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Oral microbiome diversity associates with carotid intima media thickness in middle-aged male subjects

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Abstract

Background Although there have been significant advancements in reducing the burden of cardiovascular disease (CVD) by modifying traditional CVD risk factors, substantial risks persist, particularly among male subjects who exhibit heightened susceptibility to atherosclerosis. In this context, we aim to study the link between oral microbiome and carotid intima media thickness (cIMT).

Methods The Northern Finland Birth Cohort of 1966 (mean age 46 years, $n = 869$) underwent an extensive health examination, including the measurement of cIMT. The oral microbiome was also investigated using high-throughput 16S rRNA gene sequencing.

Results Here we show that oral microbiome diversity links with atherosclerosis risk factors, namely smoking, glycemic balance, low-grade inflammation, and periodontitis. After excluding CVD-influencing factors ($n = 339$), oral microbiome genera ($p = 0.030$), Shannon index ($p = 0.001$), β-diversity Bray–Curtis ($p < 0.001$), and Jaccard ($p < 0.001$) are associated with cIMT in males, but not in the female sub-cohort. Furthermore, in the male sub-cohort ($n = 131$), the genera *Prevotella*, *Megasphaera*, and *Veillonella* associate positively with cIMT, while *Absconditabacteria*, *Capnocytophaga*, *Gemella*, *Fusobacterium*, *Neisseria*, *Aggregatibacter*, *Tannerella*, *Treponema*, *Cardiobacterium*, and *Bacteroidales* associate inversely with cIMT. We examine the involvement of serum total immunoglobulins and antibodies to phosphorylcholine (PCho) and malondialdehyde-acetaldehyde LDL (MAA-LDL) with cIMT. Subjects with high cIMT have lower levels of serum total IgA ($p = 0.009$), IgA to PCho ($p = 0.017$), and IgG to PCho ($p = 0.008$). The relative abundance of cIMT-associated genera correlates with serum IgA antibodies.

Conclusions This middle-aged birth cohort study shows that male oral microbiome diversity links to cIMT, suggesting a potential sex-specific interaction between the oral microbiome and atherosclerosis.

Plain language summary

Heart disease remains a leading cause of death worldwide and particularly affects men. In this study, we explored whether bacteria in the mouth are associated with artery thickness, a key early indicator of heart disease. We analyzed data from 869 participants, all approximately 46 years old, from the Northern Finland Birth Cohort. Among men, we found that certain groups of bacteria in the mouth were linked to increased artery thickness, a connection not observed in women. These findings suggest that the composition of bacteria in the mouth may influence heart disease risk, especially in men. Understanding this connection could lead to new heart disease prevention strategies.

Atherosclerosis is a chronic inflammatory disease and the leading cause of fatal cardiovascular events such as myocardial infarction and stroke[1]. Despite a reduction of cardiovascular disease (CVD) burden by targeting classic CVD risk factors (such as controlling LDL levels and reducing smoking), significant risk remains[2]. Accumulating evidence indicates an association between gut microbiome diversity and atherosclerosis[2]. The oral microbiome is the second most diverse microbial community, which in a dysbiotic state can cause oral diseases such as periodontitis[3,4]. A large body of evidence has supported the association of periodontitis and periodontal pathogens with atherosclerosis[4,5]; however, the specific link between the oral microbiome and atherosclerosis remains unknown[6–8].

Carotid intima media thickness (cIMT) is an established clinical marker for assessing the progression of atherosclerosis and interventions that reduce cIMT progression are associated with a decreased cardiovascular

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disease (CVD)[9]. The oral microbiome composition can be influenced by atherosclerosis-modifying factors such as aging, smoking, diabetes and periodontitis[3,10–13]. In previous case-control studies, subjects who experienced CVD events were characterized by oral microbiome compositional changes, yet no ecosystem changes such as α- or β-diversity were identified[6–8,14]. It is unknown whether a variation in the microbial community residing in the oral cavity directly associates with atherosclerosis or indirectly through disease risk factors. The northern Finland birth cohort 1966 (NFBC1966), featuring longitudinal health information resulting from detailed oral and systemic health examinations among middle-aged subjects, provided an opportunity to address this question[15].

Phosphocholine (PCho) is a small molecule present in living organisms, including *Haemophilus influenzae*, *Streptococcus* spp, *Lactobacillus* spp and the capsular polysaccharide of *Streptococcus pneumoniae*[16–19]. In atherosclerosis, low-density lipoprotein (LDL) retention along the arterial wall leads to LDL modification and the formation of PCho epitopes, which contribute to the development of atherosclerotic plaques[20,21]. Through molecular mimicry, antibodies to bacterial PCho can bind LDL-modified epitopes such as PCho[21]. Moreover, IgA antibodies play an essential role in mucosal immunity, and it has been suggested that IgA may play a role in the crosstalk between the human microbiome and atherosclerosis[21]. Notably, the exact factors eliciting the systemic antibodies to epitopes such as PCho are currently unknown.

In this study, we investigate the link between the oral microbiome and atherosclerosis, along with associated risk factors, in age-independent settings. We find that oral microbiome diversity is influenced by atherosclerosis risk factors. Furthermore, we examine the potential links between the oral microbiome and atherosclerosis, independently of mutual influencing factors. In the male sub-cohort, we show that oral microbiome diversity is associated with cIMT, whereas no such association is observed in the female sub-cohort. This work highlights a potential sex-specific interaction between the oral microbiome and atherosclerosis.

Materials and methods

Northern Finland birth cohort 1966

The Northern Finland Birth Cohort 1966 (NFBC1966) is a longitudinal and epidemiological research program comprised of infants with an expected date of birth in 1966[22,23]. In total, 12,058 (Fig. 1a) live-born infants were included in NFBC1966[22,23]. Health data were collected from the sixteenth gestational week, and the whole cohort underwent a clinical follow-up when subjects were 1, 14, 31 and 46 years old[15]. At age 46, all living cohort members with a known address were invited to an extensive health examination, which involved both a questionnaire and a clinical examination[15]. Study participation was voluntary, and written consent was obtained from participants. The study has been approved by the institutional review board of North Ostrobothnia District University Hospital (EETTMK decision number 94/2011) and followed the guidelines of the declaration of Helsinki.

The questionnaire aimed to survey subjects' lifestyle and health status through questions on any diagnosed diseases, medications, organ-specific symptoms and a family history of diseases[15]. Clinical examination covering aspects such as anthropometric measurements, blood pressure, cognitive tests, electrocardiogram (EKG) and blood tests were offered to all cohort members ($n = 5832$)[15]. A comprehensive clinical examination was conducted among a population living in the Oulu area, including an oral examination and saliva sample collection ($n = 1964$). Additionally, a random subset of this population ($n = 1156$) was invited for cIMT measurement. We analyzed the oral microbiota of subjects ($n = 972$) who underwent a carotid ultrasound and detailed oral examination. From this group, 869 subjects with available microbiome data were included in this study. The oral microbiome was examined from unstimulated saliva samples. Unstimulated saliva was collected over a period of 15 min. Samples were centrifuged at 1200 rpm for 20 min at 4 °C. The pellet and supernatant were separated, and the samples were stored at −80 °C[24]. Blood samples were collected from the antecubital vein of participants following a 12-h overnight fast. The samples were then centrifuged at 2200×*g* for 11 min and stored at −20 °C[24].

