Omega- 3 Fatty Acids and Major Depression: A Mendelian Randomization Study

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Abstract

Objective: Omega-3 fatty acids have been implicated in the aetiology of depressive disorders, though trials supplementing omega 3 to prevent depression have so far been unsuccessful. Whether this association is causal remains unclear. Methods: We used two sample Mendelian Randomization (MR) to help inform causal inference. Genetic variants associated with circulating omega-3 and omega-6 fatty acids in UK Biobank (UKBB, n=115,078) were selected as exposures. The Psychiatric Genomics Consortium (PGC) Major Depressive Disorder (MDD, n=430,775; cases=116,209; controls=314,566) and recurrent Major Depression (rMDD, n=80,933; cases=17,451; controls=62,482) genome wide association studies (GWAS) were used as outcomes. Multivariable (MVMR) models were used to account for biologically correlated lipids, such as high- and low-density cholesterol and triglycerides, and to explore the relative importance of longer chain omega 3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) using data from the Cohorts for Heart and Aging (CHARGE, n=8,866). Genetic colocalization analyses were used to approximate the probability of a shared underlying causal variant between traits. Results: Genetically predicted total omega-3 fatty acids reduced the odds of MDD (ORIVW 0.96 per standard deviation (SD, i.e. 0.22mmol/l) (95% CIs 0.93-0.98, p=0.003). The largest point estimates were observed for eicosapentaenoic acid (EPA), a long chain omega-3 fatty acid (OR_{EPA} 0.92; 95% CI 0.88 to 0.96; p=0.0002). The effect of omega-3 fatty acids was robust to MVMR models accounting for biologically correlated lipids. ‘Leave-one-out’ analyses highlighted the FADS gene cluster as a key driver of the effect. Colocalization analyses suggested a shared causal variant using the primary outcome sample, but inconclusive, meaning genomic confounding could not be fully excluded. Conclusions: This study supports a role for omega-3 fatty acids, particularly EPA, in the aetiology of depression, although pleiotropic mechanisms cannot be ruled out. The findings support guidelines highlighting the importance of EPA dose and ratio for MDD, and the importance of adequate power to detect modest effect sizes.

Introduction

The association between omega-3 fatty acids and depression has been a focus of research for decades, but uncertainty about its true nature remains. While observational studies suggest inverse associations between omega-3 fatty acids and depression,[1, 2] trials supplementing omega-3 fatty acids have yielded mixed results.[3-7] Long-chain omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are widely regarded as important for brain health.[8] As a major source of long-chain omega 3 fatty acids is fish consumption, observational associations between circulating omega-3 fatty acids and depression are heavily confounded, and reverse causality from poor dietary intake in depressed individuals is likely.[9] Since omega-3 fatty acids compete with omega-6 fatty acids for shared metabolic pathways, further analytical complexity is introduced, as high omega 6 fatty acid intake reduces longer-chain omega 3 fatty acid production.[10] High omega 6:3 fatty acid ratios- a feature of typical Western diets- have been associated with mood disorders in cross-sectional[1] and longitudinal studies.[11]

Trials using omega 3 supplements for preventing depression have yielded inconsistent results.[3, 4] Good quality trials are expensive and time consuming, especially for depression prevention. However, two recent well powered factorial trials have cast doubt over whether omega 3 fatty acids prevent depression, and by

extension their possible role in its aetiology.[6, 7] In particular, the recently published VITAL-DEP trial identified an increased risk of depression from 1g fish oil per day over 5 years (HR 1.13 (1.01-1.26), p=0.03, n=9,171.)[7] The VITAL-DEP inception and recruitment preceded recent treatment guidelines advocating EPA predominant formulations, which were based on mounting evidence.[12] The authors acknowledged this potential limitation, though it remains unclear how these factors might have influenced results.

Given that major depressive disorder (MDD) is the leading cause of disability worldwide, identifying potentially modifiable factors for depression risk may have significant beneficial effects at a population level. Mendelian randomization (MR) is increasingly used in epidemiology to investigate causality by using genetic variants as 'instrumental variables' for exposures.[13] MR exploits the random allocation of alleles at conception to reduce the impact of confounding and reverse causation.[14] Two previous MR studies have investigated omega-3 fatty acids in depression.[15, 16] One among pregnant mothers in the Avon Longitudinal Study of Parents and Children using 5 DHA SNPs (RD -0.09 (-0.23 - 0.05) p=0.20, n=2,378),[15] and another using 7 omega 3 SNPs in the Psychiatric Genomics Consortium (PGC) MDD sample (OR 0.94; 95% CI 0.87-1.17; p=0.16, n=480,359)), and the Netherlands Study of Depression and Anxiety cohort (OR 1.01, 95% CIs 0.90-1.14, p=0.83, n=2,047).[16] Although neither study identified strong evidence for a causal effect, confidence intervals meant they were unable to exclude the presence of a clinically important effect. The latest GWAS of omega fatty acids includes nearly five times the sample size of the previous largest, increasing the power to detect modest, but potentially important effects.[17] We undertook a comprehensive two sample MR study of multiple fatty acids in MDD[18] and recurrent depression (rMDD)[19] to explore whether genetically predicted omega-3 fatty acids reduced depression risk.

