ORIGINAL ARTICLE

Journal of Clinical Periodontology WILEY

Testing the association between tobacco smoking, alcohol consumption, and risk of periodontitis: A Mendelian randomization study

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Abstract

Aim: To investigate the associations of tobacco smoking and alcohol consumption with periodontitis using Mendelian randomization (MR) analysis.

Materials and methods: We used 17 single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) for the number of cigarettes per day from a genome-wide association study (GWAS) of 337,334 individuals, 109 SNPs for a lifetime smoking index from GWAS of 462,690 participants, and 33 SNPs for the number of drinks per week from GWAS of 941,280 individuals. The periodontitis GWAS included 12,289 cases and 22,326 controls. Wald ratios were obtained by dividing the SNP–periodontitis effects by SNP–exposure effects and pooled using an inverse-variance weighted model.

Results: Genetic liabilities for higher number of cigarettes per day (odds ratio [OR] per one standard deviation (1SD) increment = 1.56; 95% CI: 1.18–2.07, p-value = .0018, Q-value = .0054), lifetime smoking index (OR per 1SD = 1.26; 95% CI: 1.04–1.53, p-value = .0161, Q-value = .0242), and drinks per week (OR per 1SD = 1.41; 95% CI: 1.04–1.90, p-value = .0265, Q-value = .0265) were associated with increased odds of periodontitis. Estimates were consistent across robust and multivariable MR analyses.

Conclusions: The findings of this MR analysis suggest an association between tobacco smoking and alcohol consumption with periodontitis.

KEYWORDS
alcohol, Mendelian randomization, periodontitis, tobacco smoking

Clinical Relevance

Scientific rationale for study: The relevance of tobacco smoking and alcohol consumption as risk factors for periodontitis is unclear based on traditional observational studies.

Principal findings: By considering the relationships between genetically predicted values of smoking, alcohol, and periodontitis within a two-sample instrumental variable framework which

is less influenced by environmental confounding and reverse causation, we found that smoking and alcohol affect periodontitis.

Practical implications: This study adds to the evidence base that tobacco and alcohol play a role in periodontitis. Triangulating MR and observational studies, addressing orthogonal sources of bias, are necessary to confirm this finding.

1 | INTRODUCTION

Tobacco smoking and alcohol consumption is a major public health concern (GBD 2019 Risk Factors Collaborators, 2020). The association of smoking and alcohol with periodontitis has received particular attention because both are potentially modifiable behavioural risk factors (Wang et al., 2016; Chapple et al., 2017; Leite et al., 2018; Pulikkotil et al., 2020). Nonetheless, there is still uncertainty regarding the nature of the association of smoking and alcohol use with the development and progression of periodontitis, and ascertaining causality and whether modification of these risk factors will reduce periodontal risk is less certain. Establishing causality is important, as this is essential for recommending public policies and clinical interventions.

Literature suggests that smoking and alcohol consumption increases periodontitis risk, but the available observational data might be subject to confounding and reverse causation, making causal inference difficult (Davey Smith & Phillips, 2020). Most available evidence on the association between smoking, alcohol, and periodontitis originates from cross-sectional observational studies, although with such a design one cannot determine the temporal order (Danaei et al., 2012; Heaton et al., 2014). While prospective randomized controlled trials (RCTs) are the criterion standard of causal inference (Collins et al., 2020), performing RCTs to evaluate the effects of smoking and alcohol consumption is infeasible and unethical. Mendelian randomization (MR), which uses single nucleotide polymorphisms (SNPs) as unconfounded proxies for exposures to estimate their effect on outcomes of interest, minimizes bias affecting observational epidemiologic studies (Davey Smith et al., 2020). Conceptually, MR has analogies with RCTs, with randomization occurring at meiosis, and is an important strategy for strengthening causal inference when RCTs are not available (Burgess et al., 2018). MR is less affected by reverse causation, as genetic variants are fixed at conception. MR is also less susceptible to environmental confounding compared with conventional observational studies because genetic instrumental variables (IVs) are assumed to affect the outcome only via the exposure and to be independent of confounders. We used MR to investigate the potential causal associations of genetic liability for tobacco smoking and alcohol consumption with periodontitis.

