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Familial risk for bipolar disorder is not associated with impaired peroxisomal function: Dissociation from docosahexaenoic acid deficits

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Abstract

Bipolar I disorder is associated with deficits in the long-chain omega-3 fatty acid docosahexaenoic acid (DHA, 22:6n-3). The final biosynthesis of DHA is mediated by peroxisomes, and some heritable peroxisomal disorders are associated with DHA deficits and progressive psychopathology. The present cross-sectional study investigated whether medication-free asymptomatic and symptomatic youth with familial risk for bipolar I disorder exhibit impaired peroxisomal function using a comprehensive diagnostic blood panel. Measures of peroxisomal impairment included plasma concentrations of very long-chain fatty acids (VLCFA), branched-chain fatty acids, bile acid intermediates, and pipecolic acid, and erythrocyte plasmalogen and DHA levels. Compared with healthy subjects, significant erythrocyte DHA deficits were observed in ultra-high risk and first-episode bipolar groups, and there was a trend for lower DHA in the high-risk group. There were no significant group differences for any other measure of peroxisomal function, and erythrocyte DHA levels were not correlated with any measure of peroxisome function. These results indicate that familial risk for bipolar I disorder is not associated with impaired peroxisomal function, and that DHA deficits associated with familial bipolar disorder are not attributed to heritable defects in peroxisomal function.

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Keywords

Docosahexaenoic acid; Peroxisome; Adolescents; Very long-chain fatty acids; Plasmalogen; Familial risk

1. Introduction

Bipolar I disorder is characterized by recurrent episodes of mania and often depression, as well as inter-episode periods of euthymia (Goodwin and Jamison, 2007). The initial onset of mania, and by definition bipolar I disorder, commonly occurs during late childhood and adolescence (Perlis et al., 2009). Heritability estimates for bipolar disorder range from 59% to 87% (Smoller and Finn, 2003) and having a first-degree relative with bipolar I disorder is associated with a 14-fold increased risk for developing bipolar I disorder (Mortensen et al., 2003). Depressive symptoms frequently precede the initial onset of mania (Correll et al., 2014; Howes et al., 2011; Skjelstad et al., 2010) and are associated with increased risk for developing mania in offspring of bipolar parents (Axelson et al., 2015). While familial risk and prodromal mood symptoms may aid in the identification of youth that are at increased risk for developing bipolar I disorder (Bechdolf et al., 2014), elucidating heritable biomarkers associated with symptom progression may additionally identify pathogenic mechanisms and inform intervention strategies.

A growing body of translational evidence suggests that a deficiency in long-chain omega-3 fatty acids may represent a neurodevelopmental risk factor associated with the pathoetiology of mood and cognitive disorders (McNamara and Valentine, 2015). Cross-sectional studies have consistently observed lower erythrocyte levels of docosahexaenoic acid (DHA, 22:6 n -3), the most abundant long-chain omega-3 fatty acid in cortical gray matter, in patients with bipolar I disorder (McNamara and Welge, 2016). Erythrocyte DHA deficits coincide with the initial onset of mania (McNamara et al., 2015) and are exhibited by youth that are at elevated risk for developing bipolar I disorder (McNamara et al., 2016). However, bipolar disorder is not associated with deficits in the major fatty acid precursor of DHA, docosapentaenoic acid (DPA, 22:5 n -3), or long-chain omega-6 fatty acids including arachidonic acid (20:4 n -6) (McNamara et al., 2010, 2015). This fatty acid signature suggests that DHA deficits cannot be attributed to impaired microsomal desaturase and elongase activity, and may instead be due to impaired peroxisome-mediated biosynthesis (McNamara, 2015). Peroxisomes are organelles expressing multiple enzymes that catalyze DHA biosynthesis from its fatty acid precursors, as well as other processes including the catabolism of very long chain fatty acid (VLCFA) (Ferdinandusse et al., 2001; Su et al., 2001; Moser et al., 1999a). Heritable defects in peroxisome biogenesis and some single peroxisomal enzyme deficiencies are associated with impaired DHA synthesis (Ferdinandusse et al., 2001) and lower blood and tissue DHA levels (Martinez, 1992; Moser et al., 1999b). However, it is not currently known whether the DHA deficit observed in bipolar I disorder is due to a defect in peroxisomal function.

