 doi:10.20944/preprints202010.0145.v1

Article

Causal association between Periodontitis and Parkinson's Disease: A bidirectional Mendelian Randomization study

João Botelho [1,2,*], Vanessa Machado [1,2], José João Mendes [2] and Paulo Mascarenhas [2,3]

[1] Periodontology Department, Clinical Research Unit (CRU), Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz (IUEM), 2829-511 Almada, Portugal; vmachado@egasmoniz.edu.pt
[2] Evidence-Based Hub, CRU, CiiEM, IUEM, 2829-511 Almada, Portugal; jmendes@egasmoniz.edu.pt
[3] Center for Medical Genetics and Pediatric Nutrition Egas Moniz, IUEM, 2829-511 Almada, Portugal; pmascarenhas@egasmoniz.edu.pt
* Correspondence: jbotelho@egasmoniz.edu.pt

Abstract: Latest evidence revealed a possible association between Parkinson's disease (PD) and periodontitis. We explored the causal relationship of this association through two-sample Mendelian randomization (MR) in European ancestry populations. To this end, we used openly accessible data of genome-wide association studies (GWAS) on PD and periodontitis. As instrumental variables for periodontitis, seventeen single-nucleotide polymorphisms (SNPs) from a GWAS of periodontitis (1817 periodontitis cases vs. 2215 controls) and forty-five SNPs from a GWAS of PD (20,184 cases and 397,324 controls). Eight non-overlapping SNPs of periodontitis from an additional GWAS assisted in the validation of association being studied. Multiple approaches of MR were carried-out. There was no evidence of genetic liability of periodontitis being associated with a higher risk of PD (B= -0.0003, Standard Error [SE] 0.0003, P = 0.26). The eight independent SNPs (B= -0.0000, SE 0.0001, P = 0.99) validated this outcome. We found no association of genetically primed PD towards periodontitis (B= -0.0001, SE 0.0001, P = 0.19). This MR study found no conclusive evidence to support a bidirectional causal genetic liability between PD and periodontitis. Further GWAS studies are needed to confirm the consistency of these results.

Keywords: Parkinson's disease; Periodontitis; Periodontal disease; Mendelian Randomization; Bioinformatics; Oral Health

1. Introduction

Parkinson's disease (PD) is a neurodegenerative condition with heterogeneous clinical patterns and progression [1,2]. Despite the cause of PD is still to be fully understood, a comprehensive mendelian randomization (MR) study described the relative risk of developing PD for 12 different exposures [3]. Furthermore, the role of inflammation in PD, either by local or systemic causes, has been consistently researched [4].

A recently examined inflammatory cause was periodontitis [5], a chronic inflammatory disease of the gums with worldwide impact [6]. This condition, which can be the local manifestation of a developing immuno-physiological disruption, results in alveolar bone loss and can cause tooth loss [7]. The evidence for an association between PD and periodontitis is still to scarce, though two particular studies have reported that the progression of PD (through motor and non-motor deterioration) may frustrate oral hygiene and oral health [8,9]. Moreover, PD individuals have high risk of developing periodontitis [10–14], and if periodontitis is installed this may lead to systemic leukocytosis [5], however its consequences are still unknown.

The causal link between periodontitis and PD risk is limited in terms of evidence, with only observational studies available. To overcome the limitations of observational trials, including the lack

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2020 doi:10.20944/preprints202010.0145.v1

of randomization, we can follow a Mendelian randomization approach, using summary data from genome-wide association studies (GWAS) for causality assessment in putative exposure-outcome pathways.

Mendelian randomization (MR) uses genetic variants as instrumental variables (IVs) for the exposure of interest [15]. These IVs may be exploited to infer causal effect of the exposure if they meet three key premises: 1) strongly associated with the exposure; 2) independent of confounding factors of the observational association; and 3) associated with the outcome only via the exposure (no horizontal pleiotropy) [15–17]. Also, GWAS are increasing, underpinning the opportunity of MR approaches. For these reasons, we aimed to investigate a potential bidirectional causal relationship between periodontitis and PD in European ancestry populations using a MR approach.