Methods

Data Sources

Genetic Association Data for Fatty Acids

Single nucleotide polymorphisms (SNPs) were selected from GWASs of circulating measures of omega fatty acids from 115,078 participants in the UK Biobank (UKBB)[17] (table 1). Omega-3 fatty acid exposures included total omega-3 fatty acids, (mmol/L), percentage of omega 3 to total fatty acids (%) and DHA. Total omega-6 and its longer chain derivative linoleic acid (LA) were used as comparators. One standard deviation (SD) of each exposure corresponded to a change of 0.22 mmol/l of total omega 3, 0.08mmol/l for DHA, 1.56% for omega 3 (%), 4.45 mmol/L of total omega-6 and 3.41 mmol/L of LA.

As EPA was not measured within UKBB, we used SNP- exposure effect sizes from fatty acid GWASs from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (n=8,866), for EPA analyses, and in multivariable models with DHA.

Genetic Association Data for Depression

Outcome data were obtained by permission from the PGC MDD working group. The PGC MDD sample[18] contains genotype data from 807,533 participants (Supplementary material S3, table 1). As the instruments for

our primary analyses were derived from UKBB, we used a PGC MDD subsample with UKBB participants removed, leaving 135,458 cases and 344,901 controls for our primary analyses. We compared our findings to analyses using the complete PGC cohort (including UKBB participants) to confirm results and maximise power (246,363 cases and 561,190 controls). This also enabled us to observe whether our findings were affected by sample overlap, as this was unavoidable for our secondary outcome measure (recurrent MDD, rMDD).

Summary statistics for rMDD were obtained from a PGC subsample, based on DSM-5 diagnoses from an online mental health questionnaire in UKBB.[19] This GWAS classified rMDD cases as individuals reporting multiple depressive episodes across their lifetime (n=17,451) and controls as those with no prior episodes of depression (n=63,482).[19]

Selection of Fatty Acid Instruments

SNPs were selected as potential genetic instruments if they reached traditional genome-wide p-value thresholds in the relevant GWAS ($p< 5 \times 10^{-8}$). SNP sets were clumped to remove those in high linkage disequilibrium (threshold $r^2<0.001$), using the 1000 genomes European LD reference panel. Instruments were standardised to reflect a standard deviation change in each exposure. Full details of methods and SNP sets for all exposures are provided in supplementary material (S1/S2).

Statistical analysis

Analyses were undertaken in R 4.0.2[20]. Genetic instruments were identified using the ieugwasr package (version 0.1.5), using the publicly available GWAS data from the IEU Open GWAS project.[21]

The TwoSampleMR analysis package (version 0.5.6)[22] was used to harmonise the SNP-exposure and SNP-outcome data and derive odds ratio (OR) estimates for each SNP (Wald ratios) per standard deviation change in the exposure (SD mmol/L). OR_{Wald} estimates were pooled to generate Inverse Variance Weighted (OR_{IVW}) estimates, used as our primary method. MR-Egger, weighted median, weighted mode and MR-RAPS (Robust Adjusted Profile Score) were used as sensitivity analyses to check for consistency across methods.

Scatter-plots, forest plots and leave-one-out analyses for individual fatty acids, and a forest plot combining all fatty acid OR_{IVW} estimates, were created using the ggplot2 R package[23]. Cochran's and Rucker's Q were calculated to investigate heterogeneity across instruments in each analysis.

Validation of Findings

As many of the SNPs identified in the omega-3 fatty acid GWAS map to genes which are either of unknown relevance to omega-3 fatty acid metabolism, or thought be related to lipoprotein metabolism more broadly, we undertook several supplementary analyses to investigate consistency, biological mechanisms and potential pleiotropy. Statistical power for these analyses was variable, so the primary aim was to check consistency in magnitude of effect across results.

Firstly, we undertook reverse MR and Steiger Filtering[24] to investigate the direction of effect. Secondly, we restricted analyses to a key biologically plausible pathway, using a SNP on the *FADS* gene cluster, known to relate to omega fatty acid desaturation.[25] Thirdly, we used multivariable MR (MVMR),[26] to investigate whether the effects could be driven by pleiotropic effects on genetically correlated lipids, and to explore the comparative effects of longer chain fatty acids- EPA and DHA- on MDD. We used genetic colocalization[27] to estimate the probability of a shared causal variant in the *FADS* region underlying variation in both circulating omega 3 and MDD. Finally, we undertook a phenome wide association study (PheWAS) of the lead *FADS* SNP driving the apparent causal effect on depression (*rs174564)* to consider potential biological pathways, mediating phenotypes and sources of pleiotropy downstream of that SNP.

Further methodological details are provided in supplementary material S1.

Results

Genetic Instruments

Genetic instruments for fatty acid exposures explained between 3.2% to 9.3% of the variance of each exposure (see supplementary material S3). Mean F-statistics were between 61-241 for fatty acid exposures, and 15-6,315 for *FADS* analyses, suggesting our primary analyses were unlikely to be biased by weak instruments (Table S4).

Major Depressive Disorder

MR results are summarised in Table 2 and Figure 1, showing that genetically elevated omega 3 reduced MDD risk across all measures. Point estimates for longer chain fatty acids EPA (OR_{IVW} 0.92 (95% CI: 0.88-0.96) p=0.0002) and DHA (OR_{IVW} 0.95 (0.92-0.98) p=0.001) were larger than for total omega 3 (OR_{IVW} 0.96 (0.93-0.98) p=0.003), or omega 3 (%) (OR_{IVW} 0.96 (0.93-0.98) p=0.0002), though confidence intervals overlapped.

