Serum phosphatidylinositol as a biomarker for bipolar disorder liability

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Abstract

Objectives: Individuals with bipolar disorder (BPD) exhibit alterations in their phospholipid levels. It is unclear whether these alterations are a secondary consequence of illness state, or if phospholipids and illness risk overlap genetically. If the latter were true, then phospholipids might provide key insights into the pathophysiology of the illness. Therefore, we rank-ordered phospholipid classes by their genetic overlap with BPD risk in order to establish which class might be most informative in terms of increasing our understanding of illness pathophysiology.

Methods: Analyses were conducted in a sample of 558 individuals, unselected for BPD, from 38 extended pedigrees (average family size=14.79, range=2−82). We calculated a coefficient of relatedness for all family members of nine individuals with BPD in the sample (N=185); this coefficient was set to be zero in unrelated individuals (N=373). Then, under an endophenotype ranking value (ERV) approach, this scalar index was tested against 13 serum-based phospholipid concentrations in order to rank-order lipid classes by their respective overlap with BPD risk.

Results: The phosphatidylinositol class was significantly heritable ($h^2=0.26$, $P=6.71 \times 10^{-05}$). It was the top-ranked class, and was significantly associated with BPD risk after correction for multiple testing ($\beta=-1.18$, $P=2.10 \times 10^{-03}$, ERV=0.49).

Conclusions: We identified a peripheral biomarker, serum-based phosphatidylinositol, which exhibits a significant association with BPD risk. Therefore, given that phosphatidylinositol and BPD risk share partially common etiology, it seems that this lipid class warrants further investigation, not only in terms of treatment, but also as a promising diagnostic and risk marker.

Keywords
bipolar, family study, genetics, lipidome, phosphatidylinositol

1 | INTRODUCTION

Identifying endophenotypes for bipolar disorder (BPD) will garner greater understanding of psychiatric illnesses, including BPD, which in turn will aid in their identification, diagnosis and treatment.1–3 An endophenotype is a biomarker, or measurable characteristic, that is associated with disease.4 Crucially, an endophenotype must share some appreciable portion of its genetic etiology with disease risk.4 This requirement is important because it implies that some portion of the biological processes that underlie the endophenotype overlap...
with those that are disrupted in disease. Thus, the identification of endophenotypes for psychiatric illnesses should contribute to our understanding of illness pathophysiology. Peripheral markers, such as serum-based lipid measurements, hold great promise as endophenotypes for two reasons. First, their underlying biochemical underpinnings are relatively well understood, particularly when compared to, for example, brain- or behavior-based phenotypes. Second, peripheral markers are easily obtainable at comparatively low cost. These advantages are particularly appealing for BPD, a psychiatric illness that is ranked as one of the leading causes of disability and premature mortality worldwide, but whose physiological underpinnings are still largely unknown.

Lipids and their polyunsaturated fatty acids (PUFAs) constitute basic and essential components of all human cells, in terms of both structure, making up the major component of cell membranes, and function, playing a part in neurotransmission, receptor function, and eicosanoid biosynthesis. A number of lipidomic alterations have been noted in those with BPD and also major depressive disorder (MDD). For example, increases in plasma levels of lipid peroxidation have been noted in euthymic adults with BPD while decreases have been noted in adolescent BPD individuals. It has been shown that essential PUFAs in red blood cell membranes, including arachadonic and docosahexaenoic acid (DHA), are reduced in BPD individuals in a manic phase and in individuals with MDD, and that the fatty acid composition of phospholipids in serum is altered in those with MDD such that the arachadonic:ecosapentaeenoic acid ratios are higher.

