GENETICS OF THE RELATIONSHIP BETWEEN PERIODONTITIS AND CARDIOVASCULAR DISEASE

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ABSTRACT

Objective: It has been proposed that periodontitis and cardiovascular disease are related through a similar underlying inflammation pathway. There has also been suggestion of a genetic etiology connecting the two conditions. Further clarification of this proposed relationship has public health significance as both diseases affect over a quarter of the adult population of the United States. Using data from the Center from Oral Health Research in Appalachia cohort, we examined the relationship between periodontitis and cardiovascular disease. We then tested their association to candidate genetic loci to assess if the genetic loci may explain part of the relationship between the conditions.

Methods and Results: Three indicators of periodontitis were developed; a molecular testing-based variable (BANA, n=970) and two probing depth measurements, a liberal approximation that considered missing teeth as missing due to periodontitis (n=973) and a conservative one that did not (n=961). Personal and family history of heart disease and high blood pressure were condensed into a composite score indicative of cardiovascular disease (n=973). Four candidate single nucleotide polymorphisms (SNPs) were identified in the literature as related to both periodontitis and cardiovascular disease: rs10864294, rs4252120, rs1800795, and rs10965215. Regression models were used to test the association between periodontitis and cardiovascular disease indicators and candidate SNPs with and without

adjustment for covariates including age, sex, race, smoking status, and body mass index. Periodontitis and cardiovascular disease proxies were significantly associated with each other in unadjusted models ($p<0.050$). Heart disease score was not significantly associated with any candidate SNP, but both conservative and liberal probing depth-based indicators had a significant association with rs10864294 in models adjusted for covariates ($p=0.003$, $p=0.006$). BANA did not demonstrate a significant association with heart disease score or any candidate SNP.

Conclusions: This study yielded support of the relationship between periodontitis and cardiovascular disease, but not an extension to the genetic level. Although rs10864294 was significantly associated with the probing depth periodontitis indicators, no candidate SNP was associated with heart disease score so it is not possible to conclude that any of these candidate SNPs explain the significant relationship seen between periodontitis and cardiovascular disease indicators.

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1.0 INTRODUCTION

Oral health is an important aspect of overall well-being, and research is continually uncovering additional components to support this connection. Recent studies have suggested that this relationship may even extend to a common genetic etiology between certain systemic diseases [1]. One example involving oral health is that the underlying inflammation pathway of chronic periodontitis may involve some of the same genes as that of cardiovascular disease [2]. According to the American Academy of Periodontology, periodontitis is a disease involving chronic inflammation and deterioration of the tissues and bone in the mouth [3]. If not treated properly, chronic periodontitis can lead to the separation of the gums from the teeth, eventually resulting in the loss of teeth. The onset and development of periodontitis is influenced by many components, including general oral health, the microbial environment of the mouth, and possibly shared genetic mutations that also cause systemic diseases such as cardiovascular disease [2]. Only recently has evidence supporting the genetic commonalities between periodontitis and cardiovascular disease been published, and there are some studies that contradict the claims, even though knowledge of the general connection has been well documented [1].

A review article by Aarabi et al. summarized previous studies that looked at genetic loci related to both chronic periodontitis and coronary artery disease, a subtype of cardiovascular disease [1]. The identified loci, represented by their lead single nucleotide polymorphisms (SNPs) were identified through genome-wide association studies and are near genes associated with regulation of inflammation and the immune system: *VAMP3, CAMTA1, PLG, ANRIL, CDKN2A*, and *CDKN2B*. These genes are involved in controlling blood clotting, expression of immune system proteins in and growth of cardiovascular tissues, and demonstrate reciprocal regulation of

each other [1]. Further literature review identified another SNP, corresponding to the pro-inflammatory cytokine interleukin-6, *IL6,* that has been shown to be associated with periodontitis and cardiovascular disease independently [4, 5].

This study intends to analyze further the link between periodontitis and cardiovascular health and determine if this association is found in the initial cohort study of the Center for Oral Health Research in Appalachia (COHRA1). Using data from COHRA1, composite phenotypes of periodontitis and cardiovascular disease will be used to test the significance of these SNPs reported by Aarabi et al. and similar studies. Two specific aims have been developed to answer this question.

- The first specific aim is to perform a literature review to assess current understanding of the similarities in the underlying genetic mechanism of periodontitis and cardiovascular disease.
- The second specific aim is to perform statistical analyses to determine if the genetic commonalities identified in literature are supported by data from the COHRA1 study. This will be broken down into three main questions:
    - Are the candidate single nucleotide polymorphisms (SNPs) associated with medical or family history of cardiovascular disease?
    - Are the candidate SNPs associated with indicators of periodontal disease?
    - Are the indicators of periodontal disease associated with medical or family history of cardiovascular disease? If so, do any candidate SNPs explain part of the relationship between the periodontitis and cardiovascular disease variables?

Gaining a further understanding of the significance of these genes relating to periodontitis, cardiovascular disease, and the relationship between the two will have multiple implications.

Understanding more about this genetic relationship is a significant public health concern because of the growing health care burden associated with both cardiovascular and periodontal diseases. Results will add to the literature regarding an underlying genetic relationship between these conditions, which can help with identifying individuals at risk for hereditary periodontitis and/or cardiovascular disease to allow the appropriate management of their health. This would be important for the fields of cardiovascular genetic counseling and dentistry as it could provide another piece of family history information that may be important to consider when determining risk of cardiovascular disease. The results of this study will be disseminated through a written report as well as presentations and meetings with medical and public health professionals.

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF PERIODONTAL DISEASE

In general, periodontal disease refers to a range of chronic inflammatory diseases of the soft tissue, bone, and ligament in the mouth [2]. The mildest form, gingivitis, involves localized inflammation of the gums, leaving them red and swollen with a tendency to bleed easily. Gingivitis is often due to poor oral hygiene, which leads to and perpetuates the development of plaque and bacterial film on the teeth [2, 3]. If gingivitis is left untreated for an extended period of time, it can progress to periodontitis, a more severe condition involving the deterioration of gum tissue and attachment structures, eventually leading to tooth loss [2].

Depending on its presentation, periodontitis can be classified as either an aggressive or a chronic form. Aggressive periodontitis has an isolated, rapid loss of attachment of teeth to supporting structures and has a stronger genetic component, while chronic periodontitis has a slower disease course, with prolonged development of pockets of recession prior to tooth loss [3]. These pockets are the hallmark of the disease, and they can indicate the severity of the loss of attachment as well as provide access for bacterial infection to further inflammation [3]. Chronic periodontitis is more common in adults, while aggressive periodontitis can also be seen in children [2]. Periodontitis is considered to be generalized if at least 10 of the 32 permanent teeth are affected, but it is more often seen that both chronic and aggressive periodontitis demonstrate site specificity [2].

Due to the multifactorial aspect of periodontitis, different studies have attempted to measure the disease in different ways. Often, researchers derive the variables from the

understanding of the etiology of periodontitis at the time [6]. A common definition of periodontitis utilizes an established metric developed through a joint effort by the Centers for Disease Control and Prevention and the American Academy of Periodontology. In this definition, severe periodontitis is determined when at least two teeth have clinical attachment loss (CAL) of over 6mm and periodontal probing depth (PPD) of over 5mm, while moderate periodontitis has two sites with over 4mm CAL or two with over 5mm PPD [7].

As gingivitis progresses to periodontitis, there is a characteristic shift in the oral microbiome that triggers infection and inflammation. There are three main microbial species responsible for this shift: *Porphyromonas gingivalis* (*P. gingivalis*), *Bacteroides forsythus* (*B. forsythus*), and *Treponema denticola* (*T. denticola*) [8]. Interactions between these microbial species and others in the mouth shifts the symbiosis that maintains a state of oral health to dysbiosis that promotes the development of periodontitis [8]. These bacterial species are responsible for hydrolyzing benzoyl-DL-arginine-naphthylamide (BANA), a synthetic substrate; therefore, it is useful to test for the presence of this marker as a measure of periodontal infection [9]. The utility of testing BANA species is dependent on the sensitivity and specificity of culturing to assess the composition of the microbiome [10]. Studies have shown that the BANA test can have a similar effectiveness to DNA testing for the presence of the key microbial species involved in periodontitis, although it is not as useful a measurement after treatment for the condition [11].

Treatment for periodontitis depends on the severity of the disease at the time of diagnosis. Diagnosis is based on clinical findings on exam as well as radiographic analysis of attachment and medical and dental history [12]. Periodontal screening should be performed as a routine part of dental care, with the frequency and extent of a full evaluation depending on whether or not a patient currently or previously showed signs of the disease [12]. It is relatively easy to prevent or reverse

the early signs of periodontitis with improved oral hygiene. The first step of treatment is a thorough cleaning of the teeth, or debridement, to remove plaque and biofilm buildup above and below the gum line that may be contributing to inflammation [2]. If there are signs of attachment loss or bone loss, further steps can involve medication and surgical intervention to reduce inflammation and maintain healthy dentition [2]. Some medications used are designed to specifically target the effects of the key microbial species associated with periodontitis, such as *P. gingivalis* [13].

Of the multiple known risk factors for periodontitis, some are modifiable, and others are not. The main modifiable risk factor is cigarette smoking because of the effect smoking has on the composition of the microbial community of the mouth, namely altering the quantity of particular bacterial species [14]. In one study, comparing the oral microbiome of smokers and non-smokers with chronic periodontitis in Korea, smokers were found to have higher levels of certain bacteria, including those of the genus *Fusobacterium* which have been linked to immunosuppression and periodontal attachment loss [14]. In general, smokers have worse outcomes in terms of rate of periodontitis, tooth loss, and response to treatment [2]. The effects of this are reversible, though, as smoking cessation can lead to an improvement in periodontal health measurements [15]. A systematic review in combination with meta-analysis of individual patient data showed that smoking cessation can reduce probing depths and increase clinical attachment level after the intervention of non-surgical treatment [15]. Another risk factor for periodontitis that can be partially modifiable is type-2 diabetes mellitus, as there is approximately a three-fold increased risk of developing periodontitis in individuals with diabetes [16]. Proper glycemic control is a key factor of periodontal health for patients with diabetes due to the systemic impact [16]. Studies show that there is a reciprocal relationship as the inflammatory response of periodontitis can alter glycemic control and elevated glucose levels can propagate the dysbiosis of the oral microbiome

[17]. Individuals with periodontitis and type-2 diabetes have increased insulin resistance and treatment for periodontitis can improve HbA1C levels, a diagnostic marker of diabetes [18]. The strength of the evidence for the relationship of periodontitis and type-2 diabetes has led to the suggestion that screening for periodontitis be implemented as part of a multidisciplinary approach to treating and managing diabetes [19]. It has also been suggested that predicting the impact of periodontitis on teeth and an individual's response to treatment could be done in part based on gathering information about smoking and diabetes [20].