2 | MATERIALS AND METHODS

MR applies IVs to investigate the potential causal effect of an exposure using genetic variants as IVs for the exposure (Burgess et al., 2018). IVs are used to make causal inference in non-experimental data and have been widely used in economics (Wooldridge, 2010). Given the random allocation of genetic variants at conception, MR estimates are not biased by confounding, reverse causation, and measurement error. The most widely adopted approach is to rely on inferences from SNPs identified through genome-wide association studies (GWAS) (Burgess et al., 2018). Three core assumptions are required for a genetic variant to qualify as a valid IV: (1) the genetic variants are reliably associated with the exposure (the "relevance" assumption); (2) they are independent of confounding factors connecting the exposure with the outcome (the "exchangeability" or "no correlated pleiotropy" assumption); and (3) the genetic variants do not affect the outcome via any variable other than the exposure (the "exclusion restriction" criterion) (Hemani et al., 2018; J. Labrecque & Swanson, 2018). To satisfy the first MR assumption, we chose SNPs that were associated with our exposure variables at a level of genome-wide significance (p-value $<5 \times 10^{-8}$) and performed linkage disequilibrium (LD) clumping to ensure that the SNPs are independent by selecting only the SNP with the lowest p-value among all SNPs with an LD $r^2 \geq .001$ (Burgess et al., 2020). To further verify the first assumption, we computed the F-statistic and the proportion of the variance of phenotype explained by all SNPs (Burgess & Thompson, 2011).

We used GWAS exposure summary data from the largest available GWAS on the number of cigarettes per day, a lifetime smoking index, and the number of alcoholic drinks per week (Liu et al., 2019; Wootton et al., 2020). The GWAS of the number of cigarettes per day included 337,334 individuals from the Sequencing Consortium of Alcohol and Nicotine use (GSCAN), UK Biobank, and 23andMe (Liu et al., 2019). Cigarettes per day was defined as the average number of cigarettes smoked per day, either as a current smoker or former smoker. Any non-European samples were excluded to meet the assumption of the two-sample MR approach that the samples are homogeneous so that the SNP–exposure associations are identical across the samples (Burgess et al., 2018). The GWAS also excluded results from smaller studies when estimation results were inflated or deflated per the genomic control. We selected 17 SNPs as instruments for the number of cigarettes per day, which explained 3.7% in the phenotypical variance and had a minimum F-statistic of 29.7 (Supplementary Table 1). A secondary analysis was performed using GWAS summary data on a lifetime smoking index among UK Biobank participants. Applying a previously established methodology (Leffondré et al., 2006), the authors of the GWAS developed a model that incorporated time since onset, duration of smoking, and cigarettes per day, as well as the half-life and lag time constants to capture the non-linear risk of smoking on health (Wootton et al., 2020).

After excluding individuals who did not pass genotype exclusions and who had missing phenotype data, 462,690 individuals remained for the GWAS. The 109 SNPs selected for the lifetime smoking index explained 12.1% of the phenotypical variation; the minimum F-statistic was 29.8. The GWAS of alcohol consumption was based on the number of drinks consumed per week in 941,280 individuals from several cohorts including 23andMe, UK Biobank, and deCODE. Drinks per week was defined as the number of drinks a study participant reported drinking each week, aggregated across the types of alcohol. We selected 33 SNPs explaining 2.8% of the phenotypical variance with a minimum F-statistic of 20.8 (Liu et al., 2019).

To verify the second MR condition, that is, the IVs are not associated with confounders, we searched the PhenoScanner database (Kamat et al., 2019) for previously reported associations of instrument SNPs (and LD proxies) with potential confounders. When the PhenoScanner search revealed previously described genome-wide significant associations (p-value $<5 \times 10^{-8}$) with common causes of smoking/alcohol and periodontitis (Chapple et al., 2017), we performed multivariable MR analysis (Sanderson et al., 2019) to adjust for indirect pathways, which could have introduced correlated pleiotropy. The PhenoScanner search found associations of instruments with obesity and education-related traits. We, therefore, selected SNPs for body mass index from a GWAS (Pulit et al., 2019) of 694,649 participants and SNPs for education from a GWAS (Lee et al., 2018) of 1.131 million individuals for the multivariable MR analyses.

