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Exploring causal correlations between oily fish intake and multiple sclerosis: A two-sample Mendelian randomization study

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ABSTRACT

Background and Objectives: According to observational studies, dietary habits may influence the occurrence of multiple sclerosis (MS). There are, however, only a few Mendelian randomization (MR) studies on both. **Methods and Study Design:** This two-sample MR study's objective was to examine possible causal associations between the twenty-one dietary practices and MS. For this investigation, we employed MR analysis utilizing generally accessible statistics from genome-wide association studies (GWAS) to examine causal connections between dietary habits and MS susceptibility among persons of European descent. The IEU Open GWAS project (https://gwas.mrcieu.ac.uk/) provided these GWAS data. Pleiotropy and heterogeneity were investigated using the MR-Egger Intercept test and Cochran's Q test, respectively. MR-Egger, weighted median, inverse variance weighted (IVW), simple mode, and weighted mode were used to assess the causal relationship between 21 dietary habit levels and MS. **Results:** After removing outliers, we found a significant association between genetically induced oily fish intake and MS risk (IVW, OR: 0.557; 95% CI: 0.351-0.884; p=0.013). Extensive sensitivity analyses confirmed this result. Other dietary habits had no discernible relationship with MS risk. **Conclusions:** This Mendelian randomization (MR) analysis provides evidence of an association between dietary patterns and the risk of developing multiple sclerosis (MS). Notably, higher intake of oily fish was associated with a reduced risk of MS among individuals of European ancestry.

Key Words: dietary habits, multiple sclerosis, Mendelian randomization, genome-wide association studies, inverse variance weighted

INTRODUCTION

As an autoimmune disorder, multiple sclerosis (MS) has a predominant involvement in the spinal cord and brain.[1] The condition generally becomes apparent upon the initial stages of adulthood and ranks as the primary contributor to young adult-stage non-traumatic neurological impairments.[2] MS has been on the rise in recent years, though the precise reasons for this remain unknown due to an incomplete understanding of its etiology.

Environmental, nutritional, and lifestyle risk factors, in addition to genetic risk factors, have been shown to contribute to MS risk. A magnified worldwide focus on dietary factors' possibility as MS risk attributes has become recently realized. Numerous studies have provided substantial evidence that dietary factors can influence the risk and symptoms of MS. For example, an Iranian case-control study indicated that adhering to a healthy eating pattern

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may reduce the risk of MS. In contrast, an unhealthy eating pattern may increase the risk.[3] Several other studies have also supported these findings.[4,5] Additionally, a recent observational study found that following a healthy eating pattern was linked to a lower risk of MS.[6]

Although research suggests that adopting healthy eating habits can help prevent and manage MS symptoms, studies on the effects of individual foods on MS have produced inconsistent and inconclusive results. Several case-control papers, for instance, have established higher fish consumption's correlation with mitigated MS risk progression.[7,8] However, observational studies have found no significant link between fish consumption and the risk of MS.[9] Similarly, eating meat products was linked to an increased risk of MS in a factor-analysis study conducted in the United States,[10] but this finding was inconsistent across other studies.[11,12] Likewise, some multicenter case-control studies have found that coffee consumption may have a protective effect against MS,[13] while others have found no significant association.[14] Other potential protective and risk factors identified in observational studies include the consumption of vegetables,[4,12,15,16] fruits,[4,11-13,17-19] nuts,[17] dairy products,[17,20,21] beans,[15,18] cheese,[12,15] and alcohol.[22,23] It is important to note that nutritional epidemiological studies face difficulties in minimizing inconsistent confounders that may influence the relationship between diet and MS, and it is also essential to acknowledge that most previous dietary trials on MS have not been sufficiently robust. It is indeed crucial to amass convincing proof demonstrating that dietary behaviors can either prevent MS onset or alter its progression.

As an epidemiological methodology, Mendelian randomization (MR) employs the distinctive genotype characteristics to examine the causal connection between exposure and outcomes.[24,25] By employing instrumental variables (IVs) as genetic variants that are strongly correlated with exposure and are allocated randomly to populations during meiosis as well as conception, it emulates an environment with randomized controls. Potential confounding variables and reverse causal bias can both be circumvented using MR designs. A number of genome-wide association studies (GWAS) have uncovered evidence of inherited dietary habits.[26,27] Consequently, MR can serve as a suitable research design to evaluate the influence of dietary habits on health outcomes or diseases.[28]

By employing extensive GWAS data alongside MR analysis, we examine 21 dietary habits' correlation with the risk of MS and determine particular diets that might have a causal effect on such risk. This is a pioneer regarding dietary practices' causal association with the risk of

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acquiring MS to be investigated using an MR analysis. Our study could provide a scientific foundation for disease prevention.

MATERIALS AND METHODS

Study design

We examined the causal association of exposure against the results by employing genetic variants to be the IVs in this two-sample MR study. Figure 1 illustrates our MR design flowchart. The Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) checklist was completed for this observational study (Supplementary Table 1).