2. Materials and Methods

2.1. MR of Periodontitis on risk of PD

Periodontitis data was obtained from two meta-analysis of GWAS of periodontitis. The first was sourced from Teumer et al. review of 4,032 individuals (1,817 periodontitis cases vs. 2,215 controls) of European ancestry [18]. Seventeen single-nucleotide polymorphisms (SNPs) were proposed as being related with periodontitis based on a significant P-value (5×10^{-6} threshold) and were used as IVs. The second, from Munz et al. [19], was used for a validation analysis, in which eight SNPs (non-overlapping with the aforementioned seventeen SNPs) were deemed associated with periodontitis in 12,225 individuals with European ancestry (4,924 periodontitis cases vs. 7,301 controls). For both studies, summary statistics are available in Appendix S1.

In this analysis, instrumental SNPs in linkage disequilibrium were pruned with a clumping R^2 cut-off of 0.001 and the SNP with the lowest P-value was retained. Summary statistics of PD were from one latest GWAS study by Chang et al. (20,184 cases and 397,324 controls) [20], from which the effect sizes for SNPs of periodontitis were extracted. Summary statistics of Stage 1 but not the overall analysis contain the information on these SNPs. The study by Chang et al. [20] is, so far, one of the greatest GWAS of clinically diagnosed PD with European ancestry and publicly accessible summary statistics.

2.2. MR of PD on risk of Periodontitis

PD data was derived from one GWAS by Chang et al. (20,184 cases and 397,324 controls) [20] with respective summary statistics (Appendix S2). Forty-five SNPs were associated with the risk of PD at genome-wide significance (P value 5×10^{-8}). Summary statistics for all SNPs were available in the GWAS of periodontitis, and so were included as IVs for PD in our study.

2.3. MR Analysis

All statistical analyses were performed in R (version 3.6.1), with packages TwoSampleMR (0.4.25), MRPRESSO (1.0) and meta (4.9–7).

The presence of horizontal pleiotropic outliers were inspected using MR-PRESSO R package (pleiotropy residual sum and outlier) [21]. Next, we computed Pseudo R^2 (proportion of variance of liability explained by SNPs and F-statistic) to estimate the strength of the instruments, whenever effect allele frequency (EAF) values were present.

TwoSampleMR was run for selected SNPs individual lookup requests against multiple GWAS, harmonization of effect allele across studies, linkage disequilibrium (LD) pruning and sensitivity analyses. The option to use adequate proxy SNPs to replace exposure SNPs absent from the outcome dataset was enabled.

The causality in both conditions was tested throughout the following MR effect estimation methods: inverse-variance weighted (IVW) method (random effects) [16], weighted median [17] and MR-Egger [22], with the latter two being considered relatively robust to horizontal pleiotropy.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2020 doi:10.20944/preprints202010.0145.v1

Furthermore, we also estimated the causal effect through the MR-RAPS (robust adjusted profile score) method, due to its robustness towards weak instrument bias [23]. Horizontal pleiotropy was examined by computing the intercept and 95% confidence interval (CI) of the MR-Egger regression line [22]. We tested for heterogeneity between the causal estimates of individual SNPs using Cochran's Q statistic for the IVW and MR-Egger methods. Leave-one-out analyses were performed to ascertain that the effect was not disproportionately influenced by a single SNP. MR estimates are reported as odds ratio (OR) for the outcome per unit increase in ln(OR) of the exposure. We emphasize that the calculation of MR estimates associated with a binary exposure (unlike a continuous exposure) is more efficient for identifying presence of a causal effect than quantifying the magnitude of the causal effect [15].

Ethical consent had been provided in the original clinical studies [18–20].

3. Results

3.1. Protein-Protein Interaction Analysis

In this bidirectional MR approach, no outliers were detected using MR-PRESSO (P=0.80, P=0.39 and P=0.36 for Teumer et al., Munz et al. and Chang et al., respectively).

Using the seventeen independent SNPs for periodontitis, pseudo R^2 value was 0.119 and F-statistic was 329.1 suggesting fairly weak instruments for periodontitis in this study. Regarding the other eight SNPs, pseudo R^2 and F-statistic were not feasible to calculate due to lack of certain EAF. Likewise, from the 45 SNPs as instruments for PD, some SNPs did not have EAF values, making impossible the computation of pseudo R^2 and F-statistic.

The bidirectional MR estimates are displayed in Table 1. Using the seventeen SNPs in Teumer *et al*. as periodontitis IVs, there was no association between genetically primed periodontitis and the risk of PD (B= -0.0003, SE 0.0003, P = 0.26) based on the IVW estimate, which was supported by the MR-RAPS method (B= -0.0003, SE 0.0003, P = 0.27). Also, the MR assessment using the weighted median (B= -0.0003, SE 0.0003, P = 0.37) and MR-Egger estimate (B= -0.008, SE 0.0006, P = 0.17) reported equal outcomes. The validation using eight non-overlapping SNPs in Munz *et al*. as periodontitis IVs confirmed no causal effect of genetic susceptibility to periodontitis on the risk of PD (Table 1) (Figure 2).

