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Sex differences in GABAergic gene expression occur in the anterior cingulate cortex in schizophrenia

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ABSTRACT

GABAergic dysfunction has been strongly implicated in the pathophysiology of schizophrenia. In this study, we analyzed the expression levels of several GABAergic genes in the anterior cingulate cortex (ACC) of postmortem subjects with schizophrenia ($n = 21$) and a comparison group of individuals without a history of psychiatric illness ($n = 18$). Our analyses revealed a significant sex by diagnosis effect, along with significant differences in GABAergic gene expression based on medication status. Analyses revealed that in male groups, the expression of GABAergic genes was generally lower in schizophrenia cases compared to the controls, with significantly lower expression levels of GABA-Aα5, GABA-Aβ1, and GABA-Aε. In females, the expression of GABAergic genes was higher in the schizophrenia cases, with significantly higher expression of the GABA-Aβ1 and GAD67 genes. Analysis of the effect of medication in the schizophrenia subjects revealed significantly higher expression of GABA-Aα1-3, GABA-Aβ2, GABA-Aγ2, and GAD67 in the medicated group compared to the unmedicated group. These data show that sex differences in the expression of GABAergic genes occur in the ACC in schizophrenia. Therefore, our data support previous findings of GABAergic dysfunction in schizophrenia and emphasize the importance of considering sex in analyses of the pathophysiology of schizophrenia. Sex differences in the GABAergic regulation of ACC function may contribute to the differences observed in the symptoms of male and female patients with schizophrenia. In addition, our findings indicate that antipsychotic medications may alter GABAergic signaling in the ACC, supporting the potential of GABAergic targets for the development of novel antipsychotic medication.

1. Introduction

Schizophrenia is a widespread and debilitating disorder, with a lifetime risk of approximately 0.7% (Saha et al., 2005). Several hypotheses for the pathophysiology of schizophrenia have been proposed. These focus on the neurotransmitter systems implicated by pharmacological evidence, particularly the dopamine system (Kuepper et al., 2012; Seeman, 2013), the serotonin (5-HT) system (Meltzer et al., 2012), and more recently the glutamate system (Sodhi et al., 2008; Coyle et al., 2012; Javitt, 2012; Moghaddam and Krystal, 2012). Inadequate inhibition of these systems due to dysfunctional γ-aminobutyric acid (GABA) neurotransmission has also been proposed, and accumulating data support the GABAergic hypothesis of schizophrenia (Guidotti et al., 2005; Stan and Lewis, 2012).

The combined effect of nature (genes) and nurture (a stressful environment) is considered to underpin the causes of schizophrenia (Roth et al., 2009; Owen et al., 2010; Brown, 2011; Gejman et al., 2011; Uher, 2014). Gene expression provides a readout of both the genetic and the environmental factors that contribute to the pathophysiology of schizophrenia. Analysis of human postmortem brain is a powerful approach with which to elucidate the pathophysiological mechanisms of schizophrenia, because unlike studies of living patients, detailed molecular analyses can be performed directly in the critical brain regions of interest.

Accumulating data indicate that GABAergic function is disrupted in schizophrenia. Significant associations have been detected between variation of several GABAergic genes and schizophrenia, including the genes encoding the 67 kDa isoform of glutamic acid dehydrogenase (GAD67) (Straub et al., 2007; Zhao et al., 2007), and the GABA-A receptor subunits GABA-Aα1, GABA-Aα6 (Petryshen et al., 2005), GABA-Aβ2 (Lo et al., 2004, 2007; Yu et al., 2006; Zhao et al., 2007), and GABA-Aγ2

