The role of phospholipases A2 in schizophrenia

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A range of neurotransmitter systems have been implicated in the pathogenesis of schizophrenia based on the antidiopaminergic activities of antipsychotic medications, and chemicals that can induce psychotic-like symptoms, such as ketamine or PCP. Such neurotransmitter systems often mediate their cellular response via G-protein-coupled release of arachidonic acid (AA) via the activation of phospholipases A2 (PLA2s). The interaction of three PLA2s are important for the regulation of the release of AA – phospholipase A2 Group 2 A, phospholipase A2 Group 4 A and phospholipase A2 Group 6 A. Gene variations of these three key enzymes have been associated with schizophrenia with conflicting results. Preclinical data suggest that the activity of these three enzymes are associated with monoaminergic neurotransmission, and may contribute to the differential efficacy of antipsychotic medications, as well as other biological changes thought to underlie schizophrenia, such as altered neurodevelopment and synaptic remodelling. We review the evidence and discuss the potential roles of these three key enzymes for schizophrenia with particular emphasis on published association studies.

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Introduction

Neurotransmitters, such as dopamine, serotonin and glutamate often mediate their cellular response via G-protein-coupled activation of second messengers. One such common second messenger is arachidonic acid (AA) that is released from membrane phospholipids via the G-protein-coupled activation of phospholipase A2 (PLA2).1 Phospholipase A2 activity and AA-based signalling is also important for normal brain development and synaptic functioning.2–5 This review outlines how PLA2s, in particular PLA2G2A, PLA2G4A and PLA2G6A, may be relevant for the understanding of schizophrenia pathogenesis and its treatments.6

Method

Medline and Google Scholar (www.scholar.google.com) were used to identify articles from 1970 to 2005 using the following key words: schizophrenia, essential fatty acids (EFA), polyunsaturated fatty acid (PUFA), AA, PLA2, phospholipase A2 Group 4 A (PLA2GIV, PLA2GIIA), phospholipase A2 Group 2 A (PLA2GIA, PLA2G2A), phospholipase A2 Group 6 A (PLA2GVIA, PLA2G6A), dopamine, serotonin, G-protein-coupled receptor, eicosanoids and phospholipids. Crosschecking of references was used to identify further papers of interest.

PLA2s – naming conventions

When analysing PLA2 studies it is essential to properly define which enzymes are being examined. Phospholipases A2 are a large family of enzymes that specifically deacylate fatty acids from the stereospecifically numbered second carbon atom (sn2, thus PLA2) of the triglyceride backbone of membrane phospholipids, producing a free fatty acid and a lyso-phospholipid (Figure 1).7 Historically, PLA2s were named by activity location, that is pancreatic, cytosolic or secretory. Later naming systems also included calcium requirements, although this can be misleading. Some calcium-dependent PLA2s require calcium for catalytic activity, whereas others are constitutively active and calcium promotes binding to phospholipid membranes. The more structured classification system based on genetic relationships will be used in this review.7 In this system each PLA2 is assigned to one of (currently) 11 groups; each group may contain multiple homologues further assigned a letter. For example, phospholipase A2 Group 4 A
PLA2s and AA

PLA2s regulate both the availability of free lysophospholipids for AA membrane incorporation, and the cleavage of AA from membranes for signalling (Figures 1 and 2). The polyunsaturated fatty acid (PUFA) AA itself is a potent signalling molecule, and also the precursor for a range of messengers (eicosanoids) necessary for normal neuronal function. To limit aberrant signalling AA is usually bound to cell membranes phospholipids. In addition to creating a reservoir for later release of AA in response to appropriate signals, the ratio of saturated to unsaturated fatty acids (such as AA) also defines membrane fluidity, which alters the activity of membrane bound proteins including neurotransmitter receptors.

PLA2G4A has a 50-fold preference for phospholipids containing AA over any other PUFA, whereas PLA2G6A and PLA2G2A show no fatty acid preference. PLA2G4A initiates AA release and eicosanoid production, whereas PLA2G2A enhances the production of AA (Figure 2). Mice with the genes for PLA2G4A and PLA2G2A nullified (knocked out) do not produce eicosanoids, have reduced incorporation rates of free AA into cell membranes, yet normal AA levels.

