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Research paper

# Dysregulation of phospholipase and cyclooxygenase expression is involved in Schizophrenia

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ABSTRACT

*Background:* Schizophrenia (SZ) is a severe mental disease with highly heterogeneous clinical manifestations and pathological mechanisms. Schizophrenia is linked to abnormalities in cell membrane phospholipids and blunting of the niacin skin flush response, but the associations between these phenotypes and its molecular pathogenesis remain unclear. This study aimed to describe the PLA2/COX pathway, the key link between phospholipids and niacin flush, and to illustrate the pathogenic mechanisms in schizophrenia that mediate the above phenotypes.

*Methods:* A total of 166 patients with schizophrenia and 54 healthy controls were recruited in this study and assigned to a discovery set and a validation set. We assessed the mRNA levels of 19 genes related to the PLA2/COX cascade in leukocytes by real-time PCR. Plasma IL-6 levels were measured with an ELISA kit. Genetic association analysis was performed on *PLA2G4A* and *PTGS2* to investigate their potential relationship with blunted niacin-skin response in an independent sample set.

*Findings:* Six of the 19 genes in the PLA2/COX pathway exhibited significant differences between schizophrenia and healthy controls. The disturbance of the pathway indicates the activation of arachidonic acid (AA) hydrolysis and metabolization, resulting in the abnormalities of membrane lipid homeostasis and immune function, further increasing the risk of schizophrenia. On the other hand, the active process of AA hydrolysis from cell membrane phospholipids and decreased transcription of *CREB1*, *COX-2* and *PTGER4* may explain the reported findings of a blunted niacin response in schizophrenia. The significant genetic associations between *PLA2G4A* and *PTGS2* with the niacin-skin responses further support the inference.

*Interpretation:* These results suggested that the activation of AA hydrolysis and the imbalance in COX-1 and COX-2 expression are involved in the pathogenesis of schizophrenia and blunting of the niacin flush response.

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## Introduction

Schizophrenia (SZ) is a psychiatric disease associated with delusions, hallucinations, thought disorders and cognitive deficits [1], affecting approximately 0.5 to 1.0% of the population worldwide [2].

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**Research in context**

*Evidence before this study*

Schizophrenia is a severe psychiatric disorder with abnormalities in membrane phospholipids and the immune system. Various studies have found that skin flushing in response to niacin is abnormally blunted among patients with schizophrenia. The PLA2/COX pathway is the key link between phospholipids, chronic inflammation and the niacin flush response. However, it's still unclear how the PLA2/COX pathway works in schizophrenia and what role it plays in the progression of schizophrenia.

*Added value of this study*

We described the disturbance of PLA2/COX pathway in patients with schizophrenia in detail. The upregulation of the cPLA2 promotes the dissociation of arachidonic acid (AA) from the cell membrane and the catabolism of free AA is significantly disturbed. COX-1 was elevated significantly in schizophrenia, rather than COX-2 which was previously described. The metabolites could affect nervous system function by interfering with the release of neurotransmitters and inducing overexpression of cytokines such as IL-6. Meanwhile, downregulation of CREB1, COX-2 and the PGE2 receptor EP4, may be related to the blunting of the niacin skin flush response.

*Implications of all the available evidence*

These results suggested that the activation of AA hydrolysis and the imbalance in COX-1 and COX-2 expression are involved in the pathogenesis of schizophrenia and blunting of the niacin flush response. Different pathological processes seems to be integrated through the PLA2/COX pathway.

Although several etiological hypotheses have been proposed to explain schizophrenia, including neurotransmitter abnormalities, developmental or neurodegenerative processes and immune system disruption, none of these hypotheses completely explains the complex symptoms exhibited by different patients [3]. Due to our limited understanding of the etiology and mechanism of schizophrenia, we have not yet fully exploited these changes as potential diagnostic and therapeutic targets.

Among the many pathways under study, the phospholipase A2 (PLA2)/cyclooxygenase (COX) pathway attracted our attention as it is an important link between schizophrenia pathogenic hypotheses and niacin-endophenotypes, reflected mainly by the following three observations:

First, equilibrium of membrane phospholipids catabolized by PLA2 is related to schizophrenia. Phospholipids and essential polyunsaturated fatty acids (PUFAs) play critical roles in membrane structure and function [4]. PUFAs are particularly important in signal transduction via receptor-mediated, phospholipid-derived second messengers [5]. In 1994, Horrobin postulated the "membrane phospholipid hypothesis" of schizophrenia, which states that inappropriate levels of membrane fatty acids may be a factor contributing to schizophrenia [6]. Arachidonic acid (AA) is one of the most important omega-6 PUFAs, which determine the quantity and quality of membrane phospholipids. The abundance of AA is critical to behavioural development, pathologies of the brain [7], and membrane signal transduction [8]. Lower levels of AA have been reported in red blood cell (RBC) membranes both from medicated patients with chronic schizophrenia [9] and from never-medicated schizophrenia patients [5] than in those from control individuals, but the cause of the abnormality is unclear. The processes of AA binding to (via ACSL4-mediated catabolism) and dissociating from (via PLA2-mediated catabolism) phospholipids are in dynamic equilibrium, and abnormalities in either process could lead to changes in the functioning of the membrane and of cell signalling systems, which may be involved in the schizophrenia pathology [10].

