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Metabolomic investigation of major depressive disorder identifies a potentially causal association with polyunsaturated fatty acids

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Abstract

Background: Metabolic differences have been reported between individuals with and without Major Depressive Disorder (MDD), but their consistency and causal relevance has been unclear.

Methods: We conducted a metabolome-wide association study of MDD with 249 metabolomic measures available in UK Biobank (N = 29, 757). We then applied 2-sample bidirectional Mendelian Randomisation (MR) and colocalization analysis to identify potentially causal relationships between each metabolite and MDD.

Results: One hundred and ninety-one metabolites tested were significantly associated with MDD ($P_{FDR} < 0.05$), which reduced to 129 after adjustment for likely confounders. Lower abundance of Omega-3 fatty acid measures and a higher Omega-6: Omega-3 ratio showed potentially causal effects on liability to MDD. There was no evidence of a causal effect of MDD on metabolite levels. Furthermore, genetic signals associated with Docosahexaenoic Acid colocalized with loci associated with MDD within the FADS gene cluster. Post-hoc MR of gene-transcript abundance within the FADS cluster demonstrated a causal association with MDD. In contrast, colocalization analysis did not suggest a single causal variant for both transcript abundance and MDD liability, but the likely existence of two variants in LD with one another.

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Conclusion: Our findings suggest that decreased Docosahexaenoic Acid and increased Omega-6: Omega-3 fatty acids ratio may be causally related to MDD. These findings provide further support for the causal involvement of fatty acids in MDD.

Keywords

Metabolome; Depression; Mendelian Randomisation; Colocalization; Multi-Omics; Fatty Acids

Introduction

Major Depressive Disorder (MDD) is a debilitating condition estimated to affect 322 million people, leading to a global total of 50 million years lived with disability (1). This is due to both the high prevalence of MDD and its frequent comorbidity with other conditions (2-4), particularly cardiovascular disease (5). Most antidepressants target monoamine related pathways (6) but are ineffective in 40% of cases (7). Better understanding of the molecular mechanisms of MDD may aid in the development of more effective treatments.

Twin studies have estimated the heritability of MDD as ~37% (8) and investigating this genetic component of MDD may help to identify its pathophysiological basis. Recently, genome wide association studies (GWAS) have been increasingly successful in identifying genetic variants associated with MDD (9-11). Despite this success, the high polygenicity of MDD presents significant challenges to researchers who wish to identify actionable mechanisms lying between genetic variants and clinical phenotype (12). One means of translating genetic findings into biomarkers and biological mechanisms is through the incorporation of molecular phenotype data, such as metabolomics. These data may help to clarify the downstream functional impact of identified risk single nucleotide variant (SNVs) on molecular traits. This information can then be utilized to stratify risk, provide therapeutic targets or enable lifestyle interventions (13).

There have been several recent studies investigating the links between genetic risk to MDD and molecular phenotypes, including DNA methylation (14,15), mRNA levels (16,17) and protein markers (18,19). Metabolomics considers the role of circulating metabolites (20), including lipids, amino acids and other small molecules that have been implicated in a range of disorders and some hypothesis-driven studies of MDD (21,22). The metabolome has not been characterized to the same extent as other molecular phenotypes (23), but the recent availability of reproducible high-throughput metabolomics makes studies feasible for many thousands of individuals (24). Studying the association between metabolite levels and MDD may offer potential biomarkers and interventional targets for MDD, and provides a means to investigate MDD's comorbidity with cardiometabolic disorders (2,3). The majority of previous metabolic studies of MDD have concentrated on small numbers of metabolites (25), often used sample sizes of less than 100 (25-29), and have implemented a variety of methodological approaches all leading to somewhat heterogeneous findings (30). However, larger and more standardized studies are beginning to emerge; a recent meta-analysis of 230 metabolite levels in 15, 428 individuals (5, 283 Cases and 10, 145 Controls) found evidence of a distinct metabolomic profile associated with MDD (31).

The UK Biobank recently released a large metabolomic dataset (31), in which we conducted a comprehensive and agnostic examination of metabolite associations with MDD. Association analysis between metabolites and MDD is limited because of the susceptibility of these analyses to confounding and reverse causation. We therefore also assessed potentially causal associations between MDD, and metabolite levels using two-sample Mendelian Randomisation (MR). Finally, we sought to identify potentially causal gene targets using MR and colocalization analysis of expression quantitative trait loci (eQTL) (Figure S1).

Methods and Materials

Study Population

The UK Biobank (UKB) is a large prospective study (N = 502, 492) of participants recruited from around the United Kingdom between the ages of 40 and 69 (33). Baseline data collection, including extensive phenotyping and genotyping for a range of health outcomes, occurred between 2006 and 2010. UKB data acquisition was approved by the North West NHS Research Ethics Committee (11/NW/0382). The analysis and data acquisition for the present study were conducted under application #4844. Written informed consent was obtained for all participants.