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(Zai et al., 2009). Data from postmortem gene expression analyses have revealed reduced expression of GAD67 in several brain regions in schizophrenia, including the dorsolateral prefrontal cortex (DLPFC) (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000; Hashimoto et al., 2005, 2008a,b; Veldic et al., 2005; Woo et al., 2008; Duncan et al., 2010; Curley et al., 2011; Kimoto et al., 2014) and the anterior cingulate cortex (ACC) (Guidotti et al., 2000; Woo et al., 2004; Hashimoto et al., 2008b; Thompson et al., 2009). Moreover, differences in the expression of GABA-A receptor genes have been detected in the DLPFC in schizophrenia, such as the GABA-A receptor subunits α1 (Impagnatiello et al., 1998; Ohnuma et al., 1999; Ishikawa et al., 2004; Hashimoto et al., 2008a,b; Beneyto et al., 2011), α2 (Volk et al., 2002; Beneyto et al., 2011), α5 (Impagnatiello et al., 1998; Duncan et al., 2010; Beneyto et al., 2011), β2 (Beneyto et al., 2011), and δ (Hashimoto et al., 2008a,b). Studies of neutrotransmitter-to-receptor binding indicate that increased GABA-A receptor binding occurs in the neurons of layers II and III of the ACC (Benes et al., 1992) and in the prefrontal cortex in schizophrenia (Hanada et al., 1987; Benes et al., 1996). These data indicate that there is dysregulation of GABAergic signaling in schizophrenia. Moreover, reduced numbers of GABAergic cells expressing the glutamate receptor subunits GRIN2A and GRIK1 have been reported to occur in the ACC in schizophrenia, indicating that glutamatergic inputs may fail to adequately activate inhibitory GABAergic interneurons in schizophrenia (Woo et al., 2004, 2007).

The ACC is a critical component of the cortico-limbic circuitry in the brain, which is considered to be disrupted in schizophrenia (Benes, 2010; Beneyto and Lewis, 2011). Abnormal function of the ACC would be predicted to disrupt several aspects of emotional and cognitive processing, which are important components of the clinical presentation of schizophrenia. Data indicate altered morphological, metabolic, and neurotransmitter-related abnormalities in the ACC in schizophrenia (Clark et al., 2006). Furthermore, structural changes of the ACC have been detected using brain imaging of patients with schizophrenia (reviewed in Fornito et al., 2009). Functional imaging reveals deficits in the activation of the ACC during tests of executive function in schizophrenia patients (Minzenberg et al., 2009). For example, schizophrenia patients show impaired performance of the Stroop task, which measures information processing skills that require activation of the ACC (Krabbendam et al., 2009).

In the current study, we have tested the hypothesis that there is abnormal GABAergic gene expression in the ACC in schizophrenia. We investigated the expression levels of several GABAergic genes in the ACC of postmortem subjects with schizophrenia and a comparison (control) group of subjects without a history of psychiatric illness. Genes were prioritized for analysis if they were previously associated with schizophrenia. We report sex differences in GABAergic gene expression in the ACC in schizophrenia, which may contribute to the differences observed in the symptoms and pathophysiology of the disorder in males and females. Furthermore, we report differential GABAergic gene expression in medicated and unmedicated patients with schizophrenia, indicating that these medications may alter ACC function in schizophrenia through the GABAergic system.

2. Materials and methods

2.1. Tissue used for study

Subjects were recruited at the Mount Sinai/Bronx Veterans Administration Medical Center Department of Psychiatry Brain Bank. Postmortem brain tissue was taken from individuals with schizophrenia diagnosed by DSM-IV criteria and comparison (control) subjects with no history of psychiatric or neurological disorders (Table 1). All subjects died of natural causes, without a history of alcoholism and/or substance abuse. All subjects had thorough neurological characterization to rule out neurodegenerative disorders including Alzheimer's disease, as described previously (Oni-Orisan et al., 2008). This included evaluation for National Institute of Neurological and Communicative Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences (NINCDS-AIREN) criteria for a diagnosis of vascular dementia; NINCDS, DSMIV and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) diagnosis of dementia; Consensus criteria for Lewy body disease; unified Parkinson's disease rating scale (UPDRS); clinical criteria for frontotemporal dementia; and tests of cognitive function including the mini-mental state examination (MMSE) and clinical dementia rating (CDR). The brain tissue also underwent neuropathological examination macro- and microscopically using CERAD guidelines. All assessment, consent, and postmortem procedures were conducted as required by the Institutional Review Boards of Pilgrim Psychiatric Center, Mount Sinai School of Medicine, and the Bronx Veterans Administration Medical Center. Tissue collection was as described previously (Oni-Orisan et al., 2008). Frozen tissue was placed on slides in 20 μm sections, including the anterior cingulate cortex, and stored at –80 °C.