Second, metabolites of free AA catabolized via the COX pathway are closely related to immune function. Phospholipid hydrolysis produces free AA, the level of which has been reported to be increased in schizophrenia patients [11]. Free AA can be metabolized via three main catabolic pathways—the COX pathway, LOX pathway and CYP pathway—and the downstream metabolites are involved in a variety of biological processes, including neurotransmitter function and release, immune function, inflammation, and pain regulation. Among the downstream metabolites, the 2-series prostaglandins (PGs) catabolized by the COX pathway have been reported to be closely related to schizophrenia. The main function of PGs is to mediate inflammation in the surrounding tissues. For example, prostaglandin E2 (PGE2) and PGI2 can promote the expression of IL-6 [12,13]. The pro-inflammatory cytokines IL-6 could interfere with the actions of various neurotransmitters and correlated to features of schizophrenia [14,15]. More interestingly, the decreased membrane PUFAs, especially AA, were associated with increased secretion of IL-6 (not IL-10) in schizophrenia [16,17].

Third, the PLA2/COX pathway is involved in the niacin skin flush reaction. Skin flushing in response to niacin is abnormally blunted among a subset of patients with schizophrenia and is considered a potential marker for schizophrenia [18,19]. The biochemical basis of the niacin skin flush response is reasonably well understood. Niacin interacts with a specific G protein-coupled receptor, GPR109A (gene symbol: *HCAR2*); its activation stimulates PLA2-mediated release of AA from cell membranes. AA is then converted to vasodilatory prostaglandin molecules via a reaction catalyzed by cyclooxygenase-2 (COX-2). Dysregulation of the PLA2/COX-2 cascade pathway may affect the niacin flush response [20], and the specific etiology of niacin flush response blunting in schizophrenia remains unclear.

The PLA2/COX pathway is the key link among phospholipids, chronic inflammation and the niacin flush response. In this study, we used qPCR to systematically evaluate the transcription levels of PLA2/COX cascade-related genes in patients with schizophrenia and in normal controls. We aimed to illustrate the pathogenic mechanisms in schizophrenia that mediate the above phenotypes.

## Methods

*Participants*

Patients with schizophrenia were recruited from the Fourth People's Hospital of Wuhu in Anhui Province, China. The patients in the discovery set were drug-naive or had experienced relapse after at least 1 month without any antipsychotic drugs, and the patients in the validation set were inpatients under treatment. The inclusion criteria for participants in the SZ group were (a) diagnosis of schizophrenia according to ICD-10 (International Classification of Diseases, 10th Revision) criteria and (b) an age of 18–65 years. Age-matched healthy controls were recruited from among the staff, including doctors and nurses, in the same hospital. The inclusion criteria for participants in the healthy control (HC) group were (a) the absence of current or past symptoms of psychiatric illness (b) without a history of psychiatric diseases, and (c) an age of 18–65 years. The general exclusion criteria for participants in the SZ and HC groups were (a) the presence of a neurological disease, such as epilepsy, cerebral tumour, or severe head injury; (b) the use of nonsteroidal or steroidal anti-inflammatory drugs within the previous 14 days; (c) pregnancy; and (d) alcohol or nicotine abuse. Written informed consent was

obtained from all participants. This study has been approved by the ethics committee of Shanghai Jiao Tong University.

*Sample collection*

Blood was collected in 5-mL EDTA-treated tubes early in the morning following an overnight fast (~10−12 h). The plasma and buffy coat were separated from whole blood by centrifugation. Erythrocytes were selectively lysed with RBC lysis buffer (Tiangen, Beijing, China), and leukocytes were recovered by centrifugation. TRIzol reagent (1 mL; Invitrogen, Carlsbad, CA, USA) was added to the clear leukocyte pellet for extraction of total RNA according to the manufacturer's instructions. The plasma and RNA pellet were stored at −80°C until use. The quality and quantity of the isolated RNA were assessed by electrophoresis (Agilent 2100 Bioanalyzer, Santa Clara, CA, USA) and spectrophotometry (NanoDrop, Rockland, DE, USA), respectively.

*IL-6 enzyme-linked immunosorbent assay (ELISA)*

Plasma IL-6 levels were measured with an ELISA kit (ab178013; Abcam, Cambridge, UK) according to the manufacturer's instructions. The sensitivity of the ELISA kit was 1.6 pg/mL.

*qPCR*

One microgram (1 $\mu$g) of total RNA was reverse transcribed to cDNA using a SuperRT cDNA Synthesis Kit cDNA (DRR047A; TaKaRa, Kyoto, Japan). Gene expression levels were evaluated via real-time PCR utilizing SYBR Green Mixture (DRR081C; TaKaRa, Kyoto, Japan). The primer sequences used to amplify the target genes are listed in Supplementary Table 1. Two housekeeping genes—*ACTB* and *SDHA*—were used in the experiments [21], and the stability was evaluated. *SDHA* were chosen as the internal controls used for quantitative analysis. The relative expression levels of the target genes were calculated according to the 2-ΔΔCt method.

*Niacin-skin test and genetic analysis in an independent sample group*

An independent sample group was recruited for the niacin-skin flushing test and genotyping of *PLA2G4A* and *PTGS2*. All individuals in this sample group have undergone the niacin-skin test according to our previously reported method and the 16-points total scores were calculated to indicate the degree of flushing [19]. The tagSNPs were identified using the CHB and CHS data in 1,000 genome database (phase 3). Tagger procedure in Haploview V4.2 was used for tagSNP selection using a minor allele frequency (MAF) larger than 5% and linkage disequilibrium (LD) patterns with $r^2 > 0.8$. Genomic DNA were extracted from the whole blood. Genotyping of the selected tagSNPs was performed using the iPLEX® HS panel on the MassARRAY® System (Agena Bioscience, San Diego, CA, USA).

