BanI polymorphism of cytosolic phospholipase A2 gene is associated with age at onset in male patients with schizophrenia and schizoaffective disorder

S. Nadalin\textsuperscript{a}, G. Rubeša\textsuperscript{b}, J. Giacometti\textsuperscript{c}, M. Vulin\textsuperscript{d}, D. Tomljanović\textsuperscript{e}, J. Vraneković\textsuperscript{a}, M. Kapović\textsuperscript{a}, A. Buretić- Tomljanović\textsuperscript{a,*}

\textsuperscript{a}Department of Biology and Medical Genetics, School of Medicine, University of Rijeka, Croatia
\textsuperscript{b}Psychiatry Clinic, Clinical Medical Centre, Rijeka, Croatia
\textsuperscript{c}Department of Chemistry and Biochemistry, School of Medicine, University of Rijeka, Croatia
\textsuperscript{d}Psychiatric Hospital Lopača, Rijeka, Croatia
\textsuperscript{e}Private Psychiatric Practice, Rijeka, Croatia

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Abstract

The enzymes phospholipases A2 are believed to be involved in the pathology of schizophrenia. We investigated allelic and genotype frequencies of PLA2G4A BanI polymorphism and the rs4375 in PLA2G6A in Croatian schizophrenic patients (n = 81) and controls (n = 182), using PCR/RFLP. Genotype and allelic frequencies of both loci, alone or in combination did not show significant difference ($\chi^2$-test). Allele-wise and genotype-wise meta-analyses of BanI polymorphism in case-control and family-based studies also revealed no significant association with schizophrenia.

Multiple logistic regression analyses revealed statistically significant association between several items from PANSS general psychopathology scale and BanI polymorphism in PLA2G4A. BanI polymorphism further showed a significant impact on mean age of the onset of disease in males ($f_{A1} = 0.351$, $P = 0.021$; Spearman’s $r_{A1} = 0.391$, $P = 0.010$) indicating lower mean age at admission in homozygous A2A2 males.

1. Introduction

Schizophrenia is a severe mental disease of polygenic etiology that affects nearly 1–1.5% of the human population worldwide. Its consistent worldwide prevalence supports the hypothesis that many different genes influence susceptibility to schizophrenia. Several candidate genes, but no major contributing mutations, have been connected with schizophrenia through linkage and association studies [1–4]; therefore, it seems plausible that genetic susceptibility could be attributed to common genetic polymorphisms. Possible candidate genes can be derived from observations of biochemical alterations found in patients with schizophrenia [5]. The phospholipid membrane hypothesis of schizophrenia reported by Feldberg [6] and Horrobin [7] suggested that abnormal phospholipid metabolism is involved in development of the disorder. Because neuronal membranes consist largely of phospholipids containing long chain polyunsaturated fatty acids (LC-PUFAs), abnormalities of the neuronal phospholipids have been proposed as a possible biochemical basis for schizophrenia.

Brain phospholipids provide an interesting means for studying gene–environment interactions in pathogenesis of the disease for several reasons. Firstly, the enzymes and other proteins that regulate phospholipid metabolism are genetically determined [8]; secondly, the fatty
acid composition of cell membranes is largely influenced by nutrition [9,10] and possibly other factors (gender, metabolism) [9,11]; and finally, numerous data show altered phospholipid metabolism in patients with schizophrenia. Evidence for altered phospholipid metabolism in schizophrenia includes: (1) increased phospholipase A2 (PLA2) activity in serum, plasma, platelets, and brain tissue of schizophrenic patients [12–15]; (2) reduced levels of 20:4n-6 arachidonic acid (AA) and 22:6n-3 docosahexaenoic acid (DHA) in the membrane phospholipids of patients with schizophrenia [16–18]; (3) magnetic resonance imaging indicates an increased rate of phospholipid breakdown in the brain of unmedicated schizophrenic patients [19,20]; and (4) high percentage of schizophrenic patients show reduced flushing response to oral or topical niacin due to AA deficiency in the cell membranes [15,21,22].

