DENTITION STATUS AND THE RISK OF CHRONIC SYSTEMIC DISEASES: THE ROLE OF SYSTEMIC INFLAMMATION, AUTOIMMUNITY AND ORAL MICROBIOTA

vorgelegt von
DVM, MSc. PH
Kolade Oluseye Oluwagbemigun
geb.in Ibadan, Nigeria

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Promotionsausschuss:

Vorsitzender: Prof. Dr. Tobias Kurth

Gutachter: Prof Dr Heiner Boeing

Gutachter: Prof Dr Reinhard Busse

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Intelligent people are always ready to learn. Their ears are open for knowledge.
(Proverbs 18:15 New Living Translation)

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Abbreviations

ACPA	Anti-citrullinated protein autoantibodies
ATCC	American Type Culture Collection
BMI	Body mass index
Anti-CCP2	Second generation anti-cyclic citrullinated peptide
CI	Confidence Interval
CT	cycle threshold
CVD	Cardiovascular disease
DAGs	Directed acyclic graphs
DALYs	Disability-adjusted life-years
DMARD	Disease-modifying antirheumatic drugs
DMS	German Oral Health Study
DNA	Deoxyribonucleic acid
EPH	Ecological plaque hypothesis
EPIC-Potsdam	European Prospective Investigation into Cancer and Nutrition-Potsdam
FDI	Fédération Dentaire Internationale
gDNA	Genomic DNA
HDL	High-density lipoprotein
HR	Hazard ratio
HOMIM	Human Oral Microbe Identification Microarray
HOMINGS	Human Oral Microbe Identification using Next Generation Sequencing
HsCRP	High-sensitivity C-reactive protein
IgG	Immunoglobulin G
KPH	Keystone pathogen hypothesis
LDL	Low-density lipoprotein
NSAID	Nonsteroidal anti-inflammatory drugs
OUT	Operational taxonomic unit
PCR	Polymerase chain reaction
PCA	Principal component analysis
RR	Relative risk
SES	Socioeconomic status
SPH	Specific plaque hypothesis
T2DM	Type 2 diabetes mellitus
TNSPH	Traditional non-specific plaque hypothesis
UNSPH	Updated non-specific plaque hypothesis
WHO	World Health Organization

Abstract

Introduction: Dental diseases such as periodontitis, dental caries and endodontitis which are of polymicrobial (polybacterial) aetiology are highly prevalent. These conditions, if untreated in consort with other risk factors such as smoking lead to tooth loss. Moreover, there is evidence that the number of teeth (tooth loss) has an impact on overall health, particularly the risk of some systemic chronic diseases. Low-grade inflammation and autoimmunity are widely acknowledged as possible biological explanations. The aims of this thesis were to: 1) investigate the association between the number of teeth and chronic systemic diseases (study I), 2) to determine the relationship between the number of teeth and low-grade systemic inflammation (study II), 3) to determine the association between *Porphyromonas gingivalis* and the number of teeth, and an autoantibody(study III), and 4) to investigate whether a novel statistical method, treelet transform can yield interpretable dental disease-associated bacterial profiles. The latter investigation was done under the assumption whether the association between these bacterial profiles and the number of teeth, and low-grade systemic inflammation is dependent on the overall bacterial diversity, and to define the relative importance of these bacterial profiles as compared to other risk factors (study IV)?

Methodology: We studied participants of the EPIC-Potsdam cohort (n=27,548) who are middle-age adults recruited between 1994 and 1998 from the general population of Potsdam, Germany, and presently undergoing the sixth wave of follow-up. A total of 24,313 participants were examined longitudinally in study I and we investigated 3,086, 600, and 281 individuals cross-sectionally in study II, study III, and study IV, respectively. Information on the number of teeth and lifestyle factors were obtained through questionnaires and illness-related information was verified. Saliva was sampled for bacteria using quantitative polymerase chain reaction (qPCR) and the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS). Furthermore, levels of serum high-sensitivity C-reactive protein (hsCRP) and anti-citrullinated protein autoantibodies (ACPA) measured as the second generation anti-cyclic citrullinated peptide immunoglobulin G (anti-CCP2 IgG) were determined.

Results: In study I, after controlling for confounders, every one unit increase in the number of teeth was associated with 3% (HR: 0.97; 95% CI 0.96, 0.99; P-value= <0.01) decreased risk of myocardial infarction. In study II, there was neither linear trend nor nonlinear association between number of teeth and serum hsCRP concentrations, a marker of low-grade systemic inflammation. In study III, there was a consistent linear trend of increasing

quantities of *Porphyromonas gingivalis* DNA and increasing number of teeth (P-value for trend= 0.02). Moreover, among participants with serum hsCRP concentrations greater than 3.0 mg/L, those with high and moderate *Porphyromonas gingivalis* DNA had anti-CCP2 IgG titres that were 4 times and 34% higher than in participants with low *Porphyromonas gingivalis* DNA (P-value for trend= 0.01). In study IV, treelet transform yielded periodontitis-associated, caries-associated and endodontitis-associated bacterial profiles. After adjustment for covariates, there was a weak inverse correlation between periodontitis-associated bacteria score and the number of teeth (r= -0.19; 95% CI: -0.35; -0.02). Among participants with low bacterial diversity, periodontitis-associated bacteria score was the second most important predictor of the number of teeth and hsCRP. Periodontitis-associated bacteria score was also an influential predictor of number of teeth among participants with high bacterial diversity, albeit weaker.

Conclusions: There is a link between the number of teeth and myocardial infarction and to a less extent, stroke. The diversity of saliva bacteria community may explain the relation and we could show that *Porphyromonas gingivalis* is an important bacterial candidate for tooth loss. In this context, low grade inflammation and autoantibodies might provide the background for the action of this bacterium. These findings provide evidence in public health decision making.

Keywords: Tooth loss, periodontitis, dental caries, endodontitis, chronic systemic diseases, myocardial infarction, anti-citrullinated protein autoantibodies, low-grade systemic inflammation, high-sensitivity C-reactive protein, oral bacteria, bacterial diversity

1 Introduction

1.1 Oral health and the teeth

According to the World Health Organization (WHO), oral health is defined as "a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial well being" [1]. Oral health is also regarded as integral part of the global burden of non-communicable chronic diseases [2, 3]. Moreover, oral health is also crucial since the oral cavity is the entrance to two important systems–the gastrointestinal and the respiratory system. In fact, the oral cavity is directly connected to the systemic circulation through its plethora of blood and lymphatic vessels. Thus, bacteraemia and systemic spread of bacteria by-products and immunocomplexes might occur following clinical procedures in the oral cavity [4]. It is therefore not surprising that poor oral health is linked to many conditions and diseases in distant body organs.

The oral cavity possesses several soft tissues such as the tongue and hard tissues such as the teeth. The teeth are the most important hard tissue occupying about 20% of the structural area of the oral cavity [5]. The tooth is divided into the crown and root at the cementoenamel junction (or neck) [5]. The enamel is mineralized and inert, supported by a less mineralized, more resilient and hard dentin and a rich innervated and vascularised dental pulp. The teeth are firmly attached to the mucosal surface (gums or gingiva) of the maxillary and mandibular bone through a mineralized and avascular cementum that anchors the fibre bundles of the periodontal ligament to the tooth root. The periodontal ligament connects the cementum to the alveolar bone and ensures teeth flexibility and sensorial receptor functions, and the mineralized alveolar bone also supports the teeth [5]. In fact, the teeth are necessary for the development, stimulation and maintenance of the alveolar bone [6]. Clearly, dental health is an elemental component of the overall oral health.

The teeth and its supporting structures are affected by two main dental diseases–periodontitis (or periodontal disease or gum disease) and dental caries (or tooth decay or cavities) [7-9]. Periodontitis is a chronic inflammatory process involving the gum, alveolar bone and other tooth-supporting structures resulting in irreversible loss of periodontal ligament and alveolar bone [10, 11]. On the other hand, dental caries occurs when bacteria within dental plaque on tooth surfaces hydrolyse starches and metabolise sugars to form lactic and other acids which slowly and intermittently demineralise enamel, dentine and/or cementum [12, 13]. Intrinsic mediators, specifically internal inflammation in response to endogenous oxidative stress also breaks down the dentine [14]. Moreover, deep dental caries and apical foramen due to severe periodontitis can introduce bacteria into the dental

pulp resulting in infection and inflammation (endodontitis) [15, 16]. Microbial factors and host defences at the interface between infected radicular pulp and periodontal ligament further results into apical periodontitis [17, 18].

Periodontitis and dental caries are highly prevalent diseases with an about 3.9 billion people affected according to the 2010 Global Burden of Disease Study [2]. Furthermore, it was reported that severe periodontitis affects 11% of the global population, mostly adults with disability-adjusted life-years (DALYs) of 5,413 and dental caries affects 35% of the global population, mostly children and older adults and DALYs of 4,988 [2]. In fact, the burden of severe periodontitis increases with age to become the most predominant cause of DALYs [2]. In older adults, dental caries additionally worsen oral health-related quality of life [19-21]. Apparently, these conditions also have severe impacts on dentition status, such as the number of teeth [22-24]. Periodontitis is generally regarded as the major cause of loss of natural (or permanent) teeth in adults [22, 25], although dental caries is the leading cause of tooth loss in some populations [26-30]. Interestingly, in most European countries, a large proportion of individuals still retain some teeth until late in life with exception of socially disadvantaged groups [31] In fact, tooth loss is often mild in most people but can become severe in others. The prevalence of severe tooth loss has reduced in the last decade, although the 2010 global estimate is still 2% [2]. This severe loss of teeth might result in a state of loss of all the natural teeth, often referred to as complete edentulism [14]. In contrast to loss of few teeth, complete edentulism is more strongly influenced by societal factors [32]. Besides, progressive tooth loss also causes irreversible alveolar bone resorption and remodelling which is particularly worse among complete edentates [6].

In Germany, more than 25% of adults have or have had periodontitis and/or dental caries with about 25 million of these adults suffering from moderate to severe periodontitis alone [33]. According to the Third German Oral Health Study (DMS III) in 1997, 14% of middle-age adults and 24% of older adults have severe periodontitis while 12% of middle-age adults and 16% of older adults have dental caries. The average tooth loss was four and 18 among middle-age adults and older adults respectively, and the prevalence of edentulism was 2% and 25% of middle-age adults and older adults, respectively [34]. The follow-up study 8 years later showed a reduction in the prevalence of severe periodontitis to 4-8% of middle-age adults and 14-22% of older adults. Tooth loss decreased to three among middle-age adults and 14 among older adults, and edentulism also decreased to 1% of middle-age adults and 23% of older adults. In contrast, the prevalence of dental caries increased to 22% of middle-age adults and 45% of older adults [35]. These dental surveys showing growing proportion of adults retaining natural teeth to advanced age and edentulism becoming a rare occurrence is a commendable progress of dental health in Germany. In support of findings from the 2010 Global Burden of Disease Study [2], a shift to more dental disease as

demonstrated by dental caries will further contribute to the increasing burden of dental diseases in the ageing German population.

1.2 Oral bacterial microbiota

The microbes of the oral cavity of healthy individuals is highly complex and diverse, comprising a broad range of bacterial, archaeal, fungal and viral species, collectively termed the “oral microbiota”. The oral bacterial microbiota is well characterized when compared to other group of microbes [36]. The bacterial microbiota is quite unique in that it has a rapid regenerative ability [37], minimal influenced by genetics [38], relatively stable over time [39], and quiet similar among individuals in different geographic areas [40, 41]. It comprises the protective microbiota, transient invaders from food and other external sources, and opportunistic inhabitants [42]. The protective microbiota exert antagonism against the non-indigenous microbiota by occupying colonization sites making it difficult for non-indigenous species to become established, producing inhibitory substances, contributing to the innate immune response, and inducing low levels of circulating and secretory antibodies [43, 44]. These bacterial species usually proliferate in an attached multispecies biofilm community called dental plaque [45] because most of them require the presence of other species for attachment and/or nutrients [46].

The complex interplay within the plaque prior to the development of dental diseases has led to five main hypotheses [47]: the traditional non-specific plaque hypothesis (TNSPH), the updated non-specific plaque hypothesis (UNSPH), the specific plaque hypothesis (SPH), the ecological plaque hypothesis (EPH) and the keystone pathogen hypothesis (KPH). The TNSPH proposed that dental disease develops as a result the overgrowth of the entire plaque bacteria. The UNSPH submit that beyond the overgrowth of the entire plaque, increase in virulence and changes in the microbial composition of the plaque that leads to production of noxious products contribute to the development of dental diseases. The specific plaque hypothesis suggests that overgrowth of few specific bacteria out of the total plaque is responsible for dental disease. The ecological plaque hypothesis attests to the UNSPH, and additionally important are ecological factors such as the presence of nutrients, pH and redox potential. The KPH maintains that specific bacteria are involved in dental diseases, albeit dependent on (some of) the remaining microbiota and host immune response. Indeed, the variety or more specifically, diversity of the bacterial microbiota and its transition during disease progression is very crucial in development of dental diseases [48]. Thus, the role of the bacterial microbiota, local environment, and susceptibility of the host in the development of dental diseases can be inferred from these hypotheses [49]. In fact, the bacterial microbiota must contain virulent species with chromosomal and extra-chromosomal genetic factors to initiate disease. The abundance of these bacteria must also exceed the threshold of the host. Furthermore, these bacteria must be located at appropriate anatomical sites, and the remaining bacterial microbiota must facilitate, or somewhat not hinder the process that leads to the development of the dental disease. Besides, the oral

cavity must promote the expression of the bacterial virulence and the host must also be susceptible [50].

The diversity of the bacterial microbiota in the oral cavity is also a principal factor that is necessary for the initiation of dental diseases. Interestingly, the composition and stability of this bacterial microbiota vary according to the anatomical site [51-53]. The mucosa that covers regions such as the cheek, gingiva, palate and the floor of the mouth is characterized by a continuous desquamation of its surface epithelial cells and adhering bacteria [54]. Nevertheless, the bacteria on the papillary surface of the tongue, gingival crevice and tooth surfaces are less prone to shedding [55, 56]. Additionally, the tooth surfaces also have varying bacterial composition. The rough surfaces and protected areas are associated with the larger microbial communities when compared to those on smoother and more open surfaces that are exposed to the prevailing environmental conditions [56].

1.3 Salivary bacterial microbiota, dental diseases and the number of teeth

Bacterial microbiota and in fact species associated with periodontitis, dental caries, and endodontitis dislodge from various oral sites into the two major oral physiological fluids: the gingival crevicular fluid and the saliva. The gingival crevicular fluid is an exudate originating from plasma that passes through the gingiva to reach the gingival crevice and flows along teeth [52]. This fluid is enriched with bacteria from the gingival crevice and it finally enters the oral cavity where it mixes with the saliva [57]. The saliva is a mineral- and protein-rich oral fluid that is produced from the salivary glands and possesses several functions that are important for dental health [58]. In addition to gingival crevicular fluid, oral mucosal transudates, upper airways secretions and gastrointestinal reflux are also present in the saliva [59]. In fact, the salivary bacterial profile mirrors that of the throat, tonsils, superior surface of the tongue and other non-dental sites [53, 60] and it harbours more diverse bacterial species when compared to the teeth and gingival crevice [61]. Saliva act as a reservoir for bacterial colonization, enhances bacterial transmission in the oral cavity, and contains a specific bacterial community that helps maintain homeostasis of the mouth ecosystem [62, 63]. Moreover, the saliva is a practically optimal fluid for investigating the oral bacterial profile because its collection is simple and not invasive [64].

Epidemiological studies showed that periodontitis-associated bacteria [65-76], dental caries-associated bacteria [77-85] and endodontitis-associated bacteria [86] are accurately detected in the saliva. However, salivary bacterial composition are influenced by many factors such as age [87, 88], smoking [89] and saliva collection techniques [63]. The bacteria that are primary risk indicators of periodontitis include *Porphyromonas gingivalis*, *Treponema species, Selenomonas species*, *Campylobacter species*, *Fusobacterium species*, *Bacteroides species*, *Aggregatibacter actinomycetemcomitans*, *Prevotella species* and *Actinomyces species* [43]. Additionally, primary dental caries-associated bacteria include *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus gordonii*, and *Streptococcus oralis*, *Lactobacillus species* and *Prevotella species* [43] while *Atopobium species*, *Fusobacterium species*, *Parvimonas species*, *Dialister species*, *Prevotella species*, *Enterococcus species (such as Enterococcus faecalis)*, *Peptostreptococcus species* and some *Streptococcus species* are associated with endodontitis [4, 42, 90]. Unsurprisingly, some bacteria associated with periodontitis and dental caries have been found in endodontic lesions [91]. Salivary levels of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella intermedia* correlates with clinical periodontitis [70-74], salivary *Streptococcus mutans* [79, 80, 83, 85], *Lactobacillus* species [79, 83], *Prevotella species* [85] also correlates with clinically-diagnosed dental caries but the role of other bacteria such as non-mutans *streptococci* is still poorly understood [92].

Naturally, loss of teeth results in changes in the oral bacterial microbiota [93]. Besides, significant changes in oral bacterial microbiota occur following the loss of all natural teeth [94, 95]. The return of similar microbiota seems unachievable with denture as there is a significant difference in bacterial profile in individuals with all natural when compared to those with denture teeth [96]. In fact, the quantity of *Porphyromonas gingivalis* and *Prevotella intermedia* [97], *Porphyromonas gingivalis* and *Treponema denticola* [98], *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* [67], *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Campylobacter rectus* and *Treponema denticola* [99] is related to the number of teeth with periodontal pockets. Presumably, these teeth are eventually lost. Similarly, high levels of salivary *Streptococcus mutans* was associated with reduced a number of teeth [77, 100]. These findings suggest a relation between oral bacteria and the number of teeth. However, these findings need to be cautiously interpreted because bacteria are not naturally present in isolation (or in a few species). Therefore, beyond multiple pathogenic bacteria species, the entire oral bacterial community should also be considered. Interestingly, rapid high-throughput technologies such as the next-generation sequencing platforms have allowed comprehensive analysis of the oral bacteria [101], providing the opportunity to explore bacterial diversity [102]. The Human Oral Microbe Identification Microarray (HOMIM) and the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) are examples of this platform that determines oral bacteria up to the specie level; HOMINGS determines more bacteria when compared to the HOMIM [103].

Next-generation sequencing datasets typically have high sample dependence and high dimension (observations smaller than the number of variables). This is a challenge to analysing with conventional statistical methods that typically assume independence and low dimensionality [104]. High-dimensional data can be reduced by variable selection, variable combination, and a combination of variable selection and variable combination [105]. Indeed, variable combination procedures will not only determine important bacteria, but will also maintain the interactions and the multivariate structure of the bacterial community. Classical variable combination procedures such as principal component analysis (PCA) and factor analysis form linear transformations of variables to produce latent variables (linear combination of variables) [106] albeit they generate unstable results that are very complex and difficult to interpret [107]. However, another dimension reduction method, the treelet transform produce sparse latent variables from original variables that are easier to describe when compared to the classical methods [107,108]. From an epidemiological perspective, accurate description of the oral microbiota through this statistical method is crucial for

establishing relation with dentition status such as the number of teeth and other health parameters.

1.4 Other risk factors for tooth loss

Several risk factors, many of which are closely related to dental diseases, are linked to tooth loss [109]. One of the most important of these risk factors is smoking. Several studies reported that smokers as compared to nonsmokers are more likely to lose their teeth [110-116]. The evidence was further brought to the fore by recent findings in a large cohort of the European Prospective Investigation into Cancer and Nutrition-Potsdam (EPIC-Potsdam). It was reported that the association between smoking and tooth loss is stronger in men and younger adults. In fact, the risk of tooth loss among individuals who have quit smoking for between 10 to 20 years approached the risk among never smokers [117]. Smoking decreases intestinal uptake of calcium [118], decreases the antioxidative capacity of saliva [119], and also modifies the oral microbiota by supporting plaque-forming anaerobic bacteria [120].

Apparently, oral tissues undergo ageing-related morphological and functional changes that are unrelated to any pathological condition. These morphological changes include senescence of the periodontal ligament, gingival recession, porosity of the alveolar bone, and erosion of the enamel due to lifetime mastication [121]. Thus, age is an independent risk factor for tooth loss [122-125]. Moreover, sex differences also exist in the incidence and prevalence of tooth loss and edentulism [126]. The presence of steroid hormones in tissues adjacent to biofilm encourages the growth of certain bacteria triggering an exaggerated immune response leading to dental diseases [127]. The difference in the risk for tooth loss between women and men might also be due to sex/gender-related differences in socioeconomic status (SES) [128]. In addition, abdominal obesity worsens dental status resulting into tooth loss [129-131], although this link seems to be indirect through common behavioural and biological risk factors [132]. Independent of other risk factors, SES [124, 128, 133, 134], and childhood conditions [135] also play important role in tooth loss. Furthermore, alcohol consumption [112**,** 136-138] leads to tooth loss because the acid and sugar content supports dental caries, ethanol content causes dehydration of the mouth reducing the anti-bacterial effect of the saliva, reduces the intake of calcium and vitamin D and reduces bone mineral density [139, 140].

Food such as milk, cheese, whole grains, green tea, vegetables and fruits directly protect the teeth by preventing demineralization, interfering with bacterial attachment and/or biofilm formation, providing natural cleansing and neutralizing harmful acids in the mouth [141-144]. Moreover, flavonoid-rich foods through their anti-inflammatory properties improve the defence mechanisms of the periodontium [145, 146]. On the other hand, more natural teeth are associated with a good dietary capability and optimum nutritional intake [147]. In fact, edentates consume healthy foods when compared to dentates [148-150] and surprisingly dentures appear not to effectively alleviate the problem of reduced food intake that is associated with loss of natural teeth [151]. In addition, systemic chronic diseases

such as type 2 diabetes mellitus (T2DM) increases tooth loss due to elevated the inflammatory response among diabetics in the presence of periodontal pathogens resulting in tissue destruction and impaired wound healing [152]. Tooth loss might also be due to genetic predisposition, either due to the direct variation of some genes, such as the matrix γ-carboxyglutamate (Gla) protein gene that is associated with the number of teeth [153] or indirectly through genes that are associated with periodontitis and caries [154]. Undoubtedly, the strongest risk factor for tooth loss is unclear owing to these varying and interrelated risk factors beyond dental conditions. In fact, the relative importance of these risk factors might even be different across different populations. It was therefore not surprising that some researchers are of the opinion that focus should be given to the occurrence of tooth loss before dental caries and periodontitis [21].

1.5 Number of teeth and chronic systemic diseases

The relation between dental health and chronic systemic diseases was first reported about century ago [155]. In early and mid-1990s, there was an exponential increase in the number of articles addressing the relation dental health and chronic systemic diseases [156, 157]. The World Oral Health Report in 2003 further strengthens this evidence [158]. However, it is possible that this relation could have been confounded as a result of shared lifestyle risk factors [159, 160] and underlying inflammatory disease process [161, 162]. The most commonly reported chronic systemic diseases that is related to dental heath are cardiovascular disease (CVD), T2DM and cancer [163]. Indeed, a relation between the number of teeth and these chronic systemic diseases will be important since individuals that have lost one or more natural teeth are more likely to have had poor dental health.

Several epidemiological studies reported a link between the number of teeth and CVD, T2DM and cancer. Cross-sectional studies showed that reduced number of teeth is associated with clinical surrogate markers of CVD such as carotid artery plaque and intima-media thickness [164, 165], aortic valve sclerosis [166] and arterial stiffness [167] as well as hard end point such as and myocardial infarction [168, 169] and coronary artery disease [170, 171]. Similarly, case-control studies also showed that the number of missing teeth is associated with increased risk of myocardial infarction [172-176]. Additionally, prospective studies showed that edentates were at greater risk of coronary artery disease when compared to dentates, although no trend in risk was observed among the dentates [177]. Furthermore, cross-sectional studies [178, 179], prospective studies [180-182], and systematic review [183] showed that numbers of teeth lost is associated with stroke. A meta-analysis also suggests that a moderate association exist between number of missing teeth (and other measures of poor dental health) and CVD [184]. In fact, fewer number of teeth [185-190] and edentulousness [191] are independent predictors of CVD mortality, and also associated with improved CVD survival [192].

Prospective studies showed that the number of missing teeth [190] and total tooth loss are associated with T2DM [193]. Indeed, earlier [194] and more recent [195] intervention studies revealed that teeth extraction resulted in improved glycaemia control among individuals with T2DM. Furthermore, prospective studies also reported a relation between fewer numbers of teeth and increased risk of gastric cancer [196], pancreatic cancer [197], and lung cancer [198]. Evidence from meta-analysis also showed that tooth loss is risk factor for head and neck cancer [199, 200], oesophageal carcinoma [201], and gastric cancer [202].

Admittedly, arguments against the relation between the number of teeth and chronic systemic disease are completely justified. Most observational studies have been relatively

small sample sized and very few are prospective. Moreover, some of these studies failed to control adequately for measured confounding factors and unmeasured confounding factors such as SES and lifestyle factors, specifically smoking [177, 203, 205]. Besides, diet may play a central role (as confounder or mediator) in the relation between the number of teeth and chronic systemic diseases, especially CVD [203] since large prospective studies have showed that changes in diet [205] and CVD-related nutrients results in tooth loss [206]. Furthermore, most studies have focused on single chronic systemic disease outcome or closely-related diseases. Nevertheless, there appears to be a moderate degree of evidence that support a relation between the number of teeth and chronic systemic diseases which is stronger for CVD when compared to other diseases. Evidently, a causal relation between number of teeth and CVD is rather far-fetched.

As a matter of fact, it is important to investigate the relation between the number of teeth and multiple chronic systemic disease end points which can be competing outcomes. In this regard, significant findings, especially in large prospective studies will support previous evidences thereby ensuring that they are put in better perspective. Consequently, possible biological mechanisms can be investigated within such cohort.

