Published in final edited form as:

Psychiatr Genet. 2001 December ; 11(4): 207–212.

Association studies of cytosolic phospholipase A2 polymorphisms and schizophrenia among two independent family-based samples

Kodavali V. Chowdari[a], **Bernard Brandstaetter**[a], **Prachi Semwal**[c], **Triptish Bhatia**[d], **Smita Deshpande**[d,e], **Ravi Reddy**[a], **Joel Wood**[a], **Clarice R. Weinberg**[f], **B.K. Thelma**[c,d], and **Vishwajit L. Nimgaonkar**[a,d,b]

[a]Department of Psychiatry, University of Pittsburgh School of Medicine and Graduate School of Public Health, Pittsburgh, Pennsylvania, USA

[b]Department of Human Genetics, University of Pittsburgh School of Medicine and Graduate School of Public Health, Pittsburgh, Pennsylvania, USA

[c]Department of Genetics, University of Delhi South Campus, New Delhi, India

[d]Indo-US Project on Schizophrenia Genetics, New Delhi, India

[e]Department of Psychiatry, Dr R.M.L. Hospital, New Delhi, India

[f]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

Abstract

An association between the cytosolic phospholipase A2 locus (*cPLA2*) and schizophrenia has been reported using two polymorphic DNA markers. In an attempt to replicate these results, two independent family-based samples were ascertained from the United States and India (86 and 159 families, respectively). No significant associations were detected in either sample.

Keywords

schizophrenia; genetic; association; phospholipase A2

INTRODUCTION

Several lines of evidence indicate that schizophrenia may be associated with abnormal metabolism of membrane phospholipids, including reduced levels of arachidonic acid and docosahexaenoic acid (Horrobin *et al.*, 1995; Reddy and Yao, 1999). Increased activity of cytosolic phospholipase A2 (cPLA2) is thought to account for these findings. cPLA2 is widely distributed in human tissues, with elevated levels in the lung and hippocampus. cPLA2 preferentially hydrolyzes arachidonic acid from the sn-2 position of membrane phospholipids in a dose-dependent fashion (Clark *et al.*, 1991). The released arachidonic

Correspondence to V.L. Nimgaonkar, MD, PhD, WPIC, Room 443, 3811 O'Hara Street, Pittsburgh, PA 15213-2593, USA.

acid and its eicosanoid metabolites play an important role as regulators of *trans*-synaptic neuronal activity through modulatory effects on specific neuronal receptors, ion channels and on neurotransmitter release (Hudson *et al.*, 1993). A reduction of arachidonic acid has been found in erythrocyte membranes from patients with schizophrenia (Glen *et al.*, 1994; Peet *et al.*, 1994), particularly in those with persistent chronic negative symptoms (Glen *et al.*, 1994). Elevated cPLA2 activity in the serum has been reported among patients with schizophrenia, compared either with healthy control subjects or with psychiatric patients without schizophrenia (Gattaz *et al.*, 1987, 1990, 1995; Ross *et al.*, 1997). Magnetic resonance spectroscopy studies have also indirectly implicated cPLA2. Patients with schizophrenia had significantly reduced levels of phosphomonoesters (PMEs) and significantly increased levels of phosphodiesters (PDEs) in the dorsal prefrontal cortex, compared with healthy control subjects (Keshavan *et al.*, 1991; Pettegrew *et al.*, 1991). While PMEs are cleaved from their respective phospholipids in the presence of phospholipase C, the degradation of membrane phospholipids to PDEs is mediated by phospholipase A1 and A2.

The gene coding for cPLA2 has been localized on chromosome 1q25 (Tay *et al.*, 1995). Two *cPLA2* polymorphisms have been used for association studies of schizophrenia. A significant association was first reported using a bi-allelic *Ban*I restriction fragment length polymorphism in the first intron of the *cPLA2* gene. A significant excess of individuals homozygous for the A2 allele (*Ban*I site present) were noted among 36 unrelated Indian patients compared with 27 unrelated ethnically matched control subjects (odds ratio = 2.11, $P < 0.03$) (Lee *et al.*, 1998). The association could not be detected among 193 unrelated Caucasians and 101 unrelated healthy control subjects. In contrast, haplotype risk (HRR) analysis among 50 Caucasian patients and their parents suggested excess transmission of A2 alleles to the patients (Wei *et al.*, 1998).

