Clinical Epigenetics

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Biological aging mediates the association between periodontitis and cardiovascular disease: results from a national population study and Mendelian randomization analysis

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Abstract

Background The relationship between periodontitis and cardiovascular disease (CVD) has been extensively studied, but the role of biological aging in this relationship remains poorly understood. This study is dedicated to investigating the effect of periodontitis on the incidence of CVD and to elucidating the potential mediating role of biological aging. Furthermore, this study will seek to elucidate the causal association between periodontitis, CVD, and biological aging.

Methods We included 3269 participants from the National Health and Nutrition Examination Survey (2009–2014) with diagnostic information on periodontitis and composite CVD events. Biological aging was evaluated by utilizing both the Klemera–Doubal method's calculated biological age (KDMAge) and phenotypic age (PhenoAge). Logistic regression, restricted cubic spline (RCS) analysis, and subgroup analysis were used for data analysis. Mediation analysis was employed to explore the mediating role of biological aging. Subsequently, Mendelian randomization (MR) analyses were performed using genome-wide association study databases to explore potential causal relationships between periodontitis, CVD, and biological aging.

Results Periodontitis was associated with a higher risk of CVD. Participants with periodontitis were found to have increased levels of biological aging, and elevated levels of biological aging were associated with increased CVD risk. Mediation analyses showed a partial mediating effect of biological aging (PhenoAge: 44.6%; KDMAge: 22.9%) between periodontitis and CVD risk. MR analysis showed that periodontitis played a causal role in increasing the risk of small vessel stroke, while myocardial infarction was found to increase the risk of periodontitis. In addition, reverse MR analysis showed that phenotypic aging can increase the risk of periodontitis, and there is a two-way causal relationship between CVD and biological aging.

Conclusions Periodontitis is associated with an increased CVD risk, partially mediated by biological aging, with a complex causal interrelationship. Targeted interventions for periodontal health may slow the biological aging processes and reduce CVD risk.

Keywords Periodontitis, Cardiovascular disease, Biological aging, Causal association, Mediating effect

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Introduction

Periodontitis is a chronic, inflammatory disease of the oral cavity. It is characterized by the accumulation of plaque in the soft tissues of the periodontium and gingival margins. Periodontitis affects a large proportion of the world's population [1], and severe periodontitis has a prevalence of approximately 11% globally [2]. In addition to its impact on oral health, the systemic inflammation caused by periodontitis can affect organs, including the cardiovascular system [3, 4]. Cardiovascular disease (CVD) is a leading cause of disability and death worldwide [5]. While some studies have linked the severity of periodontitis to an increased incidence of CVD, others have yielded divergent findings, highlighting the need for further exploration [6–9]. Notably, while the relationship between periodontitis and CVD has been explored, no studies have examined potential mediators of this association.

Periodontitis and CVD are recognized as diseases of biological aging [10, 11]. Physiological changes due to biological aging may affect host-bacterial interactions, which may exacerbate the incidence and severity of periodontitis [12]. Likewise, biological aging is an independent risk factor for CVD, as biological age-related changes contribute to increased susceptibility [13]. Given the effects of biological aging on both diseases, it can be hypothesized that periodontitis may increase CVD risk by promoting biological aging.

The present study utilized data from the National Health and Nutrition Examination Survey (NHANES) to investigate the association between periodontitis and CVD. Additionally, the potential mediating effect of biological aging was explored. Subsequently, a Mendelian randomization (MR) analysis was conducted, employing data from a large genome-wide association study (GWAS) to evaluate the potential causal relationship between periodontitis, CVD, and biological aging.

Method

Study design and population

This study utilized NHANES 2009–2014 data to represent the majority of the U.S. population. Data from 2009 to 2014 were chosen because periodontitis-related exams were performed only during this period. Detailed information about NHANES recruitment procedures, population characteristics, and study design is available on the website of the Centers for Disease Control and Prevention (CDC). Only participants who underwent a complete dental examination and completed a self-reported CVD questionnaire as part of the NHANES were included in the current study. The analysis for this research included variables such as age, sex, race/ethnicity, education, body mass index (BMI), poverty income ratio (PIR), smoking status, alcohol use, diabetes, hypertension, and hyperlipidemia. This is due to their previous association with periodontitis and CVD in scientific literature, as well as their role as pivotal demographic and health factors with the potential to impact study outcomes [14, 15]. Ethic approval was not required for the secondary data analysis. The Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed in this study.

Study variables and criteria

Oral health examinations are conducted by duly licensed dentists at the Mobile Examination Center (MEC). Clinical attachment level (CAL) and probing pocket depth (PPD) were measured using a periodontal probe at six sites on each tooth, excluding the third molar, in participants aged ≥ 30 years. Periodontitis was defined according to the 2012 CDC/American Academy of Periodontology classification [16, 17] (Table S2). Participants were categorized into three groups depending on their periodontitis status: none/mild periodontitis, moderate periodontitis, and severe periodontitis [18]. Mean CAL and PPD were included in the analysis. The definitions of CVD were determined through self-reported histories of congestive heart failure (CHF), angina, myocardial infarction (MI), stroke, and coronary heart disease (CHD) in data obtained from personal interviews [19]. These questions were asked, in the home, by trained interviewers using the Computer-Assisted Personal Interviewing (CAPI) system. The Klemera–Doubal method (KDMAge) and phenotypic age (PhenoAge) were used as markers to assess biological aging. PhenoAge was determined using nine aging-related variables, including albumin level, creatinine level, blood glucose level, C-reactive protein (CRP) level, lymphocyte percentage, mean cell volume, erythrocyte distribution width, alkaline phosphatase level, and white blood cell count [20]. KDMAge was determined based on eight biomarkers: ln-CRP, blood creatinine, glycosylated hemoglobin, serum albumin, serum total cholesterol, serum urea nitrogen, serum alkaline phosphatase, and systolic blood pressure [21]. KDMAge or PhenoAge acceleration was calculated using the residuals of chronological age regression. The detailed calculation method is outlined in Supplement methods.

The covariates included in this study were as follows: age (30–59 years or ≥ 60 years); sex (male or female); race/ethnicity (non-Hispanic white, non-Hispanic black, or others); education level (lower than high school, high school or equivalent, or college and above); BMI (< 25.0, 25–29.9, or ≥ 30.0); PIR (< 1, 1–2.99, or ≥ 3); smoking status (never, ever, or current); alcohol use (never,

occasional, or frequent); hypertension (yes or no); hyperlipidemia (yes or no), and diabetes (yes or no).