Evidence also suggests a potential link between peroxisomal defects and psychopathology (Rosebush et al., 1999). While peroxisomal biogenesis disorders are associated with early

postnatal mortality, neurological features in patients with X-linked adrenoleukodystrophy (ALD), the most common single enzyme peroxisomal disorder, are progressive and can initially manifest in childhood or young adulthood (Wanders and Waterham, 2006). Adult-onset ALD patients frequently present with manic or psychotic symptoms and are initially diagnosed with bipolar I disorder or schizophrenia (Rosebush et al., 1999). While ALD is not typically associated with robust DHA deficits it is characterized by elevated VLCFA levels which are commonly 10-fold greater than healthy subjects (Moser et al., 1999a). Progressive neurological and psychiatric symptoms in ALD are associated with white matter pathology thought to result in part from VLCFA accumulation in myelin and subsequent inflammation-mediated demyelination (Powers and Moser, 1998). Although white matter pathology is also associated with the initial onset of bipolar disorder (Adler et al., 2006; Nortje et al., 2013), peroxisomal function in bipolar disorder has never been investigated.

The primary goal of the present study was to investigate whether familial risk for bipolar I disorder is associated with impaired peroxisomal function. Peroxisomal function was measured using a comprehensive diagnostic blood panel to interrogate several biomarkers associated with different single enzyme peroxisomal disorders, including VLCFA, branched-chain fatty acids, bile acid intermediates, and pipecolic acid, and erythrocyte plasmalogen and DHA levels. Based on prior evidence that adult-onset ALD patients frequently present with manic symptoms (Rosebush et al., 1999) and exhibit elevated VLCFA levels (Moser et al., 1999a), we hypothesized that first-episode manic patients would exhibit elevated VLCFA levels compared with healthy subjects. A second objective was to investigate the relationship between biomarkers of peroxisomal function and erythrocyte DHA biostatus.

2. Methods

2.1. Study participants

Study participants were recruited from inpatient units and outpatient clinics at Cincinnati Children's Hospital Medical Center and University of Cincinnati Medical Center. Demographically-matched healthy comparison subjects were recruited from the communities in which the other participants resided. The cohort consisted of 4 groups of medication-free adolescents (9–20 years of age): 1) healthy comparison subjects without a personal history of a DSM-IV Axis I disorder or first or second degree relative with mood or psychotic disorders ('Controls'; $n=19$), 2) subjects with no personal DSM-IV Axis I diagnosis and at least one biological parent with bipolar I disorder ('high-risk'; HR, $n=21$), 3) subjects with a DSM-IV Axis I diagnosis of major depressive disorder (MDD) or depressive disorder not-otherwise-specified (NOS) and at least one biological parent with bipolar I disorder ('ultra-high risk'; UHR, $n=19$), and 4) first-episode adolescent patients exhibiting mixed or manic symptoms who received a DSM-IV diagnosis of bipolar I disorder ('Bipolar', $n=20$). Erythrocyte DHA data from a subset of controls (37%), high risk (62%), and ultra-high risk (52%) subjects, but none of the first-episode bipolar patients, were used in a prior study (McNamara et al., 2016). First-episode manic adolescents had no prior exposure to mood-stabilizer or antipsychotic medications, and adolescents with MDD were antidepressant-free. DSM-IV diagnoses were determined using the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-

U-KSADS) (Geller et al., 2001). Parental diagnoses were determined using the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1995) and confirmed using the Family Interview for Genetic Studies (FIGS) (Maxwell, 1999). All diagnostic assessments were performed by psychiatrists or master's-level interviewers with established inter-rater reliabilities ($\kappa > 0.9$).

