Original Article

Assessment of causal factors for Parkinson's disease in European populations: A phenome-wide Mendelian randomisation analysis

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Background and Objectives: To assess the causality of potentially modifiable factors, including lifestyle, nutrients, lipids, anthropometric traits, and inflammatory factors of Parkinson's disease (PD), genetic instruments for modifiable factors were identified from genome-wide association studies (GWAS). **Methods and Study Design:** Genetic associations for PD (1,239 cases and 451,025 matched controls) were extracted from the UK Biobank GWAS summary statistics. The causal effects of modifiable factors on the risk of PD were estimated using the multiplicative random-effects inverse variance weighted method (IVW). **Results:** In the IVW analysis, a decreased risk for PD was causally associated with genetically predicted smoking cessation (odds ratio 0.41, [95% confidence interval] 0.32-0.78; $p<0.001$), and higher bone mineral density (0.43, 0.38 -0.71; $p<0.001$), higher concentrations of vitamin B-12 (0.56, 0.43-0.91; $p<0.001$), docosahexenoic acid (0.52, 0.37-0.71; $p<0.001$), and sIL-6R (0.69, 0.58-0.75; $p<0.001$). Instead, analysis further supported the role of apolipoprotein (a) isoform size(1.67, 1.36-1.71; $p<0.001$), being a genetically morning person (2.18, 1.12-4.72; $p<0.001$), and number of cigarette smoking (1.05, 1.01-1.08; $p<0.001$) in contributing to the risk of developing PD. **Conclusions:** Our findings provide new evidence for the potential positive causal association of cigarette smoking number and apolipoprotein (a) isoform size and the inverse causal association of vitamin B-12, docosahexaenoic acid, smoking cessation, and soluble interleukin-6 receptor with PD, which contributes to the development of new interventions for PD.

Key Words: Mendelian randomization, Parkinson's disease, lifestyle, smoking, vitamin B-12

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra.[1] The pathogenesis of PD remains largely unclear, and effective preventive approaches are lacking;[2] therefore, it is of great interest to explore the causal factors for PD. Observational studies have indicated that body mass index (BMI), higher circulating homocysteine, iron, low density lipoprotein cholesterol (LDL), total cholesterol (TC), and fibrinogen were associated with a higher risk of PD, whereas higher serum vitamin B-6, vitamin B-12, 25-hydroxyvitamin D [25(OH)D], and vitamin E concentrations, cigarette smoking number, and bone mineral density were observed to be related to a lower risk of PD.[3-13] Moreover, no significant associations were observed for serum C-reactive protein (CRP) and vitamin C concentrations.[6,14] However, observational studies found it difficult to fully account for reverse causality and are confounded by shared environmental factors, such as socioeconomic status and unmeasured lifestyle factors. Therefore, whether such observations reflect causality remains unclear.

Mendelian randomization (MR) analysis is a widely used method, which utilizes genetic variants as instrumental variables (IVs) to estimate the causal relationship between risk factors and outcomes.[15,16] Owing to genetic variants are randomly assigned at conception, they are as attractive as IVs, while unmeasured confounding factors such as socioeconomic status and lifestyle factors and reverse causation are limited in MR analysis.[17] Therefore, in the present study, MR analysis was used to precisely examine the causality between potentially modifiable factors and PD based on the European population using summary level data from genetic consortia and the UK Biobank. This is the first study with a sufficient sample

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size under MR analysis to observe that consistent risk of PD decreases with smoking cessation, higher circulating vitamin B-12, docosahexaenoic acid and soluble interleukin-6 receptor levels, and bone mineral density, but increases with genetically being a smoker and higher apolipoprotein (a) isoform size. Moreover, our results provide evidence that healthy habits and regular eating patterns, such as vitamin B-12 and docosahexaenoic acid supplementation, may relieve the progression of PD.

METHODS

Modifiable factors

Potentially modifiable factors were divided into the following categories: lifestyle, nutrients, lipids, inflammatory factors, and anthropometric traits. These factors were reported in previous observational studies, but were not verified at the genetic level. Therefore, it is necessary to precisely determine the association of these factors with PD at the genetic level. In addition, these factors selected in our MR are accessible in daily life, making it possible to make feasible preventive measures.

Data sources

Summary-level data were extracted from published genome-wide association studies (GWAS, Table 1) of the modifiable risk factors and identified genetic variants [single nucleotide polymorphisms (SNPs)] with genome-wide significant ($p<5\times10^{-8}$) associations for sleep dura-

Table 1. Mendelian randomisation analyses of potentially modifiable risk factors associated with Parkinson's disease.