PLA2G6A cleavage of phospholipids is primarily for the purpose of cellular membrane remodelling, by altering phospholipids/fatty acid ratios and modifying membrane fluidity. Loss of PLA2G6A function leads to significant reductions in the amount of AA incorporated into the cell membrane.

PLA2 in neurobiology

The neurodevelopmental hypothesis and the neurotransmitter theories of schizophrenia propose that abnormal genetic and environmental processes inter-
Brain maturation, cortical development and synaptic remodelling
Brain maturation, cortical development and synaptic remodelling involves removal of excess brain cells or parts of cells such as dendrites or axons. Key regulator proteins involved in these processes are the caspases, in particular caspase 3. Caspases manage apoptotic cellular metabolism via the cleavage of certain proteins such as PLA2G4A and PLA2G6A into alternative forms, altering their behaviour.22 Cleaved PLA2G4A proteins have a dominant-negative function, halting cellular PLA2G4A activity. Truncation of PLA2G6A upregulates its activity, allowing rapid remodelling of the cellular membrane and generation of free AA required for apoptosis.18 The remodelled membranes generates phagocytic attraction signals to allow correct disposal of the cell remnants created resulting from the apoptotic process.22,39–41

While not required within apoptotic neurons, complete loss of PLA2G4A and PLA2G2A abolishes inflammation following central nervous system (CNS) injury, and reduces neuronal apoptosis.19 Studies have shown how PLA2G4A is expressed in adjacent glial cells postinjury, but not within apoptotic neurons.42,43 Together this indicates a primary role for PLA2G6A activity in neuronal remodelling by apoptosis, while PLA2G4A and PLA2G2A act peripherally to manage inflammation.44,45

PLA2s in schizophrenia
Horrobin46,47 suggested that schizophrenia might be a prostaglandin deficiency disease and consequently formulated the Membrane Phospholipid Hypothesis of schizophrenia. This hypothesis can be examined in the light of the combined functions of these three PLA2s.

PLA2s and reduced PUFA's in schizophrenia
Red blood cell (RBC) and CNS cell membranes of medicated and drug naïve patients with schizophrenia have significantly lower levels of PUFA's, in particular AA 48–52 suggesting that this is not only a drug effect. The change in AA levels correlates to decreased membrane fluidity and to psychosis severity12,13,49,51,53 with some conflicting results.54 AA reductions could not solely be explained by environmental factors such as smoking, diet or medication.55–57 As loss of PLA2G6A function results in reduced cell membrane AA levels, variations in this gene may be responsible for these results.24

PLA2s and in vivo phospholipid metabolism
31-Phosphorus magnetic resonance imaging (31P MRS) allows measurement of membrane phospholipid dynamics in the living brain.45 31P MRS of drug naïve first episode schizophrenia patients reveals significantly increased breakdown and reduced creation of cellular membranes in never treated patients with schizophrenia (Pettegrew 1991, Fukusako 1999). Interestingly, a significant relationship between reduced peripheral RBC AA levels and in vivo brain membrane breakdown products has been demonstrated in patients with schizophrenia.48 Changes in the activity of these PLA2s, which are important for apoptosis and membrane maintenance would explain the correlation between peripheral AA levels and brain phospholipid metabolism.

Niacin insensitivity – a marker for an abnormal AA metabolism
The vitamin nicotinic acid (niacin) induces the release of inflammatory eicosanoids such as prostaglandin D2, which are produced from AA metabolism.68 Depending on the method/definitions used, 40–80% of patients with psychotic disorders and 10% of controls show an impaired sensitivity to niacin.59–62 Niacin sensitivity is also impaired in healthy first degree relatives of probands, suggesting it is a marker for underlying genetic risk.63 Niacin insensitivity remains associated with schizophrenia after exclusion of environmental factors modifying inflammation.64–67 Niacin insensitive patients have significantly lower AA levels when compared to niacin sensitive patients.68 Prospective monitoring revealed conversion from niacin insensitive to sensitive was associated with restoration of AA levels and improvement in symptomatology.68,69 Interestingly, the abolishment of AA release and resultant eicosanoid production reported previously in mice with the genes for PLA2G4A and PLA2G2A knocked out is applicable to niacin insensitivity.69,70 Niacin sensitivity could serve as a readily measurable biological marker of altered AA metabolism indicative of altered PLA2 activity.

