# Letter to the Editor

**Psychiatric Genetics** 2021, **31**:102–105

## Re-analysis of genetic polymorphism data supports a relationship between schizophrenia and microsatellite variability in *PLA2G4A*

**Craig J. Hudson**[a], **Justin X.G. Zhu**[b] and **Alexandra M. Durocher**[c], [a]Biosential Inc., Toronto, Ontario, [b]Lakeridge Health, Whitby, Ontario and [c]Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

Correspondence to Craig J. Hudson, MD FRCP(C), Biosential Inc. 100 King Street West, Suite 5700 Toronto, ON M5X 1C7, Canada
Tel: +1 800 735 4538/+519 763 0123; e-mail: craighudson@biosential.com

**Received** 13 December 2020 **Accepted** 8 March 2021

Phospholipases $A_2$ ($PLA_2$) comprise a superfamily of enzymes that regulate lipid metabolism by catalyzing fatty acid release from membrane phospholipids (Dennis, 1994). Cytosolic $PLA_2$ ($cPLA_2$) is one such isoform found in the central nervous system that regulates eicosanoid production. The activity of $cPLA_2$ has been implicated in various neural processes, including the development and modification of synapses (Farooqui *et al.*, 2000). Two decades ago, $cPLA_2$ was studied for its potential role in the pathophysiology of schizophrenia. A study by Hudson *et al.* (1997) discovered a subset of schizophrenic patients who were nicotinic acid-insensitive; that is, they did not produce a facial vasodilation response to nicotinic acid, a phenomenon normally mediated by $cPLA_2$. Based on this finding, it was hypothesized that nicotinic acid insensitivity resulted from dysfunctional $cPLA_2$ activity, prompting researchers to investigate variations in the *PLA2G4A* gene in schizophrenia.

In 1995, Tay *et al.* discovered a genetic marker on the long arm of chromosome 1, ~ 1 kilobase upstream of the promoter of *PLA2G4A*. The marker was a large microsatellite – a sequence of DNA with a repeated characteristic – consisting of adenine units (polyA). The study established length polymorphism of the microsatellite by identifying 10 alleles with different numbers of adenine units. Very short or long length microsatellites can cause replication errors, thereby increasing the probability of loss-of-function mutations in nearby genes (Leclercq *et al.*, 2010). Additionally, microsatellite sequences can influence nucleosome positioning and thereby influence transcription factor binding or epigenetic modifications (Bagshaw 2017). Indeed, in yeast polyA sequences influence gene expression (Iyer and Struhl 1995; Yang *et al.*, 2018). Length polymorphisms of the polyA microsatellite marker may therefore alter *PLA2G4A* expression or production of a functional or properly regulated $cPLA_2$ enzyme. Several groups have investigated these length polymorphisms in schizophrenia; however, this association remains unclear. Here, we revisit these studies and provide a re-analysis that indicates the polyA microsatellite as a functional marker of schizophrenia.

