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Epigenome-wide association study using peripheral blood leukocytes identifies genomic regions associated with periodontal disease and edentulism in the Atherosclerosis Risk in Communities study

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Abstract

Aim: To investigate individual susceptibility to periodontitis by conducting an epigenome-wide association study using peripheral blood.

Materials and Methods: We included 1077 African American and 457 European American participants of the Atherosclerosis Risk in Communities (ARIC) study who had completed a dental examination or reported being edentulous at Visit 4 and had available data on DNA methylation from Visit 2 or 3. DNA methylation levels were compared by periodontal disease severity and edentulism through discovery analyses and subsequent testing of individual CpGs.

Results: Our discovery analysis replicated findings from a previous study reporting a region in gene *ZFP57* (6p22.1) that was significantly hypomethylated in severe periodontal disease compared with no/mild periodontal disease in European American participants. Higher methylation levels in a separate region in an unknown gene (located in Chr10: 743,992-744,958) was associated with significantly higher odds of edentulism compared with no/mild periodontal disease in African American participants. In subsequent CpG testing, four CpGs in a region previously associated with periodontitis located within *HOXA4* were significantly hypermethylated in severe periodontal disease compared with no/mild periodontal disease in African American

participants (odds ratio per 1 SD increase in methylation level: cg11015251: 1.28 (1.02, 1.61); cg14359292: 1.24 (1.01, 1.54); cg07317062: 1.30 (1.05, 1.61); cg08657492: 1.25 (1.01, 1.55)).

Conclusions: Our study highlights epigenetic variations in *ZPF57* and *HOXA4* that are significantly and reproducibly associated with periodontitis. Future studies should evaluate gene regulatory mechanisms in the candidate regions of these loci.

KEYWORDS

DNA methylation, epigenome-wide association study (EWAS), periodontal disease, periodontitis, peripheral blood

Clinical Relevance

Scientific rationale for study: Without altering the DNA sequence, epigenetic effects (e.g., DNA methylation changes) can alter gene activity and influence host response to periodontal infections. Our well-powered study investigates individual susceptibility to periodontitis by conducting a thorough assessment of periodontitis-related DNA methylation levels in blood.

Principal findings: We identified two gene regions, *ZPF57* and *HOXA4*, that are differentially methylated in individuals with periodontitis compared with those without periodontitis.

Practical implications: Studying differential leukocyte DNA methylation patterns may point to candidate regions and the underlying gene regulatory mechanisms that play a key role in the progression and/or susceptibility to periodontitis.

1 | INTRODUCTION

Over 47% of US adults have some form of periodontitis, whose incidence rises steeply in adults aged 50 years and older (Eke et al., 2020, 2015; Eke, Dye, et al., 2012). Given the public health impact of periodontal disease (Petersen & Ogawa, 2012; Slots, 2017), studying individuals' susceptibility to periodontitis could be crucial to building effective disease prevention strategies. Although genome-wide association studies (GWAS) have discovered new genes that may contribute to the pathogenesis of periodontitis, only a few common variants identified reached genome-wide significance, highlighting the complex interplay between genetic and environmental factors in the development and progression of the disease (Divaris et al., 2013; Laine et al., 2014; Munz et al., 2017; Schaefer et al., 2010; Shungin et al., 2019). In addition to genetic factors, epigenetic modifications of DNA can affect the genetic blueprint of host responses, either directly in lesion-associated target tissue, or associated with an altered immune response. Studies have found that blood methylation levels can correlate with tissue levels, making blood-based DNA methylation an attractive target for biomarker discovery (Do et al., 2023). Therefore, assessing epigenetic alterations in whole blood may provide valuable insights into the development and progression of periodontitis and help identify individuals who are at higher risk for the disease.

In the context of periodontal disease, host response plays a critical role in an individual's susceptibility to infections (Hajishengallis, 2015), as revealed by several studies investigating DNA methylation changes in gingival tissues of patients with periodontitis. These studies have identified differential methylation patterns in genes involved in inflammation, immune response and extracellular matrix organization (de Faria Amormino et al., 2013; Schulz et al., 2016). A recent study by Kim et al. (2021) collected gingival tissue samples from 14 individuals with healthy periodontium and 14 individuals with chronic periodontitis and reported that hypermethylated CpGs in individuals with periodontitis were enriched in genes related to inflammation and immune response, while the hypomethylated CpGs were enriched in genes related to cellular and tissue development. Despite the insights gained from gingival tissue studies, the use of DNA methylation in whole blood offers subject-level sampling instead of site-level specimens. This approach enables the identification of systemic contributors to periodontitis and the reflection of immunologic susceptibility to periodontitis. Two epigenome-wide studies (EWAS) of peripheral blood leukocytes in periodontitis have been published. A cross-sectional study in adult female twins by Kurushima et al. (2019) identified several CpGs, including one in *ZNF804A*, associated with gingival bleeding and tooth mobility. A pilot study by Hernández et al. (2021), conducted in eight periodontitis patients and eight matched controls, reported statistically significant differences in peripheral blood DNA methylation in multiple regions, including the *ZNF718*, *HOXA4* and *ZFP57* genes.

To expand on previous work of periodontitis-related DNA methylation changes, we conducted both discovery analyses and subsequent testing of CpGs in specific gene regions. The discovery analysis involved an EWAS and differential methylated region (DMR) analysis to discover aberrant patterns of blood-derived DNA methylation in periodontal disease severity and edentulism among 1534 Black and White older adults who underwent a clinical dental examination as a component of the community-based Atherosclerosis Risk in Communities (ARIC) study. Additionally, we examined relationships with CpGs based on a priori knowledge of specific gene regions that may be

important for periodontitis, including the *HOXA4* and *ZFP57* genes. Our study aimed to identify CpG sites associated with periodontal disease severity and edentulism, controlling for relevant health conditions, socio-economic status, self-reported smoking and methylation-predicted pack-years.

2 | MATERIALS AND METHODS

2.1 | Study population

Study participants were enrolled in the ARIC study (RRID: SCR_021769), a prospective cohort study of cardiovascular disease risk that enrolled 15,792 participants between 1987 and 1989 from four US communities (Jackson, MS; Washington County, MD; suburban Minneapolis, MN; and Forsyth County, NC; Jackson et al., 1996; Wright et al., 2021). Participants underwent a baseline clinical examination (Visit 1) and subsequent follow-up clinical exams (Visits 2–9 completed and Visit 10 planned). Participants provided written informed consent at each visit. The ARIC study protocol was approved by institutional review boards at the four field centres and the coordinating centre.

At Visit 4 (1996–1998), participants who had at least one tooth or dental implant and were otherwise eligible and consented, underwent a clinical dental examination by trained personnel ($n = 6017$). Edentulous participants were not eligible for the dental examination. Our analysis focused on a sample of ARIC participants with available data on leukocyte DNA methylation levels collected at Visit 2 (1990–1992) or Visit 3 (1993–1995). Among the participants with methylation data, those who attended a dental examination (Naorungroj et al., 2017) ($n = 1174$) or who self-reported being edentulous ($n = 360$) during Visit 4 were eligible for our study.

2.2 | DNA methylation assessment and preprocessing

The procedures for DNA methylation assessment have been previously described (Bose et al., 2014; Navas-Acien et al., 2021). Briefly, the Illumina HumanMethylation450 BeadChip array was used for genome-wide profiling of DNA methylation in 2853 African American participants at 483,525 CpG sites and in 1104 European American participants in 482,815 CpG sites. Thus, we treated the methylation data of African American and European American participants as two separate cohorts. Details are summarized in Data S2.

2.3 | Periodontal measures

Periodontal severity was determined based on standardized dental measurements, including clinical attachment loss (CAL) calculated on the basis of the probing depth and gingival recession, which were measured at six sites on all teeth present (Beck et al., 2005; Michaud et al., 2018). To ensure comprehensive assessment, we used two different measures of periodontitis severity. The first definition, 'periodontitis (ARIC)', categorizes individuals based on the percentage of sites with CAL ≥ 3 mm into no/mild, moderate or severe periodontitis, as previously used in the ARIC study (Beck et al., 2001). The second measure, 'periodontitis (CDC-AAP)', was developed for population-based surveillance of periodontitis by the US Centers for Disease Control and Prevention–American Academy of Periodontology (CDC-AAP). This measure uses CAL, pocket depth (PD) and the site location (interproximal) to classify participants into no, mild, moderate or severe periodontitis (Eke, Page, et al., 2012). Beyond using traditional definitions of periodontal disease severity, we used edentulism as a surrogate for the most severe type of periodontitis, since tooth loss is a common outcome of periodontitis and is often used as a clinical marker of disease severity (Papapanou et al., 2018). In addition, individuals who are edentulous have likely experienced significant periodontal breakdown and tooth loss. ARIC participants who self-reported as both not having any natural teeth and not having any dental implants were classified as edentulous for this analysis.

