Using Genetics to Test the Causal Relationship of Total Adiposity & Periodontitis: *Mendelian Randomization Analyses in the Gene-Lifestyle Interactions & Dental Endpoints (GLIDE) Consortium*

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Abstract

Background: Observational relationship between obesity and periodontitis is widely known, yet causal evidence is lacking. Our objective was to investigate causal associations between periodontitis and body mass index (BMI).

Methods: We performed Mendelian randomization analysis with BMI-associated loci combined in a genetic risk score (GRS) as instrument for BMI. All analyses were conducted within the Gene-Lifestyle Interactions and Dental Endpoints (GLIDE) Consortium in 13 studies from Europe and the US including 49,066 participants with clinically assessed (7 studies, 42.1% of participants) and self-reported (6 studies, 57.9% of participants) periodontitis and genotype data (17,672/31,394 with/without periodontitis); 68,761 participants with BMI and genotype data; and 57,871 participants (18,881/38,990 with/without periodontitis) with data on BMI and periodontitis.

Results: In the observational meta-analysis of all participants, the pooled crude observational OR for periodontitis was 1.13 (95%CI:1.03, 1.24) per standard deviation increase of BMI. Controlling for potential confounders attenuated this estimate (OR=1.08; 95%CI:1.03, 1.12). For clinically assessed periodontitis, corresponding ORs were 1.25 (95%CI:1.10, 1.42) and 1.13 (95%CI:1.10, 1.17) respectively. In the genetic association meta-analysis, the OR for periodontitis was 1.01 (95%CI:0.99, 1.03) per GRS unit (one effect allele) in all participants and 1.00 (95%CI: 0.97, 1.03) in participants with clinically assessed periodontitis. The instrumental variable meta-analysis of all participants yielded an OR of 1.05 (95%CI:0.80, 1.38) per BMI standard deviation, and 0.90 (95%CI:0.56, 1.46) in participants with clinical data.

Conclusions: Our study does not support a total adiposity as a causal risk factor for periodontitis, as the point estimate is very close to the null in the causal inference analysis, with wide confidence intervals.

Key words: Mendelian randomization, BMI, Periodontitis, Casual inference, Confounding

Introduction

Periodontitis is an inflammatory disease associated with loss of supporting connective tissue and bone that surrounds teeth(1), which can result in tooth loosening and subsequent tooth loss. Periodontitis is one of two major global oral health burdens and the most prevalent in adults(2). Multiple observational studies have reported associations between periodontitis and a wide variety of systemic diseases and conditions(3), including obesity. If obesity and periodontitis are causally related, this is particularly concerning, because obesity is now at epidemic proportions, affecting almost one third of the world's population(4) and is extremely difficult to prevent and treat.

A meta-analysis summarizing results from 28 published studies reported an odds ratio (OR) of 1.35 (95% CI: 1.23, 1.47) for the association between periodontitis and obesity(5); a second meta-analysis of 12 studies reported an OR of 1.27 (1.06, 1.51) for the association between periodontitis and overweight(6). The component studies were primarily cross-sectional in design, limiting the extent to which temporal relationships can be inferred. The largest single prospective cohort study of periodontitis and anthropometric measures to date included 36,910 healthcare professionals(7); however, even large prospective observational studies are prone to confounding, including residual confounding, and reverse causality. For example, BMI is also associated with educational status, smoking and other lifestyle factors that are presumed risk factors for periodontitis and are thus putative confounders of the obesity-periodontitis associations reported previously.