Modifiable risk factor	Published genome-wide association study of the modifiable risk factor		
	Maximum sample size	No. of independent genome-wide significant SNPs†	R^2 (%) explained by the SNPs
Lifestyle			
Sleep duration	47,180	6	NA
Morning person	89,283	14	NA
Smoking (number)	86,956	4	0.5
Smoking initiation	143,023	8	0.03
Smoking cessation	64,924	1	0.19
Nutrients			
Vitamin B-6	4,763	2	NA
Folate	37,341	2	1
Vitamin B-12	4,763	10	6.3
Vitamin D	79,366	7	3.6
Vitamin C	106,147	1	NA
Vitamin E	5,006	3	1.7
Homocysteine	124,327	16	5.9
Se	4,162	4	NA
Iron	4,818	2	NA
ALA	8,866	1	NA
DPA	8,866	4	NA
EPA	8,866	3	NA
DHA	8,866	1	NA
20:0 (Saturated fatty acid)	10,129	3	NA
22:0 (Saturated fatty acid)	10,129	2	NA
24:0 (Saturated fatty acids)	10,129	2	NA
Anthropometric traits			
BMI	339,224	96	2.4
Birth weight	153,781	50	15
Height	253,288	687	36
WHRadjBMI	224,459	48	1.2
Body fat	100,716	12	0.12
Infant head circumference	29,857	2	NA
Bone mineral density	32,961	48	50
Lipids			
HDL cholesterol	188,578	71	13.7
LDL cholesterol	188,578	57	14.6
Total cholesterol	188,578	73	15
Triglycerides	188,578	39	11.7
Apolipoprotein(a) isoform size	17,503	1	NA
Lipoprotein(a) concentration	17,503	1	NA
Inflammatory factors			
CRP	66,185	39	10
Fibrinogen	120,246	39	NA
IL-1Ra	5,888	5	NA
sIL-6R	125,222	1	NA

HDL: high-density lipoprotein; LDL: low-density lipoprotein; SNP: single nucleotide polymorphism; ALA: α-linolenic acid; DPA: docosapentaenoic acid; EPA: eicosapentenoic acid; DHA: docosahexenoic acid; BMI: body mass index; WHRadjBMI: waist-to-hip ratio adjusted for body mass index; CRP: C-reactive protein; IL-1Ra: interleukin-1 receptor antagonist; sIL-6R: soluble interleukin-6 receptor.
†Number of independent genome-wide significant ($p<5\times10^{-8}$) SNPs identified in the sex-combined meta-analysis of discovery and replication samples. Independent was defined as not in linkage disequilibrium ($r^2<0.05$) with other SNPs for the same risk factor.

tion,[18] smoking (quantity, initiation, and cessation),[19] serum vitamins concentrations (vitamin B-6,[20] float,[21] vitamin B-12,[20] 25(OH) D,[22] vitamin C,[23] and vitamin E[24]), total homocysteine levels,[25] serum selenium (Se) concentrations,[26] serum iron concentrations,[27] plasma polyunsaturated fatty acids levels [(α-linolenic acid (ALA), docosapentaenoic acid (DPA), eicosapentenoic acid (EPA) and docosahexenoic acid (DHA)],[28] plasma long-chain saturated fatty acids levels (eicosanoic acid, docosanoic acid, and lignoceric acid),[29] anthropometric traits [body mass index ,[30] birth weight,[31] height,[32] waist to hip ratio adjusted for BMI (WHRadjBMI),[33] body fat,[34] infant head circumference,[35] and bone mineral density[36]), blood lipids [high density lipoprotein chol esterol (HDL), LDL, TC, and triglycerides],[37] CRP levels,[38] fibrinogen levels,[39] interleukin-1 receptor antagonist (IL-1Ra) levels,[40] and soluble interleukin-6 receptor (sIL-6R) levels via PubMed.[41] Summary statistics for the genetic variants associated with the modifiable risk factors are presented in supplemental Table.

Genetic data for the associations between SNPs related to modifiable factors and PD were obtained from the UK Biobank (http://geneatlas.roslin.ed.ac.uk/downloads/?traits=2). Some SNPs related to PD from GWAS were not included in our study due to insufficient evidence regarding their effects on the genetic susceptibility, as well as some poor quality or hardly detected SNPs. As a result, this cohort included 1,239 PD cases and 451,025 matched controls of European ancestry, and the participants provided written informed consent. Summary statistics for the genetic variants associated with modifiable risk factors and PD are presented in Supplemental Table.

Genetic variants

SNPs associated with the above modifiable risk factors were selected at thresholds for genome-wide significance ($p<5\times10^{-8}$). The correlation [linkage disequilibrium (LD)] between selected SNPs and potential confounders was assessed. In addition, we assessed LD using the SNP Annotation and Proxy search system (http://www.broadinstitute.org/mpg/snap/ldsearchpw.php) for the same reference catalog and population. The SNP with a larger *p* value was discarded if the correlation coefficient between SNPs was high ($r^2\geq0.05$). To control for confounders, the selected SNPs that were highly associated with other risk loci were not in LD.[42]

Mendelian randomisation analysis

The qualities of genetic variants used in MR analysis were as follows: 1) be truly associated with the modifiable risk factor, 2) be not associated with confounders any confounder of the modifiable risk factor and PD, and 3) be not associated with the outcomes of interest through the exposure under study (Figure 1).[43] As a result, a total of 38 potentially modifiable factors were included in this analysis. To avoid type-I errors, a Bonferroni corrected significance level (computed as 0.05, divided by 38, that is, 1.32×10^{-3}) was applied at a significance level of $p<0.05$; however, above the Bonferroni corrected significance, the threshold was considered as suggestive of evidence for a potential association.

In the present MR study, the summarized data of association [β-coefficients and standard errors (SEs)] were used, and the multiplicative random-effects inverse variance weighted (IVW) method was used as the primary approach to test the causal effect. Additional sensitivity analyses, including simple median– and weighted median–based methods were used to analyze the estimates of the causal effect of modifiable factors on PD, thereby increasing the robustness of causal findings. In addition, MR-Egger regression was used to examine whether pleiotropy was present, although this method was expected to have a low power to detect violation of assumptions.[44] The MR-Egger approach was used to plot the effect of SNPs on modifiable factors against its effect on PD and obtain an intercept term. The outcomes were not affected by pleiotropy if the intercept term was centered at the origin.

