ARTICLE

The metabolome-wide signature of major depressive disorder

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Major Depressive Disorder (MDD) is a common, frequently chronic condition characterized by substantial molecular alterations and pathway dysregulations. Single metabolite and targeted metabolomics platforms have revealed several metabolic alterations in depression, including energy metabolism, neurotransmission, and lipid metabolism. More comprehensive coverage of the metabolome is needed to further specify metabolic dysregulations in depression and reveal previously untargeted mechanisms. Here, we measured 820 metabolites using the metabolome-wide Metabolon platform in 2770 subjects from a large Dutch clinical cohort with extensive clinical phenotyping (1101 current MDD, 868 remitted MDD, 801 healthy controls) at baseline, which were repeated in 1805 subjects at 6-year follow up (327 current MDD, 1045 remitted MDD, 433 healthy controls). MDD diagnosis was based on DSM-IV psychiatric interviews. Depression severity was measured with the Inventory of Depressive Symptomatology Self-report. Associations between metabolites and MDD status and depression severity were assessed at baseline and at 6-year follow-up. At baseline, 139 and 126 metabolites were associated with current MDD status and depression severity, respectively, with 79 overlapping metabolites. Adding body mass index and lipid-lowering medication to the models changed results only marginally. Among the overlapping metabolites, 34 were confirmed in internal replication analyses using 6-year follow-up data. Downregulated metabolites were enriched with long-chain monounsaturated ($P = 6.7e{-}07$) and saturated ($P = 3.2e{-}05$) fatty acids; upregulated metabolites were enriched with lysophospholipids ($P = 3.4e{-}4$). Mendelian randomization analyses using genetic instruments for metabolites ($N = 14{,}000$) and MDD ($N = 800{,}000$) showed that genetically predicted higher levels of the lysophospholipid 1-linoleoyl-GPE (18:2) were associated with greater risk of depression. The identified metabolome-wide profile of depression indicated altered lipid metabolism with downregulation of long-chain fatty acids and upregulation of lysophospholipids, for which causal involvement was suggested using genetic tools. This metabolomics signature offers a window on depression pathophysiology and a potential access point for the development of novel therapeutic approaches.

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INTRODUCTION

Major Depressive Disorder (MDD) is a multifactorial disorder with high disease burden and chronicity in many patients. The pathophysiology of MDD is complex, with substantial molecular alterations and dysregulations of multiple pathways. Metabolomic technologies capturing simultaneously hundreds of molecules may provide a comprehensive assessment of depression pathophysiology. A review [1] summarizing metabolomic analyses in depressed patients using urine, cerebrospinal fluid, and blood samples showed that metabolites implicated in energy metabolism and neuronal integrity and transmission were altered. In a large-scale pooled analysis using the lipidomics Nightingale platform in 10,145 controls and 5283 depressed cases [2], we identified a metabolic profile (21 metabolites) for lifetime depression characterized by a shift towards less high-density lipoprotein (HDL) and more triglycerides and glycoprotein acetyls. These findings were replicated in the recent UK Biobank analysis using the same platform [3]. Such findings not only indicate pathway dysregulations that contribute to depression symptomatology development- but also help explain why comorbidities like metabolic syndrome [4], obesity [5], diabetes [6]) and cardiovascular disease [7] occur more often in depressed than non-depressed persons.

While targeted metabolomics platforms are limited by design and often overrepresented by a certain class of metabolites (like lipids on the Nightingale platform), untargeted platforms cover a larger portion of the metabolome and have the potential to uncover previously unrecognized pathobiological mechanisms. In a population-based study, the untargeted Metabolon platform was used to measure 353 unique metabolites in serum of

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1411 subjects [8]. Participants with elevated depressive symptoms as assessed with a self-report scale ($N = 72$) had decreased levels of two acylcarnitines involved in mitochondrial fatty acid oxidation. A larger pooled analysis of population-based cohorts [9] showed that self-reported symptoms of depression were associated with 8 metabolites directly derived from food or products of host/microbial food-derived products. Nevertheless, previous studies did not evaluate patients formally diagnosed with MDD using psychiatric interviews and therefore likely included relatively few participants with MDD. Furthermore, prior studies assessed metabolites only at a unique time-point, thus precluding the possibility of replicating the metabolite-depression associations.

The associations between metabolite concentrations and depression identified in observational studies may emerge from different causal pathways: 1) shared factors (e.g., lifestyle, medication use) impacting both metabolite levels and presence of depression; 2) reverse causation of subclinical depression impacting on metabolite levels via behavioral changes (e.g., reduction in physical activity, worsening of dietary habit); and 3) direct causal action of metabolite alterations on depression pathophysiological pathways. Mendelian Randomization (MR), a causal inference technique leveraging genetic variants as instruments for which random allele segregation and independent assortment limits confounding and reverse causality, can help to disentangle causes and consequences in the metabolite-depression associations. For instance, in previous MR studies, we showed that alterations in inflammatory pathways [10] and acylcarnitine metabolism [11] may have a potential causal role for the development of depression, while this was not supported for omega-3 fatty acids [12].

In the current study, we measured 820 metabolites using the Metabolon platform in subjects from a large Dutch clinical cohort ($N = 2770$) with extensive clinical phenotyping conducted at baseline and at 6-year follow up. The unprecedented sample size, broad metabolite spectrum, and longitudinal data provides a reliable, state-of the-art metabolite signature of depression. Furthermore, we applied MR analyses to examine the nature of metabolite-depression associations leveraging results from the most recent and largest genome wide association studies (GWAS) on metabolites from the Metabolon platform [13] ($N \sim 14{,}000$) and depression [14] ($N \sim 800{,}000$).

METHODS

Study design and participants

Data were from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study examining course and consequences of depressive and anxiety disorders. The NESDA baseline sample consists of 2,981 persons between 18- and 65-years, including persons with a current or remitted diagnosis of a depressive and/or anxiety disorder (74%) and healthy controls (26%). Individuals were recruited from mental health care settings, general practitioners, and the general population in the period from September 2004 to February 2007. Persons with insufficient command of the Dutch language or a primary clinical diagnosis of another severe mental disorder, such as severe substance use disorder, or a psychotic disorder, were excluded. Participants were assessed during a 4-hour clinical visit consisting of the collection of all somatic and mental health determinants in the current study as well as a fasting blood draw. Similar face-to-face assessments were repeated after two, four, six and nine years [15]. The current study uses metabolomics data from 2770 participants (2463 from NESDA baseline and 307 siblings newly recruited at the 9-year follow up, pooled as discovery cohort) and 1805 participants of the 6-year follow-up used for internal replication analyses(of which 1685 overlapped with baseline participants). Persons with an anxiety disorder without MDD were excluded from the analyses. The NESDA study was approved by the Ethical Review Boards of participating centers, and all participants signed a written informed consent form. More than 94% of the NESDA participants were from North European origin. The population and methods of the NESDA study have been described in more detail elsewhere [15, 16].