In order to determine if the association of obesity and periodontitis is causal, randomized controlled trials would ideally be employed to test whether obesity interventions reduce dental disease progression; but the large sample size requirements, given the small presumed effects, and high costs of such trials makes these unfeasible. Mendelian randomization (MR) is an epidemiological approach of causal inference that makes use of genotypes as instrumental variables(8, 9). Given that genotypes are randomly assigned during meiosis, genotypes that are known to be robustly associated with exposures of interest (e.g., BMI) can be used as surrogate markers of exposure to that factor to obtain estimates that are less prone to confounding than conventional epidemiological studies. Unlike most other

biomarkers, a person's nuclear genome is largely stable over time; thus genetic associations are also much less prone to reverse causality than non-genetic epidemiological associations. MR has, amongst several examples, been previously used to successfully define the causal relationships between triglyceride-mediated pathways and coronary heart disease(10), and between BMI and ischemic heart disease(11) as well as to suggest that C reactive protein concentration is unlikely to be causally associated with coronary heart disease(12).

The aim of this study was to investigate the causal relationship between BMI and periodontitis, by calculating observational and causal estimates for the relationship of BMI and periodontal health using clinically measured and self-reported data. To derive causal estimates, we selected three loci that are robustly associated with BMI (*FTO, MC4R* and *TMEM18*)(13), and used these as instrumental variables in the MR analysis.

Methods

Analyses were conducted in up to 68,761 individuals of European ancestry from 13 studies that had BMI dental and genotype data. Ninety-two percent of the participants were from population-based studies (up to 63,236 participants) and the rest were controls from studies (5,525 participants) where cases ascertained on type 2 diabetes (HPFS_T2D_Controls and NHS_T2D_Controls), coronary heart disease (HPFS_CHD_Controls and NHS_CHD_Controls) and cancer (NHS_Cancer_Controls). Cases were not included into our analyses. A detailed description of the participating cohorts is presented in Table 1 and the Supplementary Material. The GLIDE study was approved by Umeå Regional Ethical Review Board (Dnr 2010-387-31M and 2011-74-32M) while individual participating studies were approved by the local ethics committees.

Dental data collection and periodontitis definition

Clinical assessment of periodontal health during dental examinations by trained dental personnel was available in 7 studies with a total sample size of 20,653 participants in genetic analyses and up to 29,459 in observational analyses (Supplementary Table 1, Figures 1 & 3). In 4 studies (ARIC(14, 15), COHRA(16-18), SHIP(19) and SHIP-Trend(20)), with a total sample size of 9,359 participants, dental

examinations were performed at recruitment. In TwinGene(21), GLACIER(22) and MDC(23), dental data were obtained for participants with available genetic and phenotypic data from electronic records generated in public dental clinics across 12 Swedish counties (Scania region for MDC, Västerbotten region for GLACIER, across all 12 counties for TwinGene). Dental data were available for patients who had visited a public dental clinic at least once during the period from 1st January 2000 through 1st December 2013 for whom the patient's dentist or dental hygienist had logged relevant details on the practice's dental records system. For 6 studies in GLIDE (WGHS(24), HPFS_T2D_Controls(25), NHS_T2D_Controls(25), HPFS_CHD_Controls(26), NHS_CHD_Controls(26) and NHS_Cancer_Controls(27)) totaling 28,413 participants, self-reported periodontal health was collected using questionnaires (Supplementary Table 1).

Genotypes

Genotypes at *FTO* (rs1121980), *MC4R* (rs17782313), and *TMEM18* (rs6548238) were selected as they convey the largest known effect estimates for BMI among European-ancestry general populations(13). Genotypes were coded according to an additive genetic model. Genetic risk scores (GRS) were computed by summing the number of BMI increasing alleles. Where the index SNPs were unavailable, close proxies were selected and the GRS was constructed using these (Supplementary Table 2). Studies with available genome-wide data imputed SNPs using the CEU reference panel from HapMap2 and SNPs with low call rates (<95%) or low imputation quality (<0.3 for MACH(28) and <0.4 for IMPUTE(29)) were excluded.

Details about study-specific genotyping methods, data quality control, imputation and statistical analysis software are presented in Supplementary Table 3. In GLACIER and MDC, missing genotypes were imputed using mean imputation, as described previously(30). Conformity with Hardy-Weinberg expectations was assessed using a 2 d.f. $\chi 2$ test; all SNPs were in Hardy-Weinberg equilibrium ($P>0.1$).