The selection of dietary exposures in this study was based on two fundamental criteria to ensure both scientific rigor and biological relevance. First, all dietary traits included had to have available GWAS summary statistics from the UK Biobank (UKB), a large, prospective population-based cohort comprising approximately 500,000 participants with extensive genetic and phenotypic data. The UKB dataset is characterized by standardized data collection procedures and stringent quality control measures, which together guarantee the accuracy and reliability of genetic associations with phenotypes. Utilizing high-quality GWAS data allows for the construction of strong genetic instruments, minimizing weak instrument bias and enhancing the statistical power of MR analyses. Second, the selected dietary exposures were required to possess clear biological plausibility, supported by prior epidemiological, clinical, and experimental evidence implicating these factors in the pathogenesis of MS. Specifically, dietary components such as polyunsaturated fatty acids, red meat consumption, and alcohol intake have been shown to influence immune regulation, inflammatory responses, and metabolic pathways relevant to MS development. By integrating evidence from these diverse sources, this study ensures that the chosen exposures are both mechanistically relevant and amenable to robust genetic analysis, thereby strengthening the causal inference regarding diet and MS risk.

Exposure GWAS

This study included a total of 21 dietary exposure variables encompassing a broad spectrum of commonly consumed foods and beverages, which were systematically categorized into five main groups: meat intake (processed meat [N=13,150], pork [N=8,630], lamb/mutton [N=20,900], poultry [N=5,470], and beef [N=1,910]); fish intake (non-oily fish [N=4,458] and oily fish [N=95,507]); beverages and related habits (tea [N=37,189], coffee [N=15,854],

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weekly alcohol intake [N=26,805], alcohol intake frequency [N=169,927], water intake [N=49,416], and hot drink temperature [N=39,738]); staple foods (cereal intake [N=14,330] and bread intake [N=80,777]); and fruits, vegetables, and other foods (dried fruit [N=14,501], fresh fruit [N=62,710], salad/raw vegetable intake [N=7,252], cooked vegetable intake [N=59,215], added salt intake [N=79,294], and cheese intake [N=45,541]). The GWAS summary statistics for these dietary traits were obtained from the UK Biobank (https://www.ukbiobank.ac.uk), which provides detailed information on sample sizes, phenotype definitions, and stringent quality control procedures, thereby ensuring the reliability and consistency of the data. Further specifics, including detailed phenotype descriptions and statistical metrics, are provided in Supplementary Tables 2 and 7. This comprehensive and systematic selection of dietary exposures enables robust Mendelian randomization analyses to explore the potential causal relationships between diet and multiple sclerosis risk.

Outcome GWAS

We developed an instrumental genetic proxy for MS using information taken from the more extensively publicized GWAS from the International Multiple Sclerosis Genetics Consortium (IMSGC). The IMSGC included 68,374 controls and 47,429 MS cases, all European descent.[29]

Selection of instrumental variables in dietary habits

IVs consisting of genetic variants were employed in the MR analysis to examine the causal connection of the exposure against the outcome. Single nucleotide polymorphisms (SNPs) were most prevalent among these IVs. To determine which IVs met the criteria, we devised a sequence of procedures for quality control. First, SNPs significantly associated with each of the 21 dietary habits were chosen as IVs (p <5×10-8). Next, to assure IV independence, we establish linkage disequilibrium (LD) limits of aggregation distance>10,000 kb and r2<0.001. Then, we eliminated palindromic SNPs that exhibit intermediate allele frequencies and SNPs that were absent from the outcome dataset from each MR analysis. Fourth, each SNP was carefully reviewed in the PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk/) to ensure unconfused instrumental variables were only associated with outcomes through associated exposures.

We carefully checked each SNP to see if it was linked to any known confounders that could affect MS risk, using a strict significance cutoff ($p<5\times10^{-8}$). The analysis adjusted for

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multiple confounders including age at menarche, body mass index (BMI), obesity status, smoking habits, and socioeconomic status, as these factors may influence MS risk through immune regulation and inflammatory mechanisms. We used publicly available GWAS data to identify and exclude any SNPs significantly associated with these confounders, helping to avoid bias from horizontal pleiotropy.

This filtering step is key to meeting the core assumptions of MR, ensuring that our genetic instruments are not related to confounders and only impact MS risk through the specific dietary exposures under study. By removing SNPs that might violate these assumptions, we reduce potential bias and strengthen the confidence in our causal conclusions. Overall, this quality control process ensures that the links we find between diet and MS risk are more likely to reflect true cause-and-effect relationships, rather than being driven by confounding factors or pleiotropy. Supplementary Table 3 displays detailed summary statistics for these included SNPs. In addition, F-values were calculated to evaluate weak tool bias using the formula $(N-2) \times R^2/(1-R^2)$, where N stands for sample size and R^2 refers to the variance in dietary habits explained by the genetic instrument.[30]

MR analysis

R 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria) was utilized to conduct MR analyses, specifically the packages TwoSampleMR and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO). Inverse variance weighted (IVW) regression was employed as the principal analysis approach to ascertain the possible correlation of dietary habits with MS. This was due to the fact that IVW regression computed the exposure causation on the outcomes by means of Wald estimate of the SNP ratio related to exposure. When horizontal pleiotropic effects were absent from the IVs, the IVW method yielded the most dependable outcomes.

