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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 extended 38 extended pedigrees (average family size=14.79, range=2-82). We calculated a coefficient of relatedness for all family members of 9 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 thirteen 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.

Introduction

1 Identifying endophenotypes for bipolar disorder will garner greater understanding of psychiatric
2 illnesses, including bipolar disorder, which in turn will aid in their identification, diagnosis and
3 treatment (1-3). An endophenotype is a biomarker, or measurable characteristic, that is
4 associated with disease (4). Crucially, an endophenotype must share some appreciable portion
5 of its genetic etiology with disease risk (4). This requirement is important because it implies that
6 some portion of the biological processes that underlie the endophenotype overlap with those
7 that are disrupted in disease. Thus, the identification of endophenotypes for psychiatric illnesses
8 should contribute to our understanding of illness pathophysiology (5). Peripheral markers, such
9 as serum-based lipid measurements, hold great promise as endophenotypes for two reasons.
10 First, their underlying biochemical underpinnings are relatively well understood, particularly
11 when compared to, for example, brain- or behavior-based phenotypes. Second, peripheral
12 markers are easily obtainable at comparatively low cost (6). These advantages are particularly
13 appealing for bipolar disorder (BPD), a psychiatric illness that is ranked as one of the leading
14 causes of disability and premature mortality worldwide (7-9), but whose physiological
15 underpinnings are still largely unknown (10).

16
17 Lipids and their polyunsaturated fatty acids (PUFAs) constitute basic and essential components
18 of all human cells, both in terms of structure, making up the major component of cell
19 membranes, and function, where they play a part in neurotransmission, receptor function, and
20 eicosanoid biosynthesis (11,12). A number of lipidomic alterations been noted in those with BPD
21 and also major depressive disorder (MDD) (13). For example, increases in plasma-levels of lipid
22 peroxidation have been noted in euthymic adults with BPD (6) while decreases are noted in
23 adolescent BPD individuals (14). It has been shown that essential polyunsaturated fatty acids in
24 red blood cell membranes, including arachadonic and docosahexaenoic acid (DHA), are reduced
25 BPD individuals in a manic phase (15) and in those with MDD (16); and that the fatty acid
26 composition of phospholipids in serum is altered in those with MDD such that the
27 arachadonic/eicosapentaenoic acid ratios are higher (17,18). Accordingly, a handful of studies
28 suggest that administration of fatty acids may have benefits in the amelioration of mood
29 symptoms (19-21). Brain-based findings, both *in vivo* and *in vitro*, indicate significant elevations
30 of phosphatidylcholines in the prefrontal cortex (22), significantly reduced choline in the frontal
31 lobe (23), and reduced DHA in the orbitofrontal cortex (24) in BPD individuals. In sum, there is
32 evidence for alterations in phospholipids and their fatty acids in BPD and MDD, the direction of

1 those alterations is currently unclear, probably in part because of heterogeneity in methods and
2 patient populations. Though a recent and large study on this topic, which utilized plasma-based
3 lipid levels, documented an inverse relationship between phospholipid levels and symptoms of
4 depression (26). The present study is the first to examine the influence of genetic liability for
5 BPD on serum-based phospholipids.

6
7 Phosphatidylinositol (PI) (a membrane phospholipid that plays a crucial role in cell physiology
8 and signaling (27)) and its phosphorylated products phosphoinositides (PtdIns) are particularly
9 interesting in the context of BPD, given that lithium (Li^+), the first-line mood stabilizer treatment
10 for BPD (28), acts upon the PI signal transduction pathway (29). While there is converging
11 evidence for inositol phospholipid system dysfunction in BPD (30-38), more work is necessary in
12 order to clarify the relationship between them (39). Of course, given the link between
13 phospholipids and the mechanism of action of Li^+ , it is possible that alterations in lipid levels
14 arise as a secondary consequence of treatment for BPD. This is why establishing such a
15 peripheral marker as an endophenotype of BPD is particularly important, as alterations are
16 demonstrated as a function of genetic proximity to an affected individual, that is in unaffected
17 relatives who are not exposed to bipolar treatment regimens (5). The implication being that
18 alterations in serum phospholipid levels arise as a consequence of shared genetic etiology
19 making them and their underlying biochemical mechanisms potentially promising diagnostic
20 and/or treatment targets for BPD.