Direct evidence of altered PLA2 activity in schizophrenia
To evaluate the Membrane Hypothesis of Schizophrenia a number of studies have measured PLA2 activity in patients with schizophrenia. Most studies measuring PLA2 activity in psychotic disorders have used either a radiometric or a fluorometric method (Table 1). The fluorometric method measures all calcium-independent PLA2s, including PLA2G6A, whereas the radiometric method detects the activity of calcium-dependent PLA2s, encompassing PLA2G4A and PLA2G2A.70 Additionally, a recent study has reported on a direct measure of the PLA2G4A protein, and found increased levels of PLA2G4A in schizophrenia.71 The method used by Noponen et al.72 to detect increased PLA2 activity was novel, with the target PLA2s unconfirmed, and thus will be excluded from further discussion. Owing to the limitations in methodology activity studies will be examined in terms of PLA2 calcium requirements.

Calcium-independent PLA2 activity
Increased calcium-independent PLA2 activity in patients with schizophrenia has been replicated both peripherally and in post-mortem human brain tis-
Table 1  Biochemical analysis of phospholipase activity

<table>
<thead>
<tr>
<th>Article</th>
<th>Diagnosis and numbers*</th>
<th>Medicationb</th>
<th>Controls</th>
<th>Analysis</th>
<th>Source</th>
<th>Resultc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gattaz et al.73</td>
<td>SZ, 20, 6 Psych</td>
<td>8 DN, AF</td>
<td>21</td>
<td>Flourometric</td>
<td>Serum</td>
<td>↑ CI</td>
</tr>
<tr>
<td>Gattaz et al.74</td>
<td>SZ, 14, Psych. 8</td>
<td>6 DN, AF</td>
<td>20</td>
<td>Flourometric</td>
<td>Serum</td>
<td>↑ CI</td>
</tr>
<tr>
<td>Albers et al.79</td>
<td>SZ/SZF 10, Psych 25</td>
<td>All DN</td>
<td>10</td>
<td>Radiometric</td>
<td>Serum = CD</td>
<td></td>
</tr>
<tr>
<td>Gattaz et al.76</td>
<td>SZ, 31, Psych 31</td>
<td>Y, AF</td>
<td>31</td>
<td>Radiometric</td>
<td>Platelet</td>
<td>↑ CD</td>
</tr>
<tr>
<td>Hudson et al.80</td>
<td>SZ, 23</td>
<td>Y, AF</td>
<td>30</td>
<td>Niacin sensitivity, Radiometric</td>
<td>Serum</td>
<td>↓ CD</td>
</tr>
<tr>
<td>Katila et al.77</td>
<td>SZ, 34, Psych. 28</td>
<td>11 DN, AF</td>
<td>62</td>
<td>Flourometric</td>
<td>Plasma</td>
<td>= CI</td>
</tr>
<tr>
<td>Ross et al.70</td>
<td>SZ, 24</td>
<td>Y</td>
<td>33</td>
<td>Flourometric, Radiometric</td>
<td>Serum</td>
<td>↑ CI, = CD</td>
</tr>
<tr>
<td>Ross et al.76</td>
<td>SZ, 10, Bipolar 8</td>
<td>Y, AF</td>
<td>12</td>
<td>Flourometric</td>
<td>Brain</td>
<td>↑ CI, ↓ CD</td>
</tr>
<tr>
<td>Tavares et al.81</td>
<td>SZ, 38</td>
<td>19 DN, AF</td>
<td>28</td>
<td>Niacin sensitivity, Flourometric</td>
<td>Serum</td>
<td>↑ CI</td>
</tr>
<tr>
<td>Lasch et al.75</td>
<td>SZ/SZF 26</td>
<td>11 DN, AF</td>
<td>26</td>
<td>Flourometric</td>
<td>Serum</td>
<td>↑ CI</td>
</tr>
<tr>
<td>Macdonald et al.78</td>
<td>SZ, 29</td>
<td>Y, AF</td>
<td>27</td>
<td>Protein measurement</td>
<td>RBC</td>
<td>↑ PLA2G4A</td>
</tr>
</tbody>
</table>

*SZ, schizophrenia, SZF, schizophreniform, Psych., psychiatric Illnesses other than schizophrenia.  
 Y, yes, DN, drug naïve, AF, medication state accounted for during statistical analysis.  
 CI, calcium-independent PLA2 activity; CD, calcium-dependent PLA2 activity.

