

ORIGINAL ARTICLE

# Inflammation as a predictive biomarker for response to omega-3 fatty acids in major depressive disorder: a proof-of-concept study

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This study explores whether inflammatory biomarkers act as moderators of clinical response to omega-3 (*n*-3) fatty acids in subjects with major depressive disorder (MDD). One hundred fifty-five subjects with Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) MDD, a baseline 17-item Hamilton Depression Rating Scale (HAM-D-17) score  $\geq 15$  and baseline biomarker data (interleukin (IL)-1ra, IL-6, high-sensitivity C-reactive protein (hs-CRP), leptin and adiponectin) were randomized between 18 May 2006 and 30 June 2011 to 8 weeks of double-blind treatment with eicosapentaenoic acid (EPA)-enriched *n*-3 1060 mg day<sup>-1</sup>, docosahexaenoic acid (DHA)-enriched *n*-3 900 mg day<sup>-1</sup> or placebo. Outcomes were determined using mixed model repeated measures analysis for 'high' and 'low' inflammation groups based on individual and combined biomarkers. Results are presented in terms of standardized treatment effect size (ES) for change in HAM-D-17 from baseline to treatment week 8. Although overall treatment group differences were negligible (ES = -0.13 to +0.04), subjects with any 'high' inflammation improved more on EPA than placebo (ES = -0.39) or DHA (ES = -0.60) and less on DHA than placebo (ES = +0.21); furthermore, EPA-placebo separation increased with increasing numbers of markers of high inflammation. Subjects randomized to EPA with 'high' IL-1ra or hs-CRP or low adiponectin ('high' inflammation) had medium ES decreases in HAM-D-17 scores vs subjects 'low' on these biomarkers. Subjects with 'high' hs-CRP, IL-6 or leptin were less placebo-responsive than subjects with low levels of these biomarkers (medium to large ES differences). Employing multiple markers of inflammation facilitated identification of a more homogeneous cohort of subjects with MDD responding to EPA vs placebo in our cohort. Studies are needed to replicate and extend this proof-of-concept work.

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## INTRODUCTION

The heterogeneity of both symptoms and underlying pathophysiology confounds the development of targeted treatments for major depressive disorder (MDD).<sup>1</sup> Therefore, the discovery of biomarkers that characterize more homogeneous subgroups of patients with MDD is critical to our understanding of its pathogenesis and to the development of personalized therapies.<sup>1,2</sup>

Chronic inflammation is involved in the etiology of heart disease, stroke, cancer and diabetes,<sup>3,4</sup> and is thought to play a role in the etiology of MDD for some individuals.<sup>5</sup> Preclinical work has established that fatigue, anorexia, sleep disturbance and anhedonia are part of the behavioral component of a systemic inflammatory response.<sup>5</sup> Inflammation may cause glucocorticoid insensitivity as well as shunting of tryptophan away from monoamine production and toward production of kynurenine and its metabolites,<sup>6,7</sup> thus decreasing the synthesis of monoamine neurotransmitters, while disrupting brain glutamatergic systems.<sup>8–10</sup> Epidemiological studies demonstrate that MDD is associated with a greater prevalence of elevated markers of inflammation.<sup>11</sup> Conversely, interferon- $\alpha$  therapy-induced MDD can be successfully prevented by prophylactic antidepressant medications or pretreatment with the omega-3 polyunsaturated fatty acid (*n*-3 PUFA) eicosapentaenoic acid (EPA).<sup>12,13</sup> In a clinical trial of subjects with

treatment-resistant MDD, those with high-sensitivity C-reactive protein (hs-CRP) levels  $> 5$  had a positive response to therapy with infliximab, an anti-tumor necrosis factor- $\alpha$  antibody.<sup>14</sup>

The epidemiological literature suggests that individuals who eat diets rich in *n*-3 PUFA have less cardiovascular disease and a decreased incidence of mood disorders.<sup>15,16</sup> This led to the investigation of *n*-3 PUFA supplementation for a heterogeneous group of medical and psychiatric disorders.<sup>17,18</sup> Clinical studies investigating the efficacy of EPA, docosahexaenoic acid (DHA) and a combination of EPA+DHA as augmenting agents suggest that EPA-enriched supplementation of antidepressant medications is associated with a greater improvement in depression ratings than placebo augmentation.<sup>19–21</sup> The few trials of EPA or DHA monotherapy for the treatment of MDD have found inconsistent benefits for *n*-3 therapy.<sup>21–23</sup> We completed the first double-blind randomized monotherapy trial of EPA vs DHA vs placebo treatment of MDD; the effect sizes (ESs) were -0.179 for EPA vs placebo and -0.228 vs DHA and +0.049 for DHA vs placebo (Mischoulon *et al.*<sup>24</sup>). This finding agrees with the reviews and meta-analyses that suggest EPA or EPA+DHA (but not DHA alone) have a small ES advantage over placebo.<sup>25–27</sup> In addition, in two independent re-analyses of the Bloch and Hannestad meta-analyses, Martins *et al.* report an adjusted ES of 0.468 for studies with  $\geq 60\%$  EPA, while Lin *et al.* reported an ES of 0.58 for these