Accordingly, a handful of studies suggest that administration of fatty acids may have benefits in the amelioration of mood symptoms. Brain-based findings, both in vivo and in vitro, indicate significant elevations of phosphatidylcholines in the prefrontal cortex and reduced choline in the frontal lobe and reduced DHA in the orbitofrontal cortex in BPD individuals. In sum, there is evidence for alterations in phospholipids and their fatty acids in BPD and MDD. The direction of those alterations is currently unclear, probably in part because of heterogeneity in methods and patient populations, although a recent and large study on this topic, which utilized plasma-based lipid levels, documented an inverse relationship between phospholipid levels and symptoms of depression. The present study is the first to examine the influence of genetic liability for BPD on serum-based phospholipids.

Phosphatidylinositol (PI) (a membrane phospholipid that plays a crucial role in cell physiology and signaling) and its phosphorylated products phosphoinositides (PtdIns) are particularly interesting in the context of BPD, given that lithium (Li⁺), the first-line mood stabilizer treatment for BPD, acts upon the PI signal transduction pathway. While there is converging evidence for inositol phospholipid system dysfunction in BPD, more work is necessary to clarify this relationship. Of course, given the link between phospholipids and the mechanism of action of Li⁺, it is possible that alterations in lipid levels arise as a secondary consequence of treatment for BPD. This is why establishing such a peripheral marker as an endophenotype of BPD is particularly important, as alterations are demonstrated as a function of genetic proximity to an affected individual, that is, in unaffected relatives who are not exposed to bipolar treatment regimens. The implication is that alterations in serum phospholipid levels arise as a consequence of shared etiology, making them and their underlying biochemical mechanisms potentially promising diagnostic and/or treatment targets for BPD.

In the present study, we aimed to (1) provide evidence for shared etiology between phospholipid concentrations and BPD risk, and (2) determine which phospholipid classes might be the most informative when attempting to isolate potential diagnostic and treatment targets for BPD. We took sum concentrations of 13 phospholipid classes in a sample of 558 Mexican American individuals from 38 randomly ascertained extended pedigrees, and calculated mean-based endophenotype ranking values (ERVs) between each phospholipid class and a broad BPD phenotype (incorporating BPD types I and II). We used this broad BPD phenotype to increase the total number of included affected individuals, which in turn reduces the noise associated with any single diagnosis and maximizes power.

2 METHODS

2.1 Participants

Lipidomic and diagnostic data were available in 567 individuals from 38 pedigrees (average family size=14.79, range=2–82). The sample was 64% female and had a mean age of 49.28 years (SD=13.34, range=27–97). The lipidomic data were collected as part of the San Antonio Family Study (SAFS), and diagnostic data were also available in these same individuals as part of assessments conducted in the Genetics of Brain Structure and Function (GOBS) study. GOBS data collection occurred between 2006 and 2016. Of the 567 individuals, nine persons had received a BPD diagnosis (BPD types I (N=4) and II (N=5); see Table 1 for additional diagnostic information). Affected individuals were excluded from the analysis and therefore the analysis was performed in 558 individuals, comprising 185 participants related to an affected individual plus 373 unrelated participants (Table 2).

All participants were randomly selected from the community with the constraints that they were of Mexican American ancestry, part of a large family, and lived in the San Antonio region. All participants provided written informed consent in compliance with the institutional review board at the University of Texas Science Center of San Antonio.

2.2 Diagnostic assessment

The Mini-International Neuropsychiatric Interview (MINI), a semi-structured interview, was administered to all participants. Interviews were conducted by masters- and doctorate-level research staff, who had established reliability for diagnosing bipolar disorder (κ≥0.85). Subjects who reported possible pathology were discussed in case conference meetings with licensed psychologists and/or psychiatrists. Consensus diagnoses were determined using available medical records, the MINI, and the interviewer’s narrative.
3 | Lipid extraction and analysis procedure

The lipid extraction procedure used in this sample has been described in detail elsewhere. Lipid extraction in the SAFS is part of an ongoing longitudinal observational investigation comprising four phases of data collection during a 23-year period. The lipidomic data used in the present study were collected during the first phase, between the years 1992 and 1996. The order of the plasma samples was randomized prior to lipid extraction and analysis for each cohort. Quality control plasma samples were included at a ratio of 1:18. Total lipid extraction from a 10-mL aliquot of plasma was performed in a single-phase chloroform:methanol (2:1) extraction.