21
22 In the present study we aimed to (1) provide evidence for shared genetic etiology between
23 phospholipid concentrations and BPD risk, and (2) determine which phospholipid classes might
24 be the most informative when attempting to isolate potential diagnostic and treatment targets
25 for BP. We took sum concentrations of thirteen phospholipid classes in a sample of 558 Mexican
26 American individuals from 38 randomly ascertained extended pedigrees, and calculated mean-
27 based Endophenotype Ranking Values (40) between each phospholipid class and a broad BPD
28 phenotype (incorporating BPD Type I and II). We used this broad BPD phenotype to increase the
29 total number of included affected individuals, which in turn reduces the noise associated with
30 any single diagnosis and maximizes power.

31 32 **Methods**

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 was collected as part of the San Antonio Family Study (SAFS), 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, 9 persons had received a BPD diagnosis (BPD Type I (N = 4) and II (N = 5); see **Table 1** for additional diagnostic information). Affected individuals were excluded from the analysis and therefore analysis was run in 558 individuals, this comprised 185 individuals related to an affected plus 373 unrelateds (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.

Diagnostic Assessment

The Mini-International Neuropsychiatric Interview (MINI; (41)), 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 ($\kappa \geq 0.85$). Subjects that 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.

Lipid Extraction and Analysis Procedure

The lipid extraction procedure used in this sample has been described in detail elsewhere (see (42,43). Briefly, lipid extraction in the San Antonio Family study 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 1992-1996. 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 by a single phase chloroform:methanol (2:1) extraction (44).

Lipid analysis was performed by liquid chromatography, electrospray ionisation-tandem mass spectrometry using a Agilent 1200 liquid chromatography system combined with an Applied Biosystems API 4000 Q/TRAP mass spectrometer with a turboionspray source (350uC) and Analyst 1.5 data system (44). 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 (plasmalogen, PC(P)), lysophosphatidylcholine (LPC), lysoalkylphosphatidylcholine (lysoplatelet activating factor, LPC(O)), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylglycerol (PG) (44-46).

Multiple 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 (43).

Quantitative Genetic Analysis

All genetic analyses were performed in SOLAR (47). 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.

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 endophenotype ranking value (*ERV*). The *ERV*, an effect size ranging between 0 and 1, was

1 developed for the purpose of formally testing endophenotypic status of phenotypes and to rank
2 phenotypes by their standardized genetic covariance with a disease of interest; it is expressed as
3 follows:

$$ERV = \sqrt{h_D^2 h_E^2 |\rho_G|}$$

5
6 Where h_D^2 is the heritability of disease risk, h_E^2 is the heritability of the endophenotype, and ρ_G
7 is the genetic correlation between the two (48). The *mERV* is an extension of the *ERV* to be used
8 when the disease of interest is not sufficiently common in the data. For details on the derivation
9 of the *mERV*, see Glahn et al (2015) (40). Briefly, the *mERV* leverages the many coefficients of
10 relationship that exist in extended-pedigree data. The coefficient of relationship refers to the
11 average number of alleles held in common between individuals; for example, first-degree
12 relatives (e.g. full siblings or parents) share on average 50% of their alleles, second-degree
13 relatives (e.g. grandparents or aunts/uncles) share 25%, third-degree relatives (e.g. great
14 grandparents or great-aunts/-uncles) share 12.5%, and so on. Thus it is possible, given an
15 individual with a disease, to index all other pedigree members by their degree of relatedness to
16 that individual. This scalar can then be used to perform a fixed-effect single-degree-of-freedom
17 test, within the univariate variance components analysis outlined above, providing an estimate
18 of the standardized genetic covariance between the potential endophenotype and illness risk.
19 The *mERV* can then be used in the same way as the *ERV* to rank potential endophenotypes by
20 their degree of standardized genetic overlap with illness risk. In the present manuscript the
21 *mERV* was applied to BPD and all available lipid classes.

22
23 We Bonferroni-corrected α ($=0.05$) by the effective number of traits using the method outlined
24 by Cheverud (49), correcting for the total number of traits would be overly conservative, given
25 the extent to which they are all correlated with one and other (50). Applying this method to the
26 pairwise genetic correlations between the 13 phospholipid classes reveals that we have 10.36
27 effective traits, thus $\alpha = 0.05/10.36 = 4.82 \times 10^{-03}$.

