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Elevated delta-6 desaturase (*FADS2*) expression in the postmortem prefrontal cortex of schizophrenic patients: Relationship with fatty acid composition

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Abstract

Although emerging evidence suggests that schizophrenia (SZ) is associated with peripheral and central polyunsaturated fatty acid (PUFA) deficits, there is currently nothing known about the expression of genes that mediate PUFA biosynthesis in SZ patients. Here we determined Δ5 desaturase (*FADS1*), Δ6 desaturase (*FADS2*), elongase (*HELO1* [*ELOVL5*]), peroxisomal (*PEX19*), and Δ9 desaturase (stearoyl-CoA desaturase, *SCD*) mRNA expression, and relevant fatty acid product:precursor ratios as estimates of enzyme activities, in the postmortem prefrontal cortex (PFC) of patients with SZ (n=20) and non-psychiatric controls (n=20). After correction for multiple comparisons, *FADS2* mRNA expression was significantly greater in SZ patients relative to controls (+36%, p=0.002), and there was a positive trend found for *FADS1* (+26%, p=0.15). No differences were found for *HELO1* (+10%, p=0.44), *PEX19* (+12%, p=0.44), or *SCD* (−6%, p=0.85). Both male (+34%, p=0.02) and female (+42%, p=0.02) SZ patients exhibited greater *FADS2* mRNA expression relative to same-gender controls. Drug-free SZ patients (+37%, p=0.02), and SZ patients treated with typical (+40%, p=0.002) or atypical (+31%, p=0.04) antipsychotics, exhibited greater *FADS2* mRNA expression relative to controls. Consistent with increased Δ6 desaturase activity, SZ patients exhibited a greater 20:3/18:2 ratio (+20%, p=0.03) and a positive trend was found for 20:4/18:2 (+13%, p=0.07). These data demonstrate abnormal, potentially compensatory, elevations in Δ6 desaturase (*FADS2*) expression in the PFC of SZ patients that are independent of gender and antipsychotic medications. Greater Δ6 desaturase expression and activity could have implications for central prostaglandin synthesis and proinflammatory signaling.

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Contributors

R. McNamara designed the study and wrote the manuscript. R. Jandacek, T. Rider, and P. Tso performed the gas chromatography. Y. Liu performed the RT-PCR analysis. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

None of the authors have any actual or potential conflict of interest, including any financial, personal or other relationships with other people or organizations within three (3) years of beginning the work submitted that could inappropriately influence, or be perceived to influence, their work.

Keywords

Schizophrenia; Postmortem; Prefrontal cortex; Saturated fatty acids; Monounsaturated fatty acids; Polyunsaturated fatty acids; Stearoyl-CoA desaturase (*SCD*); Delta-5 desaturase (*FADS1*); Delta-6 desaturase (*FADS2*); Elongase (*HELO1*); Peroxisome (*PEX19*)

1. Introduction

Because mammals are incapable of synthesizing omega-6 and omega-3 fatty acids *de novo*, they are entirely dependent on dietary sources to procure and maintain adequate peripheral and central tissue concentrations. The principal polyunsaturated fatty acids (PUFA) in brain phospholipids are the omega-6 fatty acid arachidonic acid (AA, 20:4*n*-6) and the omega-3 fatty acid docosahexaenoic acid (DHA, 22:6*n*-3). The dietary omega-6 fatty acid precursor linoleic acid (LA, 18:2*n*-6) and omega-3 fatty acid precursor alpha-linolenic acid (ALA,18:3*n*-3) are converted to AA and DHA, respectively, through a series of common and competitive microsomal desaturation–elongation reactions, and DHA requires additional conversions within peroxisomes (reviewed in Sprecher and Chen, 1999). Principal human PUFA biosynthetic genes include *FADS1* (Δ5 desaturase, Cho et al., 1999a), *FADS2* (Δ6 desaturase, Cho et al., 1999b), *HELO1* [*ELOVL5*] (elongase, Leonard et al., 2000), and genes required for peroxisome assembly including *PEX19* (Götte et al., 1998). The principal enzyme mediating monounsaturated fatty acid biosynthesis from saturated fatty acids is Δ9 desaturase (stearoyl-CoA desaturase, *SCD*, Zhang et al., 1999). All of these enzymes are highly expressed in rat and human liver and brain, and their activities and expression are regulated by multiple factors including dietary PUFA composition (Cho et al., 1999a,b; Igarashi et al., 2007), gonadal hormones and insulin (*reviewed in* Brenner, 2003), as well as heritable genetic factors (Malerba et al., 2008; Nwankwo et al., 2003; Rzehak et al., 2008; Schaeffer et al., 2006; Williard et al., 2001).

An emerging body of evidence suggests that schizophrenia (SZ) is associated with peripheral and central PUFA deficits. Specifically, DHA and/or AA deficits are observed in postmortem brain tissue of SZ patients (Horrobin et al., 1991; McNamara et al., 2007; Yao et al., 2000) and in erythrocyte (red blood cell) membranes of antipsychotic-naive first-episode psychotic patients (Arvindakshan et al., 2003; Evans et al., 2003; Kale et al., 2008; Khan et al., 2002; Reddy et al., 2004). The etiology of PUFA deficits in SZ remains poorly understood, and could involve environmental factors, including dietary PUFA insufficiency (Brown et al., 1999; Henderson et al., 2006; Strassnig et al., 2005), elevated lipid peroxidation (Arvindakshan et al., 2003; Khan et al., 2002), and/or genetic factors including polymorphisms in PUFA biosynthetic genes (Malerba et al., 2008; Nwankwo et al., 2003; Rzehak et al., 2008; Schaeffer et al., 2006; Williard et al., 2001). Consistent with deficits in LA-AA and ALA-DHA biosynthesis, some studies (Evans et al., 2003; Kale et al., 2008; Reddy et al., 2004), but not all (Khan et al., 2002), have found that antipsychotic-naïve first- or early-episode psychotic patients exhibit normal erythrocyte ALA and LA levels but significantly lower DHA and AA levels. Furthermore, emerging evidence suggests that antipsychotic medications increase erythrocyte and cortical PUFA composition in SZ patients (Arvindakshan et al., 2003; Evans et al., 2003; Kaddurah-Daouk

et al., 2007; Khan et al., 2002; McNamara et al., 2007), and preclinical (Fernø et al., 2005; McNamara et al., 2009) and clinical (Kaddurah-Daouk et al., 2007; Vik-Mo et al., 2008) studies suggest that atypical antipsychotic medications increase lipid biosynthetic enzyme activity and expression.