Metabolite measures

After an overnight fast, EDTA plasma samples were collected and stored in aliquots at −80 °C until further analysis. Samples were sent in two shipments for metabolic profiling using the untargeted platform from Metabolon Inc (Durham, NC). Briefly, plasma samples were divided into four fractions; two for ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; positive ionization), one for UPLC-MS/MS (negative ionization), and one for a UPLC-MS/MS polar platform (negative ionization). Peaks were quantified using the area-under-the-curve in the spectra. To account for run-day variations, peak abundances were normalized by their respective run-day medians. Compounds were identified using an internal spectral database.

Metabolite QC

The raw metabolite data set included measures of 5181 samples and 1008 reference samples (well-characterized human plasma samples) which were used to calculate and control for technical measurement variability among a total of 1367 metabolites measured in 29 batches. One experimental sample and one reference sample with a high missingness (>5 SD + mean missingness) were excluded from the dataset. We further excluded metabolites with a missingness in over 30% of all samples. If we observed outliers or apparent measurement issues within one plate, or across several plates within one batch, all values on that plate were set to 'NA' to not affect subsequent batch correction. We batch corrected data by normalizing all samples to the batch median and then excluded those metabolites that still had a technical measurement variability of >30%. Next, we imputed missing metabolite measures. Before imputation, we tested if missingness in any of the remaining 820 metabolites accumulated in one of the three measurement waves (baseline, 6- or 9-year follow up) using a Fisher's exact test. As this was not the case, we jointly imputed all waves using a k-nearest neighbor approach ($k = 10$) [17]. Before statistical analysis, we log2 transformed the final dataset. For each metabolite, outliers larger than the mean plus five standard deviations were set to the mean plus five standard deviations. Outliers smaller than the mean minus five standard deviations were set to the mean minus five standard deviations.

Clinical assessment and covariates

Presence of current MDD (i.e.within six months prior to the interview) was assessed using the DSM-IV Composite International Diagnostic Interview (CIDI) version 2.1. Depression severity levels in the week prior to assessment were measured with the 28-item Inventory of Depressive Symptomatology (IDS) self-report [18]. Several covariates were included in the models assessing the associations between metabolites and depression. Alcohol consumption was assessed as units per week and smoking status was coded as current, ex- and never-smokers. Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) [19] and expressed as overall energy expenditure in Metabolic Equivalent Total (MET) minutes per week (MET level * minutes of activity * events per week). Body mass index (BMI) was calculated as measured weight divided by height squared. The number of self-reported current somatic diseases for which participants received medical treatment was counted. We used somatic disease categories as categorized previously [20, 21]: cardiometabolic, respiratory, musculoskeletal, digestive, neurological and endocrine diseases, and cancer. Educational level was measured as years of education. Medication use was based on medication container inspection of all medications used in the past month, classified according to the World Health Organization Anatomical Therapeutic Chemical classification, and included the antidepressant classes of selective serotonin reuptake inhibitors (SSRI, ATC code N06AB), serotonin–norepinephrine reuptake inhibitors (SNRI, N06AX16, N06AX21), tricyclic antidepressants (N06AA) and tetracyclic antidepressants (TCA, N06AX03, N06AX05 and N06AX11) An earlier large scale drug-metabolite study [22] identified three class of commonly prescribed drugs associated with widespread metabolite alterations: lipid lowering, anti-hypertensive and anti-diabetics medications. However, while the association with anti-diabetics were mainly driven by the disease of indication, the associations with anti-hypertensive medications were mainly driven by co-medication, in particular statin. Therefore we only considered the use of lipid-lowering drugs (ATC code C10) in the present study.

Instrument selection for Mendelian randomization (MR)

Summary statistics for metabolites measured with the same untargeted mass spectrometry-based platform (Metabolon HD4) were retrieved from a GWAS including up to ~14,000 samples [13] (interrogation of the GWAS results can be performed at www.omicscience.org). The Psychiatric Genomics Consortium performed an overarching meta-analysis [14] of all available GWAS datasets with depression phenotypes including established MDD diagnosis or self-declared depression, totaling 246,363 cases and 561,190 controls. Lack of sample overlap across the two discovery GWAS reduced the likelihood of MR estimates being biased toward the observational correlation [23]. Metabolites' GWAS summary statistics were processed by removing non-SNP variants, strand-ambiguous SNPs and those with MAF < 1%. Variants overlapping and allele-matching with those reported in depression GWAS were clumped (10,000 kb window, $r^2 = 0.001$, EUR population of 1000Genomes used as linkage disequilibrium reference) to identify significantly associated ($p < 5.0e{-}8$) independent SNPs.

Statistical analyses

For each of the 820 metabolites measured at the NESDA baseline wave, we ran a GEE model with the metabolite concentration as the dependent variable, and MDD status or depression severity (total IDS) as the independent variable, while correcting for education, sex, age, physical activity, smoking status, alcohol use, number of chronic diseases, and shipment. Family ID was used for clustering in the GEE models. MDD status was coded as a 3-level factor (controls, remitted MDD, current MDD), using controls as reference. In additional analyses BMI and lipid-lowering drugs use (yes/no) were added to the model to check their potential explanatory role in the metabolite-depression association.

Subsequently, for metabolites associated with current MDD and depression severity, pathway enrichment analysis was conducted using pathways pre-assigned to the metabolites by Metabolon (see sub pathways and super pathways at Supplementary Table S1). Two classes of pathways were assigned, 10 super pathways and 95 sub pathways. As only 681 metabolites were classified in one of the pathways, this set was used as the reference in the enrichment analysis. Enrichment between significant metabolites and each pathway was computed by applying the Fisher exact test to the contingency table.

In observational studies, it is difficult to clearly disentangle the potential pharmacophysiological impact of antidepressant medication from the effect of depression severity: medication use may indeed merely tag the most severe cases, most likely representing the clinical indication for treatment (confounding by indication). In this scenario, simple statistical adjustment for antidepressant use may represent an over-adjustment for depression severity. Thus, we performed different analyses to examine the potential impact of antidepressants. Firstly, SSRI use (yes/no), TCA use (yes/no) and SNRI use (yes/no) were added as covariates to the model. Furthermore, effects of antidepressants (AD) were verified by computing the associations between MDD status and metabolite concentrations while removing the antidepressant users. To further compare effects associated with antidepressant use and those associated with MDD status, SSRI, TCA or SNRI use were used as predictor in the initial model but without MDD status.