PLA2 comprises a large super-family of enzymes that catalyze the hydrolysis of the sn-2 fatty acyl bond of phospholipids to liberate free fatty acids and generate lysophospholipids. Both metabolites act as second messengers at low concentrations, whereas at high concentrations they are neurotoxic [23]. A number of studies have investigated an association between genes coding for different classes of PLA2 enzymes and the etiology of schizophrenia. Positive association has been reported between the A2 allele of the Ban1 polymorphic site in the PLA2G4A gene (cytosolic PLA2; cPLA2) and schizophrenia in a Korean population [24]. An excess of A2/A2 homozygotes has been also found in the Indian population [17], and higher transmission of the A2 allele has been reported repeatedly in the family trios of schizophrenic patients [25–27]. In the Chinese population [28], evidence for a positive association, by conditioning on genotype, between Ban1 polymorphism of the PLAG2A4 gene and two polymorphisms in other cPLA2 genes (PLAG2G4B and PLAG2G4C) and schizophrenia has been reported. The study of Junqueira et al. [29] reported allelic and genotypic associations of the calcium-independent PLA2 (iPLA2) polymorphism with schizophrenia in Brazilian populations. Other studies have reported negative associations between different PLA2 genes and schizophrenia: the poly-A repeat polymorphism of the PLA2G4A gene in a British sample [30], multiple polymorphisms in the cPLA2, synovial PLA2 (sPLA2), and iPLA2 genes in large studies by Frieboes et al. [5], investigating families from Europe and China, and Yu et al. [31] investigating Chinese parent-offspring trios.

We investigated the frequencies of two single nucleotide polymorphisms (SNPs) in PLA2 genes (rs10798059 of the cPLA2 gene and rs4375 of the iPLA2 gene) in schizophrenic patients and healthy controls in Croatian population in order to assess whether an increased risk for schizophrenia was associated with these polymorphisms. Due to largely inconsistent reports about the association of Ban1 polymorphism in cPLA2 gene with schizophrenia in different populations, we performed a meta-analysis of the case-control and family-based studies published to date attempting to clarify this issue. Based on the recent finding that cPLA2 enzyme activity correlates significantly with the presence of the A2 allele of the polymorphic Ban1 site of the cPLA2 gene [32], we further tested the hypothesis that PLA2 polymorphisms contribute to earlier onset of the disease. We also correlated data of baseline psychopathology measured via PANSS (positive and negative symptom scale) sub-scales with cPLA2 and iPLA2 allelic and genotypic variations, and their combinations.

2. Patients and methods

Eighty-one (43 males and 38 females) patients with schizophrenia (n = 61) and schizoaffective disorder (n = 20) from the Department of Psychiatry, Clinical Medical Centre, Rijeka, Croatia, and 182 healthy controls (83 males and 99 females), all Croatian citizens, were recruited for this study. Among schizophrenic patients, paranoid schizophrenia was the most common diagnosis (55/61; 90.16%). Clinical features of our patients are presented in Table 1.

The diagnosis of schizophrenia and schizoaffective disorder was confirmed by at least two psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria with the structured clinical interview. All the subjects gave informed consent for the genetic analysis, and the study was approved by the Ethics Committee of the School of Medicine, University of Rijeka, Croatia. PANSS and clinical global impression (CGI) evaluation was performed at the time of last admission, during an acute state of the illness requiring hospitalization.