1.5.1 Biological explanations of the relation between the number of teeth and chronic systemic diseases

The direct and indirect mechanisms or pathways that link poor dental health to chronic systemic diseases are metastatic infection (transient bacteraemia), metastatic injury (diffusible oral bacterial proteins or toxins) and metastatic inflammation (soluble antigen of oral bacteria inducing immunological injury) [160, 207]. For CVD, the direct systemic dissemination of oral bacteria and bacterial products that occur before, during and after tooth loss leads to the development of thrombosis and heightened atheroma formation [208] through the upregulation of the expression of platelet aggregation-associated protein [209].

Initial evidence for this possible dissemination is that individuals with nonfatal myocardial infarction harbour higher proportion of salivary levels of periodontal bacteria when compared to healthy individuals [210]. In fact, DNA of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* has been detected in atherosclerotic plaques [211, 212] and occluded arteries [213]. Furthermore, high levels of antibodies to *Porphyromonas gingivalis* [214, 215] and antibodies to combinations of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella Forsythia* and *Treponema Denticola* [216] predicted myocardial infarction independent of classical risk factors. Evidence in mice models revealed systemic dissemination of *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Fusobacterium nucleatum* into cardiovascular tissues [217]. Similarly, cariogenic *Streptococcus mutans* have been detected in aortic valve, mitral valve and aortic aneurysmal wall specimens [218] and haemorrhagic stroke patients have higher salivary *Streptococcus mutans* carrying the collagen-binding Cnm gene [219]. Furthermore, overabundance of *Fusobacterium nucleatum* and other oral bacteria have been reported in colorectal cancer [220-222].

Additionally, tooth loss is indirectly related to CVD through inflammatory processes. As dental disease progresses to a state of tooth loss, oral bacteria provoke systemic inflammatory response by the elevation of proinflammatory cytokines and acute-phase proteins [223]. Chronic activation of innate and adaptive inflammatory pathways clearly contributes to the initiation and progression of CVD [203, 224, 225]. Acute-phase proteins such as the high-sensitivity C-reactive protein (hsCRP) is elevated in response to oral bacteria [226-228] and its elevation is undoubtedly associated with incident CVD [164, 189]. Immune-mediated processes can also indirectly contribute to the pathogenesis of CVD through exhaustion of the immune system and consequent production of autoantibodies. Antibodies against phosphorylcholine and cardiolipin [229], and anti-citrullinated protein autoantibodies (ACPA) [230] have been linked to CVD. ACPA are particularly important in understanding the link between the number of teeth and CVD because *Porphyromonas gingivalis* through the actions of its unique peptidyl-arginine deiminase and gingipains

initiates early protein citrullination generating neoepitopes, which eventually results in break of immune tolerance, development of autoimmunity and the production of ACPA [231]. For T2DM, increase in levels of systemic proinflammatory mediators seems to be the major bidirectional link with tooth loss and dental diseases [232]. Furthermore, the relation between number of teeth and cancer could be as a result of infection-associated cell pathology, inflammation-associated cell pathology and microbial carcinogen metabolism [233].

1.6 Public health implication

There is no doubt that tooth loss is widespread [2, 35]. The psychosocial impact of tooth loss and subsequent denture wearing is overwhelming [234]. These consequences also include pain, discomfort, impaired mastication, speech, and sleeping, and social interactions [235-237]. Moreover, alveolar bone loss following loss of teeth is associated with premature ageing, increased wrinkles and jowl development [6]. Additionally, tooth loss affects individual earnings due to cost associated with dental care and treatment as well as a direct impact on productivity relating to absenteeism from work or school [238]. Certainly the effect of tooth loss is not only limited to the mouth, but also affect general health and overall quality of life. It was reported that the presence of nine teeth or less in the maxillary bone has a negative effect on the physical index of quality of life, which is surprisingly higher than that due to cancer. As a matter of fact, removable partial or fixed prostheses do not significantly improve the negative effect of tooth loss on psychological and physical indices of quality of life [239]. Additionally, a meta-analysis concluded that there is fairly strong evidence that tooth loss is associated with impairment of oral health-related quality of life [240].

The financial impact of tooth loss on the health services and the society is severe, and this impact is inseparable from dental diseases in general. Globally, dental diseases are the fourth most expensive disease to treat [241]. Their direct treatment cost was estimated at US$298 billion yearly, equivalent to an average of 4.6% of global health expenditure while the indirect cost was estimated at US$144 billion yearly, corresponding to economic losses within the range of the 10 most frequent global causes of death [242]. Within the European Union, economic impact and inequalities of treatment of dental diseases is also huge. This treatment services accounted for 5% of total health expenditure and 16% of private health expenditure. In fact, spending on dental treatment has steadily increased from €54 billion in 2000 to €79 billion in 2012, €84 billion in 2015 and is expected to increase to €93 billion by 2030, exceeding expenditure on CVD, cancer and dementia [243].

Similarly, in Germany approximately €22 billion representing 0.8% of the gross national income was spent on dental services in 2010. This cost has increased by 14% in the last five years [244]. Sadly, much of the burden of dental diseases is due to caries and its complications, and it is stronger in the disadvantaged, socially marginalized and vulnerable groups [245]. Interestingly, these conditions can be prevented, alleviated and cured effectively. During a shift from a treatment-oriented dental health care to a prevention-oriented one in the early 1990s, a reduction in total and public dental expenditure and improvement in dental health across all age groups was observed. Coincidentally, between 1997 and 2005, when there was a decrease in the total dental expenditure ratio from 1.2% to 0.8%, a decrease in dental contact frequency per capita from 1.3 to 1.2 and an increase in

the prevalence of dental disease were reported [246]. These data shows that dental diseases and more specifically tooth loss is surely a public health problem and it is therefore appropriate to be managed it using public health approaches [244].

Obviously, dental health can no longer be ignored and its dissociation from general health should be avoided. It is essential to protect natural teeth from being lost through dental education of the public to promote healthy oral behaviour as well as early detection and prevention of dental condition. There is strong evidence that the benefits of preventing tooth loss and dental conditions far exceed the costs of treatment, hence a considerable amount of funds can be saved. Given the high prevalence of tooth loss, the additional risk they contribute to future chronic systemic disease and its strong impact on the ageing population, the early recognition and appropriate cost-effective preventive measures addressing risk factors such as poor oral hygiene, tobacco use, excessive alcohol consumption and T2DM is paramount. To this end, a common risk factor approach has been agreed as an important strategy for the management, prevention and control of dental diseases [245]. Within this common risk factor approach, integrated preventive strategies are recommended for public health practice [247]. Data from Denmark and Sweden showed that significant reduction in the prevalence of dental diseases can be made through investment in the provision of cost-effective preventive services [243].

Taking a cue from the Fédération Dentaire Internationale (FDI)'s 'Global Goals for Oral Health 2020' [248], the German academic dentistry and the dental profession jointly issue national 'Goals for Oral Health in Germany 2020' [249]. Some of the aims were that oral health promotion and care should be integrated with other sectors that influence health and that common risk factors of oral health and general health should be addressed. It was also stated that systems and measures for oral health evaluation as well as social responsibility and ethical practices in the dental profession should be promoted. The target was that by 2020 the average missing teeth in 35-44 years old should be reduced to three, the prevalence of complete edentulism among 65-74 year olds reduced to less than 15%, and the prevalence of severe periodontitis among 35-44 and 65-74 year olds reduced to 10% and 20%, respectively. This might have contributed to the decline tooth loss [35] and reduction in the proportion of Germans with self-declared unmet needs from dental care [250]. Nevertheless, the WHO at its recent world congress emphasizes the growing problem of dental and oral diseases making a call to health policy-makers and professionals to significantly reduce the global burden by promoting greater equity, and integrating oral health promotion into the NCD prevention, control and developmental agenda [251].

1.7 Aims

In this thesis, we sought to investigate whether an association exists between the number of teeth and chronic systemic diseases in a large-scale prospective cohort study and if it does, whether we can elucidate this relation with respect to underlying biological mechanisms. Figure 1 displays some potential biological mechanisms linking the number of teeth and chronic systemic diseases.

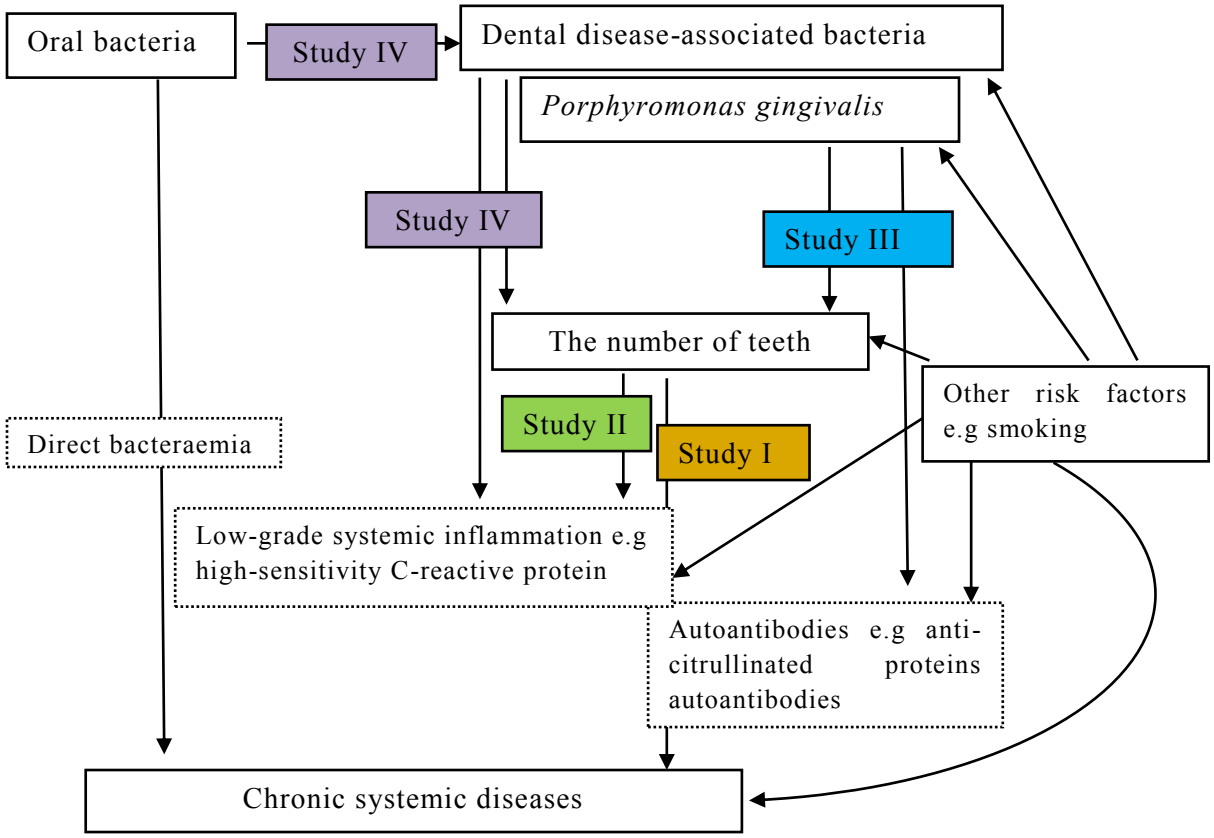

Figure 1: The number of teeth/ chronic systemic diseases association, some pathways explaining possible relationship and the focus of the present thesis depicted as study I-IV

The number of teeth is in the centre of the investigation coupled with questions regarding to the loss of teeth and their consequences. In this thesis it was only possible to address some aspects of the overall frame such as:

1. Is there an association between the number of teeth and chronic systemic diseases (study I)?

2. Is the number of teeth related to low-grade systemic inflammation (study II)?

3. Is *Porphyromonas gingivalis* associated with the number of teeth and an autoantibody (study III)?

4. Can treelet transform yield interpretable dental disease-associated bacterial profiles? If yes, is their association with the number of teeth and low-grade systemic inflammation dependent on the overall bacterial diversity and what is their relative importance as compared to other risk factors (study IV)?

2 Materials and methods

2.1 The European Prospective Investigation into Cancer and Nutrition-Potsdam study

This entire thesis is based on the participants of the European Prospective Investigation into Cancer and Nutrition-Potsdam (EPIC-Potsdam) study. The EPIC-Potsdam study is a cohort study that is part of the multicentre EPIC study. This study comprises 27,548 middle-aged participants between 35 and 64 years old recruited between 1994 and 1998 from the general population of Potsdam, Germany and adjacent communities using the general population registries (Figure 2). Boeing et al [252] presents details of study design and recruitment. Ethical approval for the study was obtained from the ethics committee of medical society of the federal state of Brandenburg, Germany and all participants gave written informed consent for participation in the study. The cohort was followed up every 2 to 3 years to obtain new lifestyle information and illness-related information. Information on incident chronic systemic diseases was linked with the local hospital and subsequent medical verification was made with the participant's physician [253].

2.2 Study I: Is there an association between the number of teeth and chronic systemic diseases?

2.2.1 Study population of study I

At baseline of the EPIC-Potsdam, anthropometric, sociodemographic and lifestyle information was collected through questionnaires and interviews. These include body mass index (BMI) calculated from body weight and height, age, sex, alcohol consumption (quantity per day), and smoking status (nonsmokers, current smokers [numbers of cigarettes smoked per day, smoking duration] and time since cessation for former smokers). Furthermore, educational attainment was expressed as vocational school or less, technical school and university. Vocational school or less implies a lower education of 10 years of school education with 2 years of additional professional training. Technical school implies 10 years of school followed by more than 2 years of professional training. Occupation was defined as higher-grade professionals, lower-grade professionals, skilled manual worker or non-manual employed, and simple manual workers. Physical activity information was obtained through a plethora of questions on occupational physical activity, cycling and sports activity. This information was used to assign participants to one of four categories (inactive, moderately inactive, moderately active, and active) of the Cambridge physical activity index [254].

In addition, dietary factors were assessed by a validated self-administered 148-item food frequency questionnaire (FFQ), aggregated into 49 separate food groups. Intake of antibiotics, vitamin and/or mineral supplements, nonsteroidal anti-inflammatory drugs

(NSAID) and hormone replacement therapy (HRT) were assessed by self-reports from interviews and FFQ. Moreover, prevalent myocardial infarction, stroke, T2DM, cancer, angina pectoris, heart failure, and transient ischaemic shock were self-reported and validated by a study physician using medical record review. Prevalent hypertension was defined as systolic blood pressure greater than 140 mm Hg or diastolic blood pressure greater than 90 mm Hg or self–reporting of a diagnosis or use of antihypertensive medication.

Importantly, at the fourth follow-up (between 2004 and 2006), participants provided information from a questionnaire on the number of teeth ("How many natural teeth do you have?") and number of teeth lost since baseline ("How many teeth have you lost since your visit to the study centre?") (Figure 2). Information on the history of periodontitis and bone loss in the mouth was also provided ("Has a dentist ever told you that you have periodontitis and /or that you are losing (or have lost) the bone around your teeth?"). The number of teeth present at baseline for each participant was calculated by the addition of the number of teeth present at the fourth follow-up and the number of teeth lost until the fourth follow-up. For this study, we considered the relation between the number of teeth and risk of four incident chronic systemic diseases: myocardial infarction, stroke, T2DM, and cancer.

2.2.2 Statistical analyses of study I

After excluding participants with missing data (n = 3,234) and implausible data (n= 1) on the number of teeth, the final analytic sample was 24,313 participants (14,953 women and 9,360 men). At first, participants were divided into five categories of the number of teeth (0, 1-17, 18-23, 24-27, and 28-32) with attention to 'healthy' number of teeth (normal presence / absence of one to four wisdom teeth) and complete edentates. We then compared some basic characteristics (or variables) across these five categories; continuous variables were expressed as the arithmetic mean (standard deviation) or median (interquartile range) while categorical variables were expressed as number and corresponding percentages.

Secondly, using Cox regression we checked whether there is interaction between categories of the number of teeth and some variables: age group (< 50years and ≥50years), sex, BMI group (<18-25 kg/m^2, >25-30 kg/m^2, and >30 kg/m^2), smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15, 15–24, and ≥25), history of periodontitis and bone loss in the mouth (yes or no) and prevalent T2DM (yes or no) in order to present sub-group analyses if any effect was significant. Further, we determined the association between the categories of the number of teeth groups (28-32 teeth as the reference) and each of the four chronic systemic diseases adjusted for covariates in four Cox regression models (unadjusted model, partial multivariable-adjusted model, full multivariable-adjusted model and competing risk analyses model). The partial

multivariable-adjusted model (model 2) was adjusted for age (continuous), sex, BMI (continuous), educational attainment (vocational school or less, technical school, and university), occupation (higher-grade professionals , lower-grade professionals, skilled manual worker or non-manual employed, simple manual workers), alcohol consumption (continuous), smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15, 15–24, and ≥25), physical activity (inactive, moderately inactive, moderately active, and active), and intake of vitamin and/or mineral supplements, antibiotics, NSAID, and HRT. The full multivariable-adjusted model (model 3) was additionally adjusted for prevalent hypertension, myocardial infarction, stroke, T2DM, cancer, angina pectoris, heart failure, transient ischaemic shock and diet (as three factors retained from factor analysis of all 49 food groups). In model 4, we conducted competing risk analyses based on model 3.

We test whether there is a linear trend in each model by employing orthogonal polynomial contrast. Additionally, we conducted analyses between the number of teeth on a continuous scale and each chronic systemic disease adjusted for covariates in model 3. Finally, using restricted cubic spline regression, we investigated nonlinear associations between the number of teeth and each chronic systemic disease adjusted for all covariates in model 3. To avoid over-fitting, we limit restricted cubic spline model to five change points (or knots) at teeth number: 0, 19, 25, 28, and 32 (5%, 27.5%, 50%, 72.5%, and 95% percentiles). For all models, we corrected for time-varying covariates by specifying linear interaction with time. Prior to these analyses, prevalent cases of each chronic systemic disease were excluded in all models of the disease. To exclude reverse causation bias in sensitivity analysis, incident cases in the first three years of follow-up were excluded.

2.3 Study II: Is the number of teeth related to low-grade systemic inflammation?

2.3.1 Study population study II

In order to investigate the role of systemic inflammation in the relation between number of teeth and chronic systemic disease, two subsamples was selected from the EPIC-Potsdam. The first subsample is 2,500 participants (1,526 women and 974 men). These participants were randomly selected in 2005. The number of teeth, anthropometric, sociodemographic and lifestyle information available on this subsample at the baseline recruitment in the EPIC-Potsdam study was used for the present study (Figure 2). Secondly, between 2010 and 2012, a subsample of 1,472 participants were randomly selected using a rectangular sampling scheme to ensure an approximately uniform sample spread across sex and three baseline age ranges (35-44 years, 45-54 years, and 55-64 years) from the 23,881 active EPIC-Potsdam participants. These selected participants were invited to the study centre for

various assessments such as whole body magnetic resonance imaging scans for the quantification of adipose and lean tissue compartments. 815 of these randomly selected participants agreed to participate in the study. In 2013, these 815 participants were reinvited and 728 (356 women and 372 men) returned for re-examination (Figure 2). During this visit, new information on the number of teeth ("How many natural teeth do you have?"), anthropometric, sociodemographic, lifestyle information and illness-related information were obtained.

High-sensitivity C-reactive protein (hsCRP), a marker of systemic inflammation was measured from the blood serum collected from the participants of the first subsample at baseline of the EPIC-Potsdam study. Similarly, hsCRP was also measured from blood serum collected from the participants of the second subsample in 2013. The measurement of the first subsample and second subsample was done in 2008 and 2013 respectively. The immunoturbidimetric assay implemented on automated analysers was used to measure hsCRP in all samples. HsCRP was expressed in milligrams per litre.

2.3.2 Statistical analyses of study II

We excluded 61 participants who are in both subsamples in order to ensure that the subsamples are independent. Thus, the final analytical sample for the first subsample and second subsample was 2,439 and 728 participants, respectively. Participants were classified into five categories of the number of teeth (0, 1-17, 18-23, 24-27, and 28-32) similar to study I. Aside from being from the same cohort, these two subsamples are also comparable in that all variables used in the analyse were defined and expressed in a similar way. Age and sex-adjusted basic characteristics were compared across these categories of number of teeth. Continuous and categorical covariates were expressed as mean (standard deviation) and number with percentages, respectively.

Generalized linear models were fitted with log-transformed hsCRP as the dependent variable in five different models. The expected geometric means for each category of the number of teeth was computed and then back-transformed for presentation. To rule out conditions related to acute inflammation and non-CVD cause, we further excluded participants with hsCRP greater than 10.0 mg/L (69 participants from the first subsample and 12 participants from second subsample). The analytical sample for these analyses was 2,370 participants in the first subsample and 716 participants in the second subsample. At first we checked for possible effect modification by including single interaction terms between the category of the number of teeth and some covariates in univariate models. These covariates include two age categories (< 50years and ≥50years for first subsample and < 65years and ≥65years for second subsample), sex, three BMI categories (<18-25 kg/m^2, >25-30 kg/m^2, and >30 kg/m^2), smoking (nonsmokers, former-smokers, current

smokers (number of cigarettes/day: <15, 15–24, and ≥25), and history of periodontitis (Yes or No). Confounding variables were selected using directed acyclic graphs (DAGs). Model 1 was unadjusted. Model 2 was adjusted for age (continuous) , sex, BMI (continuous), educational attainment (currently in training/no certificate/skill, professional school or college of higher education/university), occupation (higher-grade professionals, lower-grade professionals, skilled manual worker/non-manual employed, simple manual worker) and hours of work [full time (>=35h/week) ,part time (15-<35h/week), hourly (<15h/week), jobless or retraining, early retirement/invalidity pension and unemployed], physical activity (sport and time watching television in hours per week[continuous]), alcohol intake that includes red wine (continuous) and smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15, 15–24, and ≥25). Model 3 was adjusted for hypertension (Yes or No), T2DM (Yes or No), cancer (Yes or No) and NSAID (Yes or No). Model 4 was adjusted for tea, fruit, vegetables and legumes (continuous). We determined if the relationship between the number of teeth and hsCRP in each of these models is linear by employing orthogonal polynomial contrast.

Furthermore, we investigated the nonlinear association between number of teeth and hsCRP adjusted for all covariates in model 4 using restricted cubic spline, with five knots at teeth number: 0, 19, 25, 28, and 32. Finally, both subsamples were combined in a meta-analysis (n=3,086) in order to obtain an overall mean hsCRP for each category of the number of teeth.

2.4 Study III: Is *Porphyromonas gingivalis* associated with the number of teeth and with an autoantibody?

2.4.1 Study population of study III

Among the 728 participants of the EPIC-Potsdam who returned for re-examination in 2013 (Figure 2), 704 provided unstimulated saliva which was collected by expectoration or passive drooling into a 15mL sterile plastic tube and was immediately transferred into PSP® SalivaGene System sampling kit to preserve the DNA. The saliva was aliquotted and stored at -80°C until analysis. Previous data of these participants relating to anthropometric, sociodemographic, lifestyle and illness-related information were retrieved.

2.4.2 Isolation of genomic DNA from saliva

Genomic DNA (gDNA) was isolated from saliva samples using the QIAamp® DNA mini kit (QIAGEN AB, Sollentuna, Sweden) according to the manufacturer's protocol on DNA purification from tissues. In brief, 300 mL aliquot of each saliva sample was centrifuged at 10,000 rpm for 15 min to pellet the sample (supernatants were discarded). 180 µL of buffer

ATL and 20 µL of QIAGEN® proteinase K were consecutively added to the pelleted sample. The mixture was vortexed and incubated at 56°C for 30 minutes, and purified using ethanol-containing buffers according to manufacturer's instructions. The gDNA was eluted in nuclease-free water, followed by spectrophotometric quantification and quality assessment (A260) with NanoVue spectrophotometer (GE Healthcare) and stored at -20°C until amplification by real-time polymerase chain reaction (PCR).

2.4.3 Detection of *Porphyromonas gingivalis* with real-time polymerase chain reaction

PCR was performed on the gDNA using the universal primer 16S to detect the presence of non-specific bacterial DNA species and with *Porphyromonas gingivalis* -specific primer to detect the presence of *Porphyromonas gingivalis*. The assays were carried out using 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Duplicate samples were assayed in a total volume of 20µl, containing 40 ng of template gDNA solution, TaqMan Universal PCR Master Mix (2x) (Applied Biosystems, USA), and the specific set of primers (final concentration 18µM) and probe (final concentration 5µM) (Cybergene AB, Sweden), corresponding to 900 nM of forward and reverse primer and 250 nM of the probe at the final 1x concentration. *Porphyromonas gingivalis* primers were: forward: GCG CTC AAC GTT CAG CC, reverse: CAC GAA TTC CGC CTG C, probe: 6-FAM–CAC TGA ACT CAA GCC CGG CAG TTT CAA–TAMRA, and 16s rDNA (universal) forward: TGG AGC ATG TGG TTT AAT TCG A, reverse: TGC GGG ACT TAA CCC AAC A, probe: 6-FAM–CAC GAG CTG ACG ACA RCC ATG CA–TAMRA [255]. The Applied Biosystems 7500 Fast Real-Time PCR System was used for the amplification and detection of *Porphyromonas gingivalis* gDNA in the eluted sample. After an initial incubation step of 2 min at 50°C and denaturation for 10 min at 95°C, 40 PCR cycles (95°C for 15 s, 60°C for 1 min) were performed. The relative quantity of *Porphyromonas gingivalis* was determined as the fold-difference in mean cycle threshold (CT) value of the *Porphyromonas gingivalis* genomic DNA relative to the mean CT value of the total bacterial DNA. The *Porphyromonas gingivalis* strain American Type Culture Collection (ATCC) 33277 was used as positive control.

2.4.4 Measurement of autoantibodies and other serum biomarkers

Due to the focus of this study on *Porphyromonas gingivalis*, a related serum anti-citrullinated protein autoantibodies (ACPA) was quantified using the highly sensitive and specific second generation anti-cyclic citrullinated peptides immunoglobulin G (anti-CCP2 IgG) antibodies assay (Immunoscan CCPlus® ELISA kit, Euro-Diagnostica, Malmö, Sweden) according to the manufacturer's instructions.