Higher calcium-independent PLA2 activity has been correlated to greater severity of psychopathology70 though other positive studies have not seen this association.60,73 Katila et al.77 reported normal calcium-independent PLA2 activity in schizophrenia, though Lasch et al.75 suggest this may have been a methodological problem.

Calcium-dependent PLA2

An initial report of increased peripheral calcium-dependent PLA2 activity in schizophrenia has not been replicated.62,70,78,79 Analysis of post-mortem brain tissue found calcium-dependent PLA2 activity was decreased, rather than increased.76

Could response to antipsychotic medication be due to interactions with PLA2s?

Eight weeks of antipsychotic medication reduced the high calcium-dependent PLA2 activity of patients with schizophrenia, replicating a result seen in the original study of calcium-independent PLA2 activity.60,73 Three positive studies, and one negative study measuring calcium-independent activity utilised a portion of drug naïve patients.60,73,74,77 Statistical analysis has found no correlation between medication state and calcium-dependent PLA2 activity.52,70,78 A small population of drug naïve patients had normal calcium-dependent PLA2 activity.79

Niacin insensitivity and PLA2 activity

The increased calcium-independent PLA2 activity associated with schizophrenia is greater still in the niacin insensitive sub group.60 As reported, 8 weeks of medication reduced the abnormally high calcium-dependent PLA2 activity. Those patients who converted to niacin sensitive showed further normalisation of calcium-independent PLA2 activity.60 Although Hudson et al.80 initially found no differences in calcium-dependent PLA2 activity, niacin sensitive (normal) patients had significantly lower calcium-dependent PLA2 activity compared to controls and insensitive patients. Calcium-dependent PLA2 activity of niacin insensitive patients did not differ from controls.62

Genetic profiles of PLA2G2A, PLA2G4A and PLA2-G6A

As we are reviewing the possible involvement of genetic variation in PLA2G2A, PLA2G4A and PLA2-G6A, it is worthwhile briefly summarising their gene structure in Table 2. Regions implicated in schizophrenia in genomewide linkage studies (GWL) are recorded here, divided by the maximum 10 cm range suggested as evidence of gene–signal relationship.76

A published variant is defined as having minimum verification when it has either been reported separately at least twice, or has been genotyped in a large enough population to ensure it is truly polymorphic.83 Untranslated regions are exons that are transcribed to the messenger RNA (mRNA) and not translated into the protein while coding variants are in exons translated into amino acids. A synonymous coding variant does not change the amino acid coded for, while non-synonymous variants alter the resulting protein structure.

Association analysis of PLA2s and schizophrenia

Interest in the Membrane Phospholipid Hypothesis has fuelled association studies between PLA2 genes and schizophrenia, as summarised in Table 3.

PLA2G4A

Deletion of a promoter CA microsatellite (numerous repeats of the bases cytosine paired with an adenine) increases PLA2G4A mRNA expression by 50%.84 One CA allele, (140 repeats) has a frequency of 0.96, requiring large sample sizes to achieve sufficient statistical power to detect association.85,86 The one reported study of the CA microsatellite found no significant association with schizophrenia (Table 4).87
Table 2  Available data for PLA2 genes on dbSNP

<table>
<thead>
<tr>
<th>Gene</th>
<th>Names</th>
<th>Locus</th>
<th>Exons</th>
<th>GWL &lt; 10 cM</th>
<th>GWL &gt; 10 cM</th>
<th>Variants/ Verified</th>
<th>Coding/ Verified</th>
<th>Untranslated/ Verified</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA2G2A</td>
<td>sPLA2</td>
<td>1q35</td>
<td>6</td>
<td>No</td>
<td>1q42</td>
<td>50/40</td>
<td>8/4</td>
<td>21/17</td>
</tr>
<tr>
<td>PLA2G4A</td>
<td>cPLA2</td>
<td>1q25</td>
<td>18</td>
<td>No</td>
<td>1q21–23</td>
<td>726/407</td>
<td>5/5</td>
<td>63/27</td>
</tr>
<tr>
<td>PLA2G6A</td>
<td>iPLA2</td>
<td>22q13.1</td>
<td>16</td>
<td>No</td>
<td>22q11–12</td>
<td>494/299</td>
<td>10/8</td>
<td>91/47</td>
</tr>
</tbody>
</table>

Abbreviations: GWL, genomewide linkage studies; PLA2, phospholipases A2; PLA2G2A, phospholipase A2 Group 2 A; PLA2G4A, phospholipase A2 Group 4 A; PLA2G6A, phospholipase A2 Group 6 A.

