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Research Report

# Mendelian Randomisation Study of Smoking, Alcohol, and Coffee Drinking in Relation to Parkinson's Disease



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**Abstract.**
**Background:** Previous studies showed that lifestyle behaviors (cigarette smoking, alcohol, coffee) are inversely associated with Parkinson's disease (PD). The prodromal phase of PD raises the possibility that these associations may be explained by reverse causation.
**Objective:** To examine associations of lifestyle behaviors with PD using two-sample Mendelian randomisation (MR) and the potential for survival and incidence-prevalence biases.
**Methods:** We used summary statistics from publicly available studies to estimate the association of genetic polymorphisms with lifestyle behaviors, and from Courage-PD (7,369 cases, 7,018 controls; European ancestry) to estimate the association of these variants with PD. We used the inverse-variance weighted method to compute odds ratios ($OR_{IVW}$) of PD and 95% confidence intervals (CI). Significance was determined using a Bonferroni-corrected significance threshold ($p = 0.017$).
**Results:** We found a significant inverse association between smoking initiation and PD ($OR_{IVW}$ per 1-SD increase in the prevalence of ever smoking = 0.74, 95%CI = 0.60–0.93, $p = 0.009$) without significant directional pleiotropy. Associations in participants ≤67 years old and cases with disease duration ≤7 years were of a similar size. No significant associations were observed for alcohol and coffee drinking. In reverse MR, genetic liability toward PD was not associated with smoking or coffee drinking but was positively associated with alcohol drinking.

**Conclusion:** Our findings are in favor of an inverse association between smoking and PD that is not explained by reverse causation, confounding, and survival or incidence-prevalence biases. Genetic liability toward PD was positively associated with alcohol drinking. Conclusions on the association of alcohol and coffee drinking with PD are hampered by insufficient statistical power.

Keywords: Smoking, alcohol, coffee, Parkinson's disease, Mendelian randomisation

## INTRODUCTION

Parkinson's disease (PD) is considered as a multifactorial disease, involving genetic susceptibility and environmental factors [1]. Epidemiologic studies have revealed a striking pattern, whereby several lifestyle behaviors, including cigarette smoking, alcohol, and coffee drinking, are inversely associated with PD [2]. It remains debated whether these inverse associations are causal. The existence of a long prodromal phase in PD [3, 4] raises the possibility that they may be explained by reverse causation defined as situations where the outcome precedes and influences the exposure. For instance, the prodromal phase of PD may be characterized by a loss of appetence for cigarettes [5], and patients who later develop PD may be more successful in their attempts to quit smoking during the prodromal phase due to reduced dopamine reward [6].

Mendelian randomisation (MR) is a form of instrumental variable analysis that uses genetic variants associated with an exposure as instruments to estimate its causal association with a disease [7]. Unlike traditional epidemiologic approaches, MR analyses are not biased by confounding or reverse causation if a set of assumptions are met [8]. However, MR analyses in elderly populations may be biased due to survival bias, a form of selection bias that may arise as older persons consist of a non-random subset of the general population who have survived long enough to be included into the study [9]. This bias is a particular threat for MR studies investigating exposures that strongly affect survival [10], and is more pronounced for two-sample than one-sample MR studies [9]. MR studies that include prevalent cases may also be biased by incidence-prevalence bias, if the genetic instrument is associated with survival after disease onset [11].

As part of the COmprehensive Unbiased Risk factor Assessment for Genetics and Environment in PD (Courage-PD) consortium, we used two-sample MR to examine the association of three lifestyle behaviours (smoking, alcohol drinking, coffee drinking) with PD, and assessed whether MR findings are robust in analyses addressing the potential for survival and incidence-prevalence biases.

## MATERIAL AND METHODS

### *Study design: two-sample Mendelian randomisation*

MR uses genetic variants, mainly single nucleotide polymorphisms (SNPs), associated with an exposure to estimate its causal effect on an outcome. For SNPs to be valid instruments, three assumptions must be verified: i) SNPs should be associated with the exposure (IV1 assumption); ii) SNPs should not be directly associated with the outcome except through the exposure (IV2 assumption); and iii) SNPs should not be associated with unmeasured confounders of the exposure-outcome association (i.e., no horizontal pleiotropy, IV3 assumption) [7].

In two-sample MR, summary statistics (effect size estimates and standard errors [SE]) for the SNP-exposure and SNP-outcome associations, required to estimate the causal exposure-outcome association, come from two independent samples.