Together these findings support the hypothesis that impairments in PUFA biosynthesis contribute to the peripheral and central PUFA deficits observed in SZ patients, and that antipsychotic medications up-regulate PUFA biosynthesis. However, there is currently nothing known about the expression of genes that regulate PUFA biosynthesis in patients with SZ. As an initial evaluation, the present study determined elongase (*HELO1* [*ELOVL5*]), Δ5 desaturase (*FADS1*), Δ6 desaturase (*FADS2*), peroxisome (*PEX19*), and stearoyl-CoA desaturase (*SCD*) mRNA expression in the postmortem prefrontal cortex (PFC, Brodmann area 10) of patients with SZ (n=20) and non-psychiatric controls (n=20). Enzyme activities were estimated using relevant fatty acid product:precursor ratios. We have previously reported that SZ patients exhibit lower DHA (−20%) and AA (−10%) levels in the postmortem PFC (BA 10), and that these deficits were greater in males and partially attenuated by antipsychotic medications (McNamara et al., 2007). Moreover, we have found that the age-related decline in DHA (−22%) and AA (−15%) compositions in the non-psychiatric human postmortem PFC (BA10) are associated with reciprocal, apparently compensatory, increases in *FADS1*, *FADS2*, and *HELO1* mRNA expression (McNamara et al., 2008a). Based on these data, our specific prediction was that male and drug-free SZ patients would exhibit greater elongase and/or desaturase mRNA expression relative to non-psychiatric controls.

2. Methods

2.1. Postmortem brain tissues

Frozen, unfixed, postmortem PFC (BA10) from normal (no history of psychiatric illness) male and female controls (n=20) and male and female patients with DSM-IV defined SZ (n=20) were used. Brain tissue was generously provided by the Stanley Research Foundation Neuropathology Consortium (Torrey et al., 2000) and the Harvard Brain Tissue Resource Center. These tissues included samples used in our prior study (McNamara et al., 2007). Age at death (p=0.78), postmortem interval (p=0.41), brain weight (p=0.61), and brain pH (p=0.19) did not differ between controls and SZ patients (Table 1). At time of death, n=6 SZ patients were antipsychotic-free, n=6 SZ patients were receiving typical antipsychotic medications (fluphenazine, thiothixene, chlorpromazine, thioridazine, haloperidol), and n=8 SZ patients were receiving atypical antipsychotic medications (olanzapine, risperidone, clozapine). There were no significant differences in the age of controls, SZ-atypical, SZ-typical, and drug-free SZ subjects, $F(3,39)=0.61$, p=0.62. As a control for chronic atypical antipsychotic exposure, we also determined gene expression in a cohort (n=8) of similarly aged (p=0.49) patients with bipolar disorder receiving atypical antipsychotic medications (olanzapine, risperidone, quetiapine) at the time of death.

2.2. RT-PCR

The real-time reverse transcriptase polymerase chain reaction (RT-PCR) procedure has been described in detail previously (McNamara et al., 2006). Primers and fluorogenic probes (Midland Certified Reagent Company, Midland, TX) were designed using Primer Express v.2.0 software (Applied Biosystems, Foster City, CA) based on the human mRNA sequence. Primer and probe sequences are presented in Table 2. Each probe was conjugated to a FAM reporter at the 5′ end and a TAMRA quencher at the 3′ end. The reverse primer for probes spanned an exon–intron junction to obviate genomic DNA contamination. Each primer pair yielded a single band on agarose gels for *HELO1* (123 bp), *FADS1* (80 bp), *FADS2* (88 bp), *PEX19* (159 bp), and *SCD* (150 bp). Reverse transcription was performed using the 9600 GeneAmp thermocycler (Perkin-Elmer, Norwalk, CT). The relative quantities for mRNA were normalized to GAPDH mRNA values obtained from the same tissue sample. All samples were processed by a technician blinded to illness state.

2.3. Gas chromatography

The reliability and validity of the gas chromatography (GC) procedure has been described in detail previously (McNamara et al., 2008b). Briefly, total (triglyceride, phospholipid, and cholesteryl ester) fatty acid composition of frozen PFC samples (~100 mg, gray matter) was determined using the saponification and methylation methods originally described by Metcalfe et al. (1966). We have previously demonstrated that this method yields the same fatty acid composition as Folch (chloroform:methanol) extraction (McNamara et al., 2008b). Samples were analyzed with a Shimadzu GC-2014 equipped with an auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD). The column was a DB-23 (123–2332): 30 m (length), I.D. (mm) 0.32 wide bore, film thickness of 0.25 μM (J&W Scientific, Folsom CA). The GC conditions were: column temperature ramping by holding at 120 °C for 1 min followed by an increase of 5 °C/min from 120–240 °C. The temperature of the injector and flame ionization detector was 250 °C. A split (8:1) injection mode was used. The carrier gas was helium with a column flow rate of 2.5 ml/min. Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Analysis of fatty acid methyl esters is based on areas calculated with Shimadzu Class VP 4.3 software. Fatty acid composition data are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids, wt.% total). We have previously demonstrated that wt.% total data are highly correlated with total mass data (nmol/g) (r=0.995, $p \leq 0.0001$) (McNamara et al., 2008b). All samples were processed by a technician blinded to illness state.