Table 3  Genetic analysis of PLA2 genes in psychotic disorders

<table>
<thead>
<tr>
<th>Article</th>
<th>Model</th>
<th>Case no.</th>
<th>Ethnicity</th>
<th>Control no.</th>
<th>Analysis method</th>
<th>Associated?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hudson et al.76</td>
<td>CC</td>
<td>65</td>
<td>Ca/C, USA/C</td>
<td>65</td>
<td>Poly(A)</td>
<td>Yes</td>
</tr>
<tr>
<td>Hudson et al.76</td>
<td>T</td>
<td>44</td>
<td>Ca/C, R/C</td>
<td>88</td>
<td>Poly(A)</td>
<td>Yes</td>
</tr>
<tr>
<td>Hudson et al.76</td>
<td>CC</td>
<td>20</td>
<td>Ca/C, USA/C</td>
<td>20</td>
<td>Poly(A), Niacin sensitivity</td>
<td>Yes</td>
</tr>
<tr>
<td>Price et al.77</td>
<td>CC</td>
<td>58</td>
<td>S/C</td>
<td>56</td>
<td>Poly(A)</td>
<td>No</td>
</tr>
<tr>
<td>Doris et al.78</td>
<td>CC</td>
<td>35</td>
<td>NR</td>
<td>40</td>
<td>Poly(A)</td>
<td>No</td>
</tr>
<tr>
<td>Wei et al.79</td>
<td>CC</td>
<td>193</td>
<td>NR/C</td>
<td>101</td>
<td>Banl</td>
<td>No</td>
</tr>
<tr>
<td>Wei et al.79</td>
<td>T</td>
<td>50</td>
<td>NR/C</td>
<td>100</td>
<td>Banl</td>
<td>Yes</td>
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<tr>
<td>Peet et al.80</td>
<td>CC</td>
<td>36</td>
<td>I</td>
<td>27</td>
<td>Banl</td>
<td>Yes</td>
</tr>
<tr>
<td>Ramchand et al.81</td>
<td>CC</td>
<td>52</td>
<td>I</td>
<td>48</td>
<td>CA</td>
<td>No</td>
</tr>
<tr>
<td>Chowdari et al.82</td>
<td>T</td>
<td>86</td>
<td>USA/C, USA/AF</td>
<td>130</td>
<td>Poly(A), Banl</td>
<td>No</td>
</tr>
<tr>
<td>Chowdari et al.82</td>
<td>CC</td>
<td>86</td>
<td>USA/C, USA/AF</td>
<td>94</td>
<td>Poly(A), Banl</td>
<td>No</td>
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<tr>
<td>Chowdari et al.82</td>
<td>T</td>
<td>159</td>
<td>I</td>
<td>283</td>
<td>Banl</td>
<td>No</td>
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<tr>
<td>Frieböes et al.83</td>
<td>T</td>
<td>328</td>
<td>E/C, M, T/A</td>
<td>426</td>
<td>Poly(A)</td>
<td>No</td>
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<tr>
<td>Junqueira et al.84</td>
<td>CC</td>
<td>240</td>
<td>B</td>
<td>312</td>
<td>Banl</td>
<td>No</td>
</tr>
<tr>
<td>Junqueira et al.84</td>
<td>CC</td>
<td>240</td>
<td>B</td>
<td>312</td>
<td>Banl</td>
<td>No</td>
</tr>
<tr>
<td>Wei and Hemmings85</td>
<td>T</td>
<td>118</td>
<td>UK/C</td>
<td>236</td>
<td>Banl</td>
<td>Yes</td>
</tr>
<tr>
<td>Yu et al.86</td>
<td>T</td>
<td>168</td>
<td>M/M</td>
<td>336</td>
<td>Banl</td>
<td>No</td>
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<tr>
<td>Pae et al.87</td>
<td>CC</td>
<td>97</td>
<td>K/K</td>
<td>117</td>
<td>Banl</td>
<td>Yes</td>
</tr>
<tr>
<td>Tae et al.88</td>
<td>T</td>
<td>240</td>
<td>M/M</td>
<td>480</td>
<td>Banl</td>
<td>No</td>
</tr>
<tr>
<td>Wei and Hemmings89</td>
<td>T</td>
<td>132</td>
<td>UK/C</td>
<td>262</td>
<td>Banl</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ccGC, case/control test; T, transmission disequilibrium test.