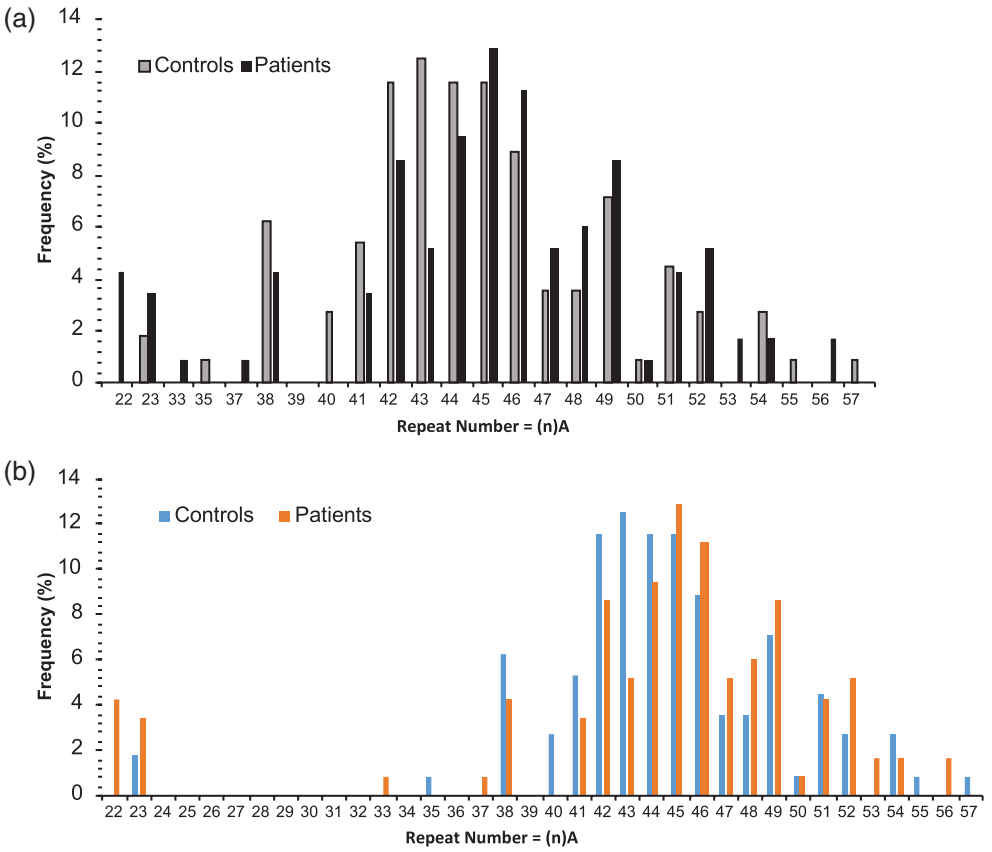
Hudson *et al.* (1996) were the first to investigate length polymorphisms of the polyA microsatellite in patients with schizophrenia. These polymorphisms occurred as 10 differently sized alleles ranging from 41 to 60 adenine residues. Because of technical limitations in the 1990s, the alleles were divided into two broad groups based on size: one group included the shorter alleles 1–6; the other group included the longer alleles 7–10. The shorter alleles were more common in healthy subjects, whereas the longer alleles were more common in subjects with schizophrenia ($n = 65$; Mann–Whitney $U$ test; $P < 0.001$). Additionally, subjects with schizophrenia were significantly more likely to have both alleles in the 7–10 range ($\chi^2$ test; $P < 0.005$). The authors then separated the schizophrenia group into two sets based on nicotinic acid sensitivity. The nicotinic acid-insensitive patients ($n = 9$) displayed longer allelic variants than the nicotinic acid-sensitive patients ($n = 11$). This finding suggested that nicotinic acid insensitivity in schizophrenia may have resulted from disrupted *PLA2G4A* expression or altered function of $cPLA_2$ due to the longer allelic variants of the polyA microsatellite.

A subsequent study by Price *et al.* (1997) sought to replicate the findings of Hudson *et al.* (1996). In the Price *et al.* (1997) study population of 58 patients and 56 unrelated controls, the authors found 24 differently sized alleles ranging from 22 to 57 adenine residues. They reported no significant association between allele length and schizophrenia. However, analyzing each allele separately, the sample size was too low for statistical evaluation; thus, Price and colleagues used a 1000-trial Monte-Carlo simulation to predict statistical significance. Additionally, the presentation of the allelic frequency data included an unmarked truncated x-axis (Fig. 1a), resulting in the data appearing as normally distributed. Here, we re-plotted the data on a complete x-axis (Fig. 1b). Our updated plot appears to have a bimodal distribution of allele frequency in both groups; however, the number of individuals with the short alleles (<27 adenines) is too few for statistical analysis.



 DOI: 10.1097/YPG.0000000000000280

**Fig. 1**




Re-analysis of *PLA2G4A* allelic variation in microsatellite region and association with schizophrenia. (a) Original graph from Price *et al.* (1997) with allelic frequency data on an unmarked truncated x-axis. Reprinted with permission from Wolters Kluwer Health, Inc. (b) New graph of data from Price *et al.* (1997) with allelic frequency data on the complete x-axis.

