Eur Arch Psychiatry Clin Neurosci (2007) 257:340–343 DOI 10.1007/s00406-007-0736-0

ORIGINAL PAPER

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Association between *Ban*I genotype and increased phospholipase A2 activity in schizophrenia

Received: 22 December 2006 / Accepted: 8 March 2007 / Published online: 14 July 2007

Abstract Phospholipases A2 (PLA2) are a family of key enzymes in the metabolism of membrane phospholipids. Several studies reported on increased blood and brain PLA2 activity in schizophrenia, which suggest a disordered phospholipid metabolism in the disease. In addition, a genetic variant of a cytosolic PLA2 gene has been reported to be associated with schizophrenia. These data indicate that variants of PLA2 encoding genes are plausible candidates for increasing the susceptibility for schizophrenia. In this study, we investigated a possible association between PLA2 activity in platelets and a polymorphic site for *Ban*I in the PLA2 (group 4A) gene on chromosome 1q25. Seventy-five schizophrenic patients (DSM-IV) and 68 healthy controls were recruited and the PCR assays were performed. A radioenzymatic assay for the cytosolic PLA_2 activity in platelets was used. The allele A2 and the genotype A2A2 were more frequent in schizophrenic patients than in controls ($p < 0.005$ and $p < 0.05$ respectively). When we assorted the subjects according to their genotypes, we found that PLA2 activity was significantly higher in patients with the A2A2 genotype (29.6 ± 5.1 pMol/mg protein/min) than in those with the A1A2 (20.8 ± 3.6 pMol/mg protein/min, $p < 0.001$) or A1A1 genotype (15.9 ± 5.1 pMol/mg protein/min, $p < 0.001$). Also in controls, carriers of the A2 allele (A1A2 and A2A2) had higher PLA2 activity than the A1A1 group ($p = 0.004$ for both). Our data suggest an association between *Ban*I genotype and PLA2G4A activity in platelets and that the presence of the allele A2 may increase risk for schizophrenia through an increment of PLA2 activity.

Key words phospholipase A2 · PLA2G4A · cPLA2 · *Ban*I polymorphism · schizophrenia

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EAPCN 736

Introduction

Increased phospholipase A2 (PLA2) activity has been reported in the blood and in brain tissue from schizophrenic patients [7–9, 10, 11, 13, 23, 25], suggesting an accelerated breakdown of membrane phospholipids in the disease. This acceleration has been mainly attributed to the increased activity of the cytosolic enzyme activity (cPLA2), which selectively cleaves arachidonic acid from natural membrane vesicles [4]. This is in line with the findings that arachidonic acid (AA) and docosahexaenoic acid are significantly depleted in membrane phospholipids from patients with schizophrenia [19] and with the clinical reports of significant reductions in positive symptoms in patients after essential fatty acids supplementation [18].

Brain phospholipids are interesting sites for the interaction between genes and environment because the enzymes and other proteins that regulate phospholipid metabolism are clearly genetically determined [11, 12]. Two polymorphisms of the cPLA2 gene (*PLA2G4A*) have been found in chromosome 1q25: the polyA polymorphism and the *Ban*I polymorphism, which occur near the promoter region and near the first intron, respectively [26]. In line with the findings of altered cPLA2 activity in schizophrenia, there are previous reports of an association between polymorphisms of *PLA2G4A* and the disease [13, 17, 19, 22, 28, 29, 30]. However, results are still contradictory and a number of studies reported a lack of association between the cPLA2 gene and schizophrenia [3, 6, 21, 24, 31].

Table 1 Allele and genotype frequencies of the *Ban*I dimorphic site in human cPLA2 gene among schizophrenic patients ($n = 75$) and in healthy controls ($n = 68$)

Group	Allelic fragment[a]		Genotype[b]		
	A1	A2	A1A1	A1A2	A2A2
Schizophrenic patients	76 (50.7%)	74 (49.3%)	23 (30.7%)	30 (40.0%)	22 (29.3%)
Controls	92 (67.6%)	44 (32.4%)	35 (51.5%)	22 (32.3%)	11 (16.2%)

[a] Allele, $\chi^2 = 7.800$; df = 1; $p = 0.005$
[b] Genotype, $\chi^2 = 7.054$; df = 2; $p = 0.029$

To clarify this issue further we investigated in the present study simultaneously the cPLA2 activity and the *Ban*I polymorphism on intron 1 of *PLA2G4A* in schizophrenic patients. We found that in schizophrenic patients PLA2 activity was higher and that the genotype A2A2 was more frequent than in controls. In addition, individuals with A2A2 genotype showed the highest PLA2 activities.