2.4. Statistical analysis

Analyses of variance (ANOVA) were performed using GBSTAT (Dynamic Microsystems, Inc., Silver Springs MD). The hypothesis that lipid biosynthetic gene expression (mRNA/GAPDH mRNA) differs by illness state was tested in a two-factor ANOVA, with Illness (Controls, SZ) and Gene (*FADS1*, *FADS2*, *HELO1*, *PEX19*, *SCD*) as main factors. Post-hoc tests (2-tailed) of simple effects were performed using the Bonferroni correction with a group-wise error rate of α=0.05 to evaluate illness state effects for the five different genes (α=0.05/5=0.01). For analysis of gender, a three factor ANOVA was used with Illness

state (Controls, SZ), Gender (male, female), and Gene as main factors. A one factor ANOVA tested the effects of antipsychotic exposure among controls, and drug-free, typical antipsychotic-treated and atypical antipsychotic-treated SZ patients. Parametric linear regression analyses were performed to determine the interrelationship between relevant fatty acids ratios and gene expression. Exploratory analyses were conducted using Student's *t*-tests (2-tailed, α=0.05), and effect size calculated using Cohen's *d*, with small, medium, and large effect sizes being equivalent to *d*-values of 0.30, 0.50, and 0.80, respectively.

3. Results

3.1. Illness and gender effects

The two-factor ANOVA found significant main effects of Illness, $F(1,196)=7.93$, $p=0.005$, and Gene, $F(4,196)=7.86$, $p\leq0.0001$, and the Illness×Fatty Acid Interaction, $F(4,196)=1.34$, $p=0.254$, was not significant. The significant main effect of illness is attributable to numerically greater expression of all PUFA biosynthetic genes, but not SCD, in SZ patients (Fig. 1A). After correction of multiple comparisons, *FADS2* mRNA expression was significantly elevated in SZ patients relative to controls (+36%, $p=0.002$, $d=1.1$) and positive trends were found for *FADS1* (+26%, $p=0.15$), *HELO1* (+10%, $p=0.44$), and *PEX19* (+12%, $p=0.44$) (Fig. 1A). *SCD* mRNA expression did not differ between groups (−6%, $p=0.85$). The three factor ANOVA found that the main effect of Gender, $F(1,177)=2.14$, $p=0.15$, the Illness×Gender interaction, $F(4,177)=2.24$, $p=0.14$, and the Illness×Gender×Gene Interaction, $F(4,177)=0.49$, $p=0.99$, were not significant. Both male (+34%, $p=0.02$, $d=1.0$) and female (+42%, $p=0.02$, $d=1.4$) SZ patients exhibited greater *FADS2* mRNA expression relative to same-gender controls (Fig. 1B). Interestingly, female SZ patients exhibited numerically greater *FADS1* (+27%, $p=0.33$) and *FADS2* (+17%, $p=0.32$) mRNA expression relative to male SZ patients.

3.2. Antipsychotic medications

Analyses of antipsychotic treatment effects found a significant main effect for *FADS2*, $F(3,39)=3.9$, $p=0.017$. Relative to controls, *FADS2* expression was greater in drug-free SZ patients (+37%, $p=0.02$, $d=1.4$) and in SZ patients treated with atypical (+31%, $p=0.04$, $d=0.8$) and typical (+40%, $p=0.002$, $d=1.4$) antipsychotic medications (Fig. 2A). *FADS2* expression in bipolar patients receiving atypical antipsychotic medications did not differ from controls (−4%, $p=0.81$), and was numerically smaller than SZ patients treated with atypical antipsychotic medications (−35%, $p=0.12$) (Fig. 2B). Analyses for antipsychotic treatment did not find a significant main effect for *FADS1*, $F(3,39)=1.9$, $p=0.15$, *HELO1*, $F(3,39)=0.6$, $p=0.59$, *PEX19*, $F(3,39)=0.3$, $p=0.86$, or *SCD*, $F(3,39)=1.1$, $p=0.37$. Furthermore, *FADS1*, $F(3,39)=0.03$, $p=0.96$, *HELO1*, $F(3,39)=0.3$, $p=0.76$, *PEX19*, $F(3,39)=0.2$, $p=0.79$, and *SCD*, $F(3,39)=1.9$, $p=0.17$, expression did not differ among controls and SZ and bipolar patients treated with atypical antipsychotic medications (Fig. 2B).

3.3. Fatty acid ratios

Consistent with increased Δ6 desaturase (*FADS2*) activity, SZ patients exhibited a greater 20:3/18:2 ratio (+20%, $p=0.03$) and a positive trend was observed for 20:4/18:2 (+13%,

p=0.07) relative to controls (Table 3). There were no significant group differences for indices of Δ5 desaturase (*FADS1*) activity (20:4/20:3, −5%, p=0.46), *HELO1* activity (22:4/20:4, +6%, p=0.37), or *SCD* activity (18:1/18:0, +4%, p=0.55; 16:1/16:0, +2%, p=0.69) (Table 3). Analysis by gender for the 20:3/18:2 ratio found a significant main effect of Illness, $F(1,39)=4.15$, p=0.049, and the main effect of Gender, $F(1,39)=0.14$, p=0.71, and the Illness×Gender interaction, $F(1,39)=0.62$, p=0.44, were not significant. The main effects of Illness and Gender, and the Illness×Gender interaction, were not significant for other fatty acid ratios. Analyses for antipsychotic treatment did not find a significant main effect for 20:3/18:2, $F(3,39)=1.8$, p=0.15, 20:4/18:2, $F(3,39)=1.1$, p=0.37, 20:4/20:3, $F(3,39)=0.6$, p=0.59, 22:4/20:4, $F(3,39)=0.3$, p=0.79, 18:1/18:0, $F(3,39)=0.3$, p=0.78, or 16:1/16:0, $F(3,39)=0.3$, p=0.83.