2.2. RNA extraction and cDNA synthesis

Tissue sections were stained in order to differentiate gray and white matter by incubation in 1% cresyl violet acetate for 2 min, submersion in 95% ethanol, 100% ethanol for 30 s, followed by immersion in xylene for 5 min. Gray matter was collected from the slides, and RNA was extracted using an RNeasy mini kit (Qiagen, Hilden, Germany). The RNA integrity number (RIN) was determined using an Agilent BioAnalyzer (Agilent Technologies, Santa Clara, California) to provide a measure of RNA quality at the UIC DNA Services Core Facility.

All RNA samples were diluted to 20 ng per microliter, followed by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California). Preamplification of cDNA was necessary due to the low starting concentration of mRNA. Equal volumes of each TaqMan assay to be used for expression analysis were combined for the preamplification reaction. 40 μl cDNA, together with 4 μl pooled assays and 44 μl TaqMan JumpStart (Sigma-Aldrich, St Louis, Missouri), was preamplified for 14 cycles as previously described (Mengual et al., 2008; Sodhi et al., 2011).

Table 1
Demographic variables of postmortem subjects included in the study.

		N	Age	pH	RIN	PMI
Control	Male	5 (0)	72.8 (14.1)	6.41 (0.27)	6.91 (0.60)	8.2 (6.7)
	Female	13 (0)	85.5 (8.5)	6.41 (0.25)	6.71 (0.84)	7.3 (6.5)
	Total	18 (0)	81.9 (11.5)	6.41 (0.25)	6.76 (0.77)	7.6 (6.3)
Schizophrenia	Male	14 (8)	80.0 (10.6)	6.60 (0.24)	6.39 (0.78)	11.9 (4.6)
	Female	7 (5)	75.4 (5.0)	6.30 (0.41)	6.61 (1.09)	13.0 (6.0)
	Total	21 (13)	78.5 (9.3)	6.50 (0.33)	6.46 (0.87)	12.2 (5.0)

Values are presented as mean with standard deviation for continuous variables in parentheses. Abbreviations: age, age at death in years; *N*, number of subjects (number on antipsychotic medication at time of death shown in parentheses); PMI, postmortem interval in hours; RIN, RNA integrity number.

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2.3. qPCR

Gene expression was measured using commercial TaqMan assays (Applied Biosystems; Table 2). The expression of GAD67 and selected GABA-A receptor subunits (Table 2) were measured in preamplified cDNA derived from the gray matter of the ACC from each subject. In addition the expression levels of the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cyclophilin (PPIA), and glucuronidase beta (GUSB) were measured. Assays for each target gene were performed in duplicate in 96-well optical plates using a Stratagene MX3000P instrument (Stratagene, La Jolla, California) and Sequence Detector Software (SDS version 1.6; PE Applied Biosystems). The relative standard curve method was used for these analyses as described previously (Sodhi et al., 2011). Briefly, standard curves were generated for each target assay and for each endogenous control assay using a calibration curve and the geometric mean of GAPDH, PPIA, and GUSB expression was used for normalization of the target genes according to Applied Biosystems instructions (Guide to Performing Relative Quantitation of Gene Expression Using Real-time Quantitative PCR, Applied Biosystems).

2.4. Statistical analysis

Statistical analysis was conducted using SPSS version 20. Any subjects with RIN < 5.0 were excluded from the analyses. For $\alpha = 0.05$, and $n = 39$, our observed power (one-tailed hypothesis) was 0.8 for a large effect size of 0.84, as determined by an online statistical calculator (www.danielsoper.com). Shapiro–Wilk tests were used to determine if data were normally distributed. Normally distributed data were analysed using multivariate ANCOVA to investigate main effects, and post hoc analyses of individual genes were performed using univariate ANCOVA. Postmortem interval (PMI), age at death, and brain pH were included as covariates due to their correlation with gene expression and/or differences in their values between diagnostic groups (data not shown). Analyses included relative gene expression as the independent variable and both diagnosis and sex as the dependent variables. Data that were not normally distributed were analysed using the non-parametric Mann–Whitney U test. Due to the potential confounding effects of antipsychotic medications, and the reports of changes in GABAergic gene expression in rodents treated with haloperidol or clozapine (Zink et al., 2004a,b), univariate ANCOVAs were also conducted to investigate the effect of medication status, including PMI, age at death, and brain pH as covariates.

