Psychiatry Research 273 (2019) 782–787

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

An investigation of calcium-independent phospholipase A2 (iPLA2) and cytosolic phospholipase A2 (cPLA2) in schizophrenia

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ARTICLE INFO

Keywords:
Schizophrenia
Calcium-independent phospholipase A2
Cytosolic phospholipase A2
Niacin insensitivity
Meta-analysis

ABSTRACT

Evidence indicates that abnormal phospholipase A2 (PLA2) levels and niacin insensitivity are present in individuals with schizophrenia. This study was designed to determine whether differences in plasma calcium-independent phospholipase A2 (iPLA2) and cytosolic phospholipase A2 (cPLA2) exist between those with schizophrenia and healthy controls, and to explore the correlation between PLA2s and the niacin skin reaction in schizophrenic patients. We performed ELISA experiments to measure the concentrations of plasma iPLA2 and cPLA2 and we conducted a series of niacin skin tests on schizophrenic patients from the Chinese Han population. In addition, a meta-analysis of the relationship between PLA2 and schizophrenia was conducted. The plasma concentration of iPLA2 in patients with schizophrenia was significantly higher than that in healthy controls while the plasma concentration of cPLA2 did not differ. The meta-analysis also revealed that the activity level of iPLA2 in individuals with schizophrenia was higher than that in healthy controls, whereas that of cPLA2 was not. Furthermore, a significant positive correlation was found between the concentration of iPLA2 and the score for the skin flushing response within 20 min. The abnormal plasma iPLA2 concentration and its relationship with the niacin skin test in schizophrenic patients has contributed to a deeper understanding of the pathology of schizophrenia, which may in turn provide new insights into the clinical diagnoses and treatment of schizophrenia.

1. Introduction

Schizophrenia is a chronic, disabling psychiatric disorder. The pathology of schizophrenia is not definite, and many hypotheses have been proposed, such as the neurodevelopmental hypothesis, the dopamine hypothesis, the glutamatergic hypothesis, the GABAergic hypothesis and the immune hypothesis (Lang et al., 2007). However, none of these hypotheses can fully explain the pathogenesis of schizophrenia.

Many studies have found that the response to the niacin skin test in schizophrenic patients is significantly weaker than that in healthy controls (NikolicLiu et al., 2007; Sun et al., 2018; Yao et al., 2016). However, patients with other psychiatric disorders, such as unipolar depression, exhibit no response difference when compared to healthy controls (Bosveld-van Haandel et al., 2006). These phenomena indicate that the pathways of skin flushing caused by niacin may correlate with the pathology of schizophrenia. The main pathway of niacin-induced flushing has been well studied, and it has been determined that niacin activates a specific G-protein-coupled receptor, GPR109A, on the membranes of dermal macrophages, keratinocytes and Langerhans cells (Benyó et al., 2006; Pike, 2005). This activation stimulates the phospholipase A2 (PLA2)-mediated release of arachidonic acid (AA) from the cell membranes (Tang et al., 2006). AA is then converted to the prostaglandins D2 (PGD2) and E2 (PGE2), which can cause vasodilatation in vascular smooth muscle cells (Murakami and Kudo, 2004). Researchers have proposed a number of influencing factors, such as the lack of certain fatty acids in the cell membrane, decreased membrane