Genotyping was performed in the Laboratory for Molecular Genetics (Department of Biology and Medical Genetics, School of Medicine, Rijeka). The 182 control individuals were blood donors who underwent no specific examination for psychiatric status. The practice with blood donation in Croatia includes providing a written statement about health status at every session. Therefore, blood donors are representative of the healthy general population, free of chronic diseases or regular medication. We analyzed two SNPs: Ban1 polymorphisms (rs10798059, A/G polymorphism: A1 versus A2 allele, respectively) located near the first intron of PLA2G4A and C/T polymorphism (rs4375) located in the intron 4 of PLA2G6A. Genomic DNA was extracted from whole blood using the NucleoSpin Blood Kit (Clontech, Saint-Germain-en-Laye, France) according to the manufacturer’s instructions. Genotyping of the SNPs was performed by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analysis using modified version of the protocol described by Meira-Lima et al. [33]. The primer
sequences were as follows: PLA2G4A-f: AAGGGA- TATTTGTAGAGGACT; PLA2G4A-r: TAGATGAT- TCGATTTATGACT; and PLA2G6A-f: GGGG TTATTTTGCTGGGTT; PLA2G6A-r: CAAGGGT- GATGGGGAGATC.

The significance of the difference in frequencies of genotypes and alleles between patients and controls was determined using the $\chi^2$-test. Probability ($P$) values less than 0.05 were considered statistically significant.

We used Spearman’s correlation, multiple forward stepwise regression analysis and a logistic regression analysis to test the possible dependence of different clinical features (mean age at first hospital admission, all items of the positive and negative symptom, and general psychopathology subscales, total PANSS and CGI scores) in patients with schizophrenia and schizoaffective disorder on several independent (predictor) variables. Predictor variables in regression analyses were mean patients’ age, their iPLA2 and cPLA2 genotypes or the presence of individual alleles of both genotypes (the presence of the allelic variant was assigned score of 1 whether homozygous or heterozygous and its absence (the presence of individual alleles of both genotypes (the presence of the allelic variant was assigned score of 1 whether homozygous or heterozygous and its absence was assigned score of 0). The analysis began with no predictor variables in the regression equation. Variables were added in subsequent steps according to defined criteria ($F = 3.0$ to enter, $F = 1$ to remove). In the first step, the predictor variable with the largest correlation with the dependent variable entered the regression equation. In the second step, if possible, the predictor variable was selected based on the highest partial correlation with the dependent variable. The possible impacts of the allelic and genotypic combinations of both loci (cPLA2/iPLA2, T/A, C/A, T/G, C/G) were analyzed using factorial ANOVA.

Statistical analyses were performed using statistical software package for Windows 2001 (by Statsoft, Inc.).

### 2.1. Meta-analysis of BanI polymorphism in cPLA2 gene

To identify studies eligible for meta-analysis, we surveyed citations in PubMed and Scopus online search engines with combination of “schizophrenia” and “phospholipase A2” keywords. The association studies of schizophrenia were also extracted from Schizophrenia Gene Database by searching genes PLA2G4A and PLA2G6A (http://www.schizophreniaforum.org/res/sczgene/default.asp) [34]. By examining all the citations and their references, up to 31 March 2008, we have identified seven case–control and three family-based studies that fulfilled the inclusion criteria: (1) investigation of PLA2G4A BanI polymorphism, (2) publication of original data in a peer-reviewed journal, (3) reporting enough data to calculate an effect size, and (4) participation of more than 50 subjects in the analysis.

A number of studies were not included in meta-analysis because they investigated another polymorphism in cPLA2 gene (poly-A repeat polymorphism) [5,30,35,36] failed to provide enough information to calculate an effect size [37] or were contained, wholly or in part, in other works [26,32,38]. In case of PLA2G6A gene, we have found only one association study between rs4375 and schizophrenia [29].

Data from each study were used to construct 2 × 2 tables in which subjects were classified by diagnostic category (patients or controls) and number of events (the presence of A2 allele or A2A2 genotype) in each category. For family-based studies, the number of events corresponded to the number of A2 alleles transmitted by heterozygous parents. The strength of association was summarized using the odds ratio (OR) in which A2 allele or A2A2 homozygosity was assigned as the risk factor based on the results of previous studies [17,24–29]. An OR > 1.0 would indicate a positive association between risk factor and schizophrenia. Case–control and family-based association studies were analyzed separately. The significance of the ORs, which were pooled, was determined using $z$-test. The heterogeneity of the group of ORs was assessed using chi-square-based Q-statistic test.