Table 1. Score results between PD and periodontitis genes identified in the network interaction.

Method	Periodontitis on PD (using Chang et al. 2017)						Parkinson's disease on Periodontitis±		
	Instrumental SNPs from Teumer et al. *			Instrumental SNPs from Munz et al. #					
	B (SE)	P-value	Q statistic / P value	B (SE)	P-value	Q statistic / P value	B (SE)	P-value	Q statistic / P value
IVW	-0.0003 (0.0003)	0.26	11.45 /0.78	-0.0000 (0.0001)	0.99	4.30 / 0.75	-0.0001 (0.0001)	0.19	46.83 / 0.36
Weighten median	-0.0003 (0.0003)	0.37	-	0.0000 (0.0002)	0.93	-	-0.0001 (0.0001)	0.31	-
MR-Egger	-0.0008 (0.0006)	0.17	10.37 / 0.80	-0.0014 (0.0046)	0.76	4.19 / 0.65	-0.0002 (0.0002)	0.24	46.39 / 0.33
MR-RAPS	-0.0003 (0.0003)	0.27	-	-0.0000 (0.0001)	0.99	-	-0.0001 (0.0001)	0.31	-

CI: confidence interval; OR: odds ratio; IVW: inverse-variance weighted; MR: Mendelian randomization; RAPS: robust adjusted profile score; SNP: single-nucleotide polymorphism

Seventeen* / Eight# SNPs were used as IVs for periodontitis respectively

Forty-five± SNPs were used as IVs for Parkinson's disease

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2020 doi:10.20944/preprints202010.0145.v1

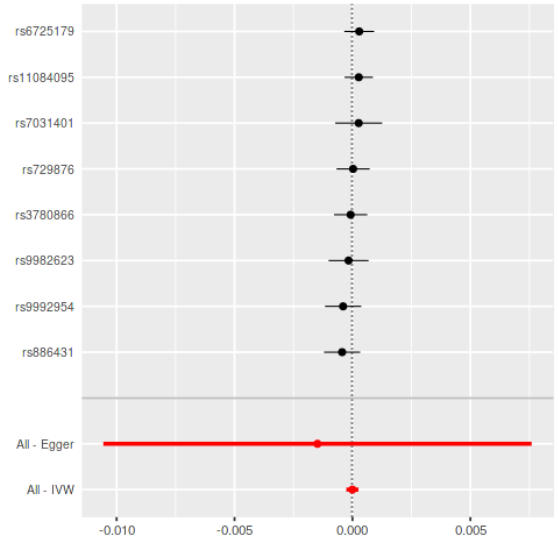

Figure 1. RNA expression of THSD4 in different brain regions according to the Consensus Human Brain Dataset.

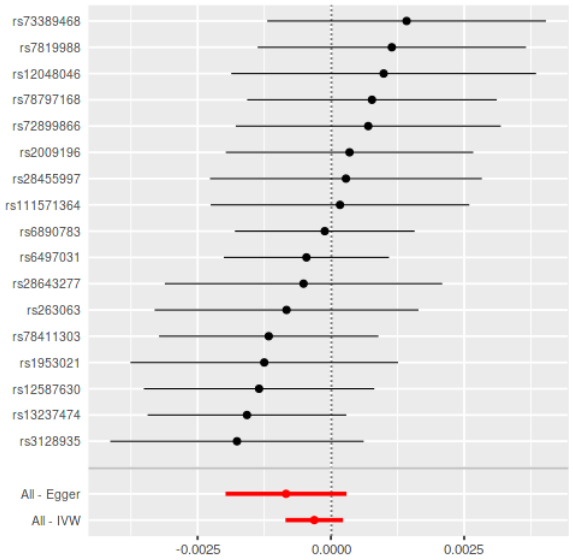

Figure 2. RNA expression of THSD4 in different brain regions according to the Consensus Human Brain Dataset.

Using the 45 SNPs as IVs for PD, there was no link between genetically primed PD and the risk towards periodontitis (B= -0.0002, SE 0.0002, $P = 0.85$) (Figure 3).