28
29 We tested the potential influence of confounding variables, in particular BMI and Major
30 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 liberal threshold of $p < 0.10$ 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 (51): 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 (41): any depressive disorder; any anxiety disorder; any alcohol use disorder; any substance use disorder.

Results

Family profiles

According to our consensus diagnoses nine individuals met the criteria of our broad BPD phenotype, 4 individuals met criteria for BPD I and 5 met 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**).

Indexing Genetic Relatedness Between BPD Liability and All Lipid Classes

The heritability of all lipid classes are shown in **Table 3** inspection of which indicates each class is significantly heritable. Also shown in **Table 3** are the β estimates from the *mERV* analysis along with the p -values of which one withstood a multiple testing correction, the PI (phosphatidylinositol) class ($\beta = -1.18$, $p = 2.10 \times 10^{-03}$, $ERV = 0.49$), this class was deemed to be significantly heritable ($h^2 = 0.26$, $se = 0.08$, $p = 6.71 \times 10^{-05}$).

Investigating The Influence of Potential Confounds: Metabolic and Psychiatric

Of the metabolic variables only Diabetes Status ($\rho_g = 0.35$, $se = 0.15$, $p = 0.03$) was significantly associated with PI levels. None of the other metabolic covariates (including BMI, Hypertension, Heart Attack, or Smoking Status) were significantly associated with PI. Of the psychiatric diagnoses only alcohol use disorders was significantly associated with PI levels ($\rho_g = -0.58$, $se = 0.28$, $p = 0.04$). None of the other psychiatric diagnoses (including depression, anxiety or

substance abuse) were significantly associated with PI. Thus, aside from diabetes status and alcohol use disorders, given the lack of significant genetic overlap between the other metabolic and psychiatric phenotypes we assumed that we need not covary for them when investigating the genetic overlap between BPD risk and PI. Most notably, neither BMI nor major depression demonstrate a shared etiology with serum levels of PI in the present sample, and accordingly neither are likely to be confounding factors in the association between BPD risk and PI that is present in the sample.

After controlling for diabetes status (in addition to age, age², sex, plus their interactions) first degree relatives of affected individuals exhibited lower levels of phosphatidylinositol than unaffected, unrelated controls (Cohen's $d = -0.53$), while 2-7th degree relatives exhibited levels intermediary between 1st degree relatives (Cohen's $d = 0.46$) and controls (Cohen's $d = -0.52$). The levels of PI in cases, unaffected relatives and unaffected unrelateds are shown in **Figure 1**. In general it appears the PI levels vary as a function of genetic proximity to an affected individual. It is important to note that cases were not included in the analyses outlined above, and thus their seemingly anomalous PI levels should not be of concern, firstly there are only 9 cases and secondly their PI levels are subject to confounding factors such as, for example, mood stabilizing medication.

Discussion

In the present study we investigated the relative genetic overlap between bipolar disorder (BPD) risk and thirteen 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 phosphatidylinositol, strongly suggests that phosphatidylinositol 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 characterised by an inositol ring, or head group, extending into the cytoplasm (52).

1 Despite their relatively low abundance compared to other membrane lipids it is the metabolism
2 of this phospholipid class which gives rise to second messengers that are major contributors to
3 the myriad aspects of cellular regulation which make up the phosphatidylinositol signal
4 transduction pathway (53,54). Phosphatidylinositol is implicated in well-characterized signal
5 transduction pathways, alterations in the molecular components of which, in particular PIP₂ and
6 PKC levels, have been previously associated with BPD (55-67). The present study, to our
7 knowledge, is the first to suggest a shared etiology between serum phosphatidylinositol levels
8 and risk for BPD. Phosphatidylinositol is a particularly interesting candidate endophenotype for
9 BPD given that lithium (Li⁺), a mood stabilizing drug and treatment of choice for BPD (68), is
10 thought to act upon the phosphatidylinositol signaling pathway. The inositol-depletion
11 hypothesis posits that lithium acts by preventing the production of phosphatidylinositol via
12 inhibition of IMPase thereby limiting turnover of inositol in the cell (29,68).