Single SNP analyses suggested the *FADS* gene cluster was a major contributor to the observed effects of omega 3 on MDD (see supplementary material S8). Omitting the *FADS* SNP (*rs174564*) in 'leave one out' analyses attenuated the effect sizes and widened the confidence intervals to include the null in all analyses, though the effect direction remained consistent, especially for the longer chain fatty acids (OR_{EPA} 0.94 (0.86-1.10) p=0.20, and OR_{DHA} 0.98 (0.93-1.03), p=0.39. See S8). UKBB sample overlap did not alter point estimates, but as expected confidence intervals were narrower using the complete PGC MDD sample (n= 807,553) compared to the sample with UKBB removed (n=480,539, see S5). Individual sensitivity plots for each fatty acid on MDD are presented in the supplementary information (S8).

We identified no evidence that genetically elevated omega-6 fatty acids altered MDD risk (OR_{IVW} per SD increase in total omega-6: 1.01 (95% CI 0.97-1.05), p=0.60, and OR_{IVW} for LA 1.01 (0.97-1.05). p=0.57), see S6.

Recurrent Major Depression (rMDD)

MR analyses using our secondary outcome measure supported the contrasting findings for omega-3 and omega-6 fatty acid measures, although confidence intervals were wider, as expected from the smaller sample

size (Table 2 and Figure 1).

Point estimates for omega-3 fatty acid measures were marginally larger for rMDD than for MDD, although not statistically different. For each SD increase in total omega-3 the odds of rMDD decreased: OR_{IVW} 0.94 (95% CIs 0.86-1.02); p=0.13), with similar findings for omega-3%: OR_{IVW} 0.91 (95% CIs 0.83-0.99); p=0.03), DHA: OR_{IVW} 0.91 per SD (95% CIs 0.83-1.00); p=0.06), and EPA: OR_{IVW} 0.91 per SD (95% CIs 0.77-1.08); p=0.27).

Consistency across Methods and Instruments

Results for all omega 3 analyses were consistent across MR methods, increasing confidence in the findings (see table 2, table S5 and individual scatter plots S8). As a large proportion of the variation in omega 3 fatty acids was explained by the *FADS2* SNP, weighted median and mode results were largely reflective of the *FADS2* Wald ratio (see S5). Heterogeneity was substantial for most analyses (total omega-3 (Q =55, p=0.01), DHA (Q=55, p=0.05) omega-3% (Q=33, p=0.39) and EPA (Q=63, p=0.01). While we did not find evidence for directional pleiotropy- with Egger intercepts close to zero- a wide confidence interval meant we were unable to rule it out.

Establishing Directionality

As all omega-3 instruments used in the primary analyses explained greater variance on the exposure than the outcome, Steiger filtering did not remove any SNPs, and results were unchanged. Reverse MR provided no evidence that genetic liability to depression lowered circulating omega-3 levels ($beta_{IVW}$ 0.03 SD per doubling of genetic risk (-0.02, 0.09) p=0.23).

Biological Pathways

As the *FADS* SNP (*rs174564*) explained a large proportion of variance in the omega-3 measures, omega-3 analyses restricted to the single *FADS* SNP were expectedly similar in direction and magnitude to the multi-SNP analyses (see table 2). Analyses restricted to the *FADS* SNP for total omega-6 suggested an inverse effect on both MDD ($OR_{rs174564}$ = 0.33(0.21-0.5), p=5.73E^{-7}) and rMDD ($OR_{rs174564}$ = 0.17 (0.01-1.99, p=0.16). The reverse was seen for LA, with a strong positive association between genetically increased LA and MDD ($OR_{rs174564}$ = 1.32 (1.16-1.49), p=5.73E^{-7}), and rMDD ($OR_{rs174564}$ = 1.42 (0.87-2.29, p=0.16 (table 2)).

Multivariable Models

Effect estimates for total omega-3 fatty acids were not altered by MVMR analyses when adjusting for other lipids (table 4.) In model 1, which accounted for total omega 6, the OR_{IVW} estimate for total omega 3 was 0.93 (0.90-0.97), p=0.0001. In the second model accounting for triglycerides and cholesterol, the was unchanged from the univariable analyses (OR_{IVW} 0.96 (0.93-0.98) p=0.001).

In MVMR model 3, point estimates for EPA were larger than for DHA, and while adjusting for DHA made little change to the effect size for EPA (MVMR OR_{EPA}0.93 (0.88-0.97) p=0.002), estimates for DHA were attenuated when adjusting for EPA (MVMR OR_{DHA} 0.98 (0.92-1.04) p=0.46, see Table 3, and S7).

Colocalization

Colocalization using our primary outcome sample were supportive of a shared causal variant between omega 3 measures and MDD (PPA = 88.9% for omega 3 and DHA, and 97.1% for EPA, see figure 2 and S9). However, this was not the case when using the sample including UKBB, where the probability for distinct causal variants increased (PPA_{H4} 27.4% vs PPA_{H3} 72.5% for Omega 3 and PPA_{H4} 16.3% vs PPA_{H3}83.6% for EPA. The strongest regional signal for MDD in the sample including UKBB was located on a neighbouring gene (*DAGLA,* r*s198457*), which is in partial LD with the *FADS2* SNP (*rs174564: rs198457* r2= 0.10). These results could be explained either by confounding by LD, or a violation of coloc's assumptions due to the presence of multiple causal variants in the region. Excessive prior variance in the outcome prevented the use of methods relaxing the single variant assumption (see supplementary material).