### *PD GWAS: Courage-PD consortium*

We used summary statistics from a GWAS (NeuroChip) [12] in 23 out of 35 studies from the Courage-PD consortium (Supplementary Material). We excluded from the analyses: samples overlapping with the international Parkinson Disease Genomics Consortium (iPDGC); Asian studies (so that estimates for SNP-exposure and SNP-PD associations come from European populations); studies that included cases only; studies with less than 50 cases and 50 controls. As the role of environmental factors may be different in carriers of Mendelian PD mutations, we also excluded participants with *GBA*/*LRRK2* mutations or with positive family history of PD. Participants' characteristics are shown in Supplementary Table 1.

In each study, the frequency of SNPs was compared in cases and controls under an additive model using

logistic regression adjusted for sex and the first four principal components. We meta-analysed summary statistics from the 23 GWAS (European ancestry: 7,369 cases, 7,018 controls) using a fixed ($I^2 \leq 25\%$) or random ($I^2 > 25\%$) effects model (Supplementary Material).

Individual studies within each genome-wide association study had received approval from a relevant institutional review board from their country, and informed consent was obtained from participants or from a caregiver, legal guardian, or other proxy.

*GWAS of exposures*

We used summary statistics (betas, SEs) from published GWAS in individuals of European descent to select SNPs associated with exposures of interest at a genome-wide significant threshold ($p < 5 \times 10^{-8}$) and with a minor allele frequency (MAF) $\geq 0.01$. To retain independent SNPs, we clumped them based on European ancestry reference data (1000 Genomes Project, $r^2 > 0.001$, genomic region = 10,000 kb). For SNPs not available in Courage-PD, we selected proxies in high linkage disequilibrium (LD) with the index SNP ($r^2 > 0.8$) according to LDlink [13] or SNIPA [14]. Summary statistics were harmonised on alleles positively associated with exposures. Ambiguous palindromic SNPs (A/T, C/G) with a MAF > 0.42 were discarded [15].

*Smoking initiation and alcohol drinking*

The GWAS and Sequencing Consortium of Alcohol and Nicotine use (GSCAN) provided summary statistics for smoking initiation ($n = 1,232,091$, 203 SNPs) and for the number of alcohol drinks per week ($n = 941,280$, 71 SNPs) in participants of European descent [16]. The proxy rs117495226 in high LD ($r^2 = 0.90$) with the index SNP rs6050446 was used.

Information on smoking status was missing in several studies from Courage-PD; therefore, we were not able to run analyses stratified by smoking status and to examine the role of SNPs associated with smoking heaviness or age at smoking cessation or initiation in smokers [17]. To overcome this limitation, we used a lifetime smoking exposure index developed using UK Biobank data (in 462,690 European descent) that allows to perform MR using summary data from samples unstratified by smoking status. Briefly, this lifetime smoking index combines several aspects of smoking, including smoking status and, among ever smokers, duration, heaviness, and cessation, and was validated using positive outcomes (e.g., lung cancer). We selected 126 SNPs associated with this index [17].

*Coffee drinking*

We used a GWAS on self-reported bitter and sweet beverage consumption, including coffee (European ancestry, $n = 370,000$, 11 SNPs) [18].

*Statistical analyses*

Statistical analyses were performed using the *TwoSampleMR*, *MRPRESSO*, and *simex* R packages (R Foundation for Statistical Computing, Vienna, Austria). *P*-values are two-sided. We examined three main exposures (smoking initiation, alcohol drinking, coffee drinking). To account for multiple testing, we used a conservative approach and applied a Bonferroni corrected significance level of 0.017 (i.e., 0.05/3). *P*-values $\leq 0.05$ but $> 0.017$ were considered as suggestive statistical evidence for an association [19].

Only SNPs available in 75% ($n = 17$) of the studies or more were retained for our analyses. For each SNP, we computed the proportion of the variance of the exposure explained by the SNP ($R^2$) [8].

For MR analyses of individual SNPs, we used the Wald ratio estimate (exponentiated ratio of the SNP-outcome association to the SNP-exposure association).

Our primary MR analyses based on multiple SNPs were conducted using the random-effects inverse-variance weighted (IVW) method that provides accurate estimates for SNPs that verify IV assumptions. Heterogeneity between genetic instruments was tested using the Cochran's Q-statistic [7].