BMI

In majority of individuals (N=40,348), weight and height were assessed using calibrated scales and wall-mounted stadiometers respectively by trained research staff; in 28,413 participants, height and weight were assessed by self-report. BMI, calculated as weight (kg) divided by squared height (m^2), was analyzed as a continuous trait in each participating study (Table 1). Participants with BMI less than 14 kg/m^2 or greater than 80 kg/m^2 were excluded from analyses on the basis that values outside this range are unlikely to be physiologically plausible. We generated age and sex stratified z-scores for BMI by subtracting mean from each individual BMI value and dividing by standard deviation within 10 year age groups (≥20 to <30, ≥30 to <40, ≥40 to <50, ≥50 to <60, ≥60 to <70, ≥70 to <80, ≥80 to <90, ≥90) in each study to minimize influence of these factors. To facilitate interpretation of z-score units of BMI we have calculated sample size weighted mean of standard deviation across all participating studies that equaled 4,62 kg/m^2 meaning that one unit change in BMI z-score would correspond to approximately 4,62 kg/m^2 change in BMI.

Periodontitis

Periodontitis was classified as present (cases) or absent (controls) using the definitions applied by each participating cohort. These included classification by Centers for Disease Control and Prevention/American Academy of Periodontology (CDC/AAP)(31), Community Periodontal Index (CPI)(32), case definition based on probing depth and/or number of deep periodontal pockets, and self-reported periodontitis. Details of periodontitis definition for each participating study are presented in Supplementary Table 2. In ARIC, in addition to CDC/AAP classification, we used the two clinical definitions based on probing depth and/or number of deep periodontal pockets used in MDC/TwinGene and COHRA (Supplementary Table 8) to investigate influence of case definition on genetic associations."

Other covariates

Complete data on age, sex, smoking status (non-smoker versus ever smoker), educational level, diabetes, tooth brushing frequency (in ARIC), reason for dental visit (in ARIC), frequency of visits to dentist, dental clinic and number of teeth was available in contributing cohorts (Supplementary Table 1 and 4).

Statistical Analysis

Software

Meta-analyses were performed using Stata 12 (StataCorp LP, TX, USA); information about statistical software used by contributing studies is presented in Supplementary Table 3.

Observational associations

The association between periodontitis and BMI z-score was assessed using unadjusted and adjusted logistic regression models. Models were adjusted for age, sex, smoking, education, oral health variables, and frequency of visits to dentist and dental clinic. Given that smoking and diabetes are the leading risk factors for periodontitis, we then repeated adjusted model to assess the association between BMI and periodontitis in participants who had never smoked and who had not been diagnosed with diabetes at the time of their examination. Associations between periodontitis or BMI and putative confounders were assessed using unadjusted logistic (dichotomous outcomes) or linear (continuous outcomes) regression. Ordinal variables (e.g., education in GLACIER or frequency of dental visit in ARIC) were analyzed using ordered logistic regression. In GLACIER, TwinGene and MDC, the duration between the date of the dental examination and the date when anthropometric and lifestyle data were collected was calculated (did not exceed 5 years) and included as a covariate in all models.

In TwinGene, the same associations as described above were tested using linear mixed-models with twin pairs fitted as a random effect using the *xtmelogit* function for periodontitis and dichotomous confounding factors and *xtmixed* function for BMI and continuous confounding variables. Because TwinGene is a national study and outcome information consequently emanates from several Swedish counties, 'county' was also fitted as a random effect in all TwinGene models.

In order to compare our observational associations with results from prior studies, we stratified our cohorts according to the WHO criteria for defining normal weight (BMI 18.5 to 24.9 kg/m^2), overweight (BMI 25.0 to 29.9 kg/m^2) and obesity (BMI $\geq$30.0 kg/m^2) and used logistic regression with covariates from adjusted model to report associations between periodontitis and obesity/overweight versus normal weight participants.