To test the third MR assumption, that is, the instrument is not associated with the outcome other than via its association with the exposure, we examined potential pleiotropy by testing for heterogeneity of the individual SNP effects using the Cochran Q and I_{GX}^2 statistics, applied the MR Egger intercept test of directional pleiotropy, the leave-one-out MR analysis to assess whether the MR estimate was driven by a single SNP, and applied various pleiotropy-robust MR methods (Hemani et al., 2018). We extracted estimates of the effects of the smoking and alcohol exposure associated variants on periodontitis from a GWAS of European studies, contributing studies of the GeneLifestyle Interactions in Dental Endpoints (GLIDE) consortium, totaling 17,353 clinical periodontitis cases and 28,210 controls (Shungin et al., 2015; Shungin et al., 2019). Periodontitis cases were classified by either the Centers for Disease and Control and Prevention/American Academy of Periodontology (Page & Eke, 2007) or Community Periodontal Index (CPI) (World Health Organization, 2013) case definition based on probing depth and/or number of deep periodontal pockets (Shungin et al., 2015).
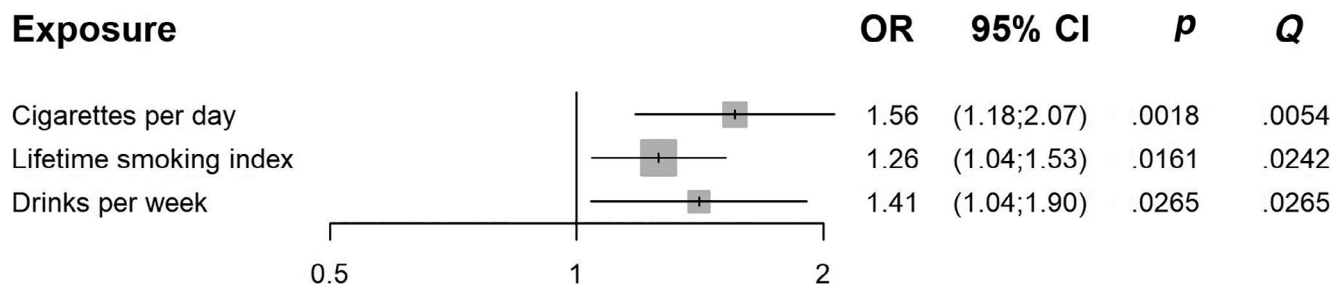
A priori statistical power was calculated according to Brion et al. (2013). Given $\alpha = 5\%$, we had ≥80% power when the expected ORs for periodontitis were ≥1.13, ≥1.07, and ≥1.15 for the number of cigarettes per day, lifetime smoking index, and the number of drinks per week, respectively.

2.1 | Statistical analyses

When genetic, exposure, and a binary outcome data are available on the individual (study participant) level ("one-sample MR"), a two-stage

residual inclusion or a logistic structural mean model can be used for IV analysis (Burgess et al., 2017). When only summary statistics (regression coefficients and standard errors) for the SNP–exposure and SNP–outcome associations are available from separate studies ("two-sample MR"), the causal effect can be estimated by first deriving SNP-specific estimates as the SNP–outcome estimate divided by the SNP–exposure estimate (Wald ratio), with standard errors derived using the Delta method, and then pooling them using multiplicative random effects inverse-variance weighted (IVW) meta-analysis (Burgess et al., 2017, 2018). This estimate can also be motivated by weighted linear regression through the origin using the precisions of the IV associations with the outcome as weights. Two-sample MR is widely used to exploit summary data from large genetic consortia to increase the statistical power of MR (Burgess et al., 2020; Davey Smith et al., 2020). IVW estimates are presented as OR per one standard deviation (1SD) increment in exposure variables. We applied the Benjamini–Hochberg procedure (by exposure variable and method across outcome) to adjust for multiple testing and presented the Q-values (Storey & Tibshirani, 2003).

Directional pleiotropy can be assessed by performing MR Egger regression (Burgess et al., 2018; Hemani et al., 2018). This is a meta-regression of SNP–outcome association estimates on the corresponding SNP–exposure association estimate after they have been oriented in the positive direction. This is identical to the standard IVW approach, except that the intercept of the regression slope is estimated, rather than being fixed to zero. We estimated penalized weighted median, robust adjusted profile score, radial regression, and MR-pleiotropy residual sum and outlier (MR-PRESSO) as pleiotropy-robust methods (Hemani et al., 2018; Slob & Burgess, 2020). The penalized weighted median method gives consistent effect estimates under the assumption that no more than 50% of the weight of the MR effect estimate comes from pleiotropic SNPs, where weight is determined by the strength of their association with the exposure. The contribution of heterogeneous SNP-specific estimates to the overall estimate is further minimized by a penalization parameter. The robust adjusted profile score model is an extension of IVW with adjustment using the profile likelihood. Radial regression uses modified second-order weights to detect and remove outlying SNPs. In MR-PRESSO, the IVW method is implemented by regression and the residual sum of squares (RSS) is calculated as a heterogeneity measure. If the RSS is decreased compared to a simulated expected distribution, then the SNP is removed from the analysis. Multivariable IVW is an extension of the standard IVW model and considers several exposures simultaneously and thus allows modelling of possible horizontal pleiotropic pathways that would violate the second MR assumption (Sanderson et al., 2019). It conditions the SNP–exposure effects on their corresponding effects on other putative risk factor traits that are on indirect pathways by regressing the summary genetic associations with the outcomes on the genetic associations with the exposure and risk factor using in a weighted regression model. In a multivariable IVW, all SNPs should fulfil the IV assumptions. In addition, each IV should associate with the exposure of interest and the risk factors. Analyses were performed using the meta (4.11.0),