RRN, not recorded in paper.

Ethnicity is expressed as country of origin/ethnicity. (a) Country of origin shorthand: USA = United States of America, Ca = Canada, S = Scotland, M = China, T = Taiwan, E = Europe, R = Italy, P = Poland, M = China, I = India, K = Korea, UK = United Kingdom. (b) Ethnicity shorthand – C = Caucasian, A = Asian, I = Indian, Af = African, B = Brazilian, M = Chinese, K = Korean.

Poly(A), adenine repeat PLA2G4A promoter variant. CA, CA microsatellite in promoter of PLA2G4A, Banl, rs10798059 in first intron of PLA2G4A, AvrII, rs4375 in fourth intron of PLA2G6A.

Abbreviation: PLA2, phospholipases A2.

Table 4  Range of Poly(A) alleles

<table>
<thead>
<tr>
<th>Studies</th>
<th>Method</th>
<th>Alleles</th>
<th>No. (A) repeats</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hudson et al.88,89</td>
<td>Electrophoresis vs known allele</td>
<td>10</td>
<td>41–60</td>
<td>Yes</td>
</tr>
<tr>
<td>Price et al.90</td>
<td>Electrophoresis vs absolute size marker and sequencing.</td>
<td>24</td>
<td>22–57</td>
<td>No</td>
</tr>
<tr>
<td>Doris et al.91</td>
<td>Electrophoresis vs absolute size marker</td>
<td>10</td>
<td>136–156</td>
<td>No</td>
</tr>
<tr>
<td>Chowdari et al.92</td>
<td>Direct sequencing</td>
<td>26</td>
<td>17–52</td>
<td>No</td>
</tr>
<tr>
<td>Frieböes et al.93</td>
<td>Electrophoresis vs absolute size marker</td>
<td>14</td>
<td>?</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviation: Poly(A), PLA2G4A promoter adenine repeat variant.

Hudson et al.88,89 reported the promoter PLA2G4A promoter adenine repeat variant (Poly(A)) (an adenine repeat) occurred as 10 size alleles, termed A1–A10, ranging from 41 to 60 repeats in length. The small differences between alleles, except for five bases between A6 and A7 made genotyping difficult, so analysis was performed using A1–A6 vs A7–A10 groupings. Association between schizophrenia and
the A7–A10 group was detected under a case/control and a transmission model.\textsuperscript{58,60} Additionally, niacin insensitivity was greater in patients with the A7–A10.\textsuperscript{61} Later methods detected completely differing alleles, and found association between schizophrenia and Poly(A) (Tables 3 and 4).\textsuperscript{54,90,91}

Alternative studies have used an adenine (A1, not cut by restriction enzyme PLA2G4A, rs10798059, intron one adenine/guanine variant (\textit{Banl})) or guanine (A2, cut by \textit{Banl}) variant in the first intron of PLA2G4A, rs10798059 (\textit{Banl}).\textsuperscript{92} The A2 allele of \textit{Banl} has been associated with schizophrenia under both case/control and transmission disequilibrium test (TDT) models (Table 3).\textsuperscript{92–96} Five additional studies have not replicated any association between the \textit{Banl} locus and schizophrenia.\textsuperscript{94,97–100} Testing of markers across the chromosome region containing PLA2G4A make it unlikely that association is due to linkage disequilibrium (LD) with a nearby gene.\textsuperscript{94}

