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Elevated Delta-6 Desaturase (*FADS2*) Gene Expression in the Prefrontal Cortex of Patients with Bipolar Disorder

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Abstract

Although evidence suggests that a dysregulation in polyunsaturated fatty acid (PUFA) homeostasis may contribute to the pathoetiology of bipolar disorder (BD), there is currently nothing known about the expression of genes that mediate long-chain PUFA biosynthesis in BD patients. In the present study we determined *FADS1* ($\Delta 5$ desaturase), *FADS2* ($\Delta 6$ desaturase), *HELO1* [*ELOVL5*] (elongase), *PEX19* (peroxisome), and *SCD* (stearoyl-CoA desaturase [$\Delta 9$ desaturase]) mRNA expression in the postmortem prefrontal cortex of non-psychiatric controls (n=12) and BD patients (n=12) by real-time reverse transcriptase polymerase chain reaction (RT-PCR). Changes in the activities of corresponding enzyme products were estimated from fatty acid product:precursor ratios. After correcting for multiple comparisons, *FADS2* mRNA expression was significantly greater in BD patients relative to controls (+27%, p=0.004). Indices of $\Delta 6$ desaturase activity, including 20:4/18:2 (+18%, p=0.15) and 20:3/18:2 (+12%, p=0.25) ratios, were numerically, but not significantly, greater in BD patients relative to controls. There were no significant group differences in *FADS1* (+17%, p=0.32), *HELO1* (+4%, p=0.81), *PEX19* (-2%, p=0.91), and *SCD* (+4%, p=0.85) mRNA expression, or indices of $\Delta 5$ desaturase (+5%, 0.59), elongase (+3%, p=0.62), and stearoyl-CoA desaturase (-11%, p=0.10) activities. These preliminary findings demonstrate that *FADS2* mRNA expression is significantly and selectively elevated in the prefrontal cortex of BD patients, and may contribute to dysregulated central PUFA biosynthesis and pro-inflammatory signaling implicated in the pathophysiology of BD.

Keywords

Bipolar disorder; manic-depressive disorder; postmortem; prefrontal cortex; delta5 desaturase (*FADS1*); delta6 desaturase (*FADS2*); elongase (*HELO1*); peroxisome (*PEX19*); stearoyl-CoA desaturase (*SCD*)

1. Introduction

The principal long-chain polyunsaturated fatty acids (LC-PUFA) in brain phospholipids are the omega-6 fatty acid arachidonic acid (AA, 20:4n-6) and the omega-3 fatty acid

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docosahexaenoic acid (DHA, 22:6n-3). 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 short-chain omega-6 fatty acid precursor linoleic acid (LA, 18:2n-6) and omega-3 fatty acid precursor alpha-linolenic acid (ALA, 18:3n-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 (Sprecher & Chen, 1999). Principal enzymes that regulate LC-PUFA biosynthesis include $\Delta 5$ desaturase (*FADS1*, Cho et al., 1999a), $\Delta 6$ desaturase (*FADS2*, Cho et al., 1999b), and elongases (*HELO1* [*ELOVL5*], Leonard et al., 2000). In addition, there are multiple *PEX* gene products required for peroxisome assembly and function (Gotte et al., 1998). The genes coding for these lipogenic enzymes have been cloned and are expressed in human cerebral cortex (McNamara et al., 2008a).

Recent studies suggest that bipolar disorder (BD) is associated with a dysregulation in PUFA homeostasis, evidenced in part by abnormalities in peripheral and central LC-PUFA composition (Chiu et al., 2003; Igarashi et al., 2010; McNamara et al., 2008b, 2010). However, the etiology of this dysregulation remains poorly understood and may involve dietary LC-PUFA insufficiency (Noaghiul & Hibbeln, 2003) and/or genetic factors including polymorphisms or mutations in PUFA biosynthetic genes (Schaeffer et al., 2006; Williard et al., 2001). Importantly, *FADS1* and *FADS2* are co-localized to chromosome 11q12-11q13.1, a locus found in a genome-wide linkage study to be associated with increased susceptibility to BD (Fallin et al., 2004). However, there is currently nothing known about the expression of these genes in BD patients. In the present study, we determined the expression of principal genes involved in LC-PUFA (*FADS1*, *FADS2*, *HELO1*, *PEX19*) and monounsaturated fatty acid (*SCD*) biosynthesis in the postmortem prefrontal cortex (BA10) of non-psychiatric controls (n=12) and age-matched patients with BD (n=12).

2. Materials and methods

2.1. Postmortem brain tissue

Gene expression was determined in frozen (unfixed) postmortem prefrontal cortex (BA10) gray matter from non-psychiatric controls (n=12) and patients with DSM-IV defined BD (n=12). Tissues were supplied by the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA, USA). There were no significant group differences in age at death, postmortem interval, brain weight, or brain pH (Table 1). At time of death, all BD patients were receiving mood-stabilizer (n=11, carbamazepine, lithium, valproic acid), antipsychotic (n=7, thiorazine, olanzapine, risperidone, quetiapine, clozapine), antidepressant (n=3, paroxetine, nefazodone) and/or anxiolytic (n=6, lorazepam, clonazepam, diltiazem, clonazepam) medications.