Sensitivity analysis

The primary analyses' robustness was evaluated through the implementation of six analyses for sensitivity. The aforementioned items were as follows: (i) weighted median regression that yields unbiased slope estimates when a minimum of 50% SNPs are valid IVs;[31] (ii) weighted mode regression that yields unbiased slope estimates when a multitude of SNPs are valid IVs;[32] (iii) Egger regression that examines horizontal pleiotropy (global directional);[33] (iv) simple mode for supplementary examination; (v) MR-PRESSO that seeks IVs depicting horizontal pleiotropy and undertakes slope recalibration following the removal of those

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searched variables;[34] and (vi) leave-one-out IVW regression, which examines the results' robustness by excluding individual instrumental variables. $p < 0.05$ denotes a statistically significant outcome. The Cochrane Q' test, a standard method for MR analysis, was used to determine heterogeneity. Pleiotropy was investigated by utilizing the MR-Egger intercept.

RESULTS

The subjects of the dietary practices varied between 335,394 and 462,346. From 47,429 MS-diagnosed subjects and 68,374 controls, the IMSGC compiled summary-level data for MS; there was little overlap between the exposed populations and outcome. SNPs associated with dietary practices were extracted utilizing the "harmonise_data" and "extract_outcome_data" functions, ensuring their validity and independence. The Supplementary Table 2 presents data regarding SNPs that are linked to twenty-one dietary practices. Between four and sixty-five SNPs were found to be efficacious. All SNPs exhibited F-statistics exceeding 10, which suggests the absence of any indication of bias due to the instrument.

Our analysis identified a protective association between higher oily fish intake and a reduced risk of MS. Initial MR analyses revealed substantial heterogeneity in this association ($p = 4.56\times10^{-7}$; Supplementary Table 5). Nevertheless, after correcting for outlier SNPs using the MR-PRESSO method, the association remained robust (IVW, OR = 0.557, 95%CI: 0.351-0.884, $p = 0.013$). Comparable results were obtained via the weighted median method following the exclusion of outliers IVW, OR=0.513, 95%CI: 0.266-0.988, $p = 0.046$), further underscoring the robustness of the association. Tests for directional pleiotropy and residual heterogeneity, including Cochran's Q statistic and the MR-Egger intercept, provided no evidence of bias after removing outlier SNPs (Supplementary Table 6). The forest plot (Figure 2A) displays the association estimates for each SNP linked to oily fish consumption and MS risk. The leave-one-out analysis (Figure 2B) indicates that no single SNP exerted disproportionate influence on the overall causal estimate. The scatter plot (Figure 2C) visualizes SNP-specific estimates across multiple MR approaches, while the funnel plot (Figure 2D) did not reveal evidence of bias.

Except for fish oil, none of the evaluated dietary exposures exhibited statistically significant associations with MS, regardless of outlier exclusion. Specifically, processed meat intake (IVW, OR = 0.801, 95%CI: 0.347-1.849, $p = 0.603$), pork (IVW, OR = 3.415, 95%CI: 0.617-18.890, $p = 0.159$), lamb/mutton (IVW, OR = 0.809, 95%CI: 0.325-2.009, $p = 0.647$), poultry (IVW, OR = 1.353, 95% CI: 0.158-11.595, $p = 0.783$), beef (IVW, OR = 0.264, 95%CI: 0.060-1.163, $p = 0.078$), and non-oily fish (IVW, OR = 0.798, 95% CI: 0.215-2.956,

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p = 0.735) showed no significant associations. Similar null findings were observed for tea consumption (IVW, OR = 0.759, 95%CI: 0.430-1.339, p = 0.340; after outlier removal: IVW, OR = 0.665, 95%CI: 0.406-1.089, p = 0.105), coffee (IVW, OR = 0.503, 95%CI: 0.247-1.024, p = 0.058), and alcohol intake measured both as drinks per week (IVW, OR = 1.046, 95%CI: 0.517-2.115, p = 0.901) and frequency (IVW, OR = 1.314, 95%CI: 0.889-1.943, p = 0.171; post-outlier removal: IVW, OR = 1.277, 95%CI: 0.977-1.671, p = 0.074). Other dietary factors, including water intake (IVW, OR = 0.916, 95%CI: 0.389-2.159, p = 0.842), hot drink temperature (IVW, OR = 1.578, 95%CI: 0.760-3.277, p = 0.221), cereal (IVW, OR = 0.955, 95%CI: 0.449-2.029, p = 0.904), bread (IVW, OR = 0.713, 95%CI: 0.166-3.054, p = 0.648; post-outlier removal: IVW, OR = 0.540, 95%CI: 0.249-1.173, p = 0.120), dried fruit (IVW, OR = 0.740, 95%CI: 0.362-1.513, p = 0.410), fresh fruit (IVW, OR = 0.789, 95%CI: 0.273-2.279, p = 0.662; post-outlier removal: IVW, OR = 0.760, 95%CI: 0.311-1.860, p = 0.549), raw vegetables or salad (IVW, OR = 0.948, 95%CI: 0.171-5.271, p = 0.951), and cooked vegetables (IVW, OR = 7.917, 95%CI: 0.312-201.014, p = 0.210; post-outlier removal: IVW, OR = 1.530, 95%CI: 0.431-5.434, p = 0.511), as well as salt (IVW, OR = 1.057, 95%CI: 0.735-1.520, p = 1.130) and cheese intake (IVW, OR = 1.038, 95%CI: 0.627-1.717, p = 0.886), likewise demonstrated no robust association with MS risk. Despite the presence of variability in the water intake exposure (Cochran's Q test p <0.05), the MR-Egger intercept outcome did not indicate any directional pleiotropy. Our MR study's findings from are summarized in Supplementary Table S4-S6 and Figure 3-4.