The MR-Egger analyses revealed there was no horizontal pleiotropy (periodontitis on risk of PD using the seventeen SNPs from the study of Teumer *et al*.: intercept 0.000, $P = 0.32$; periodontitis on PD using the eight from the study of SNPs Munz *et al*.: intercept 0.002, $P > 0.05$; and PD on risk of periodontitis: intercept 0.000, $P = 0.53$).

The Cochran's Q statistic reported minor heterogeneity among the NPS. Leave-one-out meta-analyses reported proportionately within the included SNPs (Supplementary file SXXX).

We assumed the independency of the IVs (the seventeen and the eight SNPs for periodontitis) of confounding through a comprehensive search on GWAS catalogue (https://www.ebi.ac.uk/gwas/) [24].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2020 doi:10.20944/preprints202010.0145.v1

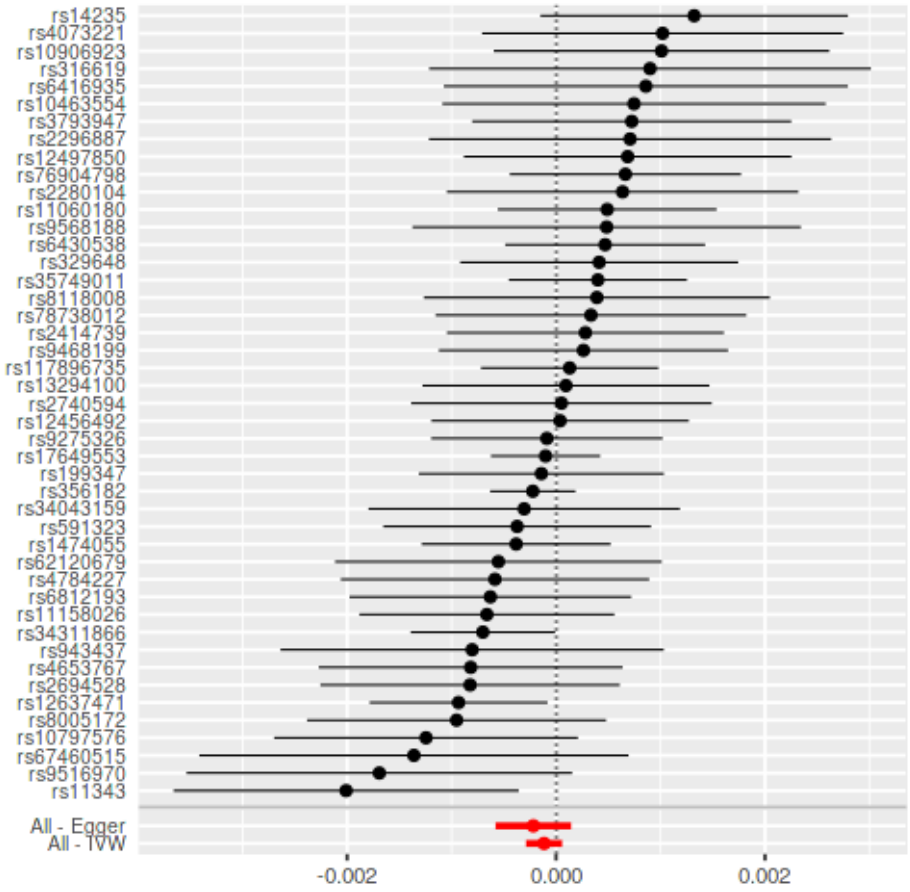

Figure 3. RNA expression of THSD4 in different brain regions according to the Consensus Human Brain Dataset.

4. Discussion

This study is the first to use a two-sample MR approach to investigate the causal relationship between PD and periodontitis in a bidirectional way, in European ancestry populations. Overall, our results do not support a bidirectional genetic liability between these two conditions. However, this should be interpreted with caution given the scarcity of GWAS studies available so far on these two diseases.

To date, periodontitis has been associated with neurological conditions, such as Alzheimer's Disease, dementia or PD [5,9,25,26]. From a biological standpoint, the association of periodontitis with these intricate illnesses may be based on the influence of systemic inflammation and the systemic spreading of periodontal pathogens products with potential affection of brain tissues [27,28]. These processes might support the reported association of *Porphyromonas gingivalis* IgG levels with compromised delayed memory and calculation [29]. Remarkably, a preclinical study in mice showed that continuous oral application of *Porphyromonas gingivalis* caused neurodegeneration and formation of extracellular $A\beta_{42}$ [30]. Still uncertain is the potential impact of leukocytosis found in PD patients with periodontitis, as persistent elevated levels of white blood cells were linked to cardiovascular or diabetic related negative events [31–33].