Genetic associations

Genetic associations with periodontitis were modeled using logistic regression. Relationships between standardized BMI, potential confounders and GRS were studied using linear regression. Genetic associations in TwinGene were tested using mixed-models, with twin pairs and Swedish counties specified as random effects using the *xtmlogit* and *xtmixed* functions respectively. Associations in studies with genome-wide data were adjusted for the first ten genetic principal components to control for population stratification (Supplementary Table 4).

Inverse variance-weighted random-effects meta-analysis implemented in the Stata software were run with the user-written command metan(33) to obtain pooled effect estimates. We also calculated between-study heterogeneity I^2 (34) in all meta-analyses. OR were meta-analyzed on the natural log scale.

Instrumental variable analysis

An MR estimate of the effect of BMI on periodontal status was calculated using GRS as instrumental variable in each study, followed by meta-analysis to obtain a pooled effect estimate. A Wald-type estimator(35) was utilized to derive MR estimates of log OR of periodontitis risk per one standard deviation (SD) unit increase in BMI by dividing log OR per allele for periodontitis by per allele increase in one SD unit of BMI. The delta method(36) was used to obtain standard errors for the log OR MR estimate followed by their exponentiation. We also formally tested for differences between instrumental and observational estimates using heterogeneity test as described elsewhere (37). The same test was used to assess heterogeneity between pooled effect estimates of BMI-GRS associations between clinically assessed and self-reported periodontitis.

Results

Analyses were conducted within the GLIDE Consortium in up to 68,761 individuals of European ancestry from 13 studies that had BMI and genotype data; 49,066 participants that had periodontitis and genotype data (17,672 / 31,394 with/without periodontitis), and 57,871 (18,881/38,990 with/without periodontitis) that had observational data on BMI and periodontitis. Main characteristics of participants are presented in Table 1 and detailed descriptions of studies are given in the

Supplementary Material. Out of 13 participating studies, 7 studies with a total sample size of 20,653 participants in genetic analyses and up to 29,459 in observational analyses had clinically assessed data on periodontitis and 6 studies with 28,413 in genetic analyses and up to 28,412 in observational analyses had self-reported data. Additional details about dental characteristics are presented in the Supplementary Table 1.

Observational associations

We examined observational associations between BMI and measures of periodontal health in up to 18,881 cases and 38,990 controls (Figure 1A-C). For a z-score unit increase in BMI (1 SD), the pooled crude observational OR for periodontitis was 1.13 (95% CI: 1.03, 1.24; Figure 1A). After models were adjusted for age, sex, smoking, educational status, oral hygiene-related behaviors, frequency of visits to dentist and dental clinic, the pooled observational association has attenuated (OR=1.08; 95%CI: 1.03, 1.12). We repeated the fully adjusted model in only those at low risk of periodontitis (12,834 cases and 16,268 controls who never smoked and were non-diabetic), but this also had no material effect on the results (OR=1.11; 95%CI: 1.04, 1.18; Figure 1A).

We further examined the same 3 models separately in the cohorts where periodontitis had been assessed objectively, during clinical examinations by trained dental practitioners (12,181 cases and 17,278 controls) and in cohorts where periodontitis was ascertained by self-report (6,700 cases and 21,712 controls; Figures 1B and 1C). In crude analysis, one z-score unit higher BMI was associated with a pooled OR of 1.25 (95%CI: 1.10, 1.42; Figure 1B) for clinically assessed periodontitis and an OR of 1.02 (95%CI: 0.96, 1.08; Figure 1C) for self-reported periodontitis. In adjusted models, the respective pooled ORs were 1.13 (95%CI: 1.10, 1.17) and 1.03 (95%CI: 0.97, 1.08). When we repeated the adjusted model in people who had never smoked and were non-diabetic, the pooled OR was 1.15 (95%CI: 1.09, 1.20) for clinically assessed periodontitis (10,380 cases and 5,025 controls) and 1.06 (95%CI: 0.91, 1.23) for self-reported periodontitis (2,454 cases and 11,243 controls).