In sensitivity MR analyses, we used other approaches that relax some IV assumptions. The *MR-Egger method* can detect directional pleiotropy and provides corrected effect estimates but has low power and requires the InSIDE (INstrument Strength Independent of Direct Effect) assumption [20]. We used the $I^2_{GX}$ statistic to quantify the strength of regression dilution bias in SNP-exposure association estimates, with values < 90% indicating violation of the NOME (No Measurement Error) assumption, in which case we used the SIMEX method to correct MR-Egger estimates [21]. The *weighted median-method* provides consistent estimates if at least 50% of the SNPs are valid instruments [22]. The *weighted mode-based method* uses the causal estimate from each SNP to calculate the modal estimate. The largest group of variants with the same causal estimate in

the asymptotic limit are considered valid instruments [23]. *MR-PRESSO* allows to detect outliers (global test), to compute an estimate corrected for horizontal pleiotropy after removing them (if p-global test < 0.05), and to test the difference between the original and updated estimates (distortion test) [24]. In addition, as two SNPs were associated with both alcohol and coffee drinking, we repeated analyses for these phenotypes after excluding them.

PD is typically a disease of old age, and survival bias may bias genetic associations and MR estimates in any direction in studies in older populations for exposures that are associated with survival into old age (Supplementary Figure 1) [9, 10]. In some situations, increasing age is associated with increasing bias; in addition, bias is likely to be more pronounced in two-sample than one-sample MR studies. As smoking and alcohol drinking are strongly associated with survival, we performed analyses stratified by median age at study of cases and controls (67 years) and examined whether MR estimates observed overall were consistent with those seen in younger participants in whom survival bias is unlikely as mortality rates are low in this group [25–27].

The consortium included prevalent and incident patients. If genetic variants have a stronger effect on survival in PD patients than controls, genetic associations may be biased [11]. We examined whether MR estimates in cases with shorter disease duration (median $\leq 7$ years) compared to controls were consistent with those obtained overall.

Finally, we tested for reverse causation by performing a reverse MR analysis where the exposure was PD genetic susceptibility and the outcome was smoking initiation, alcohol, or coffee drinking. We identified top SNPs (and corresponding association estimates) from the largest PD iPDGC GWAS as exposure instrumental variables [28], and extracted summary statistics for these SNPs from the GWAS for lifestyle behaviours [16–18]. For smoking initiation, we used summary statistics without 23andMe (N = 599,289). In this analysis, we estimated the effect of genetic liability toward PD on lifestyle behaviours in order to examine whether genetic liability toward PD is associated with smoking initiation, alcohol drinking, or coffee drinking. ORs are scaled to 1-unit increase in log odds of the prevalence of PD [29].

*Statistical power*

For each exposure, we computed the proportion of variance explained by the SNPs, the F-statistic as a measure of instrument strength [15], and statistical power for a type-I error rate of 5% and 1.7% (Supplementary Table 2) [30].

*Data availability*

Results can be reproduced using Supplementary Tables 3 and 4; no additional data available.

## RESULTS

The associations of SNPs with exposures and PD are shown in Supplementary Tables 3 and 4. Supplementary Table 5 shows the number of SNPs retained for each exposure and F-statistics.

*Smoking and PD*

Of the SNPs positively associated with smoking traits, two (1.1%) were positively associated with PD at $p < 0.05$ for smoking initiation, and five (4.4%) for the lifetime smoking index; six (3.3%) were inversely associated with PD for smoking initiation, and five (4.4%) for the lifetime smoking index (Supplementary Table 3).

Smoking initiation was significantly and inversely associated with PD ($OR_{IVW} = 0.74$, 95% CI = 0.60–0.93, $p = 0.009$; Table 1, Supplementary Figure 2). The weighted median approach also showed a significant inverse association ($p = 0.008$) while the weighted mode approach yielded slightly lower point estimates with wider CIs. The MR-Egger method did not show significant directional pleiotropy.

In sensitivity analyses, there was a suggestive inverse association for the lifetime smoking index ($OR_{IVW} = 0.54$, 95% CI = 0.29–1.00, $p = 0.050$; Table 1, Supplementary Figure 2) that became more significant after excluding two outliers identified by MR-PRESSO ($OR_{MR\text{-}PRESSO} = 0.51$, 95% CI = 0.29–0.89, $p = 0.021$). The weighted median method yielded an inverse association that was statistically significant ($p = 0.017$). MR-Egger did not show significant directional pleiotropy.