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https://doi.org/10.1016/j.psychres.2019.01.095
Received 1 August 2018; Received in revised form 24 December 2018; Accepted 29 January 2019
Available online 30 January 2019
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fluidity, decreased expression of niacin receptors and abnormal activity of PLA2s (Messamore et al., 2010; Miller and Dulay, 2008; Smesny et al., 2007; Tavares et al., 2003), but the mechanism remains unclear. We focus on PLA2s because they not only play an important role in the niacin skin reaction process, but they also participate in important physiological functions, including the development of the nervous system. Phospholipases A2 are a family of enzymes that hydrolyze the sn-2 substituent from membrane phospholipids to release a free fatty acid and a lysolipid (Gijón and Leslie, 1997). Cytosolic phospholipase A2 (cPLA2) is reported to be the main enzyme to mediate the release of AA from the membrane due to its 50-fold preference for phospholipids containing AA over any other polyunsaturated fatty acid (PUFA) (Law et al., 2006). In addition, an increased concentration of cyctosolic calcium caused by activated GPR109A may increase the activity level of cPLA2 (Messamore et al., 2003). Additionally, calcium-independent phospholipase (iPLA2) has been reported to mediate the release of AA in macrophages, which are involved in the niacin-related skin flushing reaction (Knowles et al., 2006; Nikolic et al., 2007). In addition, iPLA2 can alter phospholipids/fatty acid ratios and modify membrane fluidity (Atsumi et al., 2000; Balsinde and Dennis, 1997).

Furthermore, PLA2s are involved in many physiological activities. For example, iPLA2 affects brain maturation, cortical development, synaptic remodeling, glutamate receptor functions, long-term potentiation and depression, neuronal plasticity, and neurodegenerative processes, while cPLA2 participates in signal transduction, neurodegeneration, neurotransmitter release, and learning and memory (Allyson et al., 2012; Fitzpatrick and Baudry, 1994; Wolf et al., 2005). These functions are closely related to the hypothese regarding neurotransmitter abnormality etiology and neurodevelopmental etiology with respect schizophrenia. A study of a cadaver brain of a schizophrenic revealed increased iPLA2 activity and decreased cPLA2 activity in the temporal lobe (Ross et al., 1999). There are multiple lines of evidence suggesting that oxidative stress is increased in schizophrenia and that PLA2s are closely related to the increase in oxidative stress (Bošković et al., 2011; Sun et al., 2007). AA, which is primarily released by cPLA2, has prooxidant and proinflammatory properties, while DHA, which is mainly released by iPLA2, possesses antioxidant and anti-proinflammatory properties (Bazan, 2009; Farooqui, 2012). Considering the multifunctions of PLA2s, we suspect that PLA2s may be the junction between schizophrenia physiological phenotypes and niacin insensitivity. Hence, a systematic study of PLA2 will be helpful for the study of the pathology of schizophrenia.

Several studies have researched the level of iPLA2 associated with schizophrenia (Gattaz et al., 1990, 1995; Lasch et al., 2003; Ross, 1997; Šakić et al., 2016; Smesny et al., 2005, 2010) and the level of cPLA2 associated with schizophrenia (Barbosa et al., 2007; Hudson et al., 1999; Macdonald et al., 2004; Ross, 1997; Tavares et al., 2003). Considering the potential important functions of PLA2 in the pathology of schizophrenia, we recruited a sample set comprised of 53 schizophrenia patients and 19 healthy controls from the Chinese Han population and investigated the plasma concentrations of iPLA2 and cPLA2 using ELISA. In addition, meta-analyses of iPLA2 and cPLA2 levels among different ethnic groups have been conducted.

Because PLA2s are important enzymes in the biological pathway of the niacin skin test, plasma PLA2 concentrations were determined and niacin skin tests were performed on the schizophrenic patients in our study, with the aim of researching the association between iPLA2 or cPLA2 and niacin insensitivity.

2. Methods

2.1. Participants

A sample of 53 schizophrenic patients and 19 healthy controls were recruited from the Fourth People's Hospital of Wuhu located in Anhui province of China. The diagnostic criteria of schizophrenia were according to the International Classification of Diseases 10th Revision (ICD-10). The common exclusion criteria of the two groups were (1) the use of steroidal or non-steroidal anti-inflammatory drugs in the last two weeks as they may affect the niacin skin test by inhibiting the cyclooxygenase-2 (Funk, 2001); (2) the presence of diseases that could affect the skin flush response such as lupus erythematosus; (3) previous neurological diseases or other psychiatric disorders; (4) excessive smoking based on scores greater than 2 on the Fagerstrom Test for Nictine Dependence (FTND) and drinking based on scores greater than 7 on the Alcohol Use Disorders Identification Test (AUDIT-C); and (5) pregnancy. In addition, healthy controls were selected based on not having taken any psychotropic medications. All patients were undergoing inpatient treatment for a duration that ranged from two months to 38 years. All participants signed an informed consent, and the research was approved by the local ethics committee.