In addition to hsCRP, high density lipoprotein cholesterol (HDL) and triglyceride were measured by the enzymatic colorimeter assay and expressed in milligrams per decilitre.

Leukocyte count was measured by fluorescence flow cytometric method and expressed in gigaparticles per litre.

2.4.5 Statistical analyses of study III

We excluded 104 individuals with insufficient amount of gDNA for the detection of *Porphyromonas gingivalis*. There were no significant differences between these 104 individuals and the remaining 600 study participants with valid *Porphyromonas gingivalis* DNA and anti-CCP2 antibody results. These 600 participants were then categorized into tertiles according to the relative quantity of *Porphyromonas gingivalis* DNA. The first tertile comprised participants with a low *Porphyromonas gingivalis* DNA, second tertile with a moderate *Porphyromonas gingivalis* DNA and third tertile with a high *Porphyromonas gingivalis* DNA. Descriptive statistics were conducted in order to examine univariate correlates across these categories of *Porphyromonas gingivalis* DNA. Age and sex-adjusted basic characteristics were compared across these relative quantities of *Porphyromonas gingivalis* DNA. Continuous variables were expressed as the arithmetic mean (with standard deviation) while categorical covariates were expressed as number and percentages

We determined the relation between the categories of *Porphyromonas gingivalis* DNA and the number of teeth by negative binomial models which are appropriate for modelling overdispersed count data. In addition, Tweedie generalized linear models were applied to the relation between the categories of *Porphyromonas gingivalis* DNA and anti-CCP2 IgG. Anti-CCP2 IgG follows a Tweedie distribution because it has a point mass at zero and a continuously distributed component. Moreover, the variance function of anti-CCP2 IgG can be expressed as the dispersion parameter multiplied by the mean raised to a constant [256]. For both dependent variables (the number of teeth and anti-CCP2 IgG), we checked whether the association was modified by some covariates by including single interaction terms between categories of *Porphyromonas gingivalis* DNA and each covariate in univariate models. Strata-specific analyses were presented if the interaction term was statistically significant ($P<0.05$). These covariates include age categories (< 67years and ≥67years), sex, categories of the number of natural teeth (0, 1-17, 18-23, 24-27, and 28-32), BMI categories (<18-25 kg/m^2, >25-30 kg/m^2, and >30 kg/m^2), smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15 and 15–24), history of periodontitis (yes or no) , history of rheumatic conditions (yes or no), categories of hsCRP according to American Heart Association cut-off values (lower than 1.0 mg/L, between 1.0 and 3.0 mg/L, and values higher than 3.0 mg/L) , tertile of triglyceride/HDL ratio and leukocyte.

Furthermore, covariates that might confound the association between categories of *Porphyromonas gingivalis* DNA and these dependent variables were selected using DAGs.

These covariates were included as adjustment sets in three multivariable models in addition to the unadjusted model (model 1). Model 2 was adjusted for intrinsic covariates: age (continuous), sex and BMI (continuous). Model 3 additionally was adjusted for occupation (higher-grade professionals , lower-grade professionals, skilled manual worker/non-manual employed, simple manual worker), education (vocational training or less, technical school, university), work hours (full time (>=35h/week), part time (15-<35h/week), hourly (<15h/week), jobless or retraining, early retirement/invalidity pension and unemployed), hours of sport per week, alcohol (continuous), smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15 and 15–24). Model 4 additionally was adjusted for T2DM and cancer. The estimates were exponentiated as relative risks and interpreted as ratio of the arithmetic mean of the dependent variable between participants with high or moderate *Porphyromonas gingivalis* DNA as compared to the reference group with low *Porphyromonas gingivalis* DNA, adjusted for covariates. For all models of the number of teeth, we verified that the data are not overdispersed by the P-values of the Pearson chi-square greater than 0.05. Similarly, the power parameter in all models of anti-CCP2 IgG was set to achieve a good fit with a scaled Pearson chi-square of approximately 1. We also tested for linear trend across the categories of *Porphyromonas gingivalis* DNA.

Moreover, we assessed the robustness of these analyses by excluding participants with myocardial infarction, stroke and those who reported intake of antibiotics, NSAID for the number of teeth. In addition to the aforementioned, participants who use DMARD was also excluded for anti-CCP2 IgG. We investigated whether relation between *Porphyromonas gingivalis* DNA and these dependent variables might be nonlinear using restricted cubic spline, adjusted for all covariates in model 4 above. The spline of fold-difference of *Porphyromonas gingivalis* DNA was allowed to have four knots at the recommended 5th, 35th, 65th, and 95th percentiles. In order to ensure that the regression model is fit, we used Box-Cox power transformation to choose an optimal λ for the transformation of anti-CCP2 IgG.

2.5 Study IV: Can treelet transform yield interpretable dental disease-associated bacterial profiles? If yes, is their association with the number of teeth and low-grade systemic inflammation dependent on the overall bacterial diversity and what is their relative importance as compared to other risk factors?

2.5.1 Study population of study IV

The 281 participants for this study were randomly selected from the 704 participants who provided unstimulated saliva (Figure 2). The saliva gDNA was processed for detection and quantification of total bacteria.

2.5.2 Detection of saliva bacteria with Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS)

The total bacteria were determined from the saliva gDNA using the Human Oral Microbe Identification using Next Generation Sequencing (HOMINGS) according to a modified protocol [103, 257] at the Forsyth Institute, USA. Briefly, 10-50ng gDNA was amplified utilizing 341F/806R universal primers (V3-V4 region) and purified using AMPure beads. Next, a library of 100 ng was pooled, gel-purified, and subsequently quantified by qPCR. Finally, 12pM of the library was added to 20% Phix and sequenced on an Illumina MiSeq platform. For each sample, an average of more than 50,000 sequences of about 441 base pairs per sequence was obtained. Afterwards, species-specific 16S rRNA-based oligonucleotide probes were used in BLAST program (also called ProbeSeq for HOMINGS) written for MatLab to identify the frequency of each bacterial target (Operational Taxonomic Unit or OTU).

2.5.3 Statistical analyses of study IV

Basic characteristics were compared between men and women. Continuous and categorical variables were expressed as mean (standard deviation) and number with percentages, respectively.

2.5.3.1 Treelet transform of bacterial OTUs

We determined latent variables (or treelet components) according to the treelet transform steps [108] among the most frequent bacterial OTUs (in approximately 50 % of the study population). The initial application of treelet transform to a dataset yields a cluster tree and a coordinate system with components for the data at each cluster tree level (or cut-level). A data-driven approach was used to obtain an optimal cut-level since coordinate systems at different cut-levels are equally capable of describing the data if the number of components is large enough. In the first place, we decided to retain a favourable range between two to five treelet components. Next, successive three tenfold cross-validations with 30 and 40 Monte-Carlo repetitions was performed. The optimal cut-level that occurred more often and the corresponding number of treelet component were selected. For each bacterial OTU that load on the treelet components, we undertake extensive literature searches to identify which of the three main dental diseases these bacteria are often implicated. Thus, each treelet component was interpreted and assigned a dental disease-associated name.

Additionally, the choice of the optimal cut-level was verified in sensitivity analyses by conducting treelet transform at three cut-levels above and below the selected one (selected optimal cut-level±3). Furthermore, in three stability runs we assessed the frequency and stability of the treelet components in 80 % bootstrap-samples of the original data with 100

replications. The output of the stability run is the number of sign patterns that occurred in more than 10% of all bootstrap-samples. Changes in the sign patterns are compared to original treelet component. Therefore, a sign pattern is a retained component with loadings similar or equal to the loadings of an original extracted treelet component. The performance of the treelet transform was also compared to an established data reduction method, PCA with respect to proportion of explained variance and interpretability of bacterial OTUs loadings on retained factors. Finally, treelet component scores for all participants were calculated as follows:

$$\sum_{n=1}^{x} \text{value of standardized bacterial OTU, } x * \text{treelet component load of bacterial OTU, } x$$

Furthermore, bacterial diversity of the entire bacterial OTUs for every participant was captured using the Shannon index. This index considers abundance of each OTU such that high-abundance and low-abundance OTUs are weighted differently. This ensures that other bacteria that were not included in the treelet transform analysis are also considered. The Shannon index is the negative sum of the each OTU's proportional abundance multiplied by the natural logarithm of its proportional abundance.

$$\text{Shannon index} = -\sum \log(pi)pi, \text{ where pi is the relative frequency of each bacteria OTU.}$$

2.5.3.2 Multivariable analyses of the association between dental disease-associated bacteria scores and the number of teeth, and hsCRP

Finally, we determined the correlation between dental disease-associated bacteria scores and the number of teeth and also hsCRP stratified into two categories (low or high diversity) at the median value of the Shannon index. Model 1 is the unadjusted model and model 2 that was for age (continuous), sex, BMI (continuous), educational attainment (vocational school or less, technical school, and university), occupation (higher-grade professionals , lower-grade professionals, skilled manual worker or non-manual employed, simple manual workers), alcohol consumption (continuous), smoking (nonsmokers, former-smokers, current smokers (number of cigarettes/day: <15 and 15–24), and sports.

2.5.3.3 Relative importance of predictors of the number of teeth and hsCRP

In order to put the findings above in context of practical relevance, a random forests technique [258] with unbiased variable selection was used to assess the risk factors (variables) that are the most important predictors of the number of teeth and hsCRP. The random forests technique computes mean square error of regression (MSE) over multiple trees from random subsets of the data. Next, MSE is also computed after permuting each

variable. The differences between these MSE values are averaged and normalized by the standard error. A variable importance is measured by the percentage increase in its MSE (%IncMSE). The higher is the %IncMSE, the more important is a variable in predicting the number of teeth or hsCRP. This analysis was performed in subgroups of low and high bacterial diversity.

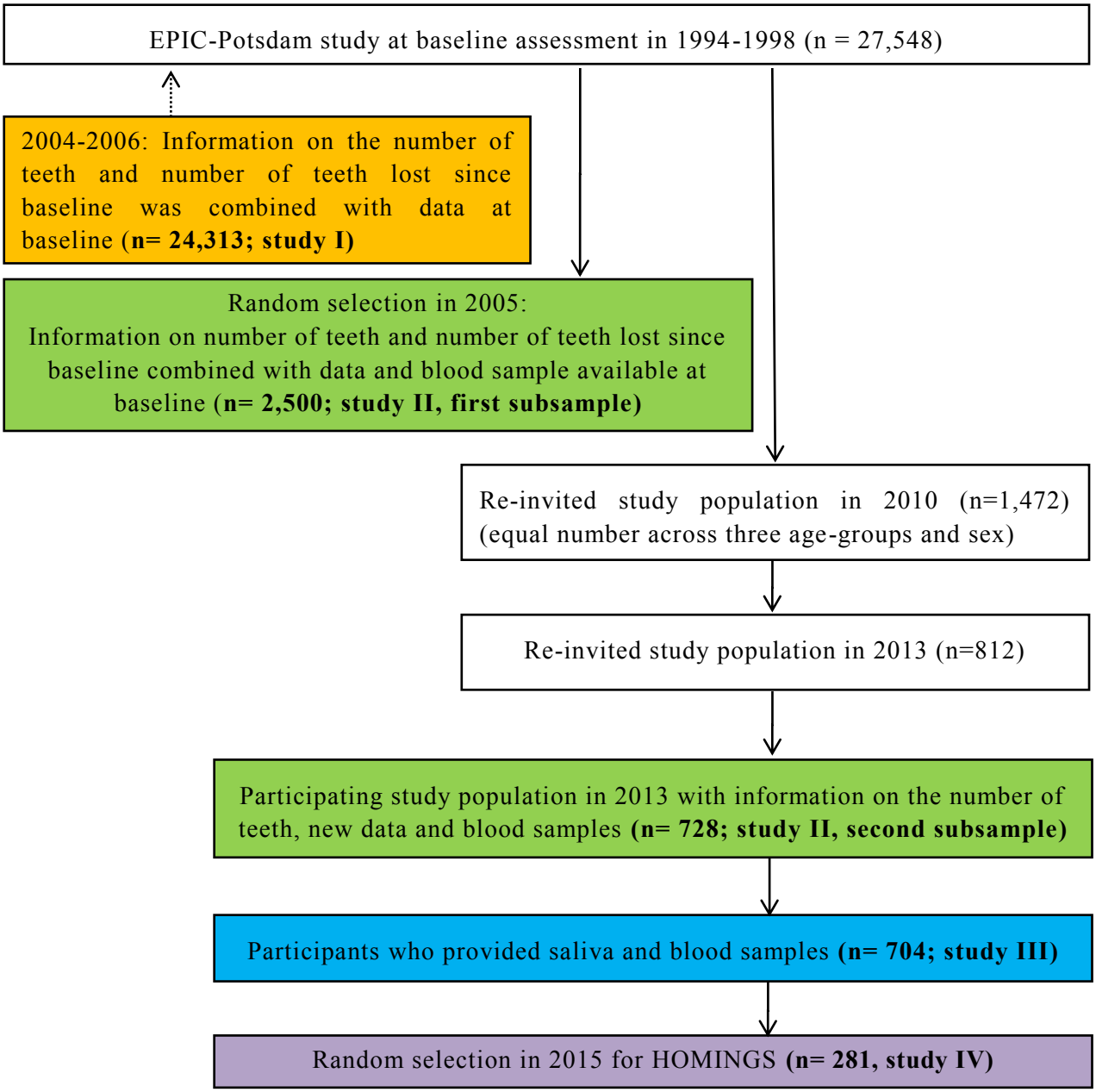

Figure 2: Flow chart of the participants of study I-IV as selected from the EPIC-Potsdam study

3 Results

3.1 Study I: Is there an association between the number of teeth and chronic systemic diseases?

3.1.1 Participant characteristics of study I

The participants comprised about 62% women. Their mean age was 50 years with women (49 years) relatively younger than men (52 years). The median number of teeth was 25 (25 in women compared to 26 in men). Table 1 shows that participants with fewer teeth were older, more likely to be current smokers but consume less alcohol, had lower physical activity, had lower educational attainment and more likely to do simple manual work. Participants with fewer teeth were also likely to have more prevalent hypertension, T2DM, myocardial infarction, stroke, cancer and periodontitis. These participants also eat less whole-grain bread. In addition, post-menopausal women with fewer teeth were less likely to take HRT. During a median of 8.3 years (range 1-13.1) follow-up, there were 233 (60 women and 173 men) incident cases of myocardial infarction, 225 (102 women and 123 men) incident cases of stroke, 987 (422 women and 565 men) incident cases of T2DM and 1,015 (536 women and 479 men) incident cases of cancer.

Table 1: Basic characteristics of participants in study I

	Number of Teeth				
	28-32	**24-27**	**18-23**	**1-17**	**0**
Number of Participants	8536	5976	3738	4366	1697
Age (years), mean (s.d)	47 (8.12)	49 (8.38)	52 (8.36)	56 (7.74)	55 (8.51)
Women, n (%)	5028(58.90)	3842 (64.29)	2427(64.92)	2676 (61.30)	983 (57.93)
Post-menopausal women, n (%)	715 (14.22)	776 (20.20)	809 (33.33)	1228 (45.89)	447 (45.47)
ANTHROPOMETRY					
BMI (kg/m^2), mean (s.d)	25.59 (4.01)	25.84 (4.08)	26.64(4.38)	27.19 (4.38)	27.32 (4.39)
EDUCATION, OCCUPATION AND LIFESTYLE FACTORS					
Vocational school or less, n (%)	2647 (31.01)	1996 (33.40)	1477(39.51)	2061 (47.21)	882 (51.97)
Simple manual worker, n (%)	183 (2.15)	119 (1.99)	132 (3.54)	213 (4.89)	119 (7.02)
Physical activity (active), n (%)	1486 (17.41)	962 (16.10)	565 (15.12)	663 (15.19)	239 (14.08)
Alcohol intake (g/dy) , mean (s.d)	15.14(19.84)	13.04(16.72)	12.9(17.11)	14.13 (19.59)	13.42 (18.72)
Current smokers, n (%)	1494 (17.50)	1069 (17.89)	740 (19.80)	1009 (23.11)	424 (24.99)
Cigarettes/day, mean (s.d)	11 (10.22)	12 (9.12)	13 (9.04)	13 (9.12)	14 (9.23)
PREVALENT DISEASE AT BASELINE					
Hypertension, n (%)	3516 (41.19)	2663 (44.56)	1943(51.98)	2427 (55.59)	982 (57.87)
T2DM, n (%)	249 (2.92)	199 (3.33)	197 (5.27)	352 (8.06)	176 (10.37)
Myocardial infarction, n (%)	75 (0.88)	73 (1.22)	62 (1.66)	1.26 (2.89)	76 (4.48)
Stroke, n (%)	60 (0.70)	49 (0.82)	44 (1.18)	67 (1.53	29 (1.71)
Cancer, n (%)	263 (3.08)	240 (4.02)	183 (4.90)	223 (5.11)	86 (5.07)
Periodontitis, n (%)	1034 (12.11)	862 (14.42)	680 (18.19)	1048 (24.00)	363 (21.39)
MEDICATION					
Multivitamin supplements, n (%)	624 (7.31)	447 (7.48)	332 (8.61)	284 (6.50)	112 (6.60)
NSAID, n (%)	154 (1.80)	126 (2.11)	111 (2.97)	169 (3.87)	69 (4.07)
Antibiotics, n (%)	46 (0.54)	34 (0.57)	15 (0.40)	21 (0.48)	8 (0.47)
HRT$^{\#}$, n (%)	208 (29.09)	217 (27.96)	178 (22.00)	251 (20.44)	68 (15.21)
DIET					
Whole grain bread (g/dy), median (IQR)	27.72 (62.67)	27.21 (59.53)	24.58(57.56)	20.25(53.84)	19.50 (58.32)

n= Number; s.d= Standard deviation; BMI=Body mass index; HRT=Hormone replacement therapy; T2DM= Type 2 diabetes mellitus; NSAID= Nonsteroidal anti-inflammatory drugs; IQR= Interquartile range; % = Percentage; $^{\#}$= Proportion of postmenopausal women**.** All variables other than number of participants, sex, and age were adjusted for sex and age. Number of participants and sex were unadjusted. Age was adjusted for sex only.

3.1.2 Multivariable analyses of the association between the number of teeth and myocardial infarction, stroke, type 2 diabetes mellitus, and cancer.

There was no significant interaction between the number of teeth and age group, and sex, BMI group, smoking status, history of periodontitis for any of the chronic systemic diseases.

The risk of myocardial infarction differed between the categories of the number of teeth. Although, there was progressive attenuation of the hazard ratio (HR) following adjustment for covariates the full multivariable-adjusted model (Table 2, myocardial infarction, model 3) showed that when compared to participants with 28-32 teeth, participants with 24-27 teeth, 18-23, 1-17 teeth and no teeth had 59% increased, 64% increased, 29% decreased and 3times increased risk of myocardial infarction, respectively. The competing risk model (Table 2, myocardial infarction, model 4) showed attenuation of all relative risk in the category of the number of teeth to 61% increased, 76% increased 10% decreased and 3times increased risk, respectively. All models showed significant linear trends of increasing risk of myocardial infarction with decreasing number of teeth. In fact, every one extra tooth was associated with three percent decreased risk of myocardial infarction (HR 0.97, 95% CI 0.96, 0.99; P-value= <0.01). Similarly, there was also overall increased risk of stroke among participants with lower number of teeth as compared to participants with 28-32 teeth. In the competing risk model of stroke, neither significant estimates nor a linear trend was observed (Table 2, stroke, model 4).

Table 2: Multivariable hazard ratio and 95% confidence interval of association between the number of teeth, myocardial infarction and stroke

Number of teeth			**Myocardial infarction**				
HR per number of tooth		**Category**	**Incident cases**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
			N	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR : HR (95% CI)**
	0.97 (0.96; 0.99)	28-32	40	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
P-value*	<0.01	24-27	44	1.65 (1.07; 2.59)	1.59 (1.02; 2.48)	1.59 (1.02; 2.48)	1.61 (0.98; 2.65)
		18-23	39	2.09 (1.32; 3.30)	1.68 (1.03; 2.73)	1.64 (1.02; 2.64)	1.76 (1.04; 3.00)
		1-17§	61	1.51 (0.72; 3.16)	0.82 (0.37; 1.80)	0.77 (0.36; 1.68)	0.90 (0.40; 2.21)
		0	49	5.99 (3.75; 9.56)	3.12 (1.84; 5.29)	2.91 (1.74; 4.86)	2.93 (1.61; 5.18)
		P-value ‡		<0.01	<0.01	0.03	0.04
Number of teeth			**Stroke**				
HR per number of tooth		**Category**	**Incident cases**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
			N	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
	0.99 (0.97; 1.01)	28-32	42	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	HR: Ref. (1.00)
P-value*	0.35	24-27	34	1.16 (0.73; 1.85)	1.00 (0.63; 1.59)	1.01 (0.64;1.62)	0.94 (0.48; 1.50)
		18-23	49	2.50 (1.63; 3.85)	1.66 (1.06; 2.58)	1.64 (1.05; 2.63)	1.50 (0.96; 2.43)
		1-17	57	2.15 (0.99; 4.65)	1.07 (0.47; 2.41)	1.06 (0.48; 2.36)	0.98 (0.42; 2.26)
		0	43	4.36 (2.63; 7.23)	2.07 (1.21; 3.54)	1.95 (1.13; 3.39)	1.82 (0.52; 3.21)
		P-value ‡		<0.01	<0.01	<0.01	0.09

Model 1: Age (continuous), sex, BMI (continuous), 1–17 teeth×time interaction; Model 2: Model 1+ education (3 categories), occupation (4 categories), smoking (5 categories), alcohol consumption (continuous), physical activity (Cambridge physical activity index), use of vitamin and/or mineral supplements,, antibiotics, hormone replacement therapy (women) and non–steroidal anti–inflammatory drugs, prevalent diseases (myocardial infarction: prevalent hypertension, angina pectoris, heart failure, transient ischaemic shock, stroke, type 2 diabetes mellitus and cancer; stroke: prevalent hypertension, angina pectoris, heart failure, myocardial infarction:, transient ischaemic shock, T2DM and cancer); Model 3: Model 2+ three retained factors from factor analysis of 49 food groups; Model 4: Model 3 + competing risk events of other three incident diseases. All models showed no violation of the proportional hazard assumption. n=Number; HR= Hazard ratio, CI= Confidence interval. * P-value for association; ‡ P-value for linear trend

Furthermore, there were linear trends of increasing risk of both T2DM and cancer with decreasing number of teeth (Table 3, model 1). However, for both diseases, linear trends were no longer observed following further covariate adjustment.

Table 3: Multivariable hazard ratio and 95% confidence interval of association between the number of teeth, T2DM and cancer

Number of teeth			**Type 2 diabetes mellitus**				
HR per number of tooth		**Category**	**Incident cases**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
			N	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
	1.00 (0.99;1.01)[§]	28-32	248	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
P-value[*]	0.96	24-27	212	1.17 (0.97; 1.42)	1.04(0.85; 1.27)	1.04 (0.85; 1.26)	1.05 (0.86; 1.29)
		18-23	174	1.62 (1.33; 1.98)	1.13 (0.91; 1.41)	1.10 (0.89; 1.35)	1.13 (0.91; 1.42)
		1-17	250	1.69 (1.21; 2.34)	0.88 (0.63; 1.24)	0.85 (0.61; 1.20)	1.06 (0.85; 1.31)
		0	103	2.04 (1.55, 2.68)	1.08 (0.81; 1.44)	1.04 (0.78; 1.36)	0.98 (0.73; 1.33)
		P-value [‡]		0.01	0.71	0.93	0.92
Number of teeth			**Cancer**				
HR per number of tooth		**Category**	**Incident cases**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
			N	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
	1.00 (0.99;1.01)[§]	28-32	299	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
P-value[*]	0.45	24-27	242	1.21 (1.03; 1.43)	1.06 (0.9; 1.26)	1.17(0.96; 1.44)	1.02 (0.86; 1.22)
		18-23	159	1.25 (1.03; 1.50)	0.90 (0.74; 1.09)	1.13 (0.88; 1.45)	0.87 (0.70; 1.07)
		1-17	218	1.53 (1.08; 2.17)	0.89 (0.62; 1.28)	1.30 (0.85; 1.98)	0.83 (0.68; 1.01)
		0	97	1.69 (1.31; 2.18)	1.01 (0.78; 1.32)	1.14 (0.83; 1.56)	1.09 (0.83; 1.43)
		P-value [‡]		<0.01	0.34	0.93	0.92

§ : Same as in Table 2; Model 1: Same as in Table 2; Model 2: Same as in Table 2; Model 3: Model 2+ three retained factors from factor analysis of 49 food groups. Model 4: Same as in Table 2. For both T2DM and cancer, all models showed no evidence that proportional hazard assumption was violated.
n=Number; HR= Hazard ratio, CI= Confidence interval * P-value for association; ‡ P-value for linear trend.

Moreover, no nonlinear association was observed between the number of teeth and myocardial infarction (P-value = 0.11) (Figure 3a). However, a significant nonlinear association between number of teeth and stroke was observed (P-value = 0.01) (Figure 3b). There was a stable increased risk of stroke between no teeth (edentulousness) and 22 teeth followed by a sharp significant decrease risk up to 32 teeth. We investigated this relationship further by dividing the data into two strata (those with the number of teeth <22 and >22) and examined the linear effects. There was a linear decline in risk of stroke with increasing number of teeth in those individuals with number of teeth >22 (HR: 0.98, 95% CI 0.97, 0.99; P-value= 0.02). In the same vein, the number of teeth showed no nonlinear associations with either T2DM or cancer (Figures 3c and 3d).

Exclusion of incident cases of all chronic systemic disease in the first three years of follow-up did not change the results of the current study.