Both for smoking initiation and the lifetime smoking index, associations tended to be stronger in younger individuals (smoking initiation: $OR_{IVW} = 0.68$, 95% CI = 0.48–0.97; lifetime smoking index: $OR_{IVW} = 0.46$, 95% CI = 0.19–1.09) than in older ones (smoking initiation: $OR_{IVW} = 0.83$, 95% CI = 0.58–1.18; lifetime smoking index: $OR_{IVW} = 0.88$, 95% CI = 0.37–2.07) and were consistent with those seen overall (Figure 1, Supplementary Table 6).

Table 1
Effect of genetically-predicted smoking on PD

| Exposure | Odds ratio (95% CI) | *p* |
|---|---|---|
| Smoking initiation (per 1-SD increase in the prevalence of ever smoking) | | |
| IVW (p-heterogeneity = 0.39) | 0.74 (0.60–0.93) | 0.009 |
| Weighted median | 0.64 (0.47–0.89) | 0.008 |
| Weighted mode | 0.58 (0.24–1.42) | 0.23 |
| MR Egger (p-pleiotropy = 0.59; $I^2_{GX}$ = 0.66) | 0.59 (0.24–1.45) | 0.25 |
| Corrected MR Egger | 0.56 (0.22–1.39) | 0.26 |
| MR-PRESSO (p-pleiotropy = 0.24) | – | – |
| Lifetime smoking index (per 1-unit)[a] | | |
| IVW (p-heterogeneity = 0.007) | 0.54 (0.29–1.00) | 0.050 |
| Weighted median | 0.37 (0.16–0.84) | 0.017 |
| Weighted mode | 0.29 (0.06–1.34) | 0.12 |
| MR Egger (p-pleiotropy = 0.93; $I^2_{GX}$ = 0.64) | 0.60 (0.05–6.77) | 0.68 |
| Corrected MR Egger | 0.60 (0.06–6.47) | 0.68 |
| MR-PRESSO (p-pleiotropy = 0.010, p-distortion = 0.77)[b] | 0.51 (0.29–0.89) | 0.021 |

CI, confidence interval; IVW, inverse variance weighted. [a]In the UK Biobank, the lifetime smoking index had a mean of 0.359 and a standard deviation (SD) of 0.694; a 1-SD increase in the lifetime smoking index is equivalent to an individual smoking 20 cigarettes a day for 15 years and stopping 17 years ago or an individual smoking 60 cigarettes a day for 13 years and stopping 22 years ago. The $OR_{IVW}$ for a 1-SD increase is $0.67^{0.694} = 0.76$. [b]Outliers: rs202645.

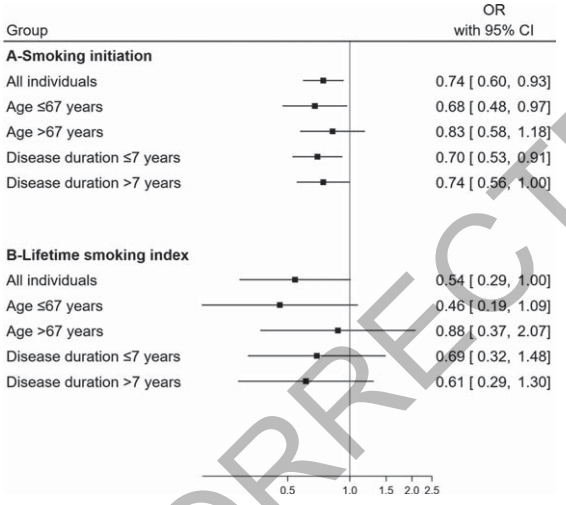


Fig. 1. Forest plot showing MR estimates for the association of smoking initiation and a lifetime smoking index with PD, overall and after stratification by age at study and disease duration in cases. OR, odds ratio; CI, confidence interval.

Associations were of a similar size in patients with shorter and longer disease duration and comparable to associations seen overall (Figure 1, Supplementary Table 7).

*Alcohol drinking and PD*

Of the SNPs positively associated with alcohol drinking, two (3.2%) were positively and three (4.8%) were inversely associated with PD at $p < 0.05$ (Supplementary Table 3).