Of the non-modifiable risk factors for periodontal disease, an area where the knowledge base has been growing is the genetic influence on disease. This risk factor is non-modifiable from the perspective that it is not possible to change an individual's genes, however it is often possible to modify the impact of genes. The development of periodontal complex traits using principal component analysis has identified ten genetic loci with genome-wide significance for certain trait groups with variable pathogen loads in a study of 975 European American adults [21]. Of these loci, the majority include genes related to the epithelial barrier-related and immune function that impact the response to microbial species [21]. Other associations that have been connected to the genetics of the immune system are the pro-inflammatory cytokines called interleukins. Polymorphisms in a number of the different types of interleukins, which all have slightly different origins and functions, have been linked to periodontitis through meta-analysis studies [22, 23]. One such example is the association with interleukin-1 (IL-1), which is an activator of cytokines and adhesion molecules used by leukocytes, that showed significant association between the IL-1β C(3953/4)T polymorphism and chronic periodontitis with an odds ratio indicating a greater risk of developing the disease for those individuals who are either homozygous or heterozygous for the T allele (OR=1.62, p=0.006) [24]. Interleukin-6 (IL-6), specifically at position -174 with a

polymorphism of a G to C substitution has been associated with both a susceptibility to chronic periodontitis as well as detection of *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) [4, 25].

There are also genetic syndromes in which periodontitis is a common clinical feature, as seen by the identification of 15 results in ClinVar, a National Institutes of Health-curated database of genetic variants related to clinical phenotypes, and 31 in the Online Mendelian Inheritance in Man database [26–28]. One such disease is Haim-Munk syndrome that, in addition to periodontitis, is associated with dermatological findings and overgrowth of the fingernails and toenails [29]. This condition is known to be caused by mutations in the cathepsin C (CTSC) gene, that is related to differentiation of skin cells and gum tissues as well as immune function [29, 30]. CTSC is also associated with Papillon-Lefevre syndrome, which has many of the same features of Haim-Munk syndrome including periodontitis and dermatologic symptoms [31].

Poor oral health in general is a large public health concern, but periodontal disease is one of the most pressing issues in dental health [32]. In adults, periodontitis is one of the major causes of tooth loss which can lead to many other health concerns including poor nutrition and infection [33]. Addressing periodontal diseases, as well as other aspects of oral health, is one of the goals of the Healthy People 2020 initiative [34]. According to the National Health and Nutrition Examination Survey (NHANES) through 2012, 46% of the adult population of the United States had periodontitis, with 8.9% having severe disease [35]. This survey is a well-established, well-validated tool for assessing trends in most aspects of health across the United States. It is possible, though, that these prevalence statistics may be lower than the actual rate, as it has been found that the NHANES protocol for evaluating periodontitis can underestimate periodontitis by as much as 50%, based on the definition utilized in the particular version of the survey [36].

2.2 OVERVIEW OF CARDIOVASCULAR DISEASE

Cardiovascular disease is a term that encompasses a broad range of medical conditions and concerns, as there are many aspects to heart health [37]. All the conditions that fall under the umbrella of cardiovascular disease have the common feature of a reduction in the ability of the heart and/or vasculature to function appropriately or effectively for blood circulation. Coronary artery disease is the most common type of cardiovascular disease, what is usually thought of with the term ‘heart disease,’ and involves the buildup of atherosclerotic plaques that restrict blood flow in arteries supplying blood to the heart [38]. A key component of heart disease is the involvement of the immune system and inflammation pathway in development of atherosclerotic plaques. Part of the buildup that blocks arteries comes from the accumulation of immune cells including macrophages and T cells recruited to the site of the fat streak that can turn into the fully formed plaque [39]. If allowed to progress without treatment, this can lead to chest pain, cramping of the heart muscle, and heart attack, also called myocardial infarction [40].

Hypertension, or chronic high blood pressure, is another prevalent heart health issue, both on its own and as an indicator of more severe cardiovascular concerns [41]. Although blood pressure naturally changes throughout the day and after taxing activities such as exercise, this condition occurs when blood regularly pumps too forcefully through the arteries [41]. An individual is considered to be ‘hypertensive’ if their blood pressure is consistently elevated to 140/90 mmHg or higher [42]. Those with blood pressure between 140-159/90-99 mmHg have stage 1 hypertension, and those with 160-179/100-109 mmHg have stage 2 hypertension [42]. Someone who has blood pressure readings consistently above normal, which is up to 120/80 mmHg, but not into the hypertensive level, is considered to be pre-hypertensive and those with a blood pressure reading greater than 180/110 mmHg are considered to be in a hypertensive crisis

[42] . Without intervention, hypertension can lead to heart attack or stroke as well as heart failure or problems with other organ systems like the kidneys [42].

There are many risk factors that contribute to the onset and progression of cardiovascular disease, some of which are controllable and some of which are not. Those that are not controllable include age, sex, race, and family history [43]. Older individuals are more likely to develop heart disease or hypertension, as are males, and African Americans tend to have more severe hypertension which leads to an elevated rate of heart disease [43]. Family history is a complicated risk factor, because it can be influenced by multiple aspects that cluster in families, including both genetic risk and shared behaviors [44]. A familial predisposition is considered when there are first degree relatives who have myocardial infarctions at an early age, 55 years for males and 65 for females, but other factors, like a seemingly autosomal dominant pattern of myocardial infarction, can be considered in assessing family history [45]. Modifiable risk factors for cardiovascular disease include behaviors shared among family members, as lifestyle choices, such as diet, exercise, and smoking, affect the chance of developing cardiovascular disease.

Another influence on the onset and progression of cardiovascular disease is the genetic component to an individual's heart health. There have been genetic polymorphisms associated with many of the individual physiological risk factors that contribute to the development of cardiovascular disease. Searching in ClinVar yields 115 results of genetic loci linked to "coronary artery disease," none of which overlap with those found for periodontitis [28, 46]. Using "hypertension," ClinVar shows 1,107 variants while the Online Mendelian Inheritance in Man database, shows 771 entries, which includes specific genes in addition to genetic disorders [47, 48]. Reviewing these results shows a large amount of overlap in entries, as well as many of the connections between cardiovascular health concerns and many other aspects of health.

Many of the loci and specific genes found to be linked with cardiovascular health phenotypes were identified using meta-analyses and association studies. In a large genome-wide association study of systolic and diastolic blood pressure in individuals of European descent, multiple loci related to hypertension, ventricular wall thickness, and stroke, as well as coronary artery disease overall, were found to be significant [49]. Genome-wide association studies have also identified genetic loci associated with the levels of circulating lipids that influence cardiovascular health [50]. Another circulatory molecule that has been related to influences on risk of cardiovascular disease is interleukin-6, a component of the immune system. Polymorphisms in this gene, -174G>C, have been associated with an increased susceptibility to coronary heart disease and severe cardiovascular events, as well as with periodontitis [5, 25, 51]. In a study from Scotland with 498 cases and 1109 controls, it was seen that those with the CC genotype had a lower risk of coronary heart disease than those with either the CG or GG genotype [51]. An analysis of data from the Cardiovascular Health Study looking at the relationship of the -174G>C loci and severe cardiac events showed that, in an elderly subset of the population, those with the -174C allele had a higher level of IL-6 which is indicative of a predisposition to atherosclerosis [5]. From an analysis of data from the Atherosclerosis Risk in Communities cohort, it has also been shown that incorporating genetic risk factors can be used in predicting incident coronary heart disease [52]. Inclusion of genetic loci nominally associated with incident coronary heart disease into a genetic risk score yielded models that acted as significant predictors of incident disease, even across races within the study [52].

Treatment of cardiovascular disease involves a variety of approaches depending on the extent of systemic involvement and the severity of the disease as well as certain predictors of response. Changing risky behaviors can have a vastly beneficial impact, even accounting for other

risk factors such as diabetes [53]. Many types of medications used in treatment of heart disease and hypertension have mechanisms of action that address different underlying causes of or possible complications from cardiovascular disease. Patients tend to have varying levels of successful response, due in part to underlying genetic differences, and as such, heart health medication has been one of the first areas in which pharmacogenetics has really taken hold [54]. Research has shown that it is possible to predict the response a patient may have to certain drugs to know if it is a useful intervention, such as the impact of particular genetic loci on the risk of developing myalgia in patients who may be prescribed a statin medication [55]. Other medications for which a response can be anticipated based on genetics include anticoagulants and antiplatelet agents, the varying responses ranging from actually responding to the treatment to potentially negative side effects [56].

There are many components to heart disease and there are many ways of measuring it, both directly and indirectly. Direct measurements include taking an individual's blood pressure or other values based on physical exam or laboratory analysis. It is also possible to create a composite phenotype to evaluate the likelihood an individual has or would develop cardiovascular disease based on related risk factors [57]. Many studies utilized this approach; however, a true standard has not yet been developed. Analyzing cardiovascular health in terms of outcomes measurements involves looking at the rate or number of major adverse cardiovascular events (MACE), which includes stroke, myocardial infarction, and death [58]. It is possible, though, to use less severe indicators of heart health as a way of predicting or inferring heart disease. One study identified a method of using blood pressure, total cholesterol, and LDL cholesterol levels to predict heart disease in those who did not have a clear diagnosis [59].

Cardiovascular health is an enormous public health issue, as heart disease is the leading cause of death in the United States [60]. Approximately 11.7% of adults in the United States, which equates to 28.4 million people, have been diagnosed with heart disease [61]. The economic burden of heart health-related concerns in 2011 was over $320 billion: the estimated direct cost of cardiovascular disease was $195.6 billion, which includes hospital and health professional services, medications, and other aspects of care, and another $124.5 billion in loss due to premature death [62]. These numbers are only expected to rise, as it is predicted that by 2030, approximately 40% of the population in the United States will have some type of cardiovascular health issue [63]. Addressing this trend and improving cardiovascular health is a major aim of public health initiatives across the country, in no small part as an attempt to curb the associated costs.