2.2. iPLA2 and cPLA2 determination

Venous blood (5 ml) was collected from each subject in a plastic syringe with EDTA at 8:00 AM after overnight fasting. Plasma was obtained after a 10 min blood centrifugation at 1600 × g. The storage temperature of plasma and whole blood samples was −80 °C. The plasma iPLA2 concentration was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone Corp., USA). The minimum detectable dose of iPLA2 was typically less than 30 pg/ml, and the inter-assay coefficient of variation was 3.7%. The plasma cPLA2 concentration was also determined using an ELISA kit (MyBioSource Corp., USA). The sensitivity of the kit was 10 pg/ml, and the inter-assay coefficient of variation was 3.0%.

2.3. Niacin skin test

A round filter paper patch (1.29 cm in diameter, Bio-Rad) was used to apply niacin in the form of aqueous methyl nicotinate (AMN, C7H7NO2, 99%, Sigma-Aldrich). Four concentrations of AMN solutions (0.1, 0.01, 0.001, and 0.0001 M) were freshly prepared. Before the test, a sticky ruler was attached to the inner side of each subject's forearm to easily locate the paper patches. Four dampened paper patches from each of the four AMN solutions were applied to neighboring sites on the forearm skin for 1 min and then removed. The skin flush response was photographed from a fixed vertical view at 5, 10, 15, and 20 min after the patches were removed, and the responses were rated according to the photos presented in Adobe Photoshop (Version CS6, Adobe) on a 4-point scale where 0 indicates no erythema, 1 indicates incomplete erythema, 2 indicates complete erythema within the area covered by the patch, and 3 indicates erythema beyond the definite area of the patch. The scores of the flush responses of each subject were rated twice by two research assistants trained by a same senior researcher and averaged to obtain a final score (Sun et al., 2018).

2.4. Statistical analysis

First, the Kolmogorov-Smirnov test was performed to evaluate the normality of our data. The chi-square test and the independent-samples *t*-test were then used to test the sociodemographic characteristics of the patients and the healthy controls. The non-parametric test was used to compare the difference in plasma PLA2 concentrations between the patients and healthy controls. The correlations between the plasma concentrations of PLA2 and the scores of the skin flush response at different times were analyzed using the Spearman correlation, and data analyses were performed using SPSS 18.0 software. The criterion of statistical significance was set at $p < 0.05$.

2.5. Meta-analysis: PLA2 for schizophrenia

We conducted a meta-analysis of PLA2 for schizophrenia. The

Table. 1
Sociodemographic characteristics of patients with schizophrenia (n = 53) and those in the healthy control group (n = 19).

	Schizophrenia	Control group	Statistics
Total (n)	53	19	
Sex (male / female)	25/28	9/10	χ^2 = 0.000; df = 1; p = 0.988
Age (mean ± SD)	37.36 ± 11.39	33 ± 9.60	t = 1.451; df = 70; p = 0.151
BMI (mean ± SD)	24.35 ± 3.53	23.58 ± 1.14	t = 1.337; df = 65.022; p = 0.186
Duration of disorder in years (mean ± SD)	11.27 ± 9.36	N.A.	

literature was selected using PubMed and Embase, and the search covered work up to March 2017 without language restrictions. This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Groups (Moher et al., 2009). Two authors (Xu and Sun) performed the search (last search: March 2017), assessed eligibility, and independently extracted data. The search strategy is presented in supplementary Table 1. A manual search of the references from major review articles was also completed. All articles were included if (1) patients with schizophrenia and healthy individuals as controls were included, (2) activity or expression of iPLA2 or cPLA2 were measured in both groups using any method, and (3) sufficient data to calculate standardized mean differences (SMDs) were included. The variables recorded from each included study were age, sex, antipsychotic status, method of PLA2 measurement and diagnoses.