In order to perform a conservative selection of the metabolites more reliably associated with depression to be carried forward for internal replications and MR we performed the following step-wise selection (Supplementary Fig. S1):

1. Metabolites significantly associated with MDD status and depression severity (applying FDR < 0.05 for both outcomes) were selected;
2. Metabolites with betas for the association with AD use that were >2 times higher than the betas for the association with MDD status were removed;
3. For the remaining metabolites, internal replication was checked by analyzing associations with depression outcomes using the 6-year follow up data (as this is done in a subset of the baseline sample, it cannot be considered a fully independent replication). Metabolites were selected and carried forward for MR analysis if nominal *P*-values in the follow-up data for MDD status or depression severity were smaller than 0.05, and directions of effect were consistent. In the 6-year follow-up sample no family relations were present so instead of GEE, linear models were used, with the same initial covariates now assessed at the 6-year follow-up.

Mendelian randomization

For the metabolites identified in baseline data and internally replicated at the follow up, and for which at least two independent associated SNPs were found in the corresponding GWAS, two-sample Mendelian randomization (2SMR) analyses based on GWAS summary statistics were performed to test the potential causal role of metabolites on lifetime depression risk. For each metabolite, genome-wide significant independent SNPs used as instruments were aligned on the positive strand for exposures (metabolites) and outcome (MDD status). 2SMR analyses were performed based on the inverse variance weighted estimator [24]. False discovery rate (FDR) *q*-values according to Benjamini-Hochberg procedure were calculated taking into account multiple testing. The robustness of significant results was tested in sensitivity analyses based on weighted median [25] and MR-Egger [26] estimators. Furthermore, heterogeneity among the included SNPs was tested via Cochran's Q test, single SNP, and leave-one-out SNP analyses. The presence of potential horizontal pleiotropy (a genetic instrument for exposure influencing the outcome by mechanisms other than exposure) was tested using the MR-Egger intercept [27] and the MR-PRESSO method [28]. Analyses were conducted using the R MR-Base package [29].

RESULTS

Demographics

Metabolites from the Metabolon platform ($N = 820$) were measured in whole blood samples from 2770 participants (2463 recruited at NESDA baseline and 307 siblings recruited at 9-year follow up). Participants had current MDD ($N = 1101$), remitted MDD ($N = 868$) or were healthy controls ($N = 801$), were 65% female, had a mean age of 43 years (sd 13) and a mean BMI of 26 (sd 5, Table 1). The three MDD status groups did not differ in the proportion of subjects using lipid-modifying drugs (Supplementary Table S2).

Table 1. Sample characteristics at baseline ($N = 2770$) and at 6-year follow-up ($N = 1802$).

	Baseline	6-year follow up
Cohort size	$n = 2770$	$n = 1802$
Sex (% women)	65%	66%
Age (mean years, SD)	43.05 (13.33)	48.21 (12.99)
BMI ((mean, SD)	25.56 (4.94)	26.22 (5.17)
Education (mean years, SD)	12.33 (3.28)	12.88 (3.32)
Physical Activity (mean MET, SD)	3734.55 (3108.02)	3942.05 (3404.76)
Alcohol use (mean drinks/week, SD)	6.23 (7.76)	4.98 (6.25)
chronic diseases (mean number, SD)	0.59 (0.86)	0.62 (0.87)
Smoking (% current smokers)	57%	41%
MDD status		
no MDD. %	29%	24%
remitted MDD. %	31%	58%
Current MDD. %	40%	18%
IDS (mean score. SD)	20.7 (14.3)	15.6 (11.9)
Use of antidepressants		
TCA. %	3%	3%
SSRI. %	16%	12%
SNRI. %	6%	6%

MET Metabolic Equivalent Total, minutes per week (MET level * minutes of activity * events per week).

Table 2. Enrichment analysis of metabolites upregulated ($n = 40$) or downregulated ($n = 39$) in depression.

		Upregulated metabolites ($n = 40$)			Downregulated metabolites ($n = 39$)		
Pathway	**Pathway size (%[a])**	***P***	**FDR**	**Overlap[b]**	***P***	**FDR**	**Overlap[b]**
Long-chain saturated fatty acid	7 (1%)	NS	NS	0	2.8E−04	1.3E−02	4 (10%)
Long-chain monounsaturated fatty acid	7 (1%)	NS	NS	0	2.8E−04	1.3E−02	4 (10%)
Hemoglobin and porphyrin metabolism	4 (0.6%)	NS	NS	0	6.7E−04	2.1E−02	3 (8%)
Lysophospholipid	16 (2.3%)	1.3E−04	1.3E−02	6 (15%)	NS	NS	0

[a]Pathway size % is the percentage of all metabolites ($n = 681$) assigned to the pathway.
[b]Overlap is the number of metabolites (%) that are both in the pathway and in the significantly up or downregulated metabolite set.

Cross sectional associations between metabolites and MDD status and depression severity at baseline

For each metabolite, a GEE model was fit (correcting for family structure) using MDD as dependent variable (MDD as 3 level factor, control group as reference) while correcting for demographics and technical covariates (see Methods). When comparing controls and persons with current MDD, 139 metabolites were significantly different (Supplementary Table S1, FDR < 5%, 92 metabolites downregulated, 47 upregulated). Most significant was methylstearate (beta = −0.26, $P = 5.6e{-9}$, FDR = 4.5e−6), downregulated in the current MDD group. There were 88 metabolites that significantly differed between the remitted and control groups (86 downregulated); 57 of these overlapped with the metabolites associated with current MDD. Betas of the 139 metabolites associated with current MDD were strongly correlated with the betas from the association with remitted MDD (Supplementary Fig. S2, r = 0.81, 99% of downregulated metabolites had same direction of effect, 68% of upregulated metabolites had same direction of effect), but most effects were stronger for current MDD (65%). When adding BMI and lipid-lowering drug use to the models, 134 of the 139 metabolites were still significantly associated with current MDD (Supplementary Table S1). For each metabolite, a GEE model was fit in the total sample using the total IDS score for depression severity as dependent variable while correcting for demographics and technical covariates. There were 126 metabolites associated with depression severity (FDR < 5%, 67 upregulated); 79 of them overlapped with those found for current MDD (57% of 139 hits). From the 139 metabolites associated with current MDD, the betas were strongly correlated with the betas from the association with depression severity (Supplementary Fig. S3, r = 0.89, 99% of downregulated metabolites with same direction of effect, 100% of upregulated metabolites with same direction of effect). For the 139 metabolites associated with current MDD, tests for sex interaction effects were performed in the metabolite-current MDD associations (Supplementary Table S1). No significant findings were present after FDR correction.