Study design and subject grouping

The cutoff values for low or high cIMT were derived from a study of 115 populations ($n = 65,774$), with a mean cIMT 0.617 mm at age 46 years old[25]. The periodontal pocket depths (PPDs) were determined from four sites of each tooth and the probing force (25 g) was calibrated by scale before examining each participant[26]. The blood value of HbA1c was used as a diabetes risk marker, and subjects with HbA1c > 42 mmol/L were classified as high HbA1c[27]. Blood pressure was classified as normal (systolic blood pressure (SBP) ≤ 129 and diastolic blood pressure (DBP) ≤ 84), moderate (SBP ≤ 159 and DBP ≤ 99), high (SBP ≥ 160 and DBP ≥ 100), and isolated (SVP ≥ 140 and DVP < 90) blood pressure. Smoking status was classified as follows: (1) never, (2) ex-smoker (quit smoking more than 6 months ago), (3) occasional smoker (smokes occasionally, 1 day a week, or quit less than 6 months ago), and (4) current smoker (smokes 2–7 days a week). The Nordic LDL cholesterol reference interval at age 30–49 is 1.4–4.7 mmol/L, such that LDL cholesterol levels >4.7 mmol were classified as high[28]. Subjects with a serum high-sensitivity C-reactive protein (hsCRP) >3 mg/L were classified as high hsCRP group.

Characterization of male and female sub-cohorts

Studying the link between atherosclerosis and the oral microbiota remains challenging, given many confounding factors (such as smoking, host age, diabetes and periodontitis) known to influence both the composition of the oral microbiota and atherosclerosis. Here, we aimed to assemble male and female sub-cohorts consisting of subjects without mutual (CVD and oral microbiome) influence factors (Fig. 1c). In male and female sub-cohorts, we excluded the following subjects: current or occasional smokers ($n = 171$), high HbA1c ($n = 34$), high CRP levels ($n = 89$) and subjects with periodontal pocket depths ≥4 mm ($n = 401$). After applying such exclusion criteria, we identified male ($n = 131$), and female ($n = 208$) sub-cohorts (Fig. 1c). It is worth mentioning that a subject may have multiple exclusion factors. A power analysis was conducted to determine the adequacy of the sample size for detecting an association between intima media thickness (IMT) and oral microbiome diversity in the male sub-cohort. The Cohen's effect size was calculated based on the mean cIMT values for sex. Using an α significance level of 0.05 and a sample size of $n = 131$ males, the power of the study was calculated using the "pwr" package in R, resulting in a power of 0.933. This power indicates that our study is highly likely to detect an existing association between cIMT and oral microbiome diversity in males.

Antigen preparation

Phosphorylcholine Keyhole Limpet Hemocyanin (PCho-KLH; PC-1013-5, Bioresearch Technologies) were commercially purchased. Malondialdehyde-acetaldehyde-modified LDL (MAA-LDL) was prepared according to previously published protocol[29–31]. Malondialdehyde (MDA) was generated by incubating a 0.5 M solution of 1,1,3,3-tetramethoxypropane (108383, Sigma-Aldrich) in 0.3% hydrochloric acid at 37 °C for 10 min. The pH was then adjusted to 6.0–7.0, and the final volume was brought to 4 mL with sterile water. For LDL modification, 900 µL of the 0.5 M MDA solution was combined with 6 mg of LDL, and the total volume was adjusted to 3 mL. The mixture was incubated at 37 °C for 3 h, followed by extensive dialysis against phosphate-buffered saline containing 0.27 mM ethylenediaminetetraacetic acid (PBS-EDTA). To prepare malondialdehyde acetaldehyde (MAA)-modified LDL, 310 µL of PBS, 140 µL of 20% acetaldehyde, 5 mg of LDL, and 300 µL of 0.5 M MDA were mixed. The pH was re-adjusted to 4.8, and the mixture was incubated at 37 °C for 2 h.

Chemiluminescence immunoassay

Microfluor 2 white plate U bottom (7105, Thermo Fisher Scientific) were coated respectively with 5 µg/mL of anti-human IgA-ALP (A9669; Sigma-Aldrich), anti-human IgM-ALP (A9794; Sigma-Aldrich), anti-human