2.2. RT-PCR

Frozen tissue was homogenized (Caframo Model RZR1 homogenizer) in Tri Reagent, and total RNA isolated and precipitated according to the manufacturer's instructions (RNeasy Lipid Tissue Mini Kit, Qiagen, Valencia, CA). The real-time reverse transcriptase polymerase chain reaction (RT-PCR) procedure, primer and probe sequences, and gene accession numbers have been described previously (McNamara et al., 2008a). 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. 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). To control for variability in RNA yield/quality, mRNA values were normalized to GAPDH mRNA values obtained from the same tissue sample (Johnston et al., 1997). All samples were processed in triplicate (and values averaged) by a technician blinded to illness state.

2.3. Gas chromatography

The gas chromatography procedure has been described in detail previously (McNamara et al., 2008b). Briefly, total fatty acid composition was determined with a Shimadzu GC-2010 (Shimadzu Scientific Instruments Inc., Columbia MD), and analysis of fatty acid methyl esters was based on area under the curve calculated with Shimadzu Class VP 4.3 software. Fatty acid identification was based on retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Primary measures were fatty acid product/precursor ratios indicative of $\Delta 6$ desaturase (20:4/18:2 and 20:3/18:2), $\Delta 5$ desaturase (20:4/20:3), elongase (22:4/20:4), and stearoyl-CoA desaturase (18:1/18:0) activities.

2.4. Statistical analysis

Group differences in individual gene expression (mRNA/GAPDH mRNA) were evaluated with unpaired *t*-tests (2-tail), and Bonferroni-adjusted for five separate comparisons ($\alpha=0.05/5 = 0.01$). Homogeneity of variance was confirmed using Bartlett's test. Effect sizes were calculated using Cohen's *d*. All analyses were performed with GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

There were no significant group differences for GAPDH mRNA expression ($p=0.54$). After correcting for multiple comparisons, *FADS2*/GAPDH mRNA expression was significantly greater in BD patients relative to controls (+27%, $p=0.004$, $d = 1.3$) (Fig. 1). Group differences in *FADS1* (+17%, $p=0.32$), *HELO1* (+4%, $p=0.81$), *PEX19* (-2%, $p=0.91$), and *SCD* (+4%, $p=0.85$) mRNA expression were not significant. *FADS2* mRNA expression did not differ between right and left hemispheres in either controls ($p=0.61$) or BD patients ($p=0.28$), and did not differ between male and female BD patients ($p=0.31$) or male and female controls ($p=0.49$). *FADS2* mRNA expression in BD patients treated with antipsychotic and mood-stabilizer medications ($n=7$) did not differ significantly from BD patients treated exclusively with mood-stabilizer medications ($n=5$) ($p=0.86$). Removal of the $n=3$ patients treated with antidepressants did not alter the greater *FADS2* mRNA expression in BD patients relative to controls (+27%, $p=0.006$). Among all subjects ($n=24$), *FADS2* mRNA expression was not correlated with tissue pH ($p=0.23$). Indices of $\Delta 6$ desaturase activity, including 20:4/18:2 (+18%, $p=0.15$) and 20:3/18:2 (+12%, $p=0.25$) ratios, were numerically greater in BD patients but were not statistically significant. Relative to controls, indices of $\Delta 5$ desaturase (20:4/20:3, +5%, $p=0.59$), elongase (22:4/20:4, +3%, $p=0.62$), and stearoyl-CoA desaturase (18:1/18:0, -11%, $p=0.10$) activities were not significantly different in BD patients.

4. Discussion

The principal finding of the present study is that patients with BD exhibit significant and selective elevations in *FADS2* mRNA expression in the postmortem frontal cortex compared with controls. *FADS2* mRNA expression did not differ between male and female BD patients, and did not differ between BD patients receiving combined antipsychotic and

mood-stabilizer medications and patients treated exclusively with mood-stabilizer medications. Although there were trends for greater indices of $\Delta 6$ desaturase activity in BD patients, these did not differ significantly from controls. These preliminary findings demonstrate that *FADS2* mRNA expression is significantly and selectively elevated in the prefrontal cortex of BD patients. Interestingly, *FADS2* mRNA expression is also significantly and selectively elevated in the prefrontal cortex of schizophrenic patients (Liu et al., 2009), suggesting that this is common to both disorders.