PheWAS

Over 400 traits were identified in the PheWAS of the lead SNP in the analyses (*rs174564)*, although many of these studies were either highly correlated or repeated measures (see supplementary material S10). As expected for a SNP encoding an important enzyme for lipid metabolism, most phenotypes were lipid measures, with other metabolic, endocrine and haematological measures the next most prevalent. The strongest of the lipid measures were included in MVMR models to account for potential bias due to horizontal pleiotropy.

Discussion

Our results suggest a protective effect of multiple genetically predicted omega-3 fatty acid measures on MDD and its recurrence, with little evidence that this is driven by the effect of depression on circulating omega 3. The importance of *FADS* gene cluster mirrors previous MR results in schizophrenia,[25] suggesting an important physiological role for the synthesis of longer omega-3 fatty acids in preventing mental illness. Effect sizes are modest, particularly as they reflect a lifetime of exposure. Supplementation trials will need to ensure adequate power to detect these effects. Our results are consistent with point estimates from previous MR studies of omega-3 fatty acids in depression, which although lower in statistical power, showed similar magnitudes of effect and consistency across methods.[15, 16] Large GWAS samples of both fatty acids and MDD provided adequate power to detect modest causal effects, highlighting the power of increasing genetic sample sizes for MR studies in nutritional psychiatry.

The *FADS* gene cluster appeared to be a strong driver of the effect. On one hand this adds weight to the biological plausibility of causal inference, being involved a rate limiting step in the elongation of short-chain fatty acids to the long-chain derivatives important for brain health (such as EPA and DHA). However, as the *FADS* gene cluster affects multiple fatty acids and other complex lipid metabolic processes,[10] it is difficult to rule out horizontal pleiotropy, in which the effect of the *FADS* SNP on MDD is mediated by mechanisms other than omega 3. Underlying pleiotropy may be suggested by the high heterogeneity statistics. To further investigate potential pleiotropy, we used MVMR models to account for other lipids, which preserved a strong effect of omega-3 on MDD. Our supplementary PheWAS of the lead *FADS* SNP identified many potential sources of pleiotropy, though some of these measures may be downstream effects of insufficient omega 3. Studies showing improved lipid profiles among omega 3 supplemented animals,[28] and the introduction of EPA treatments to reduce hypertriglyceridemia in humans[29] may support this. Colocalization analyses were suggestive of a shared causal variant between omega 3 and MDD for our primary outcome sample, though not

for all outcomes. This may be a due to multiple conditionally independent SNPs in the region, violating the assumptions of colocalization, or it could be that confounding by linkage disequilibrium is driving the MR results.

Previous research has explored the role of the *FADS* gene cluster in schizophrenia,[25] response to omega-3 supplementation,[30] and infant cognition.[31] Studies investigating the *FADs* genotype in depression have been inconclusive, though sample sizes have been comparatively small.[32,33] Using two sample MR with summary genetic data facilitates the use of very large sample sizes, and increased statistical power. However, as with all methods, MR has its limitations and assumptions, which we have attempted to mitigate. A further possible limitation is the complete sample overlap between our exposure measures and our secondary outcome, rMDD. Recent studies have downplayed the importance of sample overlap, especially with large outcome sample sizes.[34, 35] Including UKBB in our primary outcome sample (MDD) did not alter the effects, suggesting bias is minimal (see S5). Furthermore, effect sizes for EPA were extracted from the non-overlapping CHARGE consortium, and results were consistent.

Our results support the rationale underlying the VITAL-DEP trial,[7] but do not explain the reasons behind the increased MDD risk among intervention participants, as EPA dose, ratio, and age of participants (>50 years), would be unlikely to explain these results.[7] MVMR estimates suggesting a likely stronger effect for EPA than DHA, is consistent with meta-analytical evidence supporting EPA predominant formulations, potentially contributing to the unexpected results.[12] One possibility is the combination with vitamin D supplements, as estimates for the combined supplement group (HR 1.23 (1.05-1.44), n=4608), were nominally higher than for omega 3 alone (vs 1.04 (0.89-1.22, n=4563.) Harmful effects resulting from oxidised omega 3 supplements,[36] or potentially adverse effects of excess omega 3, might also contribute to these findings. Further research could consider omega 3 fatty acid toxicity at higher concentrations by using non-linear methods in one-sample MR. The same methodology could also identify whether the modest effect seen in this two-sample study masks a larger threshold effect, by the inherent assumption of a linear relationship. Future interventional research could determine whether recruiting participants with sub-optimal omega-3 fatty acid status, intake, or genetically low short- to long- chain fatty acid conversion, might be superior to universal prevention efforts in depression. Finally, in the era of environmental awareness, the question of how to generate affordable and sustainable long-chain omega 3 fatty acids for a growing global population seems pertinent.