This MR study presents a powerful and comprehensive analysis from large and fashionable datasets. Nevertheless, there is a number of potential limitations to discuss. MR computation is powerless to elucidate if the existence of one illness in a particular period of lifetime can influence on the risk of developing another illness. Also, when there is a strong genetic/environmental interaction present (as might be the case in periodontitis), MR may lead to a bogus conclusion. Further, this analysis was based on European ancestry studies due to the scarcity of GWAS studies in other

 doi:10.20944/preprints202010.0145.v1

populations, therefore limiting the generalizability of these results. Although we used the two most recent GWAS of periodontitis in the population of European ancestry [18,19], we were not able to fully quantify instrument bias through F-statistics and R^2 values.

All SNPs used as instruments for periodontitis were weakly associated with periodontitis with a cut-off P value 5×10^{-6} instead of 5×10^{-8}. Moreover, there was no overlapping SNPs between the two periodontitis GWAS, which further implies weak instruments for periodontitis. In addition, the biological mechanisms for most of these SNPs related to periodontitis are unclear.

Importantly, GWAS studies on periodontitis are prone to fail in the identification of consistent SNPs [18,19,34–36], because of the inconsistent definitions of periodontitis that are being used in different studies. Hence, studies shall employ up-to-date definitions of periodontitis following the American Academy of Periodontology/European Federation of Periodontology case definition [37], considering its variability [38].

Further MR studies using summary statistics from GWAS datasets of alpha synuclein and LRRK2 may explore the potential biological pathways between periodontitis and the risk towards PD.

Despite all aforementioned shortcomings, this study presented reasonable power to perceive a small effect on the risk of PD, which may be seen as an advantage compared to other studies [39].

5. Conclusions

In this bidirectional MR study, we found no convincing evidence supporting a bidirectional genetic liability between PD and periodontitis. Further GWAS researches are warranted to explore further the consistency of these results.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Appendix S1: "Summary statistics for Mendelian randomization analysis of potential causal effect of periodontitis on Parkinson's disease", Appendix S2: "Summary statistics for MR analysis of potential causal effect of Parkinson's disease on periodontitis", Appendix S3: "Leave-one-out meta-analysis for (A) Munz et al., (B) Teumer et al. and (C) Chang et al.".

Author Contributions: Conceptualization, J.B.; methodology, X.X.; validation, P.M.; formal analysis, J.B.; investigation, V.M. and J.B.; data curation, J.B..; writing—original draft preparation, J.B., V.M., P.M., J.J.M.; writing—review and editing, J.B., V.M., P.M., J.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work is financed by national funds through the FCT - Foundation for Science and Technology, I.P., under the project UIDB/04585/2020.

Acknowledgments: None.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tysnes, O.B.; Storstein, A. Epidemiology of Parkinson's disease. *J. Neural Transm.* **2017**, *124*, 901–905.
2. Parnetti, L.; Gaetani, L.; Eusebi, P.; Paciotti, S.; Hansson, O.; El-Agnaf, O.; Mollenhauer, B.; Blennow, K.; Calabresi, P. CSF and blood biomarkers for Parkinson's disease. *Lancet Neurol.* **2019**, *18*, 573–586.
3. Noyce, A.J.; Bandres-Ciga, S.; Kim, J.; Heilbron, K.; Kia, D.; Hemani, G.; Xue, A.; Lawlor, D.A.; Smith, G.D.; Duran, R.; et al. The Parkinson's Disease Mendelian Randomization Research Portal. *Mov. Disord.* **2019**, *34*, 1864–1872.
4. Qiu, X.; Xiao, Y.; Wu, J.; Gan, L.; Huang, Y.; Wang, J. C-Reactive Protein and Risk of Parkinson's Disease: A Systematic Review and Meta-Analysis. *Front. Neurol.* **2019**, *10*.