A polyA polymorphism in the promoter region of *cPLA2* is located 1 kB upstream of the first exon (Tay *et al.*, 1995). Among 65 cases and matched unrelated control subjects, Hudson *et al.* (1996a) detected a significant association using this polymorphism. The results were later confirmed following HRR analysis of 44 case — parent triads (Hudson *et al.*, 1996b). However, an association could not be detected in a smaller sample of 58 cases and 56 control subjects (Price *et al.*, 1997).

These results are suggestive, but the lack of consistency precludes definitive conclusions about the putative genetic association. Some of the discordant results could be due to inclusion of cases and unrelated control subjects. Such studies are prone to artifacts resulting from the population substructure. Family-based association studies, such as those based on the Transmission Disequilibrium Test (TDT), circumvent this potential problem by investigating preferential transmission of alleles to probands from heterozygous parents (Spielman *et al.*, 1994). To investigate the proposed association more thoroughly, we conducted our analyses using independent family-based US and Indian samples. Both the *Ban*I and the poly(A) polymorphisms were analyzed. In addition to the TDT, we also used the Likelihood Ratio Test (LRT) (Weinberg, 1999). Like the TDT, the LRT is insensitive to associations resulting from genetic admixture. Unlike the TDT, the LRT can usefully extract information about transmission from single parent families. In addition, the LRT can also

detect and discriminate between the effects of an inherited genotype versus a maternal genotype. Since only heterozygous transmissions are used for TDT analysis, substantial numbers of cases and parents may be discarded. Therefore, we also examined the association using the US cases and ethnically matched, unrelated control subjects.

METHODS

Clinical

US sample cases and parents—The family-based sample was ascertained from psychiatric treatment facilities within a 500 mile radius of Pittsburgh, PA. It consisted of cases and available parents. The patients were recruited from outpatient and inpatient treatment facilities in this region. Clinical information about the patients included the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger *et al.*, 1994), available hospital records and relevant interviews with relatives. This information was synthesized and a consensus diagnosis obtained (DSM-IV criteria, American Psychiatric Association, 1994). The parents of the cases provided blood samples, but psychiatric assessments were not conducted. There were 86 cases and 130 parents drawn from 86 families (44 patients with both parents and 42 patients with one parent). Only one case was selected from each family. There were 69 families of Caucasian and 17 families of African-American ethnicity. Among the cases, 47 fulfilled DSM-IV criteria for schizophrenia and the remainder were diagnosed with schizoaffective disorder. The sample included 52 men and 34 women, with a mean age of 36 years [range, 21–62, standard deviation (SD) = 8.6].

Three additional African-American cases and five Caucasian cases did not have a parent available but were included in the analyses involving unrelated control subjects. Thus, a total of 94 cases were available for these analyses. All participants provided written informed consent as required by the University of Pittsburgh Institutional Review Board (IRB).

Unrelated US controls—Cord blood samples were obtained from 94 live births at Magee-Women's Hospital, Pittsburgh, PA and served as unscreened, population-based controls. No information apart from ethnicity and gender was available for these samples. Therefore, informed consent was not required by the University of Pittsburgh IRB.

Indian sample—The sampling design and interview procedures were identical to those of the US studies. We used the Hindi version of the DIGS (Deshpande *et al.*, 1998). Patients were ascertained from psychiatric treatment facilities in the metropolitan New Delhi area. There were 159 cases and 283 parents drawn from 159 families (124 patients with both parents and 35 patients with one parent). Only one case was selected from each family. All but two of the cases were diagnosed with schizophrenia (DSM-IV criteria). The cases included 95 men and 64 women, with a mean age of 33.3 years (range, 16–59; SD 9.2). All participants provided written informed consent as required by the University of Pittsburgh IRB, the Office for Protection of Research Risks (National Institutes of Health), the ethical committees based at the University of Delhi, and Dr Ram Manohar Lohia Hospital, New Delhi.

Laboratory

Samples from the US participants were analyzed at the University of Pittsburgh (V.L.N.)and those from India at University of Delhi, South Campus (B.K.T.). Genomic DNA was extracted from whole blood by using the phenol-chloroform method.