2.4 | Covariate assessment

Modifiable risk factors associated with periodontitis and edentulism include tobacco smoking (Lazăr et al., 2022), nutrition (Martinon et al., 2021), psychological stress and depression (Spector et al., 2020), obesity, diabetes (Genco & Borgnakke, 2020) and metabolic syndrome (Madiba & Bhayat, 2018). At Visit 2 (1990–1992) and Visit 3 (1993–1995), cigarette smoking status (current, former, never) and cigarette smoking dose were measured. To account for potential confounding by leukocyte cell composition, we used a DNA methylation deconvolution procedure (see Data S2) to estimate the proportions of different cell types in each sample (Salas et al., 2022). We used the R function EstDimRMT to determine that five principal components with non-zero eigenvalues were needed to correct batch effects in each cohort (Leek et al., 2010; Plerou et al., 2002). Additionally, we included age, sex, smoking category (never, former, current), pack-years and body mass index (BMI) as covariates in the EWAS and DMR analyses. In contrast, our subsequent testing of CpGs from specific gene regions included additional covariates such as methylation-predicted pack-years smoked, ARIC field center, socio-economic status (SES) and diabetes status. Diabetes at baseline was defined as fasting plasma glucose ≥126 mg/dL, non-fasting plasma glucose ≥200 mg/dL, receiving diabetes treatment or self-report of a diabetes diagnosis by a physician. Participants with high life-course SES were identified using the lifetime SES variable, calculated based on 12 factors associated with SES (Lu et al., 2019).

2.5 | Statistical analysis

To investigate the association between DNA methylation and periodontal disease, we conducted an EWAS using multivariable logistic

regression models. This analysis aimed to identify CpG sites associated with severe versus no/mild periodontitis (ARIC definition), moderate versus no/mild periodontitis (ARIC definition) and edentulous versus no/mild periodontitis (ARIC definition), or severe versus no periodontitis (CDC-AAP definition), mild/moderate versus no periodontitis (CDC-AAP definition) and edentulous versus no periodontitis (CDC-AAP definition) per 1 SD increase in methylation level at single CpG sites. All models were adjusted for age, sex, five surrogate variables for batch effects, smoking category (never, former, current), pack-years, BMI and leukocyte cell composition. To control for potential residual confounding by smoking, we included a methylation-predicted pack-years developed based on a meta-analysis of association results between DNA methylation and cigarette smoking in individuals from 16 cohorts (Joehanes et al., 2016). The Benjamini–Hochberg method was used to calculate the false discovery rate (FDR) for multiple comparison adjustments (FDR *q*-value <.05). Lastly, we conducted EWAS separately for African American and European American participants.

We also performed a DMR analysis using the DMRcate R/Bioconductor package to identify the DMRs associated with periodontal disease status. The DMR analysis was conducted separately for African American and European American participants. A linear model adjusted for the same covariates as the EWAS was fitted using the DMRcate function along with the following parameters: lambda = 1000 and kernel adjustment $C = 2$ (Peters et al., 2015). Statistically significant DMRs were required to have a minimum of two statistically significant single CpGs and to meet the multiple testing adjustment criteria of FDR (*q*-value) <.05 (calculated using the Benjamini–Hochberg method; Benjamini & Hochberg, 1995).

Figure S1 provides a summary of the discovery analyses described above and subsequent CpG testing. CpGs belonging to the statistically significant DMRs were further evaluated using a baseline category logit model to test for the association with periodontitis status according to both the CDC-AAP and the ARIC periodontitis definitions. Forty-two CpGs were selected based on prior knowledge from both our own DMRcate results and previously published pilot study by Hernández et al. (2021) The regions of *HOXA4* and *ZFP57* genes were of interest because they were found to be significant with multiple consecutive CpGs in the same direction after controlling for cell composition in the Hernandez study model (Hernández et al., 2021). We compiled CpGs from the three DMRs identified by Hernandez et al. and by our own DMRcate and tested them in the ARIC dataset. All models controlled for age, sex, SES, BMI, diabetes status, smoking category (never, former, current), pack-years, methylation-predicted pack-years smoked, leukocyte cell composition, ARIC field centre and five surrogate variables for batch effects. Subgroup analysis was also conducted for those 58 years and older versus under, male versus female participants, and participants of higher versus lower SES. Analyses were conducted separately for African American and European American participants. In this subsequent CpG testing, we did not correct for multiple comparisons since the statistical tests for the individual CpGs were pre-specified and were not exploratory in nature, reducing the risk of obtaining spurious results due to chance.

3 | RESULTS

3.1 | Population characteristics

Table 1 summarizes the characteristics of ARIC participants included in this study by periodontal disease status defined by the CDC-AAP and by the ARIC periodontitis definitions. The average time between drawing blood and Visit 4 was 6 years. Participants with severe periodontitis were more likely to be male, Black, obese, less educated, current smoker and have lower life-course SES (Table 1). Compared with those of European ancestry, African American participants in this study were more likely to be diabetic, had low SES, were less educated, had fewer teeth and were more likely to have severe periodontitis (Table S1). African American participants were younger, with a median age of 55 years, compared with 58 years for European American participants. Of the participants, 28.6% of African Americans had severe periodontal disease, with 45.7% having no/mild periodontal disease, while 11.4% and 60.2% of European Americans had severe or no/mild periodontal disease, respectively.

3.2 | EWAS and DMRcate analysis

Using the ARIC periodontitis definition, the single CpG EWAS did not identify differentially methylated CpGs that were statistically significant after multiple comparison corrections ($q < .05$). After adjusting for methylation-predicted pack-years, we identified one statistically significant DMR (*ENSG00000231601* manual annotation Chr10: 743,992-744,958) in edentulism when compared with no/mild periodontal disease in African American participants and one statistically significant DMR (*ZFP57* 6p22.1) in severe periodontal disease compared with no/mild periodontal disease in European American participants (Table S2). The EWAS and DMRcate results did not differ from those presented above when using the CDC-AAP periodontitis definition for analysis.

3.2.1 | Zinc finger protein gene 57 (*ZFP57*)

The statistically significant DMR located on Chr6 (*ZFP57* gene), consisting of 17 CpGs, differed between European American participants with severe periodontal disease and those with no/mild periodontal disease. The 17 CpGs perfectly overlapped with a 21-CpG *ZFP57* region, which was reported by Hernandez et al. as hypomethylated when comparing periodontitis patients with age-matched and sex-matched periodontally healthy controls living in the Republic of Colombia (Hernández et al., 2021). Overlapping CpGs located in the *ZFP57* DMR region were found to be associated with a lower risk of severe periodontal disease compared with no/mild periodontal disease (ARIC definition) in European American participants (Table 2). This association was stronger in European American participants 58 years or older (Table 3). It was also comparable for male and

TABLE 1 Characteristics across periodontal disease categories among Atherosclerosis Risk in Communities (ARIC) participants, separately by race[a].

African American participants	Edentulism ($n = 309$)	Periodontitis (CDC-AAP)				Periodontitis (ARIC)		
		No ($n = 318$)	Mild ($n = 18$)	Moderate ($n = 263$)	Severe ($n = 169$)	No/Mild ($n = 183$)	Moderate ($n = 277$)	Severe ($n = 308$)
Age								
Mean (SD)	57.4 (5.56)	54.4 (5.31)	55.2 (5.99)	55.3 (5.61)	54.8 (5.24)	54.4 (5.16)	54.8 (5.62)	55.0 (5.38)
Median [min, max]	57.0 [47.0, 69.0]	53.0 [47.0, 68.0]	52.5 [49.0, 67.0]	54.0 [47.0, 70.0]	54.0 [47.0, 67.0]	53.0 [47.0, 68.0]	53.0 [47.0, 68.0]	54.0 [47.0, 70.0]
Gender, %								
Female	76.1	78.9	66.7	58.9	36.7	82.0	66.4	47.4
Male	23.9	21.1	33.3	41.1	63.3	18.0	33.6	52.6
Teeth number								
Mean (SD)	NA	16.3 (7.45)	17.2 (6.76)	17.2 (6.90)	17.6 (6.77)	17.3 (7.19)	18.8 (7.59)	16.9 (7.90)
Median [min, max]	NA	17.5 [3.00, 28.0]	18.5 [3.00, 26.0]	19.0 [2.00, 28.0]	18.0 [2.00, 28.0]	18.0 [3.00, 30.0]	20.0 [3.00, 32.0]	17.0 [2.00, 32.0]
Cigarette smoking status, %								
Current	24.9	10.1	22.2	20.5	42.0	8.2	16.6	32.5
Former	26.5	25.2	27.8	28.1	21.9	27.3	24.2	25.6
Never	48.5	64.8	50.0	51.3	36.1	64.5	59.2	41.9
Accumulated cigarette smoking packyears								
Mean (SD)	11.7 (19.8)	4.49 (10.2)	5.38 (9.07)	9.26 (16.3)	14.7 (18.1)	4.00 (9.27)	6.53 (14.5)	12.7 (16.9)
Median [min, max]	0.300 [0, 160]	0 [0, 81.0]	0.750 [0, 36.3]	0 [0, 146]	7.20 [0, 88.9]	0 [0, 81.0]	0 [0, 146]	4.15 [0, 88.9]
Drinking status, %								
Current	22.7	28.0	44.4	34.6	46.7	30.6	31.8	39.9
Former	37.2	29.9	33.3	28.9	28.4	29.5	29.6	28.9
Never	40.1	41.8	22.2	36.5	23.7	39.9	38.6	30.2
Diabetes status, %								
No	74.4	87.1	66.7	86.3	81.1	85.2	87.7	82.5
Yes	25.6	12.3	33.3	12.9	18.9	14.2	11.9	16.9
High SES status, %								
No	82.2	47.5	72.2	53.6	60.9	44.8	45.8	64.6
Yes	17.8	52.5	27.8	46.4	39.1	55.2	54.2	35.4
Education level, %								
High school (no degree) or less	55.0	20.1	44.4	32.7	34.9	18.6	23.8	38.0
High school graduate or vocational school	29.4	33.0	38.9	25.5	30.2	33.9	27.1	30.2
College or graduate School	15.2	46.9	16.7	41.4	34.9	47.5	48.7	31.8