*Statistical analysis*

Data were first tested for normality with the Kolmogorov-Smirnov (K-S) test. Normally distributed data are presented as the mean ± standard deviation (SD) values. Differences among normally distributed data were analyzed by logistic regression with adjustment for age, sex and body mass index (BMI). Correlations were analyzed by Pearson correlation analysis. All statistical analyses were performed with RStudio. The 16-points total scores for niacin-skin test were used as the quantitative trait to performed the QTL analysis on SHEsisPlus software (http://shesisplus.bio-x.cn/SHEsis.html) [22], with age, sex and BMI adjusted as covariates. A $p(q)$ value of less than 0.05 was considered to indicate a statistically significant difference.

## Results

1. Demographic characteristics
   A total of 166 patients with schizophrenia and 54 healthy controls were recruited in the qPCR and ELISA assay. According to the sample batch, 46 patients and 34 healthy controls were assigned to the discovery set, and 120 patients and 20 healthy controls were assigned to the validation set. The independent sample group involved in niacin-skin test and genotyping analysis included 169 psychiatry patients. The basic demographic characteristics of the participants are shown in Table 1.
2. mRNA expression levels of PLA2/COX-2 cascade genes in leukocytes
   Both *ACTB* and *SDHA* show good stability with coefficients of variation 2.7% and 1.7% in the discovery set respectively. *SDHA* was chosen for further analysis as its Ct value is closer to the targets than *ACTB*.
   The mRNA expression levels of 19 genes related to the PLA2/COX cascade, including receptors, phospholipases, cyclooxygenases and prostaglandin synthetases, were measured (Table 2). Among these genes, six showed significant expression abnormalities in schizophrenia patients compared with healthy controls in the discovery set (Table 2 and Fig. 1). The level of one of the phospholipases, cytosolic phospholipase A2 (cPLA2), which is encoded by the *PLA2G4A* gene and catalyzes the hydrolysis of membrane phospholipids to release AA, was increased in the SZ group. However, the level of LACS4 (gene symbol: *ACSL4*), which incorporates free AA into phospholipids, did not differ between the SZ and HC groups. The levels of both cyclooxygenases (gene symbols: *PTGS1* and *PTGS2*) differed significantly between the SZ and HC groups, exhibiting a significant negative correlation (r = -0.45, $p < 0.01$). The mRNA level of *PTGS1* was enhanced in the SZ group compared with the HC group (fold change (FC) = 2.18, $p < 0.05$), while that of *PTGS2* was reduced (FC = 0.27, $p < 0.05$). In addition, the transcription levels of another four genes (gene symbols: *PTGER4, PTGIR, TXAS,* and *TXA2R*) differed significantly between the two groups. All differences were verified in the validation set except *TXAS* (Supplementary Table 2).
3. Transcription levels of regulatory factors
   The significant changes in cyclooxygenase expression led us to consider the dynamic regulation process. The expression levels of two regulatory factors, CREB1 (gene symbol: *CREB1*) and NF-κB (gene symbol: *RELA*), were measured by qPCR. CREB1, which can regulate the expression of COX-2, was significantly reduced at the transcriptional level (Table 3 and Supplementary Fig. 1a). Furthermore, the transcription levels of *CREB1* and *PTGS2* were significantly correlated (r = 0.4066, $p = 0.0003$; Supplementary Fig. 1b).
4. Plasma IL-6 was elevated in patients with schizophrenia
   Plasma IL-6 levels were measured in 84 patients with schizophrenia and 38 healthy controls—all participants were involved in the training set and the validation set. With three outliers (numbers that are 3 standard deviations away from the mean both sides) removed, the level of IL-6 were significantly elevated in the SZ group compared with the HC group, with a FC of 1.31, $p$ value = 0.0151 (Table 4 and Supplementary Fig. 2a). IL-6 levels were positively correlated with *PTGS1* (r = 0.23, $p = 0.0154$), *PTGES2* (r = 0.22, $p = 0.0220$), *PTGER2*(r = 0.28, $p = 0.0033$), *PTGIR* (r = 0.30, $p = 0.0014$), and negatively correlated with *PTGDR2*(r = -0.19, $p = 0.0435$) (Supplementary Table 3 and Supplementary Fig. 2). All the correlations were observed with SZ group and HC group combined.
5. Diagnostic markers
   To further explore the potential diagnostic biomarkers for schizophrenia, receiver operating characteristic (ROC) curve and logistic regression analyses were used to evaluate the diagnostic

**Table 1**
Demographic characteristics

| | Discovery set | | | Validation set | | | Independent sample set for genetic analysis (N=169) |
|---|---|---|---|---|---|---|---|
| | Case (N=46) | Control (N=34) | *p* value | Case (N=120) | Control (N=20) | *p* value | |
| Sex (M/F) [a] | 20/26 | 15/19 | 0.9546 | 76/42 | 9/11 | 0.0989 | 109/60 |
| Age (years)[b] | 38.25±10.60 | 31.06±7.37 | 0.0036 | 39.44±12.75 | 35.25±11.36 | 0.1485 | 39.13±10.44 |
| BMI ($kg/m^2$)[b] | 23.62±4.16 | 22.09±2.35 | 0.1968 | 24.87±4.02 | 21.47±2.42 | 0.0001 | 25.40±4.24 |
| Smokers[c] (N, n%)[a] | 9 (19.6%) | 6 (17.6%) | 0.7551 | 19 (15.8%) | 4 (20%) | 0.6415 | 26 (15.4%) |
| Alcohol users[c](N, n%)[a] | 1 (2.2%) | 0 (0) | 0.387 | 3 (2.5%) | 1 (5%) | 0.3860 | 3 (1.7%) |

