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# Fatty acid composition and fatty acid binding protein expression in the postmortem frontal cortex of patients with schizophrenia: A case–control study

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ABSTRACT

*Background:* Abnormal levels of n-3 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA), have been found in the postmortem frontal cortex, particularly the orbitofrontal cortex, of patients with schizophrenia. Altered mRNA expression of *fatty acid binding protein* (*FABP*) *5* and *FABP7* has likewise been reported.
*Methods:* This study investigated whether PUFAs in the frontal cortex [Brodmann area (BA) 8] and mRNA expression of *FABP3*, *5*, and *7* were different between patients with schizophrenia (n = 95) and unaffected controls (n = 93).
*Results:* In contrast to previous studies, no significant differences were found in DHA between the groups. Although arachidonic acid (AA) levels were significantly decreased in the schizophrenia group, no association was found between AA and schizophrenia on logistic regression analysis. Only *FABP3* expression was significantly lower in the schizophrenia group than in the control group. Significant inverse associations were seen between only two saturated fatty acids, behenic acid and lignoceric acid, and *FABP3* expression.
*Conclusions:* We found no evidence that major PUFA levels in BA8 are involved in the etiology of schizophrenia. Although *FABP3* expression was not correlated with any of the major PUFAs, it might play a novel role in the pathology of BA8 in a subset of patients with schizophrenia.



## 1. Introduction

An emerging body of evidence in the last quarter of the twentieth century suggests that a relationship exists between polyunsaturated fatty acids (PUFAs) and schizophrenia (Mossaheb et al., 2012). The major PUFAs found in the brain are docosahexaenoic acid (DHA) (22:6 n-3) and arachidonic acid (AA) (20:4 n-6), and PUFA deficiency in brain tissue is among the pathogenic mechanisms suggested for schizophrenia. PUFA deficiency is known to change the functions of both membrane-associated proteins and cell signaling systems (Horrobin, 1998). In addition, preclinical studies have shown that n-3 PUFA administration altered functioning of the serotonergic neurotransmitter system (Chalon et al., 1998; Delion et al., 1996), a system identified to play an important role in the pathophysiology of schizophrenia.

Two meta-analyses published in 2006, however, found no conclusive evidence of symptomatic improvements from n-3 PUFA supplementation in patients with schizophrenia, although they recommended a large, well-designed clinical study be conducted to better assess the value of such treatment in schizophrenia (Freeman et al., 2006; Joy et al., 2006). A more recent meta-analysis published in 2012, which included trials using only purified eicosapentaenoic acid (EPA) (20:5 n-3) or EPA-enriched oils in schizophrenia, also showed no beneficial effects of treatment (Fusar-Poli and Berger, 2012). However, a trial conducted with adolescents and young adults who were at ultra-high risk of psychosis reported in 2010 that n-3 PUFAs not only reduced the rate of progression to first-episode psychotic disorders, but also improved positive, negative, and general symptoms of schizophrenia (Amminger et al., 2010), suggesting that interventions with PUFAs may offer a benign treatment in early schizophrenia. Moreover, two recent meta-analyses of observational studies on PUFA levels in erythrocyte membranes in schizophrenia revealed that the levels of all major PUFAs, including AA, DHA, and docosapentaenoic acid (DPA) (22:5 n-3), were decreased in

*Abbreviations:* AA, arachidonic acid; BA8, Brodmann area 8; DHA, docosahexaenoic acid; FABP, fatty acid binding protein; MF, myelin factor; PUFAs, polyunsaturated fatty acids.

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both medication-naive patients and patients taking antipsychotics (Hoen et al., 2013; van der Kemp et al., 2012), suggesting PUFA replacement as a potential treatment option for schizophrenia.

Horrobin et al. first quantified fatty acid levels of postmortem brains from patients with schizophrenia (n = 7) and controls (n = 7) in 1991 and showed decreased levels of DHA (−56%) and AA (−46%) in the frontal cortex of the patients; however, these differences were not significant when compared as fractional concentrations (i.e. percent of total fatty acids) (Horrobin et al., 1991). McNamara et al. (2007) examined the postmortem orbitofrontal cortex, specifically Brodmann area (BA) 10, and found that DHA was the only PUFA that was significantly lower, by 20%, in patients with schizophrenia (n = 21) compared with age-matched controls (n = 26). Two further postmortem brain (BA10) studies in patients with schizophrenia [n = 10 (Taha et al., 2013) and n = 15 (Tatebayashi et al., 2012)] found no differences in DHA or AA levels. Similarly, we recently reported no difference in PUFA levels in the postmortem frontal cortex (BA8) of patients with schizophrenia (n = 15), bipolar disorder (n = 15), or major depressive disorder (n = 15) when compared with unaffected controls (n = 15) (Hamazaki et al., 2015). Although nervonic acid (24:1 n-9) levels were neither significantly different nor showed significance for trend, they were 51% higher in patients with schizophrenia compared with controls. It is interesting that this difference was in the opposite direction to that reported by Amminger et al. (2012) who found a lower serum level of nervonic acid in patients with schizophrenia than in controls. It is important to note, however, that all the above mentioned postmortem studies were under-powered.