2.3 CURRENT UNDERSTANDING OF THE RELATIONSHIP BETWEEN PERIODONTAL AND CARDIOVASCULAR HEALTH

Over the course of the past few decades, there has been a steady stream of publications adding to the literature about the relationship between cardiovascular and periodontal disease. Enough data and discussion has been generated to warrant statements from a few national organizations overseeing developments in clinical practice and general understanding of their respective fields. From a joint consensus of The American Journal of Cardiology and Journal of Periodontology, there is acceptance of the possibility of periodontitis increasing the risk of cardiovascular disease, but hesitation in that there is not yet enough data to conclude a causal relationship [64]. This opinion is also found in the American Heart Association, with acknowledgement that an association between periodontal disease and a form of heart disease

called atherosclerotic vascular disease is supported by current data, however, it has not yet been shown to be causal [65].

The basis of the connection proposed between periodontitis and cardiovascular health is the underlying involvement of the inflammation pathway of the immune system in both diseases [1]. Initially, it was hypothesized that the inflammatory immune response was contributing to both more severe periodontal disease and an increased risk of coronary heart disease and other adverse cardiovascular events [66]. Part of this is due to the thought that oral infection leads to increased circulation of immune system molecules involved in the inflammation pathway [67]. One of the key factors thought to be involved with the systemic response of both diseases is C-reactive protein (CRP), an inflammatory marker associated with atherosclerosis [68]. Individuals with more severe inflammation from periodontitis have been seen to have increased CRP levels, which are associated with more pronounced cardiovascular health risks [68].

Levels of the pathogens associated with periodontitis have been found to impact the progression of the atherosclerotic process [69]. The spread of microbial species like *P. gingivalis* from the oral cavity into the blood stream seems to exacerbate any inflammation that may be present in the vasculature, as determined by measuring the level of toll-like receptor (TLR), which is another marker of the immune system that serves as an atheroprotective factor in times of microbial infection [70, 71]. This effect, though, may be mediated by genetic susceptibility and other factors that are shared risk modifiers of the disease, like diet, smoking, and diabetes [72].

Additional evidence for the connection comes from the impact that treatment of one disease has on the other. Evaluation of probing pocket depth in patients with advanced chronic periodontitis showed that those who were on statin medication had a lower burden of disease than those who were not on the medication, suggesting that the difference came from the anti-

inflammatory effect of the statin medication [73]. Patients taking statins had both lower a number of periodontal pockets and a smaller size of the pockets probed [73]. Treatment of periodontitis with antibiotics following debridement has also been shown to be associated with improved cardiovascular function in terms of measurements of pulse velocity and pressure [74]. A study comparing probing depth, bleeding, inflamed surface area, and other measurements of periodontitis and pulse pressure and velocity before and one year after treatment in 55 patients with severe untreated periodontitis disease showed reduction in those parameters that indicate more severe periodontitis and vascular health issues [74]. This further supports the idea that periodontal disease inflammation is contributing to cardiovascular disease risk as these are both anti-infective methods of treatment [74]. Using resolvins, anti-inflammatory mediators, to treat periodontitis also shows a reduction in both local and systemic inflammation that can be part of the atherosclerosis process [75].

The relationship between periodontitis and cardiovascular disease has also been analyzed from the perspective of the time it takes for periodontitis to have this proposed effect. In a prospective cohort study by Yu et al, it was found that new cases of periodontal disease, or incident periodontal disease, increase the risk for women to have a severe cardiovascular health event [76]. Studies have shown that individuals with prevalent, or on-going, periodontal disease have increased risks for cardiovascular disease, but also that there can be a relatively rapid effect of periodontal inflammation on cardiac health outcomes including major cardiovascular disease, heart attack, and stroke [76].

To assess the genetic component of the link between periodontitis and cardiovascular disease, a common approach has been using genome-wide and candidate-gene association studies, but other analyses, such as twin studies, have also been used. A Swedish twin study found evidence

of shared genetic components on the basis of a positive correlation between coronary heart disease and periodontal disease, though not with tooth loss [77]. These calculations came from analysis of more broad “like-sex” twin pairs as well as monozygotic “like-sex” twin pairs to adjust for unaccountable genetic influences [77]. That study itself did not identify any genes that may be part of that correlation, but other work has found a few specific loci that may be responsible. For example, a candidate-gene association study of chromosome 9p21.3 identified significant association for both coronary heart disease and aggressive periodontitis, mapping to the noncoding RNA ANRIL, and the *CDKN2A/CDKN2B* genes, kinases that are two of the many genes *ANRIL* modifies [33]. This study looked only at single nucleotide polymorphism markers (SNPs) in the 9p21.3 locus as it has been found to be significant in relation to atherosclerosis in more than a dozen genome-wide association studies [1]. The significance of *ANRIL* has been seen in both genome-wide association studies and candidate gene analysis [78]. Additional regions identified are chromosome 6q26 with the *PLG* gene, which is involved in binding bacteria, and chromosome 1p36 with the *VAMP3/CAMTA1* genes, both involved in the pathway of phagocytosis, and *IL6*, which is a pro-inflammatory cytokine, all of which are listed in Table 1 [1, 4, 5, 78]. These results, though not as numerous as the amount of genetic loci associated with cardiovascular disease and periodontitis individually, still demonstrate statistical significance [1]. The small number of genetic loci demonstrating statistical significance, and the lack of studies confirming proposed genetic links, are the main arguments made against the shared genetics of the relationship between periodontitis and cardiovascular disease. The statement from American Heart Association allows that there is a relationship between the two, however it has not yet been sufficiently shown to be causative or extend to a genetic level [65].

Table 1. SNPs Reported to be Associated with both Periodontal and Cardiovascular Disease

SNP	Chromosomal Location	Genes in Region
rs10864294	1p36	*VAMP3, CAMTA1*
rs4252120	6q26	*PLG*
rs1800795	7p15.3	*IL6*
rs10965215	9p21.3	*ANRIL, CDKN2A, CDKN2B*

2.4 CENTER FOR ORAL HEALTH RESEARCH IN APPALACHIA

The Center for Oral Health Research in Appalachia (COHRA) was created in 2002 as a joint effort between the University of Pittsburgh and West Virginia University to study disparities in oral health of the Northern Appalachian population in particular [79]. Appalachia spans portions of thirteen states from Mississippi to New York, with West Virginia being the only state fully encompassed by the region, as highlighted on the map in Figure 1 [79, 80]. The inhabitants of these areas are primarily Caucasian and of lower economic status, however, there is some variability. The recruitment area was intentional as a means of encompassing the social, cultural and economic aspects of rural Appalachia, though the cities and counties chosen are not necessarily fully representative of the entire county in which they are located [81]. The goal of the first cohort of COHRA (COHRA1) was to address the genetic, environmental, behavioral, and microbiological components that contribute to oral disease and tooth loss [82]. Much of the previous research out of COHRA1 has focused on caries, however data collected also included aspects of personal periodontal health and personal and family history of cardiovascular health.

Figure 1. Map of Appalachia.

From the members involved with COHRA and the data that has been collected, a multitude of analyses have been conducted. Over 85 publications have come out of COHRA group, the majority of which focus on dental caries using COHRA1 data, though some that tend more towards proof of concept for the methods used, such as using genome-wide association studies or self-report data. There have been only a few that have discussed to an appreciable extent the topics of periodontal or cardiovascular health, though they did not all use COHRA1 data. One publication that did use this data involved a genome-wide association study for genetic loci related to chronic periodontitis using probing depth in adults [83]. Ten loci with suggestive significance that had been previously associated with chronic periodontitis were identified, as were novel loci including the region near the gene *OSBPL10,* which is associated with hyperlipidemia [83]. Another project from members of the COHRA group that looked into periodontitis used Mendelian randomization analyses to incorporate body mass index-associated loci into a risk score and found total adiposity

was not a causal risk factor for periodontitis [84]. There was also one study conducted that assessed the relationship of edentulism with cardiovascular disease and sleep disordered breathing, using data from the National Health and Nutrition Examination Survey, that found edentulism to be independently associated with cardiovascular disease [85]. There has not yet been a study that uses COHRA1 data to examine the link between periodontitis and cardiovascular disease.

3.0 MANUSCRIPT

3.1 BACKGROUND

Periodontitis is a disease in which the soft tissue, bone, and ligament of the structures surrounding the teeth deteriorate due to chronic inflammation [2]. This can either be an aggressive form, involving isolated, rapid loss of attachment, or chronic, which involves a slower disease course with the development of pockets of recession [2]. The onset of periodontitis is associated with a characteristic shift in the oral microbiome in three main species: *Porphyromonas gingivalis* (*P. gingivalis*), *Bacteroides forsythus* (*B. forsythus*), and *Treponema denticola* (*T. denticola*) [8]. Measurement of periodontitis can involve analyzing the levels of these bacterial species indirectly, as they are all responsible for hydrolyzing benzoyl-DL-arginine naphthylamide (BANA) which is a useful marker of periodontal infection [6]. Clinical attachment loss (CAL) and periodontal probing depth (PPD), which are related to gum recession, are also measurements of periodontitis, as is a bleeding score based on probing of the gums [4].

Risk factors for periodontitis include modifiable influences, such as dental hygiene practices and smoking, as well as non-modifiable influences, such as age and genetic polymorphisms. The genetic component is a more recent aspect of the understanding of periodontitis, in terms of the genetic contribution to risk for the disease and disease etiology. Many of the genetic loci found to be associated with periodontitis have been linked to the immune system and inflammatory response pathway [33]. One example is the pro-inflammatory cytokine interleukin 6, which is involved in immune system response and function [25]. This corresponds to the proposed link between periodontitis and cardiovascular disease, as they are thought to share

a similar underlying inflammation pathway as well as have influences on the onset and progression of the other disease [66].