Manic and depressive symptom severity ratings were obtained using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Children's Depression Rating Scale-Revised (CDRS-R) (Poznanski et al., 1983), respectively. IQ was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1997). Pubertal development was determined using the self-report Tanner Scale (Duke et al., 1980). Subjects were excluded by a diagnosis of substance dependence within the previous 3 months, major medical or neurological illness (*e.g.*, head trauma resulting in loss of consciousness), an IQ < 70 , or had a positive urine pregnancy or toxicology test. Written informed consent was obtained from each parent or adult subject and written assent was obtained for patients younger than 18 years of age. This study was approved by the Institutional Review Boards of University of Cincinnati Medical Center and Cincinnati Children's Hospital Medical Center, and was registered at clinicaltrials.gov as NCT01237379.

2.2. Blood assays

Whole fasting venous blood (4 ml) was collected into EDTA-coated BD Vacutainer tubes, and immediately centrifuged at 4 °C for 20 min ($1500\times g$). Plasma was removed and stored at -80 °C. Erythrocytes were then washed 3 times with 0.9% NaCl and stored at -80 °C. De-identified plasma and erythrocyte samples were shipped to the Kennedy Krieger Institute, Peroxisomal Diseases Section for analysis. Saturated plasma VLCFA (C24:0 and C26:0) and branched-chain fatty acid (phytanic and pristanic acid) concentrations were determined by capillary gas chromatograph-mass spectrometry (GC-MS), as previously described (Lagerstedt et al., 2001; Steinberg et al., 2008). Plasma bile acids (cholic acid, chenodeoxy acid) and peroxisomal bile acid intermediates di- and trihydroxycholestanoic acid (DHCA and THCA) concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), as previously described (Johnson et al., 2001). Plasma pipelicolic acid concentrations were determined by GC-MS as previously described (Kok et al., 1987; Steinberg et al., 2008). Erythrocyte C16 and C18 plasmalogens were measured as dimethylacetals (DMA) and expressed as the DMA/fatty acid ratio as previously described (Björkhem et al., 1986; Moser et al., 1999b). Normal reference ranges for each analyte were obtained from the Peroxisomal Diseases Laboratory. Erythrocyte DHA composition (mg fatty acid/100 mg fatty acids) was determined in-house by gas chromatography using the direct saponification method described previously (McNamara et al., 2010, 2015, 2016). All samples were processed by a technician blinded to group assignment.

2.3. Statistical analyses

Prior to data analysis the distribution of primary outcome measures was assessed for normality ($\alpha=0.05$), and each was found to be normally distributed within each group. Group differences in demographic variables were identified using a one-way ANOVA for continuous variables and Chi-square tests for dichotomous variables. Overall group

differences in each dependent variable were evaluated with a one-way ANOVA, and individual group differences evaluated with *t*-tests adjusted for multiple comparisons ($\alpha=0.01$). Sex and ADHD effects were assessed with a two-way ANOVA. Pearson correlation coefficients were used to evaluate relationships among selected biomarkers and clinical variables. Effect sizes were calculated for differences between healthy subjects and first-episode manic patients (hypothesized to exhibit the greatest differences in outcomes) using Cohen's *d*, with small, medium, and large effect sizes being equivalent to *d*-values of 0.30, 0.50, and 0.80, respectively (Cohen, 1997). Statistical analyses were performed using GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

3.1. Study participants

Demographic and clinical characteristics of study participants are presented in Table 1. The four groups were demographically well-matched, and there were no statistically group differences in age ($p=0.9$), race ($p=0.3$), IQ ($p=0.6$), BMI ($p=0.4$), or pubertal development ($p=0.2$) (Table 1). Compared with the healthy subjects, there were more females in the high-risk (Chi-square: $p=0.04$) and ultra-high risk ($p=0.001$) groups, and there were more participants with ADHD in the high-risk (19%, $p=0.001$), ultra-high-risk (26%, $p=0.001$), and first-episode manic (45%, $p=0.001$) groups. Therefore, interactions with gender and ADHD were calculated for all outcome measures.