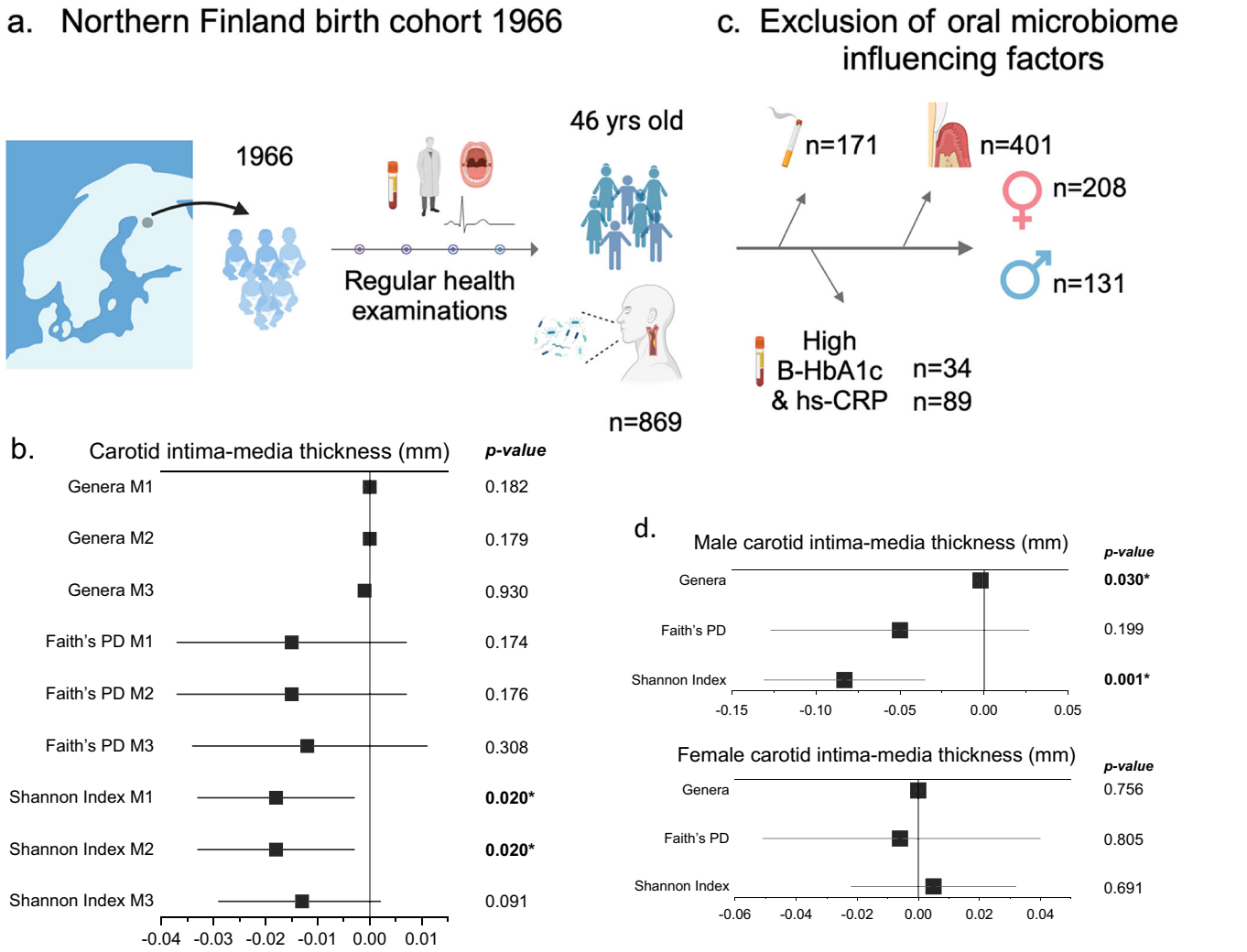

Fig. 1 | Overview of the study design and association of oral microbiome diversity and cIMT. a The Northern Finland Birth Cohort 1966 (NFBC1966) is a longitudinal cohort consisting of 12,058 infants born in the year 1966. Clinical examinations were conducted at ages 1, 14, 31 and 46 years. At the age of 46, a sub-cohort of NFBC1966 participants were invited to a detailed clinical examination. Subjects ($n = 869$) who underwent cIMT examination and available microbial samples were included in this study. **b** The association of oral microbiome diversity ($n = 869$) genera, Faith's phylogenic diversity with carotid intima media thickness were examined in linear regression model adjusted with CVD risk factors (M1: Gender, Blood pressure, BMI), (M2: M1, high LDL, high B-HBA1c, high hsCRP) and (M3: M2, Smoking, deepen periodontal pocket). **c** The exclusion factors mutual CVD and oral microbiome influencing factors smoking, high long-term glycosylated hemoglobin (B-HbA1c), high hsCRP, periodontal disease clinical markers (periodontal pocket depth ≥ 4 mm) and gender. **d** The association of oral microbiome diversity with carotid intima media thickness in male ($n = 131$) and female ($n = 208$) by linear regression model adjusted with residue CVD risk factors: BMI, LDL and blood pressure were examined. Panels (**a**) and (**c**) were created with BioRender.com. *p-value < 0.05 are bold.

IgG-ALP (A3187; Sigma-Aldrich) MAA-LDL or PC-KHL antigen in PBS-EDTA and incubated overnight at 4 °C. The plates were washed three times with PBS-EDTA and blocked with 0.5% fish gelatin from cold water fish skin (G7041, Sigma-Aldrich) in PBS-EDTA for 1 h at room temperature (RT) and washed three times with PBS-EDTA. Serum and saliva samples were incubated for 1 h in RT and washed three times with PBS-EDTA. Antibody levels were measured using alkaline phosphatase-conjugated antibodies: anti-human IgA-ALP (1:35,000, A9669; Sigma-Aldrich), anti-human IgM-ALP (1:50,000, A9794; Sigma-Aldrich), and anti-human IgG-ALP (1:30,000, A3187; Sigma-Aldrich). Chemiluminescence detection was performed using the 30% Lumi-Phos 530 substrate (P-501, Lumigen) with a Victor 3 Multilabel Counter (PerkinElmer/Wallac). Each sample was measured in duplicate, and results were recorded in relative light units (RLU) per 100 ms. The statistical differences between groups were analyzed using the independent-samples Mann–Whitney U test. The association of cIMT groups and immunoglobulins levels were examined with logistic regression model. All statistical analyses were performed with IBM's SPSS statistics 28.

Oral microbiome analysis

Total DNA was extracted from unstimulated saliva samples using a PowerSoil DNA Isolation kit (QIAGEN, Hilden, Germany) according to the National Institutes of Health, Human Microbiome Project guidelines[32]. All library preparation work and sequencing were implemented by the Institute for Molecular Medicine Finland (FIMM, University of Helsinki)[24]. Briefly, the V4 variable region of the 16S ribosomal RNA (rRNA) gene was amplified using the original 515F/806R primer pair from the Earth Microbiome Project[33]. Bar-coded amplicons were sequenced on an Illumina MiSeq platform (in a 250-bp paired-end mode) by FIMM. The microbiome sequence data analysis was performed using CLC Genomics Workbench, Microbial Genomics Module 22.0.1 (https://digitalinsights.qiagen.com/). The demultiplexed sequence data were imported to the CLC genomic workbench, and a total of 54,784,320 reads (for 869 samples) with a mean of 63,042 reads per sample were available for analysis. OTU clustering was performed against the human oral microbiome database (HOMD, version 15.22) using the 97% similarity threshold[34]. We used standard analytical workflows provided by the CLC for data quality control, OTU clustering and

Table 1 | Clinical characteristics of the study cohort

	Whole cohort	Sub-cohort with CVD risk	Sub-cohort without CVD risk	*p*-value
Phenotype	***N* (%)**			
N	869	530	339	-
Male (*n*)	382 (43.96)	251 (47.36)	131 (38.64)	-
Smokers (*n*) occasional and current	171 (19.68)	171 (32.26)	0 (0.00)	-
	Mean (SD)			
Age (years)	46 (0.00)	46 (0.00)	46 (0.00)	-
cIMT (mm)	0.602 (0.089)	0.608 (0.094)	0.594 (0.083)	0.064
BMI (kg/m^2)	26.49 (4.62)	27.08 (4.75)	25.50 (4.02)	**4.9e-7***
BP, systole (mmHg)	126.55 (15.54)	127.80 (16.09)	125.69 (14.60)	0.337
diastole (mmHg)	84.45 (10.10)	85.40 (10.27)	83.19 (9.66)	**0.006***
HDL (mmol/L)	1.55 (0.37)	1.52 (0.36)	1.60 (0.38)	**3.9e-4***
LDL (mmol/L)	3.41 (0.89)	3.46 (0.90)	3.36 (0.90)	0.179
TG (mmol/L)	1.23 (0.92)	1.29 (0.82)	1.12 (1.06)	**6.0e-6***
VFA (cm^2)	101.46 (39.88)	106.46 (41.51)	93.41 (35.55)	**2.8e-6***
Alat (U/L)	29.73 (20.96)	29.12 (19.32)	28.10 (23.50)	**0.003***
CRP (mg/L)	1.43 (2.53)	1.91 (3.19)	0.73 (0.58)	**5.3e-10***
B-HbA1c (mmol/mol)	35.29 (4.22)	35.79 (4.04)	34.38 (3.52)	**3.5e-6***
PPD ≥ 4 mm	1.70 (0.90)	4.53 (6.93)	0 (0.00)	**0.000***

The Mann–Whitney U test was used to analyze the statistical differences between the CVD sub-cohorts.
CVD cardiovascular disease, *cIMT* carotid intima media thickness, *BMI* body mass index, *BP* blood pressure, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *VFA* visceral fat area, *CRP* C-reactive protein, *PPD* periodontal pocket depth, *SD* standard deviation.
*p-value < 0.05 are bold.

the α- and β-diversity analyses. Specifically, the oral microbiome α-diversity for each subject was estimated using the following metrics: number of genera, the Shannon index and Faith's phylogenic diversity (PD). The statistical differences in the α-diversity were examined using the independent-samples Mann–Whitney U test (for two groups) and the Kruskal–Wallis test (for comparisons across more than two groups). Differences in the β-diversity between samples were visualized using the principal coordinate analysis (PCoA), based on the Jaccard and Bray–Curtis dissimilarities distances between samples. To assess community-level differences between groups, we used the PERMANOVA analysis workflow in CLC. The taxa relative abundances were calculated at the genus level, and statistical differences between groups were evaluated using Mann–Whitney U test. The association of cIMT (mm) with microbiome α-diversity and bacterial genera was examined with a linear regression model. Parameters with skewed distributions were logarithmically transformed. The association of immunoglobulins levels and bacteria genera was examined with Spearman coefficient. All statistical analyses (except the β-diversity) were performed using IBM SPSS statistics version 28. The data in this study were visualized using Genomics Module 22.0.1 (qiagen), GraphPad Prism 8.0 (GraphPad Software) and OriginPro 2022 (OriginLab Corporation).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results