Pathway enrichment

From 10 super pathways assigned to the metabolites, the 79 metabolites associated with current MDD and depression severity included 42 lipids (significantly enriched, $P = 7.2e{-4}$). From 95 sub-pathways, the 39 metabolites downregulated in current MDD were overrepresented with long-chain monounsaturated fatty acids ($P = 2.8e{-4}$), long-chain saturated fatty acids ($P = 2.8e{-4}$) and Hemoglobin and Porphyrin Metabolism ($P = 6.7e{-4}$). The 40 metabolites upregulated in current MDD were overrepresented with lysophospholipids ($P = 1.3e{-4}$, Table 2, Supplementary Table S3 for full overview).

Effects of antidepressants

From the 1101 participants with current MDD, 466 were using antidepressants (302 used SSRIs, 42 used TCAs, 122 used SNRIs). Since the most severe and chronic MDD patients are likely those who use antidepressants, a potential pharmacophysiological effect of antidepressants cannot be completely disentangled from that of depression severity. Consistently, adding directly antidepressant use as an additional covariate to the model reduced the number of significant associations: 45 of the 139 metabolites remained significantly associated with current MDD (Supplementary Table S1). To further evaluate with different approaches whether identified associations between metabolites and MDD status were driven by antidepressant use, associations between metabolites and MDD status were computed on the sample without the 466 participants that used antidepressants. From the 79 identified metabolites that were associated with current MDD and depression severity, the betas from the analysis without antidepressant users correlated strongly with the betas from the analysis including antidepressant users (Supplementary Fig. S4A, r = 0.89, 99% of betas in the same direction) showing that the identified directions of effects were not completely driven by antidepressant users. To further compare effects associated with antidepressant use and those associated with MDD status, associations between metabolites and SSRI, TCA or SNRI use were computed (Supplementary Table S1). Inspection of effect sizes (Supplementary Fig. S4B, C) showed only one outlier (5-methylthioadenosine (MTA)) with >2 times larger effect size of TCA compared to current MDD. This metabolite was removed in further analysis.

Internal replication of findings at 6-year follow up wave

We aimed to internally replicate the 78 metabolites associated with current MDD and IDS score at baseline, which were not strongly associated with antidepressant use, using 6-year follow-up data (see Supplementary Fig. S1 for an overview of the stepwise procedure leading to this selection). In 1805 respondents (1685 overlap with baseline participants) the same metabolite measures were done in whole blood samples (controls ($N = 433$), remitted MDD ($N = 1045$), current MDD ($N = 327$)). For each metabolite, a linear model was fitted using MDD status as the dependent variable (MDD as 3-level factor, control group as reference) while correcting for demographics and technical covariates. For 34 metabolites, the nominal *P*-value for the association with current MDD or depression severity was smaller than 0.05 and the directions of effects were consistent between baseline and follow up analysis (Table 3, Fig. 1). The betas of the 139 metabolites that were associated with current MDD at baseline, were strongly correlated with the same betas from 6-year follow up (Supplementary Fig. S5A, r = 0.64). A similar finding was present for the 126 metabolites associated with depression severity at baseline (Supplementary Fig. S5B, r = 0.78), suggesting general consistency of findings.

Mendelian randomization analyses

For the 34 internally replicated metabolites, 20 had GWAS summary statistics available and at least two independent associated SNPs. F-statistics (all $F > 10$, Supplementary Table S4)

Table 3. Baseline and 6-year follow up results of the 34 internally replicated metabolites.

			Baseline				6-year follow up			
Metabolite name	**Super pathway**	**Sub pathway**	**Beta CMDD**	***P*-value CMDD**	**Beta IDS**	***P*-value IDS**	**Beta CMDD**	***P*-value CMDD**	**Beta IDS**	***P*-value IDS**
(16 or 17)-methylstearate	Lip	Fatty acid, branched	−0.26	5.58E−09	−0.005	1.73E−04	−0.19	1.15E−03	−0.005	1.08E−02
10-nonadecenoate	Lip	Long chain monounsaturated fatty acid	−0.26	1.12E−08	−0.006	4.06E−05	−0.17	3.03E−03	−0.004	3.27E−02
1-stearoyl-GPC	Lip	Lysophospholipid	0.23	1.51E−07	0.006	5.16E−06	0.01	9.10E−01	0.006	7.33E−04
Sarcosine	AA	Glycine, serine and threonine metabolism	−0.24	2.06E−07	−0.006	1.61E−06	−0.19	4.97E−04	−0.002	1.99E−01
X-23782	#N/A	#N/A	−0.22	1.03E−06	−0.005	1.08E−04	−0.13	1.77E−02	−0.005	4.45E−03
(14 or 15)-methylpalmitate	Lip	Fatty acid, branched	−0.21	2.19E−06	−0.005	1.19E−04	−0.16	3.98E−03	−0.004	5.96E−02
10-undecenoate	Lip	Medium chain fatty acid	−0.22	3.79E−06	−0.006	8.88E−06	−0.04	5.12E−01	−0.004	3.26E−02
Margarate	Lip	Long chain saturated fatty acid	−0.21	8.14E−06	−0.005	1.06E−03	−0.13	1.75E−02	−0.005	1.70E−02
3-hydroxydecanoylcarnitine	Lip	Fatty acid metabolism	−0.20	9.48E−06	−0.005	2.67E−04	−0.05	3.68E−01	−0.004	4.44E−02
Phytanate	X	Food component/plant	−0.20	2.21E−05	−0.006	5.62E−06	−0.16	4.60E−03	−0.006	3.43E−03
1-stearoyl-GPE	Lip	Lysophospholipid	0.18	2.30E−05	0.006	4.78E−06	0.01	8.01E−01	0.007	1.32E−04
1-linoleoyl-GPE	Lip	Lysophospholipid	0.19	2.57E−05	0.006	1.42E−05	0.03	5.72E−01	0.007	2.50E−04
Erucate (22:1n9)	Lip	Long chain monounsaturated fatty acid	−0.19	4.35E−05	−0.005	7.27E−04	−0.07	1.88E−01	−0.004	4.72E−02
Perfluorooctanoate (PFOA)	X	Chemical	0.19	4.51E−05	0.004	3.17E−03	0.02	6.93E−01	0.002	3.75E−01
Pentadecanoate	Lip	Long chain saturated fatty acid	−0.19	5.72E−05	−0.005	5.42E−04	−0.12	3.28E−02	−0.004	4.86E−02
N-acetylneuraminate	C	Aminosugar metabolism	0.18	6.33E−05	0.007	3.01E−07	0.15	9.30E−03	0.004	3.28E−02
N-palmitoyl-sphingosine	Lip	Ceramides	0.17	1.01E−04	0.004	1.33E−03	−0.01	8.53E−01	0.006	2.77E−03
N-palmitoyl-sphinganine	Lip	Dihydroceramides	0.17	1.01E−04	0.007	1.09E−07	0.02	7.45E−01	0.005	5.33E−03
Nonadecanoate	Lip	Long chain saturated fatty acid	−0.18	1.58E−04	−0.005	7.62E−04	−0.17	3.43E−03	−0.005	7.57E−03
Isoursodeoxycholate	Lip	Secondary bile acid metabolism	0.17	3.51E−04	0.007	3.06E−06	0.09	1.17E−01	0.007	9.44E−04
N-acetylcarnosine	AA	Histidine metabolism	−0.12	6.29E−04	−0.004	3.24E−04	−0.02	7.19E−01	−0.005	1.96E−03
1-stearoyl-2-arachidonoyl-GPI	Lip	Phosphatidylinositol (PI)	0.16	6.51E−04	0.006	6.25E−06	0.06	3.21E−01	0.007	4.24E−04
Carotene diol (1)	C&V	Vitamin A metabolism	−0.15	7.54E−04	−0.005	2.47E−04	−0.07	2.03E−01	−0.005	1.50E−02
N-stearoyl-sphingosine	Lip	Ceramides	0.14	1.16E−03	0.004	1.46E−03	−0.02	7.11E−01	0.005	7.97E−03
1-stearoyl-2-linoleoyl-GPI	Lip	Phosphatidylinositol (PI)	0.15	1.29E−03	0.006	7.73E−05	0.04	5.22E−01	0.005	1.89E−02
Cholesterol	Lip	Sterol	0.14	1.49E−03	0.005	3.09E−04	0.01	9.10E−01	0.006	1.66E−03
X-13431	#N/A	#N/A	−0.15	1.51E−03	−0.006	1.59E−05	−0.12	4.05E−02	−0.004	4.85E−02
1-palmitoyl-2-linoleoyl-GPI	Lip	Phosphatidylinositol (PI)	0.15	1.56E−03	0.005	8.29E−05	0.06	3.01E−01	0.005	8.39E−03
1-palmitoyl-GPC	Lip	Lysophospholipid	0.14	1.60E−03	0.004	5.88E−03	−0.02	7.32E−01	0.006	8.35E−04
Ribitol	C	Pentose metabolism	0.14	2.84E−03	0.006	6.24E−06	0.13	2.33E−02	0.006	1.28E−03
1-stearoyl-2-linoleoyl-GPC	Lip	Phosphatidylcholine (PC)	0.14	3.60E−03	0.004	4.27E−03	0.07	2.53E−01	0.004	3.34E−02