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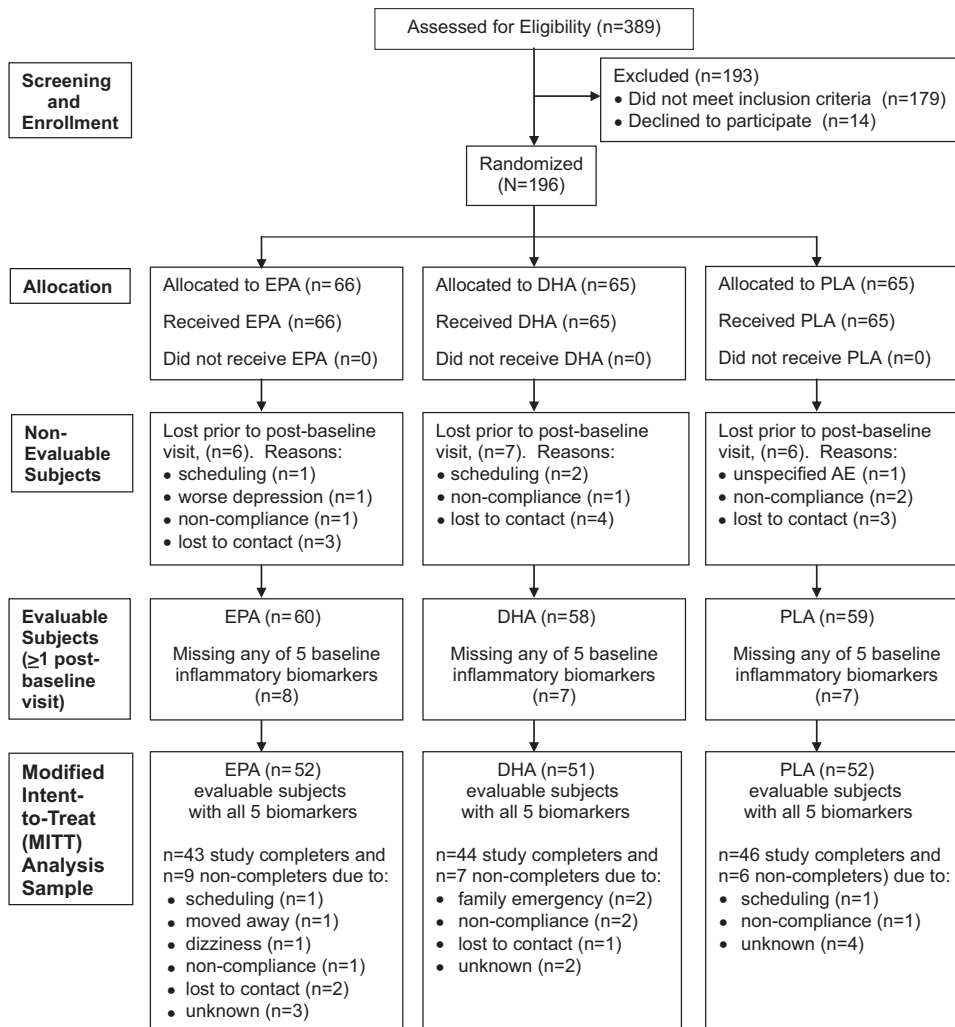


Figure 1. CONSORT statement flow diagram.

studies.<sup>28,29</sup> One postulate that reconciles the disparate data about *n*-3 therapy for MDD is that only a subset of patients with MDD benefit from *n*-3 treatment. EPA and its metabolites are important for an array of biological functions, including competing with the *n*-6 fatty acid metabolite arachidonic acid to shift synthesis away from inflammatory eicosanoids and toward the production of anti-inflammatory eicosanoids.<sup>30,31</sup> Based on the evidence that some patients with MDD have increased inflammatory markers, and data suggesting that increasing *n*-3 intake shifts eicosanoid metabolism toward production of anti-inflammatory substances, we hypothesized that PUFA monotherapy would be more effective than placebo for patients with MDD who manifest elevated markers of inflammation. We further postulated that the response to EPA would be enhanced for a more homogenous subset of patients characterized by elevated inflammatory markers.

## MATERIALS AND METHODS

This collaborative R01 was based at Massachusetts General Hospital (MGH) and Cedars-Sinai Medical Center (CSMC), and approved by their institutional review boards. All subjects reviewed and signed an informed consent form.

We recruited 389 outpatients with MDD, ages 18–80, from 18 May 2006 to 30 June 2011 through advertisements and outpatient referrals. Inclusion

criteria were a diagnosis of MDD according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) Axis I Disorders, Patient Edition (SCID I/P),<sup>32</sup> a Clinical Global Impressions-Severity score  $\geq 3$  and a baseline 17-item Hamilton Depression Rating Scale (HAM-D-17)<sup>33</sup> score  $\geq 15$ ; Figure 1).

Exclusion criteria included: pregnancy or women of child bearing potential who were not using contraception; suicidality; homicidality; unstable medical illness; history of organic mental disorders, substance use disorders, psychotic disorders or bipolar disorder; allergy to the study compounds; concurrent use of psychotropic medications, systemic corticosteroids, steroid antagonists, anticoagulants or immunosuppressant agents; electroconvulsive therapy (ECT) during the current episode; any trial of  $\geq 6$  weeks with citalopram 40 mg day<sup>-1</sup> or equivalent antidepressant during the current episode; history of *n*-3 PUFA supplement use; an average daily intake of  $\geq 3.0$  g of total *n*-3 between screening and baseline visit per the Food Processor 7.8 questionnaire (ESHA Research, Salem, OR, USA); psychotherapy; smoking  $>10$  cigarettes per day; and vitamin E supplementation or regular non-steroidal anti-inflammatory use.

Subjects were randomized in a double-blind 1:1:1 manner to two capsules of EPA-enriched mix (ProEPA Xtra, 530-mg EPA/130-mg DHA per soft gel) and two placebo capsules, four capsules of DHA-enriched mix (ProDHA, 225-mg DHA/45-mg EPA per soft gel) or four placebo (1000-mg soybean oil) capsules per day for 8 weeks (Nordic Naturals, Watsonville, CA, USA).

Subjects were evaluated every 2 weeks for 8 weeks. The primary clinical outcome measure was decrease in the HAM-D-17 score.

**Table 1.** Baseline demographic, clinical and inflammatory biomarker characteristics for *N* = 155 evaluable subjects with all five inflammatory biomarkers at baseline