DISCUSSION

The association between consumption of oily fish and MS was the most substantial finding of this research. In the event that our investigation contained any outliers, we recalculated the MR analysis and employed the revised IVW outcomes to ascertain the presence of a causal association. After outlier exclusion, causal association was identified between an increase in consumption of oily fish and a decreased MS risk. Our findings have the potential to help clinicians improve health education for MS patients by encouraging them to change their dietary habits (such as eating more fatty fish). Moreover, for people who are at high risk of developing MS, changing their eating habits may help. Consequently, this study has important implications for expanding our understanding of both risk factors and protective factors for MS. This is the comprehensive research to examine the possible causal connection of dietary factors to MS utilizing MR analysis.

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A multitude of studies provide evidence in favor of the correlation between fish consumption and the likelihood of developing MS. An investigation conducted by Kampman et al. among adolescents revealed that consuming fish at least three times weekly was related to a decreased likelihood of acquiring MS (OR = 0.55, 95%CI: 0.33-0.93, p = 0.024).[8] According to a prior systematic review, increasing fish consumption was linked to a decreased likelihood of MS progression; such a protective influence had been identified when at a minimum of 0.5 servings of fish were consumed weekly.[35] Furthermore, several studies have investigated the impact of various fish species on MS prevalence. An Australian study found that eating two servings of canned (oily) fish per week reduced the risk of focal cortical dysplasia (FCD) by approximately 40%, where FCD is a frequently observed precursor to MS.[36] Other research has discovered a negative correlation between high-fat fish consumption and MS incidence.[7] Nonetheless, few research has establish a definitive correlation between MS risk and fish consumption.[9] Variations in study designs and methodologies, such as distinct questionnaires on food frequency, modifications in diagnostic gauges regarding MS, and variations in residual confounding, may account for this discrepancy.

Several fundamental studies back up our findings. Consumption of omega-3 polyunsaturated fatty acids (n-3 PUFAs) has been identified as a significant modifiable factor associated with the riskof multiple sclerosis (MS), according to numerous studies.[37] The most prevalent source of n-3 PUFAs is found in oily fish. Animal studies have shown that n-3 PUFAs exert neuroprotective effects during aging,38 and inhibit MS-related inflammation through various mechanisms.[39,40] Research has demonstrated that n-3 PUFAs possess anti-inflammatory characteristics through the modulation of transcription factor activity, intracellular signaling pathways, and gene expression.[41] A recent review also found that n-3 PUFAs and fish oil supplementation can help reduce recurrence rates, mitigate inflammatory markers, and improve quality of life in MS patients.[42] However, researchers have differing opinions on the impact of oily fish on the pathogenesis of MS; some believe that vitamin D in oily fish reduces MS risk through internal circulation.[43,44] In conclusion, oily fish is an excellent source of vitamin D and very long-chain omega-3 polyunsaturated fatty acids (VLCn-3 PUFAs),[45,46] both of which have the potential to reduce MS susceptibility.

This investigation utilizing MR has numerous benefits. To begin, we conducted theassessment of the causal association between dietary behaviors and MS using a two-sample MR analysis, which effectively circumvented the influence of possible confounding variables on the outcomes. We then aggregated MS GWAS statistics (47,429 MS cases and 68,374 healthy controls) and utilized extensive GWAS datasets pertaining to dietary habits.

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Furthermore, the impact of stratification of population was mitigated in GWAS datasets comprising individuals of European descent. Fourth, by means of distinct statistical methodologies, we established valid IVs from the screened independent genetic variants. Finally, our positive results were robust, with no significant heterogeneity or pleiotropy. Causality should be approached with caution when interpreting the findings of our study. Further research is crucial in order to validate and extrapolate our results.

Of course, there are some limitations to this study. The MR findings are based on European populations, and extrapolated results are limited. Regarding other populations, we should exercise caution when interpreting and generalizing. Second, the biological significance of the majority of SNPs has yet to be determined. Moreover, as a result of constraints in the available data, we refrained from examining the effects of specific dietary patterns, including the Western or Mediterranean diets. As dietary practices were gathered retrospectively via an abbreviated questionnaire regarding food frequency, it is impossible to rule out recall bias and seasonal variations.

Conclusions

In summary, we conducted a methodical examination of the possible causal connection between dietary habits and MS. Based on our MR analysis, it suggests that consuming oily fish is causally linked to a decreased MS risk. By elucidating the potential influence of dietary factors on the onset of MS, our research aids in the formulation of more precise preventive measures against the disease.