Table 3. continued

Metabolite name	Super pathway	Sub pathway	Baseline			6-year follow up			
			Beta CMDD	*P*-value CMDD	Beta IDS	*P*-value IDS	Beta CMDD	*P*-value CMDD	Beta IDS	*P*-value IDS
X-24337	#N/A	#N/A	0.13	3.93E−03	0.005	4.83E−04	0.05	3.60E−01	0.006	2.67E−03
Glycodeoxycholate 3-sulfate	Lip	Secondary bile acid metabolism	0.13	5.25E−03	0.006	4.85E−05	0.12	3.75E−02	0.001	5.45E−01
X-18921	#N/A	#N/A	−0.12	7.71E−03	−0.004	3.19E−03	−0.01	8.24E−01	−0.004	3.35E−02

These metabolites were associated with MDD status and depression severity at baseline (FDR < 5%) and with MDD status or depression severity at 6-year follow up ($P < 0.05$).
#*N/A* no pathways assigned, *Lip* lipid, *AA* aminoacid, *X* xenobiotic, *C* Carbohydrate, *C&V* Cofactors and Vitamins, *CMDD* Current MDD, *IDS* Inventory of Depressive Symptoms

indicated that the strength of selected genetic instruments was adequate [30]. Table 4 shows 2SMR IVW estimates of depression risk (expressed as odds ratios [ORs] and 95% confidence intervals [95%CIs]) per SD increase in genetically-predicted log-transformed metabolite levels. An increased risk (OR = 1.09, 95% CIs = 1.05–1.13) of depression was significantly (q = 2.1e−4) associated with genetically-predicted higher levels of 1-linoleoyl-GPE (18:2) (Supplementary Fig. S5). Sensitivity analyses confirmed the robustness of this result: causal estimates obtained via weighted-median (OR = 1.09, 95% CIs = 1.05–1.13) and MR-Egger (OR = 1.09, 95% CIs = 0.98–1.21) estimators were completely in line with those from IVW analyses. Results of all MR estimators for all metabolites are reported in Supplementary Table S5. Additional analyses did not show statistically significant evidence of heterogeneity (Cochran's Q $p = 0.20$) or horizontal pleiotropy (MR-Egger intercept $p = 0.98$; MR-PRESSO global test $p = 0.45$) across SNPs indexing 1-linoleoyl-GPE (18:2) (single SNP MR Supplementary Fig. S7A; leave-one-out SNP MR Supplementary Fig. S7B). Finally, we performed a PheWAS (phenome-wide association scan) using the GWAS ATLAS Resource [31] to examine the association with other traits of the instrument's top SNP (11:61569830, rs174546), which is located in the highly pleiotropic 3'-UTR region of FADS1 (fatty acid desaturase enzyme). The PheWAS (Supplementary Table S6) reported significant GWAS association with a wide array of metabolic (e.g., fatty acids, cholesterol, triglycerides), cardiovascular (e.g., heart rate), immunological (e.g. red cell, platelet), and psychiatric (e.g., sleep duration, irritability) traits.

DISCUSSION

This analysis is the largest untargeted metabolomics study of MDD to date, performed in a clinical sample with two measurement waves. We identified new metabolites, not previously associated with MDD for which consistent associations were found using similar data from the same subjects measured after six years. Approximately half of the identified metabolites were lipids, showing specific patterns of downregulation in long-chain monounsaturated and saturated fatty acids and upregulation of lysophospholipids in depression. The other half of the metabolites were non-lipid components of a wide range of pathways discussed below. Using genetic data, a potential causal effect of lysophospholipid 1-linoleoyl-GPE on depression was confirmed.

The associations of the 139 metabolites associated in patients with current MDD were also apparent in the remitted MDD patients, with a remarkable similarity in effect sizes, particularly for the downregulated metabolites. That these seemingly persistently downregulated metabolites were largely unaffected by the state of depression could be due to either consequences (scars) of the illness or potentially represent antecedents of illness onset. The 79 metabolites associated with current MDD and depression severity were 53% lipids but also covered 7 out of 9 super pathways and 39% of all sub pathways, indicating a dysregulation of metabolites in depressed patients that does not only concern lipids but a wide spectrum of metabolic processes. The two pathways enriched in these 79 metabolites were both lipid pathways: lysophospholipids were upregulated and long chain fatty acids (both monounsaturated and saturated) were downregulated in the current MDD group. These pathways have been shown to be inter-connected by common chemical steps controlled by genetic variation within the fatty acid desaturase (FADS) locus on chromosome 11 (11q12.2–q13.1, containing the genes FADS1, FADS2 and FADS3 genes) [32]. This fatty-acid transforming metabolic pathway is responsible for the synthesis of over 100 individual polyunsaturated fatty acids (PUFA)- and long-chain PUFA-containing phospholipid and lysophospholipid molecular species that have been differently linked to innate immunity, energy homeostasis, brain development, and neurocognitive functions [33–35].