<i>Demographic characteristics</i>					
<i>Study site</i>	Cedars-Sinai Medical Center	<i>N</i> (%)			94 (60.6)
	Massachusetts General Hospital	<i>N</i> (%)			61 (39.4)
Age in years ( <i>N</i> = 148)		Mean (s.d.) [range]			46.1 (12.6) [21–73]
Sex	Female	<i>N</i> (%)			91 (58.7)
	Male	<i>N</i> (%)			64 (41.3)
Race	Caucasian	<i>N</i> (%)			104 (67.1)
	African American	<i>N</i> (%)			29 (18.7)
	Other	<i>N</i> (%)			13 (8.4)
	Prefer not to say	<i>N</i> (%)			9 (5.8)
Ethnicity ( <i>N</i> = 149)	Hispanic	<i>N</i> (%)			23 (15.4)
	Non-Hispanic	<i>N</i> (%)			126 (84.6)
Education ( <i>N</i> = 148)	High school or less	<i>N</i> (%)			39 (26.4)
	Some college or more	<i>N</i> (%)			109 (73.6)
Marital status ( <i>N</i> = 139)	Married or living together	<i>N</i> (%)			27 (19.4)
	Separated/widowed/divorced	<i>N</i> (%)			47 (33.8)
	Never married	<i>N</i> (%)			65 (46.8)
Employment status ( <i>N</i> = 148)	Employed	<i>N</i> (%)			72 (48.6)
	Homemaker	<i>N</i> (%)			8 (5.4)
	Student	<i>N</i> (%)			9 (6.1)
	Other	<i>N</i> (%)			59 (39.9)
<i>Clinical Characteristics</i>					
HAM-D-17	Mean (s.d.) [range]			19.3 (3.1) [15–30]	
Comorbid anxiety disorder ( <i>N</i> = 148)	Current	<i>N</i> (%)			40 (27.0)
	Lifetime	<i>N</i> (%)			47 (31.8)
BMI <sup>a</sup> ( <i>N</i> = 144)	Underweight	<i>N</i> (%)			6 (4.2)
	Normal Weight	<i>N</i> (%)			45 (31.2)
	Overweight	<i>N</i> (%)			45 (31.2)
	Obese	<i>N</i> (%)			48 (33.3)
<i>Distribution per inflammatory biomarker</i>					
	<i>Group</i>	<i>Median</i>	<i>Q1–Q3</i>	<i>Range</i>	<i>High inflammation definition</i>
hs-CRP (mg l <sup>-1</sup> )	Total	1.3	0.4–2.9	0.1–28.2	> 3.0 <sup>b</sup>
IL-6 (pg ml <sup>-1</sup> )	Total	1.3	0.8–2.0	0.4–9.7	> 1.92 <sup>c</sup>
IL-1ra (pg ml <sup>-1</sup> )	Total	381.8	239.9–570.2	100.5–2599.0	> 500 <sup>c</sup>
Leptin <sup>d</sup> (mg l <sup>-1</sup> )	Female	150.0	58.0–336.0	11.0–1187.0	≥ 250 <sup>c</sup>
	Male	37.0	20.5–72.5	2.0–229.0	≥ 70 <sup>c</sup>
Adiponectin <sup>d,e</sup> (mg l <sup>-1</sup> )	Female	97.0	62.0–138.0	0.04–319.0	< 80 <sup>c</sup>
	Male	59.0	32.5–93.0	0.04–220.0	< 60 <sup>c</sup>
<i>High inflammation rates by BMI category<sup>a</sup></i>					
	<i>Group</i>	<i>Underweight or normal weight N/N (%)</i>	<i>Overweight N/N (%)</i>	<i>Obese N/N (%)</i>	<i>All subjects<sup>f</sup> N/N (%)</i>
<i>High inflammation status per inflammatory biomarker</i>					
hs-CRP	Total	6/51 (11.8)	6/45 (13.3)	23/48 (47.9)	37/155 (23.9)
	Female	4/39 (10.3)	3/18 (16.7)	17/29 (58.6)	25/91 (27.5)
	Male	2/12 (16.7)	3/27 (11.1)	6/19 (31.6)	12/64 (18.8)
IL-6	Total	4/51 (7.8)	7/45 (15.6)	26/48 (54.2)	39/155 (25.2)
	Female	3/39 (7.7)	2/18 (11.1)	21/29 (72.4)	27/91 (29.7)
	Male	1/12 (8.3)	5/27 (18.5)	5/19 (26.3)	12/64 (18.8)
IL-1ra	Total	10/51 (19.6)	10/45 (22.2)	23/48 (47.9)	47/155 (30.3)
	Female	4/39 (10.3)	4/18 (22.2)	17/29 (58.6)	27/91 (29.7)
	Male	6/12 (50.0)	6/27 (22.2)	6/19 (31.6)	20/64 (31.2)
Leptin	Total	1/51 (2.0)	7/45 (15.6)	35/48 (72.9)	46/155 (29.7)
	Female	1/39 (2.6)	3/18 (16.7)	23/29 (79.3)	30/91 (33.3)
	Male	0/12 (0.0)	4/27 (14.8)	12/19 (63.2)	16/64 (25.0)
Adiponectin	Total	11/51 (21.6)	20/45 (44.4)	33/48 (68.8)	67/155 (43.2)
	Female	7/39 (18.0)	7/18 (38.9)	19/29 (65.5)	35/91 (38.5)
	Male	4/12 (33.3)	13/27 (48.2)	14/19 (73.7)	32/64 (50.0)
<i>Number of biomarkers with high inflammation</i>					
4 or 5	Total	0/51 (0.0)	2/45 (4.4)	18/48 (37.5)	21/155 (13.5)
	Female	0/39 (0.0)	0/18 (0.0)	14/29 (48.3)	15/91 (16.5)
	Male	0/12 (0.0)	2/27 (7.4)	4/19 (21.0)	6/64 (9.4)

**Table 1.** (Continued)

		High inflammation rates by BMI category <sup>a</sup>			
	Group	Underweight or normal weight N/N (%)	Overweight N/N (%)	Obese N/N (%)	All subjects <sup>f</sup> N/N (%)
2 or 3	Total	6/51 (11.8)	9/45 (20.0)	21/48 (43.8)	38/155 (24.5)
	Female	3/39 (7.7)	5/18 (27.8)	11/29 (37.9)	20/91 (22.0)
	Male	3/12 (25.0)	4/27 (14.8)	10/19 (52.6)	18/64 (28.1)
1	Total	18/51 (35.3)	22/45 (48.9)	5/48 (10.4)	50/155 (32.3)
	Female	12/39 (30.8)	8/18 (44.4)	2/29 (6.9)	24/91 (26.4)
	Male	6/12 (50.0)	14/27 (51.8)	3/19 (15.8)	26/64 (40.6)
Any of the above (1 or more)	Total	24/51 (47.1)	33/45 (73.3)	44/48 (91.7)	109/155 (70.3)
	Female	15/39 (38.5)	13/18 (72.2)	27/29 (93.1)	59/91 (64.8)
	Male	9/12 (75.0)	20/27 (74.1)	17/19 (89.5)	50/64 (78.1)
None	Total	27/51 (52.9)	12/45 (26.7)	4/48 (8.3)	46/155 (29.7)
	Female	24/39 (61.5)	5/18 (27.8)	2/29 (6.9)	32/91 (35.2)
	Male	3/12 (25.0)	7/27 (25.9)	2/19 (10.5)	14/64 (21.9)

Abbreviations: BMI, body mass index; HAM-D-17, 17-item Hamilton Depression Rating Scale; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin. <sup>a</sup>BMI was calculated as: pounds  $\times$  703.06942/inches<sup>2</sup>. Resulting BMI < 18.50 = underweight; 18.50 to < 25.00 = normal weight; 25.00 to < 30.00 = overweight; and  $\geq$  30 = obese. Because of the small number of underweight subjects ( $N=6$ ), this group was combined with the normal-weight BMI category for analyses. Height and weight data are missing for  $N=11$  subjects (5 females and 6 males). <sup>b</sup>High inflammation status for hs-CRP was based on conventionally defined level.<sup>34</sup> <sup>c</sup>High inflammation cut points for biomarkers other than hs-CRP were based on inflection points in stem-and-leaf plots, such that 'high' inflammation included the skewed end of the distribution of values. <sup>d</sup>Levels of leptin and adiponectin vary greatly by sex, so distribution data are shown separately for females and males, and thresholds for defining high inflammation were determined separately by sex. <sup>e</sup>Low values on adiponectin indicate high inflammation. <sup>f</sup>All  $N=155$  subjects with all five inflammatory biomarkers present at baseline, including 11 subjects missing BMI.