The same pathogens that are the hallmark of periodontitis have been found to contribute to the progression of atherosclerotic plaques, a component of heart disease [69]. As the pathogens circulate in the blood stream, they are capable of perpetuating inflammatory responses initiated by the immune system in the vasculature that lead to plaque development [67]. This connection is also seen at the genetic level. For example, polymorphisms in the genes that produce various pro-inflammatory cytokines, such as interleukins, have been linked to periodontitis through meta-analyses [22, 23]. The same polymorphism in one subtype of interleukin, rs1800795 in *IL6*, has also been linked to susceptibility to cardiovascular disease in separate studies [5, 51]. In a study from Scotland and an analysis from the Cardiovascular Health Study, the -174G>C mutation in *IL6* was associated with risk of coronary artery disease and severe cardiac events [5, 51].

In addition to those that look at the conditions individually, there have also been studies that directly examined the genetic link between periodontitis and cardiovascular disease. Multiple loci found to be significant for both periodontitis and cardiovascular disease, though not as many as have been implicated individually [1]. One such study is Aarabi et al, who reported a meta-analysis that yielded three single nucleotide polymorphisms (SNPs) associated with both periodontitis and cardiovascular disease [1]. Data analyzed in this and similar studies involved using meta-analyses and genome-wide association methods to find these loci associated with periodontitis and/or cardiovascular disease.

This study aims to examine the possible genetic basis of the relationship between periodontitis and cardiovascular disease, using data from the Center for Oral Health Research in Appalachia cohort 1 (COHRA1), which was a family-based study conducted in the Northern

Appalachia region examining oral health trends in the region. Variables related to periodontitis, including metrics of probing depth and BANA tests for microbial species, as well as cardiovascular disease, based on personal and family medical history, will be tested for their association with candidate SNPs identified in previous studies. Results of this study will further the understanding of the suggested genetic relationship between periodontitis and cardiovascular disease.

3.2 METHODS

3.2.1 Study Population

The data used in this study are from the first study of the Center for Oral Health Research in Appalachia, known as COHRA1, which examined the disparities in oral health in Northern Appalachia (West Virginia and Western Pennsylvania) as compared to the rest of the United States. To gather this data, recruitment sites were established at multiple sites in West Virginia and Western Pennsylvania. Across those locations, 841 households with at least one adult parent of at least one biological child between the ages of 1 and 18 were enrolled [81]. These households came from mostly rural but a few metropolitan areas, and in general, the households had a variety of compositions in terms of biological relationships and ages of household inhabitants. The COHRA1 sample comprises over 3,035 participants; however, for the analyses conducted in this study, participants were restricted to ages 18 and over with medical, oral health, and genotyping data, yielding 974 total participants. Institutional Review Board approval from the University of Pittsburgh IRB and West Virginia University IRB and informed consent were obtained.

Genetic data was collected using SNP chip genotyping from biological samples obtained from participants including blood, buccal swabs and saliva. Genotyping of 580,000 SNPs was conducted on an Illumina platform [86]. Imputation was used to analyze more than one million additional SNPs with the help of the GENEVA consortium by the Center for Inherited Disease Research.

For periodontal health, multiple variables were collected that can be used as markers or direct measurements of periodontitis. Subgingival plaque samples of the mesio-buccal sites of the four first molars and tongue scrapings were obtained for use in benzoyl-DL arginine-naphthylamide (BANA) testing, using a kit from BANAMet [82]. The results of the test were scored as categorical variables as shown in Table 2. A dichotomous yes/no variable was created for BANA results (BANA) for those participants who had results for at least three of the five sites tested. Individuals who had a total score of at least 1 based on summing all five sites were coded as yes (1) and all others as no (0).

Table 2. BANA Test Result Recording

Test Result	CFU Count of three BANA positive species	Quantitative Variable
Negative	Less than 10,000	0
Weak-Positive	More than 10,000 but less than 100,000	1
Positive	More than 100,000	2

A modified Periodontal Screening and Recording (PSR) exam that evaluated probing depth, recession, and bleeding was also performed by trained and calibrated hygienists to evaluate periodontal health [81]. These measurements, taken on participants over 17 years of age, were done for the reference teeth used for BANA measurements as well as an assessment of the whole mouth based on sectioning teeth into sextants, shown in Figure 2. For the sextants, the deepest PSR score for the teeth in each sextant was recorded, as shown in Table 3 [82]. Specific categories were created for sites indicating evidence of periodontal disease, such as the tooth being missing,

a pontic, or an implant [82]. The current study uses a proxy measurement of periodontitis by creating a dichotomous variable, with affection status defined as a probing depth measurement of at least 5.5mm for at least two teeth. A conservative approximation, periodontitis-related phenotype 1 (PSR1), was created that excluded missing measurements and coded the affection status as missing if there were not measurements for at least three of the sextants. A non-conservative approximation (PSR2) was created that considered those teeth for which no measurement was recorded to be missing due to periodontitis.

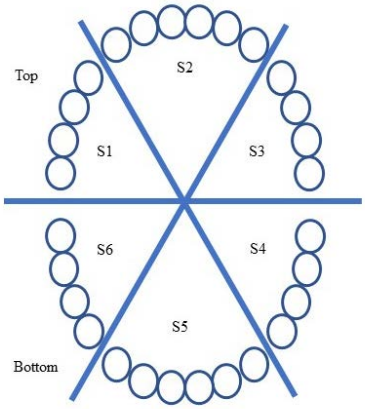

Figure 2. Sextants for PSR Exam

Table 3. PSR Score Recording for Reference Teeth and Sextants

PSR Score	Deepest Probing Depth in Sextant
1	Less than 3.5 mm
2	More than 3.5 mm but less than 5.5 mm
3	More than 5.5 mm

For cardiovascular health, the only direct measurement taken was a reading of blood pressure for participants at the beginning of their study visit. The rest of the data regarding cardiovascular health comes from the self-report health history forms that participants were asked to complete at each visit. Physical evaluation, medical history, and family history forms addressed various aspects of cardiovascular health by asking about personal medical history as well as

members of the participant's family. Blood pressure was recorded on the physical evaluation form, along with any descriptive accounting of conditions regarding the participant's heart such as medications [82]. The medical history form included questions about personal history of high blood pressure and heart disease and treatment [82]. The family history form included questions about heart disease and high blood pressure as well as which relatives were affected [82]. Aside from write-ins of personal treatment for high blood pressure or heart disease, information was recorded as yes/no responses. There was no precedent set in the literature for creating a compound phenotype of these variables, so for this study the responses were combined into a single, composite score of heart disease (HDSCORE) with a maximum possible value of 7, giving more weight to a personal history of heart disease and high blood pressure and less weight to those conditions in the family history, as outlined in Table 4. For a subset of participants who had observations from two visits, only the data for their second visit was used for all measurements, aside from carrying over the report of family history of high blood pressure or heart disease if it was only reported at the first visit. If no data was reported for either personal or family history of high blood pressure or heart disease, the participant was considered to be negative for that component of the HDSCORE variable.

Table 4. HDSCORE Variable Quantification

Variable	Score
Personal history heart disease	3
Personal history high blood pressure	2
Family history heart disease	1
Family history high blood pressure	1

3.2.2 Identification of Genetic Polymorphisms

To identify genetic loci associated with periodontitis and cardiovascular disease, search parameters were used including “periodontitis and cardiovascular disease,” “periodontitis and heart disease,” and “genetics of periodontitis and cardiovascular disease” in PubMed, a National Institutes of Health search engine [87]. From review of those results, four single nucleotide polymorphisms (SNPs) were selected based on the SNP being reported at least three times, including as part of other literature reviews, as well as the relevancy of the determination of the association with cardiovascular disease and periodontitis, based on recent reports within the past decade. Information on these SNPs is included in Table 5 [1, 88–91].

Table 5. Single Nucleotide Polymorphisms

Chromosomal Location	Base Pair Location[a]	SNP	Genes in Region	Minor Allele Frequency[b]
1p36	733260	rs10864294	*VAMP3*, *CAMTA1*	T=0.1867
6q26	160722576	rs4252120	*PLG*	C=0.1400
7p15.3	22727026	rs1800795	*IL6*	C=0.1412
9p21.3	22029446	rs10965215	*ANRIL*, *CDKN2A*, *CDKN2B*	G=0.4593
[a] Base Pair Location based on GRCh38.p7 [b] Minor Allele Frequency based on 1000 Genomes Project				

3.2.3 Statistical Analysis

Regression models were used to test the relationship between the periodontitis and cardiovascular disease variables and their association with the candidate SNPs in Table 1. Linear regression was used when the continuous HDSCORE variable was used as the outcome. Models were run using periodontitis indicators and each SNP as the predictors individually and together, both with and without adjustment for covariates of age, sex, race, smoking history, and BANA test results. Logistic regression was used when the dichotomous periodontitis indicators were the

outcome. These models were run using HDSCORE and each SNP as predictors, individually and together, with and without adjustment. Regression models were performed in STATA [92]. A post-hoc power analysis was conducted for each model using the statistical software Quanto [93].

3.3 RESULTS

This study explored the proposed phenotypic and genetic relationship between cardiovascular disease and periodontitis by modeling the phenotypic association between the two diseases and the genetic association of each disease with four candidate SNPs identified from the literature. These analyses were conducted using data from 973 adults from the COHRA1 cohort. Characteristics of the sample are shown in Table 6. The sample comprised relatively young adults, the majority of which were female (63.0%) and white (91.1%) with an average body mass index (BMI) of 29.7. For the periodontitis indicators: for the PSR1 definition, 20.2% of 961 participants had periodontitis; for the PSR 2 definition, 24.4% of 973 participants had periodontitis; for the BANA definition, 84.1% of 970 participants were positive. Of the 973 participants with an observation for HDSCORE, the mean score was 1.75, with a standard deviation of 1.2 and the majority of participants having a score of 2, as seen in Figure 3.