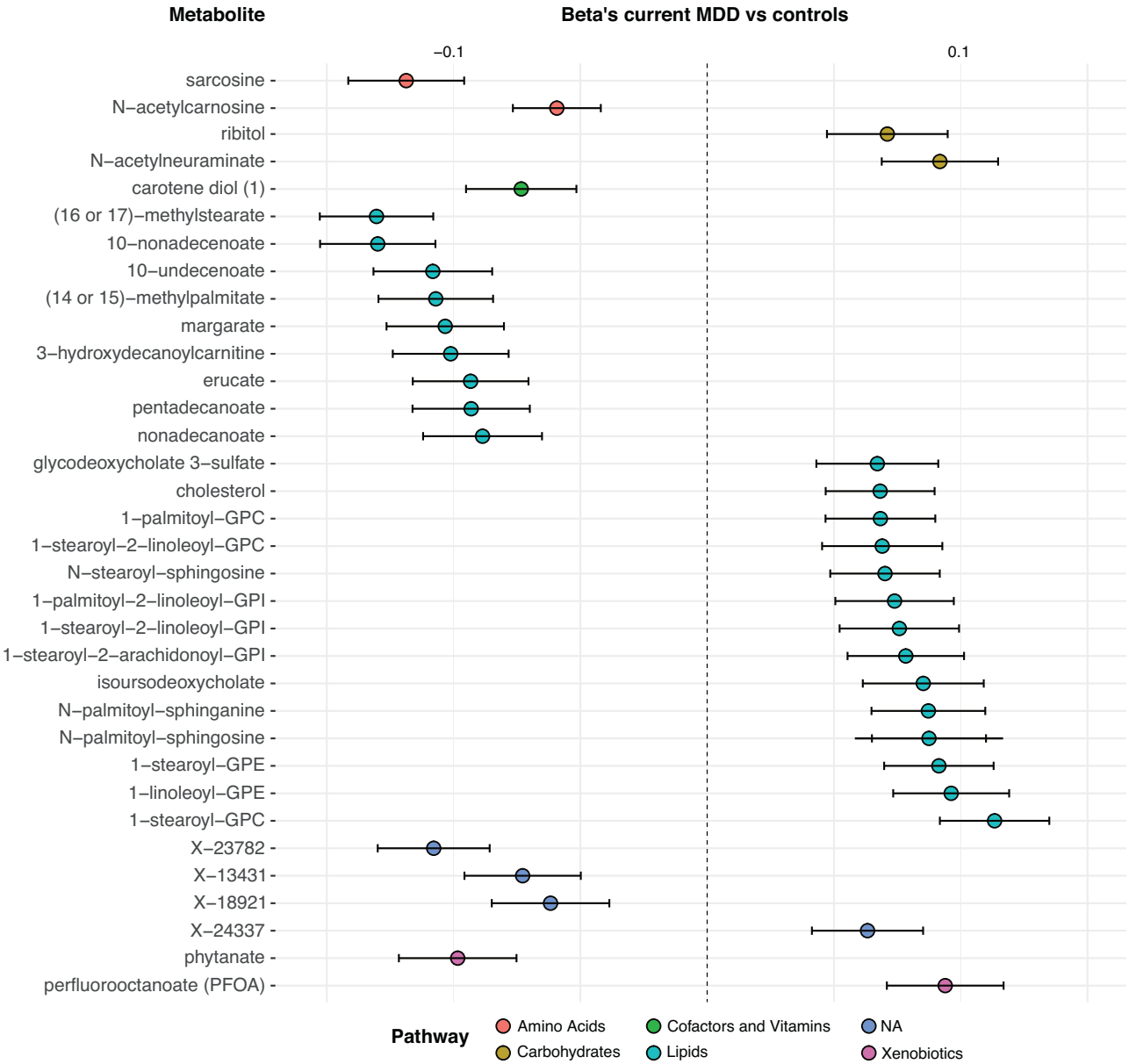

Fig. 1 Forest plot of the 34 internally replicated metabolites. Shown are standardized betas for the associations between the metabolites and current MDD at baseline. Positive beta means upregulation in the current MDD group.

Lysophospholipids have not been associated with depression in prior large-scale studies because they are not targeted by most lipid-based platforms. Out of 16 measured lysophospholipids, the 6 associated with current MDD all cluster with intercorrelations between 0.32 and 0.78. Lysophospholipids activate specific G-protein-coupled receptors and are regulators of cell growth and survival, cell-to-cell contacts and adhesion, and calcium dependent functions. Through these actions, lysophospholipids play a role in the development of the nervous system, cancer growth, and inflammation [36]. Studies in both rodent models and human plasma with small sample size ($N < 120$) have shown higher lysophospholipids in depression as compared to controls [37–40]. The lysophospholipids we identified are not well known; however two of the best-characterized lysophospholipids, lysophosphatidic acid and sphingosine-1-phosphate, are crucial in neurodegenerative diseases, especially Alzheimer's disease [41]. Dysfunction of these metabolites can lead to accumulation of amyloid-β peptides, neurofibrillary tangles and neuroinflammation [42, 43]. From these two metabolites, only sphingosine-1-phosphate was measured by the Metabolon platform we used, but it did not show an association with depression.

Two recent large-scale studies using the Nightingale platform [2, 3] both reported lower total cholesterol, and higher triglycerides, saturated fatty acids and monounsaturated fatty acids (MUFAs) in patients with lifetime diagnosis of MDD compared to controls. A comparison between these two studies and ours is not straightforward due to the differences in design (population-based sample vs clinical sample) and the difference in platforms (triglycerides and component measures of total cholesterol are not measured by the Metabolon platform, and the Nightingale platform does not measure any individual fatty acids, providing only summed measures of the MUFAs). The downregulation of MUFAs and saturated fatty acids we observed in current MDD is not consistent with these two large studies [2, 3]. However, all measured PUFAs were lower in patients with depression in our study, which was similarly found in the UKBB

Table 4. Mendelian randomization analysis results showing the association between genetically-predicted levels of 20 metabolites with GWAS data and risk of depression.