In adjusted models with obesity, overweight and normal weight categorically defined using the WHO guidelines(4), the pooled OR for periodontitis was 1.18

(95%CI: 1.03, 1.35) in obese compared with normal weight participants and the OR was 1.09 (95%CI: 1.02, 1.19; Supplementary Figure 1A) in overweight compared with normal weight participants. As above, we repeated these analyses in cohorts where periodontitis had been either clinically assessed or assessed by self-report. In obese compared with normal weight participants the pooled OR was 1.33 (95%CI: 1.18, 1.50; Supplementary Figure 1B) in the cohorts with clinically assessed periodontitis and 1.00 (95%CI: 0.78, 1.27; Supplementary Figure 1C) in the cohorts where periodontitis was defined by self-report. When overweight individuals were compared with normal weight individuals, the respective pooled ORs were 1.17 (95%CI: 1.06, 1.28; Supplementary Figure 1B) and 1.02 (95%CI: 0.96, 1.09; Supplementary Figure 1C).

Genetic associations with BMI

Most cohorts within the GLIDE consortium have previously reported on the genetic associations between the selected variants and BMI, individually or as part of larger meta-analyses. Associations of the GRS with BMI were estimated in 68,761 participants. Each copy of the effect allele was associated with a 0.07 BMI z-score units increase (95% CI: 0.05, 0.08; Figure 2A), corresponding to a combined mean BMI difference of 0.42 SD units between participants with 0 and 6 copies of the effect alleles. The increasing trend in mean BMI per GRS unit is presented in Supplementary Figure 2. In meta-analyses performed separately in cohorts with clinically assessed (40,348 participants) and self-reported (28,413 participants) periodontitis, each additional effect allele was associated with 0.06 (95%CI: 0.05, 0.08) and 0.08 (95%CI: 0.07, 0.09) BMI z-score units increase respectively (Figure 2B) with no evidence of heterogeneity between two estimates (P=0.99, heterogeneity test).

Associations with Potential Confounders

We examined the association of BMI and periodontitis with putative confounding variables (age, sex, smoking, educational status, frequency of visits to dentist, oral hygiene, time difference between dental examination date and date when anthropometric and lifestyle data was collected). Associations were observed for the majority of these comparisons (Supplementary Tables 5 & 6). By contrast, GRS was

not associated with the putative confounders, demonstrating its utility as instrumental variables (Supplementary Table 7).

Genetic associations with periodontal health

We studied associations between BMI-associated genotypes and periodontitis in 49,066 participants (17,672 cases). The pooled OR for periodontitis per additional GRS unit (effect allele) obtained from the meta-analysis of all studies was 1.01 (95%CI: 0.99, 1.03; Figure 3A). The pooled OR per risk allele in participants with clinically assessed periodontitis (10,972 cases and 9,681 controls) was 1.00 (95%CI: 0.97, 1.03; Figure 3A) and with self-reported periodontitis (6,700 cases and 21,713 controls) was 1.03 (95%CI: 0.98, 1.09).

Instrumental variable analysis

Instrumental variable estimates for studies in GLIDE are shown in Figure 4. Instrumental variable analysis yielded a pooled OR for periodontitis of 1.05 (95%CI: 0.80, 1.38) per standard deviation increase in BMI. This estimate overlapped with the observational OR of 1.13 (95%CI: 1.03, 1.24) (*P*=0.93, heterogeneity test). Meta-analyses performed separately in participants with clinically assessed periodontitis yielded a pooled OR for periodontitis of 0.90 (95%CI: 0.56, 1.46) per standard deviation increase in BMI. The OR was 1.25 (95%CI: 0.66, 2.36) in cohorts with self-reported periodontitis.