Results are presented as odds ratios (OR) (95% confi-

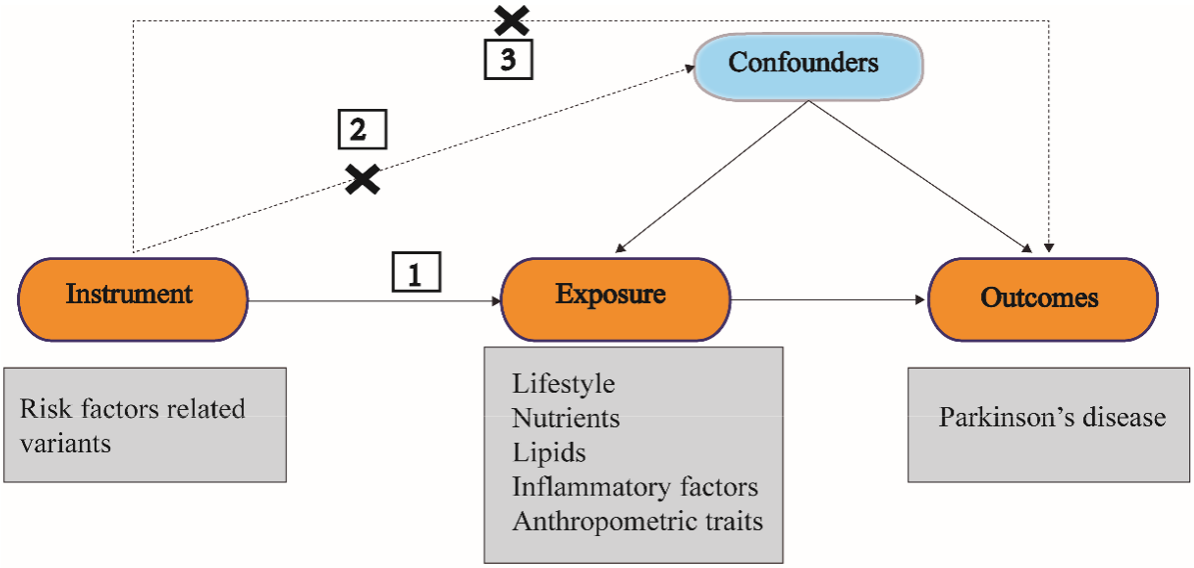

Figure 1. Principles of Mendelian randomization analysis for modifiable factor and risk of Parkinson's disease. Broken lines represent potential pleiotropic or direct causal effects between variables that would violate Mendelian randomization assumptions.

dence intervals [CI]) per genetically predicted increase in each risk factor. For the binary risk factors, the estimates represented the OR per 1unit higher log odds of the risk factor (Supplemental Table). That is, sleep duration was scaled to one hour of sleep (1 SD=0.5 hour), smoking to 10 cigarettes, vitamin D to 20% change, morning person to 5% change, vitamin B-6 to one SD (assuming one SD=47.77 pmol/mL), vitamin B-12 to one SD (assuming one SD=0.42 pg/mL), vitamin E to one SD (assuming one SD=3.4 mg/L), homocysteine to one SD (assuming one SD=0.14 pg/mL), Se to one SD (assuming one SD=0.15 μg/g), iron to one SD (assuming one SD=0.15 μmol/L), BMI to one SD (assuming one SD=5.87 kg/m^2), birth weight to one SD (assuming one SD=4.83 kg), height to one SD (assuming one SD=0.91 m), WHRadjBMI to one SD (assuming one SD=0.07 cm/cm), body fat to 1% change, infant head circumference to one SD (assuming one SD =1.6 cm), HDL to one SD (assuming one SD=17.9 mg/dL), LDL to one SD (assuming one SD =39.9 mg/dL), TC to one SD (assuming one SD=43.8 mg/dL), triglycerides to one SD (assuming one SD=81.8 mg/dL), lipoprotein(a) concentration to one SD (assuming one SD=5.64 nmol/L), CRP to one SD (assuming one SD=1 ln-mg/L)), fibrinogen to one SD (assuming one SD=0.78 g/L), sIL-6R to one SD (assuming one SD=1.5 ng/mL) and bone mineral density to one SD (assuming one SD=0.1 g/cm^2). Graphpad Prism 7 and R version 3.2.4 (R Project for Statistical Computing) were used to perform the analysis.

Ethics approval and consent to participate

Summary-level data were extracted from consortia, including DIAGRAM, CARDIoGRAMplusC4D, CKDGen, SIGN and GERFHS. All human studies were approved by their institutional ethics review committees, and all participants provided written consent.

RESULTS

IVs selection and validation

First, IVs were selected from the GWAS database of modifiable factors ($p<5\times10^{-8}$). In addition, SNPs with a larger p value in the LD ($r^2\geq0.05$) were eliminated. All selected IVs met $p<5\times10^{-8}$, indicating that the selected IVs were all modifiable factor-related loci. Second, we extracted the results for genetic variant-PD associations in the UK Biobank (Supplemental Table). We did not adopt the LD method to replace IVs because the beta and SE of all IVs were found in the UK Biobank. These findings showed that selected IVs satisfied the basic hypothesis of MR analysis: IVs were related to modifiable factors but were not associated with PD.