Review Manager Version 5.2 was used to conduct the meta-analysis and sensitivity analyses. This meta-analysis used SMDs to determine the differences in PLA2 levels between patients with schizophrenia and healthy controls. If mean values or SD values were not stated, we contacted the papers' authors to acquire the data. Effects were interpreted as small (SMD = 0.2), moderate (SMD = 0.5) or large (SMD = 0.8) (Iwata et al., 2015). We employed the inverse variance statistical method and the random effects model to adjust for study heterogeneity (O'Rourke et al., 2001), and two-sided 95% confidence intervals (CIs) were used to assess significance. The risk of bias assessment tool for nonrandomized studies was employed using the following factors: participant selection, confounding variables, measurement of exposure, blinding of outcome assessment, incomplete outcome data, and selective outcome reporting (Kim et al., 2013). Funnel plots were utilized to assess publication bias, and study heterogeneity was assessed using I^2 statistic where $I^2 \geq 50\%$ indicated significant heterogeneity. Sensitivity analyses were performed by removing each study in turn from the total and reanalyzing the remaining studies when heterogeneity was found.

3. Results

3.1. Demographic characteristics

We recruited 53 schizophrenia patients and 19 healthy controls for this study. The two groups of participants matched well in age, gender and BMI (Table 1). All of the schizophrenic participants were undergoing inpatient treatment and were being treated with atypical antipsychotics. The dosage and types of atypical antipsychotics varied with each individual.

3.2. iPLA2 and cPLA2 levels in schizophrenia: the results of ELISA and Meta-analysis

All plasma iPLA2 concentrations and cPLA2 concentrations of participants were determined using ELISA, and the data do not fit a normal distribution. The standard curve of iPLA2 was established using the five-point method, and the coefficient of determination (R^2) was 0.998. The plasma iPLA2 concentration was significantly higher in patients with schizophrenia (15.37 ± 4.89 ng/ml) compared to healthy controls (9.33 ± 3.89 ng/ml, $p < 0.001$, Fig. 1.A). The standard curve of cPLA2 was established using the six-point method and the coefficient of determination (R^2) was 0.999. No significant difference in the plasma cPLA2 concentration was found between patients with schizophrenia (764.06 ± 945.21 pg/ml) and healthy controls (639.47 ± 836.53 pg/ml, Fig. 1.B). In addition, while age and BMI exhibited significant Spearman correlations with the concentration level of iPLA2 (Age r = 0.293, p = 0.033; BMI r = 0.314, p = 0.028), there was no significant difference in iPLA2 concentration between male and female (t = 0.653, df = 45.808, p = 0.517).

Meanwhile, a meta-analysis was conducted on the cPLA2 and iPLA2 in patients with schizophrenia. The flow diagram for the literature selection is presented in supplementary Fig. 1. Eleven studies were eligible for inclusion in the meta-analysis, including six studies detecting iPLA2 (Gattaz et al., 1990, 1995; Lasch et al., 2003; Šakić et al., 2016; Smesny et al., 2005, 2010), four studies detecting cPLA2 (Barbosa et al., 2007; Hudson et al., 1999; Macdonald et al., 2004; Tavares et al., 2003) and one study detecting both iPLA2 and cPLA2 (Ross, 1997). The characteristics of these included studies are presented in supplementary Table 2. In the meta-analysis of iPLA2 in schizophrenics, one study measured the concentrations of iPLA2 (NikolicLiu et al., 2007), and the other studies measured the enzymatic activities. Furthermore, one study used platelet samples (Gattaz et al., 1995), while the other six studies used serum samples. The seven studies that measured iPLA2 included 237 patients and 283 healthy controls, and two studies divided patients into two groups according to their pathogenesis (Smesny et al., 2005, 2010). In the meta-analysis of cPLA2 for schizophrenia, one study measured the concentrations of cPLA2 (Macdonald et al., 2004), while the others measured the enzymatic activities. one used red blood cell (RBC) samples (Macdonald et al., 2004), one study used platelet samples (Barbosa et al., 2007), and the others used serum samples. The five studies that measured cPLA2 included 203 patients and 210 healthy controls. The results of the risk of bias are presented in supplementary Fig. 2 and indicate the high quality of the majority of the studies.