Subjects and methods

Subjects

A total of 75 caucasian inpatients who fulfilled DSM-IV [1] criteria for schizophrenia were recruited at the Institute of Psychiatry, Faculty of Medicine, University of São Paulo. All patients were drug-free for at least 2 weeks and had no comorbidity with organic or neurological disease. The controls were 68 healthy individuals recruited from the Blood Center from the same university hospital. The controls were examined by a trained psychiatrist to exclude past and family history of psychiatric disorder, as well as comorbidity with organic and neurological disorders. Thirty-two (43%) patients were male, with a mean age of 33.5 ± 11.0 years and 32 controls (47%) were male, with a mean age of 35.0 ± 9.3 years. Sex and age distributions were not different between patients and controls ($p = 0.81$ and $p = 0.30$, respectively).

All the subjects gave informed consent to participate in this study, which was approved by the local ethics committee (protocol number 442/01).

Genotyping

Genomic DNA was extracted from leucocytes using salting out method [15]. The *PLA2G4A* gene is mapped to chromosome 1q25 and encodes a cytolosic form of PLA2, classified into group 4 alpha. The genotyping of *Ban*I polymorphism, located in the first intron of *PLA2G4A*, was amplified by polymerase chain reaction (PCR)-based methods using forward and reverse primers (forward, 5′-TGT GCA TTT GCT CAA AGG AG-3′ and reverse, 5′-GTT CGC TGT TTT CGC TCA G-3′), with modified condition as described elsewhere [19].

Platelet isolation

Venous blood (40 ml) was collected in sodium citrate-coated tubes of 10 ml. Blood samples were homogenized in 1 ml of acid citrate dextrose solution (glucose 123.8 mM, sodium citrate 83.9 mM, citric acid 41.3 mM) and centrifuged for 15 min at 515*g* and room temperature. Supernatants (platelet-rich plasma fractions) were removed and pH adjusted to 6.5 and centrifuged for 10 min at 1,159*g* (room temperature). Pellets were re-suspended in 5 ml of wash-solution (trissodium citrate 30 mM pH 6.5, KCl 5 mM, $MgCl_2$ 1 mM, $CaCl_2$ 2 mM, 0.9% NaCl, glucose 5 mM, albumin 0.005%, apyrase 20 units/ml) and centrifuged for 8 min at 1,159*g* (room temperature). Platelet-rich pellets were then re-suspended in 500 µl of tris-sucrose solution (tris 50 mM pH 7.4, sucrose 233 mM) and stored at −70°C until determinations were performed. Prior to the radioenzymatic assays, protein levels were determined for each aliquot by a colorimetric assay (Bio-Rad DC protein assay).

PLA2 activity in platelets

The substrate used was l-alpha-1-palmitoyl-2-arachidonyl-phosphatidylcholine labeled with [l-^{14}C] in the arachidonyl tail at position *sn*-2 (^{14}C-PC) (48 mCi/mmol specific activity, New England Nuclear, Boston MA), previously diluted 1:10 (v/v) in a 0.14 g/ml solution of the antioxidant BHT (buthylated hydroxytoluene) prepared in toluol–ethanol (1:1, v/v). Prior to enzymatic reaction, ^{14}C-PC solution was nitrogen evaporated in a glass vial (total volume equivalent to 0.06 mCi per aliquot), re-suspended in 5 mg/ml human albumin solution (150 µl per aliquot), and homogenized by sonication. Platelet samples were diluted in 400 µl of Tris-sucrose solution to a concentration of 0.1 mg protein/ml, and the final volumes of the various aliquots were equalized to 1 ml by the addition of Tris buffer (50 mm, pH 8.5). The aliquots for enzymatic reactions were prepared on ice with 200 µl of diluted platelet samples (or respective blanks), 100 µl of calcium chloride ($CaCl_2$ 25 mm), 50 µl of Tris (1 m, pH 8.5) and 150 µl of the re-suspended radioactive ^{14}C-PC. Samples were then immersed in a 37°C shaking water-bath for 30 min, after which 700 µl of stop solution (hydrochloric acid-isopropanol 1:12 v/v) were added. Samples were allowed to rest for 10 min at room temperature in order to halt the enzymatic reaction. Radioactive arachidonic acid (^{14}C-AA) released by the PLA2 cleavage of ^{14}C-PC was then extracted by the *n*-heptane–silica method with Dole's reagent followed by adsorption of phospholipids on silica. Samples were homogenized in 700 µl of *n*-heptane, and after 5 min, centrifuged at 3,220*g* at room temperature for 10 min. About 500 µl of the upper phase were transferred to silica-coated tubes containing 300 µl of *n*-heptane. A second spinning was then performed for 10 min, after which 500 µl of the upper phase was added to the scintillation solution. Finally, the radioactivity of ^{14}C-AA was measured in a liquid scintillation counter (Packard Tri-Carb 2100 TR) and CPM counts/min was transformed in a measurement of the PLA_2 activity expressed in pMol/mg protein/min. All PLA2 activity determinations were performed in triplicate.