Lifestyle

There was a significant association between genetically predicted higher cigarette smoking number and higher odds of PD (OR 1.05, 95% CI 1.01 to 1.08; $p=1.34\times10^{-4}$). Genetically predicted smoking cessation was associated with a decreased risk of PD (0.41, 0.32 to 0.78; $p=6.52\times10^{-4}$). The MR-Egger method showed that smoking cessation (p=1.00) and cigarette number (p=0.423) were not influenced by directional pleiotropy (Figure 2). In addition, smoking initiation (0.71, 0.37 to 1.57; p=0.431) was not causally associated with the risk of PD.

In agreement with the IVW analysis, the genetic prediction of being a morning person was significantly associated with an increased risk for PD (2.18, 1.12 to 4.72; $p=3.51\times10^{-5}$). However, we did not observe a causal association between sleep duration (0.97, 0.95 to 1.04; p=0.857) and the risk of PD. The MR-Egger method showed that results for sleep duration (p=0.233) and genetic prediction of being a morning person analysis (p=0.828) were not influenced by directional pleiotropy (Figure 2).

Nutrients

Genetically predicted higher serum vitamin B-12 concentration was associated with a lower risk for PD (0.56, 0.43 to 0.91; $p=4.15\times10^{-6}$), and higher serum DHA concentrations were also causally associated with a lower risk of PD (0.52, 0.37 to 0.71; $p=2.58\times10^{-4}$). In addition, there was no significant causality between PD and 25(OH)D, ALA or eicosanoic acid (Figure 3).

Results for vitamin B-12 and docosahexaenoic acid were similar in sensitivity analyses, such as the simple median– and weighted median–based method. The MR-Egger method showed that the results for vitamin B-12 (p=0.782) and DHA analysis (p=0.872) were not influenced by directional pleiotropy (Figure 3).

Lipids and inflammatory factors

There was a significant association between genetically predicted higher apolipoprotein (a) isoform size and increased risk of PD (1.67, 1.36 to 1.71; $p=1.59\times10^{-4}$) (Figure 4), whereas higher genetically predicted serum sIL-6R concentrations were associated with a lower risk of PD (0.69, 0.58 to 0.75; $p=3.17\times10^{-6}$) (Figure 5). Results for apolipoprotein (a) isoform size and sIL-6R were similar in sensitivity analyses and were not influenced by directional pleiotropy. Moreover, there was no significant relationship between PD and HDL, LDL, or CRP levels (Figure 4, Figure 5).

Anthropometric traits

Genetically predicted higher bone mineral density was associated with a lower risk of PD (0.43, 0.38 to 0.71; $p=2.31\times10^{-5}$). The results for bone mineral density were consistent in the sensitivity analyses (Figure 6). In addition, there was no significant causality of PD with BMI, height, or WHRadjBMI.

DISCUSSION

A prospective community-based cohort study (n = 360) initiated in 2001 provided significant evidence that current cigarette smoking was associated with faster cognitive decline, indicating a positive role of smoking in PD progression.[45] Moreover, genetically being a smoker may have higher mortality in patients with PD.[46] However, it was reported that former and current smokers had a lower risk of PD than never smokers.[47] This discrepancy between observational studies may be linked to confounders, such as socioeconomic status and unmeasured lifestyle factors. The present MR on data from GWAS