Discussion

To our knowledge, this is the largest study on the observational relationships between BMI and periodontitis to date and the first to examine causal relationships between obesity and periodontal health. Our study included up to 68,761 participants from 13 studies from Europe and North America. In observational analyses including the full sample, one standardized unit of BMI (corresponding to approximately 4,62 kg/m^2) was associated with 13% higher risk of periodontitis (8% when adjusted for putative confounders); when these analyses were performed only in participants in whom periodontitis had been objectively assessed, each standardized BMI unit conveyed a 25% higher risk of periodontitis (13% when adjusted for putative confounders). The causal inference analyses do not support a causal association between BMI and periodontitis, as the equivalent risk of periodontitis per BMI unit

was close to the null (5%) and was not statistically significant. The MR point estimate was lower still when analyses were performed only in cohorts where periodontitis had been clinically assessed (-10%). Although the causal inference analysis does not support a causal effect of total adiposity as a causal risk factor for periodontitis, the wide confidence intervals for these estimates suggest that this study may not be powered to rule out very small effects, as discussed further below.

The relationship between obesity and periodontitis is biologically complex and poorly understood. Proinflammatory cytokines are often elevated in obesity(38) causing a state of low-grade inflammation, which may contribute to the pathophysiology of periodontitis in obese people(39). Macrophage infiltration of obese (hypertrophic) adipocytes is common and drives adipocyte remodeling and secretion of proinflammatory cytokines such as tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) (38, 40), which are implicated in disruption of tooth supporting tissues, and may cause periodontitis(41-43).

It has also been suggested that periodontitis may causally influence low-grade inflammation, as intervention studies of periodontal therapy appeared to lower CRP concentrations(44). This doesn't exclude possibility that the relationship between obesity and periodontitis (through inflammation) may be bi-directional and the direction of this relationship cannot be determined in our data.

Several epidemiological studies (7, 45, 46) have reported associations between obesity and periodontitis including a large prospective cohort study of 36,910 healthcare professionals(7), where the association was significant after controlling for age, a comprehensive smoking index, race, profession, physical activity, fruit and vegetable intake, alcohol consumption, and diabetes status at baseline, and was attenuated, but still significant when restricted to non-smokers and non-diabetics. However, observational studies are prone to confounding, bias and reverse causality even when controlled for epidemiological measures of putative confounding factors, as these themselves are not measured without error or bias. Indeed, we observed here that both BMI and periodontitis are associated with several measured factors that might confound associations (Supplementary Table 5 and 6). In our observational analyses, we noted some attenuation of the pooled OR for periodontitis

and obesity when models were adjusted for putative confounders or when analyses were performed in never-smokers and non-diabetic participants (Figure 1A-C).

Data on periodontitis in this study were obtained through clinical assessments or self-report. In the observational analyses focused on the cohorts within which periodontitis had been clinically assessed, we observed associations between BMI (or categories of normal weight, overweight and obesity) and periodontitis that compare well with published results from observational meta-analyses(5). However, no association between BMI and periodontitis was detectable in the cohorts where periodontitis had been determined by self-report (Figures 1B and C); these differences may be attributable to the higher validity of clinically assessed periodontitis data. Although we speculate that risk estimates using clinically assessed periodontitis might be more valid than self-report, there is no gold standard definition of periodontitis (47) and the GLIDE cohorts (even those with clinically assessed disease) have hence used somewhat heterogeneious case definitions, which in turn might influence our results. In ARIC, in addition to CDC/AAP classification, we used the two clinical definitions based on probing depth and/or number of deep periodontal pockets used in MDC/TwinGene and COHRA (Supplementary Table 8) to investigate influence of case definition on genetic associations. It is possible that the manner in which periodontitis is defined might influence the strength and magnitude of the genetic associations reported here. We were able to explore this possibility in the ARIC Study, which has full-mouth periodontal examinations. We calculated the two clinical definitions adopted by the GLIDE cohorts in ARIC and compared the associations of the GRS with these two classifications of periodontitis. We observed small differences in ORs between tests, but with widely overlapping confidence intervals (Supplementary Table 8). These analyses are restricted to a single cohort (as ARIC is the only cohort within the Consortium with the required data) and might not generalize to other cohorts within the GLIDE Consortium; nevertheless, the observations in ARIC suggest that differences in the definition of periodontitis are unlikely to profoundly impact the interpretation of our results. This conclusion also holds when we recalculated OR to RR using observed prevalences and the described formula (48) under the assumption that observed prevalences reflect the true background prevalences for these populations (Supplementary Table 8).