Metabolic biomarkers

Two hundred and forty-nine metabolite biomarkers were quantified in non-fasting baseline EDTA plasma samples (N = 118, 021) (33), collected at baseline assessment between April 2007 and December 2010 (34), and measured between June 2019 and April 2020 (31). Measurement was performed using the high-throughput nuclear magnetic resonance (NMR) spectroscopy profiling platform, developed by Nightingale Health Ltd, Finland. Detailed methodology of the Nightingale NMR pipeline has been described elsewhere (35-37). These biomarkers include detailed cholesterol measures, fatty acid compositions and low weight metabolites such as amino acids and ketones (Table S1). Most biomarkers were measured in absolute concentration (mmol/L), excluding apolipoproteins (A&B; g/L), diameter measures (Diameter of VLDL/LDL/HDL particles; nm) and the degree of unsaturation measure (degree; the total number of pi bonds and rings within a molecule). Prior to analysis, the levels of each metabolite biomarker were transformed to a standard normal distribution using rank-based inverse normalisation. Therefore, all reported beta coefficients from regression analyses represent the standardized change in ranked metabolite levels between MDD cases and controls.

Assessment of Major Depressive Disorder status

The MDD phenotype used in this study was derived from the online Thoughts and Feelings Questionnaire, fully completed by 126,077 participants in July 2017 (38). The questionnaire included the Composite International Diagnostic Interview Short Form (CIDI-SF), from which MDD lifetime diagnoses were derived (38) (SI: Methods & Materials). This sample (referred to as the mental-health questionnaire (MHQ) sample) comprised 37, 430 cases and 88, 647 controls of which 29,757 (8, 840 Cases and 20, 917 controls) also had metabolite levels derived from their blood samples (Table 1).

Metabolome-wide association study

Linear regression models were used to test the association of each metabolite level with the MDD phenotype (N = 29, 757), referred to as a metabolome-wide association study (MetWAS). In the basic MetWAS, only age, sex, assessment centre and NMR spectrometer were included as covariates. MetWAS analysis was also conducted with adjustment for socioeconomic status (SES), smoking status, ethnicity, educational attainment and body mass index (BMI) (SI: Methods and Materials) due to the strong and potentially confounding association between these factors with both MDD and metabolite levels (39-42). Significance was defined by FDR-corrected P values < 0.05.

Metabolite GWAS

For each metabolite a genome-wide association study (GWAS) was performed on unrelated individuals of European ancestry (N = 88, 329) using PLINK 2.0 (43). Unrelated individuals were identified as previously reported (44). In brief, related individuals were initially excluded based on a shared relatedness of up to the third-degree using kinship coefficients (> 0.044) calculated using the KING toolset. One member of each group of related individuals was then subsequently added back in, by creating a genomic relationship matrix (GRM) and selecting individuals with a genetic relatedness of less than 0.025 with any other participant. Association testing was performed on the imputed genotypes using an additive model, adjusted for age, sex, genotyping array, assessment centre, spectrometer and 10 genetic principal components (PC1-10). Variants with minor allele frequency (MAF) < 0.001, minor allele count (MAC) < 20 and an INFO score < 0.1 were removed. Insertion-deletions (indels) were included in the analysis. To account for multiple comparisons, significance was defined as the genome-wide threshold divided by the number of PCs which account for 99% of the variance within the metabolomic data ($5e^{-8}/42 = 1.19e^{-9}$), as described previously (45,46). An F statistic for the strength of the association between the sentinel SNVs and metabolite levels was calculated using the method: $F = ((N-k-1) / k) \times (r^2 / (1-r^2))$, where N = sample size, k = number of SNVs and r^2 = variance explained in metabolite levels by the genetic instruments. The r^2 statistic was calculated as follows: $r^2 = 2 \times MAF \times (1-MAF) \times beta^2$, where beta = effect size of the SNV and MAF = the SNV effect allele frequency. SNV heritability for each metabolite and the genetic correlation of the metabolite with MDD was performed using linkage disequilibrium (LD) score regression as implemented in the LDSC package (available at https://github.com/bulik/ldsc) (47,48). Pre-computed LD scores based on the 1000 Genomes project data for European ancestry (49) were used as the reference panel for regression weights in the analysis (available at https://data.broadinstitute.org/alkesgroup/LDSCORE).

Metabolite bidirectional Mendelian Randomisation analysis

Bidirectional MR was performed using the 'TwoSampleMR' R package, version 0.5.6 (50,51) to assess causality between the metabolite (exposure) and MDD (outcome) for all metabolites, and vice versa. The MR analysis used SNV effect sizes from the UKB metabolite GWAS conducted in this study and the MDD meta-analysis GWAS, performed by the Psychiatric Genetics Consortium (9) with UKB participants removed using three MR methods (Inverse variance weighted, MR Egger, and Weighted median). Genetic instruments

were identified by clumping genome-wide significant SNVs ($P < 1.19x10^{-9}$ and MAF > 0.005) using the 'clump_data' function in the R package TwoSampleMR ($r^2 = 0.001$, within a 10Mb window). Metabolites with < 15 genetic instruments after harmonisation with MDD summary statistics were omitted from the MR analysis.