The baseline body mass index (BMI) formula employed was: BMI = pounds  $\times$  703.06942/inches<sup>2</sup>. We used the standard conventions for defining BMI categories: < 18.50 = underweight; 18.50 to < 25.00 = normal weight; 25.00 to < 30.00 = overweight; and  $\geq$  30.00 = obese.

#### Biological measures and assay methodology

Blood samples for biomarkers were drawn at baseline. Plasma interleukin (IL)-1ra concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation for the IL-1ra ELISAs were 3.9 and 5.1%, respectively. Plasma concentrations of IL-6 were measured using a high-sensitivity ELISA from R&D Systems. Intra- and inter-assay coefficients of variation for the IL-6 ELISAs were 7.0 and 9.9%, respectively. Plasma hs-CRP concentrations were assessed using an immunoturbidimetric assay kit from Sekisui Diagnostics (Framingham, MA, USA). Intra- and inter-assay coefficients of variation for the hs-CRP assays were 4.4 and 5.5%, respectively. Plasma concentrations of leptin and adiponectin were measured using separate ELISAs from R&D Systems. Intra- and inter-assay coefficients of variation for the leptin ELISAs were 6.6 and 8.9%, respectively, and 5.2 and 8.7%, respectively for the adiponectin ELISAs.

#### Statistical analyses

This investigation of high inflammation as a moderator of efficacy of the primary efficacy outcome measure, HAM-D-17 score, was based on a modified intent-to-treat sample of 155 evaluable subjects with data on all five inflammatory biomarkers at baseline and at least one post-baseline visit (for HAM-D-17). Baseline comparisons across treatment groups were made by analysis of variance for continuous measures and chi-square tests for categorical variables. All five inflammatory biomarkers had highly skewed distributions, even after log transformation, so distributions of biomarker values are presented as quartile values and ranges. Spearman's rank correlations were used to describe their interrelationship.

Since conventions are not established for plasma levels of inflammatory biomarkers except hs-CRP, and values of all biomarkers were highly skewed, 'high' levels of inflammation were defined based on inflection points in stem-and-leaf plots of baseline values for all subjects entering the study. By using this method, 'high' inflammation was defined as  $>1.92$  pg ml<sup>-1</sup> for IL-6 and  $>500$  pg ml<sup>-1</sup> for IL-1ra, along with  $>3.0$  mg l<sup>-1</sup> for hs-CRP which agrees with the Centers for Disease Control and Prevention (CDC) and American Heart Association convention.<sup>34</sup> 'High' inflammation for cut points on leptin and adiponectin were based on separate stem-and-leaf plots for males and females, since leptin and

adiponectin levels differ substantially by sex.<sup>35,36</sup> The definition of 'high' inflammation for leptin was  $\geq 250$  mg l<sup>-1</sup> for females and  $\geq 70$  mg l<sup>-1</sup> for males, while high inflammation on adiponectin (primarily a biomarker of anti-inflammatory activity) was  $< 80$  mg l<sup>-1</sup> for females and  $< 60$  mg l<sup>-1</sup> for males. In this paper, subjects in the 'not high' group will be referred to as being 'low'.

Mixed model repeated measures (MMRM) analysis was carried out to examine the effect of treatment group on changes in HAM-D-17 scores from baseline to treatment week 8. Models included subjects as a random effect and treatment group, treatment week and their interaction as fixed effects. Baseline HAM-D-17 scores were included as a covariate. Since these analyses demonstrated similar results for DHA and placebo, we focused further analysis on inflammatory moderators of EPA vs placebo response. MMRM was used to test EPA vs placebo treatment effect based on each of the five biomarkers individually, and to examine whether the EPA vs placebo separation was increased by any combination of two markers. MMRM was performed to test 'high' vs 'low' levels of individual biomarkers within EPA and placebo groups; to explore whether being 'high' on particular biomarkers was responsible for EPA response or placebo non-response. An autoregressive covariance structure was used for MMRM because it provided the best fit for the data. In light of the small numbers of cases available for comparisons of subgroups, our outcome of interest was standardized treatment ES for change in HAM-D-17 from baseline to week 8 (defined as the difference in least-square mean change divided by the pooled s.d. of change), rather than the significance of differences in slopes over the entire treatment period.

Treatment response was defined as an improvement of  $\geq 50\%$  in HAM-D-17 score from baseline and remission was defined as a HAM-D-17 score  $\leq 7$ . Comparisons of response and remission rates at subjects' last study visit were computed across treatment groups using an extension of Fisher's exact test<sup>37</sup> for the modified intent-to-treat sample and for study completers.

All statistical analyses were carried out using SAS 8.2 software (SAS Institute, Cary, NC, USA 2001). A two-tailed alpha level of 0.05 was used to determine statistical significance, uncorrected for multiple comparisons as is appropriate for preliminary analyses.<sup>37</sup> Analyses were performed based on blind treatment codes.

#### RESULTS

Baseline characteristics of the sample are shown in Table 1. The three treatment groups did not differ on demographic variables, clinical characteristics, BMI or prevalence of high levels of

**Table 2.** Change in HAM-D-17 total score from baseline to treatment week 8 by number of 'high' biomarkers at baseline<sup>a</sup>