3.2. Blood analyses

We observed a significant group effect for erythrocyte DHA composition ($p=0.01$, $d=0.84$). Compared with healthy subjects, ultra-high risk (−17%, $p=0.02$, $d=0.83$) and first-episode bipolar (−20%, $p=0.009$, $d=0.97$) groups, but not the high-risk (−13%, $p=0.10$, $d=0.59$) group, exhibited significant erythrocyte DHA deficits (Fig. 1). The results of the blood peroxisomal biomarkers and normal reference ranges are presented in Table 2. We did not observe a significant main effect of group for any of the other peroxisomal biomarkers. The main effect of gender, and the gender by group interaction, were not significant for any biomarker ($ps > 0.05$). The group by ADHD interaction was also not significant for any biomarker. Among all subjects ($N=79$), erythrocyte DHA levels were not correlated with any measure of peroxisomal functional integrity including VLCFAs, branched-chain fatty acids, or bile acid intermediates ($ps > 0.05$). Although DHA was not significantly correlated with either YMRS or CDRS total scores within each group, among all subjects ($n=79$) DHA was inversely correlated with YMRS ($r=-0.3$, $p=0.01$) and CDRS ($r=-0.25$, $p=0.04$) total scores. Among all subjects and within each group, there were no significant correlations between CDRS or YMRS total scores and any other peroxisomal biomarker ($ps > 0.05$).

4. Discussion

The primary objective of this study was to investigate whether familial bipolar I disorder is associated with a defect in peroxisomal function using a validated diagnostic blood screening panel. Consistent with previous cross-sectional data (McNamara and Welge, 2016), we observed robust erythrocyte DHA deficits in first-episode bipolar patients. We

also observed erythrocyte DHA deficits in adolescents with familial risk and depressive symptoms, which is also consistent with previous findings (McNamara et al., 2016). However, compared with healthy controls and normal reference ranges, no group exhibited significant alterations in other peroxisomal biomarkers including plasma concentrations of VLCFA, branched-chain fatty acids, bile acid intermediates, pipecolic acid, or erythrocyte plasmalogens. Among all subjects, peroxisomal biomarkers were not significantly correlated with erythrocyte DHA levels or manic and depressive symptom severity scores. Together, these results indicate that familial risk for bipolar disorder is not associated with a heritable defect in peroxisomal function, and suggest that DHA deficits associated with bipolar disorder are not attributable to impaired peroxisomal function.

Based on prior evidence that adult-onset ALD patients frequently present with manic symptoms (Rosebush et al., 1999) and exhibit elevated VLCFA levels (Moser et al., 1999a), we hypothesized that first-episode manic patients would exhibit elevated VLCFA levels. However, our results indicate that plasma VLCFA levels, as well as other measures of peroxisomal function, were normal in first-episode manic patients. It is also notable that previous studies have observed both plasmalogen (Kaddurah-Daouk et al., 2012; Wood et al., 2015) and DHA (van der Kemp et al., 2012) deficits in first-episode schizophrenia patients. In the present study, however, erythrocyte plasmalogen levels in first-episode manic patients were in the normal range and did not differ significantly from healthy controls. Furthermore, we previously reported that expression of the *PEX19* gene, which is essential for peroxisome biogenesis (Götte et al., 1998), was not altered in the postmortem brain tissue from bipolar patients (Liu and McNamara, 2011). Therefore, extant evidence suggests that while psychopathology may emerge in patients with heritable defects in peroxisomal function, such defects are not evident in symptomatic or asymptomatic youth with familial risk for bipolar disorder.