Colocalization

Colocalization analysis was performed between MDD and metabolite genetic signals that showed potentially causal associations using MR, with a window of 1Mb. Lead SNVs were identified using the default clumping method ($r^2 = 0.1$) on the 'SNP2GENE' tool within FUMA (52). To avoid running multiple colocalization tests of overlapping regions, lead SNVs were ordered by significance, and iteratively filtered so that each lead SNV was > 1Mb away from any other lead SNV. For each of the final lead SNVs, all SNVs within a 1Mb window from the metabolite GWAS and the MDD GWAS were extracted from the respective summary statistics. Colocalization analysis was then performed using the R coloc package (v5.1.1) (53). The prior probabilities were set to default values: P1 (SNV is associated with the metabolite) and P2 (SNV is associated with MDD) were both set at $1e^{-4}$, and P12 (SNV is associated with both MDD and the metabolite) was set at $1e^{-5}$. An arbitrary posterior probability (PP) > 0.8 was set for evidence of each of the four hypotheses: PP.H0 - no causal variant; PP.H1 and PP.H2 - causal variant for one of the traits; PP.H3 – distinct causal variant for each trait, PP.H4 - shared causal variant for both traits.

MR and colocalization with expression Quantitative Trait Loci Data

To probe possible mechanistic pathways between exposure (metabolite) and outcome (MDD), colocalized MDD and metabolite genetic signals were investigated to identify if they localised to any causal genes within the colocalized region. For genes lying within the same LD window as co-localized MDD-metabolite genetic signals, we first identified any cis- expression quantitative trait loci (cis-eQTLs) from whole blood gene expression data (N = 948) from the Genotype Tissue Expression (GTEx) Project (47). Secondly, MR and complementary colocalization analysis was performed to assess the causality of gene expression levels (mRNA) with MDD. The MR analysis was performed using a Wald Ratio Test (55) using a single instrumental SNV, defined as the most significant eQTL present in the dataset. For this post-hoc analysis, significance was defined as $P < 0.05$. The instrumental SNVs were also used as lead SNVs in the colocalization analysis. For each lead SNV, MDD GWAS (9) and GTEx (54) summary statistics for variants within ±1Mb were extracted as inputs for the colocalization analysis.

Results

MetWAS MDD association analysis

The demographics of the MHQ sample (metabolomic and MDD data available; n = 29, 757), alongside other subsets of UKB are presented in Table 1. The basic MetWAS, without adjustment for common confounders, found that 191 (~76%) metabolites were significantly associated with MDD ($P_{FDR} < 0.05$) (Table S2: Figure S2). The most significant positive metabolite-MDD association was for Monosaturated Fatty Acids to Total Fatty Acids (%) (MFA-TFA), ($\beta = 0.177$, P_{FDR} $1.52x10^{-43}$), and the most significant negative association

was for Polyunsaturated Fatty Acids to Monounsaturated Fatty Acids ratio (PFA-MFAR: $\beta = -0.176$, $P_{FDR} = 1.85 \times 10^{-43}$). After adjustment for possible confounders, 133 (~ 53 %) metabolites were significantly associated with MDD, 129 of which (~52 %) were shared in the unadjusted model (Figure 1) (Table S3). The most significant positive metabolite-MDD association remained MFA-TFA ($\beta = 0.069$, $P_{FDR} = 2.22 \times 10^{-7}$), whilst the most significant negative metabolite-MDD association changed to Free Cholesterol to Total Lipids in Large LDL percentage (FC-TL-LDL, %) ($B = -0.070$, $P_{FDR} = 2.82 \times 10^{-7}$). Across most metabolites (N = 224, Unadjusted β range: −0.176 to 0.177), inclusion of confounders into the analysis attenuated the absolute effect sizes by an average of 61% (Adjusted β range: −0.070 to 0.069) (Figure S3).

Metabolite GWAS

Manhattan plots for all 249 GWAS can be found in the Supplementary Folder 1. All metabolites had multiple independent ($r^2 = 0.001$, within a 10Mb window) genome wide significant SNV associations ($P < 1.19 \times 10^{-9}$), all with F-statistics > 10 (Table S4). The number of significant independent SNVs ranged from 4 (Phenylalanine and Acetoacetate) to 60 (Cholesteryl Esters in Large HDL) (Table S5). SNV-heritability of metabolites varied from 2.41% (Histidine) to 19.9% (Triglycerides to Phosphoglycerides ratio). One hundred and forty-four metabolites (57%) showed a significant genetic correlation with MDD (Table S6), with absolute correlations ranging from (−) 0.073 (Omega-3 Fatty Acids to Total Fatty Acids (%)) to (+) 0.180 (Phospholipids to Total Lipids in Large HDL percentage). Of the metabolites which showed a significant genetic correlation with MDD, 119 (~ 83 %) also had significant associations with the MDD phenotype in both the unadjusted and adjusted MetWAS analysis.