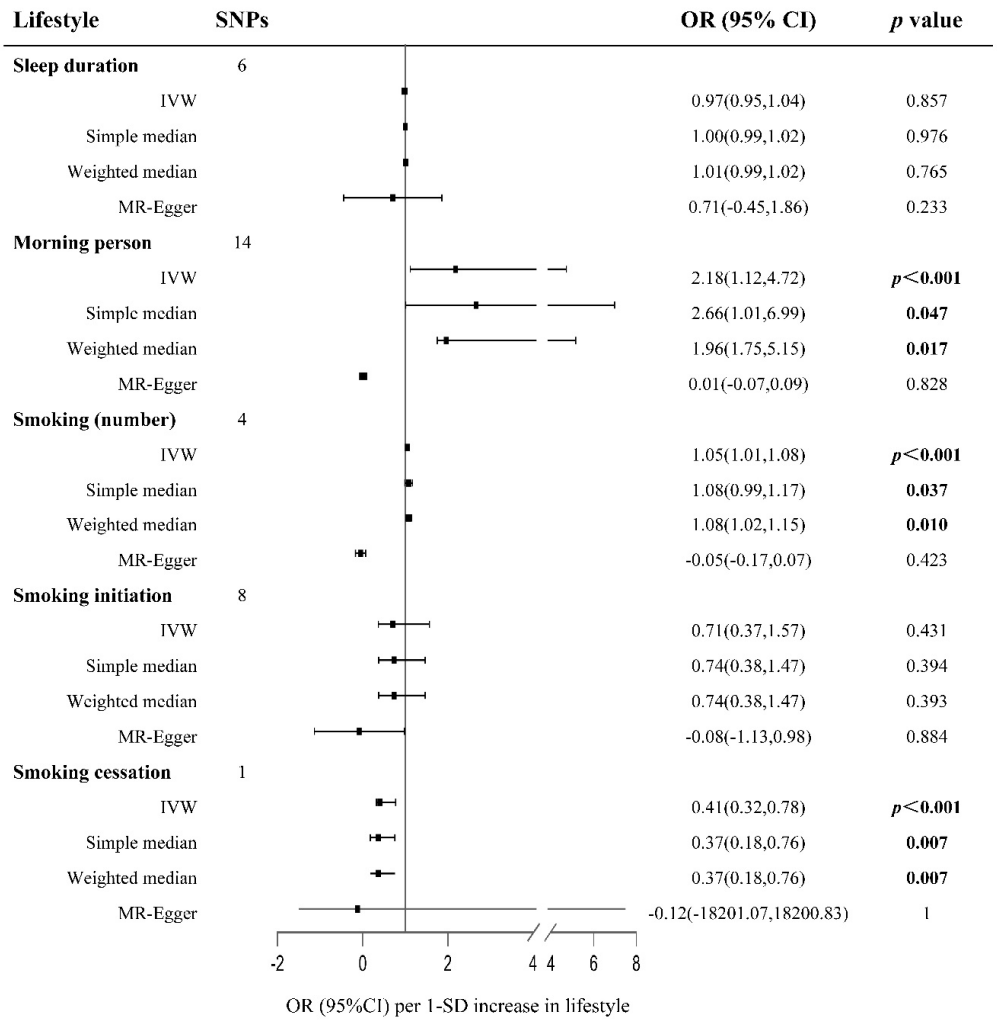

Figure 2. Odds ratios for associations between genetically predicted lifestyle and the risk of PD. In the Mendelian randomization analysis, sleep duration was scaled to one hour of sleep (1 SD: 0.5 hour), morning person to 5% change, and smoking to 10 cigarettes per day. SNPs: single nucleotide polymorphisms.

among up to 452,000 individuals supported that cigarette smoking conferred by four variants without pleiotropy was significantly associated with a high risk of PD. Few epidemiological studies have examined the association between being a morning person and PD risk. Our MR analysis found a significant association between genetically being a morning person and PD, consistently, a recent MR analysis showed that being a morning person tends to increase the risk of PD.[48] It was shown that sleep duration was positively associated with increased risk for PD, and habitual longer sleep duration may be an earlier marker of PD in a prospective study.[49] However, significant causal association between sleep duration and PD was not observed in our analysis based on the larger sample size. In line with our results, an authoritative review supported that there was no association between genetic predisposition for sleep duration and PD.[50]

A population-based cohort study reported that the risk of PD was significantly associated with a lower level intake of dietary vitamin B-6 rather than folate and vitamin B-12.[4] Another recent cohort study indicated that low serum vitamin B-12 concentration (<234 pmol/L) predicted worsening of mobility, suggesting the protective role of vitamin B-12 in PD progression.[51] Our MR analysis involving 452,264 participants provided sufficient evidence to support that low risk of PD was associated with genetically predicted high serum content of vitamin B-12 rather than vitamin B-6 or folate. This discrepancy may be related to the small sample size of the above two cohort studies. In addition, vitamin B-12 deficiency was reported to aggravate PD in patients receiving levodopa therapy by impairing S-adenosylmethionine synthesis in the substantia nigra, indicating a positive effect of vitamin B-12 on PD.[52] In addition, a well-designed cohort of Fullard ME et al showed that chronic vitamin D insufficiency did not affect dopaminergic system integrity.[53] We did not find an association between genetically determined 25(OH)D concentrations and the risk of PD based on both 4 SNPs or 5 SNPs (adding a significant variant, rs6013897). Similarly, the causal association of lower 25(OH)D concentration with the risk of PD was not found in a recent MR analysis based on 4 SNPs related to 25(OH)D concentrations.[54] It is generally thought that polyunsaturated fatty acids are beneficial as an adjuvant approach to pharmacological therapy.[55,56] Our MR supported that genetically predicted higher DHA concentration was associated with a lower risk of PD, in contrast to other polyunsaturated fatty acids, such as ALA or EPA. An MR analysis reported that PD risk was reduced by 0.997 (0.994–0.999) for every 10 mg/dL increase in se-