Conclusion

Our results provide evidence for a link between genetically predicted omega-3 fatty acids and MDD. The effect appears strongest for EPA, remains robust to biologically correlated lipids, and is not explained by reverse causality. This strengthens evidence for a causal effect of omega 3 fatty acids on MDD, although horizontal pleiotropy and confounding by linkage disequilibrium cannot be fully excluded. Further research to triangulate these findings and consider potential mediating mechanisms and phenotypes would be valuable. Future trials of omega-3 supplementation for MDD should ensure adequate power to detect modest effects, along with ensuring dose, duration, and participant selection are the most likely to benefit.

Declarations

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Further information about obtaining access to the PGC summary statistics are available from: http://www.med.unc.edu/pgc/statgen.

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Tables

Table 1: Data sources for MR Analyses

	Phenotype	GWAS study	N	Population	Data Access
Univariable MR Exposures	Total Omega 3	Borges[17]	115,078	UK Biobank	https://gwas.mrcieu.ac.uk
	Omega 3 (%)				
	DHA				
	Total Omega 6				
	LA				
	EPA	Lemaitre[37]	8,866	CHARGE Consortium*	https://www.chargeconsortium.com/main/results
Additional Exposures For Multivariable (MVMR) models	Triglycerides	Richardson[38]	441,016	UK Biobank	https://gwas.mrcieu.ac.uk
	HDL-cholesterol		403,943		
	LDL-cholesterol		440,546		
	DHA	Lemaitre[37]	8,866	CHARGE Consortium*	https://www.chargeconsortium.com/main/results
Outcomes	Major Depressive Disorder (MDD)	Howard[18]	430,775	Psychiatric Genomics Consortium (UKBB removed)*	https://www.med.unc.edu/pgc/download-results/ Access to complete MDD summary statistics requires permissions to be obtained from 23andme
			807,553	Psychiatric Genomics Consortium (Complete sample)*	
	Recurrent Major Depression (rMDD)	Coleman[19]	80,933	UK Biobank	

Further details of cohort studies are given in supplementary materials S2

Table 2: Results for two sample Mendelian Randomization using UKBB exposures and PGC MDD sample (without UKBB n=480,359), and rMDD subsample (n= 80,933)

Odds ratios for depression are given per standard deviation increase in exposure. Further details on individual SNPs, instrument strength and measures of pleiotropy are given in supplementary table S2. All SNPs in the analyses had a stronger effect on the exposure than the outcome, so repeat analysis with Steiger Filtering were identical. FADS gene analyses used a single SNP on chromosome 11 (*rs174564*). Further analyses, including weighted median and mode, and the comparison of results with and without the sample overlap for UKBB are contained in the supplementary analyses.

				Major Depressive Disorder (n=480,359)			*Recurrent Major Depression (n= 80,933)*	
	nSNP	Method		OR (95%CI)	p	nSNP	OR (95%CI)	p
OMEGA 3								
Total Omega 3	43	IVW		0.96 (0.93-0.98)	0.003	45	0.94 (0.86 -1.02)	0.13
		Egger		0.95(0.92-0.99)	0.02		0.92 (0.81 -1.05)	0.24
		MR-RAPS		0.96 (0.93-0.99)	0.01		0.94 (0.87-1.03)	0.20
		FADS (rs174564)		0.93 (0.91-0.96)	1.3E-5		0.92 (0.81-1.03)	0.16
Omega 3 %	33	IVW		0.96 (0.93-0.98)	0.0002	33	0.91 (0.83 -0.99)	0.03
		Egger		0.95 (0.92-0.98)	0.002		0.88 (0.78 -1.00)	0.07
		MR-RAPS		0.95 (0.93-0.98)	5.78E-5		0.92 (0.84-1.00)	0.05
		FADS (rs174564)		0.94 (0.92-0.97)	1.3E-5		0.93 (0.84-1.03)	0.16
DHA	40	IVW		0.95 (0.92-0.98)	0.001	40	0.91 (0.83 -1.00)	0.06
		Egger		0.94 (0.89-0.98)	0.01		0.86 (0.74 -1.02)	0.09
		MR-RAPS		0.94 (0.91-0.98)	0.001		0.92 (0.84-1.01)	0.09
		FADS (rs174564)		0.92 (0.89-0.96)	1.3E-5		0.91 (0.84-1.03)	0.16
*EPA**	41	IVW		0.92 (0.88 - 0.96)	0.0002	45	0.91 (0.77 - 1.08)	0.27
		Egger		0.91 (0.87 - 0.96)	0.002		0.85 (0.69 - 1.04)	0.13
		MR-RAPS		0.91 (0.86 - 0.95)	0.0001		0.89 (0.69 - 1.16)	0.39
		FADS (rs174564)		0.91 (0.87 - 0.95)	1.3E-5		0.89 (0.76 - 1.05)	0.16
OMEGA 6								
Omega 6	50	IVW		1.01 (0.97-1.05)	0.60	51	0.97 (0.90 -1.04)	0.44
		Egger		1.01 (0.92-1.06)	0.68		0.89 (0.79 -1.00)	0.06
		MR-RAPS		1.01 (0.97-1.04)	0.66		0.99 (0.92-1.06)	0.70
		FADS (rs174564)		0.33 (0.21 - 0.5)	5.73E-7		0.17 (0.01-1.99)	0.16
LA	50	IVW		1.01 (0.97 - 1.05)	0.57	51	0.94 (0.87 - 1.02)	0.12
		Egger		1.04 (0.96 - 1.14)	0.36		0.89 (0.78 - 1.02)	0.09
		MR-RAPS		1.00 (0.96-1.04)	0.95		0.96 (0.89-1.04)	0.32
		FADS (rs174564)		1.32 (1.16 - 1.49)	5.73E-7		1.42 (0.87 - 2.29)	0.16

Table 3: Multivariable MR Analyses
Multivariable MR analyses of Omega 3 fatty acids with other lipids sharing similar biological pathways. Results are given as odds ratios for MDD per SD increase in exposure. For further details on instruments included in model 3, please see Supplementary Materials S1.