Table 6. Summary of Covariates

Population characteristic	N	Mean (SD) or percent
Age	972	34.5 (9.3)
Sex		
Female	612	63.0%
Male	360	37.0%
Race		
White	879	91.1%
Other	86	8.9%
Smoking status		
Current smoker	308	34.2%
Never smoker	592	65.8%
BMI	812	29.7 (7.7)
PSR1		
Affected	194	20.2%
Not Affected	767	79.8%
PSR2		
Affected	237	24.4%
Not Affected	736	75.6%
BANA		
Affected	806	84.1%
Not Affected	164	15.9%
HDSCORE	973	1.75 (1.2)

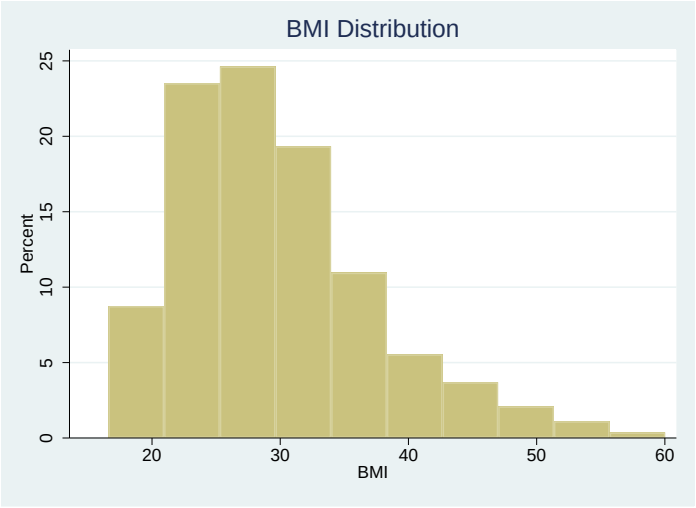

Figure 3. BMI Distribution

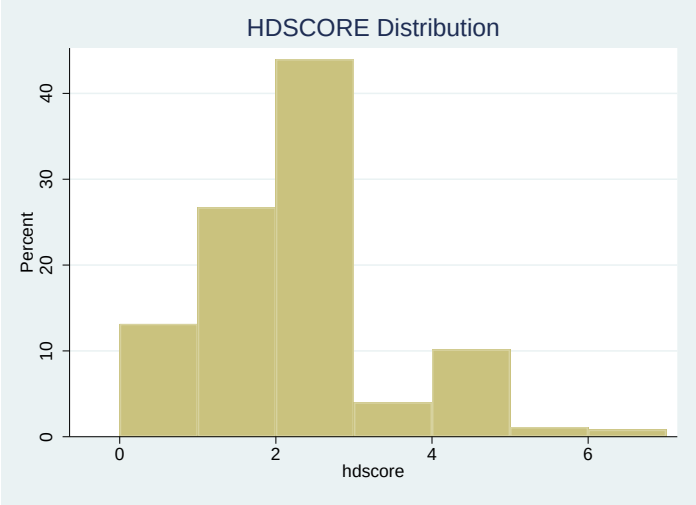

Figure 4. HDSCORE Distribution

Table 7 presents the relationships between the measures of periodontitis and cardiovascular disease. Specifically, we tested the association of HDSCORE with three indicators of periodontal disease, PSR1, PSR2, and BANA. There was a significant association between HDSCORE and both PSR1 and PSR2 in the univariate models. However, this association was not statistically significant in the covariate-adjusted model. In contrast, there was not a significant association between BANA and HDSCORE, in either univariate models or models adjusted for the covariates age, sex, race, and smoking status. Full details of the adjusted models can be seen in the supplementary tables provided in Appendix A.

Table 7. Relationship between Periodontitis and Cardiovascular Disease Variables

		Unadjusted model				Adjusted model [a]			
Dependent variable	Predictor	Beta coefficient	SE	p-value	Odds Ratio	Beta coefficient	SE	p-value	Odds Ratio
HDSCORE	PSR1	0.192	0.097	**0.048**	-	0.116	0.108	0.281	-
	PSR2	0.196	0.090	**0.030**	-	0.091	0.101	0.367	-
	BANA	0.048	0.104	0.647	-	0.094	0.112	0.402	-
PSR1	HDSCORE	0.127	0.064	**0.049**	1.135	0.084	0.078	0.280	1.088
PSR2	HDSCORE	0.130	0.060	**0.031**	1.139	0.073	0.086	0.396	1.076
BANA	HDSCORE	0.033	0.072	0.646	1.034	0.054	0.078	0.490	1.055

[a] Adjusted models include age, sex, race, smoking status, and BMI. BANA was also included as a covariate in adjusted models except for models with BANA as the dependent variable or predictor
Bold font indicates p-values less than 0.05

Four candidate SNPs identified from the literature were tested for genetic association with indicators of both periodontitis and cardiovascular diseases. The associations between these SNPs and HDSCORE are shown in Table 8. No significant genetic associations were observed for HDSCORE. This was true in both the unadjusted and adjusted models, as well as those models that included the conservative PSR1 as a covariate to test for an association between the health outcomes when the SNPs were included. Because no association was seen between any SNP and the proxy variable of heart disease, it is not possible to conclude from this sample that any of these SNPs account for the association between the proxies of periodontitis and cardiovascular disease. Based on the post-hoc power calculations conducted, we expect to have had 80% power to detect effect sizes (beta coefficients) of 0.150 to 0.225 for these SNPs. Therefore, true associations with effect sizes smaller than may have gone undetected.

Table 8. Relationship between Cardiovascular Disease Variables and Candidate SNPs

	Unadjusted model			Adjusted model [a]		
SNP	Beta Coefficient	SE	p-value	Beta Coefficient	SE	p-value
rs10864294	-0.099	0.082	0.226	-0.028	0.094	0.762
rs4252120	-0.048	0.062	0.440	-0.076	0.068	0.264
rs1800795	-0.025	0.055	0.647	-0.046	0.060	0.447
rs10965215	-0.091	0.056	0.102	-0.098	0.061	0.110
[a] Adjusted models include age, sex, race, smoking status, BANA, and BMI.						

One SNP, rs10864294, which corresponds to the *VAMP3* and *CAMTA1* genes, was significantly associated with PSR1 in the unadjusted model (p=0.031), as seen in Table 9 [88]. This relationship was also seen in the adjusted model, in which the SNP had statistical significance (p=0.003). The SNP had a similar effect on the PSR outcome odds ratios: with every additional copy of the major allele, an individual is between 0.580 and 0.714 times as likely to have periodontitis. The trends seen for each SNP in the models using PSR1 were echoed in the models using PSR2 in terms of direction of relationship indicated by the coefficient. The significance of the relationship between each SNP and PSR2 also mirrored that of PSR1 with one difference. The rs10864294 SNP did not have a statistically significant relationship with PSR2 in the unadjusted model (p=0.067), however, it did have a significant relationship with PSR2 in the adjusted model (p=0.006). Similar to PSR1, in the adjusted model, the magnitude of the effect of rs10864294 on the liberal periodontitis outcome was also greater, with an odds ratio of 0.621 as opposed to 0.762. None of the candidate SNPs were significantly associated with the dichotomous BANA indicator. For those SNPs that did not demonstrate a significant association with the periodontitis indicators, it is possible that this sample did not have the power to detect effect sizes of that magnitude, as post-hoc power calculations indicated that we expect to have 80% power to detect effect sizes of 1.35 to 1.70.

Table 9. Relationship between Periodontitis Variables and Candidate SNPs

		Unadjusted model				Adjusted model [a]			
Variable	SNP	Beta Coefficient	SE	p-value	Odds Ratio	Beta Coefficient	SE	p-value	Odds Ratio
PSR1	rs10864294	-0.337	0.157	**0.031**	0.714	-0.698	0.198	**0.000**	0.497
	rs4252120	0.087	0.130	0.502	1.091	0.166	0.152	0.273	1.181
	rs1800795	-0.024	0.113	0.831	0.976	-0.021	0.132	0.874	0.979
	rs10965215	-0.198	0.116	0.089	0.820	-0.173	0.136	0.203	0.841
PSR2	rs10864294	-0.272	0.148	0.067	0.762	-0.653	0.189	**0.001**	0.520
	rs4252120	0.063	0.120	0.602	1.065	0.152	0.142	0.285	1.164
	rs1800795	-0.780	0.105	0.460	0.458	-0.114	0.124	0.359	0.893
	rs10965215	-0.203	0.108	0.061	0.131	-0.144	0.128	0.261	0.866
BANA	rs10864294	-0.062	0.184	0.735	0.940	0.018	0.220	0.936	1.018
	rs4252120	0.035	0.136	0.798	1.036	0.036	0.155	0.815	1.037
	rs1800795	-0.076	0.124	0.539	0.927	-0.086	0.140	0.540	0.918
	rs10965215	0.039	0.124	0.750	1.040	-0.113	0.142	0.424	0.893

[a]Adjusted models include age, sex, race, smoking status, and BMI. BANA was also included as a covariate in adjusted models except for models with BANA as the dependent variable or predictor
Bold font indicates p-value less than 0.05

3.4 DISCUSSION

The goal of this study was to test the association between periodontitis and cardiovascular disease and their link to candidate genetic loci identified through literature review. The COHRA1 cohort from which the data was gathered was mostly relatively young and female, while individuals affected by periodontitis and/or cardiovascular disease are more commonly older and male [32, 38]. The majority of the participants were white, which allows for more accurate interpretation of the results as the majority of the studies looking at the genetics of cardiovascular disease and periodontitis were conducted in European populations [1].

Data collected through a dental exam and self-report forms were used to create proxy variables as indicators of these two health conditions. For periodontitis, a dichotomous variable

based on the results of testing for the microbial species associated with the disease was used (BANA). This was not found to have a significant relationship with HDSCORE or the SNPs in any model. Two additional variables were used as indicators of periodontitis, both based on the results of periodontal probing depth. One of these was a conservative approximation (PSR1) that may have been an underrepresentation of the actual number of participants in the study with periodontal disease as this variable assumed that missing teeth were not missing due to periodontitis. The other approximation (PSR2) was liberal as it considered missing teeth to be evidence of periodontitis, which may be an overrepresentation of the affected subset of the study population. For both conservative and liberal definitions of periodontitis, disease misclassification, if present, would lead to a decrease in power to detect phenotypic and genetic associations. For cardiovascular disease, a composite score was created, weighting personal history of heart disease and personal history of high blood pressure over family history of either. Combining these into one score may have reduced the power to detect associations that are not shared by both heart disease and hypertension.

In models unadjusted for covariates, the probing depth-based approximations were significantly associated with the heart disease score. This finding corresponds with the current understanding in the field that there is an association between the two diseases, as has been acknowledged by professional bodies [65, 66]. This relationship went away when the covariates of age, sex, race, smoking status, and BANA test result were added to the model, suggesting that in the COHRA1 sample, the association between periodontitis and cardiovascular disease may be confounded by these factors.