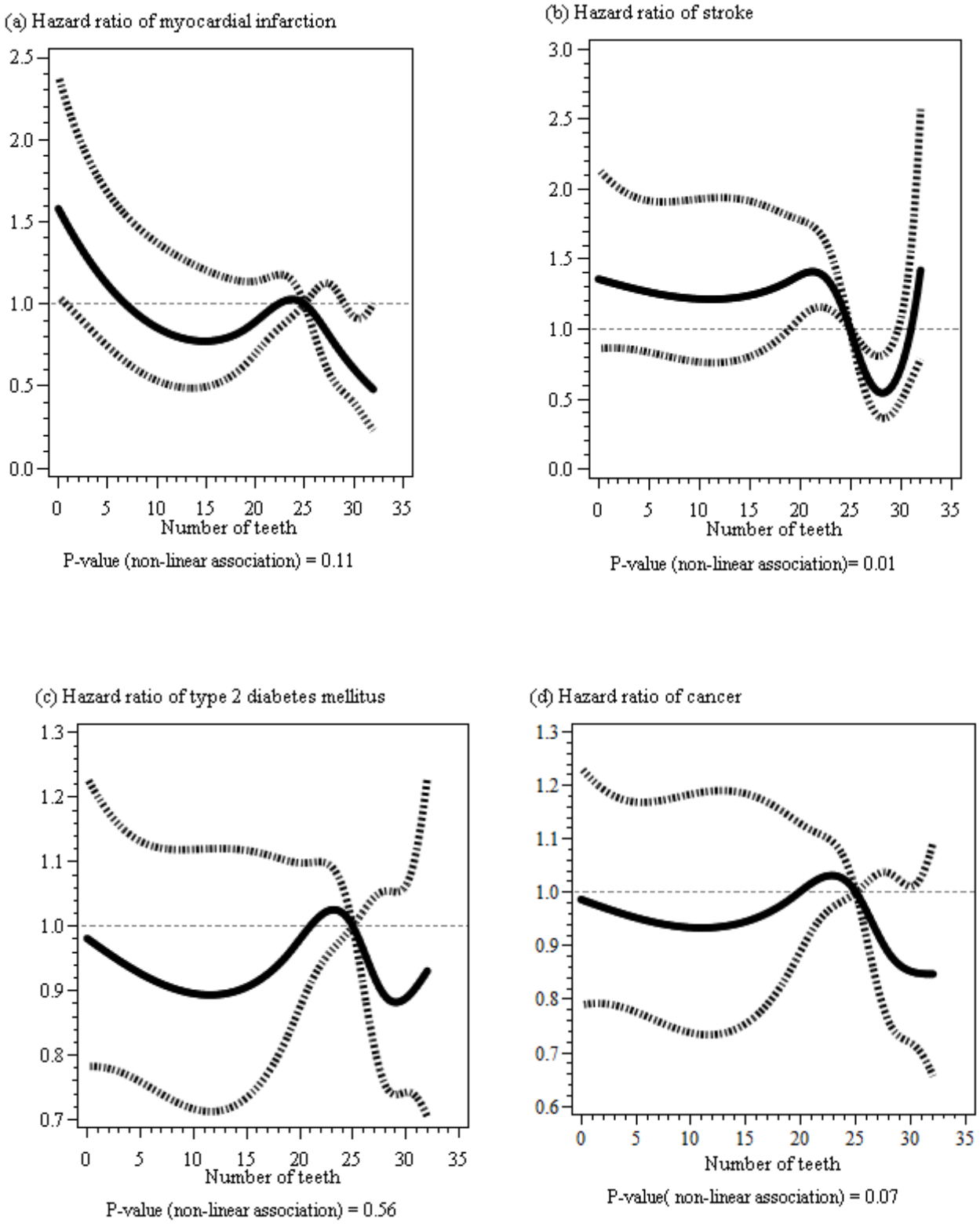

Figure 3: Hazard ratio and 95% pointwise confidence band (estimated by restricted cubic splines) of (a) myocardial infarction and (b) stroke (upper panels); (c) type 2 diabetes mellitus and (d) cancer (lower panels) per one unit increase in number of teeth.

3.2 Study II: Is the number of teeth related to low-grade systemic inflammation?

3.2.1 Participant characteristics of study II

Among the 2,439 participants of the first subsample, approximately 62% were women. The mean age was 50 years with women relatively younger than men (49 years compared to 52 years). The mean number of teeth was 22 which were similar in both sexes. Table 4 showed that those with fewer number of teeth were likely to have a higher BMI, lower number of years of formal education, more likely to be current smokers and more likely to have cancer.

Table 4: Basic characteristics of participants of study II (first subsample) according to categories of number of teeth (n= 2,439)

Participants characteristics	**Number of teeth**				
	28-32	**24-27**	**18-23**	**1-17**	**0**
Number of Participants	747	575	301	381	435
Women, n (%)	441 (59.04)	386 (67.13)	190 (63.12)	224 (58.79)	259 (59.54)
Age (years) , mean (s.d)	46 (8.20)	49 (8.15)	52 (8.15)	56 (8.20)	53 (8.13)
BMI (kg/m^2), mean (s.d)	25.91 (4.37)	25.89 (4.31)	26.24 (4.16)	26.46 (4.29)	26.60 (4.17)
EDUCATION AND LIFESTYLE FACTORS					
Vocational school or less, n (%)	223 (29.85)	192 (33.39)	109 (36.21)	174 (45.67)	223 (51.26)
Higher-grade professionals, n (%)	216 (28.92)	156 (27.13)	76 (25.25)	75 (19.69)	75 (17.24)
Sport (hours/week), mean (s.d)	2.26 (3.55)	1.97 (3.60)	1.92 (3.50)	1.87 (3.71)	1.93 (3.55)
Watching television (hours/day), mean (s.d)	1.85 (1.09)	1.83 (1.20)	2.05 (1.04)	2.16 (1.17)	2.17 (1.04)
Current smoker, n (%)	136 (18.21)	105 (18.26)	57 (18.94)	92 (24.15)	126 (28.97)
Cigarettes/day, mean (s.d)	11 (5.60)	12 (5.74)	12 (5.81)	15 (6.28)	15 (6.62)
Alcohol (grams/day), mean (s.d)	15.50 (19.95)	13.99 (19.18)	15.08 (19.26)	14.56 (19.91)	14.02 (19.40)
PREVALENT DISEASES					
Periodontitis, n (%)	253 (33.87)	196 (34.09)	111 (36.88)	158 (41.47)	43 (9.89)
Hypertension, n (%)	304 (40.70)	251 (43.65)	160 (53.16)	236 (61.94)	247 (56.78)
T2DM, n (%)	19 (2.54)	18 (3.13)	22 (7.31)	22 (5.77)	30 (6.90)
Myocardial infarction, n (%)	10 (1.34)	8 (1.39)	4 (1.33)	9 (2.36)	21 (4.83)
Stroke, n (%)	5 (0.67)	20 (0.35)	6 (1.99)	9 (2.36)	5 (1.15)
Cancer, n (%)	27 (3.61)	27 (4.70)	14 (4.65)	25 (6.56)	33 (7.59)
MEDICATION					
NSAID, n (%)	6 (0.80)	11 (1.91)	5 (1.66)	11 (2.89)	14 (3.22)
DIET					
FV (grams /day),mean (s.d)	62.38 (38.26)	62.30 (37.89)	68.18 (37.82)	66.57 (38.84)	66.70 (37.96)

n= Number; s.d= Standard deviation; BMI= Body mass index; T2DM= Type 2 diabetes mellitus; NSAID=Nonsteroidal anti-inflammatory drugs; FV= Fruit and vegetables; % = Percentage;
All variables other than number of participants, sex, and age were adjusted for sex and age.
Number of participants and sex were unadjusted. Age was adjusted for sex only.

Furthermore, about 49% of the 728 participants in the second subsample were women. The mean age was 67 years; women were relatively younger than men (66 years compared to 68 years). The mean number of teeth was 20, which was slightly lower in women compared to men (19 teeth compared to 20 teeth). Table 5 showed that participants with lower number of teeth were older and were more likely to spend more hours watching television.

Table 5: Basic characteristics of the participants of study II (second subsample) according to categories of number of teeth (n= 728)

Participants characteristics	**Number of teeth**				
	28-32	**24-27**	**18-23**	**1-17**	**0**
Number of Participants (%)	190 (26.10)	176 (24.18)	109 (14.97)	192 (26.37)	61 (8.38)
Women, n (%)	79 (41.58)	92 (52.27)	52 (47.71)	99 (51.56)	34 (55.74)
Age (years), mean (s.d)	63 (7.44)	65 (7.43)	69 (7.41)	72 (7.34)	72 (7.42)
BMI (kg/m^2), mean (s.d)	27.11 (4.41)	26.91 (4.38)	27.76 (4.28)	28.14 (4.30)	26.80 (4.30)
EDUCATION AND LIFESTYLE FACTORS					
Vocational school or less, n (%)	55 (28.95)	55 (31.25)	32 (29.36)	77 (40.10)	24 (39.34)
Higher-grade professionals, n (%)	60 (31.58)	54 (30.68)	22 (20.18)	44 (22.92)	14 (22.95)
Sport (hours/week), mean (s.d)	4.95 (7.44)	4.96 (7.30)	4.52 (7.20)	5.37 (7.34)	5.21 (7.26)
Watching television (hours/day), mean (s.d)	2.64 (1.52)	2.89 (1.46)	2.97 (1.46)	3.00 (1.52)	3.16 (1.41)
Current smokers, n (%)	14 (7.37)	10 (5.68)	16 (14.68)	28 (14.58)	8 (13.11)
Cigarettes/day, mean (s.d)	8 (6.51)	6 (6.45)	12 (6.40)	11 (6.35)	11 (6.62)
Alcohol (grams/day), mean (s.d)	13.52(15.71)	13.34(15.26)	11.81(15.24)	11.86(15.52)	10.27(15.31)
PREVALENT DISEASES					
Periodontitis, n (%)	78 (41.05)	58 (32.95)	33 (30.28)	60 (31.05)	21 (34.43)
Hypertension, n (%)	87 (45.79)	83 (47.16)	69 (63.30)	119 (61.98)	37 (60.66)
T2DM, n (%)	10 (5.26)	9 (5.11)	13 (11.93)	29 (15.10)	6 (9.84)
Myocardial infarction, n (%)	0 (0.00)	3 (1.70)	3 (2.75)	12 (6.25)	3 (4.92)
Stroke, n (%)	3 (1.58)	1 (0.57)	4 (3.67)	4 (2.08)	3 (4.92)
Cancer, n (%)	1 (0.53)	2 (1.14)	1 (0.92)	4 (2.08)	0 (0.00)
MEDICATION					
NSAID, n (%)	17 (8.95)	16 (9.09)	12 (11.01)	21 (10.94)	8 (13.11)
DIET					
FV (g /day),mean (s.d)	61.33 (2.58)	65.48 (2.61)	58.63 (3.32)	62.74 (2.53)	54.41 (4.44)

n= Number; s.d= Standard deviation; BMI= Body mass index; T2DM= Type 2 diabetes mellitus; NSAID= Nonsteroidal anti-inflammatory drugs; FV= Fruit and vegetables; % = Percentage;
All variables other than number of participants, sex, and age were adjusted for sex and age.
Number of participants and sex were unadjusted. Age was adjusted for sex only.

3.2.2 Multivariable analyses of the association between the number of teeth and hsCRP

There was no evidence of effect modification of the association between the number of teeth and hsCRP by age group, sex, BMI, smoking status and history of periodontitis in both subsamples.

Table 6 presents results for the unadjusted and multivariable-adjusted mean hsCRP across category of the number of teeth. The mean hsCRP increases with decreasing number of teeth in the unadjusted model of both subsamples but not in the combined samples. Moreover, no linear trend was observed in any adjusted model. The full multivariable-adjusted models showed that participants with 28-32 teeth, 24-27, 18-23, 1-17 and no teeth had mean hsCRP of 1.32 mg/L, 1.39 mg/L, 1.54 mg/L, 1.38 mg/L and 1.48 mg/L, respectively (model 5, first subsample); mean hsCRP of 1.64 mg/L, 1.67 mg/L, 1.73 mg/L, 1.47 mg/L and 1.87 mg/L, respectively (model 5, second subsample) and mean hsCRP of 1.49 mg/L, 1.53 mg/L, 1.64 mg/L, 1.44 mg/L, and 1.65 mg/L (model 5, meta-analyses). The increase hsCRP in the combined study was only observed between 28-32 teeth to 18-23 teeth.

Table 6: Mean of high-sensitivity C-reactive protein across categories of the number of teeth

	Number of teeth		**High-sensitivity C-reactive protein**			
			Model 1	**Model 2**	**Model 3**	**Model 4**
		n	**Mean (s.e)**	**Mean (s.e)**	**Mean (s.e)**	**Mean (s.e)**
First subsample (n=2,370*)	28-32	730	1.19 ; 0.07	1.32 ; 0.07	1.32 ; 0.07	1.32 ; 0.07
	24-27	563	1.32 ; 0.08	1.39 ; 0.07	1.39 ; 0.07	1.39 ; 0.07
	18-23	291	1.59 ; 0.11	1.55 ; 0.10	1.54 ; 0.10	1.54 ; 0.10
	1-17	367	1.56 ; 0.10	1.38 ; 0.09	1.39 ; 0.09	1.38 ; 0.09
	0	419	1.62 ; 0.09	1.49 ; 0.09	1.48 ; 0.09	1.48 ; 0.09
Ptrend			0.01	0.34	0.34	0.39
Second subsample (n=716*)	28-32	186	1.47 ; 0.07	1.65 ; 0.07	1.65 ; 0.07	1.64 ; 0.07
	24-27	174	1.54 ; 0.07	1.68 ; 0.07	1.67 ; 0.07	1.67 ; 0.07
	18-23	107	1.83 ; 0.09	1.73 ; 0.08	1.73 ; 0.08	1.73 ; 0.08
	1-17	189	1.70 ; 0.07	1.46 ; 0.07	1.47 ; 0.07	1.47 ; 0.07
	0	60	1.92 ; 0.12	1.86 ; 0.11	1.85 ; 0.11	1.87 ; 0.11
Ptrend			0.05	0.75	0.75	0.66
Meta-analysis of both subsamples (n=3,086)	28-32	916	1.33 ; 0.11	1.49 ; 0.21	1.49 ; 0.21	1.49 ; 0.21
	24-27	737	1.43 ; 0.15	1.54 ; 0.18	1.53 ; 0.17	1.53 ; 0.17
	18-23	398	1.72 ; 0.12	1.66 ; 0.07	1.64 ; 0.08	1.64 ; 0.08
	1-17	554	1.65 ; 0.08	1.43 ; 0.08	1.44 ; 0.08	1.44 ; 0.08
	0	479	1.76 ; 0.11	1.67 ; 0.20	1.65 ; 0.20	1.65 ; 0.20
Ptrend			0.27	0.69	0.66	0.66

Model 1: Unadjusted
Model 2: Adjusted for age (continuous); sex, BMI (continuous), occupation (4 categories), education (3 categories), work hours (6 categories), hours of sport per week (continuous) and hours of watching television per day (continuous), alcohol (continuous), smoking status (5 categories)
Model 3: Adjusted for hypertension (2 categories), type 2 diabetes mellitus (2 categories), cancer (2categories), nonsteroidal anti-inflammatory drugs (2 categories)
Model4: Adjusted for tea, fruit, vegetables and legumes (continuous)
n= Number; s.e= standard error,
* excluding participants with hsCRP >10mg/L from the original subsample.

Additionally, the restricted cubic spline for the full multivariable-adjusted models in both subsamples showed no nonlinear association between number of teeth and hsCRP (Figures 4a and 4b).

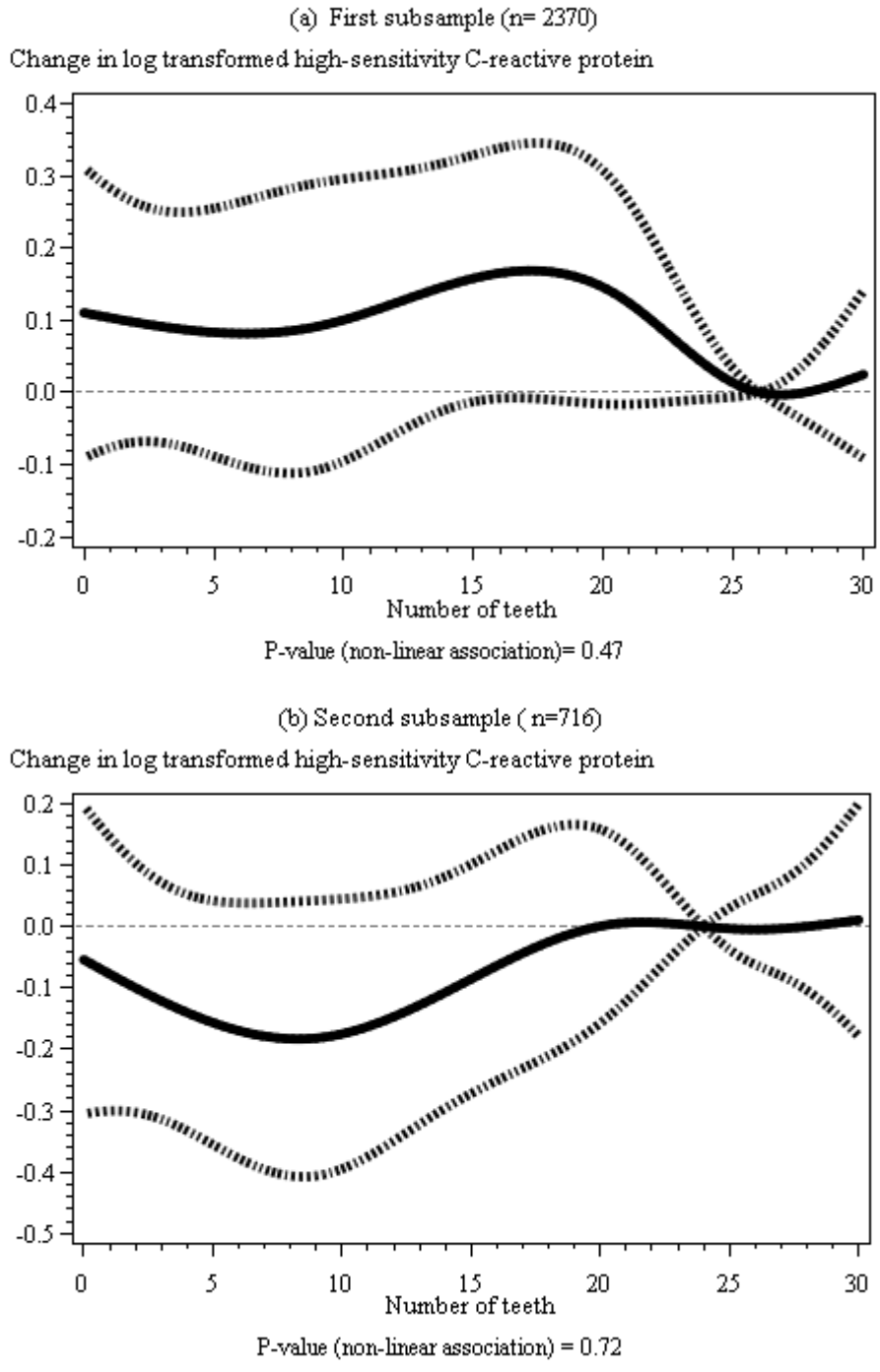

Figure 4: Functional relation (and 95% pointwise confidence band) between the number of teeth and beta coefficient (multivariate adjusted) of high-sensitivity C-reactive protein estimated by restricted cubic splines (a) first subsample and (b) second subsample

3.3 Study III: Is *Porphyromonas gingivalis* associated with the number of teeth and with an autoantibody?

3.3.1 Participant characteristics of study III

The mean age of the study population is 67 years. The study population is composed of 292 women (mean age 66 years) and 308 men (mean age 68 years). Across increasing quantities of *Porphyromonas gingivalis* DNA, there was increasing sport time, increasing alcohol intake, increasing leukocyte count and increasing proportion of participants with T2DM. Conversely, there was decreasing proportion of participants who were high-grade professionals and decreasing proportion with hypertension (Table 7).

Table 7: Basic characteristics of the participants of study III (n=600)

	Study population (n=600)	Relative quantity of P.g DNA		
		Low (n=200)	Moderate (n=200)	High (n=200)
Women, n (%)	292 (49)	94 (47)	101 (51)	97 (49)
Age (years), mean (s.d)	67 (8.32)	67 (8.05)	67 (8.81)	67 (8.12)
Body mass index (kg/m^2), mean (s.d)	27.39 (4.28)	27.38 (4.83)	27.22 (3.92)	27.56 (4.04)
Vocational training or less, n (%)	213 (36)	76 (38)	68 (34)	69 (35)
Higher-grade professionals, n (%)	157 (26)	56 (28)	54 (27)	47 (24)
Sport (hours/week), mean (s.d)	5.08 (7.53)	4.74 (6.21)	5.18 (5.69)	5.44 (9.99)
Ever-smokers, n (%)	318 (53)	112 (56)	99 (50)	107 (54)
Number of cigarettes/day [a], mean (s.d)	10 (8.86)	11 (6.85)	11 (7.39)	9 (5.94)
Alcohol consumption (g/day),mean (s.d)	12.48 (15.75)	11.51(15.43)	12.56 (15.30)	13.06 (16.54)
Periodontitis, n (%)	214 (36)	66 (33)	79 (40)	69 (35)
Hypertension, n (%)	338 (56)	119 (60)	112 (56)	107 (54)
Type 2 diabetes mellitus, n (%)	57 (10)	16 (8)	18 (9)	23 (12)
Myocardial infarction, n (%)	18 (3)	9 (5)	2 (1)	7 (4)
Stroke, n (%)	13 (2)	3 (2)	5 (3)	5 (3)
Cancer, n (%)	7 (1)	2 (1)	1 (1)	4 (2)
DMARD, n (%)	20 (3)	8 (4)	3 (2)	9 (5)
Antibiotics, n (%)	4 (1)	2 (1)	2 (1)	0 (0)
NSAID, n (%)	65 (11)	24 (12)	15 (8)	26 (13)
Triglyceride/HDL ratio, mean (s.d)	1.45 (1.51)	1.44 (1.21)	1.34 (1.10)	1.58 (2.04)
Leukocyte (Gpt/L), mean (s.d)	5.96 (1.51)	5.86 (1.46)	5.96 (1.52)	6.08 (1.56)
HsCRP (mg/L), mean (s.d)	2.94 (5.15)	3.32 (7.30)	2.77 (4.06)	2.79 (3.14)

n= Number; %= Percentage; s.d= Standard deviation; HDL= High density lipoprotein cholesterol; DMARD= Classic synthetic and biological disease-modifying antirheumatic drugs; Ever-smokers= former smokers and current smokers; NSAID= nonsteroidal anti-inflammatory drugs; P.g= *Porphyromonas gingivalis*; HsCRP= High-sensitivity C-reactive protein;

[a] Number of cigarettes of current smokers. All variables other than sex, and age were adjusted for sex and age. Sex was unadjusted and age was adjusted for sex only.

3.3.2 Multivariable analyses of the association between *Porphyromonas gingivalis* and the number of teeth, and anti-citrullinated protein autoantibodies

The association between *Porphyromonas gingivalis* DNA and the number of teeth was not modified by any of the covariates investigated. Increasing quantities of *Porphyromonas gingivalis* DNA was consistently associated with increasing number of teeth. The full-adjusted multivariable model showed that participants with moderate and high and *Porphyromonas gingivalis* DNA had an average number of teeth that was 4% and 23% higher when compared to those with low *Porphyromonas gingivalis* DNA.

Table 8: Multivariable analyses of the association between quantity of *Porphyromonas gingivalis* DNA and the number of teeth

	Number of teeth			
	Model 1	**Model 2**	**Model 3**	**Model 4**
Quantity of P.g	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
Low (n= 200)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Moderate (n= 200)	1.07 (0.92;1.25)	1.06 (0.92;1.22)	1.04 (0.91; 1.20)	1.04 (0.91; 1.20)
High (n= 200)	1.20 (1.03; 1.39)	1.22 (1.06;1.41)	1.22 (1.06; 1.41)	1.23 (1.07; 1.41)
Ptrend	0.05	0.04	0.03	0.02

Model 1: Unadjusted
Model 2: Age (continuous), sex, BMI (continuous)
Model 3: Education (3 groups), occupation (4 groups), hours of sport per week (continuous), alcohol (continuous), smoking status (4 groups)
Model 4: Type 2 diabetes mellitus and cancer
n = Number; P.g = *Porphyromonas gingivalis*; RR= relative risk; Ref. = Reference;
CI= confidence interval

Furthermore, hsCRP concentrations modified the observed differences of anti-CCP2 IgG antibody levels between the three subsets of *Porphyromonas gingivalis* DNA (P-value for interaction =<0.01). We therefore chose to present the association between *Porphyromonas gingivalis* DNA and anti-CCP2 IgG antibody levels according to the following different categories of hsCRP levels: >3.0 mg/L, 1.0-3.0 mg/L, and <1.0 mg/L. Among participants with serum hsCRP concentrations greater than 3.0 mg/L, those with high and moderate *Porphyromonas gingivalis* DNA had anti-CCP2 IgG titres that was 8 times and 37% higher than in participants with the low *Porphyromonas gingivalis* DNA. Controlling for potential confounders resulted in marked attenuation of the association such that participants with high and moderate *Porphyromonas gingivalis* DNA had about 4 times higher and 34% higher anti-CCP2 IgG titres when compared to participants with low *Porphyromonas gingivalis* DNA. There was a linear trend across tertiles of *Porphyromonas gingivalis* DNA

(Table 9). Fewer years of education [vocational training or less and technical school with relative risks of 2.61 (1.06; 6.42) and 2.83 (1.02; 7.84), respectively] and current smoking of 15–24 cigarettes per day [with relative risk of 2.97 (2.14; 11.66)] were other statistically significant predictors of elevated anti-CCP2 among participants with greater than 3.0 mg/L of hsCRP. In contrast, there were no significant predictors of elevated anti-CCP2 antibody levels in other hsCRP categories. Participants with serum hsCRP concentrations 1.0-3.0 mg/L also show somewhat higher anti-CCP2 IgG titres with increasing *Porphyromonas gingivalis* DNA.