An important limitation of the present study is that all BD patients were being treated with several medications at the time of death. In the absence of either medication-withdrawn or medication-naïve BD patients, it is not possible to evaluate the individual and/or combined effects of these medications on the present findings. This is of particular relevance because antipsychotic medications, but not mood-stabilizers (Raeder et al., 2006), up-regulate *FADS1*, *FADS2*, and *SCD* mRNA expression in human cell lines (Polymeropoulos et al., 2009). Moreover, in a previous study we found that *FADS2* expression did not differ from controls in a younger sub-group of BD patients treated with atypical antipsychotic medications (Liu et al., 2009). However, in the present study *FADS2*, and not *FADS1* or *SCD*, mRNA expression was greater in BD patients, and greater *FADS2* expression was observed in patients with and without adjunctive treatment with antipsychotics. Furthermore, we have found that chronic treatment with the atypical antipsychotic risperidone does not alter *FADS2* mRNA expression in the rat prefrontal cortex (McNamara et al., 2009). Together, these data suggest that greater *FADS2* mRNA expression cannot be attributed to prior antipsychotic exposure, though this will need to be confirmed in future studies. A second limitation is that the race of many of the controls ($n=8$) is 'unknown', so it is not possible to evaluate whether there is a racial disparity in this cohort. In our prior study, however, we found elevated *FADS2* mRNA expression in schizophrenic patients relative to a control group with a similar racial composition (Liu et al., 2009), suggesting that this variable is not influenced by race.

Pharmacological inhibition (Obukowicz et al., 1998) or mutation (Stoffel et al., 2008; Williard et al., 2001) of $\Delta 6$ desaturase activity significantly reduces the 20:4/18:2 ratio in tissue membranes. Moreover, we have previously found that *FADS2* mRNA expression is positively correlated with indices of $\Delta 6$ desaturase activity (20:4/18:2) in postmortem prefrontal cortex, and that greater *FADS2* mRNA expression is associated with greater $\Delta 6$ desaturase activity (20:4/18:2) in schizophrenic patients (Liu et al., 2009). In the present study, BD patients exhibited numerically greater fatty acid indices of $\Delta 6$ desaturase activity though these were not statistically significant. Although this study may have been underpowered to detect a statistically significant difference in $\Delta 6$ desaturase activity, this finding may also indicate that mood-stabilizer and antipsychotic medications reduce otherwise greater $\Delta 6$ desaturase enzyme activity. This is indirectly supported by our recent finding that an index of $\Delta 6$ desaturase activity (erythrocyte 20:3/18:2 ratio) was significantly elevated in medication-withdrawn BD patients (McNamara et al., 2010). Additional studies will be required to evaluate the effects of medications on $\Delta 6$ desaturase activity.

In summary, this case-control study found that *FADS2* mRNA expression, but not expression of other key lipid biosynthetic genes, is significantly elevated in the postmortem prefrontal cortex of medicated-BD patients. This finding is of particular interest in view of evidence that *FADS2* is localized to a susceptibility locus (11q12-11q13.1) for BD (Fallin et al., 2004), and that peripheral indices of $\Delta 6$ desaturase activity are significantly elevated in medication-withdrawn BD patients (McNamara et al., 2010). It is also relevant that polymorphisms within the *FADS2* gene alter LC-PUFA biosynthesis (Schaeffer et al., 2006) and increase transcription (Lattka et al., 2010). Elevated *FADS2* mRNA expression, and associated increases in omega-6 fatty acid biosynthesis, may contribute in part to elevated

pro-inflammatory signaling cascades observed in the postmortem prefrontal cortex of BD patients (Kim et al., 2010). Together these findings suggest that *FADS2* may represent a novel BD susceptibility gene warranting further investigation.

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Figure 1.

Expression (mRNA/GAPDH mRNA) of genes that regulate LC-PUFA (*FADS1*, *FADS2*, *HELO1*, *PEX19*) and monounsaturated fatty acid (*SCD*) biosynthesis in the postmortem prefrontal cortex (BA 10) of non-psychiatric controls (n=12) and bipolar patients (n=12). After correction for multiple comparisons, only *FADS2* mRNA expression is significantly altered in BD patients. Values are group mean $\pm$ S.E.M. ** $P = 0.004$.

Table 1

Comparison of subject and brain tissue characteristics

	Control (n=12)	Bipolar (n=12)	<i>p</i> -value ¹
Patient Characteristics:			
Age at death, mean $\pm$ S.D. (range)	57.9 $\pm$ 15.4 (35-80)	59.6 $\pm$ 20.3 (25-80)	0.82
Gender	7M,5F	7M,5F	-
Race ²	4C,8UN	11C,1UN	-
Cause of death			
Suicide	0	3	
Cardiopulmonary	7	4	
Accident	0	1	
Other	5	4	
Tissue Characteristics:			
Brain hemisphere	7L/5R	4L/8R	-
Brain mass (mean grams $\pm$ S.D.)	1333 $\pm$ 226	1320 $\pm$ 245	0.90
Postmortem interval (mean hours $\pm$ S.D.)	19.7 $\pm$ 6.2	21.3 $\pm$ 7.1	0.57
Tissue pH (mean $\pm$ S.D.)	6.4 $\pm$ 0.3	6.4 $\pm$ 0.2	0.91

¹Unpaired *t*-test (2-tail)²C = Caucasian, UN = Unknown