Notes: All values are the means ± SDs unless otherwise indicated.
a: *p* values were calculated by the chi-square test.
b: *p* values were calculated by the Mann-Whitney U test.
c: In this study, Smokers/Alcohol users represents the number of people who have a habit of smoking or drinking

**Table 2**
Transcription levels of PLA2/COX-2 cascade genes

| Gene | SZ (mean ± SD) | HC (mean ± SD) | FC | *p* value[a] | *q* value[b] |
|---|---|---|---|---|---|
| *HCAR2* | 1.03±0.89 | 1.30±1.02 | 0.79 | 0.0887 | 0.1684 |
| *PLA2G4A* | 1.31±0.37 | 1.08±0.42 | 1.22 | 0.0207 | 0.0561 |
| *PLA2G6* | 0.99±0.27 | 1.05±0.32 | 0.95 | 0.2175 | 0.2582 |
| *ACSL4* | 1.03±0.58 | 0.95±0.31 | 1.09 | 0.0749 | 0.1778 |
| *PTGS1* | 2.48±1.26 | 1.14±0.60 | 2.18 | 0.0005 | 0.0049 |
| *PTGS2* | 0.34±0.28 | 1.29±1.02 | 0.27 | 0.0002 | 0.0044 |
| *PTGES2* | 1.06±0.25 | 1.04±0.30 | 1.02 | 0.8436 | 0.8436 |
| *cPGES* | 0.98±0.22 | 1.01±0.16 | 0.97 | 0.6361 | 0.7109 |
| *mPGES1* | 2.03±2.20 | 1.50±1.43 | 1.35 | 0.6553 | 0.6917 |
| *PTGER2* | 0.87±0.51 | 1.05±0.31 | 0.82 | 0.1191 | 0.1885 |
| *PTGER3* | 1.83±1.46 | 1.39±1.38 | 1.31 | 0.2047 | 0.2593 |
| *PTGER4* | 0.86±0.34 | 1.07±0.39 | 0.81 | 0.0084 | 0.0320 |
| *PTGDS* | 1.02±0.74 | 1.19±0.73 | 0.85 | 0.0754 | 0.1592 |
| *HPGDS* | 0.92±0.61 | 1.10±0.52 | 0.83 | 0.1056 | 0.1824 |
| *PTGDR* | 0.93±0.66 | 1.15±0.59 | 0.81 | 0.1822 | 0.2473 |
| *PTGDR2* | 0.99±0.79 | 1.21±0.85 | 0.81 | 0.1464 | 0.2140 |
| *PTGIR* | 1.56±0.63 | 1.07±0.40 | 1.46 | 0.0049 | 0.0231 |
| *TXAS* | 0.88±0.44 | 1.07±0.40 | 0.82 | 0.0120 | 0.0381 |
| *TXA2R* | 1.76±0.82 | 1.12±0.52 | 1.57 | 0.0029 | 0.0184 |

Notes: a: *p* values were calculated by logistic regression with adjustment for age, sex and BMI. b: *q* values were calculated by FDR. *HCAR2*—Hydroxycarboxylic acid receptor 2; *PLA2G4A*—Cytosolic phospholipase A2; *PLA2G6*—Homo sapiens phospholipase A2; *ACSL4*—Fatty-Acid-Coenzyme A Ligase, Long-Chain 4; *PTGS1*—Prostaglandin G/H synthase 1; *PTGS2*—Prostaglandin G/H synthase 2; *PTGES2*—Prostaglandin E synthase 2; *cPGES*—Homo sapiens prostaglandin E synthase 3; *mPGES1*—Homo sapiens prostaglandin E synthase; *PTGER2*—Homo sapiens prostaglandin E receptor 2; *PTGER3*—Homo sapiens prostaglandin E receptor 3; *PTGER4*—Homo sapiens prostaglandin E receptor 4; *PTGDS*—Homo sapiens prostaglandin D2 synthase; *HPGDS*—Hematopoietic prostaglandin D synthase; *PTGDR*—Homo sapiens prostaglandin D2 receptor; *PTGDR2*—Homo sapiens prostaglandin D2 receptor 2; *PTGIR*—Homo sapiens prostaglandin I receptor; *TXAS*—Homo sapiens thromboxane A synthase 1; *TXA2R*—Homo sapiens thromboxane A2 receptor.

value of single and combined markers for schizophrenia. The combination of *PTGS1, PTGS2, PTGER4* and *PLA2G4A* expression in leukocytes yielded an area under the curve (AUC) of 0.974 in the discovery set and 0.992 in the validation set, thereby perfectly differentiating the SZ group from the HC group (Supplementary Fig. 3).

6. *PLA2G4A* and *PTGS2* associated with niacin-skin blunting
Two SNPs in *PTGS2* and 36 SNPs in *PLA2G4A* were finally examined on the MassARRAY platform. Results of QTL analysis for all SNPs are listed in the supplementary Table 4. Five SNPs, including rs4648250 in *PTGS2* (*P*adj-QTL = 0.004) and rs7416329 (*P*adj-QTL = 0.001), rs17591849 (*P*adj-QTL = 0.011), rs2205898 (*P*adj-QTL = 0.022), and rs10798064 (*P*adj-QTL = 0.023) in *PLA2G4A* were significantly associated with the niacin flushing degree.
We compared the total scores of niacin skin test between patients with different genotypes. The 16-points total scores of niacin-skin tests showed significant differences between different genotype groups of two SNPs, including rs10798064 in *PLA2G4A* and rs4648250 in *PTGS2* ( Fig 2).

