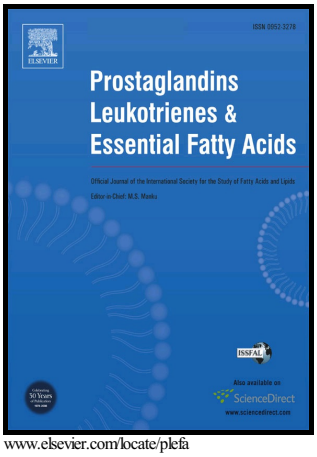

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BanI Polymorphism of Cytosolic Phospholipase A2 Gene and Somatic Symptoms in Medication-Free Acute Depressed Patients

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Abstract

Somatic symptoms are commonly seen in patients with major depressive disorder (MDD) and might be associated with inflammatory activation. Cytosolic phospholipase A2 (cPLA2) and cyclo-oxygenase-2 (COX-2) are the key enzymes in the metabolism of polyunsaturated fatty acids (PUFAs), which in turn may play an important role in inflammation and somatic symptoms in depression. This study investigated the effects of BanI polymorphism of cPLA2 gene and COX-2 rs4648308 genotypes on somatic symptoms and inflammatory marker in patients with MDD. Eighty-two patients with MDD were assessed for their psychopathology including

psychiatric and somatic symptoms, BanI polymorphism of cPLA2 and COX-2 rs4648308 genotypes and CRP levels. The results revealed that MDD patients with the cPLA2 BanI GG genotypes had higher somatic symptoms and higher levels of C-reactive protein (CRP), while no differences were found among the COX-2 rs4648308 genotypes. Inflammatory process, such as arachidonic acid cascade pathway, might help explain the effect of cPLA2 BanI polymorphism on the somatic symptoms, and may be a potential target for future investigation on treatment for MDD with somatic symptoms. However, the interpretation of the findings in this study is limited since we analyzed the data from a subset data from a larger study.

Keywords: BanI polymorphism, Cytosolic phospholipase A2 gene, cPLA2, Major depressive disorder, MDD, HDRS

1. INTRODUCTION

Depressive disorders with predominantly somatic presentation are the most common form of depression. In a clinical study, somatic symptoms including sweating, headache, urinary freuqncy, indigestion and fatigue, were documented in up to 80% of a sample of major depression [1]. About two thirds of depressed patients complained of general aches and pains, implying the close relationship between somatic symptoms and depression [2]. On the other hand, somatic symptoms have been negatively associated with eicosapentanoic acid (EPA), a type of omega-3 (or n-3) polyunsaturated fatty acids (PUFAs) with anti-inflammatory actions [3, 4], and positively associated with arachidonic acid (AA), a type of omega-6 (or n-6) PUFAs which give deriatives to pro-inflammatory cytokines [4].

Epidemiological studies [5, 6], case-control studies [7-12] , and clinical trials

[13-18] all suggest that n-3 PUFAs might play an important role in major depressive disorder (MDD). Moreover, genotypes of the key enzymes for phospholipids metabolism, such as Phospholipase A2 (PLA2) and cyclo-oxygenase-2 (COX-2), have been associated with major depressive disorder [19, 20] and Interferon (IFN)-α induced depression [21]. The major subtype of PLA2 enzymes is the cytosolic A2 (cPLA2), and the gene for cPLA2 (PLA2G4A) has also been cloned and localized to chromosome 1q25 [22, 23]. Cytosolic calcium activates cPLA2, which in turn catalyzes the release of arachidonic acid (AA) from membrane phopshpolids. The gene coding for COX-2 (also known as prostaglandin-endoperoxidase synthase 2, hence the gene is termed PTGS2), converts AA to prostaglandins (PGs), is immediately centromeric of the cPLA2 locus. These two gene loci may share a common regulatory region since they are arranged in a head-to-head configuration. Six single nucleotide polymorphisms (SNPs) present in the PTGS2/cPLA2 and a SNP4 (located in the 5'-flanking region known as rs10798059 or so-called BanI SNP) were associated with schizophrenia [24-27]. Moreover, MDD [19] and IFN-α induced depression associated somatic symptoms [21] have been associated with GG genotype of BanI polymorphism of the cPLA2 gene, while development of major depression has been associated with COX-2 rs4648308 AG genotype [21].