While the iPLA2 levels were largely enhanced in schizophrenic patients compared to healthy controls (SMD = 1.44, CI = 0.72 to 2.16, p = 0.00001), heterogeneity was significant (I^2 = 92%, $p < 0.00001$) (Fig. 2). The subgroup analysis indicated that the activities of iPLA2 in schizophrenics were largely higher than those in healthy controls (SMD = 1.60, CI = 0.70 to 2.50, $p < 0.00001$) (Supplementary Fig. 3). The result of the research regarding the concentrations of iPLA2 was consistent with our research (Šakić et al., 2016).

No single result of the nine results from the seven studies contributed to the heterogeneity based on sensitivity analyses. However, based on the total funnel plot in supplementary Fig. 4, where all reported results are positive, it can be concluded that publication bias was present.

The cPLA2 levels were not significantly different between patients with schizophrenia and the healthy controls (SMD = 0.42, CI = −0.03 to 0.83, p = 0.07) (Fig. 3). In addition, the subgroup analysis indicated that the cPLA2 activities did not differ between the two groups (SMD = 0.35, CI = -0.21 to 0.92, p = 0.22) (Supplementary Fig. 5).

No single result of the six results from the five studies contributed to the heterogeneity based on the sensitivity analyses, and no publication bias was found based on the total funnel plot in supplementary Fig. 6.

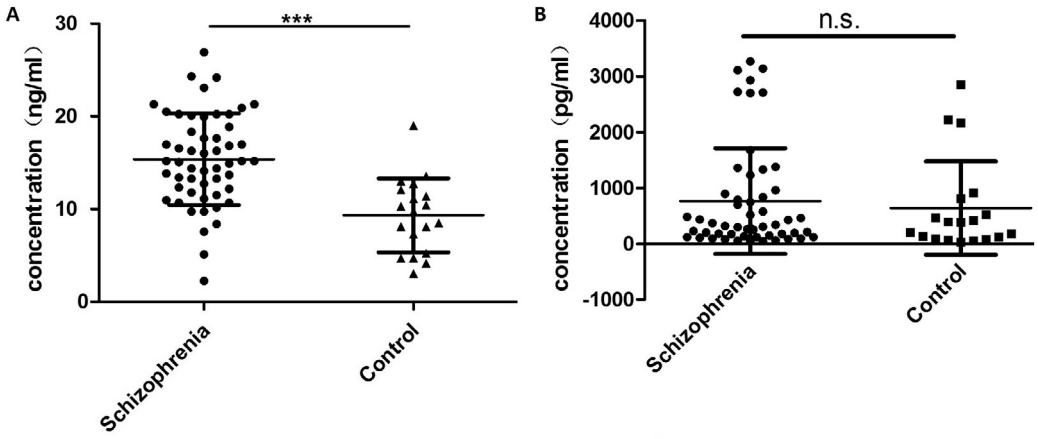

Fig. 1. Plasma iPLA2 concentrations (ng/ml) in 53 schizophrenia patients and 19 controls. B: Plasma cPLA2 concentrations (pg/ml) in 53 schizophrenia patients and 19 controls.

3.3. Correlation between iPLA2 or cPLA2 and niacin response

The 53 schizophrenia patients were administered the niacin skin test. Each patient had 21 scores for the niacin skin test: 16 raw scores (four AMN concentrations * four time points), four sum scores (of four AMN concentrations) at four time points, and one total score of all 16 raw scores. The four sum scores at four time points and the total score were used to estimate the association between the PLA2 expression level and the niacin response. The *p*-value of the Spearman correlation was decreased and the correlation coefficient increased over time between the plasma iPLA2 concentration and the niacin skin test (Fig. 4). A significant positive correlation was detected within 20 min in schizophrenic patients (Spearman $r = 0.312$, $p = 0.023$). However, the correlation between the plasma cPLA2 concentration and the niacin skin test was insignificant (data not shown).