## Discussion

We investigated the genes involved in the PLA2/COX cascade. Nineteen of them were quantified by qPCR, whereas the other two genes transcript levels in leucocytes were too low to detect. Six of the nineteen genes altered significantly in mRNA levels and five of them were verified in the validation set, indicating that this pathway is significantly dysregulated in schizophrenia patients (Fig 1). We also detected the downstream interleukin IL-6 and upstream regulatory factors CREB1, which were significantly related to the corresponding molecular. Several genetic variations of *PLA2G4A* and *PTGS2* are associated with the endophenotype of niacin-skin bluntness in psychiatry patients. The results indicated that the disruption of the PLA2/COX pathway may be involved in the pathogenesis of schizophrenia and blunting of the niacin skin flush response.

AA and other fatty acids in cell membrane phospholipids are in a dynamic equilibrium of dissociation and binding, which is important for membrane structure and function. The dissociation process is catalyzed by members of the PLA2 family. Two PLA2 genes, *PLA2G4A* and *PLA2G6*, encoding different PLA2s with different preferences for fatty acids, were investigated in our study. cPLA2, encoded by *PLA2G4A*, preferentially hydrolyzes AA attached at sn-2 position of phospholipids. According to our data, the transcription level of *PLA2G4A* was increased in the SZ group (*p* value=0.0207, *q* value=0.0561). Several previous studies have found that the cPLA2 activities in platelet [23] and expression level in red blood cell [24] were significantly higher in schizophrenia patients than in healthy controls, consistent with our results. The process of AA dissociation from the membrane is supposed to be more active in schizophrenia patients. However, the level of LACS4, which can incorporate free AA into phospholipids [25], remained unchanged in our results. A reasonable speculation is that the content of PUFAs, especially AA, on the cell membrane is reduced. A similar decrease in PUFAs has also been reported in other studies [5]. Loss of PUFAs severely impairs membrane functions, including signal transduction via receptor-mediated phospholipid-derived second messengers. Meanwhile, the PLA2 in central system modulates dopamine release and dopamine receptor sensitivity. The increased PLA2 activity in prefrontal cortex possibly contribute to hypofrontality in schizophrenia [26]. The upregulation of PLA2 accelerates the breakdown of membrane phospholipids and may play critical roles in the initial psychopathology of schizophrenia.

Cyclooxygenases catalyze the rate-limiting step in the formation of prostaglandins from AA [27]. The two main cyclooxygenases—constitutively expressed COX-1 and inducible COX-2 [28]—were evaluated in our study. Although some previous studies have suggested that the level of COX-2 is elevated in schizophrenia, we believe that the evidence for this view is insufficient. According to an RNA-seq study in prefrontal cortex tissue, the COX-2 transcription level was not significantly increased in schizophrenia [29]. Microarray data from peripheral blood mononuclear cells did not show a difference in