Table 9: Multivariable analyses of the association between the quantity of *Porphyromonas gingivalis* DNA and anti-CCP2 IgG antibody levels

		Anti-CCP2 IgG antibody levels			
		Model 1	**Model 2**	**Model 3**	**Model 4**
HsCRP levels	**Quantity of P.g**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**	**RR (95% CI)**
	Low (n = 48)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.
>3.0 mg/L (n= 151)	**Moderate (n= 49)**	1.37 (0.51; 3.67)	1.38 (0.56; 3.40)	1.26 (0.59; 2.70	1.34 (0.61; 2.96)
	High (n= 54)	8.02 (3.05; 21.10)	4.92 (1.98;12.23)	3.26 (1.45; 7.31)	3.82 (1.60; 9.11)
	Ptrend	<0.01	<0.01	0.01	0.01
	Low (n= 79)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
1.0-3.0mg/L (n= 264)	**Moderate (n= 91)**	1.05 (0.51; 2.14)	1.02 (0.50; 2.05)	1.12 (0.58; 2.19)	1.14 (0.59; 2.23)
	High (n= 94)	1.55 (0.76; 3.16)	1.40 (0.69; 2.82)	1.42 (0.72; 2.80)	1.49 (0.74; 2.97)
	Ptrend	0.39	0.55	0.57	0.50
	Low (n= 73)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
<1.0 mg/L (n= 185)	**Moderate (n= 60)**	0.83 (0.51; 1.34)	0.82 (0.50; 1.33)	0.76 (0.44; 1.31)	0.77 (0.45; 1.29)
	High (n= 52)	0.94 (0.57; 1.56)	0.93 (0.56; 1.55)	0.82 (0.46; 1.46)	0.75 (0.43; 1.32)
	Ptrend	0.74	0.72	0.59	0.49

Model 1: Unadjusted
Model 2: Age (continuous), sex, BMI (continuous)
Model 3: Education (3 groups), occupation (4 groups), work hours (6 groups), hours of sport per week (continuous), alcohol (continuous), smoking status (4 groups)
Model 4: Type 2 diabetes mellitus and cancer
n = Number; HsCRP= High-sensitivity C-reactive protein; IgG = Immunoglobulin G;
Anti-CCP2 = second generation anti-cyclic citrullinated peptides antibody;
P.g = *Porphyromonas gingivalis*; RR= Relative risk; Ref. = Reference; CI= Confidence interval

Restricted cubic spline regression showed no evidence for nonlinearity in the all the categories of hsCRP (Figures 5a-5c).In sensitivity analyses, excluding participants with myocardial infarction, stroke and those who reported intake of antibiotics, NSAID and DMARD did not change our results in all the groups of hsCRP (data not shown). Moreover, excluding participants with hsCRP of 10.0 mg/L or more (as a marker of acute inflammation) within the group with greater than 3.0 mg/L of hsCRP did not change our results. In addition, hsCRP did not mediate or confound the association between *Porphyromonas gingivalis* DNA and anti-CCP2 IgG levels.

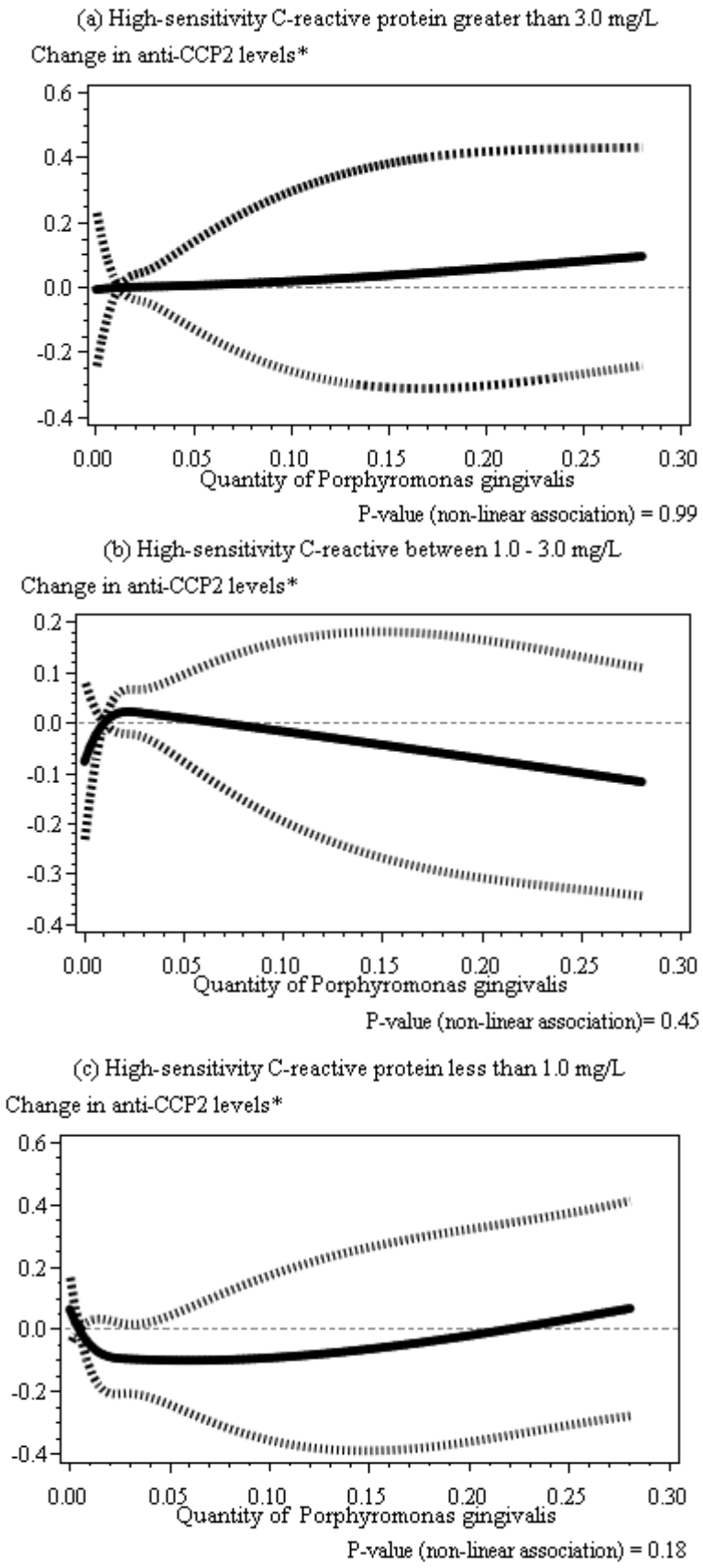

Figure 5: Functional relation (and 95% pointwise confidence band) between quantity of *Porphyromonas gingivalis* DNA and anti-CCP2 IgG antibody levels (multivariate adjusted) estimated by restricted cubic splines across three categories of high-sensitivity C-reactive protein:(a) greater than 3.0mg/L, b)1.0 - 3.0 mg/L and (c) less than 1.0 mg/L

3.4 Study IV: Can treelet transform yield interpretable dental disease-associated bacterial profiles? If yes, is their association with the number of teeth and low-grade systemic inflammation dependent on the overall bacterial diversity and what is their relative importance as compared to other risk factors?

3.4.1 Participant characteristics of study IV

The sample is composed of approximately equal proportion of women (n=139; mean age 66 years) and men (n=142; mean age 68 years). As compared to women, men were more likely to have a higher BMI, likely to do less sports, more high-grade professionals, more ever smokers, drink more alcohol, higher proportion with T2DM and also eat more cakes and cookies. The number of bacterial OTUs ranged between 66 and 296 bacterial OTUs in a single individual which was slightly more in women than in men (Table 10). A total of 530 bacterial OTUs were detected overall, of which 11, 513 and 6 were genus-level, species-level and subspecies-level OTUs, respectively (Table S1).

Table 10: Basic characteristics of the participants of study IV (n=281)

	Study population(n=281)	**Women (n=139)**	**Men (n=142)**
Age (years), mean (s.d)	67 (8.18)	66 (8.13)	68 (8.10)
Body mass index (kg/m^2), mean(s.d)	27.69 (4.31)	27.31 (4.36)	28.05 (4.29)
Vocational school or less, n (%)	95 (33.81)	47 (33.81)	48 (33.80)
Higher-grade professionals, n (%)	68 (24.20)	24 (17.27)	44 (30.99)
Sport (hours/week), mean (s.d)	4.47 (7.41)	4.87 (7.55)	4.09 (7.39)
Ever smokers, n (%)	155 (55.16)	54 (38.85)	101 (71.13)
Number of cigarettes/day[a], mean (s.d)	10 (6.49)	8 (5.92)	12 (5.90)
Alcohol consumption (g/day), mean(s.d)	13.27 (17.01)	9.85 (16.62)	16.63(16.68)
Hypertension, n (%)	153 (54.45)	69 (49.64)	84 (59.15)
Type 2 diabetes mellitus, n (%)	15 (5.34)	3 (2.16)	12 (8.45)
Antibiotics; n (%)	2 (0.71)	1 (0.72)	1 (0.72)
NSAID, n (%)	21 (7.47)	10 (7.19)	11 (7.75)
Fruit and vegetables (g/dy), mean (s.d)	77.31 (48.59)	77.24 (48.81)	77.37 (48.86)
Cakes and cookies (g/dy), mean (s.d)	48.05 (36.54)	41.58 (35.25)	54.38 (35.27)
Number of teeth, mean (s.d)	21 (8.82)	20 (8.60)	21 (9.09)
Edentates, n (%)	24 (8.54)	11 (7.91)	13 (9.15)
Bacterial OTU (min-max)	66-296	80-296	66-296

n= Number; %= Percentage; s.d= Standard deviation;

NSAID =Nonsteroidal anti-inflammatory drugs; OTU= Operational taxonomic unit;

Ever-smokers= former smokers and current smokers;

[a] Number of cigarettes of current smokers.

3.4.2 Treelet transform of bacterial OTUs

There were 174 most frequent bacterial OTUs (two genus-level, 168 species-level and four subspecies-level) (Table S2). Therefore, treelet cross-validation was performed on them. The treelet transform cross-validation procedure yielded optimal cut-levels between 32 and 92 (Table 11). Optimal cut-level of 42 and 44 each occurred twice, once each at three and four extracted components. We decided to choose the smaller number of components and smaller optimal cut-level. Thus, treelet transform on the correlation matrix of the 174 bacterial OTUs was performed with an optimal cut-level of 42 and three extracted treelet components.

Table 11: Results of 10fold cross-validations to determine the optimal cut-level for dimension reduction of 174 bacterial OTUs with treelet transform

Treelet components (n)	**30 repetitions (optimal cut-level)**			**40 repetitions (optimal cut-level)**		
Cross-validations*	**1**	**2**	**3**	**1**	**2**	**3**
2	88	92	89	72	82	83
3	48	42	45	49	44	53
4	50	51	42	52	44	55
5	37	34	40	32	36	33

*Cross-validations were done for two to five treelet components

The proportion of explained variance for the three extracted treelet components were 6.44%, 5.46% and 3.34 % for treelet component 1, treelet component 2 and treelet component 3; respectively with treelet component 1 explaining the highest proportion. The cumulative explained variance of these three treelet components was 15.24 % (Table 12).

Furthermore, treelet component 1 was loaded by eleven bacterial OTUs, out of which eight were frequently associated with primary endodontitis [4, 16, 42, 90, 259-261], therefore this component was called endodontitis-associated bacteria component. Treelet component 2 was the periodontitis-associated bacteria component because it was loaded by seven putative periodontitis-associated bacteria [70, 71, 99, 262-266] out of the eight bacterial OTUs that loaded. Finally, treelet component 3 was loaded by five bacterial OTUs, three out of which were generally associated with dental caries [71, 85, 266-268], thus called caries-associated bacteria component. All bacterial OTUs had positive loadings on all the three treelet components; the lowest loading was 0.25 (*Anaeroglobus geminatus* and *Streptococcus constellatus)*, while the highest was 0.48 (*Atopobium rimae* and *Bergeyella sp (OTU907*) (Table 12).

Table 12: Characteristics and loading patterns of three extracted treelet components generated by treelet transform on 174 bacterial OTUs

	Treelet component 1	**Treelet component 2**	**Treelet component 3**
Total explained variance (15.24%)	6.44	5.46	3.34
Loaded by original variables	11	8	5
Loading patterns			
*Prevotella buccae**	0.35		
Mycoplasma salivarium	0.35		
*Prevotella oralis**	0.34		
*Eubacterium [11][G-3] brachy**	0.31		
*Fretibacterium sp. (OTU360**	0.31		
*Prevotella baroniae**	0.30		
*Parvimonas micra**	0.27		
*Dialister pneumosintes**	0.27		
Neisseria elongata	0.27		
*Anaeroglobus geminatus**	0.25		
Streptococcus constellatus	0.25		
*Treponema denticola**		0.39	
*Treponema maltophilum**		0.39	
*Porphyromonas gingivalis**		0.39	
*Fretibacterium fastidiosum**		0.37	
Prevotella dentalis		0.36	
*Tannerella forsythia**		0.32	
*Treponema socranskii**		0.32	
*Porphyromonas endodontalis**		0.28	
*Atopobium rimae**			0.48
Bergeyella sp. (OTU907)			0.48
*Prevotella pallens**			0.46
Eubacterium [11][G-1] infirmum			0.41
*Selenomonas sputigena**			0.40
	Endodontitis-associated bacteria	**Periodontitis-associated bacteria**	**Caries-associated bacteria**

*Bacteria which are consistently associated with the dental disease;
OTU= Operational taxonomic unit

Table 13 shows the consistency of results as assessed in 80% bootstrap-samples with 100 replications in three stability runs. The first run resulted in four sign patterns. Sign pattern 1 was similar to endodontitis-associated bacteria component and was present in 14 % of the bootstrap-samples. Sign pattern 2 corresponded exactly to periodontitis-associated bacteria component and was present in 63% of the bootstrap-samples. Sign pattern 3 was analogous to caries-associated bacteria component and sign pattern 4 was in consonant with it and together the two sign patterns were present in 50 % of the bootstrap-samples. The second run resulted in two sign patterns. Sign pattern 1 corresponded exactly to periodontitis-associated bacteria component and was present in 55% of the bootstrap-samples while sign pattern 2 also fully agrees with caries-associated bacteria component and was present in 36% of the bootstrap-samples. The third run resulted in four sign patterns. Endodontitis-associated bacteria component was not described by any sign pattern. Sign pattern 1 was similar to periodontitis-associated bacteria component and sign pattern 2 corresponded exactly to it; both were present in 55% of the bootstrap-samples. Sign pattern 3 was similar caries-associated bacteria component and sign pattern 4 corresponded exactly to it; both were present in 49% of the bootstrap-samples. This sensitivity analyses showed that periodontitis-associated bacteria component is the most stable component, followed by the caries-associated bacteria component and the least stable was the endodontitis-associated bacteria component.

Table 13: Stability analyses of treelet transform on bacterial OTUs as the sign patterns and their frequencies

Treelet component	**Similar sign patterns**	
Stability run 1	**4 sign patterns**	**Frequency**
EBC	1 (-1: *Anaeroglobus geminatus*)	14%
PBC	2*	63%
CBC	3 (+2 : *Olsenella sp. OTU807*, *Prevotella oris*), 4*	13 +37→ 50%
Stability run 2	**2 sign patterns**	
EBC		
PBC	1*	55%
CBC	2*	36%
Stability run 3	**4 sign patterns**	
EBC		
PBC	1 (+1: *Prevotella sp. OTU526*), 2*	13 +42→ 55%
CBC	*1* (+2: *Olsenella sp. OTU807, Prevotella oris*) , 4*	13 + 36→ 49%

*sign patterns match exactly to the treelet components;

EBC= Endodontitis-associated bacteria component;

PBC= Periodontitis-associated bacteria component;

CBC= Caries-associated bacteria component;

OTU= Operational taxonomic unit

Additionally, the choice of the optimal cut-level was verified in sensitivity analyses by conducting a treelet transform with three treelet components, but with the cut-levels of 39, 40, 41, 43, 44, and 45. The proportion of variance within the data was between 15.24 % and 16.62 %. The bacterial targets loaded at cut-level of 39, 40, and 41were exactly the same when compared to the original analysis with a cut-level at 42. However, at levels of 43 and above, endodontitis-associated bacteria component was similarly loaded on the same bacterial target: *Bacteroidaceae [G-1] sp.(OTU272)*, and in addition three *Prevotella spp OTUs*: *Prevotella denticola*, *Prevotella nigrescens* and *Prevotella oris* was loaded on caries-associated bacteria component at cut-level 45 (Table 14). This sensitivity analysis confirmed the findings from the previous stability analyses in that the periodontitis-associated bacteria component is the most stable component. Although the caries-associated bacteria and endodontitis-associated bacteria components are less reliable, however, the changing bacteria OTUs are those often associated with the respective dental diseases.

Table 14: Sensitivity analyses of treelet transform on bacterial OTUs with optimal cut-level 39, 40, 41, 43, 44 and 45

Optimal cut-level	**Explained variance**	**Number of loading original variables**		
		EBC	**PBC**	**CBC**
39	15.24	11	8	5
40	15.24	11	8	5
41	15.24	11	8	5
42 (CHOSEN)	15.24	11	8	5
43	15.58	12 (+1: *Bacteroidaceae [G-1] sp. OTU272*)*	8	5
44	15.58	12 (+1: *Bacteroidaceae [G-1] sp. OTU272*)*	8	5
45	16.62	12 (+1: *Bacteroidaceae [G-1] sp. OTU272)**	8	8 (+3:*Prevotella denticola*, *Prevotella nigrescens, Prevotella oris*)**

*explained variance=6.78%, **explained variance=4.38%;
EBC= Endodontitis-associated bacterial component;
PBC= Periodontitis-associated bacterial component;
CBC= Caries-associated bacterial component;
OTU= Operational taxonomic unit

Moreover, we compared the treelet transform analysis with the PCA. Two factors were retained from the PCA of the 174 bacterial OTUs based on the rule of eliminating all components that explain less than 5% of the total variation. Table S3 shows the results of the PCA. The proportions of explained variance were 9.74% for factor 1 and 5.68% for factor 2 (with respective eigenvalues of 16.83% and 9.69%). The overall proportion of explained variance was 15.42%. Bacterial OTUs loadings higher than 0.25 or lower than -0.25 were considered as relevant for the interpretation of the factors, given that the lowest bacterial OTU loading from treelet transform was 0.25. Accordingly factor 1 was loaded by 82 bacterial OTUs and factor 2 was loaded by 51 bacterial OTUs (20 of which also loaded high on factor 1). Predictably, both factors comprise bacteria OTUs that are associated with all the three main dental diseases. Additionally, factor 1 include 10 bacterial OTUs of treelet component 1 (endodontitis-associated bacteria component), all bacterial OTUs of treelet component 2 (periodontitis-associated bacteria component) and three bacterial OTUs of treelet component 3 (caries-associated bacteria component), while factor 2 include three bacterial OTUs of treelet component 2 (periodontitis-associated bacteria component) and one bacterial OTU of treelet component 3 (caries-associated bacteria component).

The lower proportion of explained variance in single treelet component as compared to factors of the PCA is due to the less original variables loaded on the treelet components. However, the marginal difference (0.18%) in the total explained variance between both methods and the overall better interpretability of the treelet transform analysis indicates that for the current dataset of bacterial OTUs, treelet transform analysis is better than the PCA.

3.4.3 Multivariable analyses of the association between dental disease-associated bacteria scores and the number of teeth, and hsCRP

Table 15 shows correlation between each dental-disease associated bacterial score with the number of teeth and with hsCRP according to low diversity and high diversity. There was generally a significant weak negative correlation between all the dental disease-associated bacteria scores and the number of teeth; however only among participants with low bacterial diversity (model 1). In this strata, after adjustment for covariates, the correlation was only significant for the periodontitis-associated bacteria score (r= -0.19; 95% CI: -0.35; -0.02) and caries-associated bacteria score (r= -0.18; 95% CI: -0.35; -0.01; model 2). The coefficient of determination, r^2 suggests that 3.61% and 3.24% of the variation in number of teeth is accounted for by the periodontitis-associated bacteria and caries-associated bacteria scores, respectively. As compared to periodontitis, dental caries is strongly related to diet, especially sugar and carbohydrate-rich ones and as such exclusion of participants in the highest percentile of intake reveals attenuation of the correlation between caries-associated bacteria score and the number of teeth (r= -0.15; 95% CI: -0.27; 0.04).In contrast, there was no significant correlation between any of the bacteria scores and hsCRP (Table 15).

Table 15: Correlations and partial correlations between dental-disease associated bacterial score and the number of teeth and hsCRP

	Model 1		Model 2	
	Number of teeth	**hsCRP**	**Number of teeth**	**hsCRP**
	r (95%CI)	**r (95%CI)**	**r (95%CI)***	**r (95%CI)***
EBS				
High diversity (n=140)	-0.05 (-0.21; 0.12)	0.01 (-0.16; 0.18)	-0.03 (-0.20; 0.14)	0.07 (-0.11; 0.24)
Low diversity (n=139)	-0.21 (-0.36; -0.04)	-0.06 (-0.23, 0.10)	-0.10 (-0.27; 0.08)	-0.03 (-0.20; 0.15)
PBS				
High diversity (n=140)	0.02 (-0.16; 0.18)	0.02 (-0.14; 0.19)	0.06 (-0.12; 0.23)	-0.04 (-0.21, 0.14)
Low diversity (n=139)	-0.31 (-0.44; -0.14)	0.04 (-0.13; 0.21)	-0.19 (-0.35; -0.02)	0.05 (-0.13; 0.21)
CBS				
High diversity (n=140)	-0.06 (-0.23; 0.10)	0.05 (-0.12; 0.21)	-0.07 (-0.24; 0.10)	0.02 (-0.16; 0.19)
Low diversity (n=139)	-0.28 (-0.42; -0.11)	-0.06 (-0.22; 0.11)	-0.18 (-0.35; -0.01)	-0.05 (-0.22; 0.13)

*adjusted for age (continuous), sex, BMI (continuous), education (3 groups), occupation (4 groups) alcohol (continuous), smoking (4 groups), and sport hours (continuous);
r= Pearson's correlation coefficient; EBS= Endodontitis-associated bacterial score;
PBS= Periodontitis-associated bacterial score; CBS= Caries- associated bacterial score;
BMI= Body mass index, HsCRP= High-sensitivity C-reactive protein

Moreover, more single bacterial OTUs were generally significantly correlated with the number of teeth among participants with low bacterial diversity as compared to participants with high bacterial diversity (Figure 6a). On the other hand, except for the caries-associated bacteria score, the correlation between single bacteria OTUs and hsCRP appears to be similar when comparing the two oral bacterial diversity categories (Figure 6b).

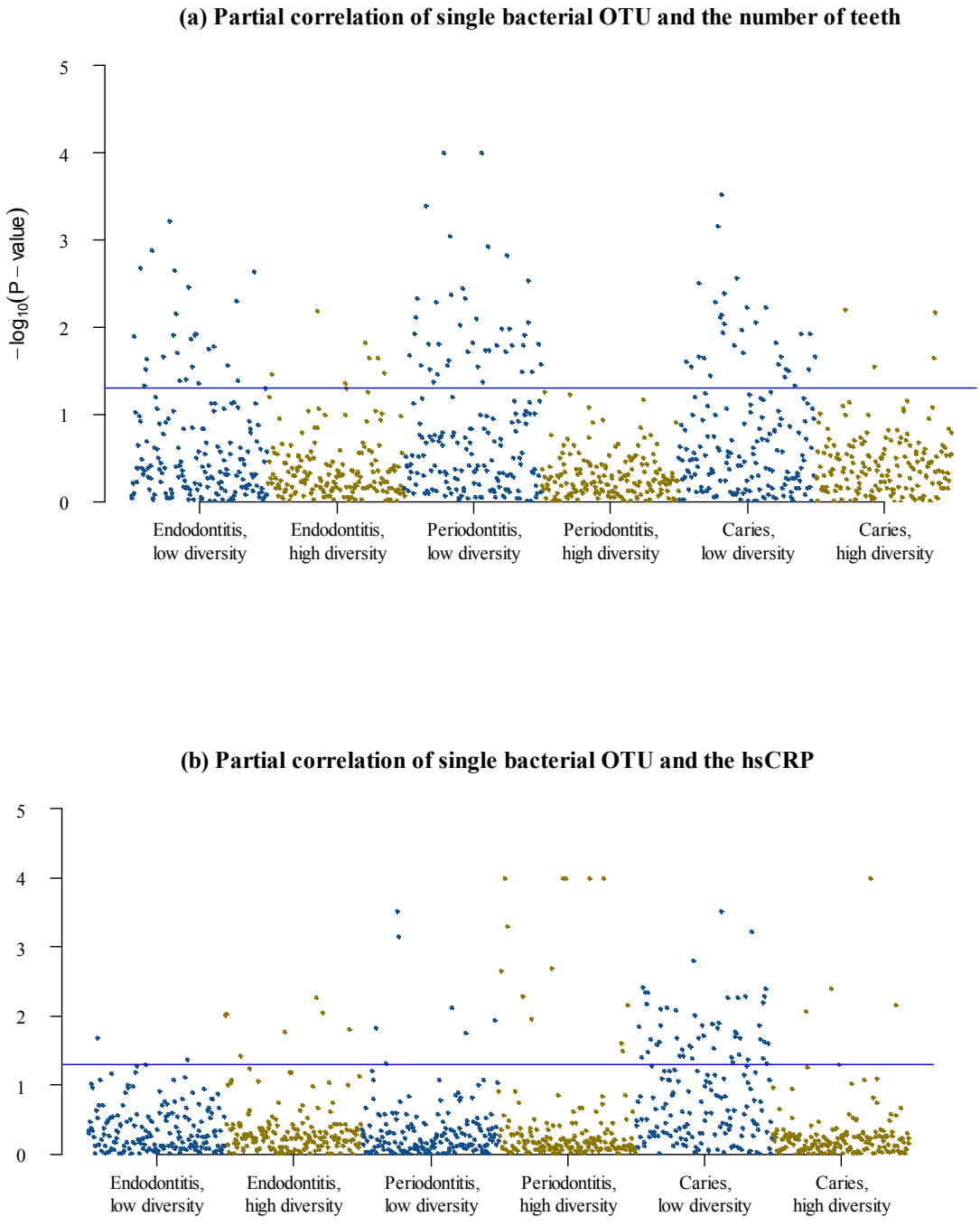

Figure 6: Plots of P-values of the partial correlation between 174 bacterial OTUs and the number of teeth as well as hsCRP (each dot represents a bacterial OTU and the blue line represents cut-off level of P-value=0.05). HsCRP= High-sensitivity C-reactive protein; OTU= Operational taxonomic unit

3.4.4 Relative importance of predictors of the number of teeth and hsCRP

Among participants with low bacterial diversity, a combination of age, periodontitis-associated bacteria score, endodontitis-associated bacteria score, caries-associated bacteria score, alcohol and smoking variables were the most influential predictors of the number of teeth (Figure 7a). All these variables (except caries-associated bacteria score) plus BMI were also important predictors for the number of teeth among participants with high bacterial diversity (Figure 7b). Overall, the importance of these variables is stronger among participants with low bacterial diversity. In general, sex, sports and socioeconomic status appeared to have little impact on the number of teeth.