				Inverse variance weighted			
Super.Pathway	**Sub.Pathway**	**Marker.name**	**nsnp**	**or**	**or_lci95**	**or_uci95**	**fdrq**
#N/A	#N/A	X-18921	5	1.00	0.98	1.02	9.37E−01
#N/A	#N/A	X-13431	5	1.02	1.00	1.03	2.89E−01
Amino acid	Glycine, serine and threonine metabolism	Sarcosine	4	0.99	0.97	1.02	8.51E−01
Amino acid	Histidine metabolism	N-acetylcarnosine	8	0.98	0.95	1.00	3.36E−01
Carbohydrate	Aminosugar metabolism	N-acetylneuraminate	4	1.03	0.96	1.11	8.51E−01
Carbohydrate	Pentose metabolism	Ribitol	3	1.01	0.98	1.04	8.51E−01
Cofactors and vitamins	Vitamin A metabolism	carotene diol (1)	8	0.99	0.97	1.01	8.51E−01
Lipid	Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	7	1.00	0.95	1.04	9.37E−01
Lipid	Lysophospholipid	1-palmitoyl-GPC (16:0)	4	1.00	0.95	1.06	9.37E−01
Lipid	Lysophospholipid	1-stearoyl-GPC (18:0)	6	0.98	0.94	1.01	7.16E−01
Lipid	Lysophospholipid	1-linoleoyl-GPE (18:2)*	4	1.09	1.05	1.13	2.05E−04
Lipid	Lysophospholipid	1-stearoyl-GPE (18:0)	9	1.00	0.98	1.02	9.37E−01
Lipid	Medium chain fatty acid	10-undecenoate (11:1n1)	3	1.02	0.96	1.08	8.51E−01
Lipid	Phosphatidylcholine (PC)	1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	7	1.05	0.99	1.10	4.08E−01
Lipid	Phosphatidylinositol (PI)	1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	11	0.98	0.95	1.01	7.28E−01
Lipid	Phosphatidylinositol (PI)	1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	4	1.02	0.96	1.09	8.51E−01
Lipid	Phosphatidylinositol (PI)	1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	6	1.02	0.97	1.06	8.51E−01
Lipid	Secondary bile acid metabolism	Isoursodeoxycholate	3	1.02	0.96	1.08	8.51E−01
Lipid	Secondary bile acid metabolism	Glycodeoxycholate 3-sulfate	5	1.00	0.99	1.02	9.96E−01
Lipid	Sterol	Cholesterol	8	0.99	0.95	1.03	8.51E−01

and NESDA studies [3, 44]. There is a lot of debate about the possible role for PUFAs in depression and in particular their potential role in the prevention of depression, with inconsistent findings in the literature. A large-scale meta-analysis of observational studies found a decrease of the PUFA omega-3 associated with depression [45], but a meta-analysis of prevention studies using omega 3 did not show positive results [46]. Recent work suggests many trials of PUFAs may have used doses too low to have a significant antidepressant or preventive effect, with 4 grams/day of eicosapentaenoic acid have both positive effects on both clinical and inflammatory outcomes [47, 48].

Two recent studies evaluated metabolites measured with the Metabolon platform in relation to depression. A population-based study [8], of 1411 adults, of whom 72 had self-reported depressed mood, identified lower levels of the medium-chain acylcarnitine laurylcarnitine in the depressed subjects compared to controls. Similarly, in the present study the medium-chain acylcarnitine 3-hydroxydecanoylcarnitine was negatively associated with MDD. Interestingly, a previous genomic-based study using MR showed that altered metabolism of the medium chain acylcarnitines octanoylcarnitine and decanoylcarnitine is potentially causal for the development of depression [11]. While measures of laurylcarnitine, octanoylcarnitine and decanoylcarnitine were not available in the present study, it is important to note that acylcarinites of similar chain length share a substantial proportion of their genetic liability, as indicated by strong genetic correlations between them [11]. The second study is a pooled analysis of population-based cohorts by van der Spek and colleagues [9] and reported 53 metabolites associated with depression severity. A total of 43 of their 53 significant metabolites were also measured in our study, and 17 were replicated. The other way around, from the 126 metabolites associated with depression severity in our study, Van der Spek et al. measured 96, and 36 were replicated (37.5%), whereas from the top 15 hits 53% were replicated ($P < 0.05$, with consistent directions of effect, Supplementary Table S7). The fatty acid findings from our study were partially replicated (47% with $P < 0.05$) and had overall consistent effect sizes (73%) but some lysophospholipid findings had opposite directions of effect. The relatively low replication rate may stem from design differences across studies. Van der Spek et al. performed a meta-analysis using 5 population-based cohorts in which 4 different self-report instruments to assess depression symptoms were used, in contrast to the clinically enriched cohort with uniform assessment of MDD via psychiatric interviews used in the current paper.

The identified association between depression and the metabolomic signature may stem from different, non-mutually exclusive, causal pathways. Shared factors (e.g., lower socioeconomic status, presence of chronic somatic diseases, use of medications) may impact both metabolite levels and depression. However, adjustment for major sociodemographic, lifestyle and health-related factors had only a marginal impact on the associations identified. A potential confounding role might be expected for BMI, whose inclusion in the statistical models partially reduced the strength of the association between depression and metabolites. However, BMI-adjusted estimates should be carefully interpreted due to the complex causal pathways between BMI, metabolite levels and depression. BMI and adiposity in general may indeed represent a confounder, a mediator, but also a consequence (collider) of metabolic alterations affecting both metabolite concentrations and depressive symptoms. Nevertheless, as opposed to previous large-scale studies using the Nightingale platform [2], 95% of the associations detected in the present study remained statistically significant after additional statistical adjustment for BMI and lipid-lowering drugs. In our study, the use of lipid-lowering drugs was uncommon and not associated with MDD status, likely explaining why adding use of lipid-lowering drug minimally impacted the findings. Similarly, the use of antidepressant medications had a limited impact on the metabolite-depression associations, as shown by substantially similar estimates for all metabolites obtained in subjects not using antidepressants. Only the level of one metabolite, 5-methylthioadenosine, was strongly connected to the use of tricyclic antidepressants. Moreover, AD use may merely be a proxy for depression severity, likely representing the clinical indication for treatment (thus, confounding by indication) [49]. In this sense, adjusting associations between depression and metabolites for AD use would introduce substantial overadjustment for depression severity. Further mechanistic and experimental medicine approach are needed to properly disentangle the specific effect of antidepressant medications. In another causal scenario, depression could impact metabolite levels via depression-related behavioral symptoms, For instance, worsening of diet may reduce the intake of fatty acids included in metabolites pathways found to be downregulated in depression, such as long-chain monounsaturated and saturated fatty acids. Alternatively, alterations in metabolite metabolism may have a direct causal action on depression pathophysiology. In a previous genomic study leveraging Mendelian randomization techniques, we showed that alteration in metabolism of medium-chain acylcarnitines, which are involved in fatty acid transport into mitochondria for beta-oxidation, may have a potential causal role for the development of depression [11]. In the present study, the medium-chain 3-hydroxydecanoylcarnitine was consistently associated with depression across different measures and assessments.