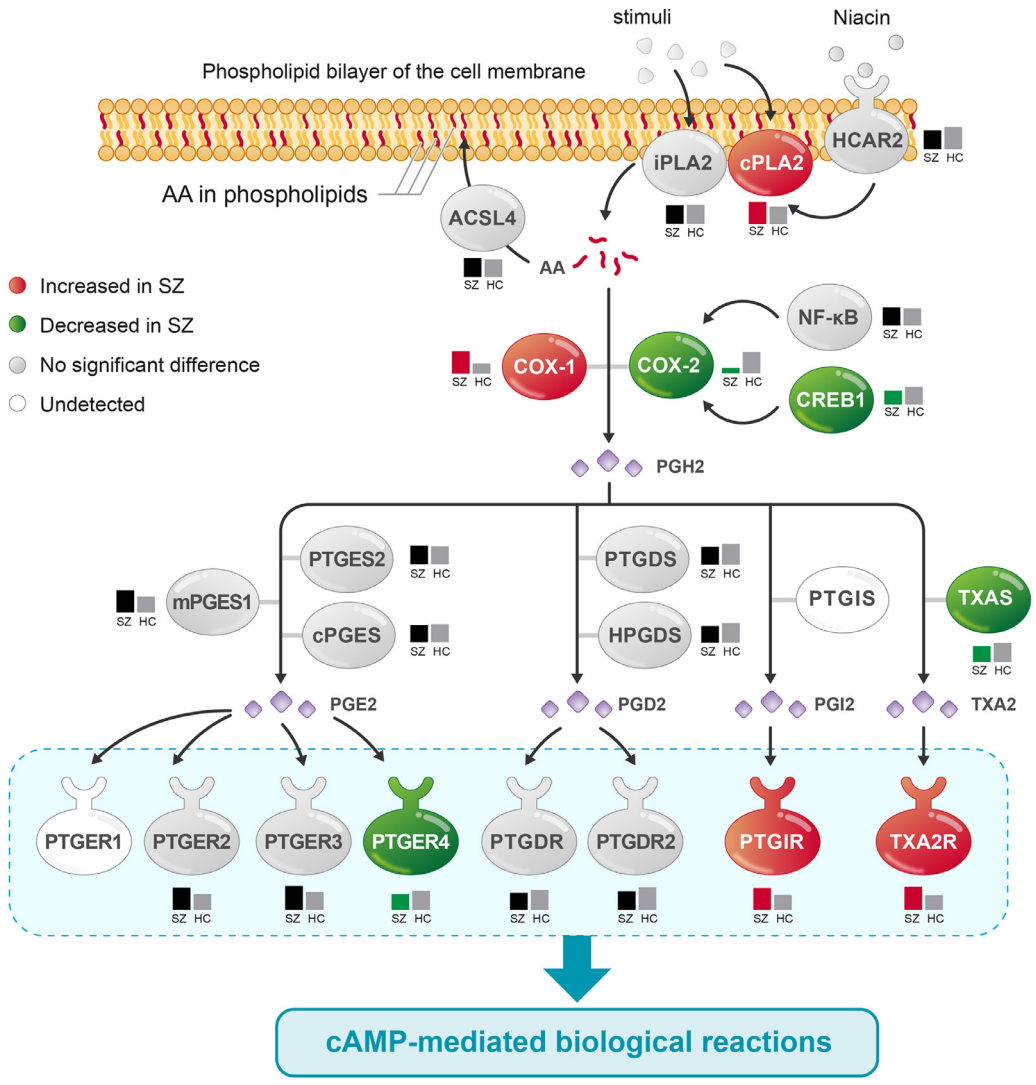


**Fig. 1.** All detected PLA2/COX pathway genes and regulatory factors and alterations in their transcription levels between patients with schizophrenia and healthy controls

**Table 3**
Transcription levels of *CREB1* and *RELA*

| Gene | SZ (mean ± SD) | HC (mean ± SD) | FC | *p* value[a] | *q* value[b] |
|---|---|---|---|---|---|
| *CREB1* | 0.80±0.37 | 1.01±0.24 | 0.79 | 0.0029 | 0.0058 |
| *RELA* | 1.17±0.42 | 1.01±0.22 | 1.16 | 0.6496 | 0.6496 |

*CREB1*—Cyclic AMP-responsive element-binding protein 1; *RELA*—RELA Proto-Oncogene, NF-KB Subunit
a: *p* values were calculated by logistic regression with adjustment for age, sex and BMI. b: *q* values were calculated by FDR.

**Table 4**
Demographic characteristics and the quantitative results of the IL-6 ELISA

| | Case (N=84) | Control (N=38) | *p* value |
|---|---|---|---|
| Sex (M/F)[a] | 50/34 | 18/20 | 0.2106 |
| Age (years)[b] | 36.57±13.76 | 32.31±8.84 | 0.0681 |
| BMI (kg/m$^2$) [b] | 29.37±9.58 | 21.72±2.24 | 0.6826 |
| IL-6 (pg/mL) [b] | 3.62±2.00 | 2.76±1.65 | 0.0151 |

Notes: All values are the means ± SDs unless otherwise indicated.
a: *p* values were calculated by the chi-square test.
b: *p* values were calculated by the Mann-Whitney U test.

COX-2 expression between SZ and HC either [30]. A plasma study showed that COX-2 transcription levels in schizophrenia patients older than 40 years were significantly lower than those in normal controls [31]. An important argument supporting the elevated level of COX-2 in schizophrenia is the benefit derived from treatment with COX-2 inhibitors. However, the meta-analysis in 2013 confirmed that COX-2 inhibitors exhibit no significant therapeutic effect in patients with schizophrenia [32], especially in patients with long disease duration [33]. Our data showed a reduction in COX-2 expression not only in the training set but also in the validation set, which we believe to be reliable.

COX-2 is an inducible protein regulated by various factors and signalling pathways. A series of pro-inflammatory stimuli could induce COX-2 expression, whereas some have an inverse effect. For example, LPS in primary human monocytes causes a rapid and specific destabilization of *COX-2* mRNA [34], and the atrial natriuretic peptide (ANP) shows a dose-related inhibition of *PTGS2* mRNA [35]. CREB1 are essential for transcription of *PTGS2* [36]. According to our results, CREB1 and COX-2 were both decreased and were positively correlated, suggesting the down-regulation of CREB1/COX-2 pathway in schizophrenia. COX-1 and COX-2 perform compensatory functions in AA metabolism. A previous study reported that *PTGS1* mRNA was upregulated along with the increase of PGE2, when *PTGS2* was knocked out [37]. The opposite alterations of *PTGS1* and *PTGS2* in our results were consistent with the previous findings, from which we further speculated that the downregulation of *PTGS2* for unclear reasons may be the cause of *PTGS1* upregulation. Meanwhile, the activation of AA dissociation caused by oxidative stress and increased PLA2

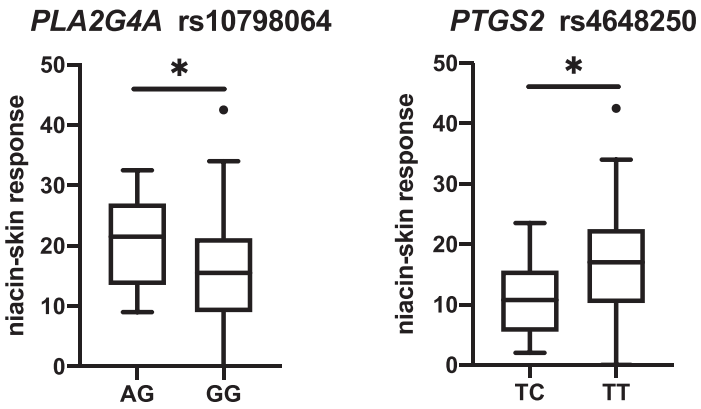


**Fig. 2.** Plot of niacin-skin response levels in different genotypes.
P-values of rs10798064 and rs4648250 were obtained by T-test.

may promote the expression of *PTGS1*. Although the role of COX-1 in mental illness has not received much attention, there are still some studies indicating that COX-1, rather than COX-2, is a key component in neurodegeneration and neuroinflammation [38,39].We take a similar view in schizophrenia.