4. Discussion

Our study was designed to research the concentrations of plasma iPLA2 and cPLA2 in schizophrenic patients. The results revealed that the concentrations of plasma iPLA2 were greater in schizophrenics compared to healthy controls while the levels of cPLA2 were not. In the meta-analysis, a subgroup analysis of prior studies regarding enzyme activity concluded that this activity promoted increased levels of iPLA2 activity in patients with schizophrenia, whereas this was not the case for cPLA2. The studies regarding concentrations, however, are too few to conduct a subgroup analysis. The heterogeneity of the meta-analysis may be caused by the different methods and sample properties used in different studies. The sensitivity analysis proved our meta-analysis results were valid and robust.

Several studies reported that increased oxidative stress and mitochondrial dysfunction were found in individuals with schizophrenia (Bošković et al., 2011; Prabakaran et al., 2004). In our preliminary work, free fatty acids (FFAs) such as palmitate and β-oxidation, which could increase oxidative stress, were significantly increased in patients with schizophrenia compared to healthy controls (Yang et al., 2017). In addition, a meta-analysis revealed that the abundance of fatty acid in red blood cell membranes was decreased in those with schizophrenia, a phenomenon that may be caused by the increased oxidative stress (Hoen et al., 2013). iPLA2 not only participates in the repair of mitochondrial injury, but it also reduces ROS-mediated apoptosis in β-cells caused by FFAs such as palmitate (Balsinde et al., 1995; Song et al., 2014). Additionally, the docosahexaenoic acid (DHA) released by iPLA2 can be metabolized into neuroprotectin D1, which has antioxidant properties (Ong et al., 2015). Based on these findings, we propose that oxidative stress level is elevated due, in part, to abnormal lipid metabolism, and as a response, the iPLA2 level in schizophrenics increases to eliminate the effects of oxidative stress. Subsequently, abnormal plasma levels of iPLA2 may affect brain maturation, gray and white matter structure and synaptic remodeling by regulating the process of membrane remodeling, neurotransmission and neuroinflammation (Ramanadham et al., 2015). However, more research is needed to clarify the relationships among iPLA2 levels, FFAs and oxidative stress in patients with schizophrenia to verify our hypothesis.

In the document retrieval process of meta-analysis, only one study researched the correlation between plasma cPLA2 activities and niacin responses in schizophrenia, while no studies have researched the correlation between iPLA2 levels and niacin responses. cPLA2 was found to