Mendelian Randomisation

In the metabolite (exposure) to MDD (outcome) MR analysis, 21 metabolites were excluded due to having too few (< 15) genetic instruments (Figure S4). MR analysis on the remaining 228 metabolites (Figure 2; Figure S5) found five with significant evidence for a causal relationship with MDD (Table 2; Table S7), all relating to long-chain polyunsaturated fatty acids (LC-PUFAs). Specifically, Omega-3 fatty acid levels (Omega-3 Fatty Acids, Omega-3 to Total Fatty Acids (%), Docosahexaenoic Acid) and the Degree of Unsaturation had a negative causal effect on MDD, whilst Omega-6: Omega-3 ratio had a positive causal effect on lifetime MDD (Figure S6). These metabolites also had strong correlations with the other metabolites that significantly associated with MDD in the MetWAS phenotypic analysis, ranging from Omega-3 Fatty Acids (significantly correlated with 79% of associated metabolites) to the Degree of Unsaturation (significantly correlated with 100% of the other MDD-associated metabolites) (Figure S7). Of the five putatively causal metabolites, only the Degree of Unsaturation showed consistency in previous observational analyses, holding a significant association and genetic correlation with MDD (Figure S8). In the MR leave-one-SNV-out analysis, three of the significant metabolites-to-MDD MR results; Degree of Unsaturation, Omega-3 Fatty Acids and Docosahexaenoic Acid were dependent on rs174528, a single SNV in the *FADS* gene cluster on chromosome 11 (Table S8, Figure S9).

In the MDD (exposure) to metabolite (outcome) MR analysis, all metabolite measures were included. In this analysis, there was no significant evidence that MDD was significantly causal to a change in any of the metabolite levels tested (β range: −0.28 to 0.26, P_{FDR} range: 0.91 to 0.99) (Table S9: Figure S10).

Colocalization

The number of lead SNVs identified across the genome for the 5 metabolites with MR $P_{FDR} < 0.05$ ranged from 20 (Omega-3 Fatty Acids to Total Fatty Acids (%)) to 30 (Docosahexaenoic Acid) (Table S10). Docosahexaenoic Acid had strong evidence for colocalization (PP.H4 = 0.89) with MDD at SNV rs2727271 (Table 2), which is an intronic variant for the *Fatty Acid Desaturase II* (*FADS2*) gene (Figure 3). The rest of the metabolites showed weak evidence of colocalization with MDD (PP.H4 < 0.2) (Table S11; Figure S11).

MR and colocalization with expression Quantitative Trait Loci Data

Transcriptomic data were used in downstream MR analysis with MDD for the genes Myelin Regulatory Factor (*MYRF)*, Flap endonuclease 1 (*FEN1)*, Transmembrane protein 258 (*TMEM258*) and Fatty Acid Desaturase 1-3 (*FADS1*/2/3) (Figure 3D), as these were both within DHA-MDD colocalized region on chromosome 11 and had eQTL data available from GTEx. mRNA-MDD MR analysis found the mRNA levels of *MYRF*, *TMEM258, FADS1*, *FADS2* and *FADS3* to have significant positive causal effect on lifetime MDD status (OR range: 1.01 to 1.12, $P = 7.58x10^{-7}$ to 0.011) (Table 3: Figure S12). In contrast, mRNA levels of *FEN1* were not significantly causal to MDD status (OR = 0.98, P = 0.62). Colocalization analysis for the FADS cluster eQTLs and MDD found minimal evidence of shared causal variant for both gene expression and MDD. Supporting evidence was strongest for the existence two independent causal variants for each trait at the same locus (PP.H3 > 0.7) (Table S12).

Discussion

This study reports a large-scale (N = 29, 757) association and causal analysis of blood metabolites with MDD. We found that 191 metabolites were significantly associated with lifetime MDD status, 129 of which withstood further adjustment for common confounders (39). These findings provide greater confidence that the significant metabolite-MDD associations observed in this study are not due solely to the effects of confounding. Furthermore, we found that 144 metabolites showed a significant genetic correlation with MDD, suggesting a shared genetic architecture between the traits. Bidirectional MR analyses found evidence for potentially causal relationships between five metabolite measures relating to long-chain polyunsaturated fatty acids (LC-PUFAs) and MDD. Complementary colocalization analyses between these metabolites and MDD, found that an Omega-3 Fatty Acid, Docosahexaenoic Acid (DHA) colocalized with MDD within the *FADS* region. Subsequent MR analysis of eQTL data of genes in the FADS cluster found evidence of causality between *MYRF, FADS1, FADS2, FADS3* and *TMEM258* mRNA abundance and MDD. However, complementary colocalization analysis found evidence that this locus contains separate independent causal variants for mRNA abundance and MDD.