Statistical analysis

We used analytic methods applicable to the NHANES design, accounting for stratification and weighting, to produce nationally representative estimates [6]. Categorical variables were expressed as percentages, and continuous variables were expressed as mean ± standard deviation (SD). Normality tests were performed to assess the distribution of continuous variables. Multiple imputations were applied for missing data [22]. Logistic regression models were used to assess associations between periodontitis, CVD, and biological aging. In addition to the crude model with unadjusted covariates, three models were built: Model 1, adjusted for age, sex, and race/ethnicity; Model 2, adjusted for education, BMI, and PIR; and Model 3, further adjusted for smoking status, alcohol use, hypertension, hyperlipidemia, and diabetes. Subgroup analyses were performed to test the robustness of the models. Restricted cubic spline (RCS) analysis was used to examine the non-linear relationship between periodontitis, CVD, and biological aging. In addition, the potential mediating effects of biological aging in the association of periodontitis with CVD were assessed using parallel mediation modeling.

MR analyses

Selection of genetic instruments for periodontitis, biological aging, and CVD

Single nucleotide polymorphisms (SNPs) were obtained from the GWAS database, and MR analysis was used to investigate the causal relationship between periodontitis, CVD, and biological aging. Details of the GWAS data used in this study are listed in Table S1. We obtained periodontitis data from three GWAS databases, including the GLIDE consortium (17,353 cases; 28,210 controls) [23], the FinnGen consortium (4784 cases; 272,252 controls) [24], and a meta-analysis from the IEU (1740 cases; 347,186 controls) [25]. Data on biological aging was drawn from a GWAS meta-analysis of 34,710 individuals of European descent in 28 cohorts, including DNA methylation (DNAm), GrimAge acceleration, DNAm Hannum age acceleration, Intrinsic epigenetic age acceleration, and DNAm PhenoAge acceleration [26]. Five CVDs were selected for study. AF data stemmed from a meta-analysis involving six European GWAS (60,620 cases; 970,216 controls) [27]. MI data were sourced from a GWAS meta-analysis of two cohort studies (61,505 cases; 577,716 controls) [28]. Ischemic heart disease (IHD) data (20,857 cases; 340,337 controls) and CHD data (10,157 cases; 351,037 controls) were retrieved from the UK Biobank. Data on stroke (40,585 cases; 406,111 controls) and its subtypes, including ischemic stroke (34,217 cases; 406,111 controls), large artery stroke (4373 cases; 406,111 controls), small vessel stroke (5386 cases; 406,111 controls), and cardioembolic stroke (7193 cases; 406,111 controls), were obtained from the MEGASTROKE consortium [29].

This MR study was conducted in accordance with the STROBE-MR guidelines [30]. All participants in the original study provided informed consent, and ethical clearance was obtained.

Statistical analysis

In the screening of instrumental variables, a slightly looser threshold of $p < 5 \times 10^{-6}$ was selected to screen SNPS, due to the limited number of SNPs meeting the strict threshold ($p < 5 \times 10^{-8}$). Independent clustering (r^2 cut off 0.001; distance window 10,000 kb) was then performed on European samples using 1000 Genomes data. After standardizing the effect alleles in the GWAS data, we used the MRPRESSO method to remove outliers, and then the Radial MR method to remove outliers again. We ensured that the F-statistic for these instruments exceeded the threshold of 10. Inverse variance weighting (IVW) was used as the main indicator. In addition, MR-Egger, weighted median, simple model, and weighted model methods were used to assess the robustness of our findings. Reverse MR analysis was used to examine the reverse causality between exposure and outcome. We performed sensitivity analyses to detect underlying pleiotropy and heterogeneity in MR estimates. Heterogeneity markers derived from the IVW approach were used to identify potential horizontal pleiotropy (Cochran Q-derived test $p < 0.05$). In addition, a leave-one-out analysis was performed to assess the influence or bias of individual SNPs on the MR estimate.

All statistical analyses were performed with R, version 4.3.3. All tests were two-tailed, and the significance level was set at $p < 0.05$. Figure 1 summarizes the population selection and research process used in this study.

Results

Population characteristics

Table 1 shows the basic characteristics of the participants. A total of 3269 adults were enrolled in this study, including 1670 males (51.1%) and 1599 females (48.9%), representing approximately 60,831,523 U.S. residents. The distribution of periodontitis was as follows: 1394 participants (42.6%) had no/mild periodontitis, 1446 (44.2%) had moderate periodontitis, and 429 (13.1%) had severe periodontitis. Of the 3269 participants, 275 were diagnosed with CVD. There were 168 cases (61.1%) of moderate periodontitis and 51 cases (18.5%) of severe periodontitis in the CVD group, 1278 cases (42.7%) of