5. Botelho, J.; Lyra, P.; Proença, L.; Godinho, C.; Mendes, J.J.; Machado, V. Relationship between blood and standard biochemistry levels with Periodontitis in Parkinson's Disease patients: data from the NHANES 2011-2012. *J. Pers. Med.* **2020**, *In press*, 1–11.
6. Tonetti, M.S.; Jepsen, S.; Jin, L.; Otomo-Corgel, J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *J. Clin. Periodontol.* **2017**, *44*, 456–462.
7. G. Caton, J.; Armitage, G.; Berglundh, T.; Chapple, I.L.C.; Jepsen, S.; S. Kornman, K.; L. Mealey, B.; Papapanou, P.N.; Sanz, M.; S. Tonetti, M. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J. Clin. Periodontol.* **2018**, *45*, S1–S8.
8. Kaur, T.; Uppoor, A.; Naik, D. Parkinson's disease and periodontitis - the missing link? A review. *Gerodontology* **2016**, *33*, 434–438.
9. Lyra, P.; Machado, V.; Proença, L.; Domingos, J.; Godinho, C.; Mendes, J.J.; Botelho, J. Parkinson's Disease, Periodontitis and patient-related outcomes: a cross-sectional study. *Medicina (B. Aires).* **2020**, *In press*.
10. Schwarz, J.; Heimhilger, E.; Storch, A. Increased periodontal pathology in Parkinson's disease. *J. Neurol.* **2006**, *253*, 608–611.
11. Einarsdóttir, E.R.; Gunnsteinsdóttir, H.; Hallsdóttir, M.H.; Sveinsson, S.; Jónsdóttir, S.R.; Olafsson, V.G.; Bragason, T.H.; Saemundsson, S.R.; Holbrook, W.P. Dental health of patients with Parkinson's disease in Iceland. *Spec. Care Dent.* **2009**, *29*, 123–127.
12. Hanaoka, A.; Kashihara, K. Increased frequencies of caries, periodontal disease and tooth loss in patients with Parkinson's disease. *J. Clin. Neurosci.* **2009**, *16*, 1279–1282.
13. Nakayama, Y.; Washio, M.; Mori, M. Oral health conditions in patients with Parkinson's disease. *J. Epidemiol.* **2004**, *14*, 143–150.
14. Van Stiphout, M.A.E.; Marinus, J.; Van Hilten, J.J.; Lobbezoo, F.; De Baat, C. Oral Health of Parkinson's Disease Patients: A Case-Control Study. *Parkinsons. Dis.* **2018**, *2018*.
15. Burgess, S.; Labrecque, J.A. Mendelian randomization with a binary exposure variable: interpretation and presentation of causal estimates. *Eur. J. Epidemiol.* **2018**, *33*, 947–952.
16. Burgess, S.; Butterworth, A.; Thompson, S.G. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data. *Genet. Epidemiol.* **2013**, *37*, 658–665.
17. Bowden, J.; Davey Smith, G.; Haycock, P.C.; Burgess, S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet. Epidemiol.* **2016**, *40*, 304–314.
18. Teumer, A.; Holtfreter, B.; Völker, U.; Petersmann, A.; Nauck, M.; Biffar, R.; Völzke, H.; Kroemer, H.K.; Meisel, P.; Homuth, G.; et al. Genome-wide association study of chronic periodontitis in a general German population. *J. Clin. Periodontol.* **2013**, *40*, 977–985.
19. Munz, M.; Richter, G.M.; Loos, B.G.; Jepsen, S.; Divaris, K.; Offenbacher, S.; Teumer, A.; Holtfreter, B.; Kocher, T.; Bruckmann, C.; et al. Meta-analysis of genome-wide association studies of aggressive and chronic periodontitis identifies two novel risk loci. *Eur. J. Hum. Genet.* **2019**, *27*, 102–113.
20. Chang, D.; Nalls, M.A.; Hallgrímsdóttir, I.B.; Hunkapiller, J.; Brug, M. van der; Cai, F.; Kerchner, G.A.; Ayalon, G.; Bingol, B.; Sheng, M.; et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat. Genet.* **2017**, *49*, 1511–1516.
21. Verbanck, M.; Chen, C.-Y.; Neale, B.; Do, R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.*

2018, *50*, 693–698.