The most important variables for predicting hsCRP among participants with low bacterial diversity were BMI, periodontitis-associated bacteria score, caries-associated bacteria score, endodontitis-associated bacteria score, age, smoking and sports in their order of importance (Figure 7c). In contrast, among participants with high bacterial diversity hsCRP was most dependent on smoking and alcohol, but also on endodontitis-associated bacteria score, socioeconomic status and sex (Figure 7d).

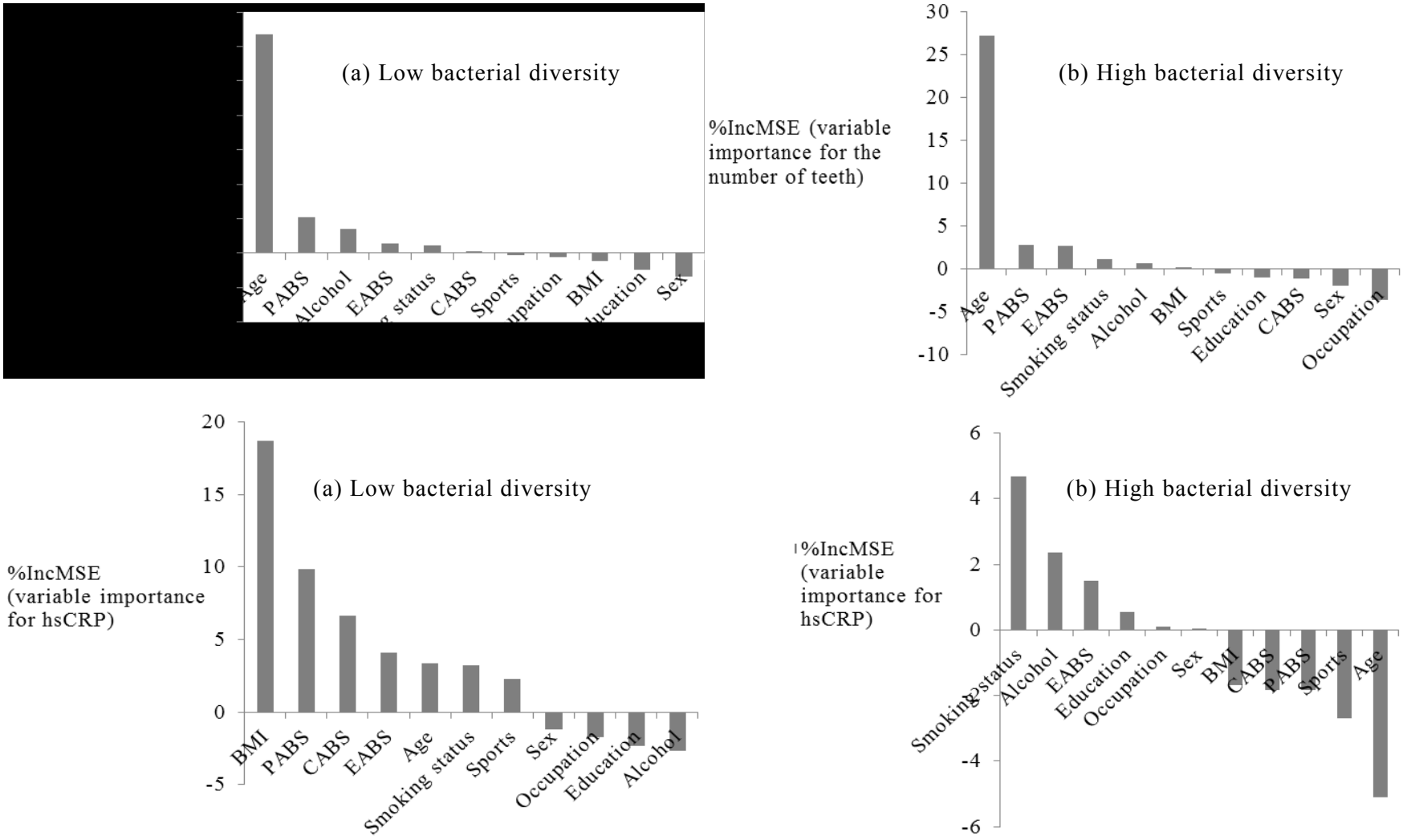

Figure 7: Variable importance plots showing the percentage increase in mean square error (%IncMSE) (upper panels for number of teeth: (a) Low bacterial diversity and (b) High bacterial diversity; lower panels for hsCRP: (c) Low bacterial diversity and (d) High bacterial diversity

PABS= Periodontitis-associated bacteria score; CABS= Caries-associated bacteria score; EABS= Endodontitis-associated bacteria score; BMI= Body mass index, HsCRP= High-sensitivity C-reactive protein

Figure 8 displays an overview of the findings of the present investigation. The symbol √ indicates the presence of an association while the symbol × indicates the absence of an association.

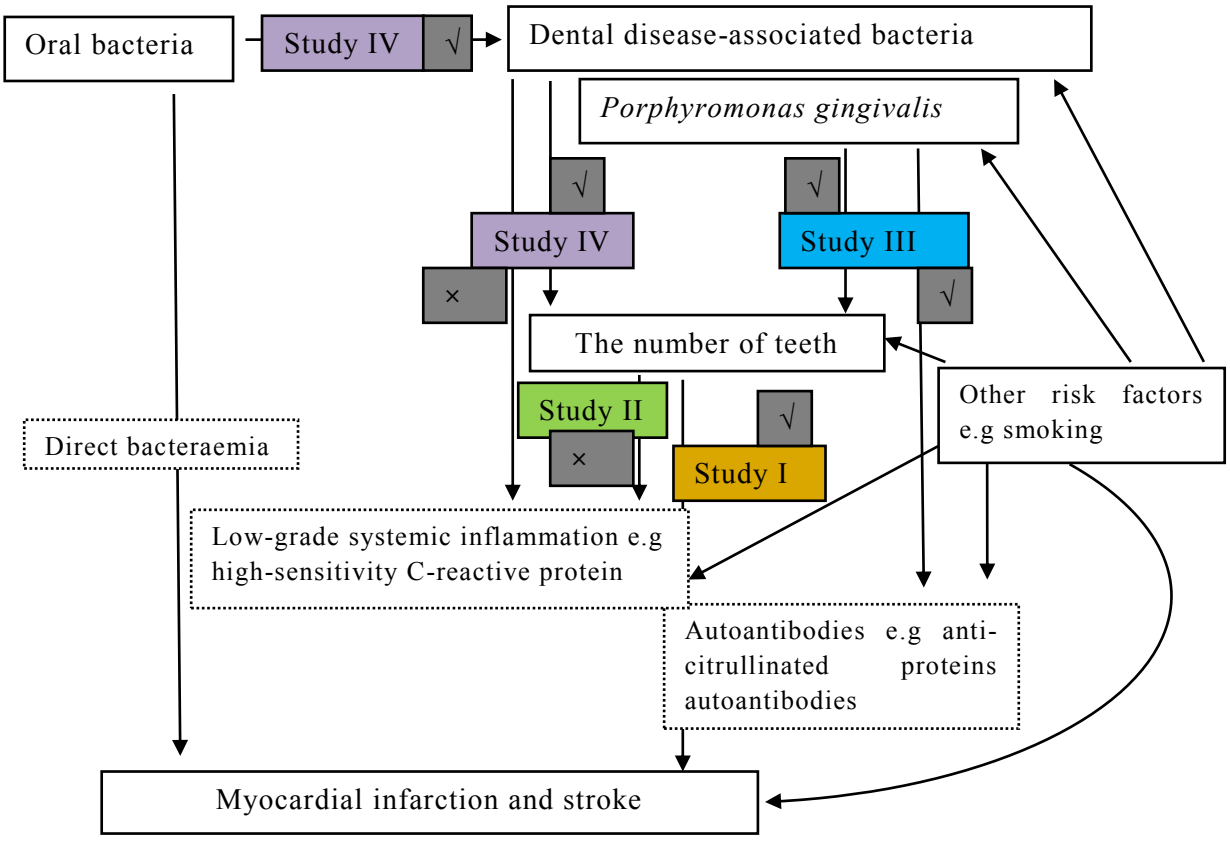

Figure 8: Summary of findings of the present investigation

4 Discussion

The aim of this thesis was to examine the relation between the number of teeth and chronic systemic diseases, and to elucidate this relation with respect to some underlying biological mechanisms within the framework of a well-characterised large prospective cohort.

Firstly, we observed that the number of teeth is strongly associated with the risk of myocardial infarction, to a lesser extent with stroke, and no association with either T2DM or cancer. Specifically, individuals with more teeth when compared to those that have fewer teeth and completes edentates, appear to have a lower risk of myocardial infarction. In addition, only in the highest number of teeth range increasing was associated with less risk of stroke. Moreover, no difference in the strength of association was observed in a subgroup analysis according to stroke types, although there were very few haemorrhagic cases when compared to ischemic cases. Few prospective epidemiological studies linking the number of teeth to myocardial infarction [187, 190] and stroke [181, 191] within European populations have been published; in this context our study has a comparatively large sample size and long follow-up. Besides, the discrepancy that we observed between the relation between number of teeth and the two CVD endpoints agrees with findings of periodontal infections-CVD association [269]. Admittedly, these findings suggest that low number of teeth and/or dental infections is one of the risk factors of CVD since the traditional CVD risk factors cannot explain all incident diseases. Further, it also reflects and supports the heterogeneity of the pathogenesis of myocardial infarction and stroke [270].

The lack of statistically significant interaction between the history of periodontitis and the number of teeth with respect to these CVD endpoints may be due to a low accuracy of the here used single self-reported question as observed in another German population [271]. A combination of several self-reported items related to periodontitis probably have provided a better ascertainment of periodontitis [272]. Similarly, our finding that was not explained by diet suggests that the number of teeth has minimal effect on the ability to eat certain foods in middle-age adults as compared to reports among the elderly [147]. Although tooth loss as a direct result of CVD is unlikely, excluding the possibility of reverse causality was critical since CVD is indirectly linked to pain in the mandible and teeth, and CVD-prone individuals may retain more teeth due precaution in dental procedures [273].

The non-significant association between the number of teeth and T2DM is partially reflected in results from prospective studies. This suggests that loss of teeth does not precede T2DM but rather uncontrolled T2DM is likely a risk factor for tooth loss [274]. The number of teeth showed no association with overall cancer, but we cannot rule out association with specific cancer sites since prospective studies within Finnish population

have linked the number of teeth to non-cardia gastric cancer [196] and pancreatic cancer [197].

Our findings do not fully establish causation between number of teeth and myocardial infarction, and even though we adjusted for various CVD risk factors, residual and unmeasured confounding cannot be completely ruled out. Nonetheless, a low number of teeth may be a surrogate measure of the bacterial load on teeth and in fact the saliva. This may contribute to bacteraemia and together with low-grade systemic inflammation play a role in the development of acute myocardial infarction [275].

As possible explanations for our findings, we investigated the role of hsCRP and ACPA as markers of low-grade systemic inflammation and autoimmunity. HsCRP did not significantly increase as the number of teeth decreased, after controlling for potential confounders. The linear trend observed in age and sex-adjusted model in one of the subsamples was eliminated after adjustment for the first set of confounders, notably BMI. This role of BMI is supported by other studies among other European populations [276, 277]. The rather consistently higher hsCRP among full edentates in our study supports findings from another German population [278]. However, we could not demonstrate the relation between the number of teeth and hsCRP observed in other European populations, even in sex-specific analyses [192, 279]. An obvious limitation in all studies that reported an association between number of teeth and hsCRP is the failure to adjust for potentially important confounders, particularly alcohol [192, 278, 279] and amount of cigarette smoking [192]. Moreover, our study population also have somewhat lower hsCRP as compared to these populations. Our data suggest that there is little for the association between the number of teeth and low-grade systemic inflammation, measured by hsCRP.

However, in our subsequent study, among individuals with hsCRP levels >3 mg/L, increasing quantities of *Porphyromonas gingivalis* DNA was associated with increasing serum ACPA, even after adjusting for potential confounding factors. These data support the aetiological hypothesis that *Porphyromonas gingivalis* infection plays a causative role in the development of ACPA [280] and that a systemic inflammatory milieu is necessary for *Porphyromonas gingivalis* to initiate and sustain processes resulting in the production of ACPA [231]. Our finding is also supported by a number of reports that also pinpoints a link between ACPA and *Porphyromonas gingivalis* infection, assessed by the presence *Porphyromonas gingivalis* at diseased sites [281] and serum anti-*Porphyromonas gingivalis* antibody levels [282-286]. Although none of these studies reported that their observed association is influenced by the degree of systemic inflammation. Moreover, it has been shown that inflammation is crucial in the induction of autoimmunity by chronic oral bacterial infections [287]. In addition, our data linking smoking [288, 289] and lower education [290] to elevated ACPA is a recognized phenomenon. Indeed, our findings linking

increasing quantities of *Porphyromonas gingivalis* DNA and elevated serum ACPA among individuals with hsCRP levels >3 mg/L is independent of these established risk factors.

Actually, the detection of *Porphyromonas gingivalis* in the saliva in this study is not conclusive evidence that the bacterium is either indicative of a disease or implicated in a disease process. However, a high *Porphyromonas gingivalis* affiliation (colonization) might indicate a previously poor dental health. Consequently, *Porphyromonas gingivalis* may be involved in future disease onset and progression through translocation into periodontal pockets. Admittedly, due to the continuous flow of the gingival crevicular fluid, saliva translocation of *Porphyromonas gingivalis* into periodontal pockets is a controversially discussed issue [291]. Nevertheless evidence from prospective studies has linked saliva colonization by *Porphyromonas gingivalis* and other periodontopathic bacteria with periodontal pockets colonization [292]. Clearly, the presence of ACPA in apparently healthy individuals is predictive of rheumatoid arthritis [280]. However, the risk of myocardial infarction is substantially increased preceding diagnosis of rheumatoid arthritis [293] and ACPA is elevated in myocardial infarction [230]. Therefore, our results suggest that *Porphyromonas gingivalis* might play a role in the risk of myocardial infarction through the development of anti-citrulline immunity.

Contrary to our expectation, increasing quantity of *Porphyromonas gingivalis* DNA was also associated with increasing number of teeth. Furthermore, it was also surprising that peridontititis did not modify this association since the disease influence salivary bacteria [294] and in fact salivary *Porphyromonas gingivalis* [97]. Although, there are conflicting evidence that gingivitis and/periodontitis [71, 295] and even plaque development [296, 297] does not influence the quantity of salivary bacteria. This suggest that the intrinsic link between saliva *Porphyromonas gingivalis* and the number of teeth is apparently much more complicated than previously thought. In fact, it is likely that the presence of multiple odontopathogenic bacteria in saliva, rather than the presence of only *Porphyromonas gingivalis* is associated with reduced number of teeth. This could be similar to the observation in periodontitis [99; 298], dental caries [299, 300], and endodontitis [301, 302].

Finally, the entire bacterial composition of the saliva was characterized using 16S rRNA gene next-generation sequencing approach and the interaction within this complex mixed consortium was statistically modelled. Interestingly, among participants with a low bacterial diversity those with high dental disease-associated bacteria score were more likely to have a low number of teeth. However, only the association of periodontitis-associated bacteria and caries-associated bacteria scores with the number of teeth remained significant after full covariate adjustment.The association between caries-associated bacteria score and the number of teeth was lost after exclusion of individuals with the highest intake of carbohydrate and sugar-rich diet. This supports the well-established link between these diets

and dental caries as compared to other bacterial-associated dental diseases [303]. Similarly, an explanation for the loss of association between endodontitis-associated bacteria score and number of teeth after covariate adjustment might be due to the acute nature of endodontitis and as a result these bacteria are only present transiently in the saliva during the disease process. In fact, the presence or absence of endodontitis seems to be unrelated to the detection of endodontitis-associated bacteria in the saliva [304]. A major cariogenic bacterium, *Streptococcus mutans* not loaded in the treelet transform analysis suggests its weak correlation with all bacteria during the variable pairing step of the treelet transform procedure. Moreover, major endodontitis bacteria, *Enterococcus faecalis* was not included in the treelet transform analysis because it was sporadically identified (1%) in our study population.

Furthermore, our data showing that alteration of the saliva bacterial diversity to a low status is associated with tooth loss is in support of earlier studies that reported that a low salivary bacterial diversity is associated with dental disease [305; 306], however, evidence from recently published data on the association between saliva bacterial diversity and dental disease vary greatly [69, 70, 84, 85, 266, 267]. One explanation for this is that these inflammatory diseases are characterised by periods of exacerbations and remissions, and thus a higher variation of saliva bacterial composition when compared to other oral niches. Besides, this discrepancy may also reflect the closer similarity in saliva carriage of oral bacteria across populations with lesser geographical distance [41]. A recent study and perhaps the largest till date reported a similar finding as ours, that low salivary bacterial diversity is associated with lower number of teeth [267]. Surprisingly, this study also reported that a low saliva bacterial diversity is associated with parameters indicative of dental health such as shallower periodontal pocket, less gingival bleeding and absence of decayed teeth [267]. Despite the comprehensive analyses of the saliva bacterial communities performed in previous studies, none of them have focused on the relationship between the colonization pattern of dominant bacteria in the presence of less dominant ones and the number of teeth.

Teeth that are severely compromised or diseased are probably more likely to harbour greater mature biofilms and dental-disease associated bacteria. Thus, they will continually despatch bacteria that become planktonic in the saliva, in order to colonise new oral niches. Therefore, the loss of these teeth results in a marked decrease in the bacteria that are shed into the saliva and hence a decreased diversity. Although one would expect a high quantity of all disease-associated bacteria within a low saliva diversity to be associated with a low number of teeth, however, periodontal bacteria as compared to other pathogenic bacteria have a relatively lower dependency on solid surfaces and are therefore likely to reside

longer in the saliva. This is one of the reasons why periodontal bacteria are more adept in their intra-oral distribution and competently colonise preferred dental surfaces [291].

Our results also showed that none of the dental disease-associated bacteria scores was associated with hsCRP. This is not surprising, considering the dearth of literature on the association between oral bacteria and hsCRP as compared to the association between clinically diagnosed dental diseases and hsCRP among European populations. One study among Norwegian men reported that the antibody level against oral bacteria varied according to the quartile levels of hsCRP [216]. This suggests that measures of both active and past oral infections are crucial in the variation of hsCRP and perhaps other markers of systemic inflammation.

Despite the weak association between periodontitis-associated bacteria score and the number of teeth, periodontitis-associated bacteria being second to age as the most crucial predictor of the number of teeth is informative, meaningful, and practically relevant. It is quite striking that independent of bacterial diversity; smoking and alcohol when compared to periodontitis-associated bacteria score had less impact on the number of teeth. This further emphasizes the strong impact of this group of oral bacteria on the number of teeth since smoking was recently reported as a risk factor for tooth loss in our study population [117]. However, socioeconomic status appears to have little impact on the number of teeth unlike reports from another German population [134]. This might suggest a lower social gradient in our study population. Overall, changes in the number of teeth being one of the undesired effects of ageing can be avoided or postponed if bacteria-associated dental diseases are controlled and other adverse lifestyle such as smoking is ceased.

Additionally, we also provide the evidence that hsCRP is at least in part influenced by the oral bacteria. It is quite surprising that among participants with low bacterial diversity, all bacterial scores in their importance were between BMI-a very strong risk factor for hsCRP [307], and less strong risk factors of hsCRP such as smoking [308, 309] and sports and/or physical activity [310]. This suggests that dental disease-associated oral bacteria influence hsCRP in a milieu of relatively limited bacterial diversity. Moreover, this finding gives insights into the role of oral bacteria, perhaps as part of a generalized infection that triggers low-grade systemic inflammation. On the contrary, among participants with high oral bacterial diversity, bacterial scores appear to be weaker predictors of hsCRP. This suggests that a high oral bacterial diversity provides colonization resistance to invasion of opportunistic and/or pathogenic bacteria. In fact, this may translate to stability of the ecological milieu of the entire body against elevation of hsCRP. However, smoking, alcohol, and socioeconomic status being more influential predictors of hsCRP in this group of individuals indicates that socioeconomically patterned lifestyle factors such as smoking and alcohol influence hsCRP [311].

5 Strengths and limitations

The present investigation has its strengths and limitations, both in study design and methods. An obvious strength is that the EPIC-Potsdam study is well-designed. The study population reflects the general population of Potsdam from which it was drawn. This indicates that our study population provides an appropriate platform to obtain data from individuals with varying numbers of teeth (including complete edentate), different saliva bacterial profiles, difference in the range of inflammatory marker and autoantibody levels, and chronic disease risk. The EPIC-Potsdam study also has rich exposure-related information and the data collection is comprehensive. We were therefore able to develop clear and relevant research aims and be confident that observed findings are close to the real-world settings.

Furthermore, a strength regarding our methods was that the exposure and/or outcome were professionally-verified, objective, quantitative, and well-defined. All participants were examined with the same methods of data collection, blood and saliva collection and sampling. The next-generation sequencing approach to detect and quantify all bacteria in the saliva is quite advantageous. This provides a rich data to model the complex interaction of the oral bacterial community. Furthermore, appropriate statistical tests were used in all investigations. In the statistical analyses of exposure-outcome associations, reverse causality was checked when it could be a possible explanation for the observed associations. Additionally, we appropriately identified adjustment covariates by causal graphs of the DAGs. This helps to understand the relationships between the variables in each study and also help control for bias of effect estimates. Moreover, this investigation applied the novel treelet transform statistical approach to oral bacteria data. The important strengths of this method are notable for the analyses of high-dimensional data, particularly in terms of interpretability of the generated components, and stability of components [108].

Despite these strengths, the interesting findings from this investigation should be interpreted with caution. This work is based on observational studies, thus we can only elucidate cause-and-effect relationships but unfortunately cannot establish causality. Although, the longitudinal design of the study I as a promising start on which subsequent studies were built provides an evidence of a temporal relationship, however, it was difficult to differentiate between cause and effect or the sequence of events in the subsequent cross-sectional studies. Notwithstanding being representative of the EPIC-Potsdam, studies III and IV have relatively small sample sizes; therefore larger confirmatory studies are necessary.

Moreover, our investigation still suffers from some biases. The “number of teeth lost since baseline” may suffer from recall bias, particularly among older participants and those

with debilitating conditions, since it was obtained retrospectively. Besides, the questions used to obtain the information on the number of teeth were not validated in our study population, nevertheless, responses to these questions was reported to have a high specificity in another German study [271]. Moreover, the number of remaining teeth was not objectively verified by trained personnel. However, epidemiological studies across different populations uniformly reported on the relatively high sensitivity and specificity of the number of teeth [312, 313]. On the contrary, self-reported periodontitis have low sensitivity, but they are still valuable in epidemiological studies [312] because the current standard methods for assessments of periodontitis are labour-intensive, expensive, and highly complex [314]. Relevant dental information such as the history of other dental diseases, oral health practises and dental care and visits were not available and so their role cannot be investigated. Furthermore, it is possible that tooth loss and myocardial infarction occur as a result of some common underlying disease process or due to genetic predisposition. The matrix γ-carboxyglutamate (Gla) protein gene is linked to the number of teeth [153] and CVD [315]. Moreover, variants of genes such as BRINP3 (bone morphogenetic protein/ retinoic acid inducible neural-specific 3) and CDKN2B-CDKN2A have been implicated in dental diseases and indirectly tooth loss, inflammation, and myocardial infarction [316-318]. However, the impact of genetics on the salivary composition of the *Porphyromonas gingivalis* and overall bacteria appears to be minimal [38, 318]. Therefore, biased effect estimates are plausible due to residual or unmeasured confounding.

The qPCR and HOMINGS used whole genomic DNA, therefore it was impossible to discriminate between dead and viable bacterial population. Complementary DNA as a template for the qPCR in a reverse transcription-PCR method (RT-qPCR) would have afforded the quantification of viable bacteria. In addition, determination of gene expression of each bacterial species in the saliva could provide insight into the functional activities of these pathogens in the community [319]. The functional activities of these bacteria in the saliva could be differentiated from the expression pattern of genes from similar bacteria in dental plaques. The host immune response is important in dental infections and restriction of nutrients is critical to preventing bacterial outgrowth [320]. Therefore, the relationship between metabolic changes such as serum metabolites and markers of pathogenic bacteria antigenic challenge such as serum antibody response can provide an insight into oral bacteria-host metabolic interaction. In fact, this can be extended to the gut microbiota in a broader microbiota-host metabolic interaction.