Meta-analyses were conducted using Comprehensive Meta-Analysis software, version 2.0 (Biostat International, Inc.).

### 3. Results

#### 3.1. Genotype and allele distribution

The frequencies of genotypes and polymorphic alleles are presented in Table 2. Based on the number of total subjects involved (81 patients and 182 controls), the
statistical power of our study was 80% in detecting a 1.5-fold change in the frequency of cPLA2 A2A2 and iPLA2 TT homozygosity (where 35.9% and 34.6% of control subjects were homozygous, respectively) and 1.3-fold change in the cPLA2-A2 and iPLA2 T-allelic frequency (the frequencies of cPLA2 A2 allele and iPLA2T allele were 59.9% and 56.9% in the control subjects, respectively). For the iPLA2 C allele, our study exhibited 80% power in detecting the 1.7-fold change in the frequency of iPLA2 CC homozygosity (where 20.9% of controls were homozygous) and 1.4-fold change in iPLA2 C allelic frequency (iPLA2 C-allelic frequency was 43.1% in the control subjects).

The allelic frequencies and genotype distribution of PLA2G6A and PLA2G4A polymorphisms were consistent with the Hardy–Weinberg equilibrium in both patients and controls. There were no significant differences in the frequencies of genotype and allele distribution between patients and controls for either polymorphism. Frequency analysis of the combinations of genotypes and alleles for both loci did not reveal significant differences between patients and controls, neither was there any significant difference in genotype and allelic frequencies between males and females.

### 3.2. Mean age at disease onset

We calculated Spearman’s coefficients of correlation and used multiple forward stepwise regression analysis to test for possible associations between mean age at first hospitalization, and cPLA2 and iPLA2 genotype and alleles, and their combinations. Mean age at first hospitalization has been considered to approximately match the age of onset of the disease. Spearman’s coefficients of correlation showed statistically significant positive association between mean age at first hospitalization in patients with schizophrenia and schizoaffective disorder and the presence of cPLA2 A1 allele ($r_{A1} = 0.232$; $t = 2.12$; $P = 0.037$) (Table 3). Multiple forward stepwise regression analysis revealed the same trend, although statistically insignificant ($\beta = 0.201$; $F = 3.326$; $P = 0.072$). The results suggested that a lower mean age at first hospitalization could be correlated to the absence of the allele A1 or the presence of A2A2 genotype in the cPLA2 gene. However, the power of the Spearman’s correlation was low, suggesting a weak association. We further tested the same association in males and females separately. In males, both multiple stepwise regression analysis and Spearman’s Rank–Order correlation showed moderate but statistically significant association between the presences of A1 allele and older age at first admission in males ($\beta_{A1} = 0.351$, $F = 5.768$, $P = 0.021$; Spearman’s $r_{A1} = 0.391$, $P = 0.010$) while no association was found in females ($P = 0.636$ and 0.822, respectively). Thus, lower age at admission was associated with A2A2 genotype in male patients (Table 3). According to our results, BanI polymorphism explains 12.33% variability in mean age at disease onset in males.

### 3.3. PANSS and CGI analysis

The investigated PLA2 alleles and genotypes of patients in the study were further correlated to all symptom items of the PANSS subscales, and total PANSS and CGI scores. Since all the items of the PANSS negative subscale, several items of the PANSS general psychopathology subscale (mannerisms and posturing, poor attention, disturbance of volition, preoccupation, social avoidance), PANSS negative score, and total PANSS and CGI scores were found to correlate positively in a statistically significant manner ($0.000 < P < 0.05$) with mean age of our patients at last admission, we calculated partial coefficients of correlation (data not shown) and performed multiple logistic regression analyses of PANSS items to control for the age effect. The results of both analyses were consistent. The multiple logistic regression results are presented in