*Ban*I polymorphism

The sequence containing the polymorphic site for BanI in the first intron of the *cPLA2* gene was amplified using the polymerase chain reaction (PCR). The primers used were CATGCCCGTAATACCAGCAC (forward) and GCAAACAAGATGAATGGGAAC (reverse)(Wei *et al.*, 1998). PCR conditions included initial denaturation at 94°C for 5 min, 35 cycles of 96°C for 20 s, 60°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min using a Perkin-Elmer 9600 thermocycler. The PCR reactions (12 included 80 ng genomic DNA, 100 μM each dNTP, 2.1 mM $MgCl_2$, 3 pmol each primer, 0.25 U *Taq* polymerase, 0.125 μl formamide and 1 × PCR buffer. The amplified 546 base-pairs PCR product was digested with *Ban*I for 2 h and electrophoresced on a 2% agarose gel. The digestion yielded a dimorphic site with two alleles: A1 (restriction site absent) and A2 (restriction site present).

Poly(A) polymorphism

The primers used were CCTCCTTTCTAGAAATTCAG (forward) and CAGAGCTTCAGTGAGCCA (reverse) (Price *et al.*, 1997). The forward primer was labeled with γ-^{32}P-ATP by T4 polynucleotide kinase. The PCR reactions (10 μl)included 60 ng genomic DNA, 250 μM dATP, dGTP and dTTP, 100 μM dCTP, 1.5 mM $MgCl_2$, 6 pmol each primer, 0.5 U Taq polymerase, 1 × Taq buffer and 0.025 μl α-^{32}P-dCTP (3000 Ci/mmol specific activity). PCR conditions included initial denaturation at 97°C for 7 min, 25 cycles of 95°C for 90s, 57°C for 40 s, 72°C for 40 s, and a final extension at 72°C for 5 min using a Perkin-Elmer 9600 thermal cycler. The PCR samples were run on 6% polyacrylamide gel electrophoresis at 1500 V/2 h. The M13 DNA sequencing ladder and a CEPH sample were used as internal controls.

Statistical analysis

The chi-square test was used for comparisons between cases and unrelated controls. We conducted TDT analysis using the Monte Carlo Extended TDT (MCETDT) (Zhao *et al.*, 1999). The ETDT calculates three likelihood ratio TDT statistics: an allele-wise statistic that assumes an allele-specific effect on transmission distortion, a genotype-wise statistic that allows for an independent effect for each parental genotype, and a goodness-of-fit statistic that assesses the adequacy of the allele-wise model compared with the genotype-wise model. The MCETDT software excludes families where parental genotypes are missing or if it is not possible to deduce transmitted alleles (Sham and Curtis, 1995). We utilized GENEHUNTER software for TDT analysis involving haplotypes (Kruglyak *et al.*, 1996). Since our US sample included a substantial number of families in which a parent was unavailable, we also used the LRT (Weinberg, 1999). Linkage disequilibrium was estimated using published software applied to the parents of the US cases (Zhao *et al.*, 2000).

RESULTS

*Ban*I polymorphism

Of the 86 US and 159 Indian families, only 42 and 116 heterozygous transmissions, respectively, could be analyzed using MCETDT. No significant transmission distortions were detected in either sample. Among the informative transmissions, 22 A1 and 20 A2 alleles were transmitted in the US sample. In the Indian sample, 59 A1 alleles were transmitted compared with 47 A2 alleles. The LRT did not yield significant evidence for an association in the US sample [inherited genotype effect, $\chi^2 = 2.12$, 2 degrees of freedom (df); maternal genotype effect, $\chi^2 = 0.24$, 2 df; interaction effect, $\chi^2 = 2.52$, 2 df]. A significant association was also not detected in the Indian sample (inherited genotype effect, $\chi^2 = 0.99$, 2 df; maternal genotype effect, $\chi^2 = 2.56$, 2 df; interaction effect, $\chi^2 = 0.54$, 2 df).

In view of the published reports involving unrelated controls, we also compared a subset of our US cases with ethnically matched neonatal controls. No significant differences in genotype distribution or allele frequencies were noted among 74 Caucasian and 20 African-American cases compared with the unrelated controls (Table 1).