Exposure

Exposure	OR	95% CI	*p*	*Q*
Cigarettes per day	1.56	(1.18;2.07)	.0018	.0054
Lifetime smoking index	1.26	(1.04;1.53)	.0161	.0242
Drinks per week	1.41	(1.04;1.90)	.0265	.0265

FIGURE 1 Estimates for the relationship of genetic liability for cigarettes per day, lifetime smoking index, and drinks per week with risk of periodontitis. Odds ratios per standard deviation increment in the exposure from single-variable inverse-variance weighted analysis

TABLE 1 Multivariable inverse-variance weighted estimates for adjusted relationships with periodontitis

Adjustment	Exposure	OR	(95% CI)	*p*-Value
Education, body mass index	Cigarettes per day	1.21	(1.04; 1.42)	.0153
Education, body mass index	Lifetime smoking index	1.22	(1.07; 1.45)	.0301
Education, body mass index	Drinks per week	1.13	(0.99; 1.29)	.0707
Drinks per week	Cigarettes per day	1.43	(1.04; 1.97)	.0299
Cigarettes per day	Drinks per week	1.37	(1.12; 1.68)	.0023

Abbreviations: CI, confidence interval; OR, odds ratio.

MendelianRandomization (0.4.3), MRPRESSO (1.0), phenoscanner (1.0), and TwoSampleMR (0.5.5) packages in R, version 4.0.3. The code is available at github.com/BaumeisterS/MR_smoking_alcohol_periodont. The study was not pre-registered.

3 | RESULTS

Phenotypical descriptive statistics of studies included in the exposure and outcome GWAS are provided in Supplementary Table 2. The MR analysis showed genetic liability for the number of cigarettes per day having an effect estimate consistent with increased odds of periodontitis (IVW OR = 1.56; 95% CI: 1.18–2.07; *p*-value = .0018; *Q*-value = .0054) (Figure 1). In a secondary analysis, genetic liability to lifetime smoking index was associated with increased odds of periodontitis. The genetically instrumented number of drinks per week was positively associated with periodontitis (IVW OR = 1.41; 95% CI: 1.04–1.90; *p*-value = .0265; *Q*-value = .0265).

There was small heterogeneity (in terms of I_{GX}^2) between Wald ratios, and the MR-Egger intercept analyses did not indicate directional pleiotropy (Supplementary Table 3). IVW leave-one-out analysis did not identify any leverage points with high influence. The IVW estimates were consistent with estimates from robust methods (Supplementary Table 4). The PhenoScanner search found associations of instruments with obesity and education-related traits. Estimates were attenuated after adjusting for potentially correlated pleiotropy by body mass index and education in multivariable IVW analysis (Table 1). In multivariable IVW analysis, assessing the genetic liabilities for cigarettes per day and drinks per week jointly, both exposures retained a direct relationship with periodontitis (Table 1).

4 | DISCUSSION

We evaluated potential associations of the genetic liability for tobacco smoking and alcohol consumption with periodontitis and found evidence that smoking and alcohol consumption were associated with increased periodontitis risk. MR estimates were consistent in magnitude and direction across exposures and analysis models, in multivariable MR analyses adjusted for education and body mass index, and in mutual exposure adjustment.

Our findings extend observational literature, suggesting that tobacco smoking and alcohol consumption increase the risk of periodontitis. A recent systematic review highlighted an association between tobacco smoking and the risk of periodontitis summarizing estimates from 12 prospective studies in 12,238 adults (Leite et al., 2018). The meta-analysis reported a pooled confounder-adjusted relative risk (RR) for smoking and periodontitis of 1.85 (95% CI: 1.50–2.20) with high heterogeneity (I^2 = 90.5%). The review further estimated that smoking accounts for about 14% of the population attributable risk. Many of the included studies had substantial attrition at follow-up, which could have induced selection bias. While all studies included in the review adjusted the analysis for observed confounding factors, a threat to the validity of the associational estimate is residual confounding; although a strong unaccounted confounder, associated with smoking and periodontitis with an RR of 2.36 (*E*-value; Mathur & VanderWeele, 2020), would be required to shift the lower CI limit of the meta-analysed RR (i.e., 1.50) to the null. Further, the available prospective studies did not investigate dose–response, cumulative smoking exposure, or change in smoking exposure over time, and did not consider time-varying

confounding, and the reliance on a single self-report possibly introduced regression dilution bias. The underlying mechanisms of smoking in the pathophysiology of periodontitis remain to be elucidated, but potentially include effect on the immune response, the microbial composition, and the healing capacity of the periodontium (Söder et al., 2002; Matthews et al., 2012; Jiang et al., 2020).