The frontal eye field (BA8) is part of the frontal cortex and is responsible for eye-tracking; in schizophrenia, dysfunction of eye-tracking is the most commonly replicated behavioral deficit [for a review, see (Levy et al., 2010)]. Two recent meta-analyses revealed large effect sizes for global and certain specific measures of eye movement dysfunction in patients with schizophrenia (O'Driscoll and Callahan, 2008) as well as in first-degree biological relatives (Calkins et al., 2008). Moreover, the responsive search score, one of the measures of eye movement dysfunction, was shown to be a very stable, biological vulnerability marker for schizophrenia in a multi-center study by the World Health Organization (Kojima et al., 2001). The reason why examining fatty acids in this particular region (BA8) is important is because Amminger et al.'s (2010) prevention trial mentioned above included first-degree relatives with a psychotic disorder (based on DSM-IV criteria) who had premorbid hypofunction. As BA8 impairment is responsible for eye movement dysfunction and can be used to screen for schizophrenia even in first-degree relatives of patients with schizophrenia (Calkins et al., 2008), we hypothesized that there might be differences in PUFA levels in BA8 between patients with schizophrenia and controls. In general, there are regional differences across the brain in PUFA composition (Diau et al., 2005) and PUFA incorporation rates (Umhau et al., 2009). Although we previously conducted a small study in this particular region (BA8), which suggested no relationship between PUFAs and psychiatric disorder (Hamazaki et al., 2015), more evidence is needed on the relationship between psychiatric disorders and fatty acid levels.

Fatty acid binding proteins (FABPs) are lipid chaperones that bind to fatty acids, such as AA and DHA, and transfer them within the cytoplasm. Ten FABP family members are known in humans, of which FABP3, 5, and 7 are localized in neural stem/progenitor cells, neurons, and glia in a cell-type specific manner [for a review, see (Matsumata et al., 2014)]. These three FABPs are regulated by complex signaling network and transcription factors, and appear to be major downstream effectors of signaling pathways such as Reelin-Dab1/Notch (Liu et al., 2010). Although all bind to long PUFAs, their preference for fatty acids is different: FABP7 shows high specificity to long PUFAs and low specificity to saturated fatty acids; FABP3 has higher affinity to short fatty acids than long PUFAs; and FABP5 binds to long PUFAs and eicosanoids (Matsumata et al., 2014).

Watanabe et al. (2007) examined human *FABP7* transcript levels in the postmortem prefrontal cortex (BA46) of patients with schizophrenia and controls and found significant up-regulation of *FABP7* mRNA in patients. Later, Shimamoto et al. (2014) examined *FABP3* and *5* transcript levels and found that *FABP5* was also significantly up-regulated in the same area. Both groups speculated that this up-regulation was due to an enduring compensatory mechanism caused by disturbances in the metabolism of n-3 PUFAs in early development. To the best of our knowledge, *FABP* mRNA levels in the postmortem frontal cortex (BA8) of patients with schizophrenia have not been reported. While examining differences in fatty acid levels in this study, we also wanted to explore whether any relationship exists between *FABP* mRNA levels and fatty acid levels. Therefore, in this study, we compared the fatty acid composition and mRNA levels of *FABP3*, *5*, and *7* in BA8 of patients with schizophrenia and unaffected controls.

## 2. Materials and methods

### 2.1. Postmortem frontal cortex samples

Brain tissue samples were obtained from the Victorian Brain Bank Network (VBBN) at the Florey Institute for Neuroscience and Mental Health. The collection of tissue was approved by the Ethics Committee of the Victorian Institute of Forensic Medicine, and the supply of tissue for the study was approved by the Tissue Access Committee of the VBBN following approval of the study by the Ethics Committee of RIKEN Brain Science Institute and the University of Toyama (No 22–56). Samples were from patients with schizophrenia (n = 95) and non-affected subjects as controls (n = 93). Samples were collected mostly from the left hemisphere (84/95 patients with schizophrenia; 83/93 controls). Gray matter blocks of the superior frontal gyrus (BA8) were cut out and rapidly frozen to −80 °C as described previously (Dean et al., 1999), and sent to RIKEN Brain Science Institute and then on to the University of Toyama.

All cadavers were stored at 4 °C within 5 h of death. Age at death, sex, postmortem interval (PMI), and brain pH were examined (Kingsbury et al., 1995). If death had been witnessed, PMI was from time of death to autopsy; if death had not been witnessed, PMI was taken as the mid-point between the times the person was last observed alive and the time found dead (maximum of 5 h) (Scarr et al., 2013). After reviewing the clinical records in each case, a psychiatric diagnosis was made based on DSM-IV criteria and using the Diagnostic Instrument for Brain Studies that allows for a consensus psychiatric diagnosis to be made after death (Hill et al., 1996; Roberts et al., 1998). For all non-psychiatric cases, case histories were extensively reviewed and family members and treating clinicians were questioned to exclude any history of psychiatric illness. A neuropsychopharmacological profile was also obtained during this review. The demographic characteristics of the study participants are shown in Table 1.

**Table 1**
Characteristics of patients with schizophrenia and controls.

| | Schizophrenia n = 95 | Control n = 93 | *p* value |
|---|---|---|---|
| Age at death (years) | 45.9 ± 17.4 | 47.9 ± 16.6 | 0.43 |
| Sex (male/female) | 70/25 | 73/20 | 0.50 |
| Postmortem interval (hours) | 41.7 ± 13.9 | 41.8 ± 13.9 | 0.98 |
| Duration of specimen period (years) | 17.2 ± 4.2 | 16.4 ± 4.5 | 0.26 |
| Brain tissue pH | 6.27 ± 0.24 | 6.16 ± 0.26 | 0.03 |
| Death by suicide (n) | 44 | 1 | <0.0001 |
| Brain hemisphere (left/right) | 84/11 | 83/10 | 1.00 |

*p* value: 2 × 2 chi-squared test for categorical variables and *t*-test for continuous variables.

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