Table 2
Analyses of GABAergic gene expression in the ACC in schizophrenia.

Gene	Protein	Locus	Assay ID#
GABRA1	GABA-Aα1	5q34	Hs00168058_m1
GABRA2	GABA-Aα2	4p12	Hs00168069_m1
GABRA3	GABA-Aα3	Xq28	Hs00168073_m1
GABRA5	GABA-Aα5	15q12	Hs00181291_m1
GABRB1	GABA-Aβ1	4p12	Hs00181306_m1
GABRB2	GABA-Aβ2	5q34	Hs00241451_m1
GABRD	GABA-Aδ	1p36	Hs00181309_m1
GABRE	GABA-Aε	Xq28	Hs00608332_m1
GABRG1	GABA-Aγ1	4p12	Hs00381554_m1
GABRG2	GABA-Aγ2	5q34	Hs00168093_m1
GABRG3	GABA-Aγ3	15q12	Hs00264276_m1
GAD1	GAD67	2q31	Hs01065893_m1
GUSB	GUSB	7q21	Hs99999908_m1
GAPDH	GAPDH	12p13	Hs99999905_m1
PP1A	Cyclophilin	7p13	Hs99999904_m1

Relative gene expression was measured using commercially designed assays (Applied Biosystems).

3. Results

3.1. Effect of diagnostic group

Data were normally distributed for all target gene transcripts except GABA-Aε. Analysis of normally distributed target genes by multivariate ANOVA indicated no significant effect of diagnosis ($F_{11,22} = 0.644$, $p > 0.05$) or sex ($F_{11,22} = 1.454$, $p > 0.05$) but revealed a significant sex by diagnosis interaction for GABAergic gene expression ($F_{11,22} = 3.049$, $p = 0.013$). To further investigate this sex by diagnosis effect, post hoc analyses were conducted on males and females separately.

Expression data in male subjects were normally distributed for every gene tested. Multivariate ANCOVA indicated no significant association between GABAergic gene expression and diagnosis in the male group ($F_{12,3} = 1.521$, $p > 0.05$), but post hoc analyses of individual genes revealed significantly decreased expression of GABA-Aα5 ($F_{1,14} = 9.504$, $p = 0.008$), GABA-Aβ1 ($F_{1,14} = 7.427$, $p = 0.016$), and GABA-Aε ($F_{1,14} = 4.843$, $p = 0.045$) (Fig. 1A). In the female subjects, all gene expression data were normally distributed except for GABA-Aε. Multivariate ANOVA of normally distributed data showed no effect of diagnosis ($F_{11,5} = 1.233$, $p > 0.05$), but post hoc tests indicated significant increases in the expression of GABA-Aβ2 ($F_{1,15} = 7.197$, $p = 0.017$) and GAD67 ($F_{1,15} = 4.545$, $p = 0.05$) (Fig. 1B). The Mann–Whitney *U* test indicated no significant differences in the expression of GABA-Aε between the diagnostic groups ($p > 0.05$).

3.2. Effect of medication status

The patient group was dichotomized into cases that were reported to be on medication at time of death and those who were not on antipsychotic medication. Analysis of gene expression by medication status was conducted in the male schizophrenia group only, due to the small number of females (<5) in the "off medication" group. ANCOVA showed significant differences between the on- and off-medication groups for the expression of several GABAergic genes (Fig. 2, Table 3). Within the male subjects, the off-medication group had significantly lower expression compared to the on-medication group for the genes encoding GABA-Aα1, GABA-Aα2, GABA-Aα3, GABA-Aβ2, GABA-Aγ2, and GAD67 (Fig. 2).