TABLE 1 (Continued)

African American participants	Edentulism ($n = 309$)	Periodontitis (CDC-AAP)				Periodontitis (ARIC)		
		No ($n = 318$)	Mild ($n = 18$)	Moderate ($n = 263$)	Severe ($n = 169$)	No/Mild ($n = 183$)	Moderate ($n = 277$)	Severe ($n = 308$)
BMI categories, %								
BMI < 18.5	1.0	0.3	0.0	0.8	0.0	0.5	0.4	0.3
BMI ≥ 18.5 and <25	14.9	16.0	22.2	17.5	20.7	12.0	19.5	19.5
BMI ≥ 25 and <30	35.0	36.8	16.7	40.3	42.0	37.2	37.5	40.6
BMI ≥ 30 and <40	40.5	39.0	44.4	32.7	33.7	41.0	34.3	34.1
BMI ≥ 40	8.7	7.9	11.1	8.4	3.6	8.7	7.9	5.5
ARIC field center, %								
Forsyth County, NC	4.9	3.1	33.3	7.2	11.2	7.7	6.9	6.8
Jackson, MS	95.1	96.9	66.7	92.8	88.8	92.3	93.1	93.2

European American participants	Edentulism ($n = 51$)	Periodontitis (CDC-AAP)				Periodontitis (ARIC		
		No ($n = 171$)	Mild ($n = 28$)	Moderate ($n = 162$)	Severe ($n = 45$)	No/Mild ($n = 224$)	Moderate ($n = 130$)	Severe ($n = 52$)
Age								
Mean (SD)	60.2 (4.74)	58.2 (5.04)	57.0 (4.95)	58.7 (5.47)	57.5 (5.36)	57.9 (5.04)	58.9 (5.50)	58.0 (5.47)
Median [min, max]	61.0 [48.0, 67.0]	58.0 [48.0, 68.0]	56.5 [49.0, 65.0]	59.0 [47.0, 67.0]	58.0 [48.0, 67.0]	58.0 [47.0, 68.0]	59.0 [47.0, 67.0]	58.0 [48.0, 67.0]
Gender, %								
Female	62.7	77.2	57.1	50.6	26.7	71.0	49.2	36.5
Male	37.3	22.8	42.9	49.4	73.3	29.0	50.8	63.5
Teeth number								
Mean (SD)	NA	23.8 (5.06)	26.0 (2.05)	22.5 (6.23)	22.1 (5.42)	24.8 (4.40)	24.0 (4.97)	17.9 (8.37)
Median [min, max]	NA	25.0 [2.00, 28.0]	27.0 [20.0, 28.0]	25.0 [1.00, 28.0]	24.0 [5.00, 28.0]	26.0 [2.00, 32.0]	26.0 [4.00, 30.0]	19.5 [1.00, 31.0]
Cigarette smoking status, %								
Current	35.3	11.1	3.6	18.5	24.4	10.3	18.5	26.9
Former	21.6	29.2	35.7	36.4	35.6	30.8	35.4	38.5
Never	43.1	59.6	60.7	45.1	40.0	58.9	46.2	34.6
Accumulated cigarette smoking packyears								
Mean (SD)	20.9 (30.4)	7.95 (14.2)	7.68 (13.2)	16.7 (23.8)	17.6 (20.0)	8.64 (16.1)	13.5 (17.1)	26.5 (30.4)
Median [min, max]	9.40 [0, 141]	0 [0, 65.8]	0 [0, 44.0]	3.28 [0, 148]	11.0 [0, 82.0]	0 [0, 84.0]	3.28 [0, 73.5]	22.9 [0, 148]

(Continues)

TABLE 1 (Continued)

European American participants	Edentulism ($n = 51$)	Periodontitis (CDC-AAP)				Periodontitis (ARIC		
		No ($n = 171$)	Mild ($n = 28$)	Moderate ($n = 162$)	Severe ($n = 45$)	No/ Mild ($n = 224$)	Moderate ($n = 130$)	Severe ($n = 52$)
Drinking status, %								
Current	27.5	59.1	67.9	59.9	68.9	61.6	60.0	61.5
Former	15.7	10.5	7.1	9.3	17.8	8.9	13.1	11.5
Never	56.9	30.4	25.0	30.9	13.3	29.5	26.9	26.9
Diabetes status, %								
No	94.1	97.1	100.0	96.9	95.6	98.2	95.4	96.2
Yes	5.9	2.9	0.0	3.1	4.4	1.8	4.6	3.8
High SES status, %								
No	58.8	13.5	7.1	16.7	11.1	11.2	13.8	26.9
Yes	41.2	86.5	92.9	83.3	88.9	88.8	86.2	73.1
Education level, %								
High school (no degree) or less	39.2	5.3	3.6	14.8	6.7	5.8	10.0	21.2
High school graduate or vocational school	47.1	44.4	53.6	41.4	37.8	46.4	42.3	30.8
College or graduate School	13.7	50.3	42.9	43.8	55.6	47.8	47.7	48.1
BMI categories, %								
BMI < 18.5	0.0	1.2	3.6	1.9	0.0	0.9	1.5	3.8
BMI ≥ 18.5 and <25	45.1	51.5	32.1	35.2	37.8	49.1	33.8	32.7
BMI ≥ 25 and <30	33.3	33.3	42.9	45.1	53.3	35.7	46.2	50.0
BMI ≥ 30 and <40	19.6	12.9	21.4	17.9	8.9	13.8	17.7	13.5
BMI ≥ 40	2.0	1.2	0.0	0.0	0.0	0.4	0.8	0.0
ARIC field center, %								
Forsyth County, NC	94.1	90.6	67.9	84.0	71.1	85.3	86.2	75.0
Minneapolis, MN	3.9	8.2	21.4	13.6	15.6	12.1	12.3	11.5
Washington County, MD	2.0	1.2	10.7	2.5	13.3	2.7	1.5	13.5

Abbreviations: BMI, body mass index; CDC-AAP, Centers for Disease Control and Prevention–American Academy of Periodontology; SES, socio-economic status.

[a]Results are reported separately by race because methylation profiling was performed separately by race. Characteristics were from the same ARIC visit as the methylation data (mostly Visit 2 or 3), periodontal disease categories were from the dental examination performed at Visit 4, and edentulism status was assessed at Visit 4.

TABLE 2 Odds ratio (OR) for CpGs in the *ZFP57*[a] differentially methylated region and using two definitions of periodontal disease severity in Atherosclerosis Risk in Communities (ARIC).[b]