In general, the MR analyses using OR and RR to quantify risk yielded confidence intervals that were overlapping and point estimates slightly below and above 1 respectively (data not shown). Thus, the overall conclusion is that, regardless of the approach used to determine risk, total adiposity is unlikely to be causally related with periodontitis, and, even if it is, the magnitude of this effect is unlikely to be of clinical significance. Also, edentulous participants were excluded from our analyses, which could have diluted observed estimates.

In the causal inference models focused on clinically assessed data and in the full cohort collection, the risk estimates were close to the null (Figures 4A and B). When these findings are placed alongside the observational data on BMI, periodontitis and putative confounding variables, we conclude that our study does not support a causal relationship between BMI and periodontitis. Nevertheless, as with many negative findings studies, particularly those where causal effects are very small in magnitude and of no clinical relevance, even very large cohort collections such as GLIDE will be underpowered to rule out an association. It has also been suggested that negative results of MR experiments are less prone to biases related to violation of MR assumptions and provide robust evidence when effects are very little of absent (49).

Unlike the vast majority of exposures that can be assessed in epidemiological studies, germline DNA variants are randomly assigned during meiosis and remain virtually unchanged across the lifespan and are usually uncorrelated with putative confounding variables. This renders associations between genotypes and periodontitis, obesity, and other outcomes less prone to confounding than purely observational studies, as we show here (Supplementary Table 7). Moreover, genotypes can be accurately measured in large sample collections, which contrasts many non-genetic exposures and confounders. Moreover, genotypes characterize life-long effects of exposures such as for BMI (50) which resolves questions about causal direction that often hamper the interpretation of epidemiological data. It is these characteristics that make genotypes powerful instruments in causal inference analyses, such as those performed here. Nonetheless, the variants studied here explain only a small proportion of the variance in total adiposity (51). Owing to this, it is possible that some aspects of adiposity that are not captured by the genetic instruments could bear stronger relationships with periodontitis than our data have

led us to conclude. Until such a time that a much more detailed genetic characterization of adiposity becomes available, it is hard to envisage how this limitation can be overcome.

Conventional observational epidemiology and MR analyses both seek to define causal relationships, although, as we emphasize above, causal inference is more challenging in the conventional observational setting owing to confounding, which MR seeks to address. Nevertheless, there are also limitations when causal inferences are made through MR studies. Although MR analyses are free from most forms of confounding, where pleiotropy exists (the concurrent, independent association of a genotype with two or more phenotypes), biological confounding can occur. Although it is difficult to completely exclude confounding by pleiotropy, we pooled three uncorrelated genotypes acting through different biological pathways (*FTO*, *MC4R* and *TMEM18*) into a GRS, which should decrease this possibility. A further limitation of MR, as with all genetic association analyses, is that it is prone to confounding by population stratification(8). To minimize this possibility, we used a standard approach (genetic principal components) to control for this type of confounding. Moreover, the low admixture of several of the participating cohorts (e.g., those form Sweden) and the ethnic homogeneity of all cohorts, means that population stratification is unlikely to be a pervasive issue in these analyses.

Lastly, it is possible that the exclusion of participants without any teeth may have diluted the observed effect estimates.