	Exposure	*GWAS*	*nSNP*	*OR*	*p*	*Conditional F*	*Q (p)*
MODEL 1	Omega 3 fatty acids	met-d-Omega_3[17]	43	0.93 (0.90-0.97)	0.0001	268	96 (0.01)
	Omega 6 fatty acids	met-d-Omega_6[17]	49	1.07 (1.02-1.12)	0.01	165	
MODEL 2	Triglycerides	ieu-b-111[38]	388	1.08 (1.03-1.13)	0.002	30	682 (9.88E-18)
	HDL cholesterol	Ieu-b-109[38]	387	1.06 (1.01 -1.10)	0.01	42	
	LDL cholesterol	Ieu-b-110[38]	388	0.98 (0.93-1.03)	0.37	43	
	Omega-3 fatty acids	met-d-Omega_3[17]	387	0.96 (0.93 -0.98)	0.001	25	
MODEL 3	EPA	Lemaitre[37]	40	0.93(0.88-0.97)	0.002	9	52 (0.05)
	DHA			0.98 (0.92-1.04)	0.46	6	

Figures

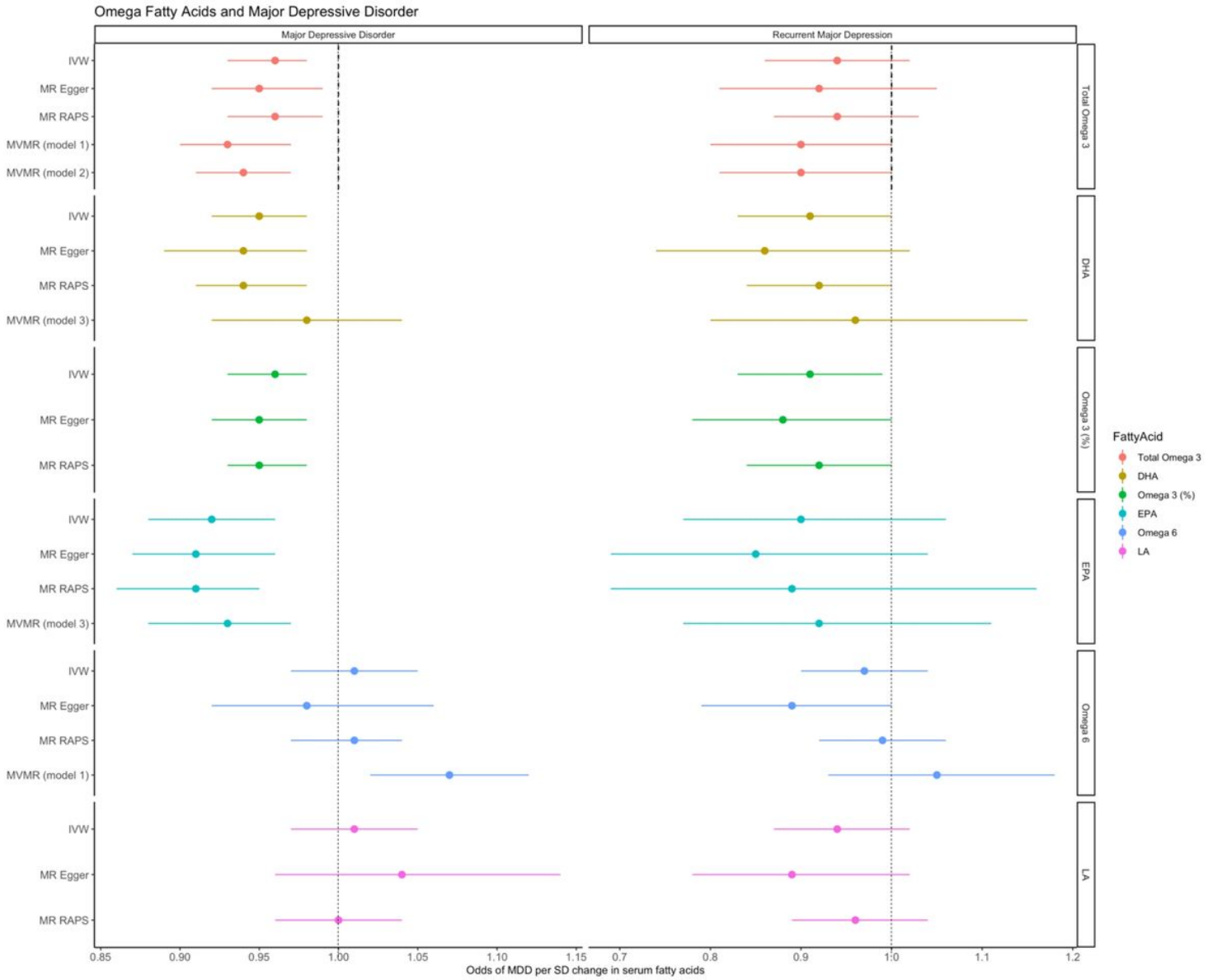

Figure 1

MR results for multiple fatty acid exposures on MDD (n=480,539) and rMDD (n=80,933). The forest plot shows OR for each fatty acid exposure on the two depression outcomes, using different methodologies: IVW (Inverse variance weighted), MR Egger and MR RAPS, with MVMR where appropriate