Lipid analysis was performed using liquid chromatography–electrospray ionization tandem mass spectrometry using an Agilent 1200 liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) combined with an Applied Biosystems API 4000 Q/TRAP mass spectrometer with a turbo-ionspray source (350 uC) and the Analyst 1.5 data system. We have previously reported the use of precursor ion and neutral loss scans on control plasma extracts to identify the predominant lipid species of the following phospholipid classes: sphingomyelin (SM), phosphatidylcholine (PC), alkylphosphatidylcholine [PC(O)], alkenylphosphatidylcholine [PC(P); plasmalogen], lysophosphatidylcholine (LPC), lysoalkylphosphatidylcholine [LPC(O); lysoplatelet activating factor], phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylglycerol (PG).

Multi-reaction monitoring (MRM) experiments were established for the major species of each lipid class identified in plasma. Relative lipid amounts were calculated by relating the peak area of each species to the peak area of the corresponding stable isotope or non-physiological internal standard. Total lipid classes were calculated from the sum of the individual lipid species within each class.

2.4 | Quantitative genetic analysis

All genetic analyses were performed in SOLAR. SOLAR implements a maximum likelihood variance decomposition to determine the proportion of variation in a phenotype due to genetic and environmental influences by modeling the covariance amongst family members as a function of genetic proximity. The simplest such decomposition is one where the additive genetic contribution of a trait is indexed by the heritability, or $h^2$. All lipid classes were subject to univariate decomposition analysis to ensure that they were significantly heritable.

2.5 | Genetic correlation between BPD and lipid classes: the mean-based endophenotype ranking value (mERV)

The mean-based endophenotype ranking value (mERV) represents an extension of the ERV. The ERV, an effect size ranging between 0 and 1, was developed for the purpose of formally testing endophenotypic status of phenotypes and to rank phenotypes by their standardized genetic covariance with a disease of interest; it is expressed as follows:

$$ERV = \sqrt{h_D^2 h_P^2}$$

where $h_D^2$ is the heritability of disease risk, $h_P^2$ is the heritability of the endophenotype, and $P_D$ is the genetic correlation between the two. The mERV is an extension of the ERV to be used when the disease of interest is not sufficiently common in the data. For
details on the derivation of the mERV, see Glahn et al. Briefly, the mERV leverages the many coefficients of relationship that exist in extended-pedigree data. The coefficient of relationship refers to the average number of alleles held in common between individuals; for example, first-degree relatives (e.g. full siblings or parents) share on average 50% of their alleles, second-degree relatives (e.g. grandparents or aunts/uncles) share 25%, third-degree relatives (e.g. great-grandparents or great-aunts/uncles) share 12.5%, and so on. Thus, it is possible, given an individual with a disease, to index all other pedigree members by their degree of relatedness to that individual. This scalar can then be used to perform a fixed-effect single-degree-of-freedom test, within the univariate variance components analysis outlined above, providing an estimate of the standardized genetic covariance between the potential endophenotype and illness risk. The mERV can then be used in the same way as the ERV to rank potential endophenotypes by their degree of standardized genetic overlap with illness risk. In the present paper, the mERV was applied to BPD and all available lipid classes.

We Bonferroni-corrected α (=0.05) by the effective number of traits using the method outlined by Cheverud, correcting for the total number of traits would be overly conservative, given the extent to which they are all correlated with one another. Applying this method to the pairwise genetic correlations between the 13 phospholipid classes reveals that we have 10.36 effective traits, thus α=0.05/10.36=4.82×10^-03.