Number of biomarkers reflecting 'high' inflammation	Change from baseline to treatment week 8					Standardized treatment effect size <sup>b</sup> at treatment week 8					Paired comparison of groups at treatment week 8			Significance of treatment-by-time interaction
	EPA	DHA	PLA	EPA vs PLA	DHA vs PLA	EPA vs DHA	EPA vs PLA	DHA vs PLA	EPA vs DHA	PLA	DHA vs EPA	PLA vs DHA		
4 or 5 Biomarkers (N = 21)	LS-mean (s.e.m.) [N]	-11.14 (1.79) [10]	-4.90 (2.17) [7]	-5.02 <sup>c</sup> (2.52) [4]	ES (95% CI)	-1.11 (-2.35 to +0.13)	+0.02 (-1.21 to +1.25)	-1.10 (-2.14 to -0.05)	t	-2.01	+0.04	-2.13	F	0.94
									df <sup>d</sup>	34.4	31.9	31.5	df <sup>d</sup>	2, 79.8
									P-value	0.052	0.972	0.041	P-value	0.396
2 or 3 Biomarkers (N = 38)	LS-mean (s.e.m.) [N]	-12.38 (1.47) [13]	-11.52 (1.35) [13]	-9.43 (1.35) [12]	ES (95% CI)	-0.59 (-1.39 to +0.21)	-0.44 (-1.23 to +0.36)	-0.17 (-0.94 to +0.60)	t	-1.48	-1.09	-0.42	F	0.70
									df <sup>d</sup>	82.2	82.1	82.1	df	2, 135
									P-value	0.142	0.279	0.672	P-value	0.498
1 Biomarker (N = 50)	LS-mean (s.e.m.) [N]	-11.76 (1.28) [13]	-7.31 (1.11) [17]	-10.80 (1.10) [20]	ES (95% CI)	-0.20 (-0.90 to +0.50)	+0.73 (+0.06 to +1.40)	-0.97 (-1.73 to -0.20)	t	-0.57	+2.23	-2.62	F	1.20
									df	122	123	120	df	2, 177
									P-value	0.569	0.027	0.010	P-value	0.303
Any (1-5) biomarkers (N = 109)	LS-mean (s.e.m.) [N]	-11.46 (0.82) [36]	-8.59 (0.77) [37]	-9.57 (0.80) [36]	ES (95% CI)	-0.39 (-0.86 to +0.08)	+0.21 (-0.25 to +0.67)	-0.60 (-1.07 to -0.13)	t	1.66	0.88	2.55	F	0.86
									df	251	249	250	df	2, 405
									P	0.099	0.381	0.017	P	0.423
No biomarker (N = 46)	LS-mean (s.e.m.) [N]	-7.78 (0.85) [16]	-11.65 (0.96) [14]	-10.85 (0.83) [16]	ES (95% CI)	+0.91 (+0.18 to +1.64)	-0.23 (-0.95 to +0.49)	+1.11 (+0.33 to +1.88)	t	+2.60	-0.63	+3.03	F	4.09
									df	215	215	215	df	2, 215
									P-value	0.010	0.528	0.003	P-value	0.018
All subjects with 5 baseline biomarkers (N = 155)	LS-mean (s.e.m.) [N]	-10.14 (0.57) [52]	-9.61 (0.57) [51]	-9.79 (0.55) [52]	ES (95% CI)	-0.09 (-0.47 to +0.30)	+0.04 (-0.34 to +0.43)	-0.13 (-0.52 to +0.26)	t	-0.44	+0.22	-0.65	F	0.17
									df	716	716	716	df	2, 716
									P-value	0.661	0.823	0.513	P-value	0.840

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ES, effect size; HAM-D-17, 17-item Hamilton Depression Rating Scale; LS, least squares; MMRM, mixed model repeated measures analysis; PLA, placebo. <sup>a</sup>HAM-D-17 was administered at baseline and at 2-week intervals during the 8-week study. MMRM analyses were performed on change from baseline to week 8 for subsets of (N = 155) evaluable subjects with all five biomarkers present at baseline, testing the significance of effects of treatment, time and treatment-by-time interaction, covarying for the baseline HAM-D-17 score. <sup>b</sup>By Cohen's *d* effect size: difference between LS-mean change/pooled s.d. for each pair of treatments (s.d. per group computed from s.e.m. of LS-mean from MMRM). A negative effect size indicates that the first group (in the comparison pair) improved more than the second one (had a larger negative LS-mean change). <sup>c</sup>Change at 8 weeks is not significantly different from zero; all other means are significantly different from zero, at  $P < 0.02$  to  $P < 0.0001$  (shown in italics). <sup>d</sup>Degrees of freedom were determined using the Satterthwaite approximation method.

**Table 3.** Change in HAM-D-17 total score from baseline to treatment week 8 for subjects treated with EPA vs PLA with high inflammation on individual biomarkers and pairs of biomarkers at baseline<sup>a</sup>

High inflammatory status on	Change from baseline to treatment week 8						Standardized treatment effect size at treatment week 8		EPA vs PLA at treatment week 8		Significance of treatment-by-time interaction			
	EPA			PLA			ES <sup>b</sup>	95% CI	t	df <sup>c</sup>	P-value	F	df	P-value
Individual biomarkers	LS-mean	s.e.m.	N	LS-mean	s.e.m.	N								
hs-CRP	-12.44	1.54	15	-7.99	1.86	8	-0.78	-1.67 to +0.11	-1.84	42.8	<i>0.073</i>	1.70	1, 90.6	0.195
IL-6	-9.78	1.44	15	-7.84	1.40	12	-0.37	-1.13 to +0.40	-0.96	54.0	0.341	0.42	1, 104	0.518
IL-1ra	-12.14	1.16	23	-9.63	1.61	11	-0.46	-1.18 to +0.27	-1.26	72.1	0.213	0.31	1, 127	0.580
Leptin	-8.99	1.29	19	-5.61	1.46	13	-0.62	-1.34 to +0.11	-1.72	63.3	0.090	1.01	1, 101	0.318
Adiponectin	-11.69	1.10	21	-8.81	1.06	21	-0.58	-1.20 to +0.04	-1.88	94.9	0.063	1.69	1, 159	0.195
<i>10 Pairs of biomarkers</i>														
hs-CRP+IL-6	-12.00	1.68	10	-4.44	2.30	4	-1.47	-2.77 to -0.17	-2.65	22.2	<i>0.015</i>	4.13	1, 51.9	<i>0.047</i>
hs-CRP+IL-1ra	-12.74	1.60	13	-7.05	3.66	2	-0.99	-2.53 to +0.54	-1.42	25.1	0.167	1.18	1, 54.2	0.281
hs-CRP+leptin	-10.56	1.91	12	-6.30	2.52	5	-0.67	-1.74 to +0.40	-1.34	28.3	0.192	0.87	1, 64.6	0.354
hs-CRP+adiponectin	-12.12	1.70	9	-3.56	2.33	4	-1.72	-3.10 to -0.34	-2.92	18.9	<i>0.009</i>	3.66	1, 47.5	0.062
IL-6+IL-1ra	-11.53	1.98	9	-7.41	2.21	6	-0.72	-1.79 to +0.35	-1.35	23.7	0.189	0.68	1, 56.1	0.415
IL-6+leptin	-10.15	1.63	12	-3.15	1.96	6	-1.30	-2.38 to -0.22	-2.74	32.1	<i>0.010</i>	3.94	1, 65.3	0.051
IL-6+adiponectin	-10.27	1.68	11	-7.46	2.03	6	-0.52	-1.53 to +0.49	-1.06	27.7	0.300	0.28	1, 65.7	0.597
IL-1ra+leptin	-10.22	1.66	14	-5.80	2.54	5	-0.73	-1.78 to +0.32	-1.43	32.1	0.162	0.60	1, 71.4	0.441
IL-1ra+adiponectin	-12.99	1.44	12	-10.44	2.33	4	-0.52	-1.67 to +0.63	-0.92	28.1	0.367	0.00	1, 52.9	0.954
Leptin+adiponectin	-9.88	1.27	15	-5.42	1.86	6	-0.92	-1.92 to +0.07	-1.99	38.2	0.054	1.22	1, 72.1	0.274