There was no significant association between alcohol drinking and PD ($OR_{IVW} = 0.68$, 95% CI = 0.39–1.18, $p = 0.17$; Table 2, Supplementary Figure 2). Exclusion of two SNPs associated with coffee drinking led to similar conclusions. In stratified analyses, there was no significant association between alcohol drinking and PD in younger individuals and patients with shorter disease duration (Supplementary Tables 6 and 7). There was a trend towards an inverse association only in prevalent PD cases ($OR_{IVW} = 0.53$, 95% CI = 0.27–1.02, $p = 0.057$).

MR analyses using a single SNP in the *ADH1B* gene (rs1229984) showed no association with PD ($OR_{Waldratio} = 0.93$, 95% CI = 0.45–1.91, $p = 0.85$; Supplementary Table 3); this gene plays an important role in explaining alcohol drinking variance and analyses based on this gene are less likely to be biased by pleiotropy due to the known role of alcohol dehydrogenase in alcohol metabolism.

*Coffee drinking and PD*

Of the SNPs positively associated with coffee drinking, one (9.1%) was positively, and another (9.1%) was inversely associated with PD at $p < 0.05$ (Supplementary Table 3).

There was a positive and non-statistically significant association of genetically-predicted coffee drinking with PD ($OR_{IVW} = 1.69$, 95% CI = 0.51–5.63, $p = 0.40$; Table 3, Supplementary Figure 2). However, after excluding two SNPs associated with alcohol drinking, the association decreased

Table 2
Effect of genetically-predicted alcohol drinking on PD

| Exposure | Odds ratio (95% CI) | *p* |
|---|---|---|
| Alcohol drinking (per 1-SD increase of ln(drinks per week)) | | |
| IVW (p-heterogeneity = 0.062) | 0.68 (0.39–1.18) | 0.17 |
| Weighted median | 0.86 (0.42–1.75) | 0.67 |
| Weighted mode | 0.86 (0.41–1.77) | 0.68 |
| MR Egger (p-pleiotropy = 0.94; $I^2_{GX}$ = 0.96) | 0.70 (0.30–1.61) | 0.40 |
| MR-PRESSO (p-pleiotropy = 0.042, p-distortion = 0.73[a]) | 0.77 (0.46–1.29) | 0.33 |
| Alcohol drinking: after exclusion of 2 SNPs associated with coffee drinking: rs1260326 and rs2472297 (per 1-SD increase of ln(drinks per week) | | |
| IVW (p-heterogeneity = 0.11) | 0.78 (0.45–1.35) | 0.37 |
| Weighted median | 0.88 (0.44–1.76) | 0.72 |
| Weighted mode | 0.85 (0.46–1.58) | 0.61 |
| MR Egger (p-pleiotropy = 0.98; $I^2_{GX}$ = 0.96) | 0.78 (0.34–1.78) | 0.56 |
| MR-PRESSO (p-pleiotropy = 0.089) | – | – |

CI, confidence interval; IVW, inverse variance weighted. [a]Outliers: rs9607814.

Table 3
Effect of genetically-predicted coffee drinking on PD

| Exposure | Odds ratio (95% CI) | *p* |
|---|---|---|
| Coffee drinking (per ln(cups per day)) | | |
| IVW (p-heterogeneity = 0.017) | 1.69 (0.51–5.63) | 0.40 |
| Weighted median | 1.51 (0.50–4.51) | 0.47 |
| Weighted mode | 1.45 (0.52–4.03) | 0.50 |
| MR Egger (p-pleiotropy = 0.73; $I^2_{GX}$ = 0.97) | 2.50 (0.21–29.59) | 0.49 |
| MR-PRESSO (p-pleiotropy = 0.035, p-distortion = 0.86)[a] | 1.86 (0.67–5.11) | 0.26 |
| Coffee drinking: after exclusion of 2 SNPs associated with alcohol drinking: rs1260326 and rs2472297 (per ln(cups per day)) | | |
| IVW (p-heterogeneity = 0.053) | 1.09 (0.26–4.45) | 0.91 |
| Weighted median | 1.06 (0.32–3.53) | 0.93 |
| Weighted mode | 1.11 (0.31–4.01) | 0.88 |
| MR Egger (p-pleiotropy = 0.35; $I^2_{GX}$ = 0.96) | 3.86 (0.22–66.63) | 0.38 |
| MR-PRESSO (p-pleiotropy = 0.12) | – | – |

CI, confidence interval; IVW, inverse variance weighted. [a] Outlier: rs574367.