We tested the potential influence of confounding variables, in particular BMI and major depressive disorder (MDD), on all lipid classes. We tested the potential genetic overlap, using a bivariate polygenic model, between the lipids and potential psychiatric and metabolic confounds. We then included those covariates with a significant genetic overlap with the lipid class, using a liberal threshold of P<.10 in order to increase confidence that important covariates were included in the univariate polygenic model described above in addition to the BPD coefficient of relationship (which was fixed in the model). We tested the following variables that were collected at the time of blood sampling as part of the SAFS assessment: body mass index (BMI); diabetes status; ever had a heart attack; smoking status; and hypertension status. In addition, we investigated the following variables from the GOBS assessment taken from the MINI: any depressive disorder; any anxiety disorder; any alcohol use disorder; any substance use disorder.

3 | RESULTS

3.1 | Family profiles

According to our consensus diagnoses, nine individuals met the criteria of our broad BPD phenotype, four individuals met the criteria for BPD I and five met the criteria for BPD II. Table 2 shows the mean age and percent female of each category of relatedness to an affected individual, as well as the unrelated group. No two affected BPD individuals fell within the same pedigree, but these individuals were related to 185 individuals (Table 2).

4 | DISCUSSION

In the present study, we investigated the relative genetic overlap between BPD risk and 13 phospholipid classes; this was in an effort to rank the phospholipids according to which might be most informative when attempting to disentangle the etiology of BPD. To our knowledge, this is the first study to investigate possible genetic overlap between BPD risk and serum phospholipid levels. The existence of
significant genetic overlap between BPD risk and phospholipid levels, and more specifically between illness risk and PI, strongly suggests that PI is not merely a secondary manifestation of either illness state or treatment but rather an endophenotypic marker of the illness with the potential for aiding early detection and diagnosis, as well as enhanced treatment regimens.

Phosphatidylinositols are membrane phospholipids found mostly on the inner leaflet of the cell and are characterized by an inositol ring, or head group, extending into the cytoplasm. Despite their relatively low abundance compared to other membrane lipids, it is the metabolism of this phospholipid class which gives rise to second messengers which are major contributors to the myriad aspects of cellular regulation that make up the PI signal transduction pathway. PI is implicated in well-characterized signal transduction pathways, alterations in the molecular components of which, in particular Phosphatidylinositol 4,5-bisphosphate (PIP$_2$) and protein kinase C levels, have been previously associated with BPD.

The present study, to our knowledge, is the first to suggest a shared etiology between serum PI levels and risk for BPD. PI is a particularly interesting candidate endophenotype for BPD given that lithium (Li$^+$), a mood-stabilizing drug and treatment of choice for BPD, is thought to act upon the PI signaling pathway. The inositol-depletion hypothesis posits that lithium acts by preventing the production of PI via inhibition of inositol monophosphatase, thereby limiting turnover of inositol in the cell.

The direction of the relationship between BPD risk and PI in the present study was negative, meaning that heightened risk for BPD was associated with low levels of the phospholipid. This is the only study, to our knowledge, to assess phospholipid levels in serum in relation to BPD risk. However, Demirkan and colleagues also showed a negative correlation between plasma-based phospholipids (phosphatidylincholine and sphingomyelin) and symptoms of depression and anxiety.