### Table 2
The frequency of rs10798059 (PLA2G4A) and rs4375 (PLA2G6A) genotypes and alleles in schizophrenic patients and healthy controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients ($N = 81$)</th>
<th>Controls ($N = 181$)</th>
<th>Genotypes</th>
<th>Patients ($N = 81$)</th>
<th>Controls ($N = 182$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1A1</td>
<td>14 (17.3%)</td>
<td>29 (16.0%)</td>
<td>TT</td>
<td>27 (33.3%)</td>
<td>63 (34.6%)</td>
</tr>
<tr>
<td>A1A2</td>
<td>42 (51.9%)</td>
<td>87 (48.1%)</td>
<td>TC</td>
<td>42 (51.9%)</td>
<td>81 (44.5%)</td>
</tr>
<tr>
<td>A2A2</td>
<td>25 (30.9%)</td>
<td>65 (35.9%)</td>
<td>CC</td>
<td>12 (14.8%)</td>
<td>38 (20.9%)</td>
</tr>
<tr>
<td>A1</td>
<td>70 (43.2%)</td>
<td>145 (40.1%)</td>
<td>T</td>
<td>96 (59.3%)</td>
<td>207 (56.9%)</td>
</tr>
<tr>
<td>A2</td>
<td>92 (56.8%)</td>
<td>217 (59.9%)</td>
<td>C</td>
<td>66 (40.7%)</td>
<td>157 (43.1%)</td>
</tr>
</tbody>
</table>

*Differences in frequency are not statistically significant ($P>0.05$).*
3.4. Meta analysis of the case–control studies

In the meta-analysis of eight case–control association studies (1451 cases and 1581 controls including current study), we found no evidence for statistically significant association between A2 allele and A2A2 genotype of the cPLA2 BanI polymorphism and schizophrenia. Pooled odd ratios were: ORA2 = 1.1 (CI = 0.9–1.3; z = 1.07; P = 0.285) and ORA2A2 = 1.1 (CI = 0.9–1.5; z = 1.00; P = 0.316), respectively (Table 5). Heterozygosity (OR = 0.98; z = −0.40; P = 0.692) and A1A1 homozygosity (OR = 0.93; z = −0.59; P = 0.56) were also not significant risk factors. The pooled heterogeneity, in both A2 allele-wise and homozygous A2A2 genotype-wise analyses, was statistically significant (P<0.05 for both), as well as in Asian studies analyzed separately (P<0.01 for both A2 allele and homozygous genotype). More consistency was found in studies that included Caucasian samples (PA2 = 0.617; PA2A2 = 0.437) in which OR estimates had similar values under both fixed-effect and random-effect models. The stability of the estimates was examined by subsequent removal and replacement of each study from the analysis. Regardless of the removed study, the 95% CIs around the pooled OR always contained the value 1.0. The relative weight of allele-wise and genotype-wise studies, under random-effect model, varied from 4.7–17.9% and 6.0–18.1%, respectively.

Neither item from the PANSS positive subscale was found to significantly correlate with mean age at last admission or genetic polymorphisms in our patients.

Table 4. Only one item in the domain of the PANSS general psychopathology symptoms (lack of judgment and insight) showed higher and statistically significant correlation with the cPLA2 BanI polymorphism than with patients’ age (P = 0.012), while mannerisms and posture showed a marginally significant association (P = 0.045). Table 4 also shows the direction of the relationships between variables (positive or negative β), and the percentage of the variation (multiple R² change) in the dependent variables that can be explained by the predictor. Negative β shows that patients not having A2 allele in their cPLA2 genotype (genotype A1A1) tended to have symptom increases during a psychotic state. Polymorphism in the PLA2G4A accounted for 4.5–7.9% of symptoms’ variability, revealing its relatively small impact.

Neither item from the PANSS positive subscale was found to significantly correlate with mean age at last admission or genetic polymorphisms in our patients.