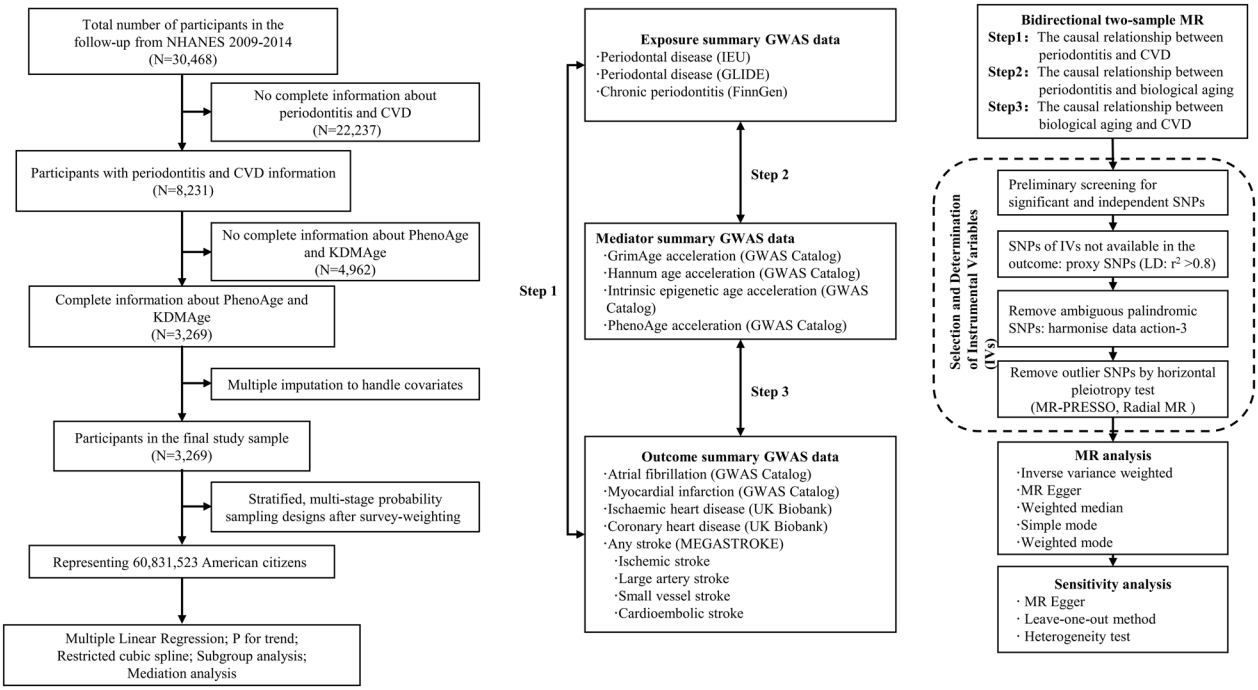

Fig. 1 Flow diagram of this study. MR, Mendelian randomization; GWAS, genome-wide association studies; NHANES, National Health and Nutrition Examination Survey; CVD, cardiovascular disease. PhenoAge, Phenotypic age; KDMAge, biological age as calculated by the Klemera–Doubal method

moderate periodontitis, and 378 cases (12.6%) of severe periodontitis in the non-CVD group. Logistic regression analysis showed the difference between the two groups (Table S3).

Associations between periodontitis and CVD

Figure 2a shows the association between periodontitis and CVD. In all models, moderate or severe periodontitis was significantly positively correlated with CVD, compared with patients with none/mild periodontitis (p for trend < 0.004). In the crude model, severe periodontitis was significantly positively correlated with CVD risk (OR 3.22; 95% confidence interval [CI] 2.17–4.79; $p < 0.001$), and in model 3, it was still established (OR 1.79; 95% CI 1.15–2.77; $p = 0.009$). Similarly, mean CAL and mean PPD were positively correlated with CVD in each model, however, in models 2 and 3, the association of mean PPD was not significant (p from 0.209 to 0.366) (Fig. 2a).

After adjusting for potential confounders, no significant non-linear relationship was found between mean CAL/PPD and CVD (Figure S1a–b). We performed stratification and interaction analyses to determine the association between mean PPD/CAL and CVD incidence (Fig. 3). For example, mean PPD was associated with stroke in participants aged 30–59 (OR 1.41; 95% CI 1.06–1.89), race of non-Hispanic white (OR 1.38; 95% CI 1.07–1.77), education of college and above (OR 1.79; 95% CI 1.25–2.57), and BMI of < 25.0 (OR 1.66; 95% CI 1.01–2.72). However, we did not find statistically significant interactions in stratified analyses for the investigation of effect modification.

Associations between biological aging and CVD

Figure 2b shows that PhenoAge and KDMAge were significantly positively correlated with CVD risk in all models ($p < 0.001$). In the crude model, PhenoAge acceleration was significantly positively correlated with CVD risk (OR 2.14; 95% CI 1.66–2.75; $p < 0.001$), which was still significant in model 1 and 2 (models1: OR 1.85; 95% CI 1.40–2.43; $p < 0.001$; model2: OR 1.60; 95% CI 1.21–2.12; $p < 0.001$), but not in model 3 (OR 1.28; 95% CI 0.96–1.70; $p = 0.088$). KDMAge acceleration obtained the same conclusion in the crude model (OR 1.30; 95% CI 1.02–1.67; $p = 0.037$), which was still significant in model 1 (OR 1.34; 95% CI 1.04–1.75; $p = 0.026$), but in model 2 and 3, this relationship was not significant (models2: OR 1.21; 95% CI 0.92–1.57; $p = 0.168$; model3: OR 0.97; 95% CI 0.74–1.27; $p = 0.823$). After adjusting for all confounders, no significant non-linear relationship was observed between PhenoAge/KDMAge and CVD (Figure S1c-d).

Associations between periodontitis and biological aging

As shown in Fig. 2c-d, there was a significant positive correlation between periodontitis and CVD risk in all

Table 1 Characteristics of the study participants

Characteristic	Overall (N = 3269)	Non-CVD (N = 2994)	CVD (N = 275)
Age, n (%)			
30–59	2213 (67.7)	2121 (70.8)	92 (33.5)
≥ 60	1056 (32.3)	873 (29.2)	183 (66.5)
Sex, n (%)			
Male	1670 (51.1)	1490 (49.8)	180 (65.5)
Female	1599 (48.9)	1504 (50.2)	95 (34.5)
Race/ethnicity, n (%)			
Non-Hispanic white	1618 (49.5)	1454 (48.6)	164 (59.6)
Non-Hispanic black	530 (16.2)	478 (16.0)	52 (18.9)
Others	1121 (34.3)	1062 (35.5)	59 (21.5)
Education, n (%)			
Less than high school	895 (27.4)	808 (27.0)	87 (31.6)
High school or equivalent	718 (22.0)	653 (21.8)	65 (23.6)
College and above	1656 (50.7)	1533 (51.2)	123 (44.7)
BMI (kg/m^2), n (%)			
< 25.0	827 (25.3)	772 (25.8)	55 (20.0)
25–29.9	1167 (35.7)	1078 (36.0)	89 (32.4)
≥ 30.0	1275 (39.0)	1144 (38.2)	131 (47.6)
PIR, n (%)			
< 1	600 (18.4)	537 (17.9)	63 (22.9)
1–2.99	1345 (41.1)	1233 (41.2)	112 (40.7)
≥ 3	1324 (40.5)	1224 (40.9)	100 (36.4)
Smoking status, n (%)			
Never smoker	1788 (54.7)	1673 (55.9)	115 (41.8)
Ever smoker	852 (26.1)	749 (25.0)	103 (37.5)
Current smoker	629 (19.2)	572 (19.1)	57 (20.7)
Alcohol use, n (%)			
Never drinker	1203 (36.8)	1069 (35.7)	134 (48.7)
Occasional drinker	1284 (39.3)	1205 (40.2)	79 (28.7)
Frequent drinker	782 (23.9)	720 (24.0)	62 (22.5)
Hypertension, n (%)			
No	2119 (64.8)	2048 (68.4)	71 (25.8)
Yes	1150 (35.2)	946 (31.6)	204 (74.2)
Hyperlipidemia, n (%)			
No	1902 (58.2)	1804 (60.3)	98 (35.6)
Yes	1367 (41.8)	1190 (39.7)	177 (64.4)
Diabetes, n (%)			
No	2900 (88.7)	2693 (89.9)	207 (75.3)
Yes	369 (11.3)	301 (10.1)	68 (24.7)
Mean CAL (mm)	1.73 ± 1.20	1.67 ± 1.14	2.36 ± 1.54
Mean PPD (mm)	1.68 ± 0.63	1.67 ± 0.62	1.81 ± 0.66
Periodontitis status, n (%)			
None/Mild	1394 (42.6)	1338 (44.7)	56 (20.4)
Moderate	1446 (44.2)	1278 (42.7)	168 (61.1)
Severe	429 (13.1)	378 (12.6)	51 (18.5)
PhenoAge (years)	49.32 ± 15.13	47.97 ± 14.53	64.00 ± 13.67
PhenoAge acceleration, n (%)			
No	1846 (56.5)	1738 (58.0)	108 (39.3)
Yes	1423 (43.5)	1256 (42.0)	167 (60.7)
KDMAge (years)	48.97 ± 14.23	47.85 ± 13.80	61.18 ± 13.08
KDMAge acceleration, n (%)			
No	1988 (60.8)	1837 (61.4)	151 (54.9)
Yes	1281 (39.2)	1157 (38.6)	124 (45.1)