**PLA2G2A and PLA2G6A**

A variant in the fourth intron of PLA2G6A rs4375 has been used to test for association with schizophrenia. Rs4375 is either a cytosine base that creates a cut site for the \textit{AvrIII} restriction enzyme or a noncut thymine. Both a case/control and a TDT model in this Brazilian population indicated association between the cyto- steine allele and schizophrenia.\textsuperscript{99} Although studies have used alternative PLA2s, such as PLA2G1B, there are no published association studies of PLA2G2A in psychotic illnesses.\textsuperscript{88,90,99}

**Discussion**

The three PLA2s regulate the levels of bound and free AA that has key functions in processes such as brain maturation, memory formation and synaptic remodelling all processes suggested to be important for schizophrenia.\textsuperscript{5,6,11,101} An increasing body of evidence suggests that AA is altered in schizophrenia in a causative manner:

(a) \textit{In vivo} 31P-MRS and \textit{post-mortem} measurements of AA levels of patients with schizophrenia confirm that AA alterations are present in the brain and peripheral tissues.\textsuperscript{48,52}

(b) AA changes are present in drug naïve patients indicates that the relationship is independent of drug treatment and associated with the onset of disease.\textsuperscript{49–51}

(c) Chronic haloperidol administration in rats reduced AA levels specifically in dopaminergic neurons, possibly due to interactions with dopamine receptors and their downstream targets, the PLA2 enzymes.\textsuperscript{57}

(d) Increased calcium-independent PLA2 activity has been replicated in both peripheral and CNS tissue, and in drug naïve patients (Table 1).\textsuperscript{50,70,74,76}

(e) Higher calcium-independent PLA2 activity correlates significantly with greater severity of psychopathology, suggesting a direct relationship between the degree of PLA2G6A activity and disease progression.\textsuperscript{79}

(f) Though numbers were small, the work of Tavares \textit{et al.}\textsuperscript{102} supports the concept of antipsychotic efficacy through modulating PLA2 activity.

(g) Niacin sensitivity requires PLA2G4A- and PLA2G2A-mediated AA release from cellular membranes. Membrane AA levels are maintained by PLA2G6A, and the separate associations of reduced AA levels and increased calcium-independent activity with niacin insensitivity is intriguing.\textsuperscript{20,25}

(h) The strengthening of association between schizophrenia and a variant in the PLA2G4A gene after stratification by niacin sensitivity is promising, but not replicated.\textsuperscript{88} Yet increased calcium-dependent PLA2 activity, which includes PLA2G4A, was not associated with niacin insensitivity. Rather it was the patient group with normal sensitivity to niacin that possessed increased calcium-dependent activity.\textsuperscript{62} Low participant numbers may explain this unexpected result, or as reported by the authors, the niacin insensitive group had the greatest activity variation, with a standard deviation twice that of the other groups.\textsuperscript{62} A repeat of this experiment in a larger population using direct measures of PLA2G4A protein level is required before further conclusions can be made.

That there are no GWL peaks for schizophrenia within the suggested 10 cM of PLA2 loci is not absolute proof of noninvolvement (Table 2).\textsuperscript{102} The population needed to detect GWL increases dramatically as the relative risk (RR) conferred by variants at that loci decrease.\textsuperscript{65} The RR estimated from replicated associations of variants in dopamine and serotonin receptors as well as the neuregulin 1 gene range from 1.2 to 2.4.\textsuperscript{103,104} If the RR of PLA2 variants is two or more, then thousands to tens of thousands of families are required to detect GWL, and if the RR is lower millions of families are needed.\textsuperscript{89} All that can be concluded is that these PLA2 genes they, like neuregulin or dopamine receptors do not contribute a high (4 +) risk for schizophrenia.

The different allele profiles found for the Poly(A) site in the PLA2G4A promotor may be due to method of set detection, as there was the greatest consistency in allele profiles when direct sequencing was used (Table 4). Interestingly the Poly(A) and \textit{Banl} variants are in significant LD, supporting the concept that association is due to an adjacent causative variant(s).\textsuperscript{48} Although the absence of the repressive CA microsatellite has a functional effect, it is not clear if a change in CA repeat numbers also modifies expression. Genotyping in sufficiently large populations to overcome the high frequency of one of the alleles would be interesting.

The association between schizophrenia and either of PLA2G4A variants in populations of different ethnicity and alternative statistical models is promis-
ing (Table 4). Yet many studies have not replicated these associations. There are a number of possible reasons for this inconsistency.