Two systematic reviews have examined the association between alcohol consumption and periodontitis (Wang et al., 2016; Pulikkotil et al., 2020). The latest meta-analysis included 23 cross-sectional and six prospective studies and produced a summary OR comparing any alcohol intake and no intake of 1.26 (95% CI: 1.11–141, $I^2 = 71.7\%$) (Pulikkotil et al., 2020). The meta-analysis by Pulikkoltil and colleagues did not report a pooled estimate restricted to prospective studies. Although the primary studies adjusted for confounders of the alcohol–periodontitis association, weak residual confounding would suffice to explain away the point estimate and lower CI limit (E-values for the OR and the lower 95% limit: 1.48 and 1.29; Mathur & VanderWeele, 2020). Also, the review did not include a dose–response meta-analysis and collapsed all quantities and frequencies of alcohol consumption into one category. Wang et al. (2016) reported on a meta-analysis of 14 cross-sectional and four prospective observational studies published until 2015. The pooled RR comparing the highest and lowest alcohol consumption groups was 1.59 (95% CI: 1.37–1.85, $I^2 = 70.8\%$). In an analysis restricted to four prospective studies, the RR was 1.28 (95% CI: 1.04–1.57) and the dose–response analysis revealed a linear relationship (Wang et al., 2016). A limiting factor is that very few of the available prospective studies (Pitiphat et al., 2003; Okamoto et al., 2006; Sankaranarayanan et al., 2020) excluded participants who had prevalent periodontitis at baseline, although this an essential principle to reduce the risk of reverse causation (Heaton et al., 2014). The possible mechanisms linking alcohol consumption and periodontitis are host defence (impaired neutrophil, macrophage, and T-cell functioning), cytokines, and necrosis factor-α levels (Szabo & Saha, 2015; Barr et al., 2016).

Although the majority of available observational data on tobacco smoking and alcohol consumption is cross-sectional, included prevalent users, and might be subject to residual confounding, findings from the observational literature support the positive associations of tobacco smoking and alcohol consumption with the risk of periodontitis detected in our MR analysis. Evidence for tobacco smoking as a causal risk factor for periodontitis seems to be stronger because more prospective observational data is available, the observational meta-analyses are less susceptible to unobserved confounding, and the multivariable MR analysis pointed to a lower potential for correlated pleiotropy.

Several limitations need to be considered. Our analysis assumes a linear relationship between the risk factors and the outcome. Quantitative estimates may be misleading if the true relationship is non-linear, although estimates are still reflective of the presence and direction of the population-averaged causal effect (Burgess et al., 2014). Another limitation is that exposure assessment rested on self-reported information, which is prone to underreporting. However, classical measurement error in the exposure does not affect asymptotic estimates from IV analysis. Future MR studies might exploit GWAS of biomarkers of smoking and alcohol exposure. By estimating SNP–exposure associations in a sample of middle-aged and older adults, we might have underestimated the denominator effect of the ratio estimator. When the effect of the SNP on exposure changes over time, the ratio estimator will represent a biased estimate of the lifetime effect of smoking and alcohol use on periodontitis (J. A. Labrecque & Swanson, 2019). One way to minimize this potential bias is to average over multiple SNP effects on phenotype (as was done here through multiplicative random effects IVW), assuming that time-dependent effects of multiple instruments average across a lifetime (J. A. Labrecque & Swanson, 2019). Even when the point estimates might be biased due to time-varying SNP–exposure associations, the MR analysis still provides a valid test of the causal null hypothesis (J. A. Labrecque & Swanson, 2019). The smoking, alcohol, and periodontitis SNP effect estimates were obtained from European studies, thus minimizing the possibility of population stratification bias and increasing the plausibility of the two-sample MR assumption that summary associations derived from comparable populations; nevertheless, caution is warranted before generalizing findings to other populations. We performed sensitivity analyses to assess and minimize heterogeneity and pleiotropy. The biologic mechanisms of the selected SNPs are unknown; however, sensitivity analyses failed to find evidence for horizontal pleiotropy. The possibility of horizontal pleiotropy introduced by observed confounders was further examined using multivariable MR. However, the multivariable analysis does not overcome bias due to other pleiotropic effects by pathways other than education or obesity. Regarding instrument selection, we used a stringent selection threshold (p-value $<5 \times 10^{-8}$) (Burgess et al., 2020) to reduce the possibility of weak instrument bias.

We provided evidence that tobacco smoking and alcohol consumption increase periodontitis risk, suggesting important public health and clinical consequences. Yet, we emphasize the importance of triangulating multiple lines of MR and observational evidence to strengthen causal inference (Munafò & Smith, 2018).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Sebastian-Edgar Baumeister, Dennis Freuer, Birte Holtfreter. Development of methodology: Sebastian-Edgar Baumeister, Dennis Freuer, Michael Nolde, Hansjörg Baurecht. Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Sebastian-Edgar Baumeister. Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Sebastian-Edgar Baumeister, Dennis Freuer, Michael Nolde, Hansjörg Baurecht. Writing, review, and/or revision of the manuscript: Sebastian E

Baumeister, Dennis Freuer, Michael Nolde, Hansjörg Baurecht, Thomas Kocher, Yeganeh Khazaei, Benjamin Ehmke, Birte Holtfreter. *Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases)*: Sebastian-Edgar Baumeister, Michael Nolde.