Continuous variables were presented as mean ± SD. Categorical variables were presented as n (%). CVD, cardiovascular disease; BMI, Body Mass Index; PIR, Poverty income ratio; PPD, pocket probing depth; CAL, clinical attachment level; PhenoAge, Phenotypic age; KDMAge, biological age as calculated by the Klemera–Doubal method; SD, standard deviation; N, numbers of participants; %, percentage

models (p for trend < 0.006). Specifically, in the crude model, moderate periodontitis was significantly positively correlated with the risk of PhenoAge acceleration (OR 1.42; 95% CI 1.22–1.65; $p < 0.001$), and the risk of severe periodontitis was higher (OR 2.72; 95% CI 2.18–3.40; $p < 0.001$). It was still significant in model 1 ($p < 0.032$). However, in models 2 and 3, the positive correlation between severe periodontitis and accelerated phenotypic aging was still significant ($p < 0.002$), but moderate periodontitis was no longer significant (p from 0.089 to 0.523). The relationship between periodontitis and KDMAge acceleration was similar. For example, in model 3, severe periodontitis was significantly positively correlated with the risk of KDMAge acceleration (OR 1.42; 95% CI 1.10–1.82; $p = 0.006$). Subsequent in-depth analysis did not find a significant non-linear relationship between mean PPD/CAL and biological aging (Figure S1e-h).

Mediation analyses

Figure 4 demonstrated the significant mediating effects of biological aging in the association between periodontitis (including periodontitis status, mean PPD, and mean CAL) and CVD (all $p < 0.01$). For example, the mediation proportion of periodontitis affecting the occurrence of CVD through PhenoAge was 46.60%, and the mediation proportion through KDMAge was 22.92%. The results of the sensitivity analysis showed that the mediation model established in this study is reliable (Figure S7).

MR analysis of periodontitis and CVD

In the MR analysis of various CVD using three different periodontitis databases (Figs. 5, 6a), we found that periodontitis (IEU) was significantly positively correlated with small vessel stroke (OR 1.15; 95% CI 1.00–1.33; $p = 0.049$), indicating its potential causal effect. No significant association was found with other MR methods. We did not detect horizontal pleiotropy (Table S5), and

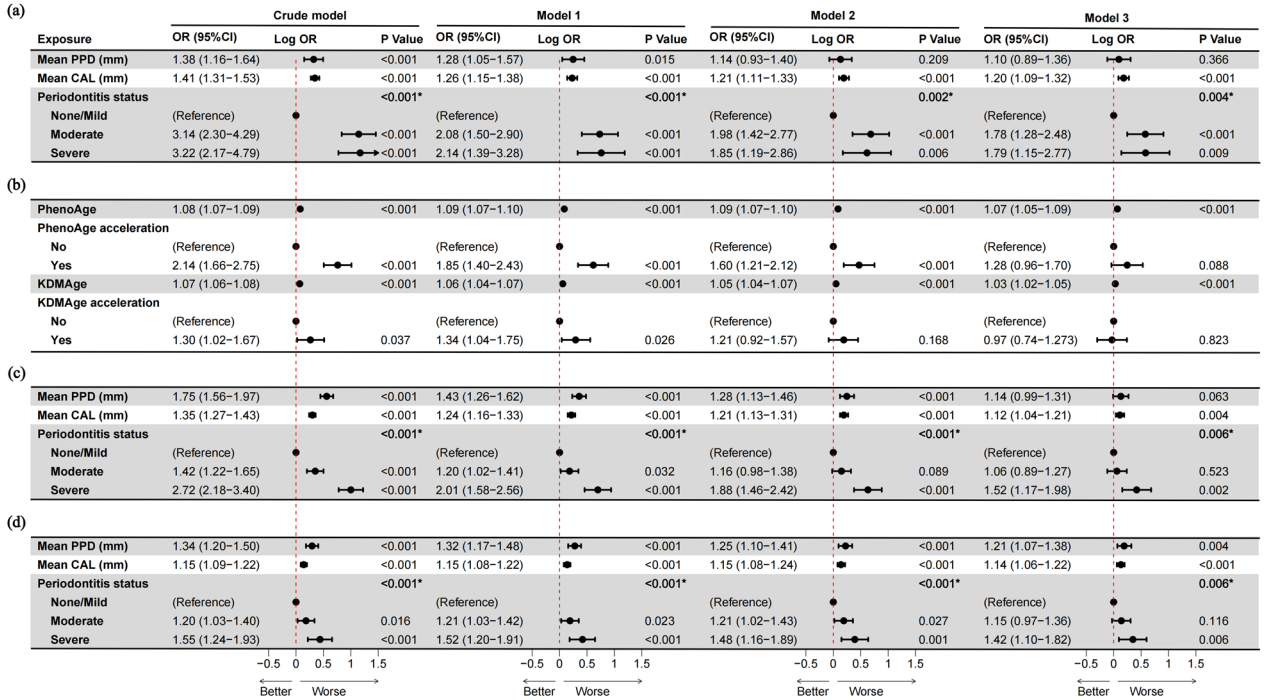

Fig. 2 Multivariable regression analysis. **a** Multivariable regression analysis between periodontitis and CVD. **b** Multivariable regression analysis between biological aging and CVD. **c** Multivariable regression analysis between periodontitis and PhenoAge. **d** Multivariable regression analysis between periodontitis and KDMAge. Model 1: adjusted for sex, age, race; Model 2: Model 1 + adjusted for education, BMI and PIR; Model 3: Model 2 + adjusted for smoking status, alcohol use, hypertension, hyperlipidemia and diabetes; BMI, Body Mass Index; PIR, Poverty income ratio; PPD, pocket probing depth; CAL, clinical attachment level; PhenoAge, Phenotypic age; KDMAge, biological age as calculated by the Klemera–Doubal method; OR, odds ratio; 95% CI, 95% confidence interval. **p*-Value for trend