4. Discussion

This is the first report of sex-specific changes of GABAergic gene expression in the ACC in schizophrenia. While the majority of GABAergic genes had reduced expression in the ACC of the male schizophrenia group relative to the male comparison subjects, there appeared to be an overall increase in the female schizophrenia group relative to female comparison group. Statistically significant reductions in the expression levels of GABA-Aα5, β1, and ε genes (GABRA5, GABRB1, and GABRE, respectively) were observed in the male schizophrenia group (Fig. 1A). In contrast, gene expression was significantly increased for GABA-Aβ1 and GAD67 (encoded by GABRB1and GAD1 respectively) in the female patients relative to the female comparison group (Fig. 1B). Therefore, our data support previous findings of GABAergic dysfunction in schizophrenia and emphasize the importance of considering sex when investigating the pathophysiology of schizophrenia by revealing significant sex differences in the relative expression of these GABAergic genes in the ACC of males and females with schizophrenia.

Although the differential expression of specific GABA-A receptor subunits has been previously reported in schizophrenia, these differences have not been consistent between the studies conducted. For example, our findings in the ACC in males of decreased expression of the GABA-Aα5 subunit in schizophrenia have previously been reported in the prefrontal cortex in schizophrenia (Duncan et al., 2010), specifically in layer 4 (Beneyto et al., 2011). Conversely, an increase in GABA-Aα5

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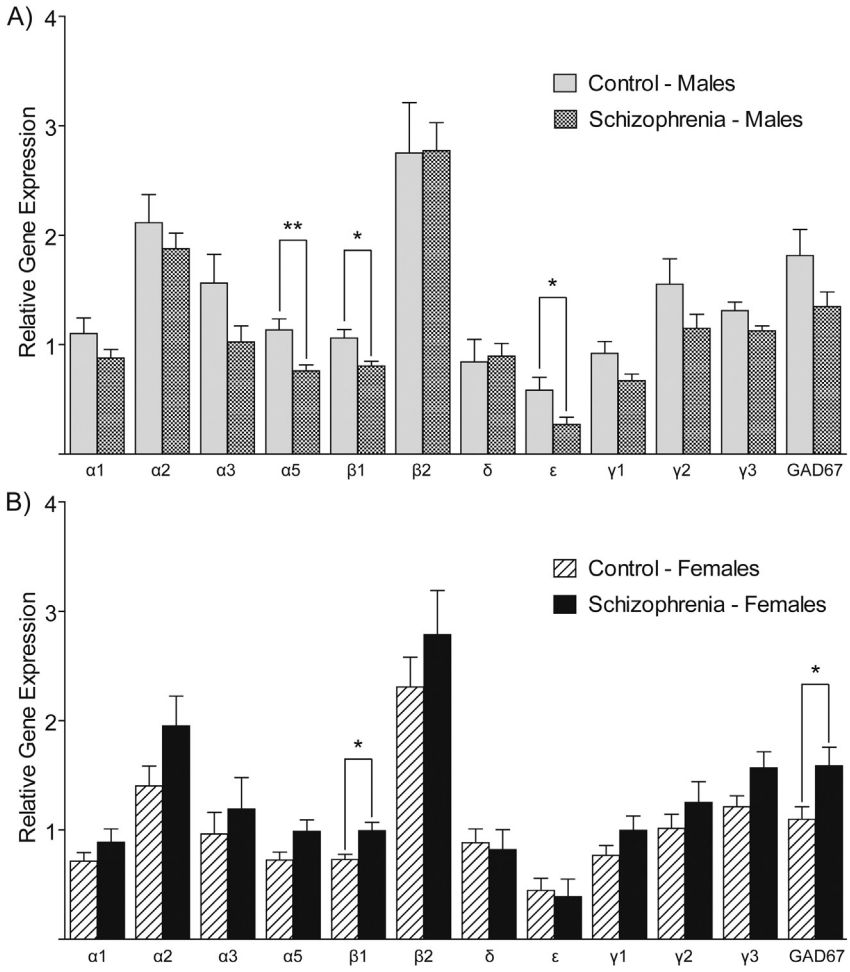