ETHICS STATEMENT

Each of the more than 80 studies contributing to the GWAS meta-analyses obtained informed consent from the study participants. This study complied with all relevant ethical regulations, including the Declaration of Helsinki, and ethical approval for data collection and analysis was obtained by each study from local boards as described in the included GWAS (Lee et al., 2018; Liu et al., 2019; Pulit et al., 2019; Shungin et al., 2019; Wootton et al., 2020).

DATA AVAILABILITY STATEMENT

The summary statistics for the smoking GWAS (Liu et al., 2019; Wootton et al., 2020) are available at https://genome.psych.umn.edu/index.php/GSCAN and https://data.bris.ac.uk/data/dataset/10i96zb8gm0j81yz0q6ztei23d (access date: 2021/03/09). The periodontitis summary data (Shungin et al., 2019) are available at https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2 (access date: 2021/03/09). The education and body mass index summary can be assessed at https://www.thessgac.org/data and https://github.com/lindgrengroup/fatdistnGWAS (access date: 2021/03/09).

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REFERENCES

Barr, T., Helms, C., Grant, K., & Messaoudi, I. (2016). Opposing effects of alcohol on the immune system. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 65, 242–251. https://doi.org/10.1016/j.pnpbp.2015.09.001

Brion, M. J., Shakhbazov, K., & Visscher, P. M. (2013). Calculating statistical power in Mendelian randomization studies. *International Journal of Epidemiology*, 42(5), 1497–1501. https://doi.org/10.1093/ije/dyt179

Burgess, S., Davies, N. M., & Thompson, S. G. (2014). Instrumental variable analysis with a nonlinear exposure–outcome relationship. *Epidemiology*, 25(6), 877–885. https://doi.org/10.1097/ede.0000000000000161

Burgess, S., Foley, C. N., & Zuber, V. (2018). Inferring causal relationships between risk factors and outcomes from genome-wide association study data. *Annual Review of Genomics and Human Genetics*, 19, 303–327. https://doi.org/10.1146/annurev-genom-083117-021731

Burgess, S., Small, D. S., & Thompson, S. G. (2017). A review of instrumental variable estimators for Mendelian randomization. *Statistical Methods in Medical Research*, 26(5), 2333–2355. https://doi.org/10.1177/0962280215597579

Burgess, S., Smith, G. D., Davies, N. M., Dudbridge, F., Gill, D., Glymour, M. M., Hartwig, F. P., Holmes, M. V., Minelli, C., Relton, C. L., & Theodoratou, E. (2020). Guidelines for performing Mendelian randomization investigations. *Wellcome Open Research*, 4(186), 186.

Burgess, S., & Thompson, S. G. (2011). Avoiding bias from weak instruments in Mendelian randomization studies. *International Journal of Epidemiology*, 40(3), 755–764. https://doi.org/10.1093/ije/dyr036

Chapple, I. L., Bouchard, P., Cagetti, M. G., Campus, G., Carra, M. C., Cocco, F., Nibali, L., Hujoel, P., Laine, M. L., Lingstrom, P., Manton, D. J., Montero, E., Pitts, N., Rangé, H., Schlueter, N., Teughels, W., Twetman, S., Van Loveren, C., Van der Weijden, F., … Schulte, A. G. (2017). Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: Consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *Journal of Clinical Periodontology*, 44(Suppl. 18), S39–S51. https://doi.org/10.1111/jcpe.12685

Collins, R., Bowman, L., Landray, M., & Peto, R. (2020). The magic of randomization versus the myth of real-world evidence. *The New England Journal of Medicine*, 382(7), 674–678. https://doi.org/10.1056/NEJMsb1901642

Danaei, G., Tavakkoli, M., & Hernán, M. A. (2012). Bias in observational studies of prevalent users: Lessons for comparative effectiveness research from a meta-analysis of statins. *American Journal of Epidemiology*, 175(4), 250–262.