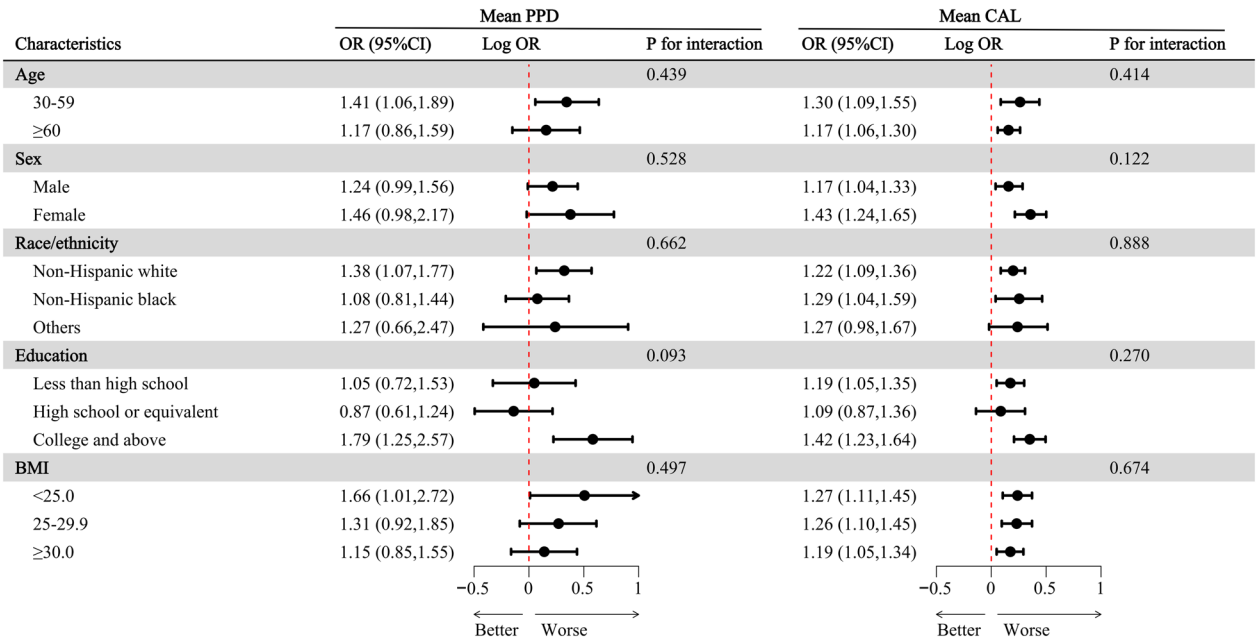

Fig. 3 Subgroup analysis of periodontitis and cardiovascular disease. Except for the stratification component itself, each stratification factor was adjusted for age, sex, race/ethnicity, education, and BMI. BMI, body mass index; OR, odds ratio; 95% CI, 95% confidence interval