**TABLE 3** Heritability and degree of bipolar relatedness for all lipid classes

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>$h^2$ (P-value)</th>
<th>$\beta$ (P-value)</th>
<th>ERV</th>
<th>Clinical covariates included*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>0.26 (6.71x10$^{-05}$)</td>
<td>-1.18 (2.10$^{-03}$)</td>
<td>0.49</td>
<td>Any alcohol use disorder; diabetes status</td>
</tr>
<tr>
<td>LPE</td>
<td>0.19 (7.82x10$^{-03}$)</td>
<td>-0.83 (0.03)</td>
<td>0.33</td>
<td>None</td>
</tr>
<tr>
<td>LPC</td>
<td>0.32 (3.10x10$^{-11}$)</td>
<td>-0.70 (0.06)</td>
<td>0.27</td>
<td>None</td>
</tr>
<tr>
<td>PC(O)</td>
<td>0.40 (4.34x10$^{-15}$)</td>
<td>0.54 (0.08)</td>
<td>0.20</td>
<td>Any anxiety disorder; any depressive disorder</td>
</tr>
<tr>
<td>PE</td>
<td>0.41 (8.69x10$^{-14}$)</td>
<td>-0.43 (0.24)</td>
<td>0.17</td>
<td>Diabetes status</td>
</tr>
<tr>
<td>PC(P)</td>
<td>0.32 (1.58x10$^{-04}$)</td>
<td>0.48 (0.21)</td>
<td>0.19</td>
<td>Any anxiety disorder</td>
</tr>
<tr>
<td>PG</td>
<td>0.38 (1.20x10$^{-14}$)</td>
<td>-0.24 (0.12)</td>
<td>0.18</td>
<td>Diabetes status; any alcohol use disorder</td>
</tr>
<tr>
<td>LPC(O)</td>
<td>0.52 (1.84x10$^{-10}$)</td>
<td>0.57 (0.70)</td>
<td>0.18</td>
<td>Diabetes status; BMI</td>
</tr>
<tr>
<td>PC</td>
<td>0.28 (3.00x10$^{-11}$)</td>
<td>-0.24 (0.17)</td>
<td>0.10</td>
<td>Any alcohol use disorder; diabetes status</td>
</tr>
<tr>
<td>PS</td>
<td>0.34 (1.01x10$^{-14}$)</td>
<td>0.21 (0.54)</td>
<td>0.08</td>
<td>Any substance use disorder; hypertension; smoker; BMI</td>
</tr>
<tr>
<td>SM</td>
<td>0.38 (1.17x10$^{-14}$)</td>
<td>0.19 (0.94)</td>
<td>0.08</td>
<td>Smoker; any alcohol use disorder; any substance use disorder; any anxiety disorder</td>
</tr>
<tr>
<td>PE(O)</td>
<td>0.38 (2.50x10$^{-06}$)</td>
<td>0.16 (0.71)</td>
<td>0.06</td>
<td>Any depressive disorder</td>
</tr>
<tr>
<td>PE(P)</td>
<td>0.45 (6.96x10$^{-09}$)</td>
<td>0.16 (0.70)</td>
<td>0.06</td>
<td>Any anxiety disorder; any depressive disorder</td>
</tr>
</tbody>
</table>

Bold indicates class surviving multiple testing correction.
BMI, body mass index; ERV, endophenotype ranking value; PI, phosphatidylcholine; LPE, lysophosphatidylethanolamine; LPC, lysophosphatidylcholine; PC(O), alkylphosphatidylcholine; PE, phosphatidylethanolamine; PC(P), alkenylphosphatidylcholine; PG, phosphatidylglycerol; LPC(O), lysoalkylphosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine; SM, sphingomyelin; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine.

*Threshold of $P$<.1.
In BPD, the technique of phosphorous-31 magnetic resonance spectroscopy (31PMRS), an imaging method that allows non-invasive measurement of biological compounds (e.g. phospholipids) in vivo, has been used to demonstrate significantly reduced choline, indicating altered phospholipid metabolism, in the frontal lobe. The phosphomonester (PME) signal in 31P MRS reflects the level of phosphocholine and phosphoethanolamine in addition to choline and myo-inositol; there is evidence to suggest that the PME signal increases during manic states and decreases during depressed states. Thus, in addition to the unknown relationship between phospholipid levels in the periphery and brain, it is possible that a second level of complexity exists where levels in both are affected by BPD illness phase. Evidence from post-mortem studies for phospholipid alterations in the brain in BPD is mixed; in some studies, alterations in phospholipids and/or their fatty acids have not been observed in BPD subjects versus controls, while in other studies such alterations have been observed. There is little consistency in terms of the focal brain region across these studies, which may explain the inconsistencies in the results. In addition, it is possible that lipidomic abnormalities in relation to affective disorders may be characterized differently in other ethnic populations. For example, non-Hispanic populations exhibit altered lipidomic profiles and associated risk for myocardial infarction relative to Hispanics. Thus, overall, it is important that the generalizability of the findings of the present study be further tested in future research.