SUPPLEMENTARY MATERIALS

All supplementary tables and figures are available upon request to the editorial office. GWAS for MS can be found in IMSGC.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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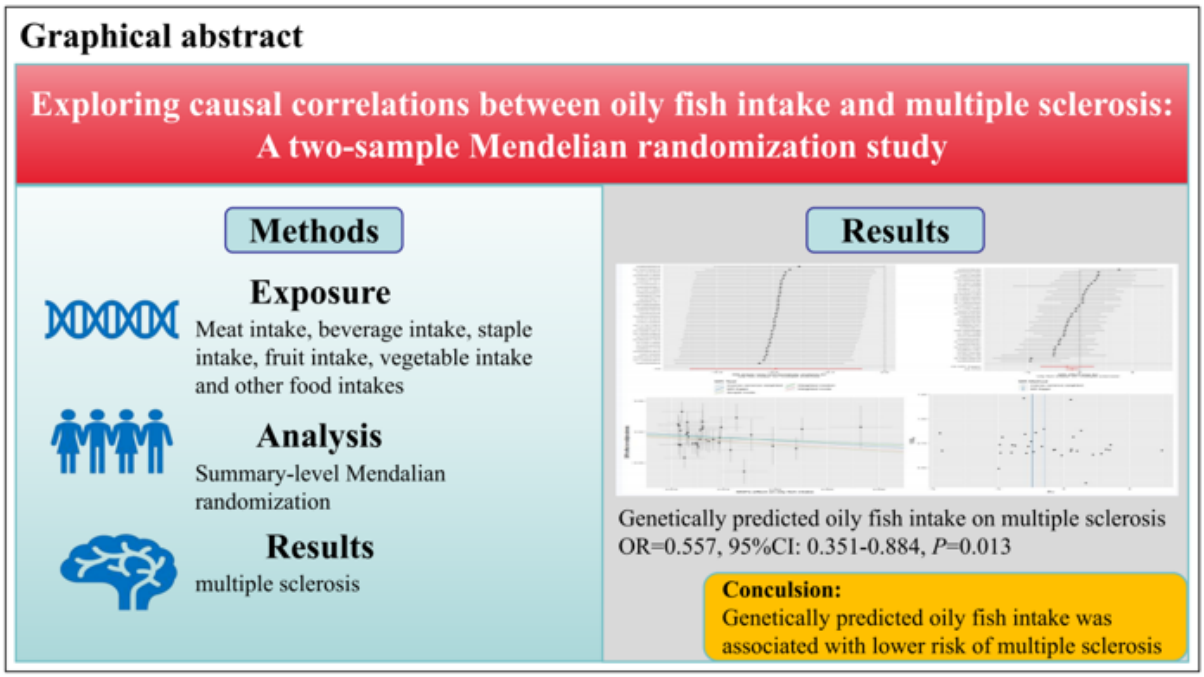

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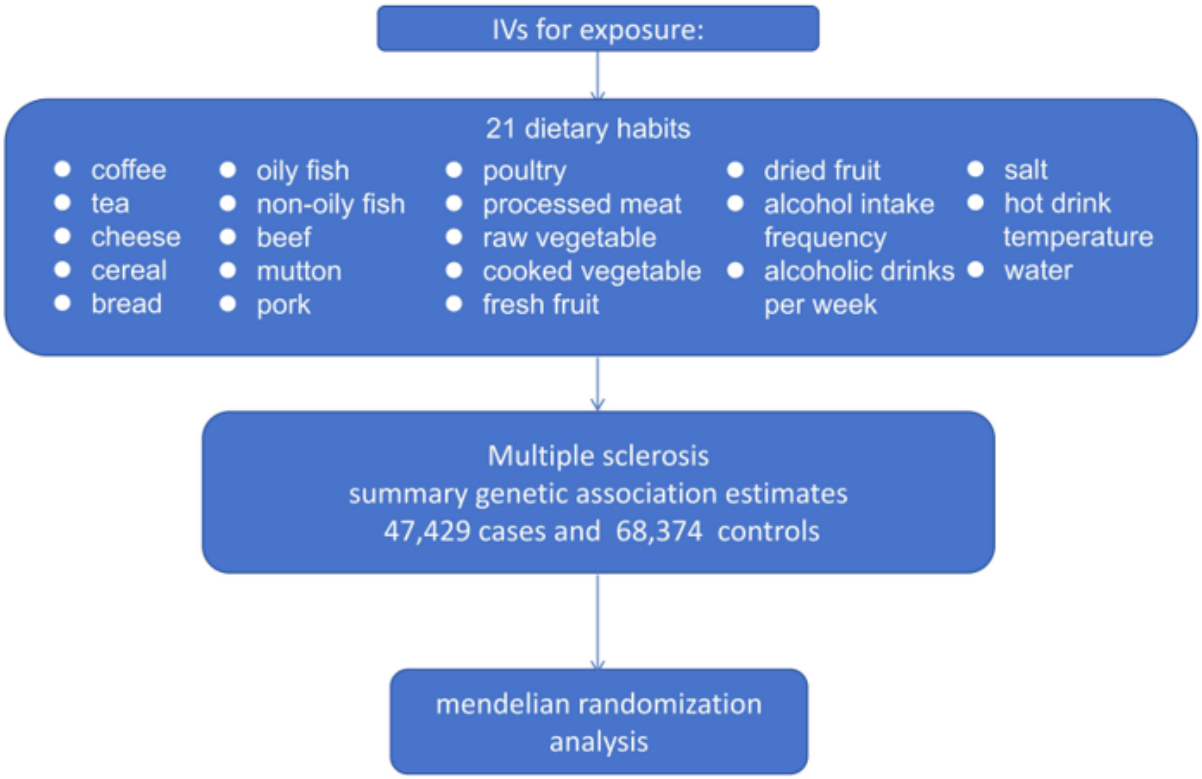