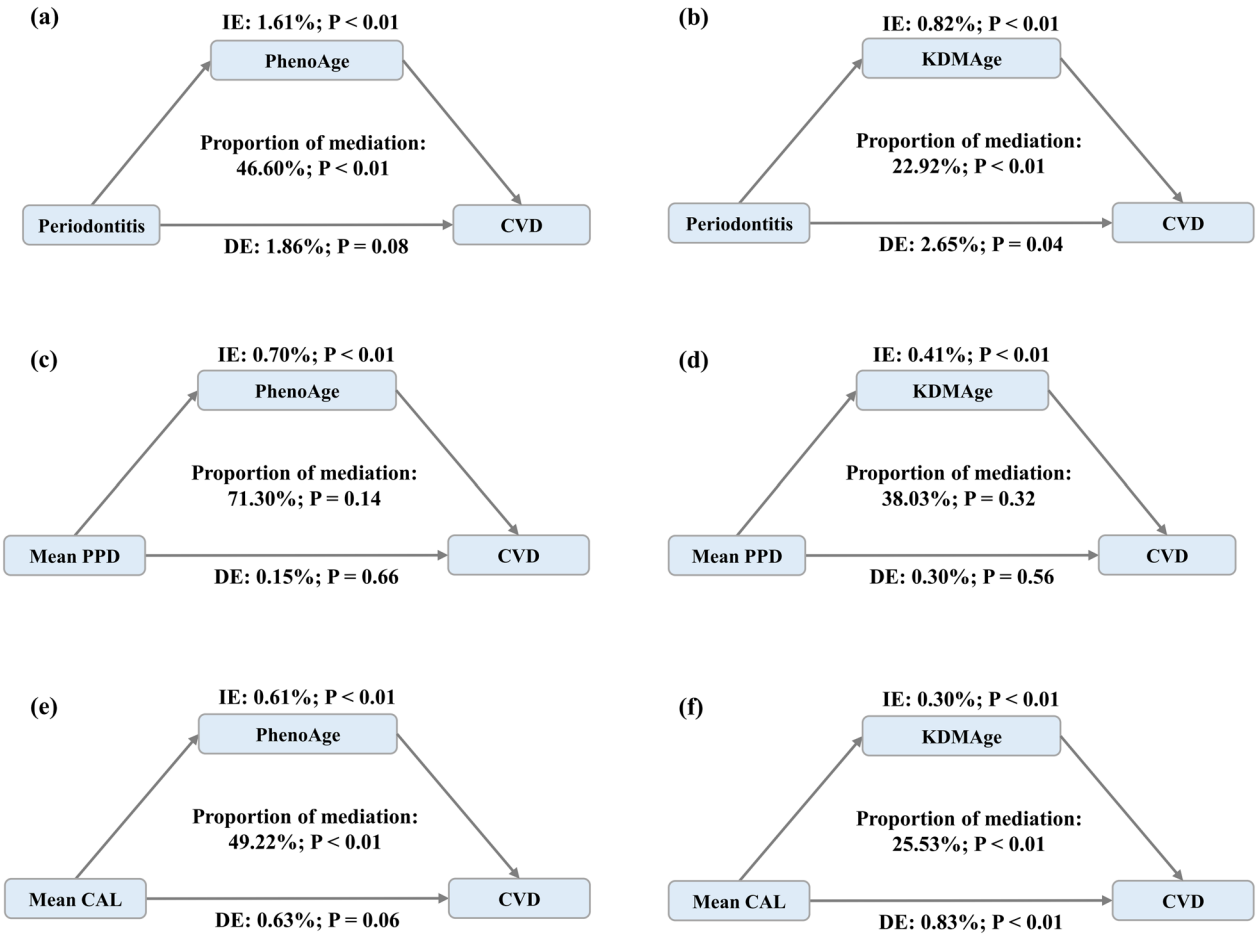

Fig. 4 Estimated proportion of the association between PD and CVD mediated by biological aging. Models were adjusted for sex, age, race, education, BMI, PIR, smoking status, alcohol use, hypertension, hyperlipidemia, and diabetes. BMI, Body Mass Index; PIR, Poverty income ratio; CVD, cardiovascular disease; PPD, pocket probing depth; CAL, clinical attachment level; PhenoAge, Phenotypic age; KDMAge, biological age as calculated by the Klemera–Doubal method; IE, the estimate of the indirect effect; DE, the estimate of the direct effect; Proportion of mediation = IE/DE + IE

leave-one-out analysis showed that the association with periodontitis was driven by all single SNPs. Heterogeneity analysis showed no significant heterogeneity among the SNPs (Table S4). We performed additional MR analysis to investigate the possibility of reverse causality (Fig. 6a and Figure S2). The potential causal effect of MI on periodontitis (FinnGen) was found, and subsequent analysis proved the reliability of this conclusion.

MR analysis of periodontitis and biological aging

The results of the MR analysis did not provide substantial support for the causal effect of periodontitis on biological aging (Fig. 6b and Figure S3). However, when examining the reverse causal effect of periodontitis on biological aging (Figure S4), we found a significant causal effect of DNAm Hannum age acceleration on periodontitis (FinnGen) (OR 1.056; 95% CI 1.01–1.11; $p=0.023$). Interestingly, we found that PhenoAge acceleration has a causal effect that potentially reduces the risk of periodontitis (FinnGen) (OR 0.965; 95% CI 0.93–1.00; $p=0.035$). Additional MR analysis and sensitivity analysis showed that our results were reliable.

MR analysis of biological aging and CVD

Figure 6c and Figure S5 show the identified potential causal effect of GrimAge acceleration on small vessel stroke (OR 0.925; 95% CI 0.87–0.99; $p=0.017$); Hannum age acceleration on AF (OR 1.019; 95% CI 1.00–1.03; $p=0.010$); and PhenoAge on AF (OR 1.014; 95% CI 1.00–1.03; $p=0.015$), MI (OR 1.026; 95% CI 1.00–1.04; $p=0.003$), CHD (OR 1.001; 95% CI 1.00–1.00; $p=0.002$), IHD (OR 1.001; 95% CI 1.00–1.00; $p=0.030$), and small vessel stroke (OR 1.073; 95% CI 1.03–1.12; $p=0.001$). When examining the reverse causal effect of biological aging on CVD (Figure S6), we found a potential causal effect of AF on Hannum age acceleration (OR 1.103; 95%

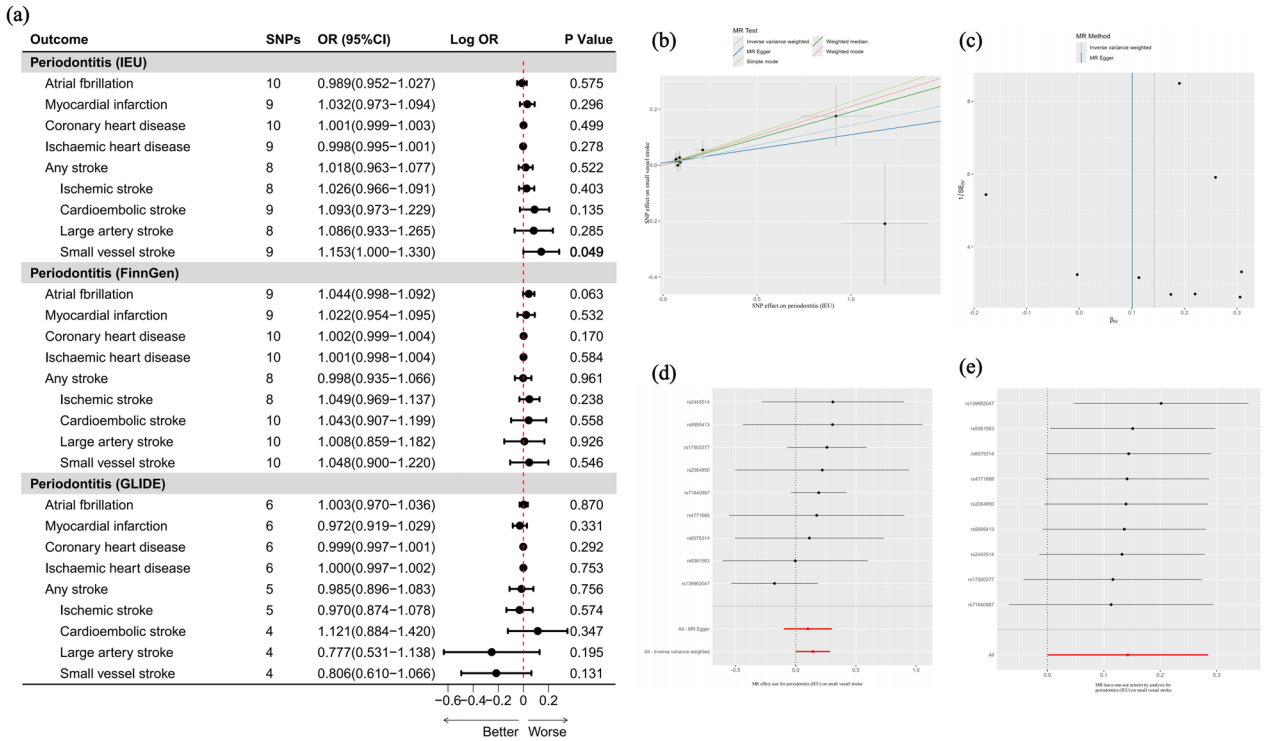

Fig. 5 Mendelian randomization (MR) estimates of the causal effects of periodontitis on cardiovascular disease. **a** MR estimates of the causal effects of periodontitis on cardiovascular disease. **b** MR scatter plot. **c** MR funnel plot. **d** MR forest plot. **e** MR leave-one-out plot

CI 1.01–1.20; $p=0.027$), cardioembolic stroke on GrimAge acceleration (OR 1.119; 95% CI 1.00–1.25; $p=0.039$), and small vessel stroke on GrimAge acceleration (OR 0.901; 95% CI 0.81–1.00; $p=0.049$). Subsequent sensitivity analyses confirmed our conclusions.