For three of the candidate SNPs, there was no statistical significance for the association between heart disease or periodontitis. rs1800795, which lies within an intron of the gene *IL6*, was

previously reported to be associated with both periodontitis and cardiovascular disease [4, 5, 94], but in this study, there was not a significant association of the SNP with either heart disease or periodontitis. As the *IL6* cytokine is a known component of the inflammation response, the reported relationship of the genetic variant to periodontitis and heart disease was biologically plausible [95]; however, it was not supported by the results of this analysis. The rs4252120 SNP, within an intron of the gene *PLG*, which has been linked to the process of bacterial binding and is important due to the involvement of pathogen-mediated inflammation in periodontitis and cardiovascular disease, was also not found to be significant in relation to either periodontitis or heart disease [78, 96, 97]. rs10965215, linked to the non-coding RNA ANRIL, which lies within an intron of the *CDKN2B* gene and is known to modify the *CDKN2A* and *CDKN2B* genes that function as tumor suppressors, did not demonstrate a significant relationship with either indicators of periodontitis or cardiovascular disease, though it has been linked to atherosclerosis and regulation of the phagocytic mediator *VAMP3* [98–102].

The only SNP that was significantly associated with indicators of periodontitis was rs10864294. Significant associations were seen for PSR1 both in the unadjusted model and the model with additional covariates. This significance was seen for the adjusted models with both PSR1 and PSR2, however, in the unadjusted model PSR2 and rs10864294 did not demonstrate a significant relationship. The results for this SNP support the idea that there is a genetic component to periodontitis. The rs10864294 SNP corresponds to the genes *VAMP3* and *CAMTA1*, linked to immune-mediated phagocytosis and tumor suppression respectively [1, 103, 104]. The C>T change of this SNP lies within the *CAMTA1* gene, and changes a transcription factor binding site, POU6F1, that involves a bipartite DNA binding motif [105, 106]. This region has also been implicated in increased periodontal pathogen colonization, which has been associated with both

periodontitis progression and vascular inflammation [107]. These results demonstrate support of the relationship between periodontitis and cardiovascular disease, but not at the genetic level. This suggests that clinical care for individuals with either periodontitis or cardiovascular disease may be improved by adding in screening for the other condition. Further research looking into this connection would clarify the most effective ways in which to make those connections. This could include adjusting analyses to demonstrate the effect of additional factors linked to periodontitis and cardiovascular disease, such as body mass index or diabetes.

There are multiple limitations of the study, many of which are a product of the dataset used for the analysis. The COHRA study had a limited recruitment region, which restricted the diversity of participants included. Additionally, the statistical analysis did not account for the relatedness among participants in the sample which could have confounded the impact of the SNPs on each health outcome based on differences in allele frequency. Although the analysis was performed in adults from the households comprising mostly unrelated parents of children not included in this study, some known and cryptic biological relationships exist among the adults of the COHRA1 sample. Another limitation based on the COHRA study protocol is the reliance on self-report data for personal and family health history, meaning it is conceivable that there was misreporting leading to inaccurate representations of the medical histories. There was also no clarification provided for the type of heart disease or number of family members with heart disease or high blood pressure. From these inexact measures of the health conditions in question, it possible that the composite variables created for periodontitis and cardiovascular disease do not adequately represent those with each disease. These sources of noise would reduce statistical power to detect associations. It is also possible that, based on the size and characteristics of the sample used, there was not adequate power to detect effects of the magnitude seen for the relationships between the

health outcomes. There are also multiple other genetic components that have been linked to both periodontitis and cardiovascular disease but were not explored in this analysis.

3.5 CONCLUSION

This study, which was designed to test the association of genetic variants identified through a literature search with both periodontitis and cardiovascular disease, demonstrated limited support for the genetic connection seen between the two health conditions. Without adjusting for covariates, there is evidence of a statistically significant association between periodontitis and cardiovascular disease. One SNP, rs10864294, corresponding to the *VAMP3* and *CAMTA1* genes involved in the phagocytosis pathway, was found to be significantly associated with periodontitis. These results support the hypothesis that there may be a genetic component to periodontitis but not to its association with cardiovascular disease, however, additional research is needed to explore this connection further. Knowledge of this potential genetic relationship would be useful to incorporate into clinical practice to more accurately assess individuals' risk of either health condition, which is especially important at this time due to the large public health burden of both periodontitis and cardiovascular disease in the United States.

3.6 ACKNOWLEDGEMENTS

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4.0 RESEARCH SIGNIFICANCE TO GENETIC COUNSELING AND PUBLIC HEALTH

Our understanding of the implications of genetics on health is rapidly expanding as more aspects of the connection between DNA and disease are elucidated. As this information becomes incorporated into clinical practice and a part of the population's general understanding of health, the crucial role of clinical genetics providers has vastly expanded. With the results of this study and others like it, genetic counselors and their colleagues have more information with which to help patients to improve health outcomes.

Cardiovascular genetic medicine is one of the areas that has recently experienced major growth. The majority of cardiovascular diseases are multifactorial and involve not only a hereditary piece, but also lifestyle and environment factors [43]. To gauge the full extent of these components, health assessments during medical appointments include gathering medical history of the patient as well as social and behavioral history and family history. Research into the link between cardiovascular disease and other medical conditions helps providers adjust the review of personal and family histories to include all relevant information. Studies such as this, that show the significance of periodontitis and single nucleotide polymorphisms in relation to cardiovascular disease, add another question to ask of patients to get a more complete picture of the risk factors contributing to their cardiovascular health.

An accurate assessment of an individual's risk for cardiovascular disease is important because of the implications for managing their health in the most effective and appropriate way. Many aspects of cardiovascular health can be improved by lifestyle changes such as a healthier

diet and more exercise, but there are also certain genetic risk factors that can indicate which medications may be most effective [56].

Genetic testing for periodontitis and cardiovascular disease, in terms of risk of developing the disease and not just determining treatment, is available. There are even direct to consumer tests for periodontal disease, meaning that there are not necessarily any health care providers involved in ordering the test or appropriately explaining the results [108]. It is difficult to ensure that medical professionals in fields other than genetics accurately understand the results and implications of genetic testing, and it is often even more difficult to ensure that understanding for the general population. Due to the availability of these tests, and the deepening knowledge base about the connections between genetics and all aspects of health, including dental health, it is more important than ever to be diligent about educating as many medical professionals as possible to be able to interpret genetic test results, and refer on to genetics specialists appropriately. Genetic counselors are uniquely trained to be able to explain genetic information to patients and providers and serve as an invaluable resource. This is especially true in situations where the genetic component of an individual's health may play a previously unknown role, such as with periodontal disease when the understanding of genetic causes is still relatively new.

With the results of this study and similar work, there is the possibility that some of those connections may be applied to treatment of periodontitis as well. In addition to being critical for proper patient care, appropriate management of these conditions has a broader impact due to the public health burden of both cardiovascular disease and periodontitis. An increasing percentage of the population with cardiovascular health problems. Heart disease has become the leading cause of death in the United States, while periodontitis is one of the major causes of tooth loss, which can exacerbate or lead to many other concerns [33]. Addressing these trends is important to

improve the health of such a large subset of the country as well as reduce the burden on the healthcare system of the direct and indirect costs of treatment, especially of those with cardiovascular disease [62].

Tackling this burden requires addressing the issue of improving access to health care, the greatest barrier of which is the prohibitive cost of services and insurance [109]. Health insurance is most often obtained through employment, meaning that individuals who are unemployed may have a harder time accessing care, and more individuals have medical insurance than they do dental, so they may not have the ability to afford care for periodontitis even if a cardiovascular health evaluation or genetic markers indicate that it is warranted [110]. Provider education and increased awareness of the importance of dental care on multiple aspects of health may be a way to improve recognition of the crucial need for broader access to dental care by validating the cost of the services. As the American Dental Association contends, the value of a clinical exam in identifying periodontal disease cannot be replaced by genetic testing or other indicators alone [111].

Research that identifies potential ways of measuring individuals' risk of developing cardiovascular disease, and periodontitis is a component of tackling this public health problem through multiple areas of the essential public health services. With the results of this study and others like it, it is possible to "evaluate [the] effectiveness… of personal…health services" to ensure that health professionals are delivering care using the most accurate information [112]. Along those lines, this research can also provide "new insights and innovative solutions to health problems" by providing additional information that can be used to address the health of those at risk for or with cardiovascular health disease and periodontitis [112].

The results of this study are also applicable on a more individual scale. There are some previously characterized, relatively rare genetic disorders involving an increased risk of periodontitis, including Haim-Munk syndrome and Papillon-Lefevre syndrome [113]. With further evidence towards the relationship between periodontitis and cardiovascular disease, it may be determined that adding cardiology evaluations for the management of these patients to fully assess their clinical picture is a necessary part of routine care. The particular SNPs evaluated in this study are not in the genes noted to cause these conditions; however, further analysis could show that these risk alleles are significant in relation to Haim-Munk syndrome, Papillon-Lefevre syndrome, and others.