Oral microbiome diversity is influenced by atherosclerosis risk factors

The clinical features of the Northern Finland Birth Cohort 1966 (NFBC1966) are presented in Table 1. At the age of 46, individuals underwent extensive clinical investigations, including oral health assessments. A total of 869 subjects with available saliva samples and cIMT data were included, and 16S rRNA oral microbiome analysis of the salivary samples was performed (Fig. 1a). The association between cIMT and oral microbiome diversity was estimated using the number of observed microbial genera, the Shannon index, and Faith's phylogenetic diversity (PD). The linear regression model shows that Shannon index associates with cIMT in models adjusted for sex, blood pressure, BMI, LDL, B-HBA1c, and hsCRP. However, after adjusting for smoking and periodontal disease markers, the model was not significant (Fig. 1b). This suggests that smoking and periodontal status could influence both atherosclerosis and oral microbiome diversity.

Next, we asked the question of how oral microbiome diversity is influenced by atherosclerosis risk factors (Supplementary Fig. 1). We found that the number of observed genera in the oral microbiome was significantly lower in subjects with poor glycemic control, as identified by high blood glycated hemoglobin (B-HbA1c, $p = 0.004$). The Shannon index of the oral microbiome was lower in the group of current smokers compared to occasional, ex-smokers, and never-smokers ($p < 0.001$). The Shannon index was also lower in subjects with low-grade inflammation identified through increased levels of high-sensitivity C-reactive protein (hsCRP, $p = 0.027$). Additionally, Faith's PD was lower in the smoking group ($p < 0.001$) and the B-HbA1c group ($p = 0.016$). Furthermore, periodontal status was associated with α-diversity, such that the number of observed genera increased with the incremental number of periodontal pockets depth 4 mm or larger (PPD ≥ 4 mm, $p < 0.001$).

Oral microbiome alfa diversity is associated with cIMT in male subjects

We aimed to further characterize the association of atherosclerosis and oral microbiome in subjects free from mutual influence factors. Additionally, sex is a known biological modifier of atherosclerosis, with male subjects being identified as more susceptible to CVD[35]. Consequently, we employed a systematic exclusion approach (Fig. 1c). A total of 530 subjects had CVD risk factors, and 339 subjects were CVD risk-free (Table 1). In addition, while there was no significant difference in α diversity between genders in the whole cohort, there were statistically significant differences in Bray–Curtis ($p = 0.004$) and Jaccard ($p = 0.003$) indices between genders. The male ($n = 131$) and female ($n = 208$) sub-cohorts consisted of non-smoking, non-diabetic subjects, and had no indications of low-grade inflammation (hsCRP) or clinical signs of periodontal disease. In a linear regression model adjusted for BMI, LDL, and blood pressure (Fig. 1d), the male sub-cohort showed an association between cIMT and bacterial genera ($p = 0.030$) as well as the Shannon index ($p = 0.001$), while in the female sub-cohort, oral microbiome diversity did not show an inverse association with cIMT. Suggesting sex-specific association of male oral microbiome with cIMT.

Oral bacteria genera and microbiome beta diversity associate with cIMT

In Table 2, we present the clinical characteristics of the whole cohort, male and female sub-cohorts according to cIMT groups. In the whole cohort, all atherosclerosis risk factors except B-HbA1c were statistically significant according to cIMT groups (Table 2). However, in the male and female sub-cohorts, after the exclusion of mutual risk factors, no differences in atherosclerosis risk factors between cIMT groups were observed (Table 2). Additionally, we found that α-diversity was statistically significant only in the male sub-cohort with oral microbiome genera ($p = 0.002$), the Shannon index ($p = 0.003$), and Faith's PD index ($p = 0.018$).

In Fig. 2a the genera level bacterial aggregated abundance according to cIMT groups in male subjects is shown. Next, we analyzed the variation of bacterial genus levels in the male sub-cohort according to cIMT (Supplementary Data 1). Furthermore, in a linear regression model, we examined the association between significant genera and cIMT (Fig. 2b). In this model, *Prevotella* ($p = 0.018$), *Megasphaera* ($p = 0.026$), and *Veillonella* ($p = 0.011$) genera were found to be positively associated with cIMT, while *Absconditabacteria* ($p = 0.008$), *Capnocytophaga* ($p = 0.008$), *Gemella* ($p = 0.017$),

Table 2 | Clinical characteristics analysis based on cIMT groups was conducted across the entire cohort, as well as the mutual oral microbiome and atherosclerosis influence factor free male and female sub-cohorts

Cohort (n = 869)				Male sub-cohort (n = 131)			Female sub-cohort (n = 208)		
	Low cIMT	High cIMT	p-value	Low cIMT	High cIMT	p-value	Low cIMT	High cIMT	p-value
Male/female (n)	215/351	167/136		74/0	57/0		0/152	0/56	
Smoking, O/C (n)	27/75	21/48		0	0		0	0	
	Mean (SD)			Mean (SD)			Mean (SD)		
cIMT (mm)	0.552 (0.04)	0.697 (0.08)	**0.000***	0.555 (0.39)	0.699 (0.09)	**0.000***	0.547 (0.04)	0.665 (0.05)	**0.000***
BMI (kg/m^2)	25.89 (4.31)	27.69 (5.04)	**1.3e-7***	26.16 (3.21)	27.16 (3.37)	0.055	24.39 (3.82)	26.04 (5.32)	0.064
BP, systole	124.50 (14.09)	130.45 (17.24)	**2.7e-6***	130.34 (12.42)	131.63 (12.42)	0.394	121.25 (14.47)	124.65 (15.46)	0.116
Diastole (mmHg)	83.74 (9.55)	85.92 (11.07)	**0.008***	86.05 (8.34)	84.45 (8.01)	0.321	81.39 (9.98)	82.72 (10.80)	0.125
HDL (mmol/L)	1.59 (0.37)	1.48 (0.35)	**4.1e-5***	1.40 (0.30)	1.38 (0.29)	0.932	1.77 (0.38)	1.64 (0.35)	0.075
LDL (mmol/L)	3.33 (0.88)	3.58 (0.89)	**4.3e-5***	3.74 (0.832)	3.63 (0.94)	0.650	3.15 (0.89)	3.15 (0.68)	0.651
TG (mmol/L)	1.16 (0.70)	1.28 (0.81)	**0.003***	1.38 (0.83)	1.54 (2.11)	0.867	0.89 (0.41)	0.94 (0.48)	0.493
VFA (cm^2)	98.86 (37.20)	106.97 (43.80)	**0.025***	91.81 (27.97)	91.55 (33.91)	0.939	92.70 (35.31)	100.85 (45.99)	0.486
Alat (U/L)	28.22 (21.48)	33.41 (25.62)	**3.0e-6***	40.64 (40.77)	36.88 (17.87)	0.543	20.82 (8.28)	21.57 (9.87)	0.677
CRP (mg/L)	1.35 (2.35)	1.69 (3.23)	**0.032***	0.69 (0.54)	0.72 (0.47)	0.549	0.74 (0.60)	0.81 (0.67)	0.316
B-HbA1c (mmol/mol)	35.21 (3.93)	35.76 (4.90)	0.231	35.08 (3.27)	34.35 (3.64)	0.229	34.00 (3.59)	34.47 (3.29)	0.372
PPD ≥ 4 mm	2.33 (4.83)	3.32 (7.35)	0.285	0	0		0	0	
Bacterial genera	60.74 (8.91)	59.96 (8.94)	0.187	61.65 (9.52)	57.49 (7.55)	**0.002***	59.18 (7.29)	58.56 (7.54)	0.788
Faith's PD	6.61 (0.25)	6.61 (0.28)	0.970	6.65 (0.23)	6.59 (0.21)	**0.018***	6.63 (0.20)	6.64 (0.23)	0.262
Shannon index	3.34 (0.38)	3.30 (0.40)	0.197	3.49 (0.29)	3.28 (0.39)	**0.003***	3.38 (0.32)	3.34 (0.37)	0.885