Fig. 1. Differential GABAergic gene expression occurs in the ACC of males and females with schizophrenia. The relative expression of each GABA-A receptor subunit and GAD67 is compared in schizophrenia and control subjects. (A) Male schizophrenia subjects have a generalized decrease in the expression of almost every gene tested relative to male controls. (B) Female schizophrenia subjects appear to have a generalized increase in the expression in almost every GABAergic gene tested. Post hoc analyses reveal significant alterations in the expression levels of specific genes and are summarized in the graphs. Values shown are mean ± SEM of the relative expression of each target gene normalized to the geometric mean of three housekeeping genes (GUSB, PPIA, and GAPDH), measured by qPCR. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

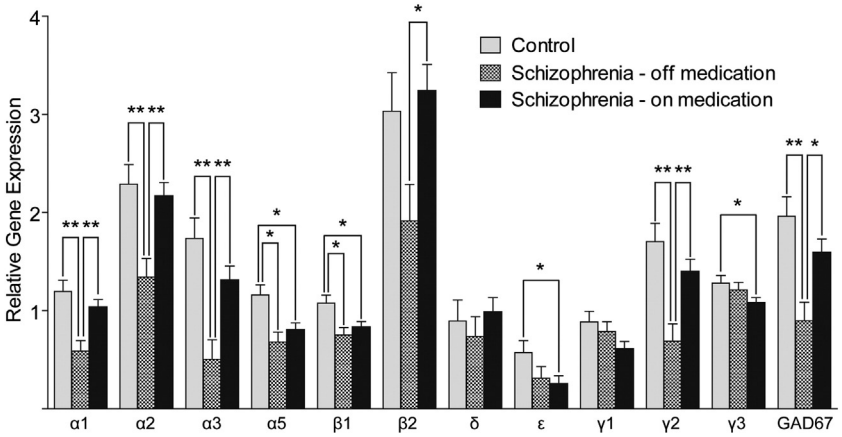

Fig. 2. Medication appears to "correct" GABAergic deficits in the ACC in male patients with schizophrenia. The relative expression of each GABA-A receptor subunit and GAD67 is compared in schizophrenia subjects on and off medication at the time of death. Male schizophrenia subjects on medication have a generalized increase in the expression of the majority of genes tested relative to the patients off medication. Post hoc analyses reveal significant alterations in the expression levels of specific genes; the off medication group had significantly lower expression compared with both the controls and the on medication groups for the genes encoding GABA-Aα1 ($p = 0.005$ and 0.006, respectively), GABA-Aα2 ($p = 0.010$ and 0.005), GABA-Aα3 ($p = 0.003$ and 0.007), GABA-Aγ2 ($p = 0.004$ and 0.008), and GAD67 ($p = 0.005$ and 0.013); expression in the control group was higher than both the on and off medication schizophrenia groups for GABA-Aα5 ($p = 0.012$ for both) and GABA-Aβ1 ($p = 0.023$ and 0.024, respectively), medicated schizophrenia cases showed higher expression of GABA-Aβ2 than the off medication group ($p = 0.015$), the control group had higher expression of GABA-Aγ3 ($p = 0.050$) and GABA-Aε ($p = 0.049$) than the medicated schizophrenia group, and there were no changes of expression for GABA-Aδ and GABA-Aγ1. Values shown are mean + SEM of the expression of each target gene normalized to the geometric mean of three housekeeping genes (GUSB, PPIA, and GAPDH), measured by qPCR. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

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Table 3
GABAergic gene expression in the ACC—effects of medication in male subjects.