3.4. Correlations between mRNA expression and fatty acids

FADS2 mRNA expression was inversely correlated with 18:2*n*-6 composition in controls (r=−0.65, p=0.002) and a negative trend was observed in SZ patients (r=−0.27, p=0.25) (Fig. 3). *FADS2* mRNA expression was not correlated with 20:4*n*-6 composition in controls (r=−0.26, p=0.26) or SZ patients (r=−0.41, p=0.06). *FADS2* mRNA expression was positively correlated with the 20:4/18:2 ratio in controls (r=+0.68, p=0.0009) but not in SZ patients (r=+0.11, p=0.65) (Fig. 3). *FADS2* mRNA expression was not correlated with the 20:3/18:2 ratio in either controls (r=+0.04, p=0.87) or SZ patients (r=+0.36, p=0.11). *FADS1* mRNA expression was inversely correlated with DHA (22:6*n*-3) composition in SZ patients (r=−0.67, p=0.001) but not in controls (r=−0.24, p=0.31). *FADS2* mRNA expression was inversely correlated with DHA composition in SZ patients (r=−0.45, p=0.04) but not in controls (r=−0.16, p=0.50). *HELO1* mRNA expression was inversely correlated with DHA composition in SZ patients (r=−0.49, p=0.03) but not in controls (r=−0.29, p=0.21). *PEX19* mRNA expression was not correlated with DHA composition in controls (r=+0.19, p=0.42) or SZ patients (r=+0.23, p=0.34).

4. Discussion

Based on our prior postmortem studies finding apparently compensatory increases in PUFA biosynthetic gene expression in response to age-related decreases in PFC (BA 10) DHA and AA composition in normal controls (McNamara et al., 2008a), and gender-specific and antipsychotic-attenuated DHA and AA deficits in the PFC (BA10) of SZ patients (McNamara et al., 2007), it was hypothesized that male and drug-free SZ patients would exhibit compensatory increases in elongase and/or desaturase mRNA expression. Contrary to this hypothesis, we found that both male and female SZ patients exhibited greater *FADS2* mRNA expression relative to same-gender controls, and that drug-free as well as antipsychotic-treated SZ patients exhibited greater *FADS2* mRNA expression relative to controls. Consistent with elevated Δ6 desaturase (*FADS2*) activity, SZ patients exhibited a greater 20:3/18:2 ratio, and a positive trend was observed for the 20:4/18:2 ratio. However, the 20:4/18:2 ratio was positively correlated with *FADS2* mRNA expression in controls, but not in SZ patients, suggesting a potential deficit in Δ6 desaturase enzyme activity. Although numerically greater *FADS1* (+26%) and *HELO1* (+10%) mRNA expression was observed in SZ patients, they did not differ significantly from controls. *FADS1, FADS2* and *HELO1*

mRNA expression were all inversely correlated with DHA composition in SZ patients but not in controls. Collectively, these data demonstrate that SZ patients exhibit greater Δ6 desaturase (*FADS2*) mRNA expression in the postmortem PFC that is independent of gender and antipsychotic medications. Moreover, these data suggest that the central PUFA deficits observed in SZ patients cannot be attributed to deficits in PUFA biosynthetic gene expression.

This postmortem study has important limitations. Firstly, there were no data available regarding the PUFA composition of the diets of the subjects used in this study, and the PUFA content in diets of SZ patients is highly variable (Brown et al., 1999; Henderson et al., 2006; Strassnig et al., 2005). A second limitation is that fatty acid ratios represent a relatively crude index of enzyme activity. However, selective deletion (Stoffel et al., 2008; Williard et al., 2001) or pharmacological inhibition (Obukowicz et al., 1998) of Δ6 desaturase is associated with a larger membrane 20:4/18:2 ratio, and we found that *FADS2* expression was positively correlated with the 20:4/18:2 ratio in controls. A third related limitation is that the immediate Δ6 desaturase product of 18:2*n*-6, 18:3*n*-6, was below the limit of reliable detection (<0.2%) in our samples, and we were therefore unable to calculate the 18:3/18:2 ratio which represents a more definitive index of Δ6 desaturase activity. Moreover, alpha-linolenic acid (18:3*n*-3) and eicosapentaenoic acid (20:5*n*-3) were below the limit of reliable detection (<0.2%), and we were therefore unable to calculate omega-3 fatty acid product:precursor ratios. Lastly, the small number of SZ patients in gender and drug subgroups may not be a representative sample in view of the heterogeneous clinical phenotype of SZ, the variety of different medications taken by SZ patients, and other potential clinical and demographic variables. Larger sample sizes will therefore be required to confirm the present findings.

The greater *FADS2* mRNA expression observed in the postmortem PFC of SZ patients may reflect a compensatory increase in response to deficits in PFC PUFA composition. For example, we previously found that the age-related decline in DHA and AA compositions in normal human postmortem PFC (BA10) were associated with reciprocal increases in *FADS1*, *FADS2*, and *HELO1* mRNA expression (McNamara et al., 2008a). In the present study, *FADS1*, *FADS2*, and *HELO1* mRNA expression were all *inversely* correlated with DHA composition in SZ patients but not in controls, suggesting that deficits in DHA composition may contribute in part to the up-regulation of *FADS2* mRNA expression in the PFC of SZ patients. However, this interpretation is not supported by a preclinical study finding that selective dietary-induced deficits in DHA composition were not associated with elevations in Δ6 desaturase mRNA expression in rat brain (Igarashi et al., 2007). This discrepancy may suggest that reductions in both DHA and AA may be required to up-regulate fatty acid biosynthetic gene expression in the mammalian brain. Indeed, both DHA and AA decrease *FADS2* promoter activity *in vitro* (Nara et al., 2002). Nevertheless, the general pattern of greater *FADS1*, *FADS2*, and *HELO1* mRNA expression in the PFC of SZ patients is consistent with a compensatory increase in response to deficits in PFC PUFA composition.