	OR per 1 SD increase in methylation level (95% CI)					
	Periodontitis (CDC-AAP definition)[c]			Periodontitis (ARIC definition)[c]		
CpG	Mild/moderate versus no	Severe versus no	Edentulism versus no	Moderate versus no/mild	Severe versus no/mild	Edentulism versus no/mild
EA participants (Visit 02)						
cg07134666	**0.69 (0.54, 0.88)**	1.09 (0.72, 1.64)	1.00 (0.66, 1.50)	0.84 (0.66, 1.07)	**0.66 (0.47, 0.92)**	1.05 (0.71, 1.55)
cg11383134	**0.65 (0.50, 0.84)**	1.04 (0.66, 1.65)	1.06 (0.66, 1.72)	**0.76 (0.60, 0.98)**	**0.65 (0.46, 0.91)**	1.12 (0.71, 1.78)
cg22494932	**0.62 (0.47, 0.82)**	1.08 (0.65, 1.79)	1.04 (0.60, 1.79)	**0.74 (0.58, 0.94)**	**0.69 (0.50, 0.96)**	1.14 (0.68, 1.90)
cg15570656	**0.70 (0.55, 0.89)**	1.09 (0.72, 1.65)	1.01 (0.66, 1.54)	0.89 (0.70, 1.13)	**0.67 (0.48, 0.94)**	1.09 (0.72, 1.63)
cg16885113	**0.68 (0.52, 0.87)**	1.09 (0.71, 1.69)	1.09 (0.70, 1.70)	0.82 (0.64, 1.05)	**0.66 (0.47, 0.92)**	1.15 (0.75, 1.76)
cg20228636	**0.70 (0.54, 0.90)**	1.13 (0.74, 1.74)	1.11 (0.72, 1.72)	0.83 (0.65, 1.06)	**0.67 (0.48, 0.94)**	1.14 (0.75, 1.73)
cg25699073	**0.65 (0.49, 0.86)**	1.16 (0.69, 1.94)	1.05 (0.62, 1.79)	**0.74 (0.58, 0.95)**	**0.69 (0.49, 0.96)**	1.10 (0.67, 1.81)
cg25978138	**0.70 (0.54, 0.91)**	0.99 (0.62, 1.58)	1.19 (0.72, 1.96)	**0.74 (0.58, 0.96)**	**0.63 (0.46, 0.88)**	1.17 (0.73, 1.90)
cg13835168	0.79 (0.63, 1.00)	1.24 (0.83, 1.85)	1.10 (0.75, 1.63)	0.97 (0.77, 1.23)	**0.70 (0.49, 0.98)**	1.12 (0.77, 1.63)
African American (AA) participants (Visit 02)						
cg07134666	1.11 (0.93, 1.31)	1.00 (0.81, 1.23)	0.96 (0.81, 1.14)	1.10 (0.90, 1.33)	1.02 (0.84, 1.24)	0.96 (0.79, 1.17)
cg11383134	1.06 (0.89, 1.25)	0.98 (0.79, 1.20)	0.96 (0.81, 1.14)	1.04 (0.85, 1.26)	0.96 (0.79, 1.17)	0.93 (0.76, 1.14)
cg22494932	1.06 (0.88, 1.26)	0.95 (0.77, 1.17)	0.94 (0.79, 1.12)	1.07 (0.87, 1.30)	0.96 (0.79, 1.18)	0.93 (0.76, 1.14)
cg15570656	1.09 (0.92, 1.29)	0.97 (0.79, 1.19)	0.93 (0.78, 1.10)	1.09 (0.90, 1.32)	1.00 (0.82, 1.22)	0.93 (0.76, 1.13)
cg16885113	1.10 (0.92, 1.30)	0.98 (0.80, 1.21)	0.97 (0.82, 1.15)	1.08 (0.89, 1.32)	1.00 (0.82, 1.21)	0.96 (0.79, 1.17)
cg20228636	1.11 (0.94, 1.32)	1.00 (0.82, 1.23)	0.95 (0.81, 1.13)	1.09 (0.90, 1.32)	1.02 (0.84, 1.25)	0.95 (0.78, 1.15)
cg25699073	1.09 (0.92, 1.30)	1.01 (0.82, 1.24)	0.99 (0.83, 1.17)	1.08 (0.89, 1.31)	1.01 (0.83, 1.23)	0.98 (0.80, 1.19)
cg25978138	1.06 (0.88, 1.26)	1.01 (0.81, 1.25)	0.92 (0.77, 1.09)	1.09 (0.88, 1.33)	0.99 (0.81, 1.22)	0.91 (0.75, 1.12)
cg13835168	1.07 (0.90, 1.27)	1.00 (0.81, 1.23)	0.94 (0.79, 1.12)	1.07 (0.88, 1.30)	1.02 (0.84, 1.24)	0.94 (0.77, 1.15)

Note: Bold OR and confidence interval (CI) values indicate statistical significance.

[a]A *ZFP57* differential methylated region (DMR) identified by our DMRcate analysis and also reported in Hernandez et al.

[b]Two definitions of pocket depth were used: Centers for Disease Control and Prevention–American Academy of Periodontology (CDC-AAP) and ARIC. See Section 2.

[c]Model adjusted for age, sex, diabetes, socio-economic status, pack-years, smoking status (never, former, current), pack-years based methylation score, body mass index, surrogate variables for batch effects and cell proportions.

female participants and participants of higher or lower SES (Table S3). In addition, there was a statistically significant decreased risk of moderate versus no/mild periodontal disease among European American participants 58 years or older (Table 3). The *ZFP57* CpGs were not significantly associated with risk of moderate or severe periodontal disease, as compared with no/mild periodontal disease in African American participants. However, increasing methylation of three *ZFP57* CpGs (cg11383134, cg15570656 and cg13835168) was associated with a significant decreased risk of severe versus no/mild periodontal disease in African American participants 58 years or older (Table 3). When we used the CDC-AAP definition to categorize periodontal disease status, increasing methylation of the *ZFP57* CpGs was found to be associated with a significant decreased risk of mild/moderate versus no periodontal disease in European American ARIC participants. The pattern of associations for those 58 years and older, male and female participants, participants of higher or lower SES, and for edentulism were all similar to what we found using the ARIC definition (Tables 2 and S3).

3.2.2 | *HOXA4*

A *HOXA4* DMR, consisting of 13 CpGs, was found to be hypermethylated in periodontitis patients with age-matched and sex-matched periodontally healthy controls (Hernández et al., 2021). This finding is consistent with Hernandez et al. Although the *HOXA4* DMR was not statistically significant in the DMRcate analyses using both of our periodontitis definitions, when we tested the CpGs contained in the *HOXA4* DMR individually, increasing methylation of cg11015251, cg14359292, cg07317062 and cg08657492 was statistically significantly associated with a higher risk of severe periodontal disease compared with no/mild periodontal disease in African American participants (Table 4). This association was comparable for African

TABLE 3 Odds ratio (OR) for CpGs in the *ZFP57*[a] differentially methylated region and periodontitis severity stratified by age at blood draw at Visit 2, Atherosclerosis Risk in Communities (ARIC) participants.

	OR per 1 SD increase in methylation level (95% CI) for periodontitis (ARIC definition)					
	Age <58[b]			Age ≥58[b]		
CpG	Moderate versus no/mild	Severe versus no/mild	Edentulism versus no/mild	Moderate versus no/mild	Severe versus no/mild	Edentulism versus no/mild
European American (EA) participants (Visit 02)						
cg07134666	1.01 (0.69, 1.46)	1.09 (0.60, 1.97)	0.84 (0.33, 2.10)	**0.59 (0.41, 0.85)**	**0.36 (0.22, 0.60)**	0.79 (0.48, 1.30)
cg11383134	0.87 (0.60, 1.26)	0.93 (0.52, 1.67)	1.03 (0.37, 2.89)	**0.58 (0.39, 0.86)**	**0.36 (0.22, 0.61)**	0.82 (0.46, 1.46)
cg22494932	0.80 (0.56, 1.16)	1.01 (0.56, 1.82)	1.10 (0.37, 3.27)	**0.59 (0.40, 0.87)**	**0.40 (0.24, 0.66)**	0.85 (0.46, 1.60)
cg15570656	1.00 (0.69, 1.45)	1.00 (0.56, 1.79)	0.84 (0.33, 2.12)	**0.69 (0.48, 0.99)**	**0.39 (0.23, 0.65)**	0.88 (0.52, 1.48)
cg16885113	0.95 (0.65, 1.37)	1.04 (0.58, 1.87)	1.01 (0.38, 2.70)	**0.60 (0.41, 0.88)**	**0.36 (0.21, 0.59)**	0.83 (0.48, 1.43)
cg20228636	0.94 (0.64, 1.36)	0.89 (0.49, 1.60)	0.90 (0.35, 2.34)	**0.63 (0.44, 0.92)**	**0.42 (0.25, 0.70)**	0.90 (0.53, 1.53)
cg25699073	0.75 (0.51, 1.09)	0.96 (0.53, 1.74)	1.17 (0.37, 3.70)	**0.64 (0.44, 0.94)**	**0.42 (0.26, 0.70)**	0.80 (0.44, 1.45)
cg25978138	0.86 (0.57, 1.30)	0.84 (0.44, 1.61)	1.10 (0.36, 3.40)	**0.61 (0.42, 0.89)**	**0.46 (0.30, 0.72)**	0.97 (0.54, 1.74)
cg13835168	1.27 (0.87, 1.85)	1.05 (0.58, 1.91)	1.01 (0.40, 2.54)	**0.66 (0.46, 0.94)**	**0.42 (0.25, 0.70)**	0.91 (0.56, 1.49)
AA participants (Visit 02)						
cg07134666	1.22 (0.97, 1.55)	1.18 (0.93, 1.50)	1.02 (0.80, 1.30)	0.81 (0.55, 1.21)	0.68 (0.46, 1.01)	0.68 (0.46, 1.00)
cg11383134	1.12 (0.89, 1.41)	1.10 (0.86, 1.40)	0.96 (0.75, 1.23)	0.83 (0.55, 1.25)	**0.65 (0.44, 0.98)**	0.70 (0.47, 1.04)
cg22494932	1.09 (0.86, 1.38)	1.06 (0.83, 1.35)	0.93 (0.73, 1.19)	1.00 (0.65, 1.53)	0.74 (0.49, 1.11)	0.77 (0.52, 1.15)
cg15570656	1.18 (0.94, 1.50)	1.16 (0.91, 1.48)	0.98 (0.76, 1.26)	0.85 (0.58, 1.26)	**0.67 (0.45, 0.99)**	**0.68 (0.46, 0.99)**
cg16885113	1.17 (0.93, 1.48)	1.13 (0.89, 1.43)	0.98 (0.77, 1.26)	0.87 (0.58, 1.29)	0.70 (0.47, 1.04)	0.73 (0.50, 1.07)
cg20228636	1.13 (0.90, 1.43)	1.15 (0.91, 1.47)	0.95 (0.74, 1.22)	0.93 (0.64, 1.36)	0.73 (0.50, 1.06)	0.75 (0.52, 1.09)
cg25699073	1.12 (0.89, 1.41)	1.13 (0.89, 1.44)	0.98 (0.77, 1.24)	0.94 (0.62, 1.42)	0.74 (0.49, 1.11)	0.79 (0.53, 1.18)
cg25978138	1.14 (0.89, 1.45)	1.05 (0.82, 1.35)	0.93 (0.73, 1.20)	0.98 (0.65, 1.47)	0.82 (0.55, 1.22)	0.74 (0.50, 1.08)
cg13835168	1.16 (0.92, 1.47)	1.19 (0.93, 1.52)	0.98 (0.76, 1.26)	0.85 (0.58, 1.23)	**0.67 (0.46, 0.99)**	0.72 (0.49, 1.03)

