Re-analysis of genetic polymorphism data supports a relationship between schizophrenia and microsatellite variability in PL2G4A

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Phospholipases A2 (PLA2) comprise a superfamily of enzymes that regulate lipid metabolism by catalyzing fatty acid release from membrane phospholipids (Dennis, 1994). Cytosolic PLA2 (cPLA2) is one such isoform found in the central nervous system that regulates eicosanoid production. The activity of cPLA2 has been implicated in various neural processes, including the development and modification of synapses (Farooqui et al., 2000). Two decades ago, cPLA2 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 cPLA2. Based on this finding, it was hypothesized that nicotinic acid insensitivity resulted from dysfunctional cPLA2 activity, prompting researchers to investigate variations in the PL2G4A 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 PL2G4A. 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 PL2G4A expression or production of a functional or properly regulated cPLA2 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 (χ2 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 PL2G4A expression or altered function of cPLA2, 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.

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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
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 cPLA2 through replication errors (Leclercq et al., 2010).

Multiple studies of serum PLA2 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 PLA2 activity with the activity of cPLA2 in the brain. PLA2 activity in blood samples can arise from either cPLA2 activity or other forms of PLA2. Indeed, most studies report increased activity or abundance of other forms of PLA2 in blood samples of patients with schizophrenia; whereas different groups identified higher, lower or no difference in cPLA2 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 cPLA2 and other forms of PLA2 did not measure cPLA2 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 cPLA2 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.

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Conflicts of interest

There are no conflicts of interest.

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