Poly(A) polymorphism

Since an association was detected in the promoter region among Caucasians, we also conducted TDT analysis using this marker in our US sample. We identified 26 different alleles and numbered them according to the number of ‘A’ repeats; thus A17 represents the allele with 17 repeats. The following alleles were detected: A17, A18, A19, A24, A27, A29, A31, A32, A33, A34, A35, A37, A38, A39, A40, A41, A42, A43, A44, A45, A46, A47, A48, A49, A51, A52. The distribution of the alleles is in the same range as those reported by Price *et al.* (1997), but differs from another report in which 10 alleles were detected (Hudson *et al.*, 1996b). TDT analysis did not reveal significant excess transmission of any allele (distribution of transmitted alleles using the aforementioned nomenclature: 0, 3, 2, 2, 0, 1, 2, 2, 1, 0, 2, 6, 10, 4, 6, 5, 4, 3, 8, 4, 1, 3, 2, 2, 0, 2; untransmitted alleles: 1, 1, 0, 0, 2, 1, 3, 0, 1, 2, 3, 5, 10, 5, 11, 8, 4, 2, 4, 6, 2, 1, 1, 1, 1, 0). The LRT was not employed for the poly(A)polymorphism because currently available software can be used only for bi-allelic markers. Although the LRT could have been used if individual alleles were analyzed, we wished to avoid multiple comparisons.

Haplotype-based analyses—We analyzed the parental transmission of haplotypes using the TDT. Thirty-three different haplotypes were transmitted in the US family-based sample. No significant evidence for transmission distortion was noted.

Linkage disequilibirum—Since genotypes for the poly(A)polymorphism were not available among the population-based neonatal controls or for the Indian sample, we estimated linkage disequilibrium (LD)between this polymorphism and the *Ban*I polymorphism among the US parents only. Significant levels of LD were present among 86 Caucasian parents for whom genotypes were available (number of inferred haplotyopes, 38; $D' \pm \text{SD} = 0.567 \pm 0.07$, $\chi^2 = 72.4$, 25 df, $P = 0.00001$). Significant LD was also observed

in the smaller sample of 18 African-American parents (19 haplotypes inferred; D' ± SD= 0.885± 0.07, χ^2= 25.8, 14 df, P = 0.028) (Lewontin, 1988; Zhao *et al.*, 2000).

Power analysis

The published association for the *Ban*I polymorphism suggests a recessive mode of inheritance and a frequency of 0.54 for the associated A2 allele among Indian controls (odds ratio = 2.11; Lee *et al.*, 1998). Power estimates for family-based samples designed to test genome-wide associations have been published (Knapp, 1999). The significance level (α) was set at 10^{-7} in these analyses. A less stringent value (α = 0.05) is permissible in replicative studies. A crude approximation suggests a reduction in the required sample size by a factor of 1/6 (M. Knapp, 1999 personal communication). Using these estimates, the Indian sample would have approximately 80% power to detect the putative association (two-tailed). The published association for the poly(A) polymorphism suggests an increased frequency of the A9 allele among cases (frequency among controls, 0.03, odds ratio = 5.0; Hudson *et al.*, 1996a). The available US sample would be unlikely to have adequate power, as only two informative transmissions for the A9 allele were detected in our sample.

DISCUSSION

Evidence is mounting for abnormal membrane phospholipid metabolism in schizophrenia (Horrobin *et al.*, 1994; Ross *et al.*, 1999). The fact that certain polyunsaturated fatty acids, particularly arachidonic acid and docosahexaneoic acid, are significantly depleted in membrane phospholipids from patients with schizophrenia, and the recent reports of significant reductions in positive symptoms in patients after essential fatty acids supplementation (Peet, 1999), makes *cPLA2* gene an interesting candidate gene in schizophrenia genetics. To date, two polymorphisms at the *cPLA2* locus have been reported to be associated with schizophrenia (Wei *et al.*, 1998; Peet *et al.*, 1998).

Our analyses do not support either of the published associations. We did not detect significant distortion of parental transmission for *Ban*I alleles in either of our family-based samples. This is unlikely to be due to exclusion of single-parent families from TDT analysis, since LRT analysis did not support the association. Comparison of our US cases with unrelated control subjects also did not suggest an association. We also analyzed a highly polymorphic $(A)_n$ marker that is localized to the promoter region and could plausibly have functional relevance. A significant association was not detected in our US sample. Although our sample lacks adequate power to investigate this highly polymorphic marker, it is noteworthy that it is in significant LD with the *Ban*I locus.