($OR_{IVW}$ = 1.09, 95% CI = 0.26–4.45, $p$ = 0.91). Stratified analyses showed no associations (Supplementary Tables 6 and 7).

MR analyses based on SNPs in two genes that are known to play an important role in caffeine metabolism (*AHR*-rs4410790, *CYP1A2*-rs2472297) showed no association with PD (rs4410790: $OR_{Waldratio}$ = 0.79, 95% CI = 0.21–2.98, $p$ = 0.72; rs2472297: $OR_{Waldratio}$ = 2.09, 95% CI = 0.47–9.16, $p$ = 0.33; Supplementary Table 3).

*Reverse MR*

Supplementary Table 8 shows the SNPs and association estimates used for reverse MR analyses. Table 4 shows the results of reverse MR analyses. There was no association between genetic liability toward PD and smoking or coffee drinking. For alcohol drinking, genetic liability toward PD was positively associated with alcohol drinking using the IVW method. MR-PRESSO identified 5 outliers; the corrected MR estimate after excluding these outliers was still in favour of a positive association.

## DISCUSSION

Based on data from the Courage-PD consortium and after exclusion of samples overlapping with iPDGC, our findings add further evidence in favour of an inverse association between smoking initiation and PD but not for alcohol and coffee drinking.

*Smoking*

According to observational studies, ever smokers have a ∼40% reduced risk of developing PD [31]. It has been argued, however, that this association may be due to reverse causation [5] as PD patients may stop smoking more easily during the PD prodromal phase than other persons due to reduced

Table 4
Reverse Mendelian randomisation using PD-associated SNPs from iPDGC [28] as genetic instruments

| Method | OR (95%CI) | *p* | p-het. | p-pleio. |
|---|---|---|---|---|
| Smoking initiation | 64 SNPs | | | |
| IVW | 1.01 (0.99–1.02) | 0.18 | < 0.001 | |
| Weighted median | 1.01 (0.99–1.02) | 0.36 | | |
| Weighted mode | 1.01 (0.99–1.03) | 0.35 | | |
| MR-Egger | 1.02 (0.99–1.05) | 0.15 | | 0.36 |
| MR-PRESSO | – | – | | < 0.001[a] |
| Lifetime smoking index | 65 SNPs | | | |
| IVW | 1.00 (0.99–1.01) | 0.83 | < 0.001 | |
| Weighted median | 1.00 (0.99–1.01) | 0.38 | | |
| Weighted mode | 0.99 (0.98–1.01) | 0.092 | | |
| MR-Egger | 1.01 (0.99–1.02) | 0.34 | | 0.23 |
| MR-PRESSO | 1.00 (0.99–1.01) | 0.56 | | < 0.001[b] |
| Alcohol drinking | 64 SNPs | | | |
| IVW | 1.02 (1.01–1.03) | 0.002 | < 0.001 | |
| Weighted median | 1.01 (0.99–1.02) | 0.14 | | |
| Weighted mode | 1.01 (0.99–1.02) | 0.21 | | |
| MR-Egger | 1.02 (0.99–1.04) | 0.097 | | 0.85 |
| MR-PRESSO | 1.01 (1.00–1.02) | 0.042 | | < 0.001[c] |
| Coffee drinking | 65 SNPs | | | |
| IVW | 1.00 (0.99–1.01) | 0.15 | < 0.001 | |
| Weighted median | 1.00 (0.99–1.01) | 0.82 | | |
| Weighted mode | 1.00 (0.99–1.01) | 0.65 | | |
| MR-Egger | 1.00 (0.99–1.01) | 0.69 | | 0.21 |
| MR-PRESSO | 1.00 (0.99–1.01) | 0.25 | | < 0.001[d] |

OR, odds ratio per 1-unit increase in log odds of the prevalence of PD; IVW, inverse-variance weighted (random-effect); CI, confidence interval; p-het., p for heterogeneity (IVW); p-pleio., p for pleiotropy (MR-Egger and MR-PRESSO). [a]No outlier detected. [b]Outliers: rs12497850, rs12600861, and rs62053943; p-distortion = NS. [c]Outliers: rs62053943, rs6476434, rs6854006, rs7134559, and rs823118; p-distortion = 0.041. [d]Outliers: rs2248244 and rs61169879; p-distortion = NS.



responsiveness to nicotine [6]. Others argued that the association seen in long-term ex-smokers [1] and the dose-effect relationship seen both in ex- and current-smokers are not in favour of this hypothesis [32].