The MR analysis provides evidence that Omega-3 LC-PUFAs may have a negative causal effect on lifetime MDD, whilst the ratio of Omega-6: Omega-3 LC-PUFAs may have a positive causal effect on lifetime MDD. Specifically, colocalization analysis provided further support for a shared causal variant between MDD and Docosahexaenoic Acid. Research on Omega-3 and Omega-6 levels and MDD has been lengthy and complex (25,56-61). Although many cross-sectional and prospective studies have observed a reduction in Omega-3 fatty acids, and an increase in the Omega-6: Omega-3 ratio in those with MDD (30,57,62), it is important to note both the considerable methodological heterogeneity and contradictory evidence (30,60,63). Clinical trial studies testing the efficacy of Omega-3 supplements in the prevention and treatment of MDD also show inconsistent findings (64-67). Of note, our findings contradict results from a two-sample MR study in 2019, which found no evidence of a significant causal effect of Omega-3 levels on MDD (68). However, this study leveraged summary statistics from smaller GWAS, and therefore may have been underpowered to detect causal effects. The enzymatic oxygenation of Omega-3 and Omega-6 fatty acids and their derived eicosanoids have opposing anti-inflammatory and pro-inflammatory effects respectively (Figure S13). Importantly, humans do not show endogenous biosynthesis of active Omega-3 and Omega-6 fatty acids (EPA and ARA respectively) and the levels of each are reliant on the intake of their precursors LA and alpha-Linoleic acid (ALA) from the diet (69). Over the last century, the ratio of Omega-6: Omega-3 has increased dramatically in many western diets (20-30:1 compared to 1:1) (70), resulting in an imbalance in pro-inflammatory and pro-resolving mediators that may contribute to MDD pathology (71). Furthermore, there is a *FADS* haplotype associated with more efficient LC-PUFA synthesis from the diet (72). Whilst this is evolutionarily advantageous in environments with limited LC-PUFA availability, it may heighten the imbalance between Omega-6 and Omega-3 levels resulting from modern western diets (72). Overall, this indicates the possibility that disproportionate dietary levels of Omega-6: Omega-3 may be contributing to MDD through increased inflammatory processes, and that these may be exacerbated by certain genetic haplotypes.

The post-hoc MR analysis found evidence of a causal association between *MYRF, FADS1, FADS2, FADS3* and *TMEM258* mRNA levels and MDD. MYRF and TMEM258 each have plausible mechanisms for their association with MDD, due to links to abnormal myelination and intestinal stress respectively (73-75). The FADS enzymes, however, are directly linked to the metabolism of both Omega-3 and Omega-6 LC-PUFAs (76,77), and have been shown to be central regulators of the ratio of their respective anti-inflammatory and proinflammatory lipid mediators (78). Therefore, this suggests FADS enzymes act as key regulatory molecules in the activation and resolution of inflammatory processes, which may be important in the pathophysiology of MDD. A recent GWAS of Bipolar Disorder of Japanese populations also found a genetic association with *FADS* intron variants (79), indicating that this association may not be specific to MDD, but rather shared across different psychiatric disorders. Although this aligns with evidence that MDD and Bipolar Disorder share genetic architecture (80), whether the disorders share the putative causal effect of FADS found in this study would require additional analysis dissecting the causal relationship of *FADS* cluster on Bipolar Disorder.

Despite these putative causal mechanisms, complementary colocalization analysis between all eQTLs and MDD found evidence that each trait had distinct causal variants in the locus. This indicates that the instrumental variable in MR exhibits horizontal pleiotropy on the outcome (a direct violation of the exchangeability assumption for MR), thus weakening the evidence for causality (81,82). Therefore, it cannot be determined with high confidence that the transcript abundance of the genes have a direct causal relationship with MDD, but rather may be commonly seen alongside the presentation of MDD. Despite these putative causal mechanisms, complementary colocalization analysis between all eQTLs and MDD found evidence that each trait had distinct, rather than shared, causal variants at the locus. This indicates that the instrumental variable for FADS gene cluster gene expression abundance may be in LD with the MDD causal variant in the region, suggesting independent genetic effects on both traits at the same locus (81,82). We cannot confidently conclude that transcript abundance of FADS region genes has a direct causal relationship with MDD.

This study overcomes various limitations that existed in previous metabolomic analyses of MDD. Firstly, this study analysed the association and causal relationship between MDD and 249 metabolites in a hypothesis-free manner, and therefore reduces the possibility of confirmation bias, which has been criticised in previous studies with an a priori focus on LC-PUFAs (25). Secondly, previous studies often had small sample sizes, each with distinct study methodology and often suffering from self-report bias (30). This study utilises a large sample size from which metabolite levels were biologically measured in the blood using NMR spectroscopy in a standardized, well-documented procedure (35,37). Our findings are also strengthened by the integration of eQTL data from GTEx v8 (54), which interrogated the role of the gene-products from the colocalized *FADS* region.