A number of other factors could also explain the failure to detect associations. These include variations in the sample characteristics, such as differing levels of population admixture between our samples and those published, or differences in the exposure to environmental etiological factors that may interact with variations in *cPLA2.* In addition, allelic heterogeneity cannot be excluded. Since only two polymorphisms were examined, it is possible that other unknown polymorphisms in the *cPLA2* gene confer risk in our samples. Therefore, further studies are required before *cPLA2* can be excluded as a susceptibility gene for schizophrenia.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health (MH01489, MH56242 and MH53459, R03 TW00730 to V.N. and Indo-US Project Agreement # N-443-645 to V.N./B.K.T.). The authors thank the following colleagues for help with ascertainment in India: S.P. Aggarwal, B.R. Agnihotri, K.M. Aggarwal, M. Batra, N. Bohra, R.K. Chadha, P.L. Chawla, A.K. Das, S.K. Das, U. Goswami, A.K. Gupta, S. Jena, R.C. Jiloha, U. Khastgir, A. Kumar, K. Kumar, A. Lal, D.N. Mandekar, H. Matai, M.N.L. Mathur, S. Mittal, D. Mohan, J. Nagpal, R. Nagpal, M. Pahwa, H.C. Raheja, R. Rastogi, R. Sagar, S. Sengupta, A.K. Sharma, P.K. Shrivastva, R.A. Singh, R.K. Singh, S. Nodiyar, P. Dwivedi, Ms Sushma, and Mrs Zutshi.

References

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th. Washington, DC: American Psychiatric Association; 1994.

Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, et al. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. Cell. 1991; 65(6):1043–1051. [PubMed: 1904318]

Deshpande SN, Mathur MNL, Das SK, Bhatia T, Sharma SD, Nimgaonkar VL. A Hindi version of the Diagnostic Interview for Genetic Studies. Schizophren Bull. 1998; 24(3):489–493.

Gattaz WF, Kollisch M, Thuren T, Virtanen JA, Kinnunen PK. Increased plasma phospholipase-A2 activity in schizophrenic patients: reduction after neuroleptic therapy. Biol Psychiatry. 1987; 22(4): 421–426. [PubMed: 3567258]

Gattaz WF, Hubner CV, Nevalainen TJ, Thuren T, Kinnunen PK. Increased serum phospholipase A2 activity in schizophrenia: a replication study. Biol Psychiatry. 1990; 28(6):495–501. [PubMed: 2223919]

Gattaz WF, Schmitt A, Maras A. Increased platelet phospholipase A2 activity in schizophrenia. Schizophren Res. 1995; 16(1):1–6.

Glen AI, Glen EM, Horrobin DF, Vaddadi KS, Spellman M, Morse-Fisher N, et al. A red cell membrane abnormality in a subgroup of schizophrenic patients: evidence for two diseases. Schizophren Res. 1994; 12(1):53–61.

Horrobin DF, Glen AI, Vaddadi K. The membrane hypothesis of schizophrenia. Schizophren Res. 1994; 13(3):195–207.

Horrobin DF, Glen AI, Hudson CJ. Possible relevance of phospholipid abnormalities and genetic interactions in psychiatric disorders: the relationship between dyslexia and schizophrenia. Med Hypotheses. 1995; 45(6):605–613. [PubMed: 8771057]

Hudson CJ, Young LT, Li PP, Warsh JJ. CNS signal transduction in the pathophysiology and pharmacotherapy of affective disorders and schizophrenia. Synapse. 1993; 13(3):278–293. [PubMed: 8497811]

Hudson CJ, Lin A, Horrobin DF. Phospholipases: in search of a genetic base of schizophrenia. Prostaglandins Leukot Essent Fatty Acids. 1996a; 55(1–2):119–122. [PubMed: 8888134]

Hudson CJ, Kennedy JL, Gotowiec A, Lin A, King N, Gojtan K, et al. Genetic variant near cytosolic phospholipase A2 associated with schizophrenia. Schizophren Res. 1996b; 21(2):111–116.

Keshavan MS, Pettegrew JW, Panchalingam KS, Kaplan D, Bozik E. Phosphorus 31 magnetic resonance spectroscopy detects altered brain metabolism before onset of schizophrenia [letter]. Arch Gen Psychiatry. 1991; 48(12):1112–1113. [PubMed: 1845229]

Knapp M. A note on power approximations for the transmission/disequilibrium test. Am J Hum Genet. 1999; 64:1177–1185. [PubMed: 10090903]

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet. 1996; 58(6):1347–1363. [PubMed: 8651312]

Lee KH, Ramchand CN, Wei J, Telang SD, Vankar GK, Shah S, Peet M. Association of the Ban I dimorphic site at the human cytosolic phospholipase A2 gene with schizophrenia. Schizophren Res. 1998; 29:128.