3.5. Meta analysis of the family-based studies

Neither in three family-based association studies, had we found evidence of the association between A2 allele of the cPLA2 BanI polymorphism and schizophrenia. The study of Wei and Hemmings [27] resulted in only one reported positive association (in the British population), while studies of Tao et al. [28] and Chowdari et al. [39] reported no association in Chinese or Indian sample. Pooled OR was 1.1 (95% CI = 0.6–2.0; z = 0.24; P = 0.812). The heterogeneity was statistically significant (Q = 6.27, d.f. = 2, P = 0.043), reflecting plausible different allelic frequencies of BanI polymorphism in investigated populations. The contribution of each study, under random-effect model, varied from 29.9% to 37.9%. 
association between the absence of expression of the disease. We found a significant
in the absence of A2 allele, i.e., A1A1 genotype (Table 4).

Significantly, higher scores for two items from the PANSS general psychopathology scale (mannerism and
ism further affected symptom severity in our sample. The effect was notable in male
patients, but not in females (Table 3).

4. Discussion

A number of studies have suggested that increased
PLA2 activity plays a role in vulnerability to schizo-
phrenia [13,15,40,41]. Recently, variation in cPLA2
activity was shown to be associated with a PLA2G4A
I polymorphism [32]. The A2 allele of the
I polymorphism has been repeatedly implicated in the patho-
genesis of schizophrenia. Pae et al. [42] reported an
association between the
I polymorphism and major depressive disorder as well. The results of our study showed no evidence of the statistically significant association between two polymorphisms in PLA2 genes and elevated risk for developing schizophrenia in Croatian population, although they argue in favour of the
I polymorphism having modulator role in the expression of the disease. We found a significant
association between the absence of
I A1 allele, i.e.,
A2A2 genotype and earlier onset of the disease as assessed by mean age at first hospitalization due to a
psychotic episode. The effect was notable in male
patients, but not in females (Table 3). 

Ban
I A2 was
not found in the British [25], US Caucasian [39], Brazilian [29] or Chinese [44] schizophrenic patients. Conflicting results were reported for Indian population [17,45]. To possibly clarify the problem of association between polymorphisms in PLA2 genes and schizophrenia, we performed a case–control and family-based
tively) (Table 2). By comparing the allelic frequencies of
rs4375 and the
I polymorphism in populations of different ethnic origin [24,25,43], we noted that allelic frequencies of both polymorphisms in our population were closer to the allelic frequencies in other European and American populations of Northern and Western European origin, and were quite different from the allelic ratios in Japanese or Korean populations (for
rs4375—74% and 26%, respectively). Similar to our
findings, Wei et al. [25] reported A1 and A2
I allelic frequencies of 36% and 64%, respectively, in unrelated
Caucasian controls, while Pae et al. [24] reported an inverse ratio of the alleles in Korean controls (67.5%
and 32.5%, respectively). Therefore, it was easier to prove a statistically significant accumulation of the pathogenic A2 allele in Korean schizophrenic patients as compared to healthy controls. An excess of
I A2 was not found in the British [25], US Caucasian [39], Brazilian [29] or Chinese [44] schizophrenic patients. Conflicting results were reported for Indian population [17,45]. To possibly clarify the problem of association between polymorphisms in PLA2 genes and schizophrenia, we performed a case–control and family-based
meta-analyses of
I polymorphism in gene PLA2G4A (Table 5) since the literature search revealed several eligible studies. The rs4375 polymorphism in PLA2G6A
gene in schizophrenia was, to our knowledge, investi-
gated only in the study of Junqueira et al. [29], and replicated in this work with opposite results. The studies in the meta-analysis included data from different populations. The heterogeneity of the groups of ORs was statistically significant in both allele-wise and