		Control		Schizophrenia Off medication		Schizophrenia On medication			
		$N = 5$		$N = 6$		$N = 8$			
Gene	Protein	Mean	SEM	Mean	SEM	Mean	SEM	$F_{2,13}$	P
GABRA1	GABA-Aα1	1.20	0.11	0.59	0.11	1.04	0.08	6.67	0.010*
GABRA2	GABA-Aα2	2.29	0.20	1.34	0.19	2.17	0.14	6.33	0.012*
GABRA3	GABA-Aα3	1.74	0.21	0.50	0.20	1.31	0.15	7.40	0.007*
GABRA5	GABA-Aα5	1.16	0.10	0.68	0.10	0.80	0.07	5.19	0.022*
GABRB1	GABA-Aβ1	1.08	0.08	0.75	0.08	0.83	0.06	3.98	0.045*
GABRB2	GABA-Aβ2	3.03	0.39	1.92	0.37	3.24	0.27	3.95	0.046*
GABRD	GABA-Aδ	0.90	0.21	0.74	0.20	0.99	0.15	0.49	0.625
GABRE	GABA-Aε	0.57	0.13	0.31	0.12	0.25	0.09	2.36	0.134
GABRG1	GABA-Aγ1	0.89	0.11	0.79	0.10	0.61	0.08	2.84	0.095
GABRG2	GABA-Aγ2	1.70	0.19	0.69	0.18	1.40	0.13	6.67	0.010*
GABRG3	GABA-Aγ3	1.28	0.08	1.21	0.08	1.08	0.05	2.96	0.088
GAD1	GAD67	1.96	0.20	0.90	0.19	1.59	0.14	6.14	0.013*

Control subjects have no history of psychiatric disorder and no antipsychotic medication treatment; schizophrenia subjects are grouped based on whether or not they were on medication at time of death. Data were analyzed by univariate ANOVA with PMI, pH, and age of death included as covariates. *$p \leq 0.05$.

expression has been reported in the same region (Impagnatiello et al., 1998). Targeting this GABA receptor subunit may be therapeutic in schizophrenia. Indeed, a GABA-A receptor positive allosteric modulator that binds to the GABA-Aα5 subunit, has been shown to reverse dopaminergic hyperactivation in a rodent model of schizophrenia (Gill et al., 2011).

The abnormal expression of GABA-Aα5 may contribute to the cognitive symptoms experienced by patients with schizophrenia. The GABA-Aα5 subunit is reported to play a role in spatial memory (Collinson et al., 2002), although global ablation of this subunit in GABRA5 knockout mice resulted in improved performance in memory and learning (Olsen and Sieghart, 2008). These data indicate that altered expression of GABA-Aα5 may disrupt the pathways necessary for optimal cognitive function. The GABA-A receptors containing the α5 subunit are located at extrasynaptic sites (Brunig et al., 2002), and these extrasynaptic GABA-A receptors are required for optimal tonic inhibitory function (Yamada et al., 2007; Brickley and Mody, 2012). Impaired tonic inhibition has previously been implicated in schizophrenia. The δ subunit of the GABA-A receptor which, like GABA-Aα5, is also predominantly within extrasynaptic GABA receptors (Zheleznova et al., 2009), is also reported to have lower expression in schizophrenia (Maldonado-Aviles et al., 2009). Moreover, our data indicated reduced levels of GABA-Aε expression in the ACC of males with schizophrenia. The role of the GABA-Aε subunit in behavior is relatively unknown, but this subunit appears to confer insensitivity to anesthesia (Thompson et al., 2002) and to treatment with benzodiazepine drugs (Thompson et al., 1998; Kasparov et al., 2001; Belujon et al., 2009). GABA-A receptors containing the GABA-Aε subunit are unusual because they exhibit spontaneous activity (Neelands et al., 1999). Furthermore, there have been indications that receptors containing GABA-Aε are located extrasynaptically and contribute to tonic inhibition (Li et al., 2013). Therefore, our GABA-Aα5 and GABA-Aε findings lend further support to the notion that altered tonic inhibition by GABA may contribute to the pathophysiology of schizophrenia (Maldonado-Aviles et al., 2009).