Davey Smith, G., Holmes, M. V., Davies, N. M., & Ebrahim, S. (2020). Mendel's laws, Mendelian randomization and causal inference in observational data: Substantive and nomenclatural issues. *European Journal of Epidemiology*, 35(2), 99–111. https://doi.org/10.1007/s10654-020-00622-7

Davey Smith, G., & Phillips, A. N. (2020). Correlation without a cause: An epidemiological odyssey. *International Journal of Epidemiology*, 49(1), 4–14. https://doi.org/10.1093/ije/dyaa016

GBD 2019 Risk Factors Collaborators. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet*, 396(10258), 1223–1249. https://doi.org/10.1016/s0140-6736(20)30752-2

Heaton, B., Applebaum, K. M., Rothman, K. J., Brooks, D. R., Heeren, T., Dietrich, T., & Garcia, R. I. (2014). The influence of prevalent cohort bias in the association between periodontal disease progression and incident coronary heart disease. *Annals of Epidemiology*, 24(10), 741–746. https://doi.org/10.1016/j.annepidem.2014.07.006

Hemani, G., Bowden, J., & Davey Smith, G. (2018). Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Human Molecular Genetics*, 27(R2), R195–r208. https://doi.org/10.1093/hmg/ddy163

Jiang, Y., Zhou, X., Cheng, L., & Li, M. (2020). The impact of smoking on subgingival microflora: From periodontal health to disease. *Frontiers in Microbiology*, 11, 66. https://doi.org/10.3389/fmicb.2020.00066

Kamat, M. A., Blackshaw, J. A., Young, R., Surendran, P., Burgess, S., Danesh, J., Butterworth, A. S., & Staley, J. R. (2019). PhenoScanner V2: An expanded tool for searching human genotype-phenotype associations. *Bioinformatics*, 35, 4851–4853. https://doi.org/10.1093/bioinformatics/btz469

Labrecque, J., & Swanson, S. A. (2018). Understanding the assumptions underlying instrumental variable analyses: Abrief review of falsification strategies and related tools. *Current Epidemiology Reports*, 5(3), 214–220. https://doi.org/10.1007/s40471-018-0152-1

Labrecque, J. A., & Swanson, S. A. (2019). Interpretation and potential biases of Mendelian randomization estimates with time-varying exposures. *American Journal of Epidemiology*, 188(1), 231–238. https://doi.org/10.1093/aje/kwy204

Lee, J. J., Wedow, R., Okbay, A., Kong, E., Maghzian, O., Zacher, M., Nguyen-Viet, T. A., Bowers, P., Sidorenko, J., Linnér, R. K., Fontana, M. A., Kundu, T., Lee, C., Li, H., Li, R., Royer, R., Timshel, P. N., Walters, R. K., Willoughby, E. A., … Cesarini, D. (2018). Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nature Genetics*, 50(8), 1112–1121. https://doi.org/10.1038/s41588-018-0147-3

Leffondré, K., Abrahamowicz, M., Xiao, Y., & Siemiatycki, J. (2006). Modelling smoking history using a comprehensive smoking index: Application to lung cancer. *Statistics in Medicine*, 25(24), 4132–4146. https://doi.org/10.1002/sim.2680

Leite, F. R. M., Nascimento, G. G., Scheutz, F., & López, R. (2018). Effect of smoking on periodontitis: A systematic review and meta-regression. *American Journal of Preventive Medicine*, 54(6), 831–841. https://doi.org/10.1016/j.amepre.2018.02.014

Liu, M., Jiang, Y., Wedow, R., Li, Y., Brazel, D. M., Chen, F., Datta, G., Davila-Velderrain, J., McGuire, D., Tian, C., Zhan, X., 23andMe Research Team, HUNT All-In Psychiatry, Choquet, H., Docherty, A. R., Faul, J. D., Foerster, J. R., Fritsche, L. G., Gabrielsen, M. E., … Vrieze, S. (2019). Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature Genetics*, 51(2), 237–244. https://doi.org/10.1038/s41588-018-0307-5

Mathur, M. B., & VanderWeele, T. J. (2020). Sensitivity analysis for unmeasured confounding in meta-analyses. *Journal of the American Statistical Association*, 115(529), 163–172. https://doi.org/10.1080/01621459.2018.1529598

Matthews, J. B., Chen, F. M., Milward, M. R., Ling, M. R., & Chapple, I. L. (2012). Neutrophil superoxide production in the presence of cigarette smoke extract, nicotine and cotinine. *Journal of Clinical Periodontology*, 39(7), 626–634. https://doi.org/10.1111/j.1600-051X.2012.01894.x

Munafò, M. R., & Smith, G. D. (2018). Robust research needs many lines of evidence. *Nature*, 553, 399–401.