Table 5

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>A2 allele OR</th>
<th>95% CI</th>
<th>A2A2 homozygosity OR</th>
<th>95% CI</th>
</tr>
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<tr>
<td>Caucasian</td>
<td></td>
<td>1.1</td>
<td>0.9–1.3</td>
<td>1.1</td>
<td>0.9–1.4</td>
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<tr>
<td>Wei et al. [25]</td>
<td>British</td>
<td>1.0</td>
<td>0.7–1.5</td>
<td>1.1</td>
<td>0.6–1.7</td>
</tr>
<tr>
<td>Chowdari et al. [39]</td>
<td>US Caucasian</td>
<td>1.1</td>
<td>0.7–1.7</td>
<td>0.9</td>
<td>0.5–1.8</td>
</tr>
<tr>
<td>Junqueira et al. [29]</td>
<td>Brazilian</td>
<td>1.2</td>
<td>0.9–1.5</td>
<td>1.4</td>
<td>0.9–1.9</td>
</tr>
<tr>
<td>Current study</td>
<td>Croatian</td>
<td>0.9</td>
<td>0.6–1.3</td>
<td>0.8</td>
<td>0.5–1.4</td>
</tr>
<tr>
<td>Asian</td>
<td></td>
<td>1.2</td>
<td>0.9–1.7</td>
<td>1.4</td>
<td>0.8–2.4</td>
</tr>
<tr>
<td>Peet et al. [17]</td>
<td>Indian</td>
<td>2.3</td>
<td>1.1–4.7</td>
<td>2.9</td>
<td>1.1–7.6</td>
</tr>
<tr>
<td>Semwal et al. [45]</td>
<td>Indian</td>
<td>0.9</td>
<td>0.7–1.2</td>
<td>0.9</td>
<td>0.7–1.3</td>
</tr>
<tr>
<td>Pae et al. [24]</td>
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<td>1.8</td>
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<td>2.8</td>
<td>1.3–5.8</td>
</tr>
<tr>
<td>Li et al. [44]</td>
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<td>0.9</td>
<td>0.6–1.3</td>
</tr>
<tr>
<td>Pooled</td>
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<td>1.1</td>
<td>0.9–1.3</td>
<td>1.1</td>
<td>0.9–1.5</td>
</tr>
</tbody>
</table>

\( ^a z = 0.88; P = 0.381; Q = 1.79, \text{ d.f.} = 3, p = 0.617 \) for heterogeneity.

\( ^b z = 0.78; P = 0.437; Q = 2.72, \text{ d.f.} = 3, p = 0.437 \) for heterogeneity.

\( ^c z = 1.11; P = 0.266; Q = 14.40, \text{ d.f.} = 3, p = 0.002 \) for heterogeneity.

\( ^d z = 1.17; P = 0.241; Q = 12.73, \text{ d.f.} = 3, p = 0.005 \) for heterogeneity.

\( ^e z = 1.07; P = 0.285; Q = 16.35, \text{ d.f.} = 7, p = 0.022 \) for heterogeneity.

\( ^f z = 1.00; P = 0.316; Q = 15.47, \text{ d.f.} = 7, p = 0.030 \) for heterogeneity.
from the general psychopathology scale (lack of judgment and insight, mannerism and posture) (Table 4). Mannerisms and posture showed only marginally significant association ($P = 0.045$). Opposite to the findings of Tao et al. [48], we should point out the significant negative association between A2 allele and symptom severity in our patients.

BanI polymorphism in PLA2G4A gene also affected mean age of disease onset in male patients in the current study. This intriguing result suggested a role of sex hormones affecting cPLA2 expression and/or activity and their implication in the etiology of schizophrenia. The neuroprotective action of female sex hormones in brain has been well established and gender-specific differences influencing the age at onset, treatment outcome and the prevalence of negative symptoms have been well recognized in schizophrenia. Recently, PLA2G4A gene was recognized as a target for estrogen-regulated gene expression, at least in bone cells, implicated in cytokine and immune functions [49]. Among numerous neuroprotective effects of estrogen, there is ability to attenuate the neurotoxic intracellular calcium influx. Since cytosolic PL2s are involved in the multiple signaling pathways (such as the phosphoinositide-protein kinase (PI-PKC) [50] and ERK1/2 pathways [51]) mediating transient calcium rise in the cytoplasm, their elevated activity may cause disturbances of the calcium homeostasis and provoke cell death and neurodegeneration. Actually, neuronal cell loss in frontal cortex was found to correlate significantly with negative symptoms in schizophrenia [52].

