one reported in MCF-7 breast cancer cell line.\textsuperscript{19}

**Gender-Dimorphic Expression of miR-30b in Mouse Brain**

In the rodent brain, the amount of estrogen bound in PFC and hippocampus and other brain regions appears to be surprisingly similar between males and females\textsuperscript{48} (and references therein). However, it is known that local production of estradiol is slightly higher in rodent female frontal cortex and hippocampus, as compared with male.\textsuperscript{48} Therefore, we examined whether any gender-dimorphic expression of miR-30b expression was present in mouse brain by measuring miR-30b expression with qRT-PCR in mouse frontal cortex and hippocampus. Interestingly, frontal cortex of female as compared with male adult C57Bl6/J mice exhibited higher miR-30b levels (figure 1A). As a control, another brain-expressed miRNA, miR-100, did not display any gender-dimorphic expression in mouse frontal cortex (supplementary file 1, figure S2). Furthermore, levels of miR-30b in female hippocampus were close to 2-fold greater as compared with male (figure 1B). Of note, hippocampal miR-30b levels also displayed a modest fluctuation in relation to estrous cycle, which was somewhat less pronounced in cortex (figure 1B).

**Female-Specific Alterations in Mature miR-30b Expression in Schizophrenia Are Influenced by Esr1 SNP Genotype**

Even though cortico-limbic structures of the human brain are thought to express high levels of ER\textsubscript{\alpha} irrespective of gender,\textsuperscript{49} there is ample evidence that estradiol differentially affects structure and function of cortico-limbic circuitry, including PFC, in males and females.\textsuperscript{50–54} Given the above and the link between schizophrenia and alterations in cortical estrogen signaling,\textsuperscript{1–7} we hypothesized that expression of estrogen-responsive miRNAs such as miR-30b could be affected in a gender-specific manner in human cerebral cortex of subjects diagnosed with schizophrenia. Toward this end, we measured miR-30b levels in 30 controls and 30 carefully matched subjects diagnosed with schizophrenia from 2 cohorts from PFC (table 2 and supplementary file 1, table S1), as well as in 12 controls and 11 schizophrenia samples from parietal cortex (Table 2 and supplementary file 1, table S2). Our analysis (see also “Methods” section) revealed a significant interaction between diagnosis and gender for miR-30b levels. Intriguingly, a significant reduction in miR-30b levels was observed only in affected females, relative to control females ($P = .0079$ and $P = .0381$ after post hoc Tukey-Kramer correction for multiple comparisons based on general mixed linear model), while levels in male cases were indistinguishable from controls (figure 2). It has to be noted, also, that unlike mouse frontal cortex and hippocampus, the difference between female and male controls was nonsignificant (figure 2). Given the heterogeneity of miR-30b data between the 3 cohorts, which was expected since qRT-PCR and PCR...
array data were combined in the initial analysis (see also "Methods" section), we wanted to determine if any individual cohort displayed robust gender- and disease-specific changes in miR-30b expression. Our analysis of miR-30b levels in each one of the 3 cohorts separately revealed an interaction between gender and diagnosis with a female-specific reduction in miR-30b levels in schizophrenia only for the larger cohort from PFC (P = .0007 and P = .0034 after post hoc Tukey-Kramer correction for multiple comparisons based on general mixed linear model). The gender-specific reductions in miR-30b levels in both analyses were not related to differences in tissue pH, RNA integrity, or other potential confounds such as PMI and age of death (data not shown and table 2 and supplementary file 1, table S1).