The findings of genetic linkage studies in depression are promising in that they are able to provide more understanding of the disorder. However, even from the studies with large sample sizes and well-designed manner, the findings are not definitive because the effect sizes of the susceptibility loci are only modest [28]. MDD is a heterogeneous complex disease and clearly contributed to by multiple genes; therefore each of the genes to be detected might only have an effect size close to the lower limit. One of the possible alternative approaches is to study the

relationship between genetic variations and symptoms with similar mechanism rather than the disorder as a whole. Since somatic symptoms are common in MDD [29], and these symptoms are similar to PG- mediated sickness behavior [30]. Taken together, the AA cascade, via the actions of PGs and other inflammatory cytokines, may be important not only in the pathogenesis of depressive disorders but also in the manifestation of somatic symptoms. In addition, depression has also been associated with increased levels of pro-inflammatory markers, such as C- reactive protein (CRP) [31], and MDD associated somatic symptoms have also been associated with inflammation [4] and higher levels of proinflammatory cytokines [32]. The aim of the study is to investigate the effect of BanI polymorphism of cPLA2 gene and COX-2 rs4648308 polymorphism on somatic symptoms in MDD with a subset data from a larger study [21]. We hypothesized patients with Ban I GG genotype will have more somatic symptoms than those with BanI AA or AG genotypes.

2. PATIENTS AND METHODS

2.1 Subjects

Eligible patients, age between 18 to 60 years, were screened with the structured Mini-International Neuropsychiatric Interview (MINI) [33] by a senior psychiatric research nurse and interviewed by certified psychiatrists. The patients were recruited from the outpatient clinic of Department of Psychiatry. Patients with a DSM-IV [34] diagnosis of MDD with a current major depressive epsiosde and a score of 17 or higher on the 21-item Hamilton Rating Scale for Depression (HDRS) [35] were enrolled in the study. All subjects were native Taiwanese and were biologically unrelated. Other minorities were excluded from this study. All patients were free of any medical illness on the comprehensive evaluations from medical history, physical

examination and laboratory tests. Those who had a recent or past history of other major psychiatric disorders were exclued. The patients were also free from any medication for at least 4 weeks prior to the study. Patients had to fully understand and sign the informed consent before enrolment. The patients were also sampled for venous blood for genotyping. The study was approved by the China Medical University Hospital Institutional Review Board.

2.2 Assessments

2.2.1 Mini-International Neuropsychiatric Interview (MINI)

MINI is a short structured clinical interview which enables researchers to make diagnoses of psychiatric disorders according to DSM-IV or ICD-10 [33]. The administration time of the interview is approximately 15 minutes and was designed for epidemiological studies and multicenter clinical trials.The information of translation, validation and instruction of Taiwanese version of MINI can be accessed on the website of Taiwanese Society of Psychiatry (http://www.sop.org.tw/dow_a.htm).

2.2.2 The Hamilton Depression Rating Scale - 21 Item (HDRS)

The Semi-structured version of the 21-item HDRS was used to assess the severity and the different clusters of depressive symptoms [35]. The HDRS items were divided into the following published clusters [36]: Core (items 1,2,7,8,10,13), Sleep (items 4,5,6), Activity (items 7,8), Anxiety (items 9,10,11,12,13), Psychic Anixety (items 9,10), Somatic (items, 11,12,13), and Delusion (items 2,15,20). The inter-rater reliability of HDRS has been described elsewhere [11].