Note: Bold OR and confidence interval (CI) values indicate statistical significance.
[a]A *ZFP57* differential methylated region (DMR) identified by our DMRcate analysis and also reported in Hernandez et al.
[b]Model adjusted for age, sex, diabetes, socio-economic status, pack-years, smoking status (never, former, current), pack-years based methylation score, body mass index, surrogate variables for batch effects and cell proportions.

American participants 58 years or older and younger than 58-year-old male and female participants, and participants of higher or lower life-course SES (Table S4). In contrast, these *HOXA4* CpGs were not significantly associated with risk of moderate or severe periodontal disease, as compared with no/mild periodontal disease in European American participants (Table 4). In addition, increasing methylation of cg14359292 and cg24169822 was associated with decreased risk of edentulism versus no periodontal disease among European American participants (Table 4; stronger association in females Table S4). Using the CDC-AAP definition, we also found significant positive associations between increasing methylation of selected *HOXA4* CpGs and periodontitis, which was largely consistent with what we found using the ARIC definition.

3.2.3 | ENSG00000231601

Using the ARIC periodontitis definition, our DMRcate analysis identified one statistically significant DMR located on Chr10 (*ENSG0000023160* gene), consisting of three CpGs (cg22954052, cg25580864 and cg05495790), which differed between African American participants with edentulism and those with no/mild periodontal disease (Table 5). Among African American participants, increasing methylation of cg22954052 and cg05495790 was significantly associated with increased risks of moderate periodontal disease, severe periodontal disease and edentulism, when compared with no/mild periodontal disease. These associations were stronger among African American participants under age 58 (Table S5). CpGs cg22954052 and cg05495790 were associated with an increased risk of edentulism versus no periodontal disease in African American participants. These CpGs were not associated with PD or edentulism in European American participants. When we used the CDC-AAP definition, increasing methylation of cg22954052 and cg05495790 was also significantly associated with increased risks of mild/moderate versus no periodontal disease and edentulism versus no periodontal disease, but not severe PD versus no PD, in African American participants.

TABLE 4 Odds ratio (OR) for CpGs in the *HOXA4*[a] differentially methylated region and periodontitis severity.

	OR per 1 SD increase in methylation level (95% CI)					
	Periodontitis (CDC-AAP definition)[b]			Periodontitis (ARIC definition)[b]		
CpG	Mild/moderate versus no	Severe versus no	Edentulism versus no	Moderate versus no/mild	Severe versus no/mild	Edentulism versus no/mild
EA participants (Visit 02)						
cg06942814	0.92 (0.70, 1.20)	1.03 (0.66, 1.61)	0.89 (0.60, 1.34)	1.03 (0.78, 1.35)	0.99 (0.66, 1.49)	0.91 (0.61, 1.35)
cg11015251	0.83 (0.61, 1.12)	0.70 (0.43, 1.13)	0.88 (0.55, 1.39)	0.97 (0.71, 1.31)	0.83 (0.55, 1.26)	0.94 (0.6, 1.45)
cg14359292	0.85 (0.65, 1.11)	0.91 (0.60, 1.39)	**0.64 (0.42, 0.99)**	1.00 (0.77, 1.30)	1.01 (0.68, 1.50)	0.70 (0.46, 1.05)
cg07317062	0.91 (0.71, 1.15)	1.10 (0.74, 1.64)	0.93 (0.64, 1.36)	1.05 (0.82, 1.33)	1.15 (0.81, 1.65)	0.99 (0.69, 1.44)
cg08657492	0.86 (0.66, 1.11)	0.94 (0.62, 1.42)	0.70 (0.47, 1.05)	1.03 (0.79, 1.33)	0.96 (0.66, 1.42)	0.75 (0.50, 1.12)
cg11410718	0.94 (0.74, 1.20)	1.00 (0.73, 1.66)	0.86 (0.58, 1.28)	1.05 (0.82, 1.35)	1.06 (0.74, 1.51)	0.89 (0.60, 1.30)
cg22997113	0.97 (0.75, 1.25)	1.11 (0.72, 1.71)	0.91 (0.61, 1.37)	1.10 (0.85, 1.43)	1.01 (0.68, 1.49)	0.92 (0.62, 1.37)
cg24169822	0.77 (0.59, 1.00)	0.83 (0.55, 1.25)	**0.59 (0.39, 0.90)**	1.03 (0.79, 1.33)	0.87 (0.59, 1.28)	0.66 (0.44, 1.01)
AA participants (Visit 02)						
cg06942814	1.20 (1.00, 1.44)	1.02 (0.81, 1.27)	0.94 (0.78, 1.13)	1.15 (0.93, 1.42)	1.18 (0.95, 1.46)	0.98 (0.79, 1.22)
cg11015251	1.18 (0.97, 1.43)	0.98 (0.78, 1.24)	0.93 (0.76, 1.13)	**1.29 (1.03, 1.61)**	**1.28 (1.02, 1.61)**	1.07 (0.85, 1.34)
cg14359292	1.18 (0.98, 1.41)	1.08 (0.87, 1.34)	0.98 (0.81, 1.17)	1.17 (0.95, 1.44)	**1.24 (1.01, 1.54)**	1.05 (0.85, 1.30)
cg07317062	1.17 (0.98, 1.40)	1.10 (0.88, 1.37)	0.98 (0.81, 1.17)	1.23 (1.00, 1.51)	**1.30 (1.05, 1.61)**	1.09 (0.88, 1.34)
cg08657492	**1.25 (1.04, 1.49)**	1.07 (0.85, 1.33)	0.99 (0.82, 1.19)	1.22 (0.99, 1.50)	**1.25 (1.01, 1.55)**	1.06 (0.86, 1.31)
cg11410718	1.15 (0.96, 1.38)	1.10 (0.88, 1.38)	0.95 (0.79, 1.15)	1.19 (0.96, 1.46)	1.20 (0.97, 1.49)	1.02 (0.82, 1.26)
cg22997113	1.14 (0.95, 1.36)	1.04 (0.83, 1.29)	0.93 (0.78, 1.11)	1.10 (0.90, 1.35)	1.13 (0.92, 1.39)	0.96 (0.78, 1.18)
cg24169822	1.20 (1.00, 1.44)	1.08 (0.86, 1.34)	0.99 (0.82, 1.19)	1.19 (0.96, 1.47)	1.23 (0.99, 1.52)	1.05 (0.85, 1.31)

Note: Bold OR and confidence interval (CI) values indicate statistical significance.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CDC-AAP, Centers for Disease Control and Prevention–American Academy of Periodontology.

[a]A *HOXA4* differential methylated region reported as periodontitis-related in Hernandez et al.

[b]Model adjusted for age, sex, diabetes, socio-economic status, pack-years, smoking status (never, former, current), pack-years based methylation score, body mass index, surrogate variables for batch effects and cell proportions.

TABLE 5 Odds ratio (OR) for CpGs in the *ENSG00000231601*[a] differentially methylated region and periodontitis severity.