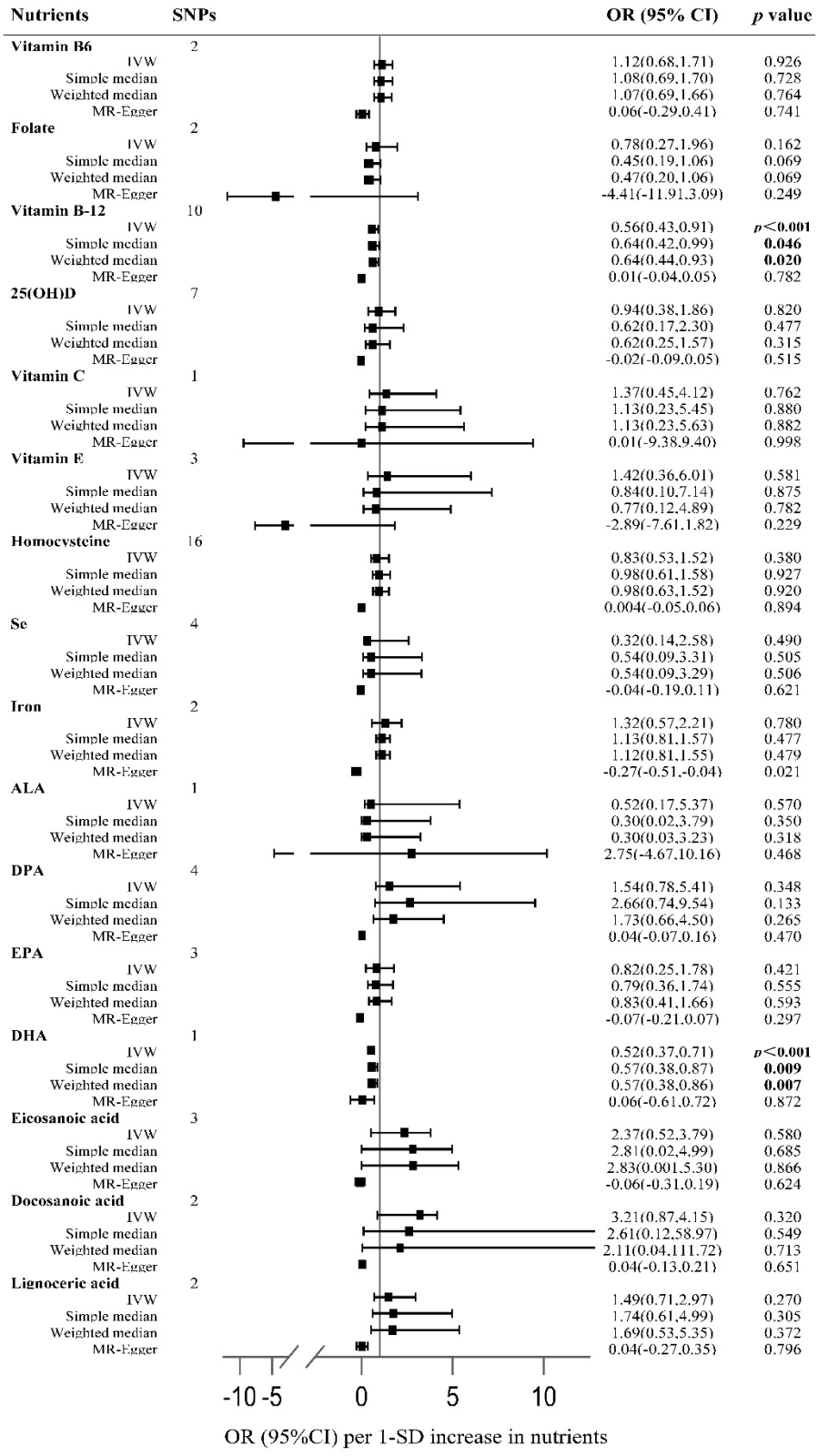

Figure 3. Odds ratios for associations between genetically predicted nutrients and PD. Estimates are 20% increase of 25-hydroxyvitamin D concentration, and 1 SD serum folate, serum vitamin B-6, serum vitamin B-12, serum vitamin C, serum vitamin E, serum selenium, serum iron, serum fatty acids and total homocysteine. SNPs: single nucleotide polymorphisms; Se: selenium.

rum iron[57] level by estimating the association between rs1799945 and serum iron concentrations in a small cohort of 278 PD patients.[58] We re-ran MR analysis using published estimates from the Dutch longitudinal projects on approximately 4,818 participants based on three variants (rs1800562, rs1799945, rs855791) or one variant (rs1799945),[59] and did not find significant results.

Lipids are involved in the formation of vast and very complex biomolecules, which makes it difficult to precisely interpret the biological significance of lipidome