This study is limited as it tests the association of MDD with plasma metabolite levels, despite MDD primarily being a brain-based disorder. However, blood samples are minimally invasive to collect, which is essential for enabling large-scale high-powered studies and affords them the most promise for future clinical applications. Another limitation to this analysis is the high LD within the *FADS* region (72), meaning that a direct violation of the exclusion-restriction assumption in MR may exist, generating false-positive results (83). However, the complementary use of MR and colocalization analysis in this study acts as a sensitivity analysis (81). Another limitation is the focus of this analysis on European ancestry, especially as the frequencies of the *FADS* haplotypes differs geographically. The divergent *FADS* haplotypes are more commonly seen in non-European populations (84), and are associated with an increased expression of *FADS1*, and a more efficient LC-PUFA synthesis (72). Consequently, the mechanistic effects hypothesised in this study may be greater in non-European ancestral groups which are not currently captured in this analysis. Additionally, although this is a large-scale metabolomic analysis, it is not wholly comprehensive for the human metabolome (85). Indeed, the recent human metabolome database (86) detailed 18,557 metabolites which had been quantified and estimated the presence of thousands more. Finally, as this study focused MDD prevalence, it may be biased by cases of chronic MDD within the cohort (87). Future work should therefore investigate whether Omega-3 Fatty Acid levels and the Omega 6: Omega 3 ratio associate with incident MDD.

In summary, this study interrogated the links between the metabolome and MDD and indicated a protective role of Docosahexaenoic Acid against MDD. Furthermore, this study also suggests a role of an increased ratio of Omega-6: Omega-3 fatty acids in the pathophysiology of MDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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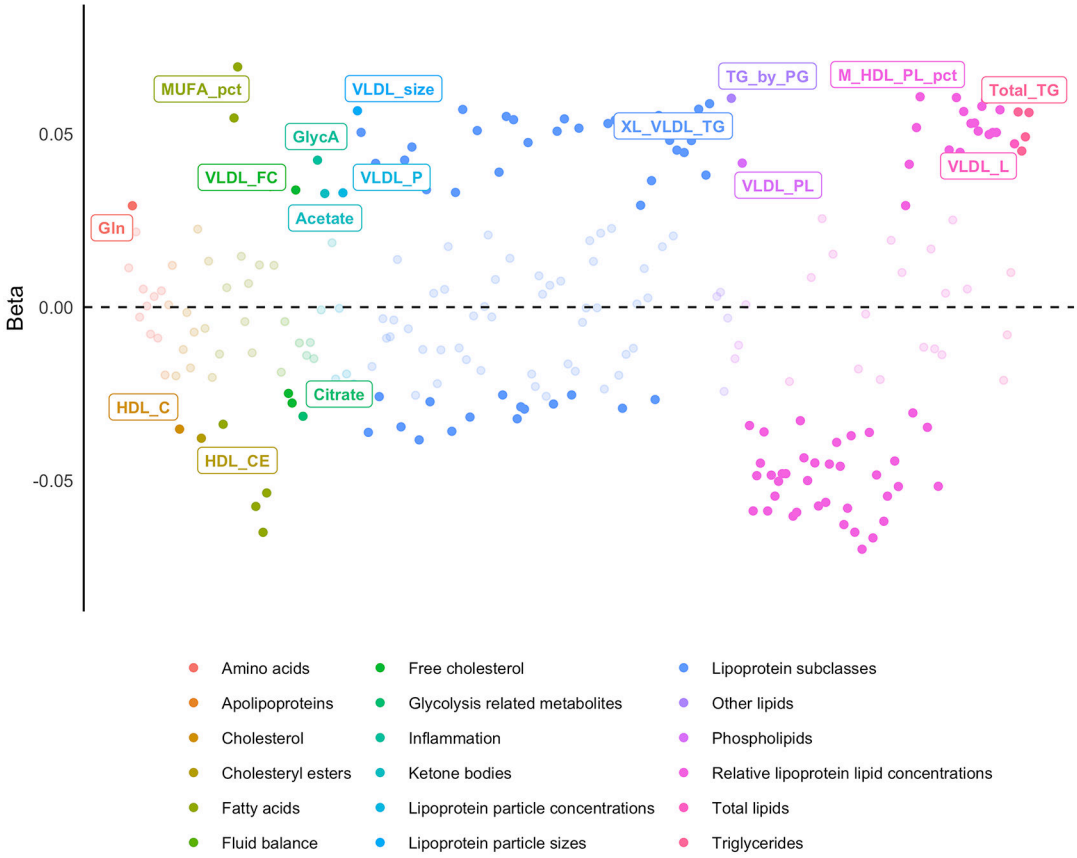

Figure 1: Adjusted MetWAS.

Standardized effect sizes for Metabolite ~ MDD + covariates. Significant associations ($P_{FDR} < 0.05$) are shaded in, and the most significant association for each metabolite group are labelled.