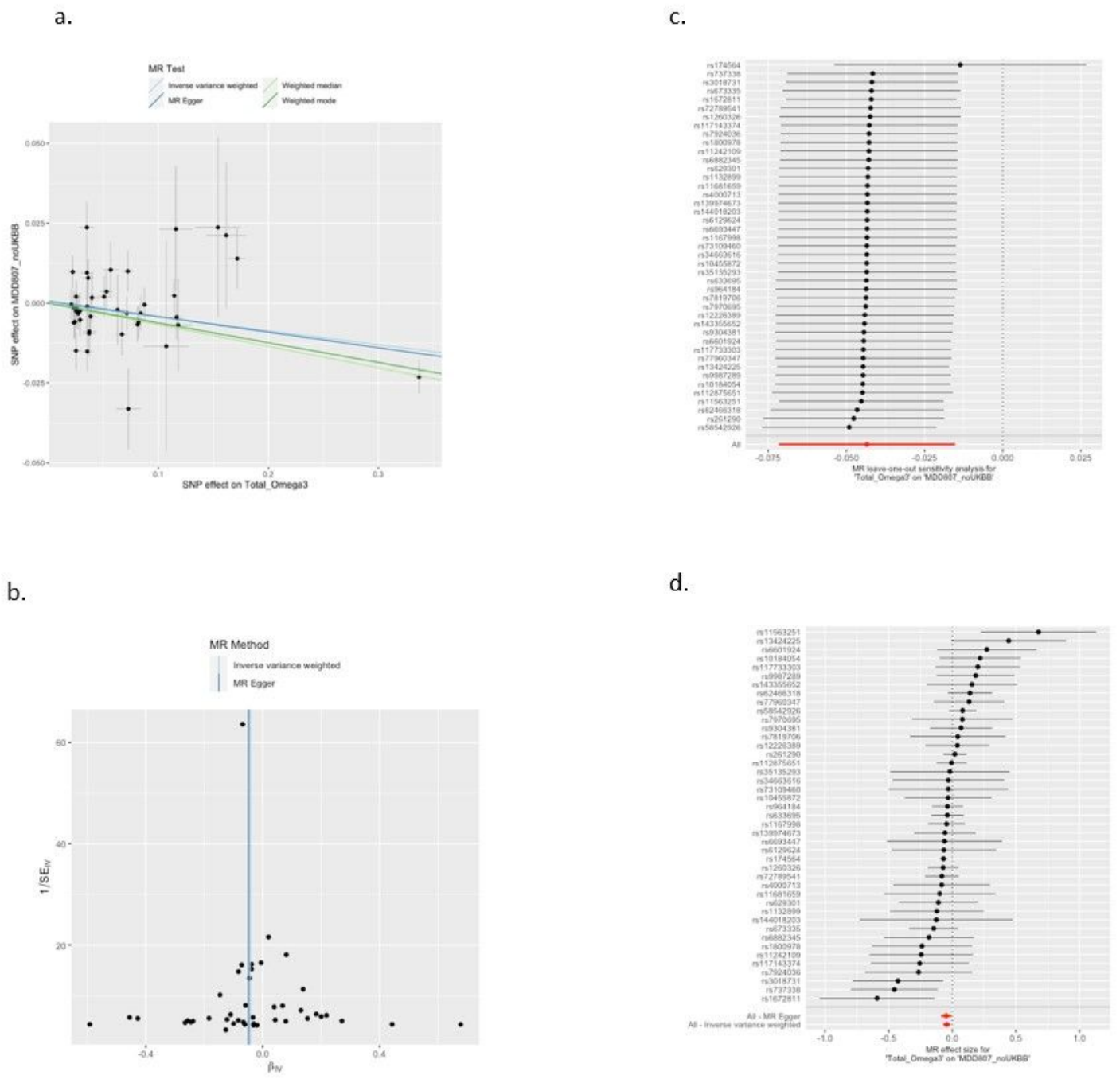

Figure 2

Individual MR sensitivity plots for total omega 3 fatty acids on MDD. Individua MRl sensitivity plots for Omega 3(%), EPA, DHA, Omega 6, and LA are provided in S7.

a. Scatter plot showing how MR estimates compare between MR methods.

b. Funnel plot depicting instrumental variable presicion. The log(odds ratios) of each IV is plotted on the x-axis (β_{IV}) against instrument strength on the y axis (1/ SE_{IV}). Asymmetry may suggest directional pleiotropy.

c. Forest plot showing individual SNP ratio estimates (SNP-outcome estimate / SNP-exposure estimate), and

d. Leave one out plot showing inverse variance weighted (IVW) estimates after omitting each SNP