To further elucidate the nature of disease- and gender-related miR-30b changes, we then focused on our larger prefrontal cohort, from which additional RNA isolates and detailed demographic information were available and in which an independent significant female-specific reduction in miR-30b levels was observed. We used total RNA samples and RNA isolates enriched for <40-nts for 7 female matched pairs to determine precursor and mature miR-30b levels. Of note, because of the overlapping sequence of pri- and pre-miR-30b (figure 3A), during our qRT-PCR measurements of miR-30b precursors (both normalized to 18S rRNA), only pri and both pre and pri (pre/pri) amplicons could be analyzed. Our results demonstrated that the observed gender-specific deficits in schizophrenia were limited to the mature form of miR-30b (figure 3B, significant or close to significant deficits of <200- and <40-nt RNA samples, respectively) because precursor forms of miR-30b (pri and pre/pri-miR-30b) remained relatively unchanged from those in controls (figure 3B). Given the fact that for this subset of 14 female brains, or 7 matched pairs, detailed demographic information about age of onset and duration of illness were available, and we genotyped them for Esr1 SNP rs2234693 that has been previously associated with schizophrenia risk and altered ERα mRNA expression.7 We hypothesized that miR-30b levels and disease-related deficits might be influenced by ERα status. Intriguingly, the degree of disease-related deficits in mature miR-30b in female subjects with schizophrenia was strongly correlated to age of disease onset and was influenced by Esr1 SNP genotype (figure 4B, <40-nt isolates). In addition, positive correlations to age of onset were observed for all forms of miR-30b molecules as well in the mature miR-30b measurements in the initial <200–nt enriched RNA samples (Pearson correlation coefficients and 2-tailed P values: r = .5803, P = .172 for pri-miR-30b; r = .6485, P = .099 for pre/pri-miR-30b; and r = .5724, P = .179 for mature miR-30b in <200-nt samples). On the other hand, no correlation was found between miR-30b...
changes and duration of illness of the disease (data not shown). Furthermore, and despite the fact that overall precursor miR-30b levels were increased or not changed in the majority of samples, the disease-related deficits in precursor miR-30b levels appeared to be also affected by Esr1 genotype in the 7 matched pairs examined (supplementary file 1, table S3). However, given the small number of pairs examined, we cannot make any solid conclusions on the effect of Esr1 SNP on precursor miR-30b expression.

We then expanded genotyping of the same Esr1 SNP to all female samples and the majority of male samples for which DNA samples were available from the additional 2 cohorts. Due to heterogeneity of data, S/C miR-30b ratios (Schizophrenia divided to Control miR-30b ratios) were again used when comparing data between different cohorts. Our reanalysis of the effect of Esr1 genotype in disease-related changes in mature miR-30b levels for all 3 cohorts revealed a significant influence of ERα on female S/C miR-30b ratios (N = 14 S/C miR-30b ratios—figure 4B). In addition, female subjects carrying the Esr1 risk allele, but not those carrying the protective allele, exhibited an overall reduction in miR-30b levels (mean ± standard error of the mean and P value based on 2-tailed 1-sample t test with 1 as the hypothetical value: 0.670 ± 0.067, P = .0011 for carriers of the risk allele [CC/CT] and 0.955 ± 0.107, P = .6987 for carriers of the protective allele [TT]—see also figure 4B). Of note, male disease-related changes in miR-30b in schizophrenia were not affected by Esr1 SNP (data not shown).

The above results taken together suggest that in human cerebral cortex, ERα receptor status could influence in a gender-specific manner the disease-related changes in miR-30b levels, which in turn are associated with the age of onset of schizophrenia. New studies in larger cohorts are needed to draw solid conclusions on the effect of estrogen signaling on the regulation of miR-30b expression.

No Effects of Chronic Antipsychotic Treatment on miR-30b Expression

In order to determine if chronic antipsychotic treatment might influence cortical miR-30b levels, we measured miR-30b expression in the cerebral cortex of mice treated with typical antipsychotic haloperidol or atypical antipsychotic clozapine. Our results showed no significant changes in cortical miR-30b levels following chronic antipsychotic treatment, with haloperidol-treated mice showing a moderate yet not significant increase in miR-30b levels (figure 5). It has to be noted, also, that
in our PFC human cohorts, the majority of cases (N = 22 of 30) were treated with typical antipsychotics, 6 cases were unmedicated, and only 2 had received atypical antipsychotics. Notably, unmedicated cases showed greater deficits in miR-30b expression than any of the 2 medicated groups (S/C miR-30b ratio mean ± SEM: 0.717 ± 0.096, 0.949 ± 0.091, and 1.109 ± 0.087 for unmedicated, haloperidol, and clozapine treatment groups, respectively). We conclude that because in both our mouse pharmacological study and in our human cohorts the mean levels of miR-30b had a trend to be lower in antipsychotic naive cortical samples, it is unlikely that the observed deficits in miR-30b expression are a result of antipsychotic treatment. However, because of the small number of unmedicated vs medicated cases and

the fact that 5 of 6 of the unmedicated cases were female, further studies are required to determine if antipsychotics could ameliorate miR-30b deficits in schizophrenia.