We have previously reported that SZ patients exhibit lower DHA (−20%) and AA (−10%) levels in the postmortem PFC (BA 10), and that these deficits were partially attenuated by

antipsychotic medications (McNamara et al., 2007). Moreover, prior clinical (Arvindakshan et al., 2003; Evans et al., 2003; Kaddurah-Daouk et al., 2007; Khan et al., 2002; McNamara et al., 2007; Vik-Mo et al., 2008) and preclinical (Fernø et al., 2005; McNamara et al., 2009) studies suggest that antipsychotic medications increase peripheral and central PUFA composition in SZ patients by increasing PUFA biosynthetic enzyme expression and/or activities. In the present study, drug-free SZ patients exhibited greater *FADS2* expression relative to controls, and bipolar patients being treated with atypical antipsychotics did not exhibit greater *FADS2* mRNA expression. Together these data suggest that greater *FADS2* mRNA expression observed on the PFC of SZ patients cannot be attributed to chronic antipsychotic medication exposure, though additional controlled studies will be required to confirm this.

The greater *FADS2* mRNA expression found in the postmortem PFC of SZ patients was not associated with greater arachidonic acid (20:4*n*-6) composition, as would be predicted from the finding that reductions in *FADS2* mRNA expression or activity decrease peripheral membrane 20:4*n*-6 composition (Obukowicz et al.,1998; Stoffel et al., 2008; Williard et al., 2001). Moreover, the 20:4/18:2 ratio was positively correlated with *FADS2* mRNA expression in controls, but not in SZ patients, suggesting a potential deficit in *FADS2* translation or Δ6 desaturase activity. However, because 20:4*n*-6 is a direct precursor for inflammatory eicosanoids, these data may have implications for cyclooxygenase- and/or lipoxygenase-mediated inflammatory eicosanoid synthesis. For example, pharmacological inhibition (Obukowicz et al., 1998) or deletion (Stoffel et al., 2008) of Δ6 desaturase activity is associated with reduced indices of inflammation and impaired eicosanoid synthesis in mice. Moreover, reductions in Δ6 desaturase activity secondary to polymorphisms in the *FADS2* gene are associated with a lower prevalence of allergic rhinitis and atopic eczema (Schaeffer et al., 2006). It is relevant, therefore, that cyclooxygenase hyperactivity has been observed in platelets of drug-naïve SZ patients (Das and Khan, 1998), though evidence for elevated CSF eicosanoid levels in SZ remains controversial (Mathé et al., 1980; Nishino et al., 1998). Additionally, it will be of considerable interest to determine whether heritable genetic variants in the *FADS2* gene (Malerba et al., 2008; Nwankwo et al., 2003; Rzehak et al., 2008; Schaeffer et al., 2006; Williard et al., 2001) contribute to elevated mRNA expression and membrane fatty acid abnormalities in SZ patients.

Although the peroxisomal assembly gene *PEX19* has been found to be essential for peroxisomal formation and function (Götte et al., 1998), and peroxisomal formation disorders are associated with deficits in peripheral and central DHA and DPA composition (Martinez, 1992), in this study SZ patients did not exhibit deficits in *PEX19* mRNA expression. However, it is possible that polymorphisms within the *PEX19* gene, and/or alterations in the expression of other peroxisomal assembly genes, may contribute to impaired peroxisomal formation and function independent of mRNA expression (Matsuzono et al., 1999). In view of evidence for DPA and/or DHA deficits in erythrocytes (Arvindakshan et al., 2003; Evans et al., 2003; Kale et al., 2008; Khan et al., 2002; Reddy et al., 2004) and the postmortem PFC (McNamara et al., 2007) of SZ patients, a comprehensive interrogation for polymorphisms in peroxisomal genes in SZ patients appears warranted.

The synthesis of oleic acid (18:1*n*-9) and palmitioleic acid (16:1*n*-7) from stearic acid (18:0) and palmitic acid (16:0), respectively, is mediated by stearoyl-CoA desaturase (*SCD*) (Ntambi and Miyazaki, 2004), and we have previously found that *SCD* mRNA expression is positively correlated with indices of SCD activity (18:1/18:0, 16:1/16:0) in postmortem brain tissue (McNamara et al., 2008a). In the present study, *SCD* mRNA expression, and indices of SCD activity (18:1/18:0, 16:1/16:0), did not differ between SZ patients and controls. Interestingly, a recent clinical study found that SZ patients treated with olanzapine exhibited greater *SCD* mRNA expression in whole blood relative to untreated SZ patients (Vik-Mo et al., 2008). However, in the present study *SCD* mRNA expression in the PFC did not differ significantly between drug-free and antipsychotic-treated SZ patients. Indeed, negative trends were found for PFC *SCD* mRNA expression in SZ (−41%, p=0.13) and bipolar patients (−29%, p=0.26) treated with atypical antipsychotic medications. However, a positive trend was observed for *SCD* mRNA expression in SZ patients treated with typical antipsychotic medications (+42%, p=0.052).

In summary, the present study found greater Δ6 desaturase (*FADS2*) mRNA expression and indices of activity in the postmortem PFC of SZ patients. Greater *FADS2* mRNA expression in SZ patients was not gender-specific and was not attenuated by antipsychotic medications, suggesting that the gender-specific and antipsychotic-attenuated DHA and AA deficits found in the postmortem PFC of SZ patients (McNamara et al., 2007) cannot be attributed to deficits in desaturase mRNA expression. Greater Δ6 desaturase expression and associated activity could potentially promote inflammatory eicosanoid synthesis (Obukowicz et al., 1998; Stoffel et al., 2008), as well as cardiovascular (Warensjö et al., 2008) and metabolic symptoms (Rimoldi et al., 2001) frequently observed in SZ patients. Remarkably, it was hypothesized over 25 years ago that elevated Δ6 desaturase-mediated omega-6 fatty acid biosynthesis and prostaglandin production could contribute to the pathoaetiology of SZ (Horrobin and Huang, 1983). It is also notable that *FADS2* mRNA expression was inversely correlated with DHA composition in SZ patients but not in controls, suggesting that increasing PFC DHA composition may be an effective strategy to normalize central *FADS2* expression in SZ patients. Nevertheless, more definitive isotope tracer studies are warranted to evaluate antemortem Δ6 desaturase activity in SZ patients prior to and following treatment with antipsychotic medications.