The statistical differences between cIMT groups were analyzed using the Mann–Whitney U test.

O/C occasional/current smokers, *CVD* cardiovascular disease, *cIMT* carotid intima media thickness, *BMI* body mass index, *BP* blood pressure, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *VFA* visceral fat area, *CRP* C-reactive protein, *PPD* periodontal pocket depth, *Faith's PD* Faith's Phylogenetic diversity, *SD* standard deviation.

*p-value < 0.05 are bold.

Fusobacterium ($p = 0.002$), *Neisseria* ($p = 0.019$), *Aggregatibacter* ($p = 0.002$), *Tannerella* ($p = 0.003$), *Treponema* ($p = 0.004$), *Cardiobacterium* ($p = 0.012$), and *Bacteroidales* ($p = 0.010$) genera were found to be inversely associated with cIMT. In addition, the variation of bacterial genus levels in the female sub-cohort according to cIMT was analyzed (Supplementary Data 2); here, *Parascardovia* genera with only 9% of presence in female subjects were statistically significant. Moreover, in male sub-cohort β-diversity (Fig. 2c) analysis based on the Bray–Curtis dissimilarity ($p < 0.001$) and Jaccard distance ($p < 0.001$) revealed significant sample clustering according to the high and low cIMT groups.

Association of total IgA and antibodies to PC-KLH with cIMT and the oral microbiome

Antibodies that target modified LDL epitopes play an important role in atherogenesis, but factors that trigger the humoral response are not fully understood[21]. In the male sub-cohort ($n = 131$; Fig. 3a), we quantified serum levels of total IgA, IgG, and IgM, as well as antibodies to MAA-LDL and PCho-KLH adducts. We found that subjects with high cIMT had lower levels of total serum IgA ($p = 0.009$), IgA to PCho-KLH ($p = 0.017$), and IgG to PCho-KLH ($p = 0.008$). Interestingly, we did not observe significant associations between these factors and cIMT in the female risk-free sub-cohort (Supplementary Fig. 2). In a linear regression model, we found that total IgA ($p = 0.008$), serum IgA to PC-KLH ($p = 0.019$), and IgG to PC-KLH ($p = 0.010$) were associated with cIMT levels (Fig. 3b).

We further analyzed the relationship between oral microbiome composition and total serum and antibodies to oxidized epitopes (Fig. 3c). Serum total IgA antibodies correlated positively with *Catonella* ($p = 0.005$), and total IgM with *Neisseria* ($p = 0.018$). IgA antibodies to PC-KLH correlated positively with *Treponema* ($p = 0.006$), *Parvimonas* ($p = 0.041$), *Bacteroidales* ($p = 0.001$), and *Peptococcus* ($p < 0.001$). From the antibody-associated genera, *Neisseria*, *Treponema*, and *Bacteroidales* also associated with cIMT, suggesting that those genera may link with atherogenesis through the humoral immune response.

Discussion

This study illustrates that the diversity of the oral microbiome correlates with carotid intima media thickness. Although prior research has extensively established a connection between periodontitis and periodontal pathogens with atherosclerosis[5,36], the relationship between oral microbiome diversity and atherosclerosis has yet to be substantiated. Notably, the oral microbiome diversity is influenced by cardiovascular disease (CVD) risk factors, posing challenges in determining whether atherosclerosis itself is directly associated with oral microbiome diversity or mediated through these disease risk factors such as smoking, diabetes, periodontitis, and systemic inflammation[13,37–39]. After excluding subjects with mutual influence factors, we discovered inverse associations between oral microbiome α-diversity and mixed β-diversity with cIMT in male subjects. While definitive conclusions cannot be drawn at this stage, our findings suggest the association of oral microbiome diversity with male cIMT in sex-specific manner.

Sex is known to be an important biological variable in atherosclerosis, with females exhibiting significantly lower cIMT levels and CVD incidence[35]. Our findings show that host sex did influence the oral microbiome β-diversity but not α-diversity. Interestingly, we observe a clear link between the oral microbiome diversity and cIMT in the middle-aged male sub-cohort, but not in female sub-cohort. Accumulating evidence suggests that sex impacts the differences in immune responses between male and female[40]. In addition, sex contributes to gut microbiome-induced autoimmune disease[41]. However, how potentially sex-tailored oral mucosal immunity contributes to atherosclerosis remains unknown.

In the male sub-cohort, we observe positive associations between the genera *Prevotella*, *Megasphaera*, and *Veillonella*, and cIMT. Conversely, we find inverse associations between the genera *Absconditabacteria*, *Capnocytophaga*, *Gemella*, *Fusobacterium*, *Neisseria*, *Aggregatibacter*, *Tannerella*, *Treponema*, *Cardiobacterium*, and Bacteroidales genera, and cIMT. These findings suggest potential links between specific oral taxa with atherosclerosis. Previous investigations into the connection between oral