Previous MR studies examined the association between smoking and PD using iPDGC data. One study (9,581 cases, 33,245 controls) assessed several risky behaviours in relation with PD [33], and reported a significant inverse association for smoking initiation (213 SNPs; OR per log odds of ever smoking = 0.71, 95% CI = 0.57–0.90). In another study (37,688 cases with some overlap with the previous study, 18,618 UK Biobank proxy-cases, 1.4 million controls), there was a trend towards an inverse association between ever-smoking and PD that was not statistically significant (OR per log odds of ever-smoking = 0.94, 95% CI = 0.88–1.01) [28]. Recently, another MR study used the same dataset to assess the role of several tobacco behaviours (smoking initiation, continuation, heaviness, and age at initiation). They showed that smoking continuation (current versus former smokers) was inversely associated with PD (OR per doubling of odds for smoking continuation = 0.64, 95%CI = 0.46–0.89, $p = 0.008$) but smoking initiation, heaviness, or age at initiation were not. However, they used only 87 SNPs for smoking initiation and analyses were not stratified by smoking status for analyses of smoking characteristics among smokers [34]. The iPDGC PD MR Portal, a platform which offers MR analyses for a total of 5,839 GWAS versus the largest iPDGC GWAS available with some overlap with the two previous studies, also reports some evidence to support a protective effect of current smoking using a genetic instrument that included 12 SNPs [35]. These studies are not independent as they relied on overlapping datasets and did not examine associations in younger subjects or in those with shorter disease duration to assess whether survival or incidence-prevalence bias may have distorted MR estimates.

Our study replicates these findings and add further evidence in favour of an association between smoking initiation and lower PD risk; in addition, our findings show that genetic liability toward PD is not associated with smoking initiation which does not support the hypothesis of reverse causation for smoking. We further show that survival into old age and incidence-prevalence bias are unlikely explanations

for the inverse association between smoking and PD. Since smoking is a major risk factor of mortality, differential survival could potentially bias the association between smoking-related SNP and PD; however, the observation that associations in younger participants were consistent with those seen overall is against this hypothesis and supports an association between smoking and PD [9, 25–27]. In addition, the finding that associations tended to be weaker in older participants than in younger ones is in agreement with some previous observational studies that showed age-dependent associations between smoking and PD with no association in the oldest age groups [36, 37]. This pattern could be interpreted in two different ways: i) survival bias may have diluted association estimates in older subjects; ii) alternatively, there may exist age-related etiologic heterogeneity in PD, and smoking may play a weaker role in PD at older ages where other factors may be more important. There was no marked difference in ORs according to disease duration, thus suggesting that incidence-prevalence bias is unlikely.

The consistency of MR findings with those from traditional epidemiologic studies and reports of smoking-by-gene interactions [38, 39] support a causal association between smoking and PD. However, the underlying mechanisms remain poorly understood. Although cigarette smoke includes a wide range of chemical components, a role for nicotine in PD was suggested by studies showing that it reduced neuronal damage in culture systems and protected against nigrostriatal dopaminergic damage in parkinsonian animal models [40]. In addition, among five compounds present in cigarette smoke (anabasine, cotinine, hydroquinone, nicotine, nornicotine), nicotine and hydroquinone inhibited alpha-synuclein aggregation [41].

Previous randomised clinical trials of nicotine for motor symptoms in PD failed to show an effect [42–44], but their design may have been hampered by small sizes and short follow-up. In addition, these trials were conducted in PD patients and examined disease progression rather than prevention. Additional well-designed neuroprotection trials are needed.

*Alcohol drinking*

A meta-analysis of the relation between alcohol and PD reported an inverse association in case-control studies but not in cohort studies, with marked heterogeneity across studies [45]. Two of the cohort studies reported positive associations, while three reported inverse associations, but none of them was statistically significant.

A previous MR study that examined several risky behaviours in relation with PD using iPDGC data (9,581 cases, 33,245 controls) did not provide evidence in favour of an association for the number of drinks per week (70 SNPs; OR = 1.15, 95% CI = 0.87–1.53) or alcohol consumption (7 SNPs; OR = 1.39, 95% CI = 0.11–17.56) [33]. In contrast, another MR study that used the most recent iPDGC dataset showed an inverse and significant association with the number of drinks per weeks (OR = 0.79, 95%CI = 0.65–0.96, $p = 0.021$; 33 SNPs) [28]. We did not find a significant association with alcohol; however, given the low power of the study to detect an association with this exposure, we cannot draw firm conclusions.