Given the limited sample sizes, we combined the data from Price *et al.* (1997) with that of a study by Chowdari *et al.* (2001). The latter study identified 26 different alleles, ranging from 17 to 52 adenine repeats, in 75 patients and 75 parents and investigated these allelic frequencies in schizophrenia using a transmission disequilibrium test. We pooled the data from these two studies, which gave an allele range between 17 and 57 repeated adenines. We then grouped the data according to the number of repeat adenines: 'short' alleles (17–27 adenines), 'medium' alleles (28–47 adenines) and 'long' alleles (48–57 adenines) (Fig. 2). We then combined the short and long alleles into an 'extreme' length group comprised of 21 allelic variants. The rationale for grouping the alleles was to increase the numbers in each group to achieve sufficient *n* for statistical comparison. By grouping the frequencies in this manner, we had 105 alleles in the extreme length group and 339 alleles in the medium group, representing 133 schizophrenic patients (75 related to controls and 58 unrelated to the controls) and 131 control individuals (75 related to the patients and 56 unrelated individuals). We compared 'extreme' versus 'medium' allelic frequencies in schizophrenia patients and healthy controls utilizing a $2 \times 2$ Fisher's exact test. The proportion of patients with schizophrenia was significantly higher in the extreme category [$P = 0.0059$; odds ratio (OR) = 1.91; 95% confidence interval (CI), 1.18–3.12], suggesting that *PLA2G4A* polyA microsatellites that are extremely long or extremely short may be associated with schizophrenia. Two other studies also evaluated the association between polyA microsatellite polymorphisms and schizophrenia, but these did not report the length of polyA sequences (Doris *et al*, 1998; Frieboes *et al.*, 2001). Therefore, we could not include their data in the re-analysis.

Our re-analysis of previous studies indicated that the polyA microsatellite near the promoter region of the *PLA2G4A* gene may, indeed, serve as a functional marker for schizophrenia. We showed that length polymorphisms of this microsatellite, with relatively short or long sequences of polyA repeats, correlate with schizophrenia. These extreme length microsatellites could contribute to

**Fig. 2**

| Allele size | Number of alleles in control group | Number of alleles in schizophrenic group | Total number of alleles |
|---|---|---|---|
| 17 | 1 | 0 | 1 |
| 18 | 4 | 3 | 7 |
| 19 | 2 | 2 | 4 |
| 20 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 |
| 22 | 0 | 5 | 5 |
| 23 | 2 | 4 | 6 |
| 24 | 2 | 2 | 4 |
| 25 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 |
| 27 | 2 | 0 | 2 |
| 28 | 0 | 0 | 0 |
| 29 | 2 | 1 | 3 |
| 31 | 5 | 2 | 7 |
| 32 | 2 | 2 | 4 |
| 33 | 2 | 2 | 4 |
| 34 | 2 | 0 | 2 |
| 35 | 6 | 2 | 8 |
| 36 | 0 | 0 | 0 |
| 37 | 11 | 7 | 19 |
| 38 | 27 | 15 | 42 |
| 39 | 9 | 4 | 13 |
| 40 | 20 | 6 | 26 |
| 41 | 19 | 9 | 18 |
| 42 | 21 | 14 | 35 |
| 43 | 19 | 9 | 28 |
| 44 | 25 | 19 | 44 |
| 45 | 23 | 19 | 42 |
| 46 | 13 | 14 | 27 |
| 47 | 8 | 9 | 17 |
| 48 | 7 | 9 | 16 |
| 49 | 11 | 12 | 23 |
| 50 | 1 | 1 | 2 |
| 51 | 6 | 5 | 11 |
| 52 | 5 | 8 | 13 |
| 53 | 0 | 2 | 2 |
| 54 | 3 | 2 | 5 |
| 55 | 1 | 0 | 1 |
| 56 | 0 | 2 | 2 |
| 57 | 1 | 0 | 1 |
| TOTAL | 262 | 191 | 444 |

Combined allelic frequency data from Price *et al.* (1997) and Chowdari *et al.* (2001) grouped into short, medium and long alleles. Short and long allele groups (orange shading) were combined for statistical comparison with medium group (grey shading) to assess differences between control individuals and schizophrenic patients.

the schizophrenia phenotype by impairing the expression of *PLA2G4A* by altering nucleosome position (Bagshaw 2017) or causing the production of a dysfunctional or improperly regulated $cPLA_2$ through replication errors (Leclercq *et al.*, 2010).