Abbreviations: CI, confidence interval; EPA, eicosapentaenoic acid; ES, effect size; HAM-D-17, 17-item Hamilton Depression Rating Scale; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; LS, least squares; MMRM, mixed model repeated measures analysis; PLA, placebo. <sup>a</sup>HAM-D-17 was administered at baseline and at 2-week intervals during the 8-week study. MMRM analyses were performed on change from baseline to week 8 for subsets of ( $N=155$ ) evaluable subjects with all five biomarkers present at baseline, testing the significance of effects of treatment, time and treatment-by-time interaction, covarying for the baseline HAM-D-17 score. <sup>b</sup>By Cohen's  $d$  effect size: difference between LS-mean change/pooled s.d. for each pair of treatments (s.d. per group computed from s.e.m. of LS-mean from MMRM). A negative effect size indicates that the EPA group improved more than the PLA group (had a larger negative LS-mean change). <sup>c</sup>Degrees of freedom were determined using the Satterthwaite approximation method.  $P$  values in italics are  $<0.05$ .

inflammation except that there were more 'high' IL-1ra subjects in the EPA group (Supplementary Table 1). There were no site differences. One third of each treatment group fell into the obese BMI category and another third into the overweight category, regardless of sex.

The prevalence of 'high' inflammation in the total sample ranged from 24% for hs-CRP to 43% for adiponectin (Table 1), with rates varying by BMI and sex. In obese subjects, the prevalence of 'high' inflammatory markers ranged from 48% for hs-CRP to 73% for leptin. Approximately, 90% of obese subjects had at least one biomarker in the high range. Obese women had a greater prevalence of high hs-CRP, IL-6 and IL-1ra biomarkers than obese men, and were more than twice as likely to have 4–5 biomarkers of high inflammation than obese men. Spearman's correlation scores among the biomarkers were higher for female than for male subjects (Supplementary Table 2).

Classifying the treatment sample by the number of 'high' biomarkers of inflammation led to important observations about treatment response (Table 2). Although overall treatment group differences for the entire sample were negligible ( $ES=-0.13$  to  $+0.04$ ), subjects with one or more 'high' biomarker of inflammation improved more on EPA than placebo ( $ES=-0.39$ ) or DHA ( $ES=-0.60$ ) and less on DHA than placebo ( $ES=+0.21$ ). Subjects randomized to EPA treatment with one or more 'high' biomarkers consistently had a  $>11$ -point decrease in HAM-D-17 scores by treatment week 8, while subjects randomized to placebo treatment were progressively less responsive as the number of high biomarkers of inflammation increased, resulting in an increasing EPA-placebo gradient of separation (from  $ES=-0.20$  associated with one marker of 'high' inflammation, to  $ES=-0.59$  for 2–3 'high' markers, to  $ES=-1.10$  for the subjects with 4–5 markers of 'high' inflammation). Conversely, subjects without any 'high' marker of inflammation were less responsive to EPA than to placebo

or DHA ( $ES=+0.91$ ). Most placebo-responding subjects fell into this low inflammation group (Supplementary Table 3). Remission and response rates were consistent with our continuous data: among subjects with 4–5 markers of 'high' inflammation, remission rates were 40% for EPA, 14% for DHA and 25% for placebo, while remission rates for subjects without any markers of 'high' inflammation were: 19% for EPA, 43% for DHA and 44% for placebo. Response rates followed a similar pattern. Inflammatory biomarkers did not consistently differentiate DHA from placebo in any analysis.

Table 3 indicates a benefit to employing a combination of biomarkers to define the 'high' inflammation group. EPA vs placebo ESs for subjects with high inflammation on individual biomarkers ranged from  $-0.368$  to  $-0.775$ . By contrast, EPA vs placebo ESs for subjects with 'high' inflammatory status on 5 of the 10 possible pairs of biomarkers were  $-0.924$  or higher. Three pairs (hs-CRP plus IL-6, hs-CRP plus adiponectin and IL-6 plus leptin) had ESs between  $-1.297$  and  $-1.718$ .

Table 4 describes the impact of 'high' vs 'low' levels of individual inflammatory biomarkers on treatment response for the EPA and placebo groups. Subjects treated with EPA who were categorized as 'high' on hs-CRP, IL-1ra or 'low' on adiponectin demonstrated a moderate ES for differences in HAM-D-17 score improvement compared to subjects who were 'low' on these biomarkers. For placebo-treated subjects (bottom of Table 4), being classified as 'high' for hs-CRP or IL-6 (moderate ES difference) or leptin (large ES difference) was associated with a decreased response to placebo when compared to subjects who were classified as being low on hs-CRP, IL-6 or leptin.

## DISCUSSION

In this proof-of-concept study, we identified an EPA-responsive subgroup of subjects with MDD, based on biomarkers of

**Table 4.** Change in HAM-D-17 score from baseline to treatment week 8 by high vs low inflammation status on each of five biomarkers at baseline for subjects treated with EPA or placebo, based on MMRM analysis<sup>a</sup>