Data analyses

All comparisons of genotype and allele frequencies in this study were made using the χ^2-square test. Probability (p) values of <0.05 were considered statistically significant (two-tailed). Unpaired Student's *t*-tests were used for comparisons between groups.

Results

Hardy–Weinberg equilibrium for the distribution of genotypes was estimated using HWE program [16] and the results were within the expected equilibrium (data not shown). The genotype and allele distributions in schizophrenia patients were significantly different from those of the controls (Table 1). The PCR products, digested by the restriction enzyme *Ban*I, showed two individual alleles, A1 (cut) and A2 (uncut). The distribution of those alleles were 51% for

Table 2 PLA2G4A *Ban*I genotypes and cPLA2 activity (in pMol/mg protein/min) in platelets in schizophrenic patients ($n = 75$) and healthy controls ($n = 68$)

Genotype	PLA2 activity	
	Schizophrenic patients ($n = 75$)	Controls ($n = 68$)
A1A1	15.9 ± 5.1	13.5 ± 4.7
A1A2	20.8 ± 3.6[a]	18.4 ± 3.8[c]
A2A2	29.6 ± 4.6[b]	18.5 ± 4.4[c]

[a] $p < 0.001$ compared to A1A1
[b] $p < 0.001$ compared to A1A1 and $p < 0.001$ compared to A1A2
[c] $p = 0.004$ compared to A1A1

A1 and 49% for A2 in patients and 68% for A1 and 32% for A2 in healthy controls ($p = 0.005$). In addition, schizophrenic patients had a significant excess of A2A2 homozygote genotype as compared to controls ($p = 0.03$) (Table 1).

Platelet $cPLA_2$ activity was significantly increased in schizophrenics (21.8 ± 6.8 pMol/mg protein/min) as compared to healthy controls (15.9 ± 5.0 pMol/mg protein/min, $p = 0.02$). When subjects were assorted according to their genotypes, we found that subjects with the A2A2 genotype had significantly higher platelet cPLA2 activity (29.6 ± 5.1 pMol/mg protein/min) than those with the A1A2 (20.8 ± 3.6 pMol/mg protein/min, $p < 0.001$) or A1A1 genotypes (15.9 ± 5.1 pMol/mg protein/min, $p < 0.001$). Also in controls, the carriers of the A2 allele (A1A2 and A2A2) had higher cPLA2 activity than the A1A1 group ($p = 0.004$ for both) (Table 2).

Discussion

The present finding of increased platelet cPLA2 activity in schizophrenia is in line with our previous results [8, 9, 25] and suggests an accelerated breakdown of membrane phospholipids in schizophrenia. The genotype and allele distributions of *Ban*I polymorphism of *PLA2G4A* were significantly different between patients and controls, which is in line with previous studies showing a positive association of A2 allele with schizophrenia [17, 19, 30]. Obviously the present sample is too small to investigate any association between genotypes and the disease. This was not, however, the aim of the present study, which was designed to investigate the association between *Ban*I genotypes and cPLA2 activity in schizophrenia. Thus, our finding of an association of A2 with schizophrenia has, at best, a confirmatory character regarding data from the literature.

Rybakowski et al. [22] reported an association between the allele A2 and disturbances of eye movement in schizophrenic patients, which are discussed as possible susceptibility markers for schizophrenia [27]. This association suggests that the allele A2 could be transmitted as a specific endophenotype of schizophrenia. Although the functional significance of the *PLA2G4A* *Ban*I polymorphism has not yet been clarified, our data indicate an effect of the A2 allele on regulatory factors of cPLA2 activity.