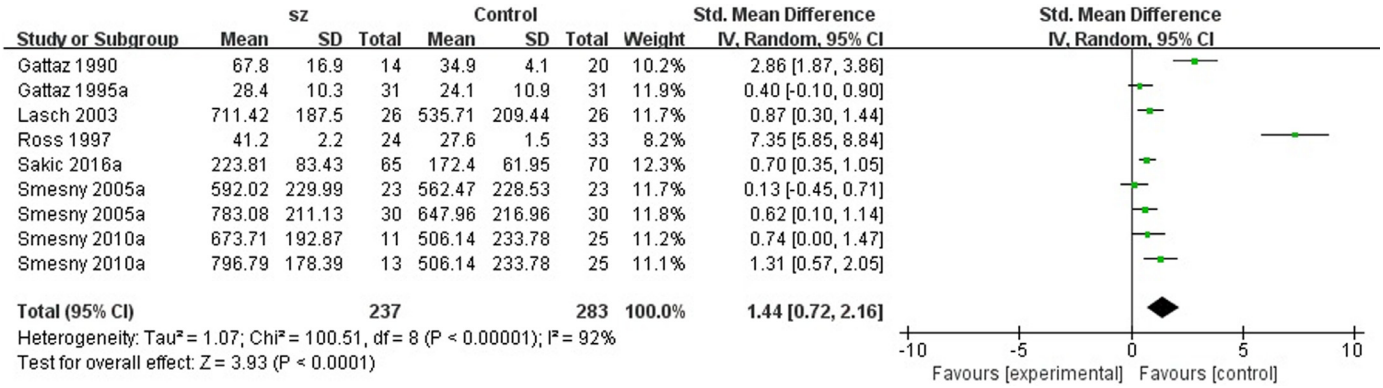

Fig. 2. Group differences in calcium-independent PLA2 levels between patients with schizophrenia and healthy controls. CI - confidence interval; IV - inverse variance; Std - standardized.

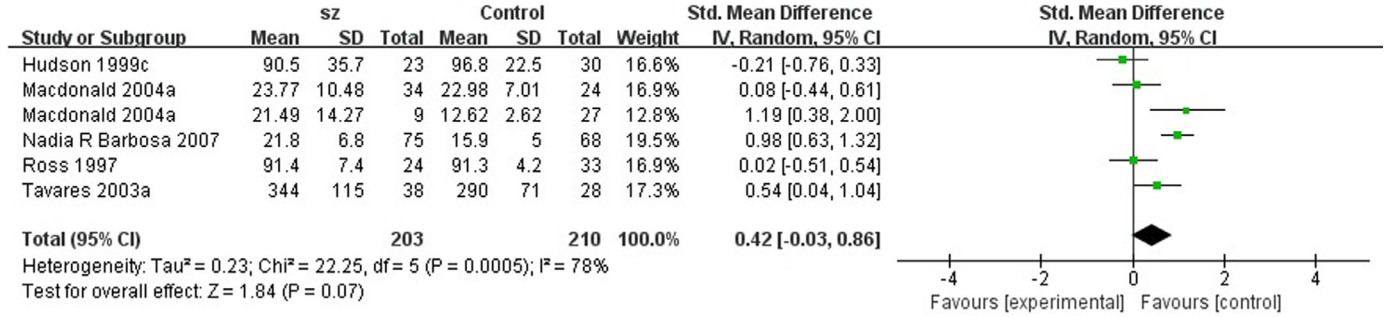

Fig. 3. Group differences in cytosolic PLA2 levels between patients with schizophrenia and healthy controls. CI - confidence interval; IV - inverse variance; Std - standardized.

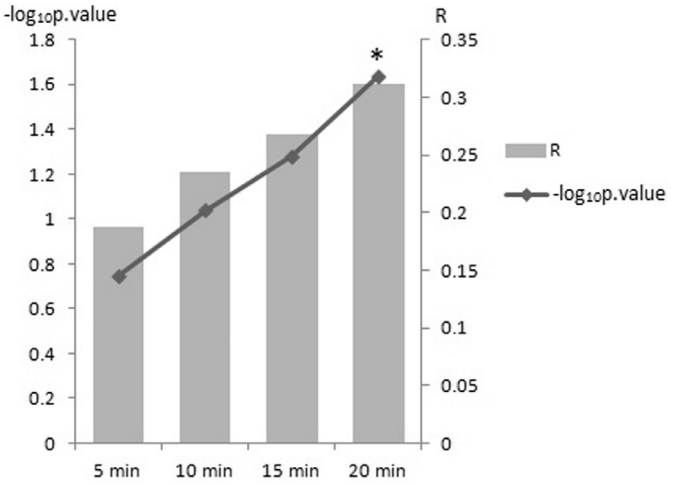

Fig. 4. The correlation between the scores of the niacin skin test and the iPLA2 concentration in schizophrenic patients ($n = 53$). R is the Spearman correlation coefficient, and the $-\log_{10} p$. value is the negative log ten operation of the p-value of the Spearman correlation between the scores of the niacin skin test and the plasma iPLA2 concentration.