Okamoto, Y., Tsuboi, S., Suzuki, S., Nakagaki, H., Ogura, Y., Maeda, K., & Tokudome, S. (2006). Effects of smoking and drinking habits on the incidence of periodontal disease and tooth loss among Japanese males: A 4-yr longitudinal study. *Journal of Periodontal Research*, 41(6), 560–566. https://doi.org/10.1111/j.1600-0765.2006.00907.x

Page, R. C., & Eke, P. I. (2007). Case definitions for use in population-based surveillance of periodontitis. *Journal of Periodontology*, 78(7 Suppl), 1387–1399. https://doi.org/10.1902/jop.2007.060264

Pitiphat, W., Merchant, A. T., Rimm, E. B., & Joshipura, K. J. (2003). Alcohol consumption increases periodontitis risk. *Journal of Dental Research*, 82(7), 509–513. https://doi.org/10.1177/154405910308200704

Pulikkotil, S. J., Nath, S., Dharamarajan, L., Jing, K. T., & Vaithilingam, R. D. (2020). Alcohol consumption is associated with periodontitis. A systematic review and meta-analysis of observational studies. *Community Dental Health*, 37(1), 12–21. https://doi.org/10.1922/CDH_4569Pulikkotil10

Pulit, S. L., Stoneman, C., Morris, A. P., Wood, A. R., Glastonbury, C. A., Tyrrell, J., Yengo, L., Ferreira, T., Marouli, E., Ji, Y., Yang, J., Jones, S., Beaumont, R., Croteau-Chonka, D. C., Winkler, T. W., GIANT Consortium, Hattersley, A. T., Loos, R. J. F., Hirschhorn, J. N., … Lindgren, C. M. (2019). Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Human Molecular Genetics*, 28(1), 166–174. https://doi.org/10.1093/hmg/ddy327

Sanderson, E., Davey Smith, G., Windmeijer, F., & Bowden, J. (2019). An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. *International Journal of Epidemiology*, 48(3), 713–727. https://doi.org/10.1093/ije/dyy262

Sankaranarayanan, R., Saxlin, T., Knuuttila, M., Ylöstalo, P., & Suominen, A. L. (2020). Alcohol use and the development of periodontal pockets: An 11-year follow-up study. *Journal of Periodontology*, 91(12), 1621–1631. https://doi.org/10.1002/jper.19-0602

Shungin, D., Cornelis, M. C., Divaris, K., Holtfreter, B., Shaffer, J. R., Yu, Y. H., Barros, S. P., Beck, J. D., Biffar, R., Boerwinkle, E. A., Crout, R. J., Ganna, A., Hallmans, G., Hindy, G., Hu, F. B., Kraft, P., McNeil, D. W., Melander, O., Moss, K. L., … Franks, P. W. (2015). Using genetics to test the causal relationship of total adiposity and periodontitis: Mendelian randomization analyses in the Gene-Lifestyle Interactions and Dental Endpoints (GLIDE) Consortium. *International Journal of Epidemiology*, 44(2), 638–650. https://doi.org/10.1093/ije/dyv075

Shungin, D., Haworth, S., Divaris, K., Agler, C. S., Kamatani, Y., Keun Lee, M., Grinde, K., Hindy, G., Alaraudanjoki, V., Pesonen, P., Teumer, A., Holtfreter, B., Sakaue, S., Hirata, J., Yu, Y. H., Ridker, P. M., Giulianini, F., Chasman, D. I., Magnusson, P. K. E., … Johansson, I. (2019). Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data. *Nature Communications*, 10(1), 2773. https://doi.org/10.1038/s41467-019-10630-1

Slob, E. A., & Burgess, S. (2020). A comparison of robust Mendelian randomization methods using summary data. *Genetic Epidemiology*, 20, 1–17.

Söder, B., Jin, L. J., & Wickholm, S. (2002). Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. *Journal of Clinical Periodontology*, 29(5), 384–391. https://doi.org/10.1034/j.1600-051x.2002.290502.x

Storey, J. D., & Tibshirani, R. (2003). Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences*, 100(16), 9440–9445.

Szabo, G., & Saha, B. (2015). Alcohol's effect on host defense. *Alcohol Research: Current Reviews*, 37(2), 159–170.

Wang, J., Lv, J., Wang, W., & Jiang, X. (2016). Alcohol consumption and risk of periodontitis: A meta-analysis. *Journal of Clinical Periodontology*, 43(7), 572–583. https://doi.org/10.1111/jcpe.12556

Wooldridge, J. M. (2010). *Econometric analysis of cross section and panel data*. MIT Press.

Wootton, R. E., Richmond, R. C., Stuijfzand, B. G., Lawn, R. B., Sallis, H. M., Taylor, G. M. J., Hemani, G., Jones, H. J., Zammit, S., Davey Smith, G., & Munafò, M. R. (2020). Evidence for causal effects of lifetime smoking on risk for depression and schizophrenia: A Mendelian randomisation study. *Psychological Medicine*, 50(14), 2435–2443. https://doi.org/10.1017/s0033291719002678

World Health Organization. (2013). *Oral health surveys: Basic methods*. World Health Organization.

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