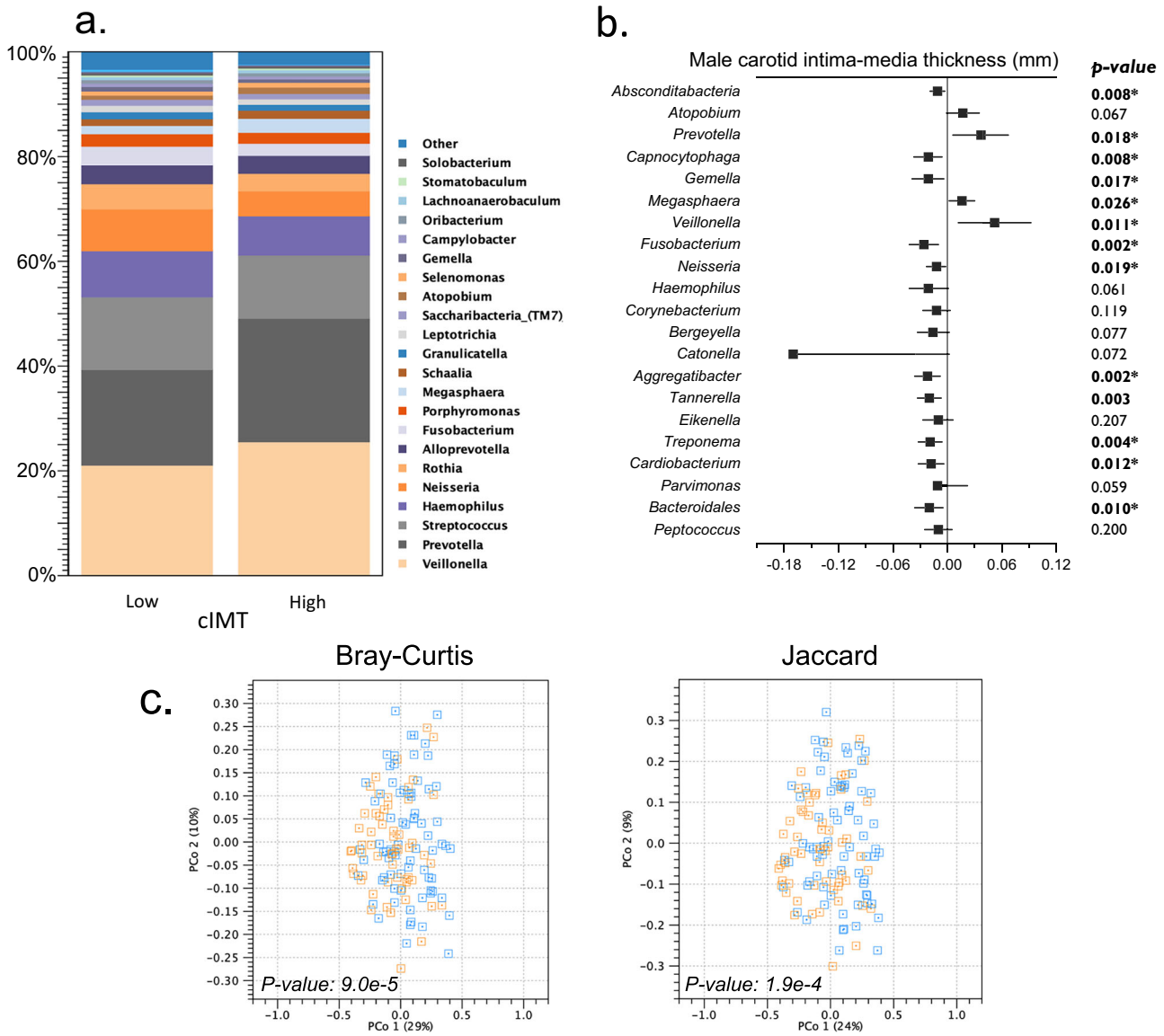

Fig. 2 | Oral microbiota in the male sub-cohort. a The relative abundance of the bacterial taxa at the genus level for the oral microbiota in subjects with a low ($n = 74$) and high ($n = 57$) carotid intima media thickness (cIMT). The taxa are sorted according to the relative abundance. **b** The association of male cIMT group ($n = 131$) with statistically significant oral genera was examined with linear regression model adjusted with residue CVD risk factors: BMI, LDL and blood pressure. Genera with over 70% presence in the male cohort was selected. **c** The community-level differences between male ($n = 131$) cIMT groups were examined using the PERMANOVA analysis. The oral microbial β-diversity between cIMT groups visualized using the principal coordinates analysis (PCoA) based on the Bray–Curtis dissimilarity and Jaccard distances between samples. Each point represents a single sample. *p-value < 0.05 are bold.

microbiome diversity and atherosclerosis primarily use CVD as the primary outcome[6–8,42]. However, in this study, we examine the relationship between oral microbiome diversity and atherosclerosis clinical marker cIMT. Our data suggest that oral microbiome diversity may play a relevant role in atherosclerosis progression.

In the past three decades, accumulating evidence demonstrates that periodontal diseases associate with CVD[5,36]. In this study, we establish a link between the oral microbiome diversity and cIMT in a periodontitis-free male subpopulation. Indicating an association between the oral microbiome diversity and atherosclerosis independent of periodontitis. This finding raises the question of how oral microbiome compositional changes may contribute to systemic health. Recent studies show that mandibular lymph nodes intercept *Listeria monocytogenes* and activate immune response in distal sites[43]. We can speculate that microbiome diversity may alter mucosal immunity, thus increasing susceptibility to systemic diseases.

The IgA antibodies play a critical role in mucosal immunity and accumulated evidence suggests the IgA as a potential link between mucosal immunity and atherosclerosis; however, little is known about the functional role of IgA antibodies in atherosclerotic plaque formation[21]. Here, we demonstrate the atheroprotective association of serum IgA and IgG to PCho with cIMT. This finding agrees with previous study[44]; however, serum IgA antibodies to PCho may also predict CVD events[18]. Natural antibodies to modified LDL epitopes such as antibodies to PCho constitute up to 30% of human natural antibodies[45]. Yet, the factors driving antibody production remain unclear. In the current study, low oral microbiome diversity and the level of IgA to PCho are associated with a high cIMT. Moreover, we find a positive correlation between specific oral bacterial taxa and serum IgA antibodies to PCho. Given that the oral microbiome diversity is thought to stabilize during adulthood and early old age[12]. It is plausible that the long-term activation of humoral immunity to oral microbiome PAMP epitopes such as PCho may contribute to atherogenesis. Furthermore, IgA antibodies may interact with microbial antigens present in the plaque, potentially influencing immune responses and plaque stability. Future research should focus on elucidating how changes in the oral microbiome might influence