	OR per 1 SD increase in methylation level (95% CI)					
	Periodontitis (CDC-AAP definition)[b]			Periodontitis (ARIC definition)[b]		
CpG	Mild/moderate versus no	Severe versus no	Edentulism versus no	Moderate versus no/mild	Severe versus no/mild	Edentulism versus no/mild
EA participants (Visit 02)						
cg22954052	1.11 (0.86, 1.43)	1.33 (0.87, 2.04)	0.88 (0.60, 1.29)	1.08 (0.84, 1.40)	1.06 (0.74, 1.51)	0.84 (0.58, 1.23)
cg25580864	1.10 (0.84, 1.43)	0.98 (0.64, 1.49)	1.01 (0.66, 1.53)	1.03 (0.78, 1.34)	1.17 (0.77, 1.76)	1.01 (0.67, 1.52)
cg05495790	1.12 (0.86, 1.44)	**1.76 (1.16, 2.67)**	0.85 (0.56, 1.31)	1.22 (0.94, 1.58)	1.21 (0.85, 1.72)	0.84 (0.56, 1.27)
AA Participants (Visit 02)						
cg22954052	**1.23 (1.02, 1.48)**	1.18 (0.94, 1.49)	**1.29 (1.07, 1.56)**	**1.54 (1.24, 1.91)**	**1.44 (1.16, 1.79)**	**1.56 (1.25, 1.94)**
cg25580864	1.17 (0.96, 1.44)	1.11 (0.87, 1.43)	1.22 (0.99, 1.50)	1.16 (0.91, 1.46)	**1.28 (1.01, 1.63)**	**1.30 (1.03, 1.65)**
cg05495790	**1.34 (1.10, 1.63)**	1.22 (0.95, 1.56)	**1.38 (1.13, 1.69)**	**1.43 (1.13, 1.80)**	**1.36 (1.08, 1.73)**	**1.53 (1.21, 1.94)**

Note: Bold OR and confidence interval (CI) values indicate statistical significance.

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CDC-AAP, Centers for Disease Control and Prevention–American Academy of Periodontology.

[a]A de novo *ENSG00000231601* differential methylated region (DMR) identified by our DMRcate analysis.

[b]Model adjusted for age, sex, diabetes, socio-economic status, pack-years, smoking status (never, former, current), pack-years based methylation score, body mass index, surrogate variables for batch effects and cell proportions.

4 | DISCUSSION

To our knowledge, this is the first large-scale epigenome-wide DNA methylation study of clinically assessed periodontitis. Using epigenome-wide analyses in 1534 ARIC participants, we identified differential methylation patterns comparing various clinically assessed periodontal disease classifications, suggesting that epigenetic changes may contribute to variation in susceptibility to periodontitis. Our DMR analysis replicated findings from a previous study reporting an association between DNA methylation levels measured in whole blood at CpG sites in the gene *ZFP57* and periodontitis using two clinically assessed periodontal disease definitions. We also found DNA methylation differences near *ENSG00000231601* (Chr10) between African American participants with edentulism and those with no or mild periodontal disease.

To account for the complexity of measuring periodontitis, our analysis used two periodontitis classifications: the ARIC definition and the CDC-AAP definition. As a chronic disease, periodontitis can present with a wide range of symptom severity and progression. We observed stronger associations using the ARIC definition for each of the identified regions. The ARIC definition for 'severe' is based on having extensive periodontal disease (30% of teeth with attachment loss ≥3 mm), while the CDC-AAP definition for 'severe' requires a greater degree of attachment loss (>6 mm) but fewer teeth. Our results suggest that the genetic regions identified may be more strongly linked to extensive periodontal disease.

Overall, we found comparable results across gender and SES, but greater associations among participants 58 years or older, likely due to a higher prevalence of extensive periodontal disease among older ARIC participants. Our analysis also revealed significant associations between DNA methylation patterns in the *HOXA4* gene and an increased risk of severe periodontal disease in African American participants. These associations were consistent across various African American subgroups, including those stratified by age, sex and SES. As a transcription factor, *HOXA4* plays a crucial role in cell differentiation and embryonic development by regulating the expression of various target genes (Akam, 1998; Krumlauf, 1994). The observed associations are in line with previous studies that have linked DNA methylation in *HOXA4* to other diseases, such as cancer and heart disease (Elias et al., 2018; Lillvis et al., 2011). Although the specific role of HOXA4 in inflammatory diseases such as periodontitis and heart disease has not been fully elucidated, it is plausible that the gene could play a role in these conditions given its involvement in developmental processes and its ability to regulate the expression of various target genes (Aonuma et al., 2022; Lillvis et al., 2011). However, further studies are warranted to replicate our findings and investigate this periodontal connection. The differences we observed between African Americans and European Americans may be attributed to population-specific factors. Most African American participants are from the Jackson Field Center. Participants from this region have experienced very different exposures during their lifetimes and have limited access to health and dental care (compared with other ARIC participants). These factors, which can influence periodontal disease development and progression, may have impacted the findings in a manner similar to the differences we observed with different disease classifications. Consequently, it is not possible to determine whether our results are due to genetic effects or to correlates of socio-economic factors.

Prior to the present study, there were only two published studies of genome-wide DNA methylation of peripheral blood leukocytes in periodontitis. Kurushima et al. (2019) conducted an epigenome-wide study using Illumina DNA methylation450K data and a retrospective dental phenotype collection of self-reported dental mobility and gingival bleeding information obtained from 528 older female twins in the Twins UK cohort (mean age: 58 years, age range: 19–82 years; compared with African American ARIC participants mean age: 55 years, age range: 47–70 years; European American ARIC participants mean age: 58 years, age range: 47–68 years; Kurushima et al., 2019). This study detected a hypomethylated region in *ZNF804A* in individuals who experienced gingival bleeding, in conjunction with an increased level of ornithine, a metabolite related to gingival inflammation, in their blood metabolomics profile. There were no concordances between our results and the results of Kurushima et al., possibly due to clear differences in study design, including the use of clinically examined versus self-reported periodontitis data. Hernández et al. (2021) performed an analysis to identify differentially methylated positions and regions with a small number of clinically diagnosed periodontitis patients and gingivally healthy controls (Hernández et al., 2021). Results of Hernandez et al. identified the genes *ZNF718*, *HOXA4* and *ZFP57* related to systemic immune-related epigenetic patterns in periodontitis (Hernández et al., 2021), including a *ZFP57* region of 21 CpGs that was found to be hypomethylated when comparing periodontitis patients with age-matched and sex-matched periodontally healthy controls. Although Hernandez et al. also carried out clinical examination, their study participants were much younger than ours (age range: 25–55 years), suggesting a more extreme comparison of phenotype in Hernandez et al. Consistent with Hernandez et al., our study identified a *ZFP57* DMR to be periodontitis-related. In addition, the 17-CpGs *ZFP57* DMR we identified perfectly overlapped with the 21-CpG *ZFP57* DMR identified by Hernandez and colleagues.

Existing evidence supports the relevance of some of the identified *ZFP57* CpGs for periodontitis. *ZFP57* is an imprint regulator, and DNA methylation in this exact *ZFP57* region has been shown to be environmentally modulated in human blood and related to transient neonatal diabetes, Parkinson's disease, and post-traumatic stress disorder (Bak et al., 2016; Henderson et al., 2021; Vinkers et al., 2021). The zinc finger proteins (ZNFs) are also important transcription factors. They are involved in tissue development, and their alterations may promote chronic conditions, including oral cancer and periodontitis (Agnihotri & Gaur, 2022). Recent studies have shown ZNFs playing a role in the progression of periodontitis (Hong et al., 2016). For instance, *ZNFA20* can inhibit NF-κβ pathway activation via inflammatory cytokines (Hong et al., 2016; Wertz et al., 2004) and thus support the disruption of local inflammatory responses by *Porphyromonas gingivalis*, the key pathogen of periodontitis (Matsui et al., 2018;

Ohshima et al., 2019). The replication of the *ZNF57* DMR in our study suggests a potential sequence of events in periodontitis development, where hypomethylation of the *ZNF57* DMR may upregulate the expression of *ZNF57*, which is related to antigen presentation and immune response regulation.

Observed DNA methylation levels could precede periodontal disease development or be the consequence of periodontal disease. We cannot establish whether the associations observed between differential methylation in whole blood and periodontitis are the cause or the consequence of the disease. Although the ARIC study is prospective and the methylation data were obtained up to 6 years before the dental examination, a dental examination was not performed concurrently with the blood draw, such that we likely studied the combination of prevalent and incident periodontal disease. Moreover, we could not detect single CpG associations at the stringent epigenome-wide significance level, and the use of the 450K array, which covers only ~2% of human CpG sites, may limit the discovery. In subsequent CpG testing, the limited number of severe periodontitis cases in certain subgroups could have reduced the statistical power to detect significant associations, even though we included all available subjects with DNA methylation data. Additionally, the new findings were not replicated in independent samples. Finally, the use of edentulism as a surrogate for the most severe type of periodontitis may not be entirely accurate. Edentulism can result from a variety of causes, including periodontal disease, dental caries and poor access to dental care. Therefore, unless patients have specifically reported losing their teeth due to periodontal disease, the assumption that edentulism is a direct result of severe periodontitis may not be entirely valid. Consequently, our analysis cannot distinguish between DNA methylation patterns of those who are edentulous due to periodontitis and other causes.