2.3 Laboratory Assessment

Genomic DNA was extracted from peripheral blood using DNA extraction kits (Qiagen, Hilden, Germany). The genotyping of cPLA2 BanI and COX-2 rs4648308 polymorphisms were performed by PCR-based restriction fragment length polymorphism (RFLP) analysis with forward and reverse primers (cPLA2 BanI: forward, 5'-TGTGCATTTGCTCAAAGGAG-3', and reverse, 5'-ATCTTGGCTCACTGCAACCT-3'; COX-2 rs4648308: forward, 5'-CGACACTGTGTTGGAAAATGTCT-3', and reverse, 5'-AATTTCCTCCTCTTTGAACTTCTTAACTTTGGGCC-3') to generate a PCR product of 342 and 171 base pairs (bp), respectively. The reaction volume was 25μl in each 0.2ml PCR tube, with reaction component concentrations of 200μM for each dNTP, 2mM MgSO4, 20mM Tris-HCl (pH 8.8), 10mM KCl, 0.1% gelatin, 0.4μM of each primer, and 2.5U Taq DNA polymerase (BioLabs, Beverly, MA, USA), and 50-100ng of genomic DNA. PCR was performed with an initial denaturation temperature of 94°C for 3 minutes, followed by 40 cycles of 95°C for 45 seconds, 60°C for 1 minute, and 72°C for 1 minute, and a final elongation at 72°C for 10 minutes. The PCR products were completely digested with 5 units for each SNP (BioLabs, Beverly, MA, USA). Then we separated DNA fragments on 2% agarose gels followed by ethidium bromide staining. Positive and negative controls were implemented in all gels.

The patients enrolled in the study were assessed for the level of inflammatory marker, CRP. CRP levels were measured by nephelometry, a latex particle-enhanced immunoassay (TBA-200FR, Tokyo, Japan), using a fully automatic biochemical analyzer (Unicel DxC 800 Synchron Clinical System; Beckman Coulter, Fullerton,

CA, USA) at the Clinical Laboratory Department of the China Medical University Hospital. The inter- and intra-assay coefficients of variations (CVs) were <2.0% and <1.9%, respectively. The lower detection limit of the assay was 0.01 mg/dL.

2.4 Statistical Analysis

All statistical analysis was carried out with the Statistical Package for the Social Science (SPSS), version 17.0 for windows.The Chi-square test was applied to examine Hardy-Weinberg equilibrium (HWE) and to assess whether the distribution of the allele frequencies differed in male and female MDD groups. The HWE is a principle stating an ideal condition that allele and genotype frequencies in a population will remain constant form generation to generation in absence of other evolutionary influences, e.g., mate choice, mutation, selection, genetic drift, gene flow and meiotic drive. The Mann-Whitney and the Kruskal-Wallis tests were applied as non-parametric tests when the data distribution was not Gaussian. The Kruskal-Wallis test was used to study the effect of the genotypes (AA, AG, and GG genotypes) of cPLA2 BanI polymorphism and COX-2 rs4648308 polymorphism on the 7 symptomatologic clusters and the total score of HDRS. Multivariate linear regression was used to examine the associations between somatic symptoms and demographic variables, BanI and COX-2 rs4648308 genotypes, and CRP levels. The Bonferroni method was used to adjust p values in post hoc analysis owing to multiple comparisons. All p values were two-tailed and $p< 0.01$ was considered statistically significant.

3. RESULTS

Eighty-two patients were enrolled in the study, with 21 males and 61 females. The frequencies of alleles and genotypes of the cPLA2 BanI polymorphism in this

group were 70.7% for A, 29.3% for G, 51.2% for AA, 39.0% for AG, and 9.8% for GG. Genotype distributions in male, female, and all patients with MDD were all in the HWE for BanI polymorphism, while only the COX-2 rs4648308 polymorphism violated the HWE test. There were no differences in age and gender between the A allele carriers (AA and AG genotypes) and GG genotype for BanI polymorphism (Table 1) and among the COX-2 rs4648308 AA, AG and GG genotypes (Table 2, Supplementary Table S1). There were also no differences in mean HDRS cluster scores of the BanI polymorphism genotypes, except for Somatic clustser; BanI GG genotype had higher Somatic cluster score than BanI AA and AG genotypes ($p=0.001$). BanI GG genotype also had higher CRP level than the other 2 genotypes ($p=0.004$) (Table 1). On the other hand, there were no differences in mean HDRS cluster scores and CRP level among the COX-2 rs4648308 genotypes (Table 2). Moreover, no significant correlation was found between HDRS symptom clusters and CRP (Data not shown). Meanwhile, regression analysis showed BanI polymorphism is the main predictor of somatic sympotms in MDD ($p<0.001$, Table 3).