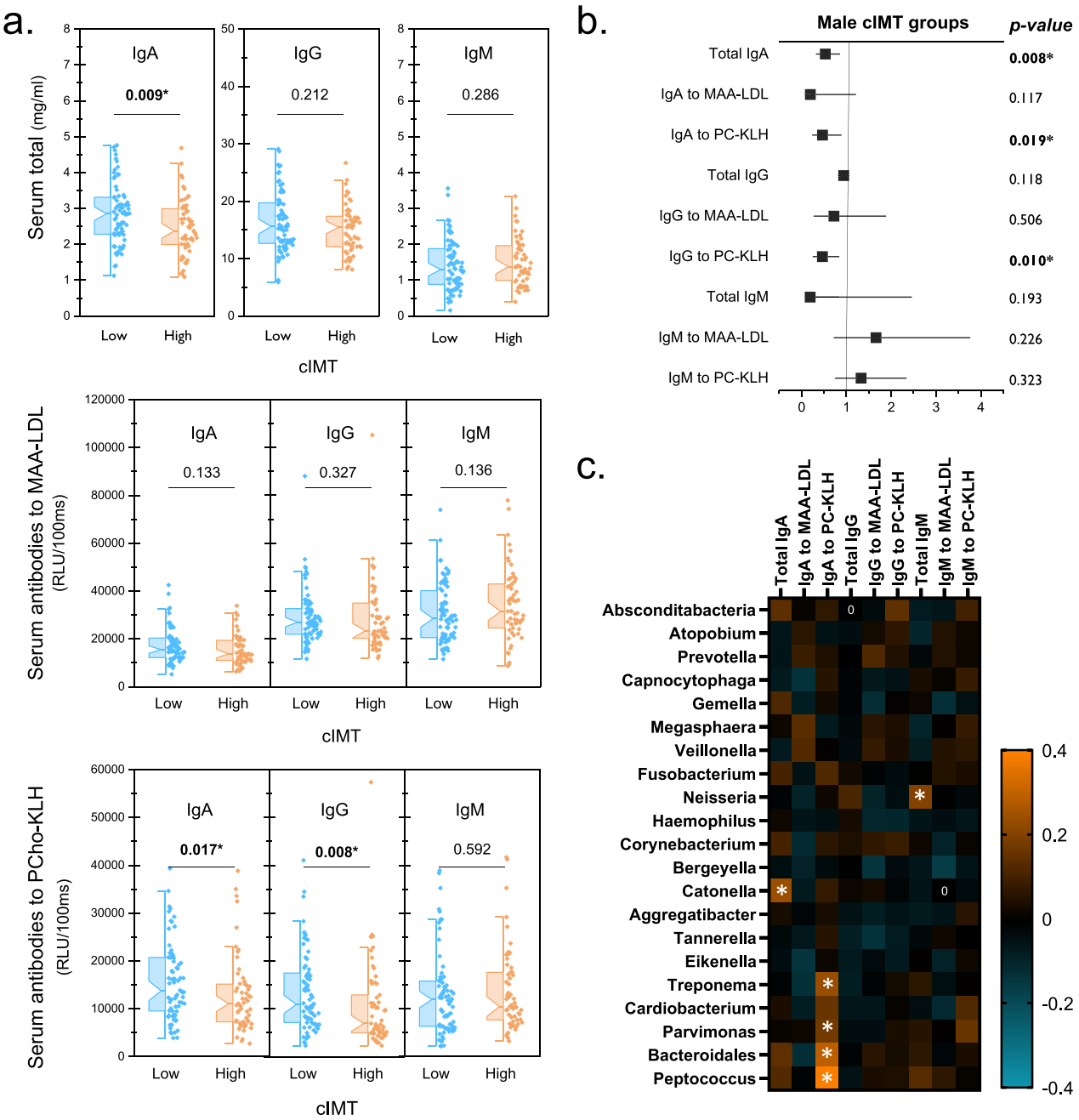

Fig. 3 | Analysis of the systemic humoral immune response in relation to carotid intima media thickness and the oral microbiome. a Serum levels of total IgA, IgG, and IgM binding to malondialdehyde-acetaldehyde LDL (MAA-LDL) and phosphorylcholine (Pcho-KLH) were compared across male ($n = 131$) carotid intima media thickness (cIMT) groups. The box plots show the median and interquartile ranges, and statistical differences between cIMT groups were analyzed using the Mann–Whitney U test. **b** Associations between immunoglobulin levels and male ($n = 131$) cIMT groups were evaluated using a logistic regression model adjusted for residual cardiovascular disease (CVD) risk factors: BMI, LDL, and blood pressure. *p-value < 0.05 are bold. **c** For the correlation analysis, the genus bacterial taxa that associate with the cIMT in male sub-cohort ($n = 131$) were selected. Correlations examined using Spearman coefficient. *p-values ≤ 0.05.

IgA serum antibody levels, thereby contributing to our understanding of the interplay between mucosal immunity and cardiovascular disease.

The main limitation of our study is the availability of data from one timepoint regarding the oral microbiome and cIMT status. In future health examinations involving this cohort, our intention is to investigate the relationship between the oral microbiome and cIMT in advanced age, thereby assessing long-term associations. Another limitation of this study is relatively small sample ($n = 339$) size of CVD risk-free sub-cohort. The lack of population diversity in our study may limit the broader interpretation of our results at a global level. Lifelong diet influences both atherosclerosis progression and oral microbiome composition[46,47]. Future research should aim to include detailed dietary assessments to better understand how different lifelong dietary patterns affect oral microbiome diversity and composition. This additional data could provide more comprehensive insights into the interactions between diet, the oral microbiome, and cardiovascular disease (CVD) risk factors. Furthermore, this study is limited by its low microbial resolution. Future investigations could employ a higher metagenomic level analysis to elucidate specific strain-level virulence factors, genes, or pathways that may contribute to atherosclerosis progression. The principal strength of this study lies in the comprehensive characterization of the

birth cohort. By virtue of this meticulous characterization, we are able to highlight the complexity of clinical microbiome research, thereby underscoring the significance of conducting a comprehensive oral health examination alongside a thorough systemic health assessment in oral microbiome studies. Age influences atherosclerosis progression, another strength of this study is that we can control these modifying factors by birth cohort setting[48].

To summarize, among the entire cohort ($n = 869$), we demonstrate that CVD risk factors such as smoking, diabetes risk marker B-HbA1c, hsCRP and periodontitis influence oral microbiome diversity. Notably, in male human subjects ($n = 131$) oral microbiome diversity is associated with atherosclerosis clinical marker cIMT. Finally, we find that certain atherosclerosis-associated bacteria correlated with serum IgA antibodies to PCho, suggesting a potential role of oral mucosal immunity in the crosstalk between atherosclerosis and the oral microbiome, independent of periodontal disease.

Data availability

The NFBC1966 data are available via the infrastructure for populations study at the University of Oulu. The NFBC1966 data are subject to the European Union General Data Protection Regulation (679/2016) and the Finnish Data Protection Act. Personal data availability is limited to the cohort participant's written informed consent at her/his latest follow-up study. For more information, please contact the NFBC project center (NFBCprojectcenter@oulu.fi) and visit the cohort website (http://www.oulu.fi/nfbc). The source data for all figures can be found in Supplementary Data 3.