have a 50-fold preference for phospholipids containing AA over any other polyunsaturated fatty acid (Clark et al., 1990), whereas iPLA2 can hydrolyze AA from the cell membrane (Nikolic et al., 2007). Given the role of cPLA2 and iPLA2 in the biological pathway of the niacin response, we administered the niacin skin test to schizophrenic patients and found a significant positive correlation between the iPLA2 concentration and the skin flush response at 20 min. The increased correlation coefficient with time suggests that the influence of iPLA2 with respect to the niacin response increases over time. In other words, the difference in dissociated arachidonic acid caused by different iPLA2 concentrations is magnified with time. Accordingly, the magnified difference of AA then causes larger differences in the skin flush response. Thus, while the correlation between the concentration of iPLA2 and niacin responses becomes stronger over time, no significant correlation was found between the cPLA2 concentration and the skin flush response at four time points. In a study published in 2003, the activity of cPLA2 increased in niacin insensitive patients (9 subjects) compared with niacin sensitive patients (13 subjects) (Tavares et al., 2003). However, these results were not repeated in our study, which employed a larger sample set. Finally, we have excluded the excessive smoking samples according to the evidence that nicotine may either increase or decrease PLA2 activity (Marin et al., 1997; Sastry and Hemontolor, 1998).

We have previously reported that patients with schizophrenia exhibit a weaker niacin skin response than do healthy controls (Sun et al., 2018). Furthermore, in this study, we simultaneously found both an increased iPLA2 concentration in schizophrenics and a positive correlation between iPLA2 and the niacin skin test. Combining these two findings, we infer that the increased concentration of iPLA2 is not the key factor influencing the decreased niacin response in schizophrenics. Future studies should systematically research this biological pathway and perform a multivariate analysis to elucidate the mechanism of niacin insensitivity in patients with schizophrenia.

In this study, we simultaneously determined the plasma iPLA2 concentration and cPLA2 concentration between schizophrenic patients and healthy controls, and concluded that the variation tendency in concentration is consistent with the variation tendency of activity discovered in the meta-analysis. In addition, we explored the relationship between the expression level of the plasma iPLA2 and cPLA2 and niacin-induced skin flush. Although some studies have researched the association between iPLA2 or cPLA2 and schizophrenia or between the niacin response and schizophrenia, the relationship between the increased iPLA2 level and the niacin insensitivities in schizophrenics remained unclarified. Our work demonstrated a significantly positive correlation between the concentration of iPLA2 and the niacin response associated with schizophrenia.

However, the sample size of healthy controls in this study was not large enough. Compared to other studies, the number of patients in the disease group in our study was comparable, whereas the number of healthy controls was smaller (Šakić et al., 2016). Furthermore, the healthy controls were not involved in the niacin skin test. Nonetheless, the correlation between niacin-induced skin flush and the concentration of PLA2s discovered in patients with schizophrenia remains meaningful. In addition, without drug cleaning, we could not eliminate the effects of drugs on the plasma concentration of PLA2s in schizophrenics. Some studies indicated the antipsychotic medications could decrease the PLA2 activity or its expression in schizophrenia (Gattaz et al., 1987; Kerr et al., 2013; Tavares et al., 2003). That said, some studies did not find any correlation between the medication state and PLA2 activity (Gattaz et al., 1995; Ross, 1997). The medication used in patients with schizophrenia is confounded, which causes the confounding factor in our results.

Acknowledgments

We thank Professor Chunbo Li for his support of the meta-analysis.

Declaration of Conflicting Interests

The author declares that there are no conflicts of interest.

Funding

This study was supported by Ministry of Science and Technology of China (2016YFC1306900, 2016YFC1306802), the National Natural Science Foundation of China (81771440), the Program for NSFC International (Regional) Cooperation and Exchange (81361120389), Grants of Shanghai Brain-Intelligence Project from STCSM

(16JC1420500), Grants of Shanghai Key Laboratory of Psychotic Disorders (13dz2260500), and Science and Technology Plan Project of Wuhu, Anhui Province (2016ZD17).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.01.095.

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