6 Implications for public health and conclusion

One of the most desirable goals in public health and health promotion is to assess risk, ascertain health status, influence disease onset and progression, and proffer appropriate interventions. Thus, this thesis provides fundamental data to support the development of public health action.Dental diseases are highly prevalent in adults but they are rarely diagnosed early. Unfortunately, when symptoms become clinically visible and diagnosis is made, treatment options such as teeth extraction or root canal therapy are extensive, expensive, and highly invasive. The impact of different dental conditions and tooth loss on oral health-related quality of life, overall well-being, and general health is affirmative [243, 321]. Similarly, myocardial infarction inflicts a serious burden on quality of life [322]. Worryingly, in Germany, the burden of tooth loss and dental disease [35], and CVD when compared to other Western European countries [323] are still substantial.

Noninvasive methods such as identification of lifestyle risk factors such as smoking, and less diverse bacteria in the saliva might provide practical approaches to identifying individuals at high risk of dental disease and tooth loss. Rapid detection of bacteria beyond those associated with periodontitis using the recently developed periodontitis-chip would be advantageous [324]. This will be of considerable importance among individuals who have challenges in maintaining routine dental hygiene such as older adults and those with developmental disabilities as well individuals who have barriers or poor access to seeking dental care. Encouragingly, a recent systematic review of prospective randomized controlled studies reported some improvements in hsCRP levels and endothelial function after periodontal therapy [325]. Despite the lack of evidence of an effect on CVD hard end points, individuals at high risk of CVD can benefit from such dental intervention.

Interestingly, dental care benefits of the statutory health insurance in Germany cover dental hygiene and dental treatment such as endodontic treatment and non-surgical periodontal treatment. The target of these treatments is usually on the dental plaques, however, planktonic bacteria shows varying degree of susceptibility to antimicrobial agents and photodynamic therapy when compared to plaque bacteria [326]. Therefore, effective control of saliva bacteria should also be improved. One additional option to these lethal therapeutic actions should be the disruption of the saliva bacteria towards a highly diverse community. The application of oral prebiotics and probiotics may be beneficial [327]. Additionally, since dental disease-associated bacteria appear to be present in saliva before the colonization of the teeth, a better understanding of the acquisition of these bacteria may lead to unique approaches for intervention [328]. However, these isolated interventions may not produce sustainable improvements in dental health [245].

More evidence from prospective cohort and randomized controlled studies which are sparse in Germany [329] are still necessary for effective and endurable primary prevention. At the population level, reduction or elimination of risk can be achieved through routine documentation of trend of tooth loss, salivary bacterial diversity, and ACPA and hsCRP levels within a preventive programme of the statutory health insurance. Furthermore, since the social determinants of dental diseases and CVD share common origin, it is important to develop effective social policies to general health promotion programs. Similarly, qualitative data from dental public health research are also significant. In addition, incorporating elements of evidence-based public health approaches such as community engagement would be beneficial [330]. Besides, interprofessional collaboration between dentists and medical practitioners should be promoted in such a way that oral-systemic health issues are addressed during patient visits. Finally, increase in total dental expenditure and integration of dental health in overall general health promotion might reduce the burden of myocardial infarction.

In conclusion, there is a link between the number of teeth and myocardial infarction and to a less extent, stroke. The diversity of saliva bacteria community may explain the relation and we could show that *Porphyromonas gingivalis* is an important bacterial candidate for tooth loss. In this context, low grade inflammation and autoantibodies might provide the background for the action of this bacterium. These findings provide evidence in public health decision making.

Summary

Dental diseases leading to tooth loss have severe impact on normal functional capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing, and as if these were not enough, these dental conditions have also been linked to chronic systemic diseases thereby affecting general health. To achieve a better understanding of the role of dentition status on chronic systemic diseases, there is a need to investigate the potential link between these conditions in a well-characterised population and also to investigate possible biological mechanisms that might elucidate disease pathogenesis. Additionally, the identification of poor dentition status and related biomarker of pathogenesis could help to detect individuals at high-risk of some chronic systemic diseases.

Therefore, the present thesis investigated the relation between dentition status as measured by the number of teeth and chronic systemic diseases and the role of some possible biological mechanisms within the EPIC-Potsdam cohort. Lower numbers of teeth were largely associated with a higher risk myocardial infarction and to some extent with stroke. Decreasing number of teeth was not associated with elevated levels of hsCRP, a marker of low-grade inflammation. However, we observed a relationship between increasing quantity of an important periodontal pathogen, *Porphyromonas gingivalis* in the saliva and elevated levels of ACPA in the presence of high hsCRP. Further, we demonstrated that in the presence of an overall low bacterial diversity, high saliva periodontitis-associated bacteria and caries-associated bacteria were also associated with a reduced number of teeth. These bacteria were also important predictors of the number of teeth and hsCRP among individuals with low bacterial diversity.

Collectively, these findings based on epidemiological observations indicate that tooth loss may be an additional risk factor for CVD. Low-grade inflammation, autoimmunity and the less diverse polybacterial oral community appear to play important roles. The quality of our studies and data, the strength of association, and consistency of results are remarkable. However, these findings need to be investigated further in other German populations. Additional evidence will strengthen potential public health actions.

Zusammenfassung

Zahnerkrankungen, die zu Zahnverlust führen, haben starke Auswirkungen auf die normale Funktionsfähigkeit hinsichtlich des Beißens, Kauens, Lächelns, Sprechens und psychosozialen Wohlbefindens. Außerdem konnte der Zustand der Zähne mit chronischen systemischen Erkrankungen verbunden werden, welche den allgemeinen Gesundheitszustand beeinflussen. Um ein besseres Verständnis zur Rolle des Zahnstatus auf chronische systemische Erkrankungen zu erhalten, sollte die mögliche Verbindung dieser Konditionen in einer gut beschriebenen Population erforscht werden und mögliche biologische Mechanismen untersucht werden, die die Pathogenese der Erkrankung verdeutlichen. Zusätzlich könnte die Identifizierung des schlechten Zahnstatus sowie krankheitsspezifischer Biomarker dazu beitragen, Individuen mit hohem Risiko für chronische systemische Erkrankungen zu ermitteln.

Daher untersuchte die hier vorliegende Doktorarbeit die Verbindung zwischen dem Zustand der Zähne, gemessen an der Anzahl der Zähne, und den chronischen, systemischen Erkrankungen innerhalb der EPIC-Potsdam Kohorte. Sie geht außerdem auf die Rolle möglicher biologischer Mechanismen ein. Eine niedrige Anzahl an Zähnen wurde weitgehend mit einem höheren Risiko für Herzinfarkt oder auch Schlaganfall in Verbindung gebracht. Sie konnte nicht mit einem erhöhten hsCRP-Wert assoziiert werden, einem Marker für leichte Entzündungen. Wir beobachteten einen Zusammenhang zwischen zunehmenden Mengen des wichtigen periodontalen Bakterien, *Porphyromonas gingivalis* im Speichel und erhöhtem ACPA-Werten im Umfeld hoher hsCRP-Wert. Weiterhin konnten wir zeigen, dass im Kontext insgesamt niedriger bakterieller Vielfalt hohe Parodontitis -assoziierte Bakterien und karies-assoziierte Bakterien auch mit der niedrigen Anzahl an Zähnen in Verbindung gebracht werden können. Diese Bakterien waren auch wichtige Prediktoren für die Anzahl der Zähne und hsCRP bei Individuen mit niedriger bakterieller Vielfalt.

Zusammengefasst zeigen diese drei auf epidemiologischen Beobachtungen basierenden Ergebnisse, dass Zahnverlust ein zusätzlicher Risikofaktor für Herz-Kreislauf-Erkrankungen sein könnte. Leichte Entzündung, Autoimmunität und eine weniger artenreiche orale Bakteriengemeinschaft scheinen eine wichtige Rolle zu spielen. Die Qualität unserer Studie und unserer Daten, die Stärke der Assoziation und die Konsistenz der Ergebnisse sind bemerkenswert. Schließlich sollten diese Ergebnisse weiter in anderen deutschen Populationen untersucht werden. Zusätzliche Untersuchungen könnten die öffentliche Gesundheit stärken.

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Supplementary tables

Table S1: 530 bacterial OTUs detected in the study population

1.	*Burkholderia genus (OTU)*	45.	*Aggregatibacter actinomycetemcomitans*
2.	*Escherichia genus (OTU)*	46.	*Aggregatibacter paraphrophilus*
3.	*Kingella genus (OTU1)*	47.	*Aggregatibacter sp. (OTU458)*
4.	*Kingella genus (OTU2)*	48.	*Aggregatibacter sp. (OTU512)*
5.	*Moraxella genus (OTU1)*	49.	*Aggregatibacter sp. (OTU513)*
6.	*Moraxella genus (OTU2)*	50.	*Agrobacterium tumefaciens*
7.	*Sanguibacter genus (OTU)*	51.	*Alloiococcus otitis*
8.	*Sphingomonas genus (OTU)*	52.	*Alloprevotella rava*
9.	*Staphylococcus genus (OTU1)*	53.	*Alloprevotella sp. (OTU308)*
10.	*Staphylococcus genus (OTU2)*	54.	*Alloprevotella sp. (OTU473)*
11.	*Staphylococcus genus (OTU3)*	55.	*Alloprevotella sp. (OTU474)*
12.	*Abiotrophia defectiva*	56.	*Alloprevotella sp. (OTU912)*
13.	*Acinetobacter_baumannii*	57.	*Alloprevotella sp. (OTU913)*
14.	*Actinobaculum sp. (OTU183)*	58.	*Alloprevotella sp. (OTU914)*
15.	*Actinomyces cardiffensis*	59.	*Alloprevotella tannerae*
16.	*Actinomyces georgiae*	60.	*Alloscardovia omnicolens*
17.	*Actinomyces gerencseriae*	61.	*Anaerococcus lactolyticus*
18.	*Actinomyces graevenitzii*	62.	*Anaeroglobus geminatus*
19.	*Actinomyces israelii*	63.	*Arcanobacterium haemolyticum*
20.	*Actinomyces johnsonii*	64.	*Arsenicicoccus sp. (OTU190)*
21.	*Actinomyces massiliensis*	65.	*Atopobium minutum*
22.	*Actinomyces meyeri*	66.	*Atopobium parvulum*
23.	*Actinomyces naeslundii*	67.	*Atopobium rimae*
24.	*Actinomyces odontolyticus*	68.	*Atopobium sp. (OTU199)*
25.	*Actinomyces oricola*	69.	*Atopobium sp. (OTU416)*
26.	*Actinomyces radicidentis*	70.	*Atopobium sp. (OTU810)*
27.	*Actinomyces sp. (OTU169)*	71.	*Atopobium vaginae*
28.	*Actinomyces sp. (OTU170)*	72.	*Bacillus clausii*
29.	*Actinomyces sp. (OTU171)*	73.	*Bacteroidaceae[G-1] sp. (OTU272)*
30.	*Actinomyces sp. (OTU172)*	74.	*Bacteroidales[G-2] sp. (OTU274)*
31.	*Actinomyces sp. (OTU175)*	75.	*Bacteroidales[G-3] sp. (OTU911)*
32.	*Actinomyces sp. (OTU178)*	76.	*Bacteroides heparinolyticus*
33.	*Actinomyces sp. (OTU180)*	77.	*Bacteroides tectus*
34.	*Actinomyces sp. (OTU181)*	78.	*Bacteroides zoogleoformans*
35.	*Actinomyces sp. (OTU414)*	79.	*Bacteroidetes[G-3] sp. (OTU280)*
36.	*Actinomyces sp. (OTU446)*	80.	*Bacteroidetes[G-3] sp. (OTU281)*
37.	*Actinomyces sp. (OTU448)*	81.	*Bacteroidetes[G-3] sp. (OTU365)*
38.	*Actinomyces sp. (OTU525)*	82.	*Bacteroidetes[G-3] sp. (OTU503)*
39.	*Actinomyces sp. (OTU848)*	83.	*Bacteroidetes[G-3] sp. (OTU899)*
40.	*Actinomyces sp. (OTU877)*	84.	*Bacteroidetes[G-4] sp. (OTU509)*
41.	*Actinomyces sp. (OTU896)*	85.	*Bacteroidetes[G-5] sp. (OTU505)*
42.	*Actinomyces sp. (OTU897)*	86.	*Bacteroidetes[G-5] sp. (OTU507)*
43.	*Actinomyces timonensis*	87.	*Bacteroidetes[G-5] sp. (OTU511)*
44.	*Actinomyces viscosus*	88.	*Bacteroidetes[G-6] sp. (OTU516)*

89.	*Bdellovibrio sp. (OTU039)*	133.	*Catonella sp. (OTU451)*
90.	*Bergeyella sp. (OTU319)*	134.	*Caulobacter sp. (OTU002)*
91.	*Bergeyella sp. (OTU322)*	135.	*Centipeda periodontii*
92.	*Bergeyella sp. (OTU907)*	136.	*Chloroflexi[G-1]sp. (OTU439)*
93.	*Bergeyellasp. (OTU900)*	137.	*Clostridiales[F-1][G-1] sp. (OTU093)*
94.	*Bifidobacteriaceae[G-2]sp. (OTU407)*	138.	*Clostridiales[F-1][G-2] sp. (OTU402)*
95.	*Bifidobacterium animalis subsp. animalis*	139.	*Clostridiales[F-2][G-1] sp. (OTU075)*
96.	*Bifidobacterium animalis subsp. lactis*	140.	*Clostridiales[F-2][G-1] sp. (OTU075)*
97.	*Bifidobacterium breve*	141.	*Clostridiales[F-2][G-2] sp. (OTU085)*
98.	*Bifidobacterium dentium*	142.	*Clostridiales[F-2][G-3] sp. (OTU366)*
99.	*Bifidobacterium longum*	143.	*Clostridiales[F-2][G-3]sp. (OTU381)*
100.	*Bifidobacterium scardovii*	144.	*Clostridiales[F-3][G-1] sp. (OTU876)*
101.	*Brevundimonas diminuta*	145.	*Corynebacterium diphtheriae*
102.	*Bulleidia extructa*	146.	*Corynebacterium durum*
103.	*Butyrivibrio sp. (OTU094)*	147.	*Corynebacterium matruchotii*
104.	*Campylobacter concisus*	148.	*Corynebacterium mucifaciens*
105.	*Campylobacter curvus*	149.	*Corynebacterium sp. (OTU184)*
106.	*Campylobacter gracilis*	150.	*Corynebacterium urealyticum*
107.	*Campylobacter sp. (OTU044)*	151.	*Cryptobacterium curtum*
108.	*Campylobacter sputorum*	152.	*Delftia acidovorans*
109.	*Campylobacter ureolyticus*	153.	*Desulfobulbus sp. (OTU041)*
110.	*Capnocytophaga gingivalis*	154.	*Desulfomicrobium orale*
111.	*Capnocytophaga granulosa*	155.	*Desulfovibrio fairfieldensis*
112.	*Capnocytophaga haemolytica*	156.	*Desulfovibrio sp. (OTU040)*
113.	*Capnocytophaga leadbetteri*	157.	*Dialister invisus*
114.	*Capnocytophaga ochracea*	158.	*Dialister micraerophilus*
115.	*Capnocytophaga sp. (OTU323)*	159.	*Dialister pneumosintes*
116.	*Capnocytophaga sp. (OTU324)*	160.	*Dialister sp. (OTU119)*
117.	*Capnocytophaga sp. (OTU332)*	161.	*Dialister sp. (OTU502)*
118.	*Capnocytophaga sp. (OTU334)*	162.	*Dietzia sp. (OTU368)*
119.	*Capnocytophaga sp. (OTU335)*	163.	*Dolosigranulum pigrum*
120.	*Capnocytophaga sp. (OTU336)*	164.	*Eggerthella lenta*
121.	*Capnocytophaga sp. (OTU338)*	165.	*Eggerthia catenaformis*
122.	*Capnocytophaga sp. (OTU380)*	166.	*Eikenella corrodens*
123.	*Capnocytophaga sp. (OTU412)*	167.	*Eikenella sp. (OTU011)*
124.	*Capnocytophaga sp. (OTU863)*	168.	*Enterococcus faecalis*
125.	*Capnocytophaga sp. (OTU864)*	169.	*Erysipelothrichaceae[G-1] sp. (OTU904)*
126.	*Capnocytophaga sp. (OTU878)*	170.	*Erysipelothrichaceae[G-1] sp. (OTU905)*
127.	*Capnocytophaga sp. (OTU901)*	171.	*Erysipelothrix tonsillarum*
128.	*Capnocytophaga sp. (OTU902)*	172.	*Erythromicrobium ramosum*
129.	*Capnocytophaga sp. (OTU903)*	173.	*Eubacterium limosum*
130.	*Capnocytophaga sputigena*	174.	*Eubacterium[11][G-1] infirmum*
131.	*Cardiobacterium hominis*	175.	*Eubacterium[11][G-1] sulci*
132.	*Cardiobacterium valvarum*	176.	*Eubacterium[11][G-3] brachy*

177.	*Eubacterium[11][G-5] saphenum*	221.	*Lachnospiraceae[G-2] sp. (OTU088)*
178.	*Eubacterium[11][G-6] minutum*	222.	*Lachnospiraceae[G-2] sp. (OTU096)*
179.	*Eubacterium[11][G-6] nodatum*	223.	*Lachnospiraceae[G-3] sp. (OTU100)*
180.	*Eubacterium[11][G-7] yurii*	224.	*Lachnospiraceae[G-5] sp. (OTU080)*
181.	*Filifactor alocis*	225.	*Lachnospiraceae[G-5] sp. (OTU455)*
182.	*Finegoldia magna*	226.	*Lachnospiraceae[G-6] sp. (OTU090)*
183.	*Fretibacterium fastidiosum*	227.	*Lachnospiraceae[G-7] sp. (OTU086)*
184.	*Fretibacterium sp. (OTU360)*	228.	*Lachnospiraceae[G-7] sp. (OTU163)*
185.	*Fretibacterium sp. (OTU361)*	229.	*Lachnospiraceae[G-8] sp. (OTU500)*
186.	*Fretibacterium sp. (OTU362)*	230.	*Lactobacillus brevis*
187.	*Fusobacterium gonidiaformans*	231.	*Lactobacillus coleohominis*
188.	*Fusobacterium necrophorum*	232.	*Lactobacillus fermentum*
189.	*Fusobacterium nucleatum subsp. animalis*	233.	*Lactobacillus iners*
190.	*Fusobacterium nucleatum subsp. nucleatum*	234.	*Lactobacillus jensenii*
191.	*Fusobacterium nucleatum subsp. polymorphum*	235.	*Lactobacillus kisonensis*
192.	*Fusobacterium nucleatum subsp. vincentii*	236.	*Lactobacillus parafarraginis*
193.	*Fusobacterium periodonticum*	237.	*Lactobacillus reuteri*
194.	*Fusobacterium sp. (OTU205)*	238.	*Lactobacillus salivarius*
195.	*Gardnerella vaginalis*	239.	*Lactobacillus sp. (OTU052)*
196.	*Gemella bergeri*	240.	*Lactobacillus vaginalis*
197.	*Gemella haemolysans*	241.	*Lactococcus lactis*
198.	*Gemella morbillorum*	242.	*Lautropia mirabilis*
199.	*Gemella sanguinis*	243.	*Leptothrix sp. (OTU024)*
200.	*GN02[G-1] sp. (OTU871)*	244.	*Leptothrix sp. (OTU025)*
201.	*GN02[G-1] sp. (OTU872)*	245.	*Leptotrichia goodfellowii*
202.	*GN02[G-2] sp. (OTU873)*	246.	*Leptotrichia hongkongensis*
203.	*Granulicatella elegans*	247.	*Leptotrichia shahii*
204.	*Haemophilus ducreyi*	248.	*Leptotrichia sp. (OTU212)*
205.	*Haemophilus parahaemolyticus*	249.	*Leptotrichia sp. (OTU215)*
206.	*Haemophilus parainfluenzae*	250.	*Leptotrichia sp. (OTU217)*
207.	*Haemophilus sp. (OTU872)*	251.	*Leptotrichia sp. (OTU218)*
208.	*Helicobacter pylori*	252.	*Leptotrichia sp. (OTU219)*
209.	*Johnsonella ignava*	253.	*Leptotrichia sp. (OTU221)*
210.	*Johnsonella sp. (OTU166)*	254.	*Leptotrichia sp. (OTU223)*
211.	*Jonquetella anthropi*	255.	*Leptotrichia sp. (OTU392)*
212.	*Kingella kingae*	256.	*Leptotrichia sp. (OTU417)*
213.	*Kingella oralis*	257.	*Leptotrichia sp. (OTU462)*
214.	*Kingella sp. (OTU459)*	258.	*Leptotrichia sp. (OTU463)*
215.	*Lachnoanaerobaculum orale*	259.	*Leptotrichia sp. (OTU498)*
216.	*Lachnoanaerobaculum saburreum*	260.	*Leptotrichia sp. (OTU847)*
217.	*Lachnoanaerobaculum sp. (OTU083)*	261.	*Leptotrichia sp. (OTU879)*
218.	*Lachnoanaerobaculum sp. (OTU089)*	262.	*Leptotrichia wadei*
219.	*Lachnoanaerobaculum sp. (OTU496)*	263.	*Leptotrichiaceae[G-1] sp. (OTU210)*
220.	*Lachnoanaerobaculum umeaense*	264.	*Leptotrichiaceae[G-1] sp. (OTU220)*

265.	*Lysinibacillus fusiformis*	309.	*Parvimonas sp. (OTU110)*
266.	*Megasphaera micronuciformis*	310.	*Peptococcus sp. (OTU167)*
267.	*Megasphaera sp. (OTU123)*	311.	*Peptococcus sp. (OTU168)*
268.	*Mitsuokella sp. (OTU131)*	312.	*Peptoniphilus asaccharolyticus*
269.	*Mitsuokella sp. (OTU521)*	313.	*Peptoniphilus indolicus*
270.	*Mobiluncus mulieris*	314.	*Peptoniphilus lacrimalis*
271.	*Mogibacterium diversum*	315.	*Peptoniphilus sp. (OTU375)*
272.	*Mogibacterium pumilum*	316.	*Peptoniphilus sp. (OTU386)*
273.	*Mogibacterium timidum*	317.	*Peptoniphilus sp. (OTU836)*
274.	*Mycobacterium leprae*	318.	*Peptostreptococcaceae[11][G-1] sp. (OTU383)*
275.	*Mycoplasma buccale*	319.	*Peptostreptococcaceae[11][G-2] sp. (OTU091)*
276.	*Mycoplasma faucium*	320.	*Peptostreptococcaceae[11][G-3] sp. (OTU382)*
277.	*Mycoplasma fermentans*	321.	*Peptostreptococcaceae[11][G-3] sp. (OTU495)*
278.	*Mycoplasma genitalium*	322.	*Peptostreptococcaceae[11][G-4] sp. (OTU103)*
279.	*Mycoplasma hominis*	323.	*Peptostreptococcaceae[11][G-4] sp. (OTU369)*
280.	*Mycoplasma lipophilum*	324.	*Peptostreptococcaceae[11][G-5] sp. (OTU493)*
281.	*Mycoplasma orale*	325.	*Peptostreptococcaceae[11][G-7] sp. (OTU081)*
282.	*Mycoplasma pneumoniae*	326.	*Peptostreptococcaceae[11][G-7] sp. (OTU106)*
283.	*Mycoplasma salivarium*	327.	*Peptostreptococcaceae[13][G-1] sp. (OTU113)*
284.	*Neisseria bacilliformis*	328.	*Peptostreptococcaceae[13][G-2] sp. (OTU790)*
285.	*Neisseria elongata*	329.	*Peptostreptococcus anaerobius*
286.	*Neisseria flavescens*	330.	*Peptostreptococcus stomatis*
287.	*Neisseria gonorrhoeae*	331.	*Porphyromonas asaccharolytica*
288.	*Neisseria lactamica*	332.	*Porphyromonas catoniae*
289.	*Neisseria meningitidis*	333.	*Porphyromonas endodontalis*
290.	*Neisseria pharyngis*	334.	*Porphyromonas gingivalis*
291.	*Neisseria sicca*	335.	*Porphyromonas sp. (OTU275)*
292.	*Neisseria sp. (OTU018)*	336.	*Porphyromonas sp. (OTU277)*
293.	*Neisseria sp. (OTU020)*	337.	*Porphyromonas sp. (OTU278)*
294.	*Neisseria sp. (OTU499)*	338.	*Porphyromonas sp. (OTU279)*
295.	*Neisseria sp. (OTU523)*	339.	*Porphyromonas sp. (OTU284)*
296.	*Neisseria subflava*	340.	*Porphyromonas sp. (OTU285)*
297.	*Neisseria weaveri*	341.	*Porphyromonas sp. (OTU395)*
298.	*Olsenella profusa*	342.	*Porphyromonas uenonis*
299.	*Olsenella sp. (OTU807)*	343.	*Prevotella baroniae*
300.	*Olsenella sp. (OTU809)*	344.	*Prevotella bivia*
301.	*Olsenella uli*	345.	*Prevotella buccae*
302.	*Oribacterium sinus*	346.	*Prevotella buccalis*
303.	*Oribacterium sp. (OTU102)*	347.	*Prevotella dentalis*
304.	*Oribacterium sp. (OTU108)*	348.	*Prevotella denticola*
305.	*Ottowia sp. (OTU894)*	349.	*Prevotella enoeca*
306.	*Paenibacillus sp. (OTU786)*	350.	*Prevotella fusca*
307.	*Parascardovia denticolens*	351.	*Prevotella histicola*
308.	*Parvimonas micra*	352.	*Prevotella intermedia*