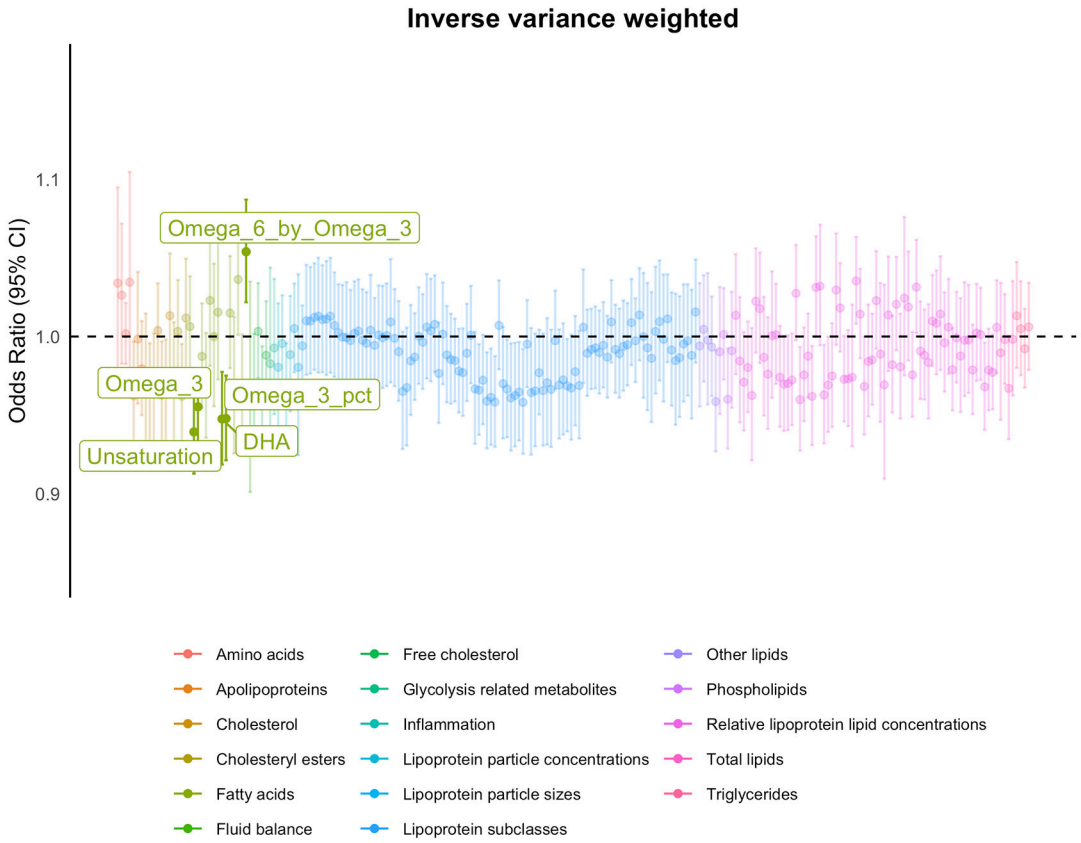

Figure 2: Metabolite to MDD Mendelian Randomisation (MR) analysis.

The odds ratios of metabolite on lifetime MDD status given by the inverse variance weighted MR method, coloured by metabolite group. Significant MR findings ($P_{FDR} < 0.05$) are filled in and labelled. DHA; Docosahexaenoic Acid, Unsaturation; Degree of Unsaturation, Omega_3_pct; Omega-3 to Total Fatty Acids (%), Omega-3; Omega-3 Fatty Acids, Omega_6_by_Omega_3; Omega-6: Omega-3 Ratio.