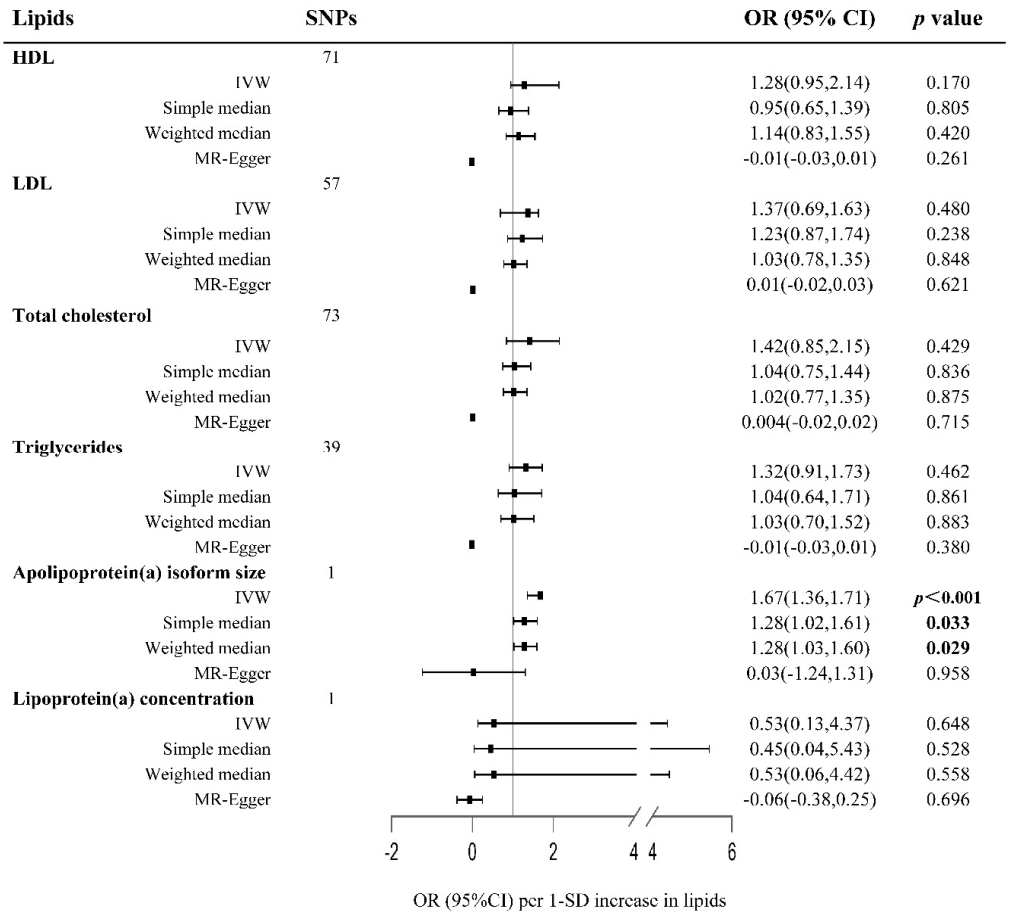

Figure 4. Odds ratios for associations between genetically predicted lipids and Parkinson's disease. Estimates are per approximate 1 SD increase of triglycerides, total cholesterol, LDL, HDL, and lipoprotein (a) concentration. SNPs: single nucleotide polymorphisms; HDL: high density lipoprotein; LDL: low density lipoprotein.

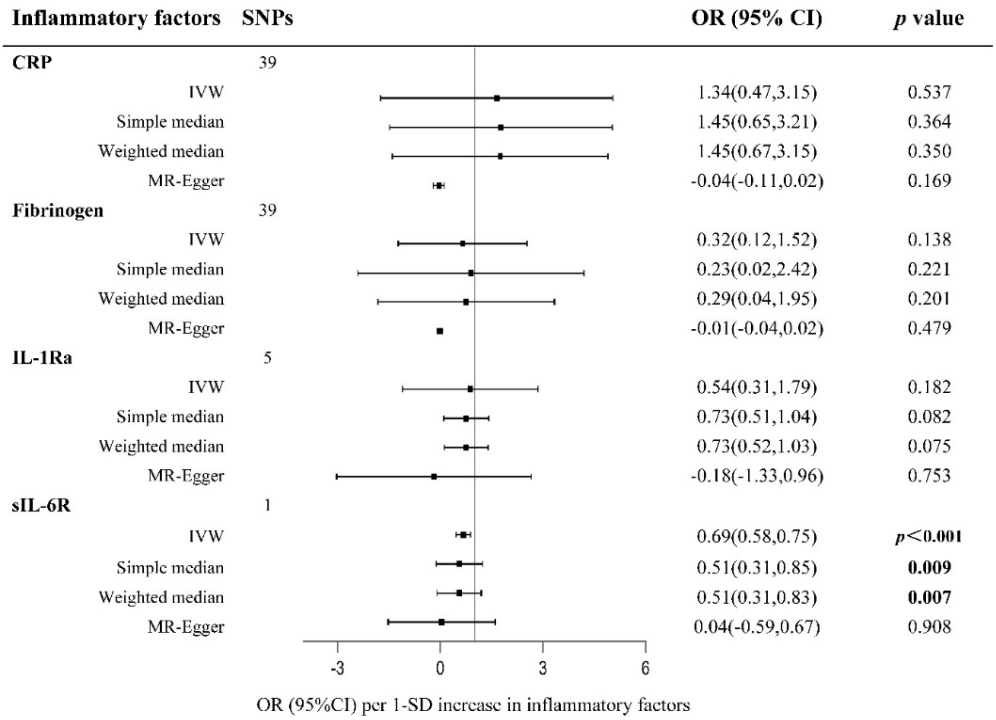

Figure 5. Odds ratios for associations between genetically predicted inflammatory factors and PD. Estimates are per approximate 1 SD increase of sIL-6R, fibrinogen, and C-reactive protein. SNPs: single nucleotide polymorphisms; IL-1Ra: Interleukin-1 receptor antagonist; sIL-6R: soluble interleukin-6 receptor.