Lewontin RC. On measures of gametic disequilibrium. Genetics. 1988; 120(3):849–852. [PubMed: 3224810]

Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, et al. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. Arch Gen Psychiatry. 1994; 51(11):849–859. discussion, 863–864. [PubMed: 7944874]

Peet, M. New strategies for the treatment of schizophrenia: omega-3 polyunsaturated fatty acids. In: Peet, M.Glen, I., Horrobin, DF., editors. Phospholipid spectrum disorder in psychiatry. Carnforth, UK: Marius Press; 1999. p. 189-194.

Peet M, Laugharne JD, Horrobin DF, Reynolds GP. Arachidonic acid: a common link in the biology of schizophrenia? [letter]. Arch Gen Psychiatry. 1994; 51(8):665–666. [PubMed: 8042917]

Peet M, Ramchand CN, Lee J, Telang SD, Vankar GK, Shah S, Wei J. Association of the Ban I dimorphic site at the human cytosolic phospholipase A2 gene with schizophrenia. Psychiatr Genet. 1998; 8(3):191–192. [PubMed: 9800222]

Pettegrew JW, Keshavan MS, Panchalingam K, Strychor S, Kaplan DB, Tretta MG, Allen M. Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naive schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy [see comments]. Arch Gen Psychiatry. 1991; 48(6):563–568. [PubMed: 1898445]

Price SA, Fox H, St Clair D, Shaw DJ. Lack of association between schizophrenia and a polymorphism close to the cytosolic phospholipase A2 gene. Psychiatr Genet. 1997; 7(3):111–114. [PubMed: 9323323]

Reddy, R., Yao, J. Membrane-protective strategies in schizophrenia: conceptual and treatment issues. In: Peet, M.Glen, I., Horrobin, DF., editors. Phospholipid spectrum disorder in psychiatry. Carnforth, UK: Marius Press; 1999. p. 75-88.

Ross BM, Hudson C, Erlich J, Warsh JJ, Kish SJ. Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium-independent phospholipase A2. Arch Gen Psychiatry. 1997; 54(5):487–494. [PubMed: 9152103]

Ross BM, Turenne S, Moszczynska A, Warsh JJ, Kish SJ. Differential alteration of phospholipase A2 activities in brain of patients with schizophrenia. Brain Res. 1999; 821(2):407–413. [PubMed: 10064828]

Sham PC, Curtis D. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Ann Hum Gene. 1995; 59(Part 3):323–336.

Spielman RS, McGinnis RE, Ewens WJ. The transmission/disequilibrium test detects cosegregation and linkage. Am J Hum Genet. 1994; 54(3):559–560. [PubMed: 8116627]

Tay A, Simon JS, Squire J, Hamel K, Jacob HJ, Skorecki K. Cytosolic phospholipase A2 gene in human and rat: chromosomal localization and polymorphic markers. Genomics. 1995; 26(1):138–141. [PubMed: 7782073]

Wei J, Lee K-H, Hemmings GP. Is the cPLA2 gene associated with schizophrenia? Mol Psychiatry. 1998; 3:480–482. [PubMed: 9857971]

Weinberg CR. Allowing for missing parents in genetic studies of case-parent triads. Am J Hum Genet. 1999; 64:1186–1193. [PubMed: 10090904]

Zhao JH, Sham PC, Curtis D. A program for the monte carlo evaluation of significance of the extended transmission/disequilibrium test [letter]. Am J Hum Genet. 1999; 64(5):1484–1485. [PubMed: 10205285]

Zhao JH, Curtis D, Sham PC. Model-free analysis and permutation tests for allelic associations. Hum Heredity. 2000; 50(2):133–139. [PubMed: 10799972]

TABLE 1

Genotypes for the *Ban*I polymorphism among US cases and controls

Ethnicity	Group	*n*	Genotypes			Frequencies	
			A1/A1	A1/A2	A2/A2	A1	A2
Caucasian	Cases	74	10	37	27	0.39	0.61
	Controls	74	14	32	28	0.41	0.59
African-American	Cases	20	3	8	9	0.35	0.65
	Controls	20	4	9	7	0.42	0.58

Alleles A1 and A2 represent the absence and presence of the *Ban*I restriction site, respectively.