Discussion

In the present study, we report that miR-30b is an estrogen-induced miRNA that is expressed at higher levels in mouse female frontal cortex and hippocampus, as compared with male. Furthermore, we show that levels of mature miR-30b are, in comparison with gender-matched controls, decreased in the cerebral cortex of female, but not male subjects with schizophrenia. Preliminary findings, based on observations obtained in a small subset of female case-control pairs, suggest that an ERα variant previously shown to be linked to schizophrenia,7 influences disease-related changes in miR-30b expression, which in turn are associated with age of disease onset. These findings, if confirmed in larger studies and for miRNAs other than miR-30b, highlight a possible link between the regulation of estrogen-sensitive small RNAs and gender-specific alterations in miRNA expression in schizophrenia.

This emerging link could be bidirectional. In addition to the role for estradiol or ERα with regard to altered miRNA expression in schizophrenia (table 1), conversely it is possible that miRNAs could influence estrogen pathways: At least 2 schizophrenia-related miRNAs are reported to target ERα; these include miR-219, which is affected by N-methyl-D-aspartic acid receptor hypofunction55 and increased in PFC of subject with schizophrenia,30 and miR-206, an miRNA for which an SNP had been linked to the disease.33 On a similar note, miR-130b, which is encoded by schizophrenia-related region 22q1147 as well as many of the schizophrenia-altered miRNAs included in table 1 (let-7d, miR-7, miR-19a, miR-20a, miR-20b, miR-29b, miR-92, miR-106b, miR-107, miR-128a, and miR-181a) have also been shown
to regulate estrogen signaling or estradiol secretion.\textsuperscript{56-60} The complete extent of the interplay between the miRNA and estrogen pathway and its implication to psychiatric disease remains to be determined.

Furthermore, a previous study that had reported alterations in a subset of miRNAs in the PFC of schizophrenia cases included miR-30b in the list of miRNAs that were found to be significantly reduced,\textsuperscript{31} which would suggest that deficits in the expression of this miRNA affect a substantial portion of subjects with schizophrenia. In addition, the same study had suggested that changes in miRNA expression in the PFC of subjects with schizophrenia were mainly at the level of mature miRNA,\textsuperscript{31} which is in agreement with our findings. However, gender-specific or \textit{Esr1} SNP genotype–related effects were not examined in any of the previous postmortem studies that have reported disease-related alterations in miRNA expression.\textsuperscript{29-32} Meta-analysis of miRNA expression levels in previously published studies is needed to further test the hypothesis whether estrogen-mediated regulation of miRNAs altered in schizophrenia is part of the underlying molecular mechanisms.

Of note, our study faces several limitations. These include the aforementioned small sample size that precludes any definitive conclusions on the role of \textit{ER\textsubscript{\alpha}} variants on miR-30b expression and biogenesis or on the possible association between miR-30b levels and age of disease onset. Furthermore, we have no information pertaining to estrogen levels and menstrual cycle stage of the female postmortem cases. These factors could play a role because in the mice of the present study, miR-30b levels showed a weak but still discernable association with estrous cycle. In addition, no information relative to alcohol or tobacco abuse were available, both of which have been shown to affect miRNA expression.\textsuperscript{61-64}

Interestingly, in our previous study, we found that expression of another member of the miR-30 family, miR-30a, is developmentally regulated and together with miR-195 displays the most pronounced inhibitory effect on brain-derived neurotrophic factor, a neurotrophic factor and potential key regulator of PFC function in healthy and diseased brain.\textsuperscript{34} Moreover, miR-30a-3p, which is derived from the same precursor as miR-30a-5p but is expressed in lower levels in human PFC,\textsuperscript{34} has been reported to be reduced by qRT-PCR but not microarray.\textsuperscript{34} In addition, a recent study identified miR-30c, another member of miR-30 family, as one of the most altered miRNAs in response to lithium treatment in mice.\textsuperscript{65} Intriguingly, at the time this article was under revision, a novel study found significant association between an SNP in another member of miR-30 family (miR-30e) and schizophrenia.\textsuperscript{66} Given the fact that miRNAs of the same family share high sequence similarities, it is of particular interest that several different members of the miR-30 family have been linked to human brain disorders. Of note, miR-30b is predicted with high probability to target at least 20 genes implicated in schizophrenia, including metabotropic glutamate receptors \textit{GRM3} and \textit{GRM5}\textsuperscript{67} (supplementary file 1, table S4). Whether or not, fine-tuning the expression of these and other targets plays a role in the putative protective effects of miR-30b in female subjects remains to be determined.

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**Supplementary Material**

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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**References**