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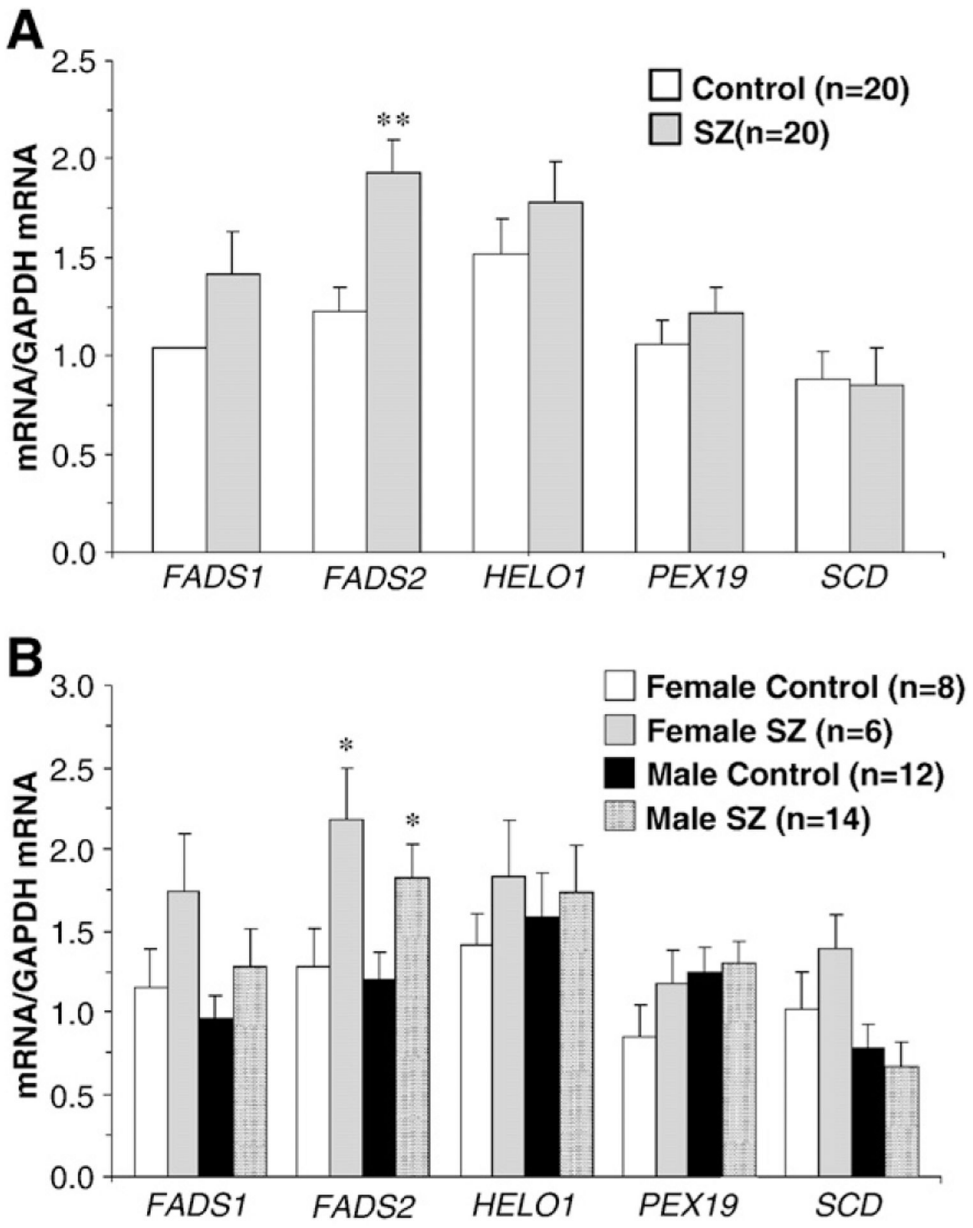

Fig. 1.

Lipid biosynthetic enzyme mRNA expression (mRNA/GAPDH mRNA) in the postmortem PFC (BA 10) of SZ patients (n=20) and non-psychiatric controls (n=20) before (A) and after (B) segregation by gender. *FADS1* (Δ5 desaturase), *FADS2* (Δ6 desaturase), *HELO1* [*ELOVL5*] (elongase), *PEX19* (peroxisome assembly), and *SCD* (Δ9 desaturase, stearoyl-CoA desaturase). Values are group mean±S.E.M. ** p=0.002 vs. controls in (A), * $P \leq 0.05$ vs. same-gender controls in (B).