In the absence of stimulation, COX-1-mediated AA metabolism favours the production of thromboxane and PGs, which have proinflammatory properties and affect the function of the nervous system. When the PLA2/COX-1 pathway is activated, PGs accumulate in patients with schizophrenia [40-42]. Previous studies have reported that PGE2 induces IL-6 mRNA expression and protein synthesis in a variety of cells, such as human astrocytoma [43], synovial fibroblasts [44] and macrophages [45,46]. Similarly, PGI2 could also promote IL-6 release [13]. According to our data, the IL-6 were positively correlated with *PTGS1, PTGES2, PTGER2* and *PTGIR* (Supplementary table 3). Our results imply that the cascade of COX-1-mediated AA dissociation/PGs /IL-6 secretion is activated in schizophrenia patients. IL-6 has been proposed as a candidate molecule that transmits inflammatory information to the CNS [47], which may contribute to the immune abnormalities in schizophrenia. In addition, IL-6 affect the release and the function of neurotransmitters, inducing serotonin and dopamine activity elevated in the hippocampus and prefrontal cortex [14]. Overexpressed IL-6 can exert a neurotoxic effect and inhibit hippocampal neurogenesis [48,49]. Transgenic mice with IL-6 overexpression in the brain develop severe neurologic symptoms characterized by tremor and ataxia and show deficits in avoidance learning [50].

TXA2 is the major AA derivative synthesized via the COX-1 pathway [51]. TXA2 can enhance dopamine release and suppress GABAergic transmission by stimulating the TXA2 receptor TXAR2 [52,53]. Upregulation of COX-1 and TXAR2 reported in this study may interfere with neurotransmitter delivery and is relevant to the pathogenesis of schizophrenia.

Blunting of the niacin flush response in schizophrenia patients has been consistently observed in many studies with the root cause unclear. The PLA2/COX pathway constitutes the molecular mechanism underlying niacin flushing. In this study, the abnormalities of gene expression levels of PLA2/COXs pathway were observed in schizophrenia, and the genetic variations of *PLA2G4A* and *PTGS2* were evidenced to be associated with the endophenotype of niacin-skin bluntness. Based on these findings, the speculation is proposed on the mechanism of niacin-skin bluntness in schizophrenia.

The level of AA in the cell membrane may decrease due to the overexpression of cPLA2. When the epidermal cells are stimulated by niacin, the precursors of PGs are insufficient, which are possibly associated with the blunting of skin flush response in schizophrenia patients. Consistent with our speculation, lower AA levels were reported in the niacin-blunted SZ patients than in niacin non-blunted patients [54].

COX-2 is the main enzyme involved in the niacin skin flush response [55,56]. Aspirin is an inhibitor of COXs. According to the previous studies, taking aspirin would reduce niacin-induced flushing in healthy adults [57,58]. The down-regulation of CREB1 and COX-2 in our results were speculated to produce similar effects as taking aspirin.

PGE2 is the main prostaglandin that causes skin flushing. It has four receptors (EP1-4) that mediate diverse biological functions [59]. Among them, EP4 is the main receptor regulating the vasodilatory response [60], which was significantly decreased in schizophrenia according to our results. Insufficient expression of EP4 may result in decreased PGE2 signalling capacity in capillary cells and reduced cAMP production, which may be the mechanism underlying the blunting of the niacin flush response.

In addition, genetic data further supports the above inferences. Five SNPs in *PLA2G4A* and/or *PTGS2* are significantly associated with niacin-skin bluntness, and two of them show significant association in niacin test scores between patients with different genotype. As shown in Fig 2, psychiatric patients with more G allele in rs1079806 or more C allele in rs4648250 have more blunted niacin responses. Similar results on other SNPs in *PLA2G4A* and *PTGS2* have been reported before [61]. These results indicate the important roles of cPLA2 and COX-2 in blunted niacin responses of patients with psychiatric disorders. However, it is unknown whether these variations are related to their abnormal gene expressions, which need more research to clarify.

In summary, the enhanced dissociation mediated by cPLA2 may result in loss of AA from membrane phospholipids. COX-1-mediated catabolism of free AA is enhanced, and the metabolites can not only affect the transmission of neurotransmitters but also promote the release of cytokines, produce inflammation and affect the function of the nervous system. These changes are closely related to the occurrence and development of schizophrenia. On the other hand, the increase in the level of cPLA2 and decreases in the levels of CREB1, COX-2 and EP4 may explain the blunting of the niacin flush response (Fig. 3). Investigation of these processes may reveal the key factor causing this blunting and provide more evidence for the mechanism of disease.

This study has some limitations. We studied the PLA2/COX cascade in leukocyte mRNA levels. It is necessary to verify our findings in brain and further to detect other molecular levels, such as proteins and metabolites. The multi-level research can help us interpret the role of the cascade in schizophrenia systematically. Considering the fact that schizophrenia is a highly heterogeneous disease and the PLA2/COX pathway is very complex, studies in larger populations and different geographic groups are needed. The genetic associations shown in this study still needs to be further studied. It is also necessary to verify the correlation between these gene variation, expression level, protein function and the niacin-skin responses in the same sample set.