In the present study, there was a gap in time between the collection of the lipidomic data and the occurrence of the psychiatric assessments. There is relatively little known about the longitudinal variability of serum phospholipid levels. There is evidence to suggest that phospholipids vary as a function of age, and as a consequence we residualized the phospholipid traits for age (in fact for age, age$^2$, sex, and their interactions) at the time of data collection. We consider this a potential strength of the study as it suggests that variation in PI reflects an early etiological step in the development of BPD. We cannot investigate this in detail in the present sample, with the limited number of affected cases available, but it is possible that the present results hint that alterations in PI reflect an “at-risk” condition for BPD. Certainly, longitudinal studies of other peripheral markers, in this case markers of inflammation, support an etiological role of inflammation in risk for MDD.

Two potential criticisms might be leveled at the present study regarding the affected individuals. The first criticism is that no two affected individuals with BPD occur in the same family, thus negating the idea that genetic factors underlie the illness. However, the heritability of BPD is not in question, having been established by numerous family-based studies and large genome wide association studies previously. Also, it is not improbable that not two affected individuals would be part of the same family. Rather, what we would expect is that an individual with an affected relative would have an increased risk for developing BPD compared to an individual without an affected relative. This risk may not necessarily be represented phenotypically as the full manifestation of the disorder within the relative’s lifetime (or indeed by the time of assessment), but may influence the expression of phenotypes related to the disorder. The second criticism is that only nine affected individuals were included in the present study, but importantly these individuals originated from extended pedigrees. Therefore, because the question under investigation in this study was one of genetic liability, this sample, comprising multiple extended pedigrees encapsulating many degrees of relatedness, provides the statistical power needed to adequately test hypotheses about putative pleiotropy between phospholipids and BPD risk. Indeed, an advantage of large, extended pedigrees such as this (where family sizes varied between 2 and 82 individuals) is that many unaffected relatives, encapsulating many degrees of relatedness, are available for a small number of cases. That being said, in subsequent work where we attempt to fine-tune our hypotheses regarding PI and its role in BPD, we may need greater numbers of probands.
The ERV is the product of three terms: the square root of the heritability of the endophenotype, the square root of the heritability of the disease, and the absolute value of the genetic correlation between the two. Similarly, the power of the ERV is a function of all three of these components, in the same way that the power of a genetic correlation is. While the heritability of the endophenotypes (i.e. the phospholipid class) can be directly estimated in the present sample, the heritability of BPD cannot, given that we have nine affected individuals. For a single endophenotype, the heritability of BPD is not identifiable (in the statistical sense) with this method. However, in principle with enough endophenotypes, the heritability of BPD may be estimable from the method due to the constraints on the parameter spaces of both the heritability and the genetic correlation, but it would generally be difficult to resolve. The total ERV, which our inference of genetic correlation/pleiotropy is based upon, is well estimated in the design. One of the substantial benefits of this method of calculating the ERV is that it does not require affected relatives of cases and thus is very useful for studying genetic determinants (shared via endophenotypes) of low-frequency diseases.

In summary, the findings presented here highlight PI as having a significant genetic overlap with BPD risk. While it has been previously demonstrated that those with BPD exhibit altered levels of phospholipids, this is the first study to highlight a shared etiology between BPD and phospholipids. It is unlikely that the association between PI and BPD risk in the present study arose as an artifact of lithium treatment, as affected individuals were excluded from all genetic analyses. Rather, the serum level of this lipid appears to vary in unaffected individuals as a function of genetic relatedness to a BPD individual and therefore the present study highlights the potential utility of serum-level measurements of PI as an indicator of illness risk. Moreover, this study suggests that the well-characterized PI signaling pathway may be an interesting avenue of research for BPD, potentially providing testable hypotheses for research aiming to improve diagnostic markers and/or treatment targets for BPD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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