Multiple studies of serum $PLA_2$ activity and its association with schizophrenia have been performed with conflicting outcomes (Law *et al.* 2006; Xu *et al.* 2019). Multiple difficulties arise in correlating serum, plasma or platelet $PLA_2$ activity with the activity of $cPLA_2$ in the brain. $PLA_2$ activity in blood samples can arise from either $cPLA_2$ activity or other forms of $PLA_2$. Indeed, most studies report increased activity or abundance of other forms of $PLA_2$ in blood samples of patients with schizophrenia; whereas different groups identified higher, lower or no difference in $cPLA_2$ activity in blood samples of schizophrenic patients compared with samples from healthy controls (see meta-analysis in Xu *et al.* 2019). Furthermore, even studies that differentiated between $cPLA_2$ and other forms of $PLA_2$ did not measure $cPLA_2$ activity or the amount of protein produced from the *PLA2G4A* gene specifically. Analysis of cPLA2 activity in postmortem brain tissue revealed decreased activity in specific brain regions of patients with schizophrenia (Ross *et al.* 1999). Similar to the analysis of $cPLA_2$ activity in the blood, which gene product is responsible for the differences remains unknown.

Consequently, we argue that the association between potentially decreased cPLA2 activity and schizophrenia pathophysiology needs additional study. In particular, a loss-of-function or decreased function of *PLA2G4A* would explain the nicotinic acid insensitivity in a subset of patients with schizophrenia. It is, therefore, important to revisit the investigation of this potential genetic predisposition for schizophrenia and the mechanistic consequences thereof, especially in the nicotinic acid-insensitive subtype of this complex disease.

## Acknowledgements

The authors thank Nancy R. Gough, Ph.D., BioSerendipity LLC., Elkridge, MD, for assisting in drafting the manuscript. The authors thank Drs. Andrew D. Paterson and Pingzhao Hu of The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada, for assistance with statistical analysis. Dr. Craig J. Hudson holds several patents pertaining to Central Nervous System Signal Transduction.

### Conflicts of interest

There are no conflicts of interest.

## References

Bagshaw ATM (2017). Functional mechanisms of microsatellite DNA in eukaryotic genomes. *Genome Biol Evol* **9**:2428–2443.

Chowdari KV, Brandstaetter B, Semwal P, Bhatia T, Deshpande S, Reddy R, *et al.* (2001). Association studies of cytosolic phospholipase A2 polymorphisms and schizophrenia among two independent family-based samples. *Psychiatr Genet* **11**:207–212.

Dennis EA (1994). Diversity of group types, regulation, and function of phospholipase A2. *J Biol Chem* **269**:13057–13060.

Doris AB, Wahle K, MacDonald A, Morris S, Coffey I, Muir W, Blackwood D (1998). Red cell membrane fatty acids, cytosolic phospholipase-A2 and schizophrenia. *Schizophr Res* **31**:185–196.

Farooqui AA, Ong WY, Horrocks LA, Farooqui T (2000). Brain cytosolic phospholipase A2: localization, role, and involvement in neurological diseases. *Neuroscientist* **6**:169–180.

Frieboes RM, Moises HW, Gattaz WF, Yang L, Li T, Liu X, *et al.* (2001). Lack of association between schizophrenia and the phospholipase-A2 genes cPLA2 and sPLA2. *Am J Med Genet* **105**:246–249.