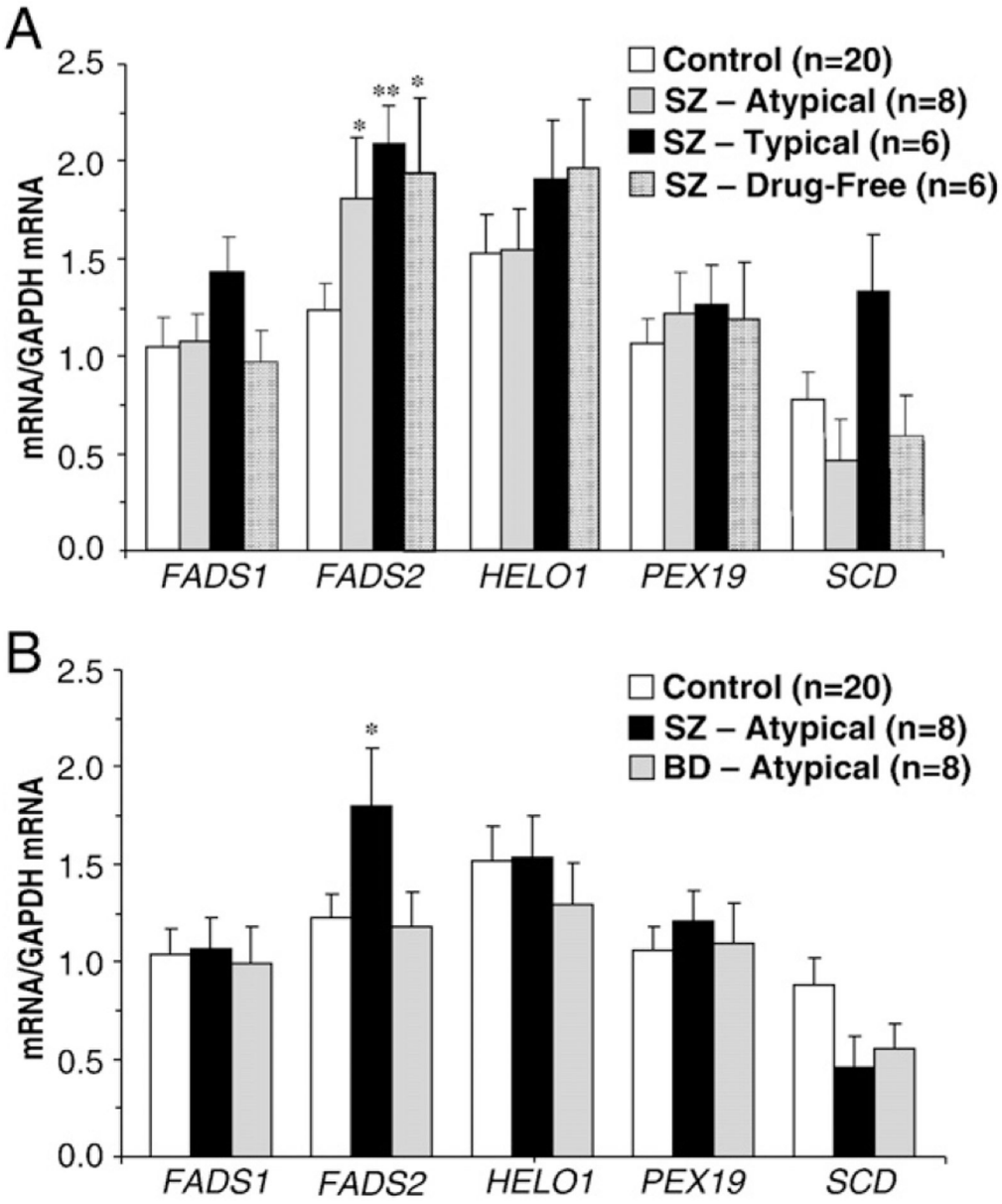

Fig. 2.

(A) Lipid biosynthetic enzyme mRNA expression (mRNA/GAPDH mRNA) in the postmortem PFC (BA 10) of non-psychiatric controls (n=20), SZ patients treated with atypical antipsychotics (n=8), SZ patients treated with typical antipsychotics (n=6), and drug-free SZ patients (n=6). (B) Lipid biosynthetic enzyme mRNA expression in non-psychiatric controls (n=20), SZ patients treated with atypical antipsychotics (n=8), and bipolar disorder (BD) patients treated with atypical antipsychotics (n=8). Values are group mean±S.E.M. *P≤0.05, **P≤0.01 vs. controls.