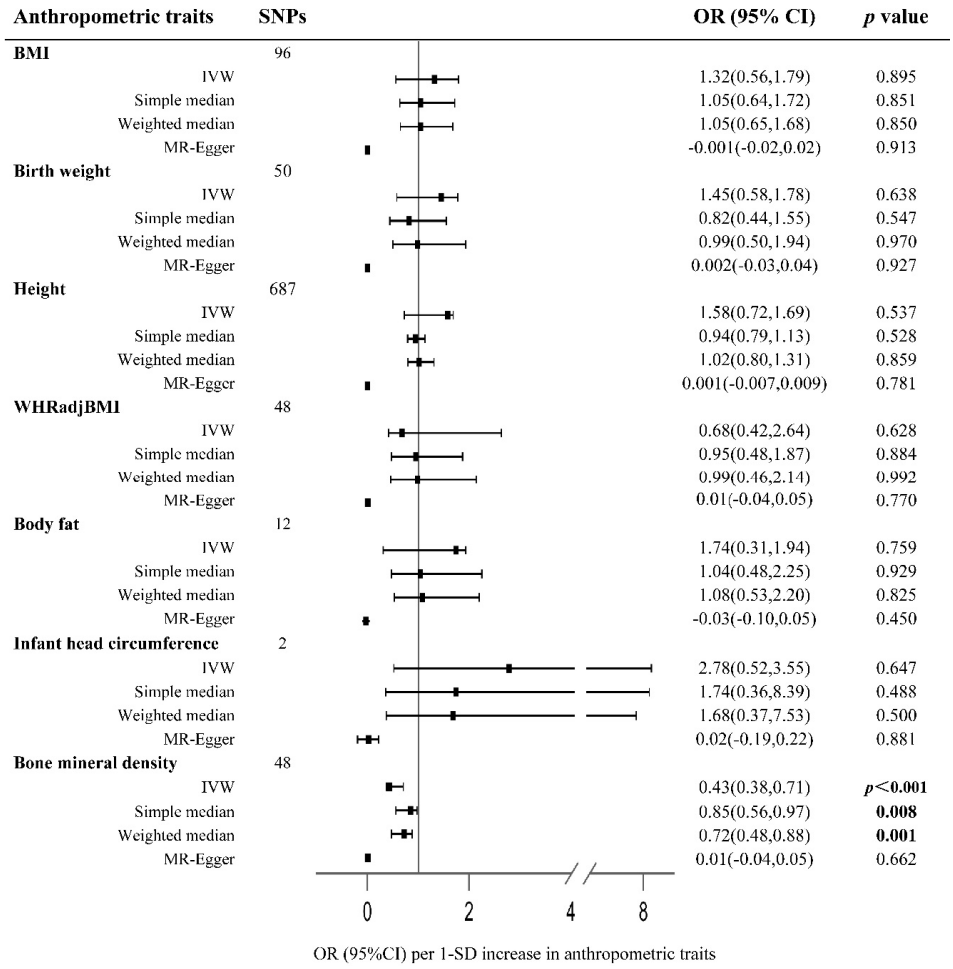

Figure 6. Odds ratios for associations between genetically predicted anthropometric traits and PD. Estimates are per approximate 1 SD increase of continuous risk factors. SNPs: single nucleotide polymorphisms; BMI: Body mass index; WHRadjBMI: Waist-to-hip ratio adjusted for body mass index.

abnormalities. Therefore, the effect of HDL, LDL, and triglycerides on the risk of PD was controversial in observational studies.[60-62] In a case-control study by Baum et al, it was reported that polymorphism in exon 3 of the LDL receptor-related protein (C766T) was not associated with PD risk.[63] Two prospective studies reported that there was no association between cholesterol intake and PD risk.[64,65] Of note, an MR analysis enrolling 111,194 Danishes also observed no association between genetically determined LDL concentrations and PD risk,[66] which is consistent with our MR analysis. There is limited epidemiological evidence for the relationship between apolipoprotein (a) isoform size and lipoprotein(a) concentration and PD risk. Our MR analysis found a significant causal association between apolipoprotein(a) isoform size and PD risk. However, our data did not support a causal association between lipoprotein (a) concentration and PD risk based on one variant (rs3777392), which was mostly associated with lipoprotein (a) concentration and could be used to precisely explore the causality between lipoprotein (a) and PD. It was reported that IL-6 markedly upregulated gp130 expression in some tissues.[67] In particular, IL-6 could cause an increased expression of gp130 in the damaged nerve.[68] Thus, IL-6 with its receptor may mediate nerve repair by elevating the gp130 signal transduction system. Of note, IL-6R is vital for peripheral nerve repair, which was also proven in a previous study by Hirota H et al. Our data supported a causal association between higher serum sIL-6R concentration and a lower PD risk.[69]

A prospective cohort study of 8,105 elderly women with PD (n = 73 with PD) showed that they had decreased hip bone mineral density and an increased hip fracture risk.[70] Our MR also supported a causal association between higher bone mineral density and lower risk of PD. A close relationship between delta nerve fibers and bone mineral density may be a potential mechanism.[71] No causality was found between body height and PD in our analysis when body height conferred by 697 genetic variants was used and 350 SNPs strongly associated with body height were analyzed. In addition, no association was observed between BMI and PD risk when 96 genetic variants most strongly associated with BMI were used. Our MR analysis result is different from a previous MR analysis,[72] which reported that a higher BMI led to a lower risk of PD. This discrepancy in metabolic factors and disease risk may be related to reverse causation bias or confounding.[73,74]

Strengths and limitations

A large number of participants were included, multiple potentially modifiable factors in relation to PD were assessed, and multiple methods were used in the current study that contribute to more precisely examine causal

associations. Of note, our MR analysis newly identified that genetic smoking cessation and higher circulating vitamin B-12, DHA, and sIL-6R concentrations are causally associated with a lower risk of PD. However, there are still some limitations to this discussion. First, pleiotropy may not be excluded because our selected SNPs may have a shared genetic basis, which suggests a causal association between modifiable factors and PD risk. Second, misclassification is unavoidable due to the different diagnostic criteria for PD in the UK Biobank, although the cases of participants met the standard criteria for probable PD. Third, there may be potential non-linear associations between some modifiable factors and PD.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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