13 The direction of the relationship between BPD risk and phosphatidylinositol in the present study
14 was negative, meaning that heightened risk for BPD was associated with *low* levels of the
15 phospholipid. This is the only study, to our knowledge, to assess phospholipid levels in serum in
16 relation to BPD risk. However, Demirkan and colleagues also showed a negative correlation
17 between plasma-based phospholipids (phosphatidylcholine and sphingomyelin) and symptoms
18 and depression and anxiety (26). Therefore while our results are seemingly in keeping with the
19 previous literature they are not necessarily what the inositol-depletion hypothesis might
20 predict, where Li⁺ theoretically works to decrease *high* levels of inositol in BPD subjects. Of
21 course the present study, like that of Demirkan and colleagues (26), relies on peripheral indices
22 of lipid levels. This allows us only to speculate on the ways in which these findings might be
23 interpreted in the brain. There is surprisingly little information in the literature regarding either
24 the direct origin of phosphatidylinositol in circulation or the relationship between levels in the
25 periphery and in the brain. Serum phosphatidylinositol is likely hepatic in origin as majority of
26 circulating lipids in lipoproteins are generated from the liver. The level of PI's is dependent on
27 the availability of myo-inositol, which in turn is synthesized from glucose. The enzyme
28 responsible for all these steps are found in liver cells as well as other organs. There is evidence
29 to suggest that lipids, and their fatty acids are shuttled to the brain from the liver where they
30 play crucial roles in neuro-development, -inflammation and -protection (69-71).

1 Future work might attempt to pin down the levels of phospholipids in brain and even the
2 relationship between those levels in brain and the periphery. One such line of research might
3 utilize phosphorous-31 magnetic resonance spectroscopic (31P MRS), an imaging method that
4 allows non-invasive measurement of biological compounds (e.g. phospholipids) in vivo. In BPD
5 this technique has been used to demonstrate significantly reduced choline, indicating altered
6 phospholipid metabolism, in the frontal lobe (23). The PME (phosphomonesters) signal is 31P
7 MRS reflects the level of phosphocholine and phosphoethanolamine in addition to choline and
8 myo-inositol (72), there is evidence to suggest that the PME signal increase during manic states
9 and decrease during depressed states (73-76). Thus in addition to the unknown relationship
10 between phospholipid levels in the periphery and brain it is possible that a second level of
11 complexity exists where levels in both are affected by BPD illness phase. Evidence from post-
12 mortem studies for phospholipid alterations in brain in BPD is mixed where some have not
13 observed alterations in phospholipids and/or their fatty acids in BPD subjects versus controls
14 (77-79) while others have (24,80). There is little consistency in terms of the focal brain region
15 across these studies, which may explain the inconsistencies in the results. In addition, it is
16 possible that lipidomic abnormalities in relation to affective disorders may be characterized
17 differently in other ethnic populations. For example, non-Hispanic populations exhibit altered
18 lipidomic profiles and associated risk for myocardial infarction relative to Hispanics (81). Thus
19 overall, it is important that the generalizability of the findings in the present manuscript should
20 be further tested in future research.

21 In the present manuscript there was a gap in time between the collection of the lipidomic data
22 and the occurrence of the psychiatric assessments. There is relatively little known about the
23 longitudinal variability of serum phospholipid levels. There is evidence to suggest that
24 phospholipids vary as a function of age (82), and as a consequence we residualized the
25 phospholipid traits for age (in fact for age, age², sex, plus their interactions) at the time of data
26 collection. We consider this a potential strength of the manuscript as it suggests that variation in
27 phosphatidylinositol reflects an early etiological step in the development of BPD. We cannot
28 investigate this in detail in the present sample, with the limited number of affected cases
29 available, but it is possible that the present results hint at alterations in phosphatidylinositol
30 reflect an “at-risk” condition for BPD. Certainly longitudinal studies of other peripheral markers,
31 in this case markers of inflammation, support an aetiological role of inflammation in risk for
32 major depressive disorder (83-85).

Two potential criticisms might be leveraged 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 GWAs previously (86). Also, it is not improbable that no 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 are included in the present study, but importantly these individuals originate 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 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 (40). That being said, in subsequent work where we attempt to finesse our hypotheses regarding phosphatidylinositol 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 affecteds. 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 phosphatidylinositol 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 genetic etiology between the two. It is unlikely that the association between phosphatidylinositol 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 unaffecteds as a function of genetic relatedness to a BPD individual and therefore the present study highlights the potential utility of serum-level measurements of phosphatidylinositol as an indicator of illness risk. Moreover, this study suggests that the well-characterized phosphatidylinositol 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.