353.	*Prevotella loescheii*	397.	*Propionibacterium sp. (OTU194)*
354.	*Prevotella maculosa*	398.	*Propionibacterium sp. (OTU915)*
355.	*Prevotella marshii*	399.	*Proteus mirabilis*
356.	*Prevotella melaninogenica*	400.	*Pseudomonas aeruginosa*
357.	*Prevotella micans*	401.	*Pseudomonas otitidis*
358.	*Prevotella multiformis*	402.	*Pseudomonas sp. (OTU032)*
359.	*Prevotella multisaccharivorax*	403.	*Pseudoramibacter alactolyticus*
360.	*Prevotella nigrescens*	404.	*Pyramidobacter piscolens*
361.	*Prevotella oralis*	405.	*Rothia aeria*
362.	*Prevotella oris*	406.	*Rothia dentocariosa*
363.	*Prevotella oulorum*	407.	*Rothia mucilaginosa*
364.	*Prevotella pallens*	408.	*Scardovia inopinata*
365.	*Prevotella pleuritidis*	409.	*Scardovia wiggsiae*
366.	*Prevotella saccharolytica*	410.	*Selenomonas artemidis*
367.	*Prevotella salivae*	411.	*Selenomonas dianae*
368.	*Prevotella scopos*	412.	*Selenomonas flueggei*
369.	*Prevotella shahii*	413.	*Selenomonas noxia*
370.	*Prevotella sp. (OTU292)*	414.	*Selenomonas sp. (OTU133)*
371.	*Prevotella sp. (OTU293)*	415.	*Selenomonas sp. (OTU134)*
372.	*Prevotella sp. (OTU296)*	416.	*Selenomonas sp. (OTU136)*
373.	*Prevotella sp. (OTU300)*	417.	*Selenomonas sp. (OTU137)*
374.	*Prevotella sp. (OTU301)*	418.	*Selenomonas sp. (OTU138)*
375.	*Prevotella sp. (OTU304)*	419.	*Selenomonas sp. (OTU143)*
376.	*Prevotella sp. (OTU305)*	420.	*Selenomonas sp. (OTU146)*
377.	*Prevotella sp. (OTU306)*	421.	*Selenomonas sp. (OTU149)*
378.	*Prevotella sp. (OTU309)*	422.	*Selenomonas sp. (OTU388)*
379.	*Prevotella sp. (OTU310)*	423.	*Selenomonas sp. (OTU442)*
380.	*Prevotella sp. (OTU315)*	424.	*Selenomonas sp. (OTU478)*
381.	*Prevotella sp. (OTU317)*	425.	*Selenomonas sp. (OTU501)*
382.	*Prevotella sp. (OTU376)*	426.	*Selenomonas sputigena*
383.	*Prevotella sp. (OTU396)*	427.	*Simonsiella muelleri*
384.	*Prevotella sp. (OTU443)*	428.	*Slackia exigua*
385.	*Prevotella sp. (OTU472)*	429.	*Sneathia amnionii*
386.	*Prevotella sp. (OTU475)*	430.	*Sneathia sanguinegens*
387.	*Prevotella sp. (OTU515)*	431.	*Solobacterium moorei*
388.	*Prevotella sp. (OTU526)*	432.	*SR1[G-1] sp. (OTU345)*
389.	*Prevotella sp. (OTU820)*	433.	*SR1[G-1] sp. (OTU874)*
390.	*Prevotella veroralis*	434.	*SR1[G-1] sp. (OTU875)*
391.	*Propionibacterium acidifaciens*	435.	*Stenotrophomonas maltophilia*
392.	*Propionibacterium acnes*	436.	*Stomatobaculum longum*
393.	*Propionibacterium avidum*	437.	*Stomatobaculum sp. (OTU097)*
394.	*Propionibacterium propionicum*	438.	*Stomatobaculum sp. (OTU373)*
395.	*Propionibacterium sp. (OTU192)*	439.	*Stomatobaculum sp. (OTU910)*
396.	*Propionibacterium sp. (OTU193)*	440.	*Streptococcus agalactiae*

441.	*Streptococcus anginosus*	486.	*Treponema sp. (OTU232)*
442.	*Streptococcus constellatus*	487.	*Treponema sp. (OTU234)*
443.	*Streptococcus cristatus*	488.	*Treponema sp. (OTU235)*
444.	*Streptococcus downei*	489.	*Treponema sp. (OTU236)*
445.	*Streptococcus intermedius*	490.	*Treponema sp. (OTU238)*
446.	*Streptococcus mutans*	491.	*Treponema sp. (OTU239)*
447.	*Streptococcus parasanguinis II*	492.	*Treponema sp. (OTU242)*
448.	*Streptococcus sanguinis*	493.	*Treponema sp. (OTU246)*
449.	*Streptococcus sobrinus*	494.	*Treponema sp. (OTU247)*
450.	*Streptococcus sp. (OTU064)*	495.	*Treponema sp. (OTU249)*
451.	*Streptococcus sp. (OTU066)*	496.	*Treponema sp. (OTU250)*
452.	*Streptococcus sp. (OTU068)*	497.	*Treponema sp. (OTU252)*
453.	*Streptococcus sp. (OTU069)*	498.	*Treponema sp. (OTU253)*
454.	*Streptococcus sp. (OTU431)*	499.	*Treponema sp. (OTU254)*
455.	*Streptococcus sp. (OTU486)*	500.	*Treponema sp. (OTU255)*
456.	*Streptococcus sp. (OTU487)*	501.	*Treponema sp. (OTU256)*
457.	*Syntrophomonadaceae[8][G-1] sp. (OTU435)*	502.	*Treponema sp. (OTU257)*
458.	*Tannerella forsythia*	503.	*Treponema sp. (OTU258)*
459.	*Tannerella sp. (OTU286)*	504.	*Treponema sp. (OTU260)*
460.	*Tannerella sp. (OTU808)*	505.	*Treponema sp. (OTU262)*
461.	*Tannerella sp. (OTU916)*	506.	*Treponema sp. (OTU263)*
462.	*TM7[G-1] sp. (OTU346)*	507.	*Treponema sp. (OTU268)*
463.	*TM7[G-1] sp. (OTU347)*	508.	*Treponema sp. (OTU269)*
464.	*TM7[G-1] sp. (OTU348)*	509.	*Treponema sp. (OTU270)*
465.	*TM7[G-1] sp. (OTU349)*	510.	*Treponema sp. (OTU271)*
466.	*TM7[G-1] sp. (OTU352)*	511.	*Treponema sp. (OTU490)*
467.	*TM7[G-1] sp. (OTU353)*	512.	*Treponema sp. (OTU508)*
468.	*TM7[G-1] sp. (OTU488)*	513.	*Treponema sp. (OTU517)*
469.	*TM7[G-2] sp. (OTU350)*	514.	*Treponema sp. (OTU518)*
470.	*TM7[G-3] sp. (OTU351)*	515.	*Treponema vincentii*
471.	*TM7[G-4] sp. (OTU355)*	516.	*Turicella otitidis*
472.	*TM7[G-5] sp. (OTU356)*	517.	*Veillonella atypica*
473.	*TM7[G-5] sp. (OTU437)*	518.	*Veillonella denticariosi*
474.	*Treponema amylovorum*	519.	*Veillonella dispar*
475.	*Treponema denticola*	520.	*Veillonella parvula*
476.	*Treponema lecithinolyticum*	521.	*Veillonella rogosae*
477.	*Treponema maltophilum*	522.	*Veillonella sp. (OTU780)*
478.	*Treponema parvum*	523.	*Veillonella sp. (OTU917)*
479.	*Treponema pectinovorum*	524.	*Veillonellaceae[G-1] sp. (OTU129)*
480.	*Treponema putidum*	525.	*Veillonellaceae[G-1] sp. (OTU135)*
481.	*Treponema socranskii*	526.	*Veillonellaceae[G-1] sp. (OTU145)*
482.	*Treponema sp. (OTU226)*	527.	*Veillonellaceae[G-1] sp. (OTU148)*
483.	*Treponema sp. (OTU227)*	528.	*Veillonellaceae[G-1] sp. (OTU155)*
484.	*Treponema sp. (OTU228)*	529.	*Veillonellaceae[G-1] sp. (OTU483)*
485.	*Treponema sp. (OTU230)*	530.	*Veillonellaceae[G-1] sp. (OTU918)*

Table S2: 174 bacterial OTUs present in approximately 50% of the study population

1.	*Kingella genus (OTU1)*	45.	*Eggerthia catenaformis*
2.	*Staphylococcus genus (OTU3)*	46.	*Eikenella corrodens*
3.	*Abiotrophia defectiva*	47.	*Erysipelothrichaceae[G-1] sp. (OTU905)*
4.	*Actinobaculum sp. (OTU183)*	48.	*Eubacterium[11][G-1] infirmum*
5.	*Actinomyces cardiffensis*	49.	*Eubacterium[11][G-1] sulci*
6.	*Actinomyces gerencseriae*	50.	*Eubacterium[11][G-3] brachy*
7.	*Actinomyces meyeri*	51.	*Eubacterium limosum*
8.	*Actinomyces sp. (OTU172)*	52.	*Filifactor alocis*
9.	*Actinomyces sp. (OTU178)*	53.	*Fretibacterium fastidiosum*
10.	*Actinomyces sp. (OTU181)*	54.	*Fretibacterium sp. (OTU360)*
11.	*Actinomyces sp. (OTU448)*	55.	*Fusobacterium nucleatum subsp. animalis*
12.	*Actinomyces naeslundii*	56.	*Fusobacterium nucleatum subsp. nucleatum*
13.	*Actinomyces odontolyticus*	57.	*Fusobacterium nucleatum subsp. polymorphum*
14.	*Aggregatibacter sp. (OTU458)*	58.	*Fusobacterium nucleatum subsp. vincentii*
15.	*Alloprevotella rava*	59.	*Fusobacterium periodonticum*
16.	*Alloprevotella sp. (OTU308)*	60.	*Gemella haemolysans*
17.	*Alloprevotella sp. (OTU473)*	61.	*Gemella sanguinis*
18.	*Alloprevotella tannerae*	62.	*Gemella morbillorum*
19.	*Alloscardovia omnicolens*	63.	*Granulicatella elegans*
20.	*Alloprevotella sp. (OTU914)*	64.	*Haemophilus ducreyi*
21.	*Anaeroglobus geminatus*	65.	*Haemophilus parahaemolyticus*
22.	*Atopobium parvulum*	66.	*Haemophilus parainfluenzae*
23.	*Atopobium rimae*	67.	*Lachnoanaerobaculum orale*
24.	*Bacteroidaceae[G-1] sp. (OTU272)*	68.	*Lachnoanaerobaculum saburreum*
25.	*Bacteroidales[G-2] sp. (OTU274)*	69.	*Lachnoanaerobaculum umeaense*
26.	*Bergeyella sp. (OTU322)*	70.	*Lachnospiraceae[G-2] sp. (OTU096)*
27.	*Bergeyella sp. (OTU907)*	71.	*Lachnospiraceae[G-3] sp. (OTU100)*
28.	*Bifidobacterium dentium*	72.	*Lachnospiraceae[G-5] sp. (OTU455)*
29.	*Campylobacter concisus*	73.	*Lachnospiraceae[G-8] sp. (OTU500)*
30.	*Campylobacter gracilis*	74.	*Lactobacillus reuteri*
31.	*Campylobacter sp. (OTU044)*	75.	*Lautropia mirabilis*
32.	*Capnocytophaga gingivalis*	76.	*Leptotrichia hongkongensis*
33.	*Capnocytophaga granulosa*	77.	*Leptotrichia shahii*
34.	*Capnocytophaga leadbetteri*	78.	*Leptotrichia sp. (OTU212)*
35.	*Capnocytophaga sputigena*	79.	*Leptotrichia sp. (OTU218)*
36.	*Cardiobacterium hominis*	80.	*Leptotrichia sp. (OTU219)*
37.	*Cardiobacterium valvarum*	81.	*Leptotrichia sp. (OTU221)*
38.	*Centipeda periodontii*	82.	*Leptotrichia sp. (OTU392)*
39.	*Clostridiales[F-2][G-2] sp. (OTU085)*	83.	*Leptotrichia sp. (OTU417)*
40.	*Clostridiales[F-2][G-1] sp. (OTU075)*	84.	*Leptotrichia sp. (OTU498)*
41.	*Corynebacterium durum*	85.	*Leptotrichia sp. (OTU879)*
42.	*Corynebacterium matruchotii*	86.	*Leptotrichia wadei*
43.	*Dialister invisus*	87.	*Leptotrichia sp. (OTU223)*
44.	*Dialister pneumosintes*	88.	*Leptotrichia sp. (OTU215)*

89.	*Megasphaera micronuciformis*	132.	*Rothia mucilaginosa*
90.	*Mycobacterium leprae*	133.	*Scardovia wiggsiae*
91.	*Mycoplasma salivarium*	134.	*Selenomonas flueggei*
92.	*Neisseria bacilliformis*	135.	*Selenomonas noxia*
93.	*Neisseria elongata*	136.	*Selenomonas sp. (OTU136)*
94.	*Neisseria flavescens*	137.	*Selenomonas sp. (OTU138)*
95.	*Neisseria meningitidis*	138.	*Selenomonas sp. (OTU146)*
96.	*Olsenella sp. (OTU807)*	139.	*Selenomonas sp. (OTU149)*
97.	*Oribacterium sinus*	140.	*Selenomonas sp. (OTU478)*
98.	*Oribacterium sp. (OTU108)*	141.	*Selenomonas sputigena*
99.	*Parvimonas micra*	142.	*Selenomonas sp. (OTU134)*
100.	*Peptostreptococcaceae[11][G-4] sp. (OTU369)*	143.	*Solobacterium moorei*
101.	*Peptostreptococcus stomatis*	144.	*Stomatobaculum longum*
102.	*Peptococcus sp. (OTU167)*	145.	*Stomatobaculum sp. (OTU097)*
103.	*Porphyromonas endodontalis*	146.	*Streptococcus anginosus*
104.	*Porphyromonas gingivalis*	147.	*Streptococcus constellatus*
105.	*Porphyromonas sp. (OTU275)*	148.	*Streptococcus intermedius*
106.	*Porphyromonas sp. (OTU395)*	149.	*Streptococcus mutans*
107.	*Porphyromonas sp. (OTU278)*	150.	*Streptococcus parasanguinis II*
108.	*Porphyromonas sp. (OTU279)*	151.	*Streptococcus sanguinis*
109.	*Prevotella baroniae*	152.	*Streptococcus sp. (OTU064)*
110.	*Prevotella buccae*	153.	*Streptococcus sp. (OTU066)*
111.	*Prevotella dentalis*	154.	*Streptococcus sp. (OTU068)*
112.	*Prevotella denticola*	155.	*Streptococcus sp. (OTU431)*
113.	*Prevotella histicola*	156.	*Streptococcus sp. (OTU486)*
114.	*Prevotella maculosa*	157.	*Tannerella forsythia*
115.	*Prevotella melaninogenica*	158.	*Tannerella sp. (OTU286)*
116.	*Prevotella nigrescens*	159.	*TM7[G-1] sp. (OTU346)*
117.	*Prevotella oralis*	160.	*TM7[G-1] sp. (OTU349)*
118.	*Prevotella oris*	161.	*TM7[G-1] sp. (OTU352)*
119.	*Prevotella oulorum*	162.	*TM7[G-1] sp. (OTU353)*
120.	*Prevotella pallens*	163.	*TM7[G-3] sp. (OTU351)*
121.	*Prevotella salivae*	164.	*TM7[G-5] sp. (OTU437)*
122.	*Prevotella sp. (OTU300)*	165.	*TM7[G-1] sp. (OTU348)*
123.	*Prevotella sp. (OTU305)*	166.	*Treponema denticola*
124.	*Prevotella sp. (OTU309)*	167.	*Treponema maltophilum*
125.	*Prevotella sp. (OTU315)*	168.	*Treponema socranskii*
126.	*Prevotella sp. (OTU472)*	169.	*Veillonella dispar*
127.	*Prevotella sp. (OTU526)*	170.	*Veillonella parvula*
128.	*Prevotella veroralis*	171.	*Veillonella rogosae*
129.	*Prevotella intermedia*	172.	*Veillonella sp. (OTU917)*
130.	*Prevotella sp. (OTU317)*	173.	*Veillonella denticariosi*
131.	*Rothia dentocariosa*	174.	*Veillonella atypica*

Table S3: Characteristics and loading patterns of two extracted factors generated by PCA on 174 bacterial OTUs

		Factor 1	Factor 2
	Total explained variance (15.42%)	**9.74**	**5.68**
	Loaded by original variables	**82**	**51**
1.	*Tannerella forsythia*	0.71**	
2.	*Bacteroidaceae[G-1] sp.(OTU272)*	0.65	
3.	*Treponema socranskii*	0.65**	
4.	*Campylobacter gracilis*	0.64	-0.29
5.	*Dialister pneumosintes*	0.64*	
6.	*Eubacterium[11][G-3] brachy*	0.63*	
7.	*Fretibacterium sp.(OTU360)*	0.59*	
8.	*Fretibacterium fastidiosum*	0.58**	0.28**
9.	*Parvimonas micra*	0.56*	
10.	*Prevotella baroniae*	0.56	
11.	*Prevotella nigrescens*	0.55	
12.	*TM7[G-1] sp.OTU349*	0.53	
13.	*Prevotella oris*	0.52	-0.3
14.	*Fusobacterium nucleatum subsp. animalis*	0.51	
15.	*Prevotella maculosa*	0.51	
16.	*Prevotella oralis*	0.51*	
17.	*Porphyromonas gingivalis*	0.49**	0.29**
18.	*Selenomonas noxia*	0.49	
19.	*Peptostreptococcaceae[11][G-4] sp.(OTU369)*	0.48	0.29
20.	*Prevotella dentalis*	0.48**	
21.	*Selenomonas sputigena*	0.48***	
22.	*Dialister invisus*	0.47	
23.	*Bacteroidales[G-2] sp.(OTU274)*	0.46	
24.	*Cardiobacterium hominis*	0.46	
25.	*Centipeda periodontii*	0.46	
26.	*Filifactor alocis*	0.46	0.33
27.	*TM7[G-5] sp.(OTU437)*	0.46	
28.	*Treponema maltophilum*	0.46**	
29.	*Fusobacterium nucleatum subsp nucleatum*	0.45	
30.	*Porphyromonas endodontalis*	0.45**	
31.	*Selenomonas sp.(OTU134)*	0.45	
32.	*Actinomyces sp.(OTU178)*	0.44	
33.	*Corynebacterium matruchotii*	0.44	
34.	*Prevotella buccae*	0.44*	
35.	*Prevotella sp.(OTU300)*	0.44	
36.	*Eikenella corrodens*	0.43	
37.	*Olsenella sp.(OTU807)*	0.43	

38.	*Treponema denticola*	0.43**	0.28**
39.	*Anaeroglobus geminatus*	0.42*	
40.	*Fusobacterium nucleatum subsp. vincentti*	0.42	
41.	*Lachnoanaerobaculum saburreum*	0.42	
42.	*Lachnospiraceae[G-8] sp.(OTU500)*	0.42	0.27
43.	*TM7[G-1] sp.(OTU346)*	0.42	
44.	*Actinobaculum sp.(OTU183)*	0.41	
45.	*Lautropia mirabilis*	0.40	
46.	*Mycobacterium leprae*	0.40	
47.	*Prevotella denticola*	0.40	-0.27
48.	*Streptococcus constellatus*	0.40*	
49.	*Tannerella sp.(OTU286)*	0.39	0.35
50.	*Capnocytophaga leadbetteri*	0.38	
51.	*Prevotella intermedia*	0.38	0.36
52.	*Erysipelothrichaceae[G-1] sp.(OTU905)*	0.37	
53.	*Neisseria elongata*	0.37*	
54.	*Prevotella oulorum*	0.37	
55.	*Prevotella sp.(OTU526)*	0.37	
56.	*Eubacterium[11][G-1] infirmum*	0.36***	
57.	*Actinomyces gerencseriae*	0.35	
58.	*Actinomyces naeslundii*	0.35	
59.	*Cardiobacterium valvarum*	0.35	
60.	*Leptotrichia shahii*	0.35	
61.	*Prevotella sp.(OTU317)*	0.35	
62.	*Actinomyces sp.(OTU448)*	0.33	-0.32
63.	*Streptococcus anginosus*	0.33	-0.40
64.	*TM7[G-1] sp.(OTU353)*	0.32	
65.	*Alloprevotella tannerae*	0.31	0.29
66.	*Gemella morbillorum*	0.30	0.30
67.	*Leptotrichia hongkongensis*	0.30	-0.29
68.	*Leptotrichia wadei*	0.29	-0.29
69.	*Rothia dentocariosa*	0.29	-0.46
70.	*Bergeyella sp.(OTU907)*	0.28***	
71.	*Capnocytophaga gingivalis*	0.27	
72.	*Leptotrichia sp. (OTU212)*	0.27	
73.	*Prevotella sp.(OTU472)*	0.27	
74.	*Streptococcus sanguinis*	0.27	
75.	*Leptotrichia sp.(OTU392)*	0.26	0.26
76.	*Leptotrichia sp.(OTU879)*	0.26	
77.	*Actinomyces meyeri*	0.25	
78.	*Lachnospiraceae[G-3] sp. (OTU100)*	0.25	
79.	*Leptotrichia sp.(OTU498)*	0.25	
80.	*Streptococcus intermedius*	0.25	
81.	*Atopobium rimae*		
82.	*Bifidobacterium dentium*		

83.	*Eggerthia catenaformis*	
84.	*Porphyromonas sp.(OTU395)*	
85.	*Selenomonas flueggei*	
86.	*Actinomyces cardiffensis*	
87.	*Capnocytophaga granulosa*	
88.	*Corynebacterium durum*	
89.	*Leptotrichia sp.(OTU219)*	
90.	*Veillonella parvula*	-0.44
91.	*Peptococcus sp. (OTU167)*	0.50
92.	*Neisseria bacilliformis*	
93.	*Veillonella dispar*	-0.51
94.	*Atopobium parvulum*	-0.29
95.	*Fusobacterium nucleatum subsp polymorphum*	
96.	*Streptococcus mutans*	-0.48
97.	*Staphylococcus genus (OTU3)*	
98.	*Clostridiales[F-2][G-1] sp.(OTU075)*	
99.	*Prevotella sp. (OTU315)*	0.34
100.	*Kingella genus (OTU1)*	
101.	*Abiotrophia defectiva*	
102.	*Scardovia wiggsiae*	-0.41
103.	*Bergeyella sp.(OTU322)*	0.33
104.	*Streptococcus sp. (OTU431)*	
105.	*Streptococcus sp. (OTU064)*	-0.42
106.	*Gemella sanguinis*	
107.	*Streptococcus parasanguinis II*	-0.36
108.	*Lachnospiraceae[G-2] sp. (OTU096)*	
109.	*Leptotrichia sp. (OTU223)*	
110.	*TM7[G-1] sp. (OTU348)*	
111.	*Actinomyces sp.(OTU172)*	
112.	*Selenomonas sp. (OTU146)*	
113.	*Aggregatibacter sp.(OTU458)*	
114.	*Gemella haemolysans*	
115.	*Neisseria flavescens*	
116.	*Leptotrichia sp. (OTU218)*	
117.	*Stomatobaculum longum*	
118.	*Streptococcus sp. (OTU066)*	
119.	*Granulicatella elegans*	
120.	*Oribacterium sinus*	
121.	*Alloprevotella sp.(OTU473)*	0.28
122.	*Capnocytophaga sputigena*	
123.	*Leptotrichia sp. (OTU417)*	
124.	*Porphyromonas sp. (OTU278)*	0.42
125.	*Selenomonas sp. (OTU138)*	
126.	*Actinomyces odontolyticus*	
127.	*Alloscardovia omnicolens*	

128.	*Haemophilus parahaemolyticus*	
129.	*Campylobacter sp. (OTU044)*	0.38
130.	*Neisseria meningitidis*	
131.	*Alloprevotella rava*	
132.	*Peptostreptococcus stomatis*	0.59
133.	*Prevotella veroralis*	
134.	*Leptotrichia sp. (OTU221)*	
135.	*Streptococcus sp. (OTU486)*	
136.	*Campylobacter concisus*	
137.	*Streptococcus sp. (OTU068)*	-0.46
138.	*TM7[G-3] sp. (OTU351)*	0.29
139.	*Clostridiales[F-2][G-2] sp. (OTU085)*	
140.	*Prevotella histicola*	
141.	*Rothia mucilaginosa*	-0.38
142.	*TM7[G-1] sp. (OTU352)*	0.31
143.	*Actinomyces sp.(OTU181)*	
144.	*Prevotella sp. (OTU309)*	
145.	*Selenomonas sp. (OTU149)*	
146.	*Veillonella denticariosi*	
147.	*Alloprevotella sp.(OTU308)*	0.28
148.	*Lachnoanaerobaculum orale*	
149.	*Lachnoanaerobaculum umeaense*	0.35
150.	*Lachnospiraceae[G-5] sp. (OTU455)*	
151.	*Selenomonas sp. (OTU136)*	
152.	*Selenomonas sp.(OTU478)*	
153.	*Haemophilus parainfluenzae*	
154.	*Oribacterium sp.(OTU108)*	
155.	*Prevotella sp. (OTU305)*	
156.	*Lactobacillus reuteri*	
157.	*Haemophilus ducreyi*	
158.	*Mycoplasma salivarium*	
159.	*Eubacterium_limosum*	
160.	*Solobacterium moorei*	0.30
161.	*Veillonella sp. (OTU917)*	-0.31
162.	*Leptotrichia sp. (OTU215)*	0.49
163.	*Alloprevotella sp. (OTU914)*	0.42
164.	*Porphyromonas sp. (OTU279)*	0.51
165.	*Stomatobaculum sp. (OTU097)*	0.38
166.	*Eubacterium[11][G-1] sulci*	0.48
167.	*Porphyromonas sp. (OTU275)*	0.50
168.	*Megasphaera micronuciformis*	
169.	*Veillonella rogosae*	0.48
170.	*Fusobacterium periodonticum*	0.69
171.	*Prevotella salivae*	
172.	*Prevotella pallens*	0.45***

173.	*Veillonella atypica*	-0.26	-0.45
174.	*Prevotella melaninogenica*	-0.34	

*Treelet component 1 (endodontitis-associated bacteria component);
**Treelet component 2 (periodontitis-associated bacteria component);
***Treelet component 3 (caries-associated bacteria component)

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