Discussion

Using the NHANES and GWAS databases, this study investigated the complex relationship between periodontitis, CVD, and biological aging. The results indicated that periodontitis was a risk factor for CVD and that aging plays a mediating role in this association. Gene-level predictive analysis further confirmed the causal effect of periodontitis on small-vessel stroke and revealed the causal effect of biological aging on periodontitis and specific CVD. Simultaneously, the study found that CVD may exacerbate the progression of biological aging.

We observed that an increase in the degree of periodontitis was associated with an increased risk of CVD, and similar results were obtained when further examining the relationship between mean PPD/mean CAL and CVD, which was consistent in different subgroups. Additionally, MR analysis revealed the potential causal effect of periodontitis on small vessel stroke. A number of epidemiologic studies have suggested a significant positive association between PD and CVD, such as CHD and stroke [31–33]. The possible mechanisms for this association include the systemic inflammation caused by periodontitis. Patients with periodontitis often have elevated levels of inflammatory markers in the blood, such as CRP and white blood cell count. These biomarkers play a key role in the pathophysiological mechanism of CVD [3, 34, 35]. On the other hand, pathophysiological studies have revealed the potential role of oral bacteria in the formation of atherosclerosis [36–38]. In addition, further supporting the pathological link between periodontitis and CVD is the observation of pathological changes similar to CVD, such as the formation of atherosclerotic plaques, in animal experimental models following the induction of periodontitis [39, 40].

We investigated the relationship between periodontitis, CVD, and aging markers. The results indicated that the progression of periodontitis is significantly associated with biological aging. This suggests that periodontitis may not only affect oral health but accelerate the systemic aging process. The presence of periodontitis may aggravate the aging of organisms and even increase all-cause mortality [11, 41]. These findings provide strong evidence for the important role of periodontitis in the mechanism of systemic aging. Furthermore, biological aging was found to be linked to a higher risk of CVD, which aligned with previous research [42, 43]. Our findings also suggest

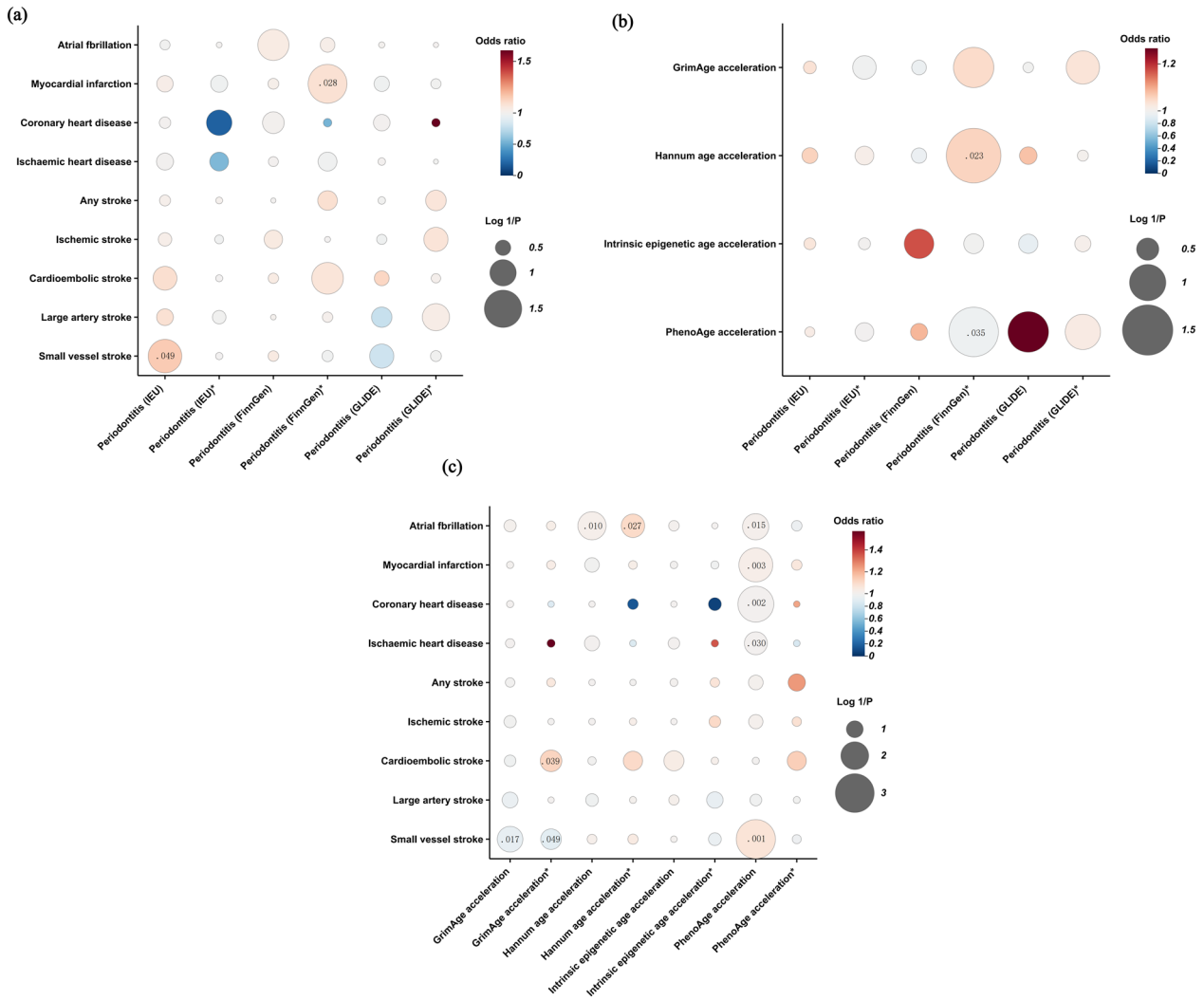

Fig. 6 Bubble plots about Mendelian randomization (MR) estimate the causality of periodontitis, biological aging, and cardiovascular disease (CVD). **a** MR analyses the causal relationship between periodontitis and CVD. **b** MR analyses the causal relationship between periodontitis and biological aging. **c** MR analyses the causal relationship between biological aging and CVD. The analysis uses exposure as the horizontal axis and outcome as the vertical axis. *Reverse MR analysis

a potential causal relationship between biological aging and periodontitis, as well as a reciprocal causal effect between aging and CVD. In other words, aging contributes to the development of periodontitis and CVD and can also be a potential consequence of CVD. These results underscore the intricate interplay between periodontitis, CVD, and biological aging.