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References

1. Libby, P. et al. Atherosclerosis. *Nat. Rev. Dis. Prim.* **5**, 56 (2019).
2. Tang, W. H. W., Bäckhed, F., Landmesser, U. & Hazen, S. L. Intestinal microbiota in cardiovascular health and disease. *J. Am. Coll. Cardiol.* **73**, 2089–2105 (2019).
3. Lamont, R. J., Koo, H. & Hajishengallis, G. The oral microbiota: dynamic communities and host interactions. *Nat. Rev. Microbiol.* **16**, 1 (2018).
4. Hajishengallis, G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nat. Rev. Immunol.* **15**, 30–44 (2015).
5. Schenkein, H. A., Papapanou, P. N., Genco, R. & Sanz, M. Mechanisms underlying the association between periodontitis and atherosclerotic disease. *Periodontol 2000* **83**, 90–106 (2020).
6. Koren, O. et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc. Natl Acad. Sci. USA* **108**, 4592–4598 (2011).
7. Kato-Kogoe, N. et al. Characterization of salivary microbiota in patients with atherosclerotic cardiovascular disease: a case-control study. *J. Atheroscler. Thromb.* **29**, 60608 (2022).
8. Fak, F., Tremaroli, V., Bergstrom, G. & Backhed, F. Oral microbiota in patients with atherosclerosis. *Atherosclerosis* **243**, 573–578 (2015).
9. Willeit, P. et al. Carotid intima-media thickness progression as surrogate marker for cardiovascular risk. *Circulation* **142**, 621–642 (2020).
10. Lockhart, P. B. et al. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association? A scientific statement from the American Heart Association. *Circulation* **125**, 2520–2544 (2012).
11. Xiao, E. et al. Diabetes enhances IL-17 expression and alters the oral microbiome to increase its pathogenicity. *Cell Host Microbe* **22**, 120–128.e4 (2017).
12. Martino, C. et al. Microbiota succession throughout life from the cradle to the grave. *Nat. Rev. Microbiol.* **20**, 707–720 (2022).
13. Yu, G. et al. The effect of cigarette smoking on the oral and nasal microbiota. *Microbiome* **5**, 3 (2017).
14. Jie, Z. et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat. Commun.* **8**, 845 (2017).
15. Nordström, T. et al. Cohort profile: 46 years of follow-up of the Northern Finland Birth Cohort 1966 (NFBC1966). *Int. J. Epidemiol.* **50**, 1786–1787j (2022).
16. Potter, M. Antigen-binding myeloma proteins in mice. *Ann. N. Y. Acad. Sci.* **190**, 306–321 (1971).
17. Kolberg, J., Høiby, E. A. & Jantzen, E. Detection of the phosphorylcholine epitope in streptococci, *Haemophilus* and pathogenic *Neisseriae* by immunoblotting. *Microb. Pathog.* **22**, 321–329 (1997).
18. Kankaanpää, J. et al. IgA antibodies to phosphocholine associate with long-term cardiovascular disease risk. *Atherosclerosis* **269**, 294–300 (2018).
19. Binder, C. J. et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between *Streptococcus pneumoniae* and oxidized LDL. *Nat. Med.* **9**, 736–743 (2003).
20. Sage, A. P., Tsiantoulas, D., Binder, C. J. & Mallat, Z. The role of B cells in atherosclerosis. *Nat. Rev. Cardiol.* **16**, 180–196 (2019).
21. Porsch, F., Mallat, Z. & Binder, C. J. Humoral immunity in atherosclerosis and myocardial infarction: from B cells to antibodies. *Cardiovasc. Res.* **117**, 2544–2562 (2021).
22. Rantakallio, P. The longitudinal study of the Northern Finland birth cohort of 1966. *Paediatr. Perinat. Epidemiol.* **2**, 59–88 (1988).
23. University of Oulu. *Northern Finland Birth Cohort 1966*. http://urn.fi/urn:nbn:fi:att:bc1e5408-980e-4a62-b899-43bec3755243 (2020).
24. Mella, M. A. et al. Compensatory IgM to the rescue: patients with selective IgA deficiency have increased natural IgM antibodies to MAA-LDL and no changes in oral microbiota. *Immunohorizons* **5**, 170–181 (2021).
25. van den Munckhof, I. C. L. et al. Relation between age and carotid artery intima-medial thickness: a systematic review. *Clin. Cardiol.* **41**, 698–704 (2018).
26. Tegelberg, P. et al. Association of hyperglycaemia with periodontal status: results of the Northern Finland Birth Cohort 1966 study. *J. Clin. Periodontol.* **48**, 25–37 (2021).
27. Tuomi, T. et al. *Type 2 Diabetes, Current Care Guidelines*. Working group appointed by the Finnish Medical Society Duodecim, the Finnish Society of Internal Medicine, the Medical Advisory Board of the Finnish Diabetes Society (The Finnish Medical Society Duodecim, 2020). https://www.kaypahoito.fi/hoi50056.
28. Rustad, P. et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scand. J. Clin. Lab. Invest.* **64**, 271–284 (2004).
29. Turunen, S. P. et al. Recognition of *Porphyromonas gingivalis* gingipain epitopes by natural IgM binding to malondialdehyde modified low-density lipoprotein. *PLoS ONE* **7**, e34910 (2012).
30. Akhi, R. et al. Cross-reactive saliva IgA antibodies to oxidized LDL and periodontal pathogens in humans. *J. Clin. Periodontol.* **44**, 682–691 (2017).
31. Kyrklund, M. et al. Humoral immune response to heat shock protein 60 of *Aggregatibacter actinomycetemcomitans* and cross-reactivity with malondialdehyde acetaldehyde-modified LDL. *PLoS ONE* **15**, e0230682 (2020).
32. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
33. Caporaso, J. G. et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl Acad. Sci. USA* **108**, 4516–4522 (2011).
34. Chen, T. et al. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database* **2010**, baq013 (2010).
35. Man, J. J., Beckman, J. A. & Jaffe, I. Z. Sex as a biological variable in atherosclerosis. *Circ. Res.* **126**, 1297–1319 (2020).
36. Tonelli, A., Lumngwena, E. N. & Ntusi, N. A. B. The oral microbiome in the pathophysiology of cardiovascular disease. *Nat. Rev. Cardiol.* **20**, 386–403 (2023).
37. Sabharwal, A. et al. The salivary microbiome of diabetic and non-diabetic adults with periodontal disease. *J. Periodontol.* **90**, 26–34 (2019).
38. Tuganbaev, T., Yoshida, K. & Honda, K. The effects of oral microbiota on health. *Science* **376**, 934–936 (2022).

39. Wade, W. G. Resilience of the oral microbiome. *Periodontol 2000* **86**, 113–122 (2021).
40. Wilkinson, N. M., Chen, H.-C., Lechner, M. G. & Su, M. A. Sex differences in immunity. *Annu. Rev. Immunol.* **40**, 75–94 (2022).
41. Markle, J. G. M. et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* **339**, 1084–1088 (2013).
42. Bouzid, F. et al. A potential oral microbiome signature associated with coronary artery disease in Tunisia. *Biosci. Rep.* **42**, BSR20220583 (2022).
43. Barreto de Alburquerque, J. et al. Microbial uptake in oral mucosa–draining lymph nodes leads to rapid release of cytotoxic $CD8^+$ T cells lacking a gut-homing phenotype. *Sci. Immunol.* **7**, eabf1861 (2022).
44. Fiskesund, R. et al. IgM phosphorylcholine antibodies inhibit cell death and constitute a strong protection marker for atherosclerosis development, particularly in combination with other auto-antibodies against modified LDL. *Results Immunol.* **2**, 13–18 (2012).
45. Chou, M. Y. et al. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J. Clin. Invest.* **119**, 1335–1349 (2009).
46. Jeong, S. Y. et al. Effects of diet on 10-year atherosclerotic cardiovascular disease risk (from the DASH Trial). *Am. J. Cardiol.* **187**, 10–17 (2023).
47. Augimeri, G. et al. Mediterranean diet: a potential player in the link between oral microbiome and oral diseases. *J. Oral. Microbiol.* **16**, 2329474 (2024).
48. Wang, J. C. & Bennett, M. Aging and atherosclerosis. *Circ. Res.* **111**, 245–259 (2012).

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Author contributions

Rami Akhi: Contributed to conception, design, data acquisition, data analysis and interpretation, drafted the manuscript. Anton Lavrinienko: Contributed to conception, design, data acquisition, data analysis and interpretation, edited manuscript. Miia Hakula: Contributed to data acquisition and edited manuscript. Leo Tjäderhane: Contributed to data acquisition and interpretation. Rasmus Hindström: Contributed to data acquisition and interpretation. Antti Nissinen: Contributed to data acquisition and interpretation. Chunguang Wang: Contributed to data acquisition and interpretation edited manuscript. Juha Auvinen: Contributed to data acquisition and interpretation. Arja M Kullaa: Contributed to data acquisition and interpretation, edited manuscript. Pekka Ylöstalo: Contributed to data acquisition and interpretation, edited manuscript. Tuula Salo: Contributed to data acquisition and interpretation. Kari Kaikkonen: Contributed to data acquisition, data analysis and interpretation, edited manuscript. Janne J. Koskimäki: Contributed conception, design, data acquisition, data acquisition, data analysis and interpretation, edited manuscript. Sohvi Hörkkö: Contributed conception, design, data acquisition, data acquisition, data analysis and interpretation, edited manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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