There is convincing evidence that phospholipid metabolism is abnormal in schizophrenia and cPLA2 has been shown to be elevated in these patients (review in [14]). In the present study, we investigated the enzyme activity in platelets, which are considered useful peripheral models for neurons, as both share some membrane and receptor properties [2, 5]. Blood cells have frequently been used to draw analogies to brain tissue regarding membrane abnormalities in neuropsychiatric disorders [20].

When we assorted the subjects according to their PLA2G4A *Ban*I genotypes, we found that cPLA2 activity in platelets was significantly higher in patients with the A2A2 genotype (29.6 ± 4.6 pMol/mg protein/min) than in those with the A1A2 (20.8 ± 3.6 pMol/mg protein/min) or A1A1 (15.9 ± 5.1 pMol/mg protein/min) ($p < 0.05$). The $cPLA_2$ enzyme has a number of properties that are crucial for signal transduction, such as migration to the membrane when activated by a variety of signals (for instance changes in intracellular calcium concentration), the production of prostaglandins and other lipid-based neuronal messengers, as well as the regulation by dopamine and glutamate receptors (review in [14]). Thus, increased PLA2 activity could contribute to a disordered brain function in schizophrenia. Taken together, our finding of an association between the PLA2G4A *Ban*I genotype and PLA2 activity suggests that individuals with the allele A2 may present a higher risk for schizophrenia due to increased PLA2 activity.

Acknowledgements This study was supported by FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo. The Laboratory of Neuroscience receives financial support from the Associação Beneficente Alzira Denise Hertzog da Silva (ABADHS). We are indebted to Dr. Emmanuel Dias Neto and Dr. Elida Ojopi for suggestions to this work.

References

1. American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th edn. American Psychiatric Press, Washington, DC
2. Barradas MA, Mikhailidis DP (1993) The use of platelets as models for neurons: possible applications to the investigation of eating disorders. Biomed Pharmacother 47:11–18
3. Chowdari KV, Brandstaetter B, Semwal P, Bhatia T, Deshpande S, Reddy R, Wood J, Weinberg CR, Thelma BK, Nimgaonkar VL (2001) Association studies of cytosolic phospholipase A2 polymorphisms and schizophrenia among two independent family-based samples. Psychiatr Genet 11:207–212
4. Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, Knopf JL (1991) A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. Cell 65:1043–1051