Limitations in the interpretation of the association data concerning cPLA2 genotype, PL2s enzymatic activity and phospholipid composition of the cellular membranes are the confounding effects of environmental factors, for instance, smoking or diet [53–55]. Nicotine has been recognized as an inhibitor of the cPLA2s activity affecting neuronal response by modulating AA release [56]. This finding may be associated with higher rate of cigarette smokers among schizophrenic patients. The synthesis of LC-PUFAs in the body depends on the dietary intake of essential linoleic (18:2(n-6)) and alpha-linolenic (18:3(n-3)) fatty acids, but limiting factor could also be their conversion rate to LC-PUFAs, mediated by desaturases and elongases. The conversion process is more effective in females than males (possibly connected to higher rate of $\beta$-oxidation of fatty acids in males) [9,11].

Brain phospholipids are highly enriched in DHA and AA comprising over 90% of brain essential fatty acids. New data suggested that group VI iPLA2 primarily mediates the release of DHA [57,58]. Cellular membranes use LC-PUFAs as structural components that enable optimal fluidity for transport processes, but LC-PUFAs also form a pool of signaling molecules, making both cPLA2 and iPLA2 necessary for maintaining phospholipid homeostasis in cellular membranes.
and appropriate cellular responses to internal or external stimuli. Other enzymes may be involved as well, for instance, sPLA2, phospholipases C and D are probably involved in the interplay with various PLA2s [23,59–62]. The higher activity of PLA2s or DHA deprivation may lead to imbalance in DHA/AA ratio in the membrane causing changes in its morphology, fluidity, neurotransmitter release efficacy, monoamine transporter function and trafficking, receptor functions, and downstream processes of the signal transduction [63–66]. The growing body of evidence suggests the involvement of n-3 PUFA metabolism in neurodevelopmental and neurodegenerative processes, neurotransmission and maintenance of the synaptic plasticity, memory formation, apoptotic cell death and other processes. Therefore, the impact of increased or reduced phospholipid breakdown in cellular membranes, and downstream mechanisms, which are both modulated by a number of environmental factors and particular genetic polymorphism(s) in phospholipase A2 genes, can be expected to influence phenotypic expression in other psychiatric diseases as well (Alzheimer disease [67] or affective disorders [68,69]). Increased iPLA2 activity has been demonstrated in serum of patients with first-episode psychosis [41], in serum of patients with bipolar disorder and a history of psychosis [70] and in temporal cortex of schizophrenic patients [71]. Increment and decrease of cPLA2 activity were determined in different brain areas in schizophrenic patients [71], an increase also in plasma of dyslexics [72]. Furthermore, the prolonged use of atypical antipsychotics, mood-stabilizers, and antidepressant drugs was shown to affect similar targets suppressing common biochemical pathway(s) [50].

In conclusion, although neither polymorphism investigated in our study could be associated with an elevated risk for schizophrenia, we demonstrated a statistically significant association between the reported pathogenic A2A2 genotype and an earlier mean age at disease onset in males. These results suggest that the Ban1 polymorphism in gene PLA2G4A confers susceptibility to developing schizophrenia or schizoaffective disorder at early, prodromal phase of the illness and imply a role of sex hormones. The PLA2G4A Ban1 polymorphism accounted for 12.33% of the age variability at first hospitalization in men, indicating the presence of other contributing genetic (such as apolipoprotein E polymorphism [73]) or environmental factors. Ban1 polymorphism also affected severity of the particular symptoms from the general psychopathology scale in our sample. Our results imply modifier role for the cPLA2 polymorphism in etiology and expression of schizophrenia and schizoaffective disorder. However, our results should be taken with caution owing to small number of subjects in the study and need confirmation from other studies. Larger studies exploring both genetic and biochemical markers of disturbed phospholipid metabolism and controlling for the contribution of nicotine usage, age, gender or diet could be helpful in elucidating the relationship between PLA2s’s activity and pathogenesis of schizophrenia.

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