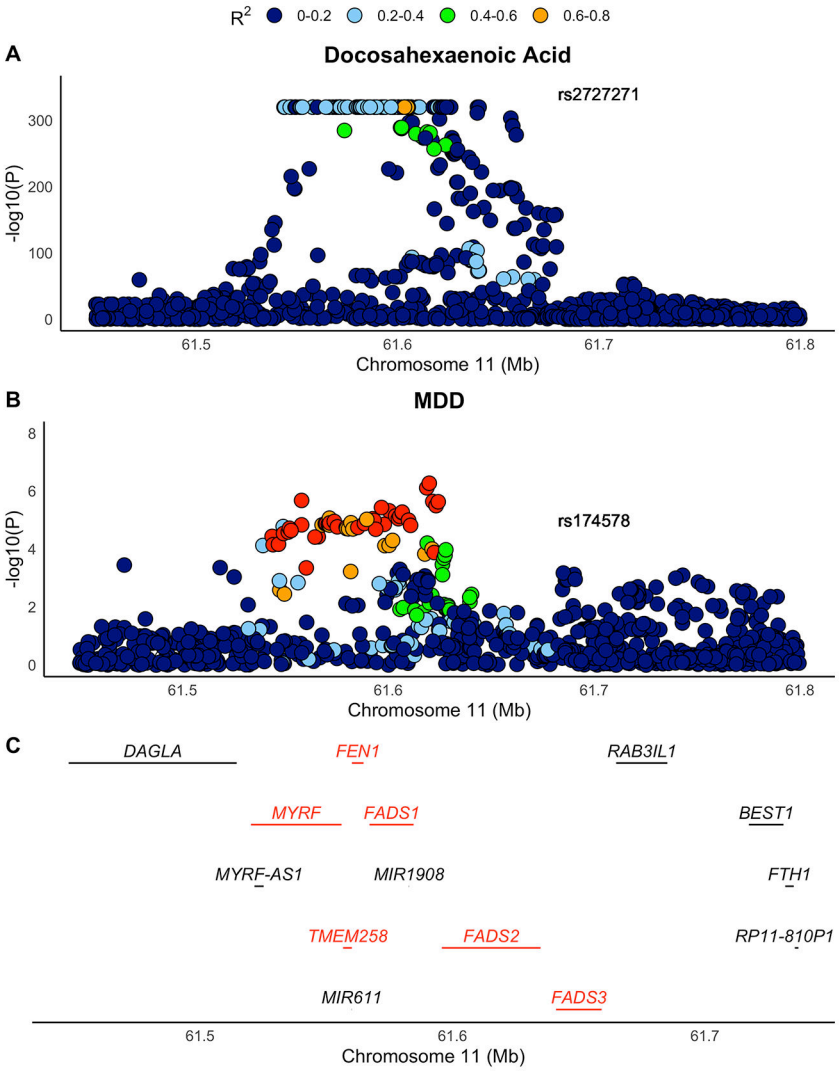

Figure 3: Colocalization of Docosahexaenoic Acid with MDD.

A) The association of SNVs in the FADS cluster in the Docosahexaenoic Acid GWAS. B) The association of SNVs in the FADS cluster in the MDD GWAS (Without UKB). C) The genes present in the FADS cluster. Genes colored red are those which were present in the GTEx data and consequently tested for a causal relationship with MDD in the post-hoc analysis.

Table 1:
Demographics of MDD Cases and Controls.

The demographics of MDD cases and controls are displayed (rows: MDD cases and MDD controls). Age, BMI, and SES are displayed as the mean values within the cohorts. % Ethnicity shows the ratio of White, Mixed and Other populations (Supplementary Information: Materials and Methods: Covariates).

	MDD controls	**MDD cases**
N	20917	8840
Age	56.67 (SD = 7.67)	54.12 (SD = 7.55)
BMI	26.48 (SD = 4.18)	27.26 (SD = 5.12)
% Female	49.95	68.82
SES	−1.92	−1.37
% Never Smoked	59.72	52.58
% Ethnicity	97:0.45:2.2	97:0.7:2
% Attended University/College	46.36	45.67

Table 2:
Significant Metabolite to MDD MR results.

All three MR methods for the five metabolites which showed a significant causal relationship with MDD in any MR method. Colocalization analysis was performed for each metabolite for multiple lead SNVs, the top colocalization result for each metabolite is reported in this table under PP.H4. The full table of colocalization results is found in Table S10. (Egger = MR Egger, WM = Weighted Median, IVW= Inverse Variance Weighted).

Metabolite	Method	N (SNPS)	Beta	SE	P	P_{FDR}	PP.H4
Degree of Unsaturation	Egger	25	−0.063	0.021	$6.20x10^{-3}$	0.89	0.026
	WM	25	−0.069	0.016	$1.20x10^{-5}$	0.0015	
	IVW	25	−0.063	0.015	$1.80x10^{-5}$	0.0041	
Omega-3 Fatty Acids	Egger	23	−0.044	0.021	$5.10x10^{-2}$	0.89	0.13
	WM	23	−0.068	0.016	$1.30x10^{-5}$	0.0015	
	IVW	23	−0.046	0.013	$3.30x10^{-4}$	0.025	
Docosahexaenoic Acid	Egger	24	−0.066	0.025	$1.60x10^{-2}$	0.89	0.89
	WM	24	−0.078	0.019	$4.50x10^{-5}$	0.0034	
	IVW	24	−0.054	0.016	$6.80x10^{-4}$	0.039	
Omega-3 Fatty Acids To Total Fatty Acids (%)	Egger	23	−0.057	0.021	$1.30x10^{-2}$	0.89	0.16
	WM	23	−0.063	0.018	$4.80x10^{-4}$	0.022	
	IVW	23	−0.054	0.014	$2.20x10^{-4}$	0.025	
Omega-6 Fatty Acids to Omega-3 Fatty Acids ratio	Egger	18	0.062	0.026	$3.10x10^{-2}$	0.89	0.15
	WM	18	0.067	0.019	$4.70x10^{-4}$	0.022	
	IVW	18	0.052	0.016	$8.80x10^{-4}$	0.04	

Table 3:
Expression quantitative trait loci to MDD Wald Ratio MR Results.

These results indicate a positive causal effect of *FADS1, FADS2, FADS3, MYRF* and *TMEM258* on lifetime MDD status. OR: Odds Ratio, CI: Confidence Intervals.

Gene	SNV	OR	LOW CI (95%)	HIGH CI (95%)	MR_P
FADS1	rs174567	1.08	1.05	1.10	$7.58x10^{-7}$
FADS2	rs968567	1.01	1.01	1.02	$6.33x10^{-5}$
FADS3	rs2524288	1.05	1.01	1.10	0.010995
FEN1	rs10897119	0.98	0.89	1.07	0.617075
MYRF	rs198462	1.03	1.01	1.05	0.000607
TMEM258	rs7943728	1.12	1.06	1.19	$5.78x10^{-5}$