5. Di Luca M, Colciaghi F, Pastorino L, Borroni B, Padovani A, Cattabeni F (2000) Platelets as a peripheral district were to study pathogenetic mechanisms of Alzheimer disease: the case of amyloid precursor protein. Eur J Pharmacol 405:277–283
6. Frieboes RM, Moises HW, Gattaz WF, Yang L, Li T, Liu X, Vetter P, Macciardi F, Hwu HG, Henn F (2001) Lack of association between schizophrenia and the phospholipase-A (2) genes cPLA2 and sPLA2. Am J Med Genet 105:246–249
7. Gattaz WF, Kollisch M, Nevalainen TJ, Thuren T, Kinnunen PKJ (1987) Increased plasma phospholipase-A_2 activity in schizophrenia: reduction after neuroleptic therapy. Biol Psychiatry 22:421–426
8. Gattaz WF, Hubner CK, Nevalainen TJ, Thuren T, Kinnunen PKJ (1990) Increased serum phospholipase-A_2 activity in schizophrenia: a replication study. Biol Psychiatry 28:495–501
9. Gattaz WF, Schmitt A, Maras AM (1995) Increased platelet phospholipase A_2 activity in schizophrenia. Schizophr Res 16:1–6
10. Glen AI, Glen EM, Horrobin DF, Vaddadi KS, Spellman M, Morse-Fisher N, Ellis K, Skinner FS (1994) A red cell membrane abnormality in a subgroup of schizophrenic patients: evidence for two diseases. Schizophr Res 12:53–61
11. Horrobin DF (1998) The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. Schizophr Res 30(3):193–208
12. Horrobin DF, Bennett CN (1999) New gene targets related to schizophrenia and other psychiatric disorders: enzymes, binding proteins and transport proteins involved in phospholipid and fatty acid metabolism. Prostaglandins Leukot Essent Fatty Acids 60:111–167
13. Hudson CJ, Kennedy JL, Gotowiec A, Lin A, King N, Gojtan K, Macciardi F, Skorecki K, Meltzer HY, Warsh JJ, Horrobin DF (1996) Genetic variant near cytosolic phospholipase A2 associated with schizophrenia. Schizophr Res; 21(2):111–116
14. Law MH, Cotton RGH, Berger GE (2006) The role of phospholipase A2 in schizophrenia. Mol Psychiatry 11:547–556
15. Miller SA, Dykes DD, Polesky HF (1988) A single salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 16:1215
16. Ott J (1988) Utility programs for analysis of genetic linkage. Columbia University, New York
17. Pae CU, Yu HS, Lee KU, Kim JJ, Lee CU, Lee SJ, Jun TY, Lee C, Paik IH (2004) *Ban*I polymorphism of the cytosolic phospholipase A2 gene may confer susceptibility to the development of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 28:739–741
18. Peet M (1999) New strategies for the treatment of schizophrenia: omega-3 polyunsaturated fatty acids. In: Peet M, Glen I, Horronin DF (eds) Phospholipid spectrum disorder in psychiatriy. Marius Press, Carnforth, UK, pp 189–194
19. Peet M, Ramchand CN, Lee J, Telang SD, Vankar GK, Shah S, Wei J (1998) Association of the *Ban*I dimorphic site at the human cytosolic phospholipse A2 gene with schizophrenia. Psychiatr Genet 8:191–192
20. Pettegrew JW, Keshavan MS, Panchalingan K, Strychor S, Kaplan DB, Tretta MG, Alen M (1991) Alterations in brain high-energy phosphate and membrane phospholipids metabolism in first-episode, drug naïve schizophrenics. Arc Gen Psychiatriy 48:563–568
21. Price SA, Fox H, St Clair D, Shaw DJ (1997) Lack of association between schizophrenia and a polymorphism close to the cytosolic phospholipase A2 gene. Psychiatr Genet 7:111–114
22. Rybakowski JK, Borkowska A, Czerski PM, Dmitrzak-Weglarz M, Hauser J (2003) The study of cytosolic phospholipase A2 gene polymorphism in schizophrenia using eye movement disturbances as an endophenotypic marker. Neuropsychobiology 47:115–119
23. Smesny S, Kinder D, Willhardt I, Rosburg T, Lasch J, Berger G, Sauer H (2005) Increased calcium-independent phospholipase A2 activity in first but not multiepisode chronic schizophrenia. Biol Psychiatry 57:399–405
24. Tao R, Yu Y, Zhang X, Guo Y, Shi J, Zhang X, Xie L, Liu S, Ju G, Xu Q, Shen Y, Wei J (2005) Cytosolic PLA2 genes possibly contribute to the etiology of schizophrenia. Am J Med Genet B Neuropsychiatr Genet 137(1):56–58
25. Tavares H, Yacubian J, Talib LL, Barbosa NR, Gattaz WF (2003) Increased phospholipase A2 activity in schizophrenia with absent response to niacin. Schizophr Res 61:1–6
26. Tay A, Simon JS, Squire J, Hamel K, Jacob HJ, Skorecki K (1995) Cytosolic phospholipase A2 gene in human and rat: chromosomal localization and polymorphic markers. Genomics 26:138–141
27. Turetsky BI, Calkins ME, Light GA, Olincy A, Radant AD, Swerdlow NR (2007) Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophr Bull 1:69–94
28. Wei J, Hemmings GP (2004) A study of a genetic association between the PTGS2/PLA2G4A locus and schizophrenia. Prostaglandins Leukot Essent Fatty Acids 70:413–415
29. Wei J, Hemmings GP (2005) A study of the combined effect of the CLDN5 locus and the genes for the phospholipid metabolism pathway in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 73(6):441–445
30. Wei J, Lee KH, Hemmings GP (1998) Is the cPLA2 gene associated with schizophrenia? Mol Psychiatry 3:480–481
31. Yu YQ, Tao R, Wei J, Xu Q, Liu SZ, Ju GZ, Shi JP, Zhang X, Xie L, Shen Y (2004) No association between the PTGS2/PLA2G4A locus and schizophrenia in a Chinese population. Prostaglandins Leukot Essent Fatty Acids 71:405–408