Reduced GAD67 expression is a consistent finding in schizophrenia (Akbarian et al., 1995; Guidotti et al., 2000; Volk et al., 2000; Woo et al., 2004; Hashimoto et al., 2005, 2008a,b; Thompson et al., 2009; Duncan et al., 2010; Curley et al., 2011; Kimoto et al., 2014); however, we observed increased GAD67 expression in the ACC of the female schizophrenia group. It is possible that this difference may be due to medication because we observed that the expression of GAD67 was significantly higher in patients who were on medication compared with those who were off medication (Fig. 2; Table 3). Indeed, elevated GAD67 expression has been detected in the ACC of rodents after treatment with antipsychotic drugs (Zink et al., 2004a,b).

The differential gene expression in males and females with schizophrenia may contribute to the sex differences observed in this disorder. While the frequency of schizophrenia is similar for both sexes (Saha et al., 2005), male patients have an earlier age of onset compared with female patients (Goldstein et al., 1990; Faraone et al., 1994; Szymanski et al., 1995; Leung and Chue, 2000; Eranti et al., 2013; van der Werf et al., 2014). Sex differences have also been noted in response to antipsychotic treatment (Usall et al., 2007; Smith, 2010), perhaps because male schizophrenia patients exhibit a greater level of negative symptoms which are relatively unresponsive to current antipsychotic treatments (Chue and Lalonde, 2014). The increased levels of GABA-Aβ1 (and perhaps also GAD67) in the ACC of female patients may contribute to their increased vulnerability to depressive symptoms compared with male schizophrenia patients (Goldstein and Link, 1988; Andia et al., 1995; Hafner, 2003).

As with any study using postmortem human tissue, there are limitations to this work. First, while the sample size has sufficient power to detect relatively large effect sizes when the entire group was considered, this statistical power would be reduced after subgrouping the subjects by sex. This postmortem cohort differs from others previously tested because there is very little substance abuse in these subjects, and a relatively high average age (Table 1). It is beyond the scope of the current study to determine whether the differences reported here are also present in the earlier stages of schizophrenia. The results were also not corrected for multiple comparisons. Therefore, these findings may require further validation through replication in other postmortem schizophrenia cohorts.

Potential confounding effects of antipsychotic medication in the schizophrenia group were explored by comparing male patients who were medicated with male patients who were unmedicated at their time of death. Treatment of male rats with haloperidol and clozapine leads to increased expression of GAD67 and GABA-A receptors in the ACC (Zink et al., 2004a,b). Our data indicate that a similar effect may occur in human subjects treated with antipsychotic drugs (Fig. 2). Higher expression levels of GAD67 and the GABA-A subunits α1, α2, α3, β2, and γ2 were detected in patients who were on-medication while male patients who were off-medication had lower levels of GABAergic gene expression. Therefore, antipsychotic medication may "correct" the expression levels of GABAergic genes to the levels observed in the male controls (Fig. 2; Table 3). It was beyond the scope of this study to fully determine whether medication was the sole cause of differences in GABAergic gene expression observed, and we were also unable to determine the effect of medication in females because only two female schizophrenia cases were off medication at time of death. Therefore, the extent to which GABAergic targets are

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modulated by currently prescribed antipsychotic medication requires further investigation. Given the sex differences reported here, future investigations of the effects of medication on GABAergic gene expression in females could reveal interesting data.

Our ongoing studies will determine whether the differences in GABAergic gene expression we have observed are specific to particular cell types or cortical layers. The current data provided an overview of GABAergic gene expression across the cortex because we included all gray matter layers in our analyses. Previous studies have indicated differential GABA receptor binding in schizophrenia in specific cortical layers of the ACC (Benes et al., 1992).

In summary, our data show that sex differences in the expression of GABAergic genes occur in the anterior cingulate cortex in schizophrenia and that antipsychotic medications may influence the regulation of GABAergic genes in this critical brain region. These data indicate that molecular pathways, including the GABAergic genes, contribute to the pathophysiology and treatment of schizophrenia and provide further support to the notion that the GABAergic system contains novel targets for antipsychotic drug development.

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Contributors

MS designed the study, VH provided the post-mortem tissue used in the study, and JB performed the experimental procedures and wrote the initial draft of the manuscript. GB conducted the statistical analyses, GB and MS wrote the manuscript, and VH provided critical comments. All authors approve the final manuscript.

Conflict of interest

All authors declare they have no conflicts of interest.

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