A second objective was to investigate the relationship between biomarkers of peroxisomal function and erythrocyte DHA biostatus. Defects in peroxisome biogenesis are associated with negligible DHA synthesis (Ferdinandusse et al., 2001) and robust blood and tissue deficits (Martinez, 1992; Martinez et al., 1994; Moser et al., 1999b). Peroxisomal disorders associated with heritable defects or deficiencies in single enzymes that catalyze DHA biosynthesis, including straight-chain acyl-CoA oxidase, D-bifunctional protein, 3-ketoacyl-CoA thiolase, and sterol carrier protein X, are also associated with impaired DHA synthesis (Ferdinandusse et al., 2001). Defects or deficiencies in the latter enzymes are also associated with elevated levels of VLCFA and/or branched-chain fatty acids and bile acid intermediates (Moser et al., 1999b; Wanders and Waterham, 2006). In the present study, we did not observe alterations in any of the latter measures and did not observe correlations between these measures and erythrocyte DHA levels. Together, these findings suggest that the DHA deficits repeatedly observed in bipolar patients are not a result of defects or deficiencies in single peroxisomal enzymes that catalyze DHA biosynthesis. While not directly evaluated in the present study, erythrocyte DHA deficits may instead result from a dietary deficiency in preformed DHA. This is supported by the observations that bipolar patients consume less DHA (Evans et al., 2014) and that dietary supplementation with fish oil, which contained preformed DHA, is sufficient to increase erythrocyte DHA in bipolar patients (Wozniak et

al., 2007). Additionally or alternatively, DHA deficits may result from increased catabolism though this mechanism remains to be evaluated.

This study has some notable limitations. First, the relatively small number of individuals in each group ($n=19-21$) may have been underpowered to achieve statistical significance. However, patients with peroxisomal disorders typically exhibit VLCFA (C26:0) concentrations that are 6- to 18-fold greater than healthy subjects (Moser et al., 1999b), and the current sample size would have > 0.99% power to detect a difference of this magnitude if bipolar disorder was associated with a similar defect in peroxisomal function. Moreover, the mean VLCFA levels observed in first-episode manic patients and healthy subjects were both within normal reference ranges and similar to means observed in larger sample size (Moser et al., 1999b). Second, although this study investigated validated blood biomarkers used in the diagnosis of peroxisomal disorders, more definitive measures of DHA biosynthesis (i.e., radio-labelled fatty acid studies using fibroblasts) were not used. Third, we did not administer a diet questionnaire to determine the contribution of habitual dietary patterns to the observed findings. Strengths of this study include the investigation of a wide range of different measures of peroxisomal function, and the well-characterized cohort of medication-free asymptomatic and symptomatic offspring with a parent with bipolar I disorder.

In summary, the present cross-sectional study provides evidence that familial risk for bipolar disorder is not associated with defects in peroxisomal function. Specifically, we demonstrate that asymptomatic and symptomatic offspring with a parent with bipolar I disorder do not exhibit abnormalities in multiple different indices of peroxisomal function compared with healthy controls as well as normal reference ranges. However, it remains to be determined whether defects in peroxisomal function emerge following chronic illness and/or in response to psychotropic medications. The results additionally suggest that erythrocyte DHA deficits observed in symptomatic youth with familial risk for bipolar disorder are not attributable to defects or deficiencies in single peroxisomal enzymes that catalyze DHA biosynthesis.

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Fig. 1.

Erythrocyte membrane DHA composition in healthy comparison (HC), high-risk (HR), ultra high-risk (UHR), and first-episode bipolar (BP) groups. DHA levels are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). Values are group mean $\pm$ S.E.M. * $P=0.02$, ** $P=0.009$ vs. HC.

Table 1

Demographic and clinical characteristics.