Figure 1. The MR study flowchart that reveals the causality between dietary habits and MS risk. MR, Mendelian randomization; MS, multiple sclerosis

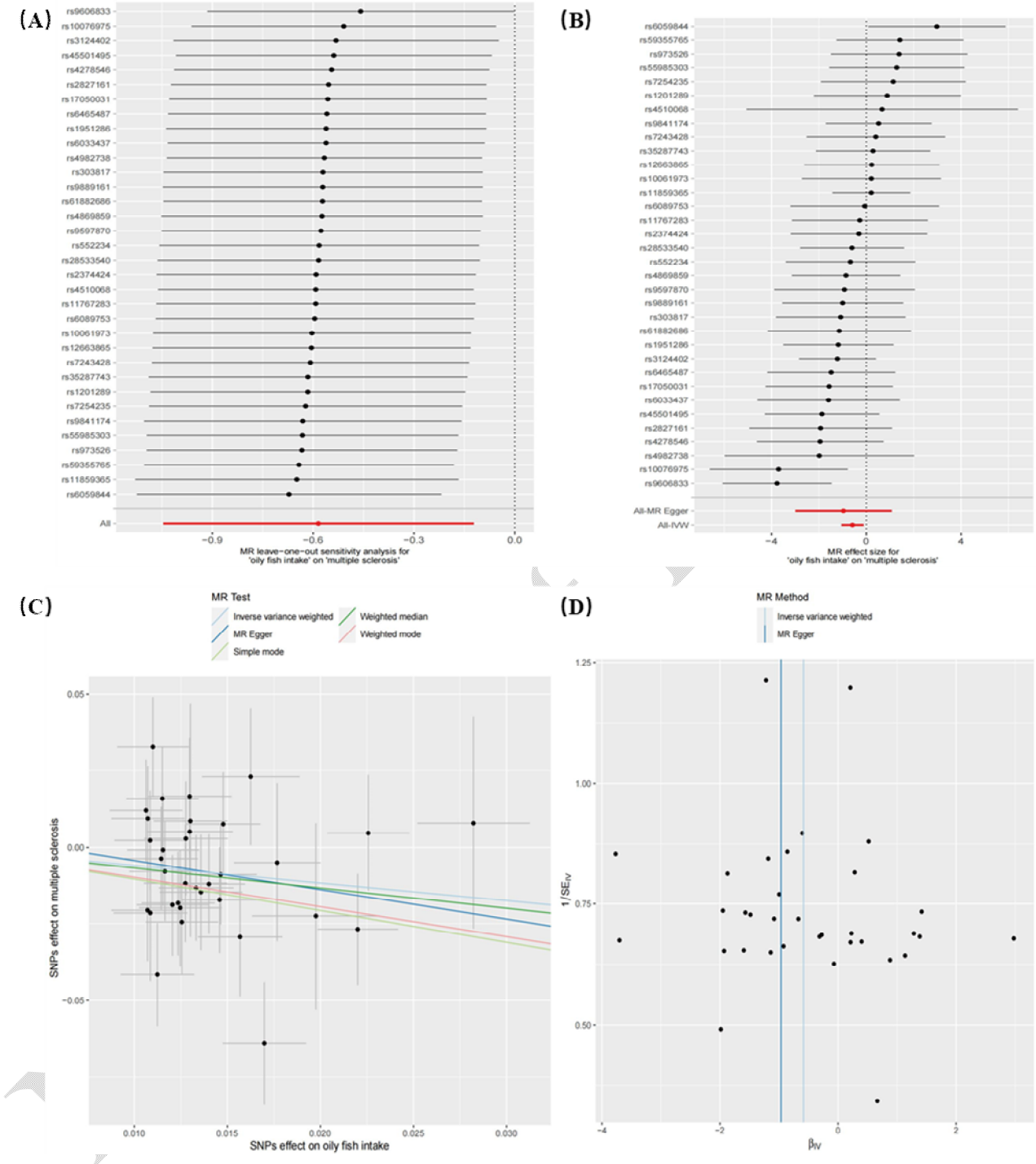

Figure 2. The connection between the SNP associated with oily fish consumption and MS risk. (A) A forest plot illustrating a two-sample MR analysis; the increase in oily fish consumption corresponds to a higher log-to-value OR of MS, as denoted by the black dots. The causal estimates for every SNP combination, as computed through diverse MR algorithms, are denoted by the red dots. (B) Results of a "leave-one-out" sensitivity analysis; the log-to-value OR of MS rises with oily fish ingestion, as indicated by the black dots. The causal estimates for every SNP combination, as computed through diverse MR algorithms, are denoted by the red dots. (C) A scatter diagram illustrating the results of a two-sample MR analysis; the line's slope denotes the causal estimates of the MR approach, and each black dot corresponds to a single SNP. (D) A funnel diagram representing MR analysis involving two samples; β denotes the regression coefficient and SE the standard error. MR, Mendelian randomization; MS, multiple sclerosis; SNP, single nucleotide polymorphisms; OR, odds ratio; SE, standard error