APPENDIX A: SUPPLEMENTAL TABLES

Table 10. Relationship between Periodontitis and HDSCORE adjusted for covariates

Dependent	Predictor	Beta Coefficient	SE	P-value	OR
HDSCORE	PSR1	0.116	0.108	0.281	-
	Age	0.034	0.005	0.000	-
	Sex	-0.008	0.090	0.925	-
	Race	0.324	0.146	0.027	-
	Smoking	-0.057	0.094	0.544	-
	BANA	0.099	0.113	0.383	-
	BMI	0.026	0.006	0.000	-
HDSCORE	PSR2	0.091	0.101	0.367	-
	Age	0.033	0.005	0.000	-
	Sex	-0.020	0.089	0.820	-
	Race	0.331	0.146	0.024	-
	Smoking	-0.060	0.093	0.520	-
	BANA	0.094	0.112	0.403	-
	BMI	0.028	0.006	0.000	-
HDSCORE	BANA	0.094	0.112	0.402	-
	Age	0.034	0.005	0.000	-
	Sex	-0.031	0.088	0.728	-
	Race	0.344	0.145	0.018	-
	Smoking	-0.049	0.092	0.597	-
	BMI	0.028	0.006	0.000	-
PSR1	HDSCORE	0.084	0.078	0.280	1.088
	Age	0.043	0.010	0.000	1.044
	Sex	-0.686	0.188	0.000	0.504
	Race	0.830	0.395	0.036	2.293
	Smoking	0.506	0.203	0.013	1.659
	BANA	0.154	0.250	0.538	1.166
	BMI	0.004	0.013	0.752	1.004
PSR2	HDSCORE	0.070	0.074	0.344	1.073
	Age	0.057	0.010	0.000	1.058
	Sex	-0.608	0.178	0.001	0.545

Table 10 Continued					
	Race	0.980	0.378	0.009	2.665
	Smoking	0.702	0.191	0.000	2.018
	BANA	-0.011	0.228	0.963	0.990
	BMI	-0.002	0.012	0.862	0.998
BANA	HDSCORE	0.073	0.086	0.396	1.076
	Age	-0.018	0.011	0.101	0.982
	Sex	-0.405	0.208	0.052	0.667
	Race	-0.839	0.415	0.044	0.432
	Smoking	-0.467	0.204	0.022	0.627
	BMI	-0.004	0.013	0.757	0.996

Table 11. Relationship between HDSCORE and candidate SNPs adjusted for covariates

Dependent	Predictor	Beta Coefficient	SE	p-value
HDSCORE	rs10864294	-0.028	0.094	0.762
	Age	0.035	0.005	0.000
	Sex	-0.028	0.089	0.755
	Race	0.345	0.164	0.036
	Smoking	-0.045	0.093	0.626
	BANA	0.094	0.113	0.405
	BMI	0.028	0.006	0.000
HDSCORE	rs4252120	-0.076	0.068	0.264
	Age	0.034	0.005	0.000
	Sex	-0.030	0.088	0.737
	Race	0.341	0.146	0.020
	Smoking	-0.046	0.092	0.616
	BANA	0.094	0.112	0.399
	BMI	0.028	0.006	0.000
HDSCORE	rs1800795	-0.046	0.060	0.447
	Age	0.034	0.005	0.000
	Sex	-0.030	0.090	0.736
	Race	0.358	0.147	0.015
	Smoking	-0.061	0.093	0.514
	BANA	0.092	0.115	0.425
	BMI	0.029	0.006	0.000

Table 11 Continued				
HDSCORE	rs10965215	-0.098	0.061	0.110
	Age	0.033	0.005	0.000
	Sex	-0.032	0.088	0.714
	Race	0.340	0.145	0.019
	Smoking	-0.053	0.092	0.562
	BANA	0.085	0.112	0.445
	BMI	0.028	0.006	0.000

Table 12. Relationship between CVD and candidate SNPs adjusted for covariates and PSR1

Dependent	Predictor	Beta Coefficient	SE	p-value
HDSCORE	rs10864294	-0.042	0.096	0.665
	Age	0.034	0.005	0.000
	Sex	-0.006	0.091	0.945
	Race	0.337	0.166	0.043
	Smoking	-0.053	0.095	0.579
	BANA	0.100	0.114	0.381
	BMI	0.027	0.006	0.000
	PSR1	0.107	0.109	0.324
HDSCORE	rs4252120	-0.085	0.068	0.215
	Age	0.034	0.005	0.000
	Sex	-0.007	0.090	0.939
	Race	0.320	0.147	0.030
	Smoking	-0.055	0.094	0.555
	BANA	0.099	0.113	0.379
	BMI	0.027	0.006	0.000
	PSR1	0.121	0.108	0.261
HDSCORE	rs1800795	-0.041	0.061	0.502
	Age	0.034	0.005	0.000
	Sex	-0.008	0.091	0.932
	Race	0.337	0.148	0.023
	Smoking	-0.071	0.095	0.458
	BANA	0.097	0.116	0.403
	BMI	0.028	0.006	0.000
	PSR1	0.117	0.110	0.284

Table 12 Continued				
HDSCORE	rs10965215	-0.092	0.062	0.136
	Age	0.033	0.005	0.000
	Sex	-0.011	0.090	0.904
	Race	0.320	0.146	0.029
	Smoking	-0.063	0.094	0.504
	BANA	0.090	0.113	0.426
	BMI	0.027	0.006	0.000
	PSR1	0.113	0.108	0.296

Table 13. Relationship between indicators of periodontitis and SNPs adjusted for covariates

Dependent	Predictor	Beta Coefficient	SE	p-value	OR
PSR1	rs10864294	-0.698	0.198	0.000	0.498
	Age	0.046	0.010	0.000	1.047
	Sex	-0.709	0.191	0.000	0.492
	Race	1.305	0.447	0.003	3.689
	Smoking	0.500	0.205	0.015	1.648
	BANA	0.157	0.252	0.534	1.170
	BMI	0.004	0.013	0.770	1.004
PSR1	rs4252120	0.157	0.152	0.301	1.170
	Age	0.046	0.010	0.000	1.047
	Sex	-0.689	0.188	0.000	0.502
	Race	0.855	0.395	0.031	2.350
	Smoking	0.501	0.203	0.014	1.651
	BANA	0.163	0.250	0.513	1.177
	BMI	0.006	0.013	0.616	1.006
PSR1	rs1800795	-0.027	0.131	0.835	0.973
	Age	0.046	0.010	0.000	1.047
	Sex	-0.646	0.191	0.001	0.524
	Race	0.996	0.417	0.017	2.706
	Smoking	0.531	0.206	0.010	1.700
	BANA	0.125	0.255	0.625	1.133
	BMI	0.009	0.013	0.469	1.009
PSR1	rs10965215	-0.182	0.136	0.180	0.834
	Age	0.046	0.010	0.000	1.047
	Sex	-0.676	0.189	0.000	0.508

Table 13 Continued					
	Race	0.861	0.395	0.029	2.366
	Smoking	0.497	0.204	0.015	1.644
	BANA	0.159	0.250	0.525	1.172
	BMI	0.006	0.013	0.634	1.006
PSR2	rs10864294	-0.651	0.190	0.001	0.521
	Age	0.058	0.010	0.000	1.060
	Sex	-0.630	0.180	0.000	0.533
	Race	1.422	0.428	0.001	4.144
	Smoking	0.692	0.192	0.000	1.997
	BANA	-0.015	0.230	0.948	0.985
	BMI	-0.003	0.012	0.830	0.997
PSR2	rs4252120	0.145	0.142	0.306	1.156
	Age	0.059	0.010	0.000	1.061
	Sex	-0.612	0.178	0.001	0.542
	Race	0.999	0.378	0.008	2.716
	Smoking	0.695	0.191	0.000	2.004
	BANA	-0.003	0.228	0.988	0.997
	BMI	0.000	0.012	0.986	1.000
PSR2	rs1800795	-0.118	0.124	0.338	0.888
	Age	0.060	0.010	0.000	1.061
	Sex	-0.556	0.181	0.002	0.574
	Race	1.125	0.396	0.004	3.081
	Smoking	0.719	0.194	0.000	2.052
	BANA	-0.073	0.232	0.753	0.929
	BMI	0.003	0.012	0.792	1.003
PSR2	rs10965215	-0.152	0.128	0.235	0.859
	Age	0.060	0.010	0.000	1.062
	Sex	-0.597	0.178	0.001	0.550
	Race	1.012	0.378	0.007	2.750
	Smoking	0.696	0.191	0.000	2.007
	BANA	-0.003	0.228	0.988	0.997
	BMI	-0.001	0.012	0.964	0.999
BANA	rs10864294	-0.650	0.190	0.001	0.522
	Age	0.058	0.010	0.000	1.059

Table 13 Continued					
	Sex	-0.646	0.179	0.000	0.524
	Race	1.422	0.428	0.001	4.144
	Smoking	0.709	0.191	0.000	2.032
	BMI	-0.002	0.012	0.835	0.998
BANA	rs4252120	0.138	0.142	0.328	1.148
	Age	0.059	0.010	0.000	1.060
	Sex	-0.629	0.177	0.000	0.533
	Race	0.999	0.377	0.008	2.716
	Smoking	0.712	0.190	0.000	2.038
	BMI	0.000	0.012	0.993	1.000
BANA	rs1800795	-0.125	0.123	0.312	0.883
	Age	0.059	0.010	0.000	1.061
	Sex	-0.570	0.180	0.002	0.565
	Race	1.131	0.395	0.004	3.099
	Smoking	0.740	0.193	0.000	2.096
	BMI	0.003	0.012	0.785	1.003
BANA	rs10965215	-0.168	0.127	0.188	0.846
	Age	0.059	0.010	0.000	1.061
	Sex	-0.613	0.177	0.001	0.542
	Race	1.013	0.377	0.007	2.755
	Smoking	0.712	0.190	0.000	2.037
	BMI	0.000	0.012	0.968	1.000

Table 14. Relationship between indicators of periodontitis and SNPs adjusted for covariates and HDSCORE

Dependent	Predictor	Beta Coefficient	SE	p-value	OR
PSR1	rs10864294	-0.698	0.198	0.000	0.497
	Age	0.043	0.011	0.000	1.044
	Sex	-0.710	0.191	0.000	0.492
	Race	1.284	0.447	0.004	3.612
	Smoking	0.505	0.205	0.014	1.658
	BANA	0.146	0.253	0.564	1.157
	BMI	0.001	0.013	0.941	1.001
	HDSCORE	0.083	0.078	0.291	1.086