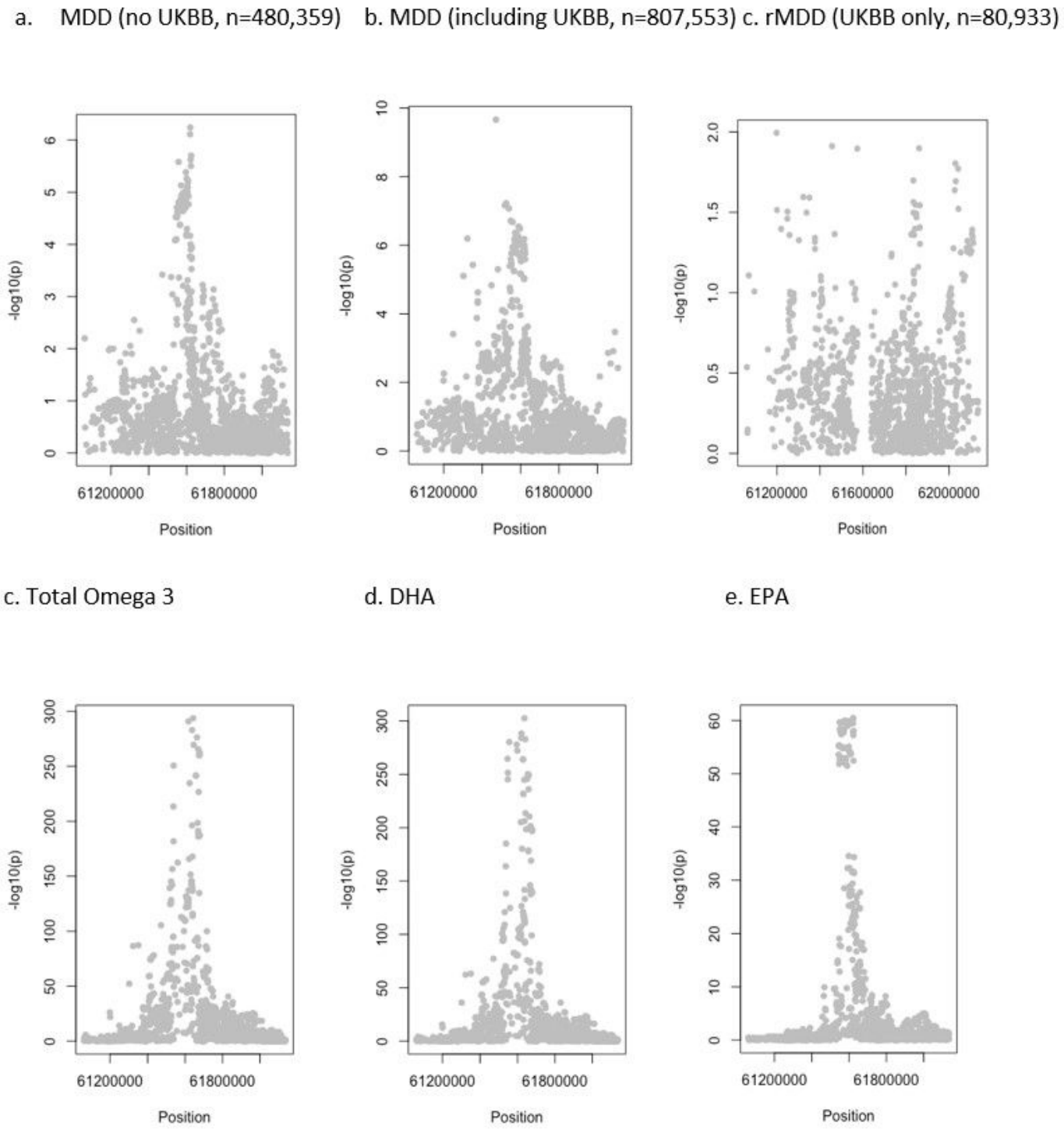

Figure 3

Genetic colocalization plots comparing MDD and omega 3. Funnel plots of the two MDD outcomes (excluding UKBB (a.) and including UKBB (b.)) and the three omega 3 exposure measures, appear consistent with the hypothesis of a shared underlying variant between traits. Colocalization analyses supported this hypothesis

for our primary outcome sample (MDD without UKBB (a.) and each of the omega 3 measures (PPA_{H4} 88.9% for total omega 3 (c.) and DHA (d.), and 97.1% for EPA (e.)). However, despite similar appearances in the plots, results for the MDD sample including UKBB suggested a higher probability that the traits were associated but had distinct causal variants (PPA_{H3} 72.5% for total omega-3 and DHA, and 83.6% for EPA,) with the emergence of a further variant in partial LD with the lead *FADS* SNP (*rs198457*) in the region, located on the *DAGLA* gene, which is in partial LD with the FADS2 variant (r^2 0.1). Excluding the *DAGLA* variant in sensitivity analyses yielded similar results to colocalization using the primary outcome (S9a). Results for rMDD suggested a causal variant for omega traits only, reflective of the low power for these analyses (PPA_{H1} 91.9% for omega 3 and DHA, 92.8% for EPA.) See S9 for further details.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OmegasupplementarymaterialFinal.docx
- Phewas.xlsx