The SNP gender interaction for BanI polymorphism was significant for the HDRS somatic cluster ($p<0.003$, Supplementary Table S2), where females with BanI GG genotype had higher Somatic cluster scores than the females with BanI AA or AG genotypes. On the other hand, there was no significance of COX-2 rs4648308- gender interaction on HDRS symptom clusters (Data not shown).

4. DISCUSSION

The major finding of this study is that MDD patients with BanI polymorphism of cPLA2 gene GG genotypes had more somatic symptoms than patients with BanI A allele carriers (AA or AG genotypes), which was concordant to our initial hypothesis.

The somatic symptoms included effects of autonomic overactivity, “butterflies,” indigestion, stomach cramps, belching, diarrhea, palpitations, hyperventilations, paresthesia, sweating, flushing, tremor, headache, urinary frequency, loss of appetite, heaviness in limbs, back or head, backaches, headache, muscle aches, loss of energy, and fatigability. Although the exact function of BanI polymorphism cPLA2 gene still requires further investigation, PLA2 are enzymes involved in phospholipids metabolism, cell membrane remodeling and intracellular signaling, while cPLA2 is responsible for release of AA and EPA from cell membrane. Moreover, BanI polymophism has been associated with increased cPLA2 activity in both patients with psychiatric disorders including MDD [19] and schizophrenia [26], which further implies MDD may be associated with disordered metabolism of phospholipids and inflammatory process. In addition, cPLA2 BanI polymorphism may be associated with somatic symptoms in MDD via the changes in EPA levels. PLA2 enzyme deacylates EPA from membrane phospholipids, thus an increased PLA2 enzyme activity in BanI GG genotypes may lead to depletion changes in EPA levels [21], which contributes to perception of somatic complaints in MDD [4]. On the other hand, although polymorphism of COX-2 rs4648308 genotypes have been associated with development of MDD, no association was found between COX-2 rs4648308 polymorphism and somtiac symptoms in MDD in this study. Moreover, although the

distribution of the COX-2 rs4648308 in the study sample violated HWE, some authors have argued that this is acceptable, since "cases" in study samples cannot be considered "general population" [37].

Somatic symptoms are common in depression; while prostaglandin administration associated sickness behavior including malaise, fatigue, and loss of appetite are similar to somatic symptoms in depression [30, 38]. The endogenous metabolism of PGs are modulated by PUFAs [39], and cPLA2 is the key enzyme to catalyze the release of AA, the precursor of PGE2, from membrane phospholipids [40]. AA is then converted to PGE2 by COX-2. The AA-derived PGs and other pro-inflammatory cytokines may then enhance painful sensations and hypersensitivity of sickeness repsonse on the neurons, glia and endothelial cells [41], which may be presented as somatic presentations in patients with depression. Moreover, blood AA level has also been positively associated with somatic symptoms in a clinical study [4].

The second major finding of this study is that MDD patients with BanI polymorphism of cPLA2 gene GG genotypes had higher CRP levels than patients with BanI A allele carriers (AA or AG genotypes). Depression has been associated with higher levels of CRP [31], while a clinical study showed that CRP significantly decreased in patients with MDD after receiving anti-depressant treatments [42]. Moreover, Rapaport et al had reported that 'high' inflammation group, including higher levels of CRP, of patients with MDD responded better to EPA treatment when compared to the 'low' inflammation group in MDD [43]. Hence, BanI GG genotype with an elevated CRP level in MDD may be an indicator for better response to EPA treatment.

We speculate that n-3 PUFAs are able to improve somatic symptoms, since docosahexaenoic acid (DHA) and EPA may lead to reduced eicosanoids and cytokines production of the neurons [44] by antagonizing the actions of AA, reducing PGE2 synthesis, inactivating IL-1 induced neurotransmitter release of nucleus accumbens [44]. In addition, changes in the n-3 to n-6 PUFAs ratio may affect monoamine neurotransmission [45], and possibly increase the risk of developing depression [46] and somatic symptoms. Thus, the balance between the n-3 PUFAs and n-6 PUFAs may be crucial in manifestation of somatic symptoms in depression.