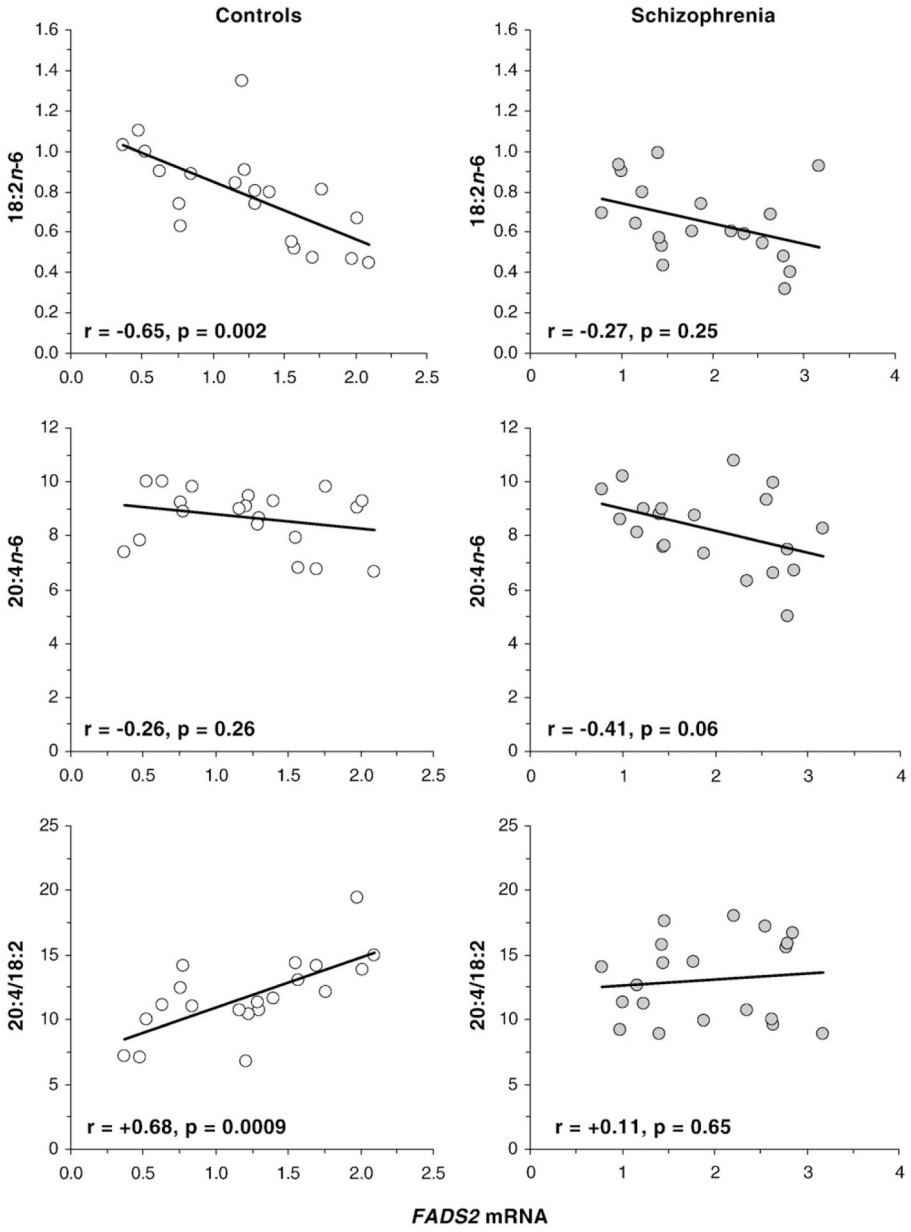

Fig. 3.

Linear regression analyses of *FADS2* mRNA expression (mRNA/GAPDH mRNA) and 18:2*n*-6 composition (top panels), 20:4*n*-6 composition (middle panels), and the 20:4/18:2 ratio (bottom panels) in controls (n=20) and patients with SZ (n=20). Note that the 20:4/18:2 ratio is positively correlated with *FADS2* mRNA expression in controls but not in SZ patients. Pearson correlation coefficients and associated p-values (two-tailed) are presented.

Table 1

Comparison of subject and brain tissue characteristics.

	Control (*n*=20)	SZ (*n*=20)	*p*-value[a]
Patient characteristics			
Age at death, mean±S.D. (range)	45.7±11.7 (29–65)	44.7±11.6 (25–65)	0.78
Gender	12M,8F	14M,6F	-
Race[b]	12C,8UN	16C,4A	-
Cause of death			
Suicide	0	3	
Cardiopulmonary	11	9	
Accident	3	3	
Other	6	5	
Tissue characteristics			
Brain hemisphere	12L/8R	9L/11R	-
Brain mass (mean grams±S.D.)	1456±162	1432±122	0.61
Postmortem interval (mean hours±S.D.)	23.4±7.9	26.1±11.9	0.41
Tissue pH (mean±S.D.)	6.3±0.3	6.2±0.3	0.19

[a] Unpaired *t*-test (2-tail).

[b] C=Caucasian, A=Asian, UN=Unknown.

Table 2

RT-PCR primer and probe sequences.

Gene (accession number)		Primer and probe sequences[a]
HELO1 (NM_021814)	F	5′-CACACTGCTGTCTCTGTATATGTTCTG-3′
	R	5′-AGGACACGGATAATCTTCATATCTGAT-3′
	P	5′-TCAGGGCACACGCACCGCAG-3′
FADS1 (NM_013402)	F	5′-TCCGCAAAGACCCAGACATC-3′
	R	5′-CTGTTTCCCAAGCTCCACAGA-3′
	P	5′-TGCATCCCTTCTTCTTTGCCTTGGG-3′
FADS2 (NM_004265)	F	5′-TTACAACATCACCAAATGGTCCAT-3′
	R	5′-GAAGGCATCCGTTGCATCTT-3′
	P	5′-CCAGCGGGTCATCGGGCACTAC-3′
PEX19 (NM_002857)	F	5′-GATCACAGAAAAGTATCCAGAATGGTT-3′
	R	5′-CGAGCCTTTTGAGTGGTTTCAC-3′
	P	5′-CAGTTTGAGGCAGAGACCCCCACAGAC-3′
SCD (NM_005063)	F	5′-CATAATTCCCGACGTGGCTTT-3′
	R	5′-AGGTTTGTAGTACCTCCTCTGGAACA-3′
	P	5′-TTCTTCTCTCACGTGGGTTGGCTGCTT-3′
GAPDH (NM_002046)	F	5′-CCACCCATGGCAAATTCC-3′
	R	5′-TGGGATTTCCATTGATGACAAG-3′
	P	5′-CGTTCTCAGCCTTGACGGTGCCA-3′

[a]Forward primer (F), Reverse primer (R), Probe (P).

Table 3

Fatty acid product: precursor ratios.

Fatty acid ratio[a]	Control (*n*=20)	SZ (*n*=20)	Δ	*d*	*p*-value[b]
Δ5-desaturase (*FADS1*)					
20:4/20:3	8.3±0.4	7.9±0.4	−5%	0.15	0.46
Δ6-desaturase *(FADS2)*					
20:3/18:2	1.4±0.1	1.7±0.1	+20%	0.64	0.03
20:4/18:2	11.4±0.6	13.1±0.7	+13%	0.42	0.07
Elongase *(HELO1)*					
22:4/20:4	0.67±0.0	0.71±0.0	+6%	0.29	0.37
Δ9-desaturase (*SCD*)					
18:1/18:0	0.93±0.0	0.97±0.0	+4%	0.20	0.55
16:1/16:0	0.03±.00	0.03±.00	+2%	0.13	0.69

Δ, Percent difference from controls.

d, Cohen's *d*-values.

[a] Values are group mean±S.E.M.

[b] Unpaired *t*-test (two-tailed).