Table 14 Continued					
PSR1	rs4252120	0.166	0.152	0.273	1.181
	Age	0.043	0.010	0.000	1.044
	Sex	-0.689	0.188	0.000	0.502
	Race	0.831	0.396	0.036	2.296
	Smoking	0.504	0.204	0.013	1.656
	BANA	0.154	0.250	0.539	1.166
	BMI	0.003	0.013	0.792	1.003
	HDSCORE	0.089	0.078	0.254	1.093
PSR1	rs1800795	-0.021	0.132	0.874	0.979
	Age	0.043	0.011	0.000	1.044
	Sex	-0.646	0.191	0.001	0.524
	Race	0.971	0.418	0.020	2.642
	Smoking	0.536	0.206	0.009	1.708
	BANA	0.116	0.255	0.650	1.123
	BMI	0.006	0.013	0.624	1.006
	HDSCORE	0.085	0.079	0.284	1.089
PSR1	rs10965215	-0.173	0.136	0.203	0.841
	Age	0.044	0.011	0.000	1.045
	Sex	-0.677	0.189	0.000	0.508
	Race	0.838	0.396	0.034	2.312
	Smoking	0.500	0.204	0.014	1.649
	BANA	0.151	0.251	0.546	1.163
	BMI	0.003	0.013	0.792	1.003
	HDSCORE	0.081	0.078	0.298	1.085
PSR2	rs10864294	-0.653	0.189	0.001	0.520
	Age	0.056	0.010	0.000	1.057
	Sex	-0.630	0.180	0.000	0.533
	Race	1.406	0.429	0.001	4.079
	Smoking	0.697	0.193	0.000	2.008
	BANA	-0.024	0.230	0.916	0.976
	BMI	-0.005	0.012	0.684	0.995
	HDSCORE	0.071	0.074	0.338	1.074
PSR2	rs4252120	0.152	0.142	0.285	1.164
	Age	0.057	0.010	0.000	1.058
	Sex	-0.612	0.178	0.001	0.542

Table 14 Continued					
	Race	0.980	0.379	0.010	2.664
	Smoking	0.699	0.191	0.000	2.012
	BANA	-0.011	0.228	0.962	0.989
	BMI	-0.003	0.012	0.825	0.997
	HDSCORE	0.074	0.074	0.318	1.077
PSR2	rs1800795	-0.114	0.124	0.359	0.893
	Age	0.057	0.010	0.000	1.059
	Sex	-0.556	0.181	0.002	0.574
	Race	1.107	0.397	0.005	3.025
	Smoking	0.723	0.194	0.000	2.061
	BANA	-0.079	0.232	0.733	0.924
	BMI	0.001	0.012	0.941	1.001
	HDSCORE	0.066	0.075	0.377	1.069
PSR2	rs10965215	-0.144	0.128	0.261	0.866
	Age	0.058	0.010	0.000	1.059
	Sex	-0.597	0.178	0.001	0.550
	Race	0.993	0.379	0.009	2.698
	Smoking	0.700	0.191	0.000	2.015
	BANA	-0.010	0.228	0.966	0.990
	BMI	-0.003	0.012	0.818	0.997
	HDSCORE	0.069	0.074	0.355	1.071
BANA	rs10864294	0.018	0.220	0.936	1.018
	Age	-0.019	0.011	0.088	0.981
	Sex	-0.417	0.210	0.048	0.659
	Race	-0.734	0.447	0.101	0.480
	Smoking	-0.499	0.205	0.015	0.607
	BMI	-0.004	0.013	0.752	0.996
	HDSCORE	0.073	0.086	0.401	1.075
BANA	rs4252120	0.036	0.155	0.815	1.037
	Age	-0.018	0.011	0.100	0.982
	Sex	-0.407	0.208	0.051	0.666
	Race	-0.821	0.416	0.048	0.440
	Smoking	-0.467	0.204	0.022	0.627
	BMI	-0.004	0.013	0.756	0.996
	HDSCORE	0.073	0.086	0.393	1.076

Table 14 Continued					
BANA	rs1800795	-0.086	0.140	0.540	0.918
	Age	-0.016	0.011	0.162	0.984
	Sex	-0.355	0.212	0.094	0.701
	Race	-0.783	0.417	0.060	0.457
	Smoking	-0.425	0.209	0.043	0.654
	BMI	-0.003	0.013	0.809	0.997
	HDSCORE	0.071	0.088	0.419	1.074
BANA	rs10965215	-0.113	0.142	0.424	0.893
	Age	-0.019	0.011	0.081	0.981
	Sex	-0.409	0.209	0.050	0.664
	Race	-0.839	0.415	0.043	0.432
	Smoking	-0.471	0.204	0.021	0.624
	BMI	-0.004	0.013	0.785	0.996
	HDSCORE	0.065	0.086	0.448	1.068

Table 15. Post-Hoc Power Calculations showing effect sizes necessary to achieve 80% power for each combination of condition and SNP

Outcome	SNP	Effect
HDSCORE	rs10864294	0.225
	rs4252120	0.225
	rs1800795	0.200
	rs10965215	0.150
PSR1	rs10864294	1.550
	rs4252120	1.550
	rs1800795	1.500
	rs10965215	1.400
PSR2	rs10864294	1.500
	rs4252120	1.500
	rs1800795	1.450
	rs10965215	1.350
BANA	rs10864294	1.700
	rs4252120	1.700
	rs1800795	1.600
	rs10965215	1.400

APPENDIX B: INSTITUTIONAL REVIEW BOARD APPROVALS

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Mary Marazita, PhD
From: IRB Office
Date: 10/31/2016
IRB#: REN16100205 / IRB0506048
Subject: Coordinating Center for Genetic Factors Contributing to Oral Health Disparities in Appalachia

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Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(8)(c)

Please note the following information:

Approval Date: 10/31/2016
Expiration Date: 11/17/2017

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Mary Marazita , PhD
From: IRB Office
Date: 5/3/2017
IRB#: REN17040241 / IRB020773
Subject: Genetic Factors Contributing to Oral Health Disparities in Appalachia.

Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(9)

Please note the following information:

Approval Date: 5/3/2017
Expiration Date: 5/17/2018

This approval is for analysis of data only.

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

Approval Letter Expedited

Action Date	06/27/2017
To	Daniel McNeil
From	WVU Office of Research Integrity and Compliance
Approval Date	10/11/2013
Expiration Date	06/26/2018
Subject	Protocol Approval Letter
Protocol Number	1309099825R004
Title	Genetic Factors Contributing to Oral Health Disparities in Appalachia

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The above-referenced research study was reviewed by the West Virginia University Institutional Review Board IRB and was approved in accordance with 46 CFR 46.101b.

It has been determined that this study is of minimal risk and meets the criteria as defined by the expedited categories listed below:

- Category 5. Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).

Documents reviewed and/or approved as part of this submission:

List of personnel 6-26-17.pdf: 2017-06-26-04:00

COHRA1 KC Amendment Form 10-13-14.docx: 2015-02-03-05:00

COHRA-1 BRAAN approval letter 10-22-12.pdf: 2015-02-03-05:00

Form H017.2012.12.04 - Continuing Review Form COHRA1 10-2-2014.docx: 2015-02-03-05:00

HIPPA document H-24094.docx: 2015-02-03-05:00

COHRA-1 BRAAN protocol 9-27-13.pdf: 2015-02-03-05:00

Documents for use in this study are available in the WVUkc system in the Notes and Attachments section of your protocol.

The Office of Research Integrity and Compliance is here to provide assistance to you from the initial submission of an IRB protocol and all subsequent activity. Please feel free to contact us by phone at 304.293.7073 with any question you may have. Thank you.

WVU Office of Research Integrity and Compliance

Date:06/27/2017

Signed:

Johnathan M. Herczyk
IRB Administrator

wing regulations apply:

encountered in this research study must be reported to the

2. Any modifications to the study protocol or informed consent form must be reviewed and approved by the IRB prior to implementation via submission of an amendment.

3. You may not use a modified informed consent form until it has been approved and validated by the IRB.

BIBLIOGRAPHY

1. Aarabi G, Zeller T, Seedorf H, Reissmann DR, Heydecke G, Schaefer AS, et al. Genetic susceptibility contributing to periodontal and cardiovascular disease. J Dent Res. 2017;96:610–617. doi:10.1177/0022034517699786.

2. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017;3:17038. doi:10.1038/nrdp.2017.38.

3. Types of Gum Disease | Perio.org. https://www.perio.org/consumer/types-gum-disease.html. Accessed 16 Aug 2017.

4. Trevilatto PC, Scarel-Caminaga RM, de Brito RB, de Souza AP, Line SRP. Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. J Clin Periodontol. 2003;30:438–442. doi:10.1034/j.1600-051X.2003.20016.x.

5. Jenny NS, Tracy RP, Ogg MS, Luong LA, Kuller LH, Arnold AM, et al. In the elderly, interleukin-6 plasma levels and the -174G>C polymorphism are associated with the development of cardiovascular disease. Arterioscler Thromb Vasc Biol. 2002;22:2066–2071.

6. Jacob P S. Measuring periodontitis in population studies: a literature review. Revista odonto ciencia. 2011.

7. Rozier RG, White BA, Slade GD. Trends in oral diseases in the U.S. population. J Dent Educ. 2017;81:eS97–eS109. doi:10.21815/JDE.017.016.

8. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30–44. doi:10.1038/nri3785.

9. Loesche WJ, Giordano J, Hujoel PP. The utility of the BANA test for monitoring anaerobic infections due to spirochetes (Treponema denticola) in periodontal disease. J Dent Res. 1990;69:1696–1702. doi:10.1177/00220345900690101301.

10. Ivic-Kardum M, Beader N, Staudt-Skaljac G. Diagnostic Methods for Evaluation of Microbial Flora in Periodontitis. Acta Stomatol Croat. 2000.

11. Andrade JA de, Feres M, Figueiredo LC de, Salvador SL, Cortelli SC. The ability of the BANA test to detect different levels of P. gingivalis, T. denticola and T. forsythia. Braz Oral Res. 2010;24:224–230. doi:10.1590/S1806-83242010000200016.

12. Preshaw PM. Detection and diagnosis of periodontal conditions amenable to prevention. BMC Oral Health. 2015;15 Suppl 1:S5. doi:10.1186/1472-6831-15-S1-S5.

13. Kataoka S, Baba A, Suda Y, Takii R, Hashimoto M, Kawakubo T, et al. A novel, potent dual inhibitor of Arg-gingipains and Lys-gingipain as a promising agent for periodontal disease therapy. FASEB J. 2014;28:3564–3578. doi:10.1096/fj.14-252130.

14. Moon JH, Lee JH, Lee JY. Subgingival microbiome in smokers and non-smokers in Korean chronic periodontitis patients. Mol Oral Microbiol. 2015;30:227–241. doi:10.1111/omi.12086.

15. Chambrone L, Preshaw PM, Rosa EF, Heasman PA, Romito GA, Pannuti CM, et al. Effects of smoking cessation on the outcomes of non-surgical periodontal therapy: a systematic review and individual patient data meta-analysis. J Clin Periodontol. 2013;40:607–615. doi:10.1111