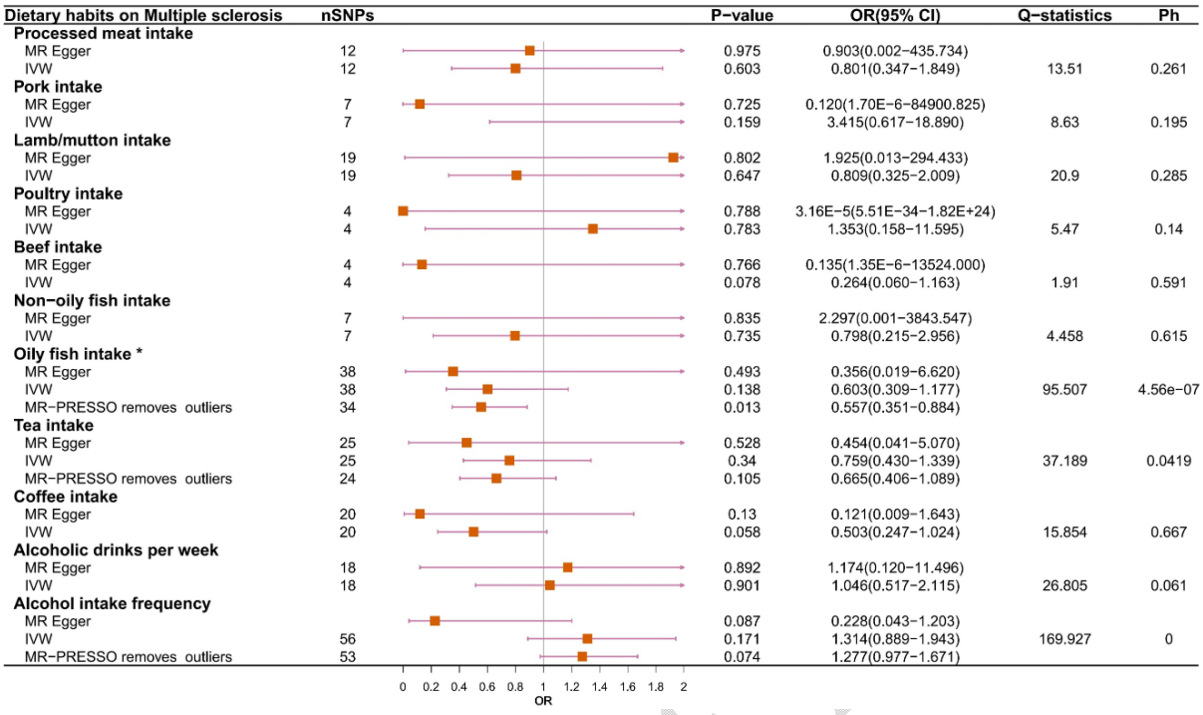

Figure 3. Forest plot using the IVW (inverse variance weighted), MR-Egger and MR-PRESSO to visualize the causal effects of dietary habits (processed meat intake, pork intake, lamb/mutton intake, poultry intake, beef intake, non-oily fish intake, oily fish intake, tea intake, coffee intake, alcoholic drinks per week, alcohol intake frequency) on MS. IVW, inverse variance weighted; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; OR, odds ratio; CI, confidence interval