22. Bowden, J.; Davey Smith, G.; Burgess, S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **2015**, *44*, 512–525.
23. Zhao, Q.; Wang, J.; Hemani, G.; Bowden, J.; Small, D.S. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. **2018**.
24. Welter, D.; MacArthur, J.; Morales, J.; Burdett, T.; Hall, P.; Junkins, H.; Klemm, A.; Flicek, P.; Manolio, T.; Hindorff, L.; et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* **2014**, *42*, D1001–D1006.
25. Leira, Y.; Domínguez, C.; Seoane, J.; Seoane-Romero, J.; Pías-Peleteiro, J.M.; Takkouche, B.; Blanco, J.; Aldrey, J.M. Is Periodontal Disease Associated with Alzheimer's Disease? A Systematic Review with Meta-Analysis. *Neuroepidemiology* 2017, *48*, 21–31.
26. Nadim, R.; Tang, J.; Dilmohamed, A.; Yuan, S.; Wu, C.; Bakre, A.T.; Partridge, M.; Ni, J.; Copeland, J.R.; Anstey, K.J.; et al. Influence of periodontal disease on risk of dementia: a systematic literature review and a meta-analysis. *Eur. J. Epidemiol.* **2020**.
27. Ide, M.; Harris, M.; Stevens, A.; Sussams, R.; Hopkins, V.; Culliford, D.; Fuller, J.; Ibbett, P.; Raybould, R.; Thomas, R.; et al. Periodontitis and cognitive decline in Alzheimer's disease. *PLoS One* **2016**, *11*, 1–9.
28. Dominy, S.S.; Lynch, C.; Ermini, F.; Benedyk, M.; Marczyk, A.; Konradi, A.; Nguyen, M.; Haditsch, U.; Raha, D.; Griffin, C.; et al. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* **2019**, *5*, eaau3333.
29. Noble, J.M.; Borrell, L.N.; Papapanou, P.N.; Elkind, M.S.V.; Scarmeas, N.; Wright, C.B. Periodontitis is associated with cognitive impairment among older adults: Analysis of NHANES-III. *J. Neurol. Neurosurg. Psychiatry* **2009**, *80*, 1206–1211.
30. Ilievski, V.; Zuchowska, P.K.; Green, S.J.; Toth, P.T.; Ragozzino, M.E.; Le, K.; Aljewari, H.W.; O'Brien-Simpson, N.M.; Reynolds, E.C.; Watanabe, K. Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice. *PLoS One* **2018**, *13*, e0204941.
31. Lee, C. Do; Folsom, A.R.; Nieto, F.J.; Chambless, L.E.; Shahar, E.; Wolfe, D.A. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: Atherosclerosis Risk in Communities Study. *Am. J. Epidemiol.* **2001**, *154*, 758–764.
32. Hegde, R.; Awan, K.H. Effects of periodontal disease on systemic health. *Disease-a-Month* **2019**, *65*, 185–192.
33. Tong, P.C.; Lee, K.F.; So, W.Y.; Ng, M.H.; Chan, W.B.; Lo, M.K.; Chan, N.N.; Chan, J.C. White Blood Cell Count Is Associated with Macro- and Microvascular Complications in Chinese Patients with Type 2 Diabetes. *Diabetes Care* **2004**, *27*, 216–222.
34. Divaris, K.; Monda, K.L.; North, K.E.; Olshan, A.F.; Reynolds, L.M.; Hsueh, W.C.; Lange, E.M.; Moss, K.; Barros, S.P.; Weyant, R.J.; et al. Exploring the genetic basis of chronic periodontitis: A genome-wide association study. *Hum. Mol. Genet.* **2013**, *22*, 2312–2324.
35. Schaefer, A.S.; Richter, G.M.; Nothnagel, M.; Manke, T.; Dommisch, H.; Jacobs, G.; Arlt, A.; Rosenstiel, P.; Noack, B.; Groessner-Schreiber, B.; et al. A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Hum. Mol. Genet.* **2009**, *19*, 553–562.
36. Shungin, D.; Haworth, S.; Divaris, K.; Agler, C.S.; Kamatani, Y.; Keun Lee, M.; Grinde, K.; Hindy, G.; Alaraudanjoki, V.; Pesonen, P.; et al. Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data. *Nat. Commun.* **2019**, *10*.

37. Tonetti, M.S.; Greenwell, H.; Kornman, K.S. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J. Periodontol.* **2018**, *45*, S149–S161.
38. Botelho, J.; Machado, V.; Proença, L.; Mendes, J.J. The 2018 periodontitis case definition improves accuracy performance of full-mouth partial diagnostic protocols. *Sci. Rep.* **2020**, In press.
39. Sun, Y.Q.; Richmond, R.C.; Chen, Y.; Mai, X.M. Mixed evidence for the relationship between periodontitis and Alzheimer's disease: A bidirectional Mendelian randomization study. *PLoS One* **2020**, *15*, 1–9.