Despite the limitations, several strengths of this study warrant attention, including the use of clinically examined periodontal phenotypes, large sample size, ethnic diversity and rigorous analytical methods. We also adjusted for SES, diabetes status, imputed cell composition, self-reporting smoking history and a methylation-predicted pack-years smoked to further reduce concern about confounding. Additionally, we used two definitions of periodontal disease to address the complexity of disease classification. Analyses in individuals of different ancestries support the need for future large-scale EWAS of periodontitis to investigate ethnicity-specific and age-specific periodontitis-related CpGs.

Along with previously published EWAS, our results suggest that gene regulation mechanisms may influence the susceptibility to and severity of periodontitis. Our well-powered EWAS and DMRcate analysis in participants drawn from the general population identified several genomic regions for which differential DNA methylation sites were associated with periodontitis. This study supports leukocyte DNA methylation for the evaluation of epigenetic patterns in periodontitis. Our study, in conjunction with a prior pilot study, implicates *ZFP57* as a promising candidate for future studies to illuminate the underlying gene regulatory mechanisms linking differential DNA methylation to periodontal disease.

AUTHOR CONTRIBUTIONS

Dominique S. Michaud and Elizabeth A. Platz designed the study. Jiayun Lu assisted with preparation of the dataset. Dominique S. Michaud supervised all research activities. Naisi Zhao conducted the statistical analyses. Naisi Zhao drafted the manuscript. Dominique S. Michaud, Flavia Teles, Elizabeth A. Platz, Karl T. Kelsey and Devin C. Koestler interpreted the data and provided critical to the manuscript. Eric Boerwinkle and Jan Bressler contributed to data collection and provided critical manuscript revisions. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Dr. Kelsey is a founder and scientific advisor for Cellintec, which, however, had no role in this research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Atherosclerosis Risk in Communities (ARIC) Study. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from https://sites.cscc.unc.edu/aric/distribution-agreements with the permission of the Atherosclerosis Risk in Communities (ARIC) Study.

ETHICS STATEMENT

This study reports analyses conducted solely using de-identified data from the ARIC study. The ARIC study protocol was approved by the Institutional Review Boards at each study site. The ARIC participants had given written informed consent.

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REFERENCES

Agnihotri, R., & Gaur, S. (2022). The role of zinc finger proteins in various oral conditions. *The Scientific World Journal*, *2022*, 1–11.

Akam, M. (1998). Hox genes: From master genes to micromanagers. *Current Biology*, *8*(19), R676–R678.

Aonuma, T., Moukette, B., Kawaguchi, S., Barupala, N. P., Sepúlveda, M. N., Frick, K., Tang, Y., Guglin, M., Raman, S. V., Cai, C., Liangpunsakul, S., Nakagawa, S., & Kim, I.-M. (2022). MiR-150 attenuates maladaptive cardiac remodeling mediated by long noncoding RNA MIAT and directly represses profibrotic Hoxa4. *Circulation: Heart Failure*, *15*(4), e008686.

Bak, M., Boonen, S. E., Dahl, C., Hahnemann, J. M. D., Mackay, D. J. D. G., Tümer, Z., Grønskov, K., Temple, I. K., Guldberg, P., & Tommerup, N. (2016). Genome-wide DNA methylation analysis of transient neonatal diabetes type 1 patients with mutations in ZFP57. *BMC Medical Genetics*, *17*(1), 1–8.

Beck, J. D., Eke, P., Heiss, G., Madianos, P., Couper, D., Lin, D., Moss, K., Elter, J., & Offenbacher, S. (2005). Periodontal disease and coronary heart disease: A reappraisal of the exposure. *Circulation*, *112*(1), 19–24.

Beck, J. D., Elter, J. R., Heiss, G., Couper, D., Mauriello, S. M., & Offenbacher, S. (2001). Relationship of periodontal disease to carotid artery intima-media wall thickness: The Atherosclerosis Risk in Communities (ARIC) study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *21*(11), 1816–1822.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300.

Bose, M., Wu, C., Pankow, J. S., Demerath, E. W., Bressler, J., Fornage, M., Grove, M. L., Mosley, T. H., Hicks, C., North, K., Kao, W. H., Zhang, Y., Boerwinkle, E., & Guan, W. (2014). Evaluation of microarray-based DNA methylation measurement using technical replicates: The Atherosclerosis Risk In Communities (ARIC) study. *BMC Bioinformatics*, *15*(1), 1–10.

de Faria Amormino, S. A., Arão, T. C., Saraiva, A. M., Gomez, R. S., Dutra, W. O., da Costa, J. E., de Fátima Correia Silva, J., & Moreira, P. R. (2013). Hypermethylation and low transcription of TLR2 gene in chronic periodontitis. *Human Immunology*, *74*(9), 1231–1236.

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., Liu, Y., Newman, A. B., Beck, J. D., & Offenbacher, S. (2013). Exploring the genetic basis of chronic periodontitis: A genome-wide association study. *Human Molecular Genetics*, *22*(11), 2312–2324.

Do, W. L., Sun, D., Meeks, K., Dugué, P.-A., Demerath, E., Guan, W., Li, S., Chen, W., Milne, R., Adeyemo, A., Agyemang, C., Nassir, R., Manson, J. A. E., Shadyab, A. H., Hou, L., Horvath, S., Assimes, T. L., Bhatti, P., Jordahl, K. M., … Conneely, K. N. (2023). Epigenome-wide meta-analysis of BMI in nine cohorts: Examining the utility of epigenetically predicted BMI. *American Journal of Human Genetics*, *110*(2), 273–283.

Eke, P., Dye, B., Wei, L., Thornton-Evans, G., Genco, R., & CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina, Chapel Hill, USA). Gordon Douglass (Past President, American Academy of Periodontology), Roy Page (University of Washin). (2012). Prevalence of periodontitis in adults in the United States: 2009 and 2010. *Journal of Dental Research*, *91*(10), 914–920.

Eke, P. I., Borgnakke, W. S., & Genco, R. J. (2020). Recent epidemiologic trends in periodontitis in the USA. *Periodontology 2000*, *82*(1), 257–267.

Eke, P. I., Dye, B. A., Wei, L., Slade, G. D., Thornton-Evans, G. O., Borgnakke, W. S., Taylor, G. W., Page, R. C., Beck, J. D., & Genco, R. J. (2015). Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. *Journal of Periodontology*, *86*(5), 611–622.

Eke, P. I., Page, R. C., Wei, L., Thornton-Evans, G., & Genco, R. J. (2012). Update of the case definitions for population-based surveillance of periodontitis. *Journal of Periodontology*, *83*(12), 1449–1454.

Elias, M. H., Azlan, H., Sulong, S., Baba, A. A., & Ankathil, R. (2018). Aberrant DNA methylation at HOXA4 and HOXA5 genes are associated with resistance to imatinib mesylate among chronic myeloid leukemia patients. *Cancer Reports*, *1*(2), e1111.

Genco, R. J., & Borgnakke, W. S. (2020). Diabetes as a potential risk for periodontitis: Association studies. *Periodontology 2000*, *83*(1), 40–45.

Hajishengallis, G. (2015). Periodontitis: From microbial immune subversion to systemic inflammation. *Nature Reviews Immunology*, *15*(1), 30–44.

Henderson, A. R., Wang, Q., Meechoovet, B., Siniard, A. L., Naymik, M., de Both, M., Huentelman, M. J., Caselli, R. J., Driver-Dunckley, E., & Dunckley, T. (2021). DNA methylation and expression profiles of whole blood in parkinson's disease. *Frontiers in Genetics*, *12*, 640266.

Hernández, H. G., Hernández-Castañeda, A. A., Pieschacón, M. P., & Arboleda, H. (2021). ZNF718, HOXA4, and ZFP57 are differentially methylated in periodontitis in comparison with periodontal health: Epigenome-wide DNA methylation pilot study. *Journal of Periodontal Research*, *56*(4), 710–725.

Hong, J. Y., Bae, W. J., Yi, J. K., Kim, G. T., & Kim, E. C. (2016). Anti-inflammatory and anti-osteoclastogenic effects of zinc finger protein A 20 overexpression in human periodontal ligament cells. *Journal of Periodontal Research*, *51*(4), 529–539.

Jackson, R., Chambless, L. E., Yang, K., Byrne, T., Watson, R., Folsom, A., Shahar, E., & Kalsbeek, W. (1996). Differences between respondents and nonrespondents in a multicenter community-based study vary by gender and ethnicity. *Journal of Clinical Epidemiology*, *49*(12), 1441–1446.

Joehanes, R., Just, A. C., Marioni, R. E., Pilling, L. C., Reynolds, L. M., Mandaviya, P. R., Guan, W., Xu, T., Elks, C. E., Aslibekyan, S., Moreno-Macias, H., Smith, J. A., Brody, J. A., Dhingra, R., Yousefi, P., Pankow, J. S., Kunze, S., Shah, S. H., McRae, A. F., … London, S. J. (2016). Epigenetic signatures of cigarette smoking. *Circulation. Cardiovascular Genetics*, *9*(5), 436–447.