(a) The associations so far may have been false positives due to population heterogeneity.
(b) The tested variants may not in perfect LD with the causative variant(s), increasing false negatives.

Using a population large enough to detect association under varying models of LD and RR, and utilising niacin sensitivity or RBC AA levels to reduce heterogeneity should limit these problems.

Additionally, replication inconsistency may be due to the underlying spectrum of disease variants. The association studies discussed have follow the common disease, common variant (CDCV) hypothesis that for each ‘disease’ gene there are one or few risk variants with minor allele frequency > 10%. The alternative common disease, rare variant (CDRV) hypothesis proposes that there a large pool of rare (minor allele frequency < 10%) or even unique risk variants.106–108 Interestingly, association between a common variant and a disease can be due to LD with high penetrance rare variants present in only a few of the total study population.109

It is likely that both paradigms contribute as a spectrum of common and rare deleterious variants has been seen in both Mendelian and complex disorders.110–112 However most studies do not characterise the rare variants present in the study population nor have the statistical power to test them.85

Conclusion

The collected evidence discussed point towards the involvement of AA and PLA2s in the pathogenesis of schizophrenia. The reported aberrations in AA levels, PLA2 enzyme activities and in vivo brain membrane breakdown specific to schizophrenia are biological processes mediated by PLA2G6A, PLA2G4A and PLA2G2A. As AA levels and metabolism is tightly associated with the activity of PLA2G4A, PLA2G2A and PLA2G6A, measurement of AA may reduce schizophrenia heterogeneity (‘a PLA2s-AA endophenotype’). The topical Niacin skin flush test or RBC AA level measurements have the potential to identify such an endophenotype.45,50,62,113,114

As reported, these three PLA2 enzymes lie down-stream of activation of neurotransmitter pathways implicated in schizophrenia, such as dopaminergic, serotonergic or glutamatergic systems. It is plausible that antipsychotic efficacy can be modulated by PLA2 genetic variants. Feedback may occur, as changes in AA release by PLA2s will alter the membrane saturated:unsaturated fats ratio, altering neurotransmitter receptors dynamics.12,13 Thus, PLA2 genetic variants with altered activity have implications in both the release and the reception of neuronal signals. Environmental modification of AA metabolism could, in a background of deleterious PLA2 variants may lead to a run away failure of brain function and development. Detection of these variants will ultimately require models that take into account both rare and common risk variants. If sampling of variation occurs in a large study population, association tests using the variants found should allow evaluation of both the CDCV and CDRV hypotheses.

In summary, the PLA2G4A gene has been associated with schizophrenia and reduced niacin sensitivity. There are as yet to be replicated studies indicating PLA2G4A protein levels and specific activity are increased in, and the PLA2G6A gene is associated with, schizophrenia. That mice null for PLA2G2A and PLA2G4A genes are viable could explain the high frequency of schizophrenia, as negative variants in these genes would be under reduced selective pressure due to compensation by other PLA2s. The lack of normal inflammation in these mice indicates that this compensation is incomplete, and such PLA2 activity shortfalls in humans may result in schizophrenia.

Abbreviations

AA, arachidonic acid; AMPAR, glutamate receptor; Banl, PLA2G4A, rs10798059, intron one adenine/guanine variant; bp, DNA base pairs; CDCV, common disease common variant; CDRV, common disease rare variant; CNS, central nervous system; EFA, essential fatty acids; G-proteins, guanosine triphosphate binding proteins; GWL, genomewide linkage studies; LD, linkage disequilibrium; LTP, long-term potentiation; mRNA, messenger RNA; PLA2, phospholipase A2; PLA2GIVA, PLA2G4A, phospholipase A2 Group 4 A; PLA2GIA, PLA2G2A, phospholipase A2 Group 2 A; PLA2Gvia, PLA2G6A, phospholipase A2 Group 6 A; Poly(A), PLA2G4A promoter adenine repeat variant; PUFA, polysaturated fatty acid; 31P MRS, 31-phosphorus magnetic resonance imaging; RBC, red blood cells; TDT, transmission disequilibrium test.

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Web Resources

The URLs for data presented herein is as follows dbSNP, http://www.ncbi.nlm.nih.gov/SNP/

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