Leveraging summary statistics from large-scale GWAS, we applied Mendelian randomization in the present study to examine the potential causal role in depression onset of 20 metabolites (13 lipids, 2 carbohydrates, 2 amino acids, 1 cofactor/vitamin and 2 unidentified). Results from Mendelian randomization suggest that higher levels of 1-linoleoyl-GPE (18:2), or the mechanism translating genetic variation to higher levels of this metabolite, are potentially causally involved in the development of depression. 1-linoleoyl-GPE (18:2) is a lysophospholipid, part of the cluster of lysophospholipids mentioned above. The precursor of this lysophospholipid is 18:2 fatty acid which is comprised of two isomers with double bond locations at n-6 and n-9 positions, respectively. The n-6 isomer represents the majority of this precursor and is largely obtained from dietary sources [50]. However, the n-9 isomer of this fatty acid involves the activity of desaturases such as FADS1. Therefore, similar to other PUFA-containing lysophospholipids, phospholipids, free fatty acids and endocannabinoids, the metabolism 1-linoleoyl-GPE (18:2) is tightly regulated by the FADS1 (fatty acid desaturase) gene. The genetic instrument indexing 1-linoleoyl-GPE (18:2) includes the SNP rs174546 in the 3′-UTR regulatory region of FADS1. Findings from a recent genomic sequencing study showed that this variant was the top signal in the association between the FADS locus and 52 lipids containing fatty acids [32]. Consistently, the wide-range examination of previous GWAS results (pheWAS) performed in the present study showed that rs174546 is associated with a wide array of metabolic, cardiovascular, immunological, or psychiatric traits. This biological complexity suggests caution in interpreting MR results using instruments including variants from the FADS locus that could index different metabolites. A previous MR-based study suggested a potential causal role in depression for polyunsaturated fatty acids such as omega-3, indexed by FADS SNPs [51]. This conclusion is in contrast with different large-scale RCTs showing no effect of omega-3 supplementation in the prevention of depression [52, 53]. Rather than attributing the complex FADS genetic signal to a specific metabolite, a more cautious interpretation of MR results points to a potentially broader involvement in depression pathophysiology of FADS-regulated metabolic processes, which may be involved in various metabolic, cardiovascular and immunological functions. Deeper

mechanistic studies are needed to disentangle the specific metabolite effector relevant for depression.

The major strengths of our study included it being the largest study of metabolomics in MDD to date, along with using data from two time points, with untargeted metabolomics and deep characterization of MDD diagnosis and depression symptom severity. A potential limitation was that the internal replication was based on a subset of the sample used for the discovery, measured 6 years later, and therefore cannot be considered a fully independent replication. Although our results were partially replicated in a similar previous study [9] many of the identified metabolites will need to be confirmed in other large samples, ideally with similar clinical assessments. The cross-sectional design of our study did not permit drawing inferences on causality, other than those suggested by the MR analysis. Furthermore, estimates from MR describe a potential average lifetime causal effect unable to distinguish specific critical windows or acute events. Finally, our genetic instruments were derived from GWAS based on samples of participants of European ancestry, so results may not generalize to different populations.

In conclusion, this study confirmed previously established metabolite pathways involved in depression and identified associations with some novel metabolites. The untargeted whole-metabolome approach demonstrated that a wide range of metabolites are dysregulated in depression, involving various metabolites interconnected around the FADS metabolic pathways. This may represent the biological substrate connecting depression with different cardiometabolic health outcomes. Furthermore, genomics-based analyses suggested a potential causal effect of this pathway in the pathophysiology of depression. This metabolomic signature is a promising target for treatment development. Nevertheless, future mechanistic studies are required to unravel the exact causal mechanisms across the complex network of processes around the FADS pathways.

DATA AVAILABILITY

The data used to support the findings of this study are available upon reasonable request from NESDA, Amsterdam: nesda@amsterdamumc.nl. Information on how to request the study data, including the data sharing policy, can be found at https://www.nesda.nl/nesda-english/.

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AUTHOR CONTRIBUTIONS

RJ and YM performed analysis and wrote the manuscript. DS, GK and MA performed pre processing of the metabolite data. XH, BD, AR, RK and BP designed the study and helped writing and interpreting.

COMPETING INTERESTS

AJR has received consulting fees from Compass Inc., Curbstone Consultant LLC, Emmes Corp., Evecxia Therapeutics, Inc., Holmusk, Johnson and Johnson (Janssen), Liva-Nova, Neurocrine Biosciences Inc., Otsuka-US; speaking fees from Liva-Nova, Johnson and Johnson (Janssen); and royalties from Guilford Press and the University of Texas Southwestern Medical Center, Dallas, TX (for the Inventory of Depressive Symptoms and its derivatives). He is also named co-inventor on two patents: U.S. Patent No. 7,795,033: Methods to Predict the Outcome of Treatment with Antidepressant Medication, Inventors: McMahon FJ, Laje G, Manji H, Rush AJ, Paddock S, Wilson AS; and U.S. Patent No. 7,906,283: Methods to Identify Patients at Risk of Developing Adverse Events During Treatment with Antidepressant Medication, Inventors: McMahon FJ, Laje G, Manji H, Rush AJ, Paddock S. M.A. and G.K. are co-inventors (through Duke University/Helmholtz Zentrum München) on patents on applications of metabolomics in diseases of the central nervous system and hold equity in Chymia LLC and IP in PsyProtix and Atai that are exploring the potential for therapeutic applications targeting mitochondrial metabolism in depression. RKD is funded by National Institute on Aging [U19AG063744, U01AG061359, 1RF1AG058942, 1RF1AG057452, RF1AG051550, and R01AG046171] and National Institute of Mental Health [R01MH108348]. This funding enabled consortia that she leads including the Mood Disorder Precision Medicine Consortium, the Alzheimer's Disease Metabolomics Consortium, and the Alzheimer Gut Microbiome Project that contributed to acylcarnitine discoveries. She is an inventor on key patents in the field of Metabolomics and hold equity in Metabolon, a biotech company in North Carolina. In addition, she holds patents licensed to Chymia LLC and PsyProtix with royalties and ownership. The funders listed above had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the paper; and decision to submit the paper for publication. BWD has received research support from Acadia, Compass, Aptinyx, NIMH, Sage, Otsuka, and Takeda, and has served as a consultant to Greenwich Biosciences, Myriad Neuroscience, Otsuka, Sage, and Sophren Therapeutics. All the other authors declare no conflict of interest.

ADDITIONAL INFORMATION

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