Mediation analysis showed that biological aging plays a mediating role in the relationship between periodontitis and CVD. This provides a new mechanistic explanation for the link between the two diseases. Periodontitis has long been considered a manifestation of aging. Previous research has shown that periodontitis may accelerate the aging process by inducing systemic chronic inflammation, thereby increasing an individual's susceptibility to different adverse outcomes. Additionally, PhenoAge and KDMAge have been found to be associated with increased activation of pro-inflammatory pathways [20, 44]. Inflammation and immune dysfunction are important factors in the pathogenesis of CVD [45]. The findings from our study indicate that periodontitis may hasten aging, resulting in systemic disruption and ultimately elevating the risk of CVD.

This study reveals a complex relationship between periodontitis, CVD, and aging, a finding that has significant implications for both clinical practice and public health policy. From a clinical perspective, these findings indicate that periodontal health is a crucial factor in the

prevention of CVD and the management of the aging process. It is therefore recommended that healthcare professionals incorporate oral health into the routine assessment of their patients. Furthermore, the findings of this study provide a crucial scientific foundation for the formulation of public health policies aimed at promoting enhanced periodontal health education and interventions in the context of aging, with the objective of reducing the prevalence of CVD and improving the overall health and quality of life of the elderly population.

This study was the first to investigate the relationship between periodontitis and CVD together with the mediating role of biological aging. By integrating multiple databases, the representativeness of the research findings was enhanced. In the data analysis process, we combined mean PPD and mean CAL as supplements. Additionally, we investigated the non-linear correlation between periodontitis, CVD, and aging, offering a new perspective on the intricate interconnections between the three. To guarantee the dependability of the periodontitis and CVD associations, we conducted a subgroup analysis. Simultaneously, we examined the mediating effect of aging markers and validated the conclusion's reliability through sensitivity analysis. The use of genetic variants in the GWAS database for MR analysis allowed for the more accurate elimination of confounding factors and enabled causal inferences, ensuring the robustness of the research results.

There were several limitations in our study. The observational design of the NHANES dataset limited our ability to establish causal relationships. Additionally, reliance on self-reported cardiovascular events introduced potential recall bias. Although MR analysis was used to verify this association, this analysis was limited by several assumptions, for example, that there is no pleiotropic association between genetic variants and phenotypes and the assumption of a linear genetic relationship. MR studies did not reveal the mediating role of biological aging between periodontitis and CVD, and we found some conclusions contrary to our cross-sectional study, such as that GrimAge acceleration and stroke are causal and protective factors. This may be due to the complex and diverse risk factors of biological aging. Despite adequate quality control, it is still difficult to completely confound SNPs, and the differences in conclusions from different GWAS databases indicate the heterogeneity of the population. Accordingly, future studies will endeavor to elucidate the causal relationship between periodontitis, CVD, and aging through the utilization of MR analysis.

In summary, this research highlights the importance of periodontal health in systemic health and emphasizes the need for effective periodontitis management to maintain overall health. Such management may have significant implications for delaying aging and reducing CVD risk. Future research can delve deeper into the underlying mechanisms of the observed effects and explore targeted interventions aimed at helping a broader range of patients achieve dual protection for both their oral and systemic health.

Conclusion

This study identified periodontitis as a risk factor for CVD and suggested a mediating role for biological aging. Mendelian randomization further revealed the complexity of the causal relationship. Effective management of periodontitis could serve as an alternative to delaying the biological aging process and reducing the risk of cardiovascular events.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-024-01732-9.

Supplementary Material 1: Supplementary Methods.

Supplementary Material 2: Table S1. Data source of Mendelian randomization Analysis. Table S2. Classification of periodontitis. Table S3. Weighted logistic regression of cardiovascular disease (CVD). Table S4. Heterogeneity test for Mendelian randomization. Table S5. Pleiotropy test for Mendelian randomization. Table S6. Mendelian randomization (MR) analysis (five methods) results. Figure S1. Restricted cubic spline analysis. Figure S2. Reverse Mendelian randomization estimates of the causal effects of periodontitis on cardiovascular disease. Figure S3. Mendelian randomization estimates of the causal effects of periodontitis on biological aging. Figure S4. Reverse Mendelian randomization estimates of the causal effects of periodontitis on biological aging. Figure S5. Mendelian randomization estimates of the causal effects of biological aging on cardiovascular disease. Figure S6. Reverse Mendelian randomization estimates of the causal effects of biological aging on cardiovascular disease. Figure S7. Sensitivity analysis of the mediating effect of biological aging.

Acknowledgements

This investigation utilized data from NHANES and GWAS Database. We sincerely appreciate all participants and investigators for their valuable contribution in making statistics accessible and publicly available. We thank Ms. Courtney at Scribbr for her professional editing and proofreading services.

Author contributions

Zhaoqi Zhang and Xingru Zhao: data analysis, writing manuscripts; Shang Gao and Kai Yang: find literature review and data interpretation; An Li and Ke Deng: preliminary cleaning and collation of data; Wei Liu and Mi Du: research design, funding, modification of manuscripts. All authors provided critical feedback and approved the final version of the manuscript.

Funding

This paper was supported in part by Taishan Scholar Youth Expert Plan (No. tsqn201909186).

Availability of data and materials

Data from the cross-sectional study are available on the CDC website (https://www.cdc.gov/nchs/nhanes/). GWAS data from sources available in the public domain (Table S1).

Declarations

Ethics approval and consent to participate
The study used NHANES database and the large publicly available GWAS database, which has received approval from their relevant ethical review board and participants.

Competing interests
The authors declare no competing interests.

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Received: 13 July 2024 Accepted: 19 August 2024
Published online: 24 August 2024

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