## Conclusion

In this study, we detected the transcription levels of PLA2/COX pathway-related genes and put forward a hypothesis that the activation of AA hydrolysis and imbalance in the levels of COX-1 and COX-2 may be involved in the pathogenesis of schizophrenia and blunting of the niacin flush response. Our results provide additional insight for etiological research on schizophrenia and help to optimize clinical strategies and facilitate precise diagnosis. Further research is required to explore PLA2/COXs pathway in schizophrenia and other mental illness.

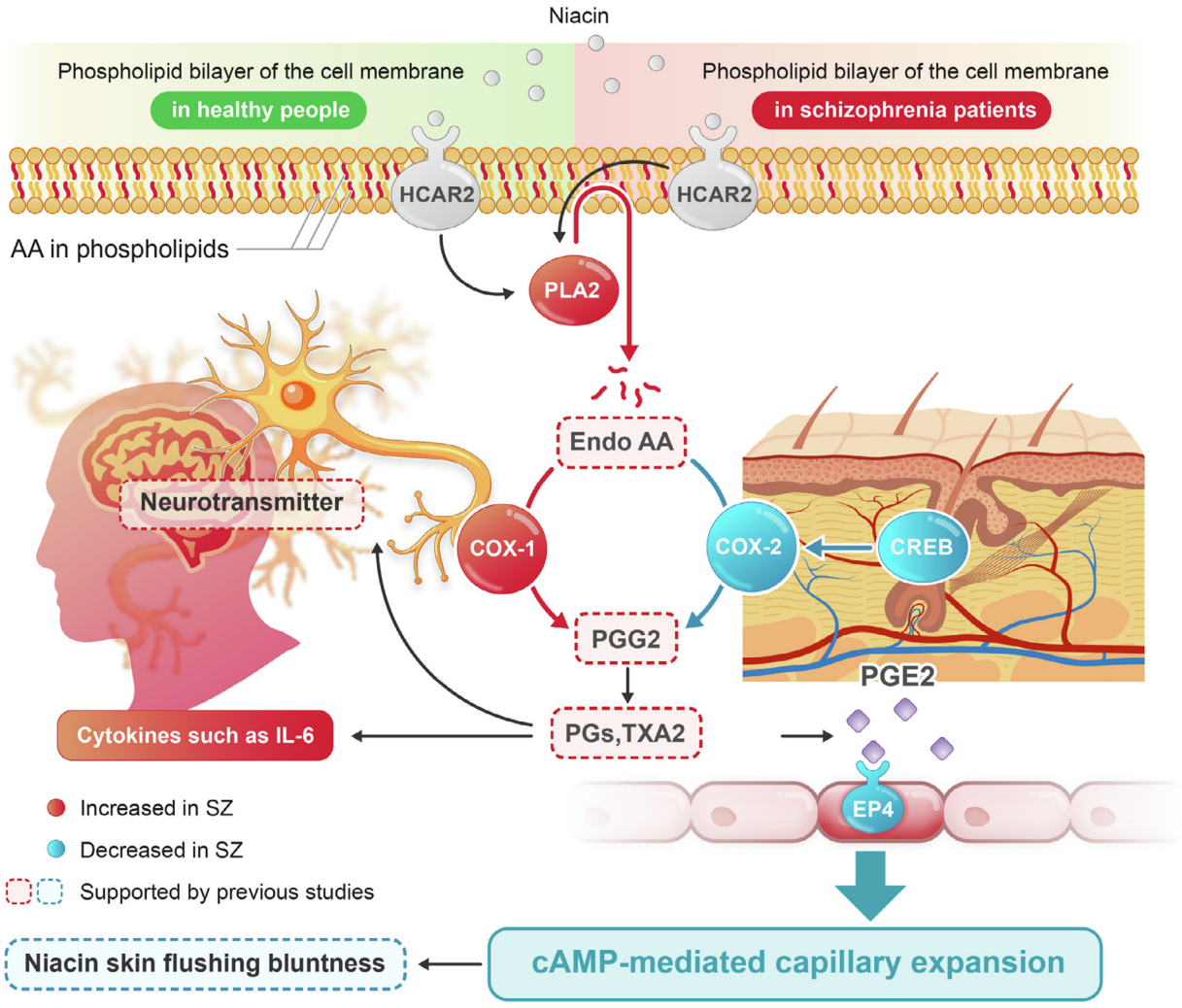


**Fig. 3.** Schematic illustrating the role of the PLA2/COX cascade in the pathogenesis of schizophrenia and blunting of the niacin flush response. The PLA2/COX pathway is significantly disrupted in patients with schizophrenia: the level of cPLA2, which primarily mediates the dissociation of AA, is significantly increased, and catabolism of free AA is enhanced by COX-1. The metabolites affect nervous system function by interfering with the release of neurotransmitters and inducing overexpression of cytokines such as IL-6. However, down-regulation of CREB1 and COX-2, as well as the decrease in the level of the PGE2 receptor EP4, may be related to the blunting of the niacin skin flush response.

## Author contributions

XY, ML, and CW designed the study. TY, YS, SQ, JZ, XL and CW contributed to the recruitment of the subjects. JJ, TY, YQ, LS, XH, DW, GC and YG collected the blood samples and clinical information. XY, ML and JJ performed the experiments and acquired the data. XY, ML, JJ, XH, and LS contributed to the data analysis. XY, ML and JJ participated the results interpretation. XY, ML wrote the manuscript.

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## Declaration of interests

The authors declare no conflict of interest.

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## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103239.

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