The present study also showed the effect of BanI genotype on somatic symptoms is more prominent in females than in males. This may imply that cPLA2 gene has different effect on gender in MDD. In clinical practice, female patients with MDD seem to have more unexplained somatic symptoms than in male patietns with MDD [47]; however, there is no appropriate biological explanation for the difference. The findings in the present study may provide a possible explanation for the gender difference in the prevalence of somatic symptoms in MDD. It is important to note that the cPLA2 BanI polymorphism only accounted for only 20% (R2) of the variation of somatic symptoms in the whole group, and 24% (R2) of the variation of somatic symptoms in the female patients with MDD. Therefore, other variations for somatic symptoms in MDD are still to be explained by the effects of other genes. Furthermore, the interaction between this susceptibility gene and the environment factors, such as perinatal insults, deprivation of parental care since young age, exposure to conflicts and violence, substance use, head injury, and stressful life events, should not be underestimated [48].

One of the limitation of the this study was that we did not include healthy controls in this study for comparison. Hence, we were not able to examine the effect

of cPLA2 BanI polymorphism with somatic symptoms in the healthy control group. Moreover, the interpretation of the findings in this study is limited because we analyzed the data from a subset data from a larger study with a small sample size . Hence, the small sample size may account for the lack of genotypic association of COX-2 rs4648308 in MDD patients, and limit generalization of this study findings. However, one of the strengths of this study is that the population in this study is of homogenous nature, where the differences of somatic symptoms and CRP levels of both groups are modest, despite statistical significance in the Ban I genotype analysis. In addition, our sample was also unequal in both sexes and the male sample was small (n=21). Thus, the lack of significance of BanI polymorphism on the male's Somatic cluster needs to be interpreted with caution. The environmental factors were also not assessed to examine the interaction with the cPLA2 gene.

5. CONCLUSIONS

The present study provided that BanI polymorphism of cPLA2 gene, but not COX-2 rs4648308 polymorphism, may be associated with somatic symptoms in patients with MDD in the Taiwanese population, and this effect may perhaps be via inflammatory actions such as the AA cascade pathway. Since depression is heterogeneous in terms of etiology and symptomatologic presentation, our result implies that the effect of specific genotype might only be specific to certain symptomatologic cluster in the genetic association studies in depression. We propose that our findings should be replicated with a larger sample size, where future work is required to establish the assocation between BanI polymophism and somatic symptomsm in MDD, and whether BanI GG genotype with an elevated CRP is a predictor for better response to EPA treatment.

Author Contributions: Prof. Su created the concept, designed the study, received the research funding and prepared and revised the manuscript. Drs. Chang & Guu performed the clinical assessments and prepared the manuscript. Profs. Su, Walczewska, and Gałecki designed the study, performed the literature search and manuscript preparation and revision. All the authors have full access to all the data and take responsibility for the integrity and accuracy of this study.

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Summery

Cytosolic phospholipase A2 (cPLA2) and cyclo-oxygenase-2 (COX-2) are the key enzymes in the metabolism of polyunsaturated fatty acids (PUFAs), which in turn may play an important role in inflammation and somatic symptoms in depression. This study investigated the effects of BanI polymorphism of cPLA2 gene and COX-2 rs4648308 genotypes on somatic symptoms in patients with MDD. Eighty-two paitents with MDD were assessed for psychiatric and somatic symptoms, BanI polymorphism of cPLA2 and COX-2 rs4648308 genotypes and CRP levels. The results revealed that MDD patients with the cPLA2 BanI GG genotypes had higher somatic symptoms and higher levels of C-reactive protein (CRP), while no differences were found among the COX-2 rs4648308 genotypes. Inflammatory process, such as arachidonic acid cascade pathway, might help explain the effect of cPLA2 BanI polymorphism on the somatic symptoms, and may be a potential target for future investigation on treatment for MDD with somatic symptoms. However, the interpretation of the findings in this study is limited since we analyzed the data from a subset data from a larger study.