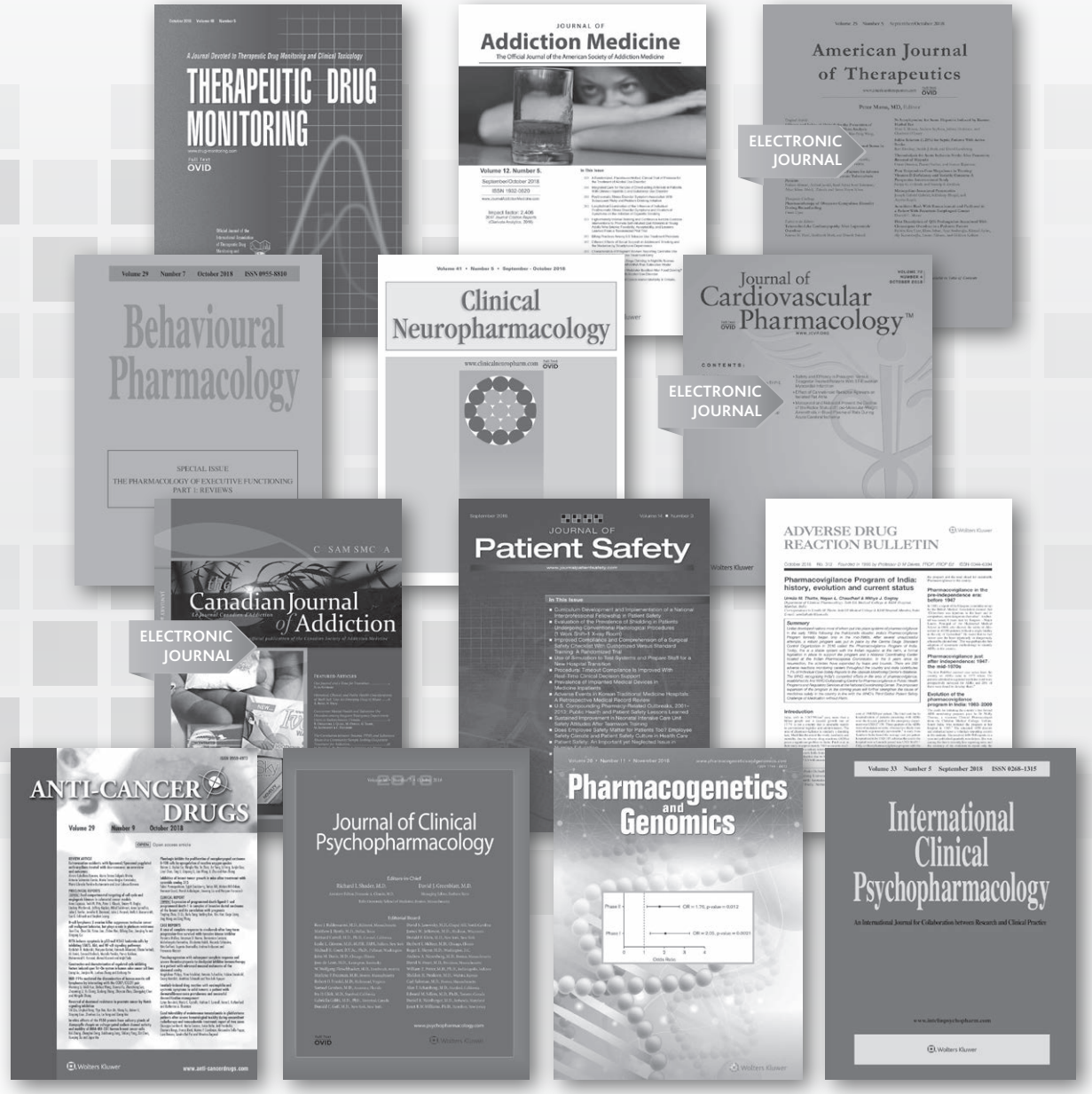
Hudson CJ, Kennedy JL, Gotowiec A, Lin A, King N, Gojtan K, *et al.* (1996). Genetic variant near cytosolic phospholipase A2 associated with schizophrenia. *Schizophr Res* **21**:111–116.

Hudson CJ, Lin A, Cogan S, Cashman F, Warsh JJ (1997). The niacin challenge test: clinical manifestation of altered transmembrane signal transduction in schizophrenia? *Biol Psychiatry* **41**:507–513.

Iyer V, Struhl K (1995). Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. *EMBO J* **14**:2570–2579.
Law MH, Cotton RG, Berger GE (2006). The role of phospholipases A2 in schizophrenia. *Mol Psychiatry* **11**:547–556.
Leclercq S, Rivals E, Jarne P (2010). DNA slippage occurs at microsatellite loci without minimal threshold length in humans: a comparative genomic approach. *Genome Biol Evol* **2**:325–335.
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.
Ross BM, Turenne S, Moszczynska A, Warsh JJ, Kish SJ (1999). Differential alteration of phospholipase A2 activities in brain of patients with schizophrenia. *Brain Res* **821**:407–413.
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.
Xu C, Yang X, Sun L, Yang T, Cai C, Wang P, *et al*. (2019). An investigation of calcium-independent phospholipase A2 (iPLA2) and cytosolic phospholipase A2 (cPLA2) in schizophrenia. *Psychiatry Res* **273**:782–787.
Yang J, Cai H, Liu J, Zeng M, Chen J, Cheng Q, Zhang L (2018). Controlling AOX1 promoter strength in Pichia pastoris by manipulating poly (dA:dT) tracts. *Sci Rep* **8**:1401.

DOI: 10.1097/YPG.0000000000000280

# Essential Titles in PHARMACOLOGY




Search articles, register for eAlerts, view abstracts, and SUBSCRIBE online at **shop.lww.com**.

Wolters Kluwer

8-R195