Kim, H., Momen-Heravi, F., Chen, S., Hoffmann, P., Kebschull, M., & Papapanou, P. N. (2021). Differential DNA methylation and mRNA transcription in gingival tissues in periodontal health and disease. *Journal of Clinical Periodontology*, *48*(9), 1152–1164.

Krumlauf, R. (1994). Hox genes in vertebrate development. *Cell*, *78*(2), 191–201.

Kurushima, Y., Tsai, P.-C., Castillo-Fernandez, J., Couto Alves, A., el-Sayed Moustafa, J. S., le Roy, C., Spector, T. D., Ide, M., Hughes, F. J., Small, K. S., Steves, C. J., & Bell, J. T. (2019). Epigenetic findings in periodontitis in UK twins: A cross-sectional study. *Clinical Epigenetics*, *11*(1), 27.

Laine, M. L., Jepsen, S., & Loos, B. G. (2014). Progress in the identification of genetic factors in periodontitis. *Current Oral Health Reports*, *1*(4), 272–278.

Lazăr, L., Dakó, T., Cozma, A., & Lazăr, A.-P. (2022). The influence of smoking on the periodontal biome. A review. *Acta Stomatologica Marisiensis Journal*, *5*(1), 6–11.

Leek, J. T., Scharpf, R. B., Bravo, H. C., Simcha, D., Langmead, B., Johnson, W. E., Geman, D., Baggerly, K., & Irizarry, R. A. (2010). Tackling the widespread and critical impact of batch effects in high-throughput data. *Nature Reviews Genetics*, *11*(10), 733–739.

Lillvis, J. H., Erdman, R., Schworer, C. M., Golden, A., Derr, K., Gatalica, Z., Cox, L. A., Shen, J., Vander Heide, R. S., Lenk, G. M., Hlavaty, L., Li, L., Elmore, J. R., Franklin, D. P., Gray, J. L., Garvin, R. P., Carey, D. J., Lancaster, W. D., Tromp, G., & Kuivaniemi, H. (2011). Regional expression of HOXA4 along the aorta and its potential role in human abdominal aortic aneurysms. *BMC Physiology*, *11*(1), 1–11.

Lu, J., Zaimi, I., Barber, J. R., Joshu, C. E., Prizment, A. E., Beck, J. D., Platz, E. A., & Michaud, D. S. (2019). SES and correlated factors do not

explain the association between periodontal disease, edentulism, and cancer risk. *Annals of Epidemiology*, *38*, 35–41.

Madiba, T. K., & Bhayat, A. (2018). Periodontal disease-risk factors and treatment options. *South African Dental Journal*, *73*(9), 571–575.

Martinon, P., Fraticelli, L., Giboreau, A., Dussart, C., Bourgeois, D., & Carrouel, F. (2021). Nutrition as a key modifiable factor for periodontitis and main chronic diseases. *Journal of Clinical Medicine*, *10*(2), 197.

Matsui, S., Zhou, L., Nakayama, Y., Mezawa, M., Kato, A., Suzuki, N., Tanabe, N., Nakayama, T., Suzuki, Y., Kamio, N., Takai, H., & Ogata, Y. (2018). MiR-200b attenuates IL-6 production through IKKβ and ZEB1 in human gingival fibroblasts. *Inflammation Research*, *67*(11), 965–973.

Michaud, D. S., Lu, J., Peacock-Villada, A. Y., Barber, J. R., Joshu, C. E., Prizment, A. E., Beck, J. D., Offenbacher, S., & Platz, E. A. (2018). Periodontal disease assessed using clinical dental measurements and cancer risk in the ARIC study. *Journal of the National Cancer Institute*, *110*(8), 843–854.

Munz, M., Willenborg, C., Richter, G. M., Jockel-Schneider, Y., Graetz, C., Staufenbiel, I., Wellmann, J., Berger, K., Krone, B., Hoffmann, P., van der Velde, N., Uitterlinden, A. G., de Groot, L. C., Sawalha, A. H., Direskeneli, H., Saruhan-Direskeneli, G., Guzeldemir-Akcakanat, E., Keceli, H. G., Laudes, M., … Schaefer, A. S. (2017). A genome-wide association study identifies nucleotide variants at SIGLEC5 and DEFA1A3 as risk loci for periodontitis. *Human Molecular Genetics*, *26*(13), 2577–2588.

Naorungroj, S., Slade, G., Divaris, K., Heiss, G., Offenbacher, S., & Beck, J. (2017). Racial differences in periodontal disease and 10-year self-reported tooth loss among late middle-aged and older adults: The dental Aric study. *Journal of Public Health Dentistry*, *77*(4), 372–382.

Navas-Acien, A., Domingo-Relloso, A., Subedi, P., Riffo-Campos, A. L., Xia, R., Gomez, L., Haack, K., Goldsmith, J., Howard, B. V., Best, L. G., Devereux, R., Tauqeer, A., Zhang, Y., Fretts, A. M., Pichler, G., Levy, D., Vasan, R. S., Baccarelli, A. A., Herreros-Martinez, M., … Cole, S. A. (2021). Blood DNA methylation and incident coronary heart disease: Evidence from the strong heart study. *JAMA Cardiology*, *6*(11), 1237–1246.

Ohshima, J., Wang, Q., Fitzsimonds, Z. R., Miller, D. P., Sztukowska, M. N., Jung, Y. J., Hayashi, M., Whiteley, M., & Lamont, R. J. (2019). Streptococcus gordonii programs epithelial cells to resist ZEB2 induction by Porphyromonas gingivalis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(17), 8544–8553.

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., Flemmig, T. F., Garcia, R., Giannobile, W. V., Graziani, F., Greenwell, H., Herrera, D., Kao, R. T., Kebschull, M., Kinane, D. F., Kirkwood, K. L., Kocher, T., Kornman, K. S., Kumar, P. S., … Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Periodontology*, *89*, S173–S182.

Peters, T. J., Buckley, M. J., Statham, A. L., Pidsley, R., Samaras, K., V Lord, R., Clark, S. J., & Molloy, P. L. (2015). De novo identification of differentially methylated regions in the human genome. *Epigenetics & Chromatin*, *8*(1), 1–16.

Petersen, P. E., & Ogawa, H. (2012). The global burden of periodontal disease: Towards integration with chronic disease prevention and control. *Periodontology 2000*, *60*(1), 15–39.

Plerou, V., Gopikrishnan, P., Rosenow, B., Amaral, L. A. N., Guhr, T., & Stanley, H. E. (2002). Random matrix approach to cross correlations in financial data. *Physical Review E*, *65*(6), 066126.

Salas, L. A., Zhang, Z., Koestler, D. C., Butler, R. A., Hansen, H. M., Molinaro, A. M., Wiencke, J. K., Kelsey, K. T., & Christensen, B. C. (2022). Enhanced cell deconvolution of peripheral blood using DNA methylation for high-resolution immune profiling. *Nature Communications*, *13*(1), 761.

Schaefer, A. S., Richter, G. M., Nothnagel, M., Manke, T., Dommisch, H., Jacobs, G., Arlt, A., Rosenstiel, P., Noack, B., Groessner-Schreiber, B., Jepsen, S., Loos, B. G., & Schreiber, S. (2010). A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Human Molecular Genetics*, *19*(3), 553–562.

Schulz, S., Immel, U. D., Just, L., Schaller, H.-G., Gläser, C., & Reichert, S. (2016). Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis. *Human Immunology*, *77*(1), 71–75.

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

Slots, J. (2017). Periodontitis: Facts, fallacies and the future. *Periodontology 2000*, *75*(1), 7–23.

Spector, A. M., Postolache, T. T., Akram, F., Scott, A. J., Wadhawan, A., & Reynolds, M. A. (2020). Psychological stress: A predisposing and exacerbating factor in periodontitis. *Current Oral Health Reports*, *7*(3), 208–215.

Vinkers, C. H., Geuze, E., van Rooij, S. J., Kennis, M., Schür, R. R., Nispeling, D. M., Smith, A. K., Nievergelt, C. M., Uddin, M., Rutten, B. P. F., Vermetten, E., & Boks, M. P. (2021). Successful treatment of post-traumatic stress disorder reverses DNA methylation marks. *Molecular Psychiatry*, *26*(4), 1264–1271.

Wertz, I. E., O'rourke, K. M., Zhou, H., Eby, M., Aravind, L., Seshagiri, S., Wu, P., Wiesmann, C., Baker, R., Boone, D. L., Ma, A., Koonin, E. V., & Dixit, V. M. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. *Nature*, *430*(7000), 694–699.

Wright, J. D., Folsom, A. R., Coresh, J., Sharrett, A. R., Couper, D., Wagenknecht, L. E., Mosley, T. H., Jr., Ballantyne, C. M., Boerwinkle, E. A., Rosamond, W. D., & Heiss, G. (2021). The ARIC (Atherosclerosis Risk in Communities) study: JACC focus seminar 3/8. *Journal of the American College of Cardiology*, *77*(23), 2939–2959.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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