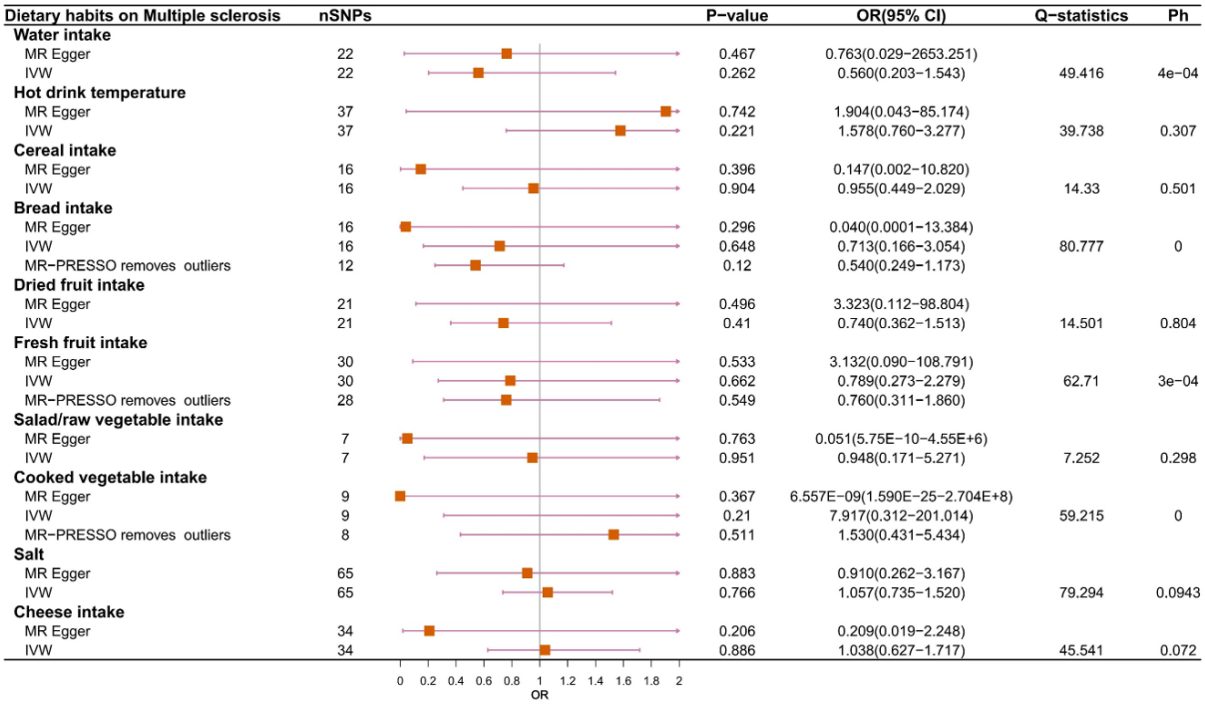

Figure 4. Forest plot using the IVW (inverse variance weighted), MR-Egger and MR-PRESSO to visualize the causal effects of dietary habits (water intake, hot drink temperature, cereal intake, bread intake, dried fruit intake, fresh fruit intake, salad/raw vegetable intake, cooked vegetable intake, salt, cheese intake) on MS. IVW, inverse variance weighted; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; OR:odds ratio; CI, confidence interval

Table 1. The study procedure

Project	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Anthropometric measurements	√						√
7-day 24-h fluid intake questionnaire	√	√	√	√	√	√	√
Water from food			√	√	√		
Determination of 24-h urine biomarkers			√	√	√		
Determination of plasma biomarkers				√			
Physical activity monitoring	√	√	√	√	√	√	√
Temperature and humidity record	√	√	√	√	√	√	√

√, The project was taken on that day.

Table 2. The characteristics of participants

Group	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Total	20.8±1.0	178.6±4.8	71.2±7.3	22.3±1.8
PAEE				
Gp1	20.5±0.8	176.0±3.5	68.9±5.58	22.2±1.1
Gp2	20.9±1.2	177.6±4.4	68.3±5.2	21.6±1.4
Gp3	21.3±1.1	180.7±6.6	72.5±7.9	22.2±1.4
Gp4	20.5±1.0	180.3±3.0	75.1±8.8	23.1±2.8
F	1.309	2.435	2.149	1.193
p	0.285	0.080	0.110	0.325
MET				
Gm1	20.5±0.9	177.2±4.1	68.4±5.7	21.7±1.3
Gm2	20.8±0.9	180.5±7.6	74.6±8.1	22.9±1.9
Gm3	21.0±1.3	177.0±2.7	68.1±3.1	21.7±0.8
Gm4	20.9±1.1	180.1±3.3	74.1±9.3	22.8±2.7
F	0.366	1.552	2.749	1.365
p	0.778	0.217	0.056	0.268

Values were shown as means±SD.

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Table 3. Water intake of participants with different PAEE and MET levels

Group	TWI (mL)	TDF (mL)	WFF (mL)	EFI (mL)	NEFI (mL)
Total	2771 (1066)	1653 (857)	1088 (570)	329(389)	1314 (620)
PAEE					
Gp1	2413 (1586)	1422 (989)	1052 (465)	172 (419)	1227 (673)
Gp2	2599 (814)	1681 (596)	972 (516)	211 (143)	1404 (651)
Gp3	3019 (787)	1602 (487)	1222 (508)	403 (544)	1241 (435)
Gp4	3421 (1042)	2109 (848)	1179 (683)	574 (427) †,‡	1439 (939)
χ^2 (K-W test)	6.083	6.129	2.641	13.902	0.307
p (K-W test)	0.108	0.106	0.450	0.003*	0.959
Z (J-T test)	2.414	2.425	0.977	3.582	0.123
p (J-T test)	0.016**	0.015**	0.329	<0.001**	0.902
MET					
Gm1	2532 (847)	1398 (371)	1052 (456)	179 (210)	1227 (552)
Gm2	3305 (1015) §	2172 (665) §	1130 (427)	375 (601) §	1583 (794)
Gm3	2356 (980) ¶	1534 (483) ¶	890 (452)	304 (340)	1156 (373)
Gm4	3282(711) §,††	2007(1069) §	1136(561)	721(507) §,††	1187(827)
χ^2 (K-W test)	11.787	11.658	3.888	14.757	6.270
p (K-W test)	0.008*	0.009*	0.273	0.002*	0.099
Z (J-T test)	1.583	1.549	0.775	3.020	-0.932
p (J-T test)	0.113	0.121	0.439	0.003**	0.351

TWI: total water intake; TDF: total drinking fluids; WFF: water from food; EFI: exercise-related fluid intake; NEFI: non-exercise-related fluid intake.

Values were shown as medians (QR).

$^{*}p<0.05$ there were statistically significant differences between different PAEE or MET groups; $^{**}p<0.05$ there was statistically significant trend with the PAEE or MET level increase.

$^{\dagger}p<0.05$ compared with Gp1; $^{\ddagger}p<0.05$ compared with Gp2; $^{\S}p<0.05$ compared with Gm1; $^{\P}p<0.05$ compared with Gm2; $^{\dagger\dagger}p<0.05$ compared with Gm3.

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