In summary, our Mendelian randomization analysis does not support a causal effect of total adiposity as a causal risk factor for periodontitis, suggesting that the epidemiological association between these variables may be confounded. Given that the observational effect estimates are relatively constant across cohorts, it is likely that if confounding underlies this association, that the confounders are consistent across studies. Identifying these putative confounding factors may yield targets for intervention that help prevent or treat periodontitis.

Supplementary Data

Supplementary Material is available at *IJE* online

Contributors

DS, PWF and IJ wrote the manuscript. DS, MCC, KD, BH, JRS, and Y-HY performed analyses in the contributing studies and DS performed all meta-analyses. All co-authors undertook revisions and commented on the manuscript.

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Figure Legends (Main Text)

Figure 1. Meta-analysis forest plots of observational associations between BMI and periodontitis. Model “Observational”: unadjusted logistic regression model of periodontitis and standardized BMI; “Observational adjusted”: adjusted for age, sex, smoking, educational status, oral hygiene behaviors, frequency of visits to dentist and dental clinic; “Never Smoked Non Diabetics adjusted”: model “Observational adjusted” in subsample of never smoked non diabetic individuals. (A) Meta-analysis forest plot including all studies in the analysis; (B) meta-analysis forest plot including studies with periodontitis assessed during clinical examinations by trained dental personnel; (C) meta-analysis forest plot including studies with self-reported periodontal health data. The ORs are for 1 standard deviation unit increase in BMI.

Figure 2. Meta-analysis forest plots of the associations of *FTO*, *MC4R* and *TMEM18* genotypes combined into genetic risk score (GRS) with standardized BMI (SD units). (A) all participants and (B) participants divided into 2 subgroups by method of periodontal health data collection. The changes in BMI z-score are presented per risk allele (1 GRS unit increase).

Figure 3. Meta-analysis forest plots of the associations of *FTO*, *MC4R* and *TMEM18* genotypes combined into genetic risk score (GRS) with periodontitis. (A) all participants and (B) participants divided into 2 subgroups by method of periodontal health data collection. The ORs are per risk allele (1 GRS unit increase).

Figure 4. Meta-analysis forest plots of instrumental variable causal estimates using genetic risk score (GRS). (A) meta-analysis forest plot including all studies in the analysis; (B) participants divided into 2 subgroups by method of periodontal health data collection. The ORs are for 1 standard deviation unit increase in BMI.

Figure Legends (Supplementary Figures)

Supplementary Figure 1. Meta-analysis forest plots of observational associations between periodontitis and obesity (BMI >30.0 kg/m^2)/overweight (BMI 25.0 to 29.9 kg/m^2) versus normal weight participants (BMI 18.5 to 24.9 kg/m^2). (A) in all participants; (B) in participants with periodontitis assessed during clinical examinations by trained dental personnel; and (C) self-reported periodontal health. All models were adjusted for age, sex; smoking, educational status, oral hygiene behavior, frequency of visits to dentist and

dental clinic.

Supplementary Figure 2. Meta-analysis mean BMI and 95% confidence intervals per each genetic risk score and distribution of genetic risk scores in studies of GLIDE consortium. Left y-axis represents meta-analysis sample size per each genetic risk score on the histogram. Meta-analysis mean BMI (dots) and 95%CI (extensions) per each genetic risk score are referenced to the right y-axis.

"Key Messages" box:

- Observational relationship between total adiposity and periodontitis is widely known, yet causal evidence is lacking.

- We performed a Mendelian randomization meta-analysis as part of the Gene-Lifestyle Interactions and Dental Endpoints (GLIDE) Consortium with BMI-associated loci combined in a genetic risk score as the instrument for BMI in 49,066 participants with clinically-assessed and self-reported periodontitis, genotype and other relevant data.

- Our study does not support a causal relationship between total adiposity and periodontitis, as the point estimate for this relationship in the Mendelian randomization analysis is very close to the null and with wide confidence intervals.