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7

8 **Table 1.** Clinical Characteristics of the Sample

9

	Number in Affecteds (N=9)	Number in Relateds (N=185)	Number in Unrelateds (N=373)
Any depressive disorder ^a	9	54	137
Any anxiety disorder ^a	5	18	38
Any alcohol use disorder ^a	6	77	113
Any substance use disorder ^a	1	32	32
Diabetes medication ^b	0	5	26
Lipid medication ^b	0	1	6
Hypertension medication ^b	0	8	27
Diabetes status ^b	0	9	45
Heart attack ^b	0	1	2

Heart surgery ^b	0	1	0
Smoker ^b	2	58	77

1 ^a collected at the time of GOBS assessment

2 ^b collected at the time of lipid data collection

3

4 **Table 2.** Means (and standard deviations) for age and percentage of females by degree of
5 relatedness to an individual with BP.

6

Degree of Relatedness	N	Mean Age (SD)	% Female
-	9	34.65 (4.05)	67
1 st	31	45.84 (13.12)	55
2 nd	21	56.29 (8.85)	76
3 rd	52	46.09 (12.16)	63
4 th	40	45.26 (11.83)	45
5 th	33	36.50 (7.16)	58
6 th	8	42.82 (8.04)	50
Unrelated	373	51.66 (13.36)	67

7

8

9 **Table 3.** Heritability and degree of bipolar relatedness for all lipid classes.

10

Lipid Class	h^2 (<i>p</i> -value)	β (<i>p</i> -value)	ERV	Clinical covariates Included*
PI	0.26 (6.71x10⁻⁰⁵)	-1.18 (2.10⁻⁰³)	0.49	Any alcohol use disorder; Diabetes status
LPE	0.19 (7.82x10 ⁻⁰³)	-0.83 (0.03)	0.33	None
LPC	0.32 (3.10x10 ⁻¹¹)	-0.70 (0.06)	0.27	None
PC(O)	0.40 (4.34x10 ⁻¹⁵)	0.54 (0.08)	0.20	Any anxiety disorder; Any depressive disorder
PE	0.41 (8.69x10 ⁻¹⁴)	-0.43 (0.24)	0.17	Diabetes status
PC(P)	0.32 (1.58x10 ⁻⁰⁴)	0.48 (0.21)	0.19	Any anxiety disorder
PG	0.38 (1.20x10 ⁻¹⁴)	-0.24 (0.12)	0.18	Diabetes status; Any alcohol use disorder
LPC(O)	0.52 (1.84x10 ⁻¹⁰)	0.57 (0.70)	0.18	Diabetes status; BMI
PC	0.28 (3.00x10 ⁻¹¹)	-0.24 (0.17)	0.10	Any alcohol use disorder; Diabetes status; BMI
PS	0.34 (1.01x10 ⁻¹⁴)	0.21 (0.54)	0.08	Any substance use disorder; Hypertension; Smoker; BMI
SM	0.38 (1.17x10 ⁻¹⁴)	0.19 (0.94)	0.08	Smoker; Any alcohol use disorder; Any substance use disorder; Any anxiety disorder
PE(O)	0.38 (2.50x10 ⁻⁰⁶)	0.16 (0.71)	0.06	Any depressive disorder
PE(P)	0.45 (6.96x10 ⁻⁰⁹)	0.16 (0.70)	0.06	Any anxiety disorder; Any depressive disorder

Bold indicates class surviving multiple testing correction

* Threshold of $p < 0.1$

- 1 PI – phosphatidylcholine; LPE – lysophosphatidylethanolamine; LPC – lysophosphatidylcholine; PC(O) – alkylphosphatidylcholine; PE –
- 2 phosphatidylethanolamine; PC(P) – alkenylphosphatidylcholine; PG – phosphatidylglycerol; LPC(O) – lysoalkylphosphatidylcholine; PC –
- 3 phosphatidylcholine; PS – phosphatidylserine; SM – sphingomyelin; PE(O) – alkylphosphatidylethanolamine; PE(P) – alkenylphosphatidylethanolamine

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