Variable ^a	Control (n=19)	High risk (n=21)	Ultra-high risk (n=19)	Bipolar (n=20)	P-value ^b
Age mean (SD) years	13.9 (2.6)	14.0 (2.9)	14.0 (3.1)	14.2 (1.9)	0.99
Sex (% Female)	47	62	79	60	0.001
Race (% White)	64	76	58	78	0.26
Tanner score, mean (SD)	3.9 (1.1)	3.5 (0.8)	3.7 (0.9)	4.1 (0.8)	0.25
BMI, mean (SD)	21.6 (4.3)	24.4 (5.7)	23.7 (8.2)	25.6 (7.1)	0.39
WASI, mean (SD)	104.4 (15.4)	104.2 (12.7)	102.2 (7.1)	97.88 (15.5)	0.64
ADHD (%)	0	19	26	45	0.001
YMRS, mean (SD)	1.3 (1.5)	3.3 (2.9)	10.9 (3.9)	28.8 (4.8)	0.0001
CDRS, mean (SD)	11.5 (8.9)	22.5 (5.6)	44.9 (9.3)	36.6 (7.6)	0.0001

^aValues are group mean $\pm$ S.D., or group percentage.

^bOne-way ANOVA or Chi-square test.

Table 2

Blood peroxisomal biomarkers.

Biomarker ^a	Normal range	Control (n=19)	High risk (n=21)	Ultra-high risk (n=19)	Bipolar (n=20)	F-value ^b	P-value ^b	d ^c
Erythrocyte Plasmalogen (ratio)								
C16DMA/C16:0	0.079–0.128	0.1 (0.01)	0.1 (0.01)	0.1 (0.01)	0.1 (0.01)	0.18	0.91	0.14
C18DMA/C18:0	0.199–0.284	0.2 (0.04)	0.2 (0.03)	0.2 (0.03)	0.2 (0.02)	0.07	0.97	0.07
Bile Acids (pmoles/10 UL)								
Cholic acid	0.02–46.60	1.5 (1.4)	2.2 (1.5)	1.7 (1.4)	2.6 (2.1)	0.57	0.33	0.05
Chenodeoxy acid	0.22–97.42	4.1 (2.6)	4.1 (2.8)	3.6 (3.4)	4.3 (1.7)	1.16	0.83	0.19
DCHA	0.00–0.10	0.06 (0.04)	0.07 (0.04)	0.07 (0.06)	0.07 (0.06)	1.24	0.86	0.54
THCA	0.00–0.11	0.19 (0.4)	0.34 (0.4)	0.30 (0.4)	0.42 (0.5)	0.99	0.39	0.55
VLCFA (ug/ml)								
Hexacosanoic acid (C26:0)	0.05–0.41	0.21 (0.05)	0.21 (0.09)	0.19 (0.05)	0.19 (0.03)	0.40	0.51	0.18
Hexacosanoic acid (C26:1)	0.01–0.36	0.1 (0.01)	0.1 (0.02)	0.1 (0.01)	0.1 (0.02)	0.24	0.86	0.12
Lignoceric acid (C24:0)	6.87–28.31	17.7 (3.6)	16.6 (2.2)	16.4 (2.6)	16.9 (3.5)	0.69	0.56	0.22
Behenic acid (C22:0)	8.43–33.51	20.0 (4.3)	19.2 (2.7)	18.9 (3.6)	18.8 (4.0)	0.42	0.74	0.30
C26:0/C22:0 ratio	0.002–0.018	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.30	0.96	0.02
C24:0/C22:0 ratio	0.64–1.04	0.89 (0.04)	0.86 (0.05)	0.87 (0.06)	0.90 (0.05)	1.94	0.13	0.37
Branched-Chain Fatty Acids (ug/ml)								
Pristanic acid	0.01–0.3	0.04 (0.01)	0.04 (0.02)	0.04 (0.01)	0.04 (0.01)	0.53	0.86	0.31
Phytanic acid	0.05–3.0	0.4 (0.1)	0.4 (0.2)	0.4 (0.1)	0.3 (0.1)	1.01	0.39	0.65
Pipecolic acid (umol/L)	0.1–4.0	1.4 (0.6)	1.2 (0.6)	1.2 (0.9)	1.1 (0.6)	1.22	0.79	0.63

^aValues are group mean ± S.D. (Abbreviations provided in text).^bOne-way ANOVA.^cCohen's *d* for Control vs. Bipolar.