Alternatively, reverse MR showed that genetic liability toward PD is positively associated with alcohol drinking, thus suggesting that persons at higher PD risk are more prone to drink alcohol, independently of whether they actually develop PD. This finding warrants further investigation and replication in further studies.

*Coffee drinking*

Previous studies, both case-control and cohort, have shown an inverse association between coffee drinking or caffeine intake and PD, with a dose-effect relationship, that was present after adjustment for smoking or in never-smokers [46].

A previous MR study that assessed several risky behaviours in relation with PD using iPDGC data (9,581 cases, 33,245 controls) did not provide evidence in favour of an association for the number of coffee cups per day (4 SNPs; OR = 1.03, 95% CI = 0.65–1.63). We did not find a significant association with coffee drinking, but given the low power of the study to detect an association with this exposure, we cannot draw firm conclusions. However, we did find that genetic liability toward PD was not associated with coffee drinking which does not support the hypothesis of reverse causation for coffee drinking.

*Strengths and weaknesses of the study*

Strengths of this study include the assessment of several lifestyle behaviours and use of data from a large consortium in which PD cases were carefully assessed by experienced movement disorders

specialists. The MR design represents another strength as it avoids bias from reverse causation and confounding [8]. This approach, however, relies on a number of assumptions. We cannot exclude that our findings might have been affected by weak instrument bias. However, in MR analyses, the F-statistic was >10 for all exposures and bias from weak instruments is expected to be towards the null in a two-sample setting [47]. Pleiotropy is an important concern for MR analyses, and recent guidelines recommend using multiple methods that make different assumptions to assess the robustness of findings [15]. We used a number of approaches developed to address this issue, including the weighted median and mode, MR-Egger, and MR-PRESSO. Population stratification is unlikely to be a major concern for our analyses, as we restricted analyses within the Courage-PD consortium to individuals of European descent, cases were compared to controls from each site, and analyses were adjusted for principal components. Another strength of our study is that individual data on age at study and disease duration were available, which allowed us to run stratified analyses. Although stratification inevitably leads to a loss of statistical power, we were able to examine whether associations were consistent in younger participants and in those with shorter disease duration. As PD is a disease of old age, it is possible that genetic associations are biased by survival bias, but analyses in younger participants are unlikely to be biased as they did not reach the age where mortality rates are high [9].

The main limitation of our study pertains to its statistical power. Our power calculations showed that our sample size was sufficient to detect ORs in the range of 0.4–0.6 for a type-1 error rate of 1.7% to 5%, for smoking and alcohol but not for coffee drinking. The highest power was noted for smoking initiation; the study was underpowered to detect weaker associations for coffee and alcohol drinking, hence limiting our ability to draw firm conclusions for these exposures. Please note that our study examined a limited number of exposures (selected *a priori* based on existing literature) and is not exploratory, and that the Bonferroni correction is conservative. Our aim was to assess whether MR findings were consistent with those from observational studies using different sets of genetic instruments and methods, and to perform subgroup sensitivity analyses to examine the robustness of our findings. One limitation of our analyses for smoking is that we were not able to stratify analyses by smoking status and to compare results in ever and never smokers using genetic instruments for smoking intensity or cessation [48]. We addressed this issue by using a lifetime smoking exposure index that takes into account several aspects of smoking and was developed in order to allow two-sample MR using data unstratified by smoking status [17].

## CONCLUSIONS AND FUTURE RESEARCH

Using an independent dataset, our study confirms previous MR findings adds further evidence in favour of a protective effect of smoking on PD and shows that this association is not explained by survival or incidence-prevalence bias. For alcohol and coffee drinking, larger studies and stronger genetic instruments are needed.

The number of PD cases is predicted to double between 2015–2040 [49], and the identification of neuroprotective strategies is a major goal. Our findings may help prioritise neuroprotective approaches for PD. Further research is necessary to improve genetic instruments for some of the exposures examined here, to understand the pathways involved in these associations, to determine whether these findings are corroborated in non-European populations, whether PD patients subgroups should be more specifically targeted, and whether there are critical periods of exposure.

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