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Table 1 Demographic Data and Hamilton Depression Rating Scale Scores and the cPLA2 BanI Polymorphism Genotypes (A allele and GG genotype)

	Genotype		
	A allele (n=74)	**GG (n=8)**	**P**
Age, yrs (SD)	36.9(12.1)	35.3(8.3)	.713
Gender, Male N (%)	19(26%)	2(25%)	----
HDRS Total	27.3(5.4)	29.3(7.2)	.344
HDRS Core	15.1(2.4)	15.8(2.3)	.472
HDRS Sleep	3.5(1.7)	3.4(2.5)	.847
HDRS Activity	3.8(1.4)	3.9(1.6)	.822
HDRS Psychic Anxiety	4.5(1.6)	4.1(1.6)	.525
HDRS Somatic	4.5(1.7)	5.8(.7)	.001*
HDRS Delusion	3.8(1.7)	4.8(2.3)	.173
CRP	0.8(0.2)	0.9(0.2)	.004*

N=82 in this analysis

Note: CRP, high-sensitivity C-reactive protein; HDRS, Hamilton Depression Rating Scale; N, number; SD, standard deviation; yrs, years.

The Chi-square test was applied to examine Hardy-Weinberg equilibrium. T-test was applied to compare the HDRS cluster scores between the two groups.

The Bonferroni method was used to adjust *p* values in post hoc analysis owing to multiple comparisons, * indicates statistical signifiance where $p<0.01$.

Table 2 Demographic Data and Hamilton Depression Rating Scale Scores and the COX-2 rs4648308 Genotypes

	Genotype			P
	AA (n=7)	**GG (n=14)**	**AG (n=61)**	
Age, yrs (SD)	42.6(13.7)	37.9(11.8)	35.7(11.4)	0.312
HDRS Total	30.6(4.9)	28.9(6.6)	26.8(5.3)	0.129
Gender, Male N(%)	0(0%)	6(7.3%)	15(18.3%)	0.132
HDRS "Core"	15.4(2.0)	16.1(2.6)	14.9(2.3)	0.239
HDRS Sleep	4.6(1.3)	3.3(1.8)	3.4(1.7)	0.220
HDRS Activity	4.1(1.7)	4.1(1.6)	3.7(1.3)	0.452
HDRS Psychic Anxiety	4.0(2.4)	4.2(1.6)	4.6(01.5)	0.508
HDRS Somatic	4.3(2.4)	5.1(1.5)	4.6(1.6)	0.421
HDRS Delusion	5.0(1.4)	4.6(1.9)	3.6(1.8)	0.043
CRP	0.9(0.2)	0.8(0.2)	0.8(0.2)	0.277

N=82 in this analysis.

Note: CRP, high-sensitivity C-reactive protein; HDRS, Hamilton Depression Rating Scale; N, number; SD, standard deviation; yrs, years.

The Chi-square test was applied to examine Hardy-Weinberg equilibrium.

T-test was applied to compare the HDRS scores in both groups.

The Bonferroni method was used to adjust *p* values in post hoc analysis owing to multiple comparisons, statistical significance is indicated when $p<0.01$.

* indicates statistical significance of $p<0.01$

Table 3 Predictors of Somatic Symptoms in MDD

	Somatic	
	Beta	T
Gender	.056	.531
Age	.036	.345
Ban I Genotype	0.429	4.081***
COX-2 rs4648308	-.113	-1.088
CRP	-.057	-.548

N= 82 in this analysis.

Note: CRP, high-sensitivity c-reactive protein; MDD, major depressive disorder; Som, somatic symptoms.

Linear regression is used to examine the preidctors of somatic symptoms.

*** indicates statistical significance of $p<0.001$

Highlights

- We investigated the effects of BanI polymorphism of cPLA2 gene and COX-2 rs4648308 genotypes on somatic symptoms and inflammatory marker in patients with MDD.
- Patients with Major depressive disorder (MDD) with the cPLA2 BanI GG genotypes had higher somatic anxiety symptoms and higher levels of C-reactive protein (CRP).
- Female patients with MDD with the cPLA2 BanI GG genotypes had higher somatic anxiety symptoms and higher levels of C-reactive protein (CRP).
- Inflammatory process, such as arachidonic acid cascade pathway, might help explain the effect of cPLA2 BanI polymorphism on the somatic anxiety symptoms, and may be a potential target for future investigation on treatment for MDD with somatic symptoms.