Treatment group and inflammatory marker	Change from baseline to treatment week 8		High vs low inflammation at treatment week 8 (95% CI)	High vs low inflammation at treatment week 8	Significance of high/low-by-time interaction
	High inflammation	Low inflammation			
<b>EPA</b>					
hs-CRP	LS-mean (s.e.m.) [N]	-9.50 (0.78) [37]	-0.497 (-1.105 to +0.111)	t = -1.55; df = 125; P = 0.124	F = 2.04; df = 1, 188; P = 0.155
IL-6	LS-mean (s.e.m.) [N]	-10.17 (0.78) [37]	+0.04 (-0.556 to +0.644)	t = +0.17; df = 127; P = 0.869	F = 0.01; df = 1, 189; P = 0.913
IL-1ra	LS-mean (s.e.m.) [N]	-8.72 (0.89) [29]	-0.667 (-1.230 to -0.104)	t = -2.39; df = 123; P = 0.018	F = 3.10; df = 1, 188; P = 0.080
Leptin	LS-mean (s.e.m.) [N]	-10.50 (0.83) [33]	+0.246 (-0.320 to +0.813)	t = +0.82; df = 121; P = 0.412	F = 0.23; df = 1, 187; P = 0.633
Adiponectin	LS-mean (s.e.m.) [N]	-8.86 (0.87) [31]	-0.633 (-1.201 to -0.065)	t = -2.24; df = 125; P = 0.027	F = 2.71; df = 1, 189; P = 0.102
<b>Placebo</b>					
hs-CRP	LS-mean (s.e.m.) [N]	-10.18 (0.71) [44]	+0.458 (-0.301 to +1.217)	t = +1.25; df = 122; P = 0.215	F = 0.24; df = 1, 185; P = 0.626
IL-6	LS-mean (s.e.m.) [N]	-10.37 (0.74) [40]	+0.490 (-0.163 to +1.142)	t = +1.50; df = 126; P = 0.136	F = 0.91; df = 1, 188; P = 0.342
IL-1ra	LS-mean (s.e.m.) [N]	-9.82 (0.74) [41]	+0.002 (-0.663 to +0.667)	t = +0.01; df = 122; P = 0.996	F = 0.03; df = 1, 186; P = 0.865
Leptin	LS-mean (s.e.m.) [N]	-10.91 (0.72) [39]	+1.042 (+0.382 to +1.702)	t = +3.20; df = 129; P = 0.002	F = 4.71; df = 1, 185; P = 0.031
Adiponectin	LS-mean (s.e.m.) [N]	-10.55 (0.85) [31]	+0.379 (-0.179 to +0.938)	t = +1.35; df = 124; P = 0.181	F = 1.13; df = 1, 187; P = 0.289

Abbreviations: CI, confidence interval; EPA, eicosapentaenoic acid; HAM-D-17, 17-item Hamilton Depression Rating Scale; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; LS, least squares; MMRM, mixed model repeated measures analysis. <sup>a</sup>MMRM analyses were performed on change from Baseline to Week 8 for subsets of (N = 155) evaluable subjects with all five biomarkers present at baseline, testing the significance of effects of high/low inflammatory status, time and high/low-by-time interaction, covarying for the baseline HAM-D-17 score. <sup>b</sup>By Cohen's d effect size: difference between LS-mean change/pooled s.d. for each pair of treatments (s.d. per group computed from s.e.m. of LS-mean from MMRM). A negative effect size indicates that the group with high inflammation improved more than the low inflammation group (had a larger negative LS-mean change). P values in italics are < 0.05.

inflammation. Subjects identified as being 'high' on any of the five biomarkers that we measured were more likely to respond to EPA than placebo (Table 3). Individuals categorized as high on two or more biomarkers of inflammation demonstrated even greater EPA-placebo separation of HAM-D-17 scores (Tables 2 and 3). To explore the reasons for this EPA-placebo separation we asked two questions:<sup>1</sup> Are elevations in specific biomarkers associated with a greater likelihood of response to EPA?<sup>2</sup> Are elevations in specific biomarkers associated with less response to placebo? We demonstrate that subjects categorized as 'high' on IL-1ra or hs-CRP or 'low' on adiponectin (which reflects high inflammation) have a greater decrease in HAM-D-17 scores in response to EPA than subjects categorized as having 'low' inflammation on these biomarkers (ES: -0.667, -0.497 and -0.633, respectively; Table 4). Conversely, subjects categorized as 'high' on hs-CRP, IL-6 or leptin were less responsive to placebo than subjects categorized as 'low' on these biomarkers (ES: +0.458, +0.490 and +1.042, respectively). The latter observation extends a secondary analysis by Raison *et al.*<sup>14</sup> Our preliminary findings suggest that employing an inflammatory biomarker panel in future studies might identify a more homogenous group of subjects responsive to EPA and less responsive to placebo.

Our findings could explain contradictory data about the efficacy of n-3 therapy for subjects with MDD.<sup>25-27</sup> If EPA supplementation only benefits MDD subjects with inflammation as part of their syndrome,<sup>38</sup> then there can be only a small ES improvement in depression ratings associated with EPA therapy for a heterogeneous cohort of subjects with MDD, since it is ineffective for the majority of subjects randomized to EPA treatment. Furthermore, if the antidepressant mechanism of action for EPA is complementary to traditional antidepressant therapy, this would explain why there is increased therapeutic response associated with the combination therapy of traditional antidepressants with EPA.

A possible explanation for the greater effectiveness of EPA over DHA for individuals with high inflammation may be that these 2 n-3's differ in their influence on the inflammatory cascade. EPA, but not DHA, can decrease the production of arachidonic acid by inhibiting delta-5-desaturase activity, compete with AA as a substrate for phospholipase A2, and can be converted into anti-inflammatory prostaglandins and leukotrienes,<sup>39</sup> thus explaining its potentially unique antidepressant properties when contrasted with DHA.

An intriguing finding was that EPA was less effective than placebo or DHA for subjects 'low' on all five biomarkers (Table 2). This agrees with Raison *et al.*<sup>14</sup> who reported that subjects with 'low' hs-CRP levels did worse with infliximab treatment than placebo. We believe that these results support the proposition that anti-inflammatory therapy is only beneficial as a treatment for inflammation-driven MDD and is ineffective and potentially harmful for individuals whose MDD is due to a different physiological disturbance. Such a postulate explains the Warner-Schmidt *et al.*<sup>40</sup> report that anti-inflammatory agents attenuated the biochemical and behavioral response to SSRIs in a mouse model of MDD and the behavioral response of SSRIs in humans. If one extends this postulate to antidepressant therapies in general, it explains why Gueorguieva *et al.*<sup>41</sup> observed that 24% of duloxetine-treated subjects became worse over the course of the duloxetine trials. These data highlight the need for targeted therapies to avoid ineffective treatment and unnecessary exposure to side effects.

Subjects' sex and weight strongly influenced biomarkers in this study. Since women have higher circulating levels of leptin and adiponectin than men, we analyzed our data separately for these biomarkers.<sup>35,36</sup> Ninety-three percent of the obese women and 89.5% of the obese men had at least one high marker of inflammation, while 86% of the obese women and 74% of the obese men had two or more high markers of inflammation. (The adipokines were the most consistently positive markers of

inflammation in this cohort). Obese women were more likely to have 4–5 elevated markers of inflammation than obese men (Table 1). Less than a third of the obese men had elevations in hs-CRP, IL-6 or IL-1ra, while ~60% or more of the obese women had elevations in these markers. In addition, the five biomarkers were more highly intercorrelated for female subjects than for male subjects (Supplementary Table 2). However, men and women did not differ in treatment-response patterns based on inflammatory biomarkers. Our findings are consistent with the literature suggesting that obesity is associated with chronic inflammation, and that a significant number of MDD patients with elevated biomarkers of inflammation are obese.<sup>42–45</sup> Future studies should explore the relationship between weight, sex, biomarkers of inflammation and other variables of interest such as the hypothalamic-pituitary-adrenal (HPA) axis and gonadal hormones.

Our choice of IL-6, IL-1ra and hs-CRP as biomarkers of inflammation was based on a review of the psychiatric and autoimmune literature investigating inflammatory biomarkers, the stability of the biomarkers in plasma and the availability of reliable assay systems. We measured adipokines because:<sup>1</sup> there is a relationship between inflammation, MDD and obesity;<sup>2</sup> leptin stimulates the production of CRP and CRP binds and modifies the actions of leptin;<sup>46,3</sup> leptin and adiponectin are complementary biomarkers of adipocyte function; and<sup>4</sup> at least with respect to metabolic function, adiponectin is a marker of anti-inflammatory activity.<sup>35,36</sup> We thought it prudent to include at least one anti-inflammatory marker in our panel. Each of the inflammatory biomarkers in this study played a role in enhancing response to EPA and/or decreasing response to placebo. Further investigation is needed to elucidate the complementary effects of these and other inflammatory biomarkers.

As with any proof-of-concept study, there are limitations. Despite a sample size of 155 subjects, the number of subjects with high biomarkers of inflammation is relatively small for a three-arm study. Our analysis of the relationship between the number and combinations of 'high' inflammatory markers and treatment response is exploratory and needs replication. However, our results strongly suggest that it is important for psychiatry to go beyond measuring a single marker of inflammation and assuming it is sufficient for determining the presence of an inflammatory process. Another unique characteristic of this study is that we employed stem-and-leaf plots to determine cutoff points for categorizing subjects as 'high' on an inflammatory marker. We did so because our biomarker data were not normally distributed and could not be transformed to meet assumptions for parametric analyses. Although one might criticize this approach as 'arbitrary', our cutoff point of >3 for hs-CRP determined by this method matches the established cutoff point for an elevated CRP by the CDC and American Heart Association.<sup>34</sup> Further investigations employing the cut points we have identified and similar assay methodology are warranted.

In conclusion, this proof-of-concept study employed biomarkers of inflammation to identify a subset of patients who were responsive to EPA monotherapy. We found that subjects with specific combinations of inflammatory markers were more likely to respond to EPA treatment and less likely to respond to placebo treatment. Our preliminary data agree with others who have suggested that obese subjects with MDD are more likely to have 'high' inflammatory biomarkers. In future studies, we hope to replicate our preliminary findings and extend them by investigating the influence of other important biological measures (for example, *n-3/n-6* ratios, HPA and estrogen and other inflammatory markers) and clinical characteristics (for example, early life trauma and current life stress levels). Biologically classifying patients may pave the way for individualized depression treatment and may inform future study designs.

## DISCLAIMER

The NIMH and Nordic Naturals had no further role in the study design, collection, analysis and interpretation of data, writing of the report or the decision to submit the paper for publication.

## CONFLICT OF INTEREST

DM has received research support from the Bowman Family Foundation, Fisher Wallace, Nordic Naturals, Methylation Sciences (MSI) and PharmsRx. He has received honoraria for speaking from Pamlab, and the Massachusetts General Hospital Psychiatry Academy. He has received royalties from Lippincott Williams & Wilkins for the book 'Natural Medications for Psychiatric Disorders: Considering the Alternatives'. AAN has served as a consultant to: Appliance Computing (Mindsite), Brain Cells, Brandeis University, Bristol-Myers Squibb, Clintara, Dainippon Sumitomo (now Sunovion), Eli Lilly and Company, EpiQ, Forest, Novartis, PamLabs, PGx Health, Shire, Schering-Plough, Sunovion, Takeda Pharmaceuticals, Teva and Targacept. He has consulted through the MGH Clinical Trials Network and Institute (CTNI): Astra Zeneca, Brain Cells, Dainippon Sumitomo/Sepracor, Johnson and Johnson, Labopharm, Merck, Methylation Sciences, Novartis, PGx Health, Shire, Schering-Plough, Targacept and Takeda/Lundbeck Pharmaceuticals. AAN received honoraria or travel expenses including CME activities from: APSARD, Belvoir Publishing, Boston Center for the Arts, University of Texas Southwestern Dallas, Hillside Hospital, American Drug Utilization Review, American Society for Clinical Psychopharmacology, Bayamon Region Psychiatric Society, San Juan, PR, Baystate Medical Center, Canadian Psychiatric Association, Columbia University, Douglas Hospital/McGill University, IMEDEX, International Society for Bipolar Disorders, Israel Society for Biological Psychiatry, John Hopkins University, MJ Consulting, New York State, Massachusetts Association of College Counselors, Medscape, MBL Publishing, Physicians Postgraduate Press, Ryan Licht Sang Foundation, Slack Publishing, SUNY Buffalo, University of Florida, University of Miami, University of Wisconsin, University of Pisa and SciMed. AAN is a presenter for the Massachusetts General Hospital Psychiatry Academy (MGHPA). The education programs conducted by the MGHPA were supported through Independent Medical Education (IME) grants from the following pharmaceutical companies in 2008: Astra Zeneca, Eli Lilly and Janssen Pharmaceuticals; in 2009 Astra Zeneca, Eli Lilly and Bristol-Myers Squibb. No speaker bureaus or boards since 2003. AAN owns stock options in Appliance Computing and Brain Cells. Additional income is possible from Infomedic.com depending on overall revenues of the company, but no revenue has been received to date. Through MGH, AAN is named for copyrights to: the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery-Asberg Depression Scale exclusively licensed to the MGH Clinical Trials Network and Institute (CTNI). AAN has received grant/research support through MGH from AHRQ, Cephalon, Forest, Mylan, NIMH, PamLabs, Pfizer Pharmaceuticals, Takeda, Elan and Shire. PJS works part-time both as Senior Research Associate in the Department of Psychiatry and Behavioral Sciences at the Emory University School of Medicine, Atlanta, Georgia; as well as Principal Statistician in the Department of Psychiatry of the School of Medicine at the University of California, San Diego. She has no other direct or indirect affiliations or financial interests in connection with the contents of this paper. MHR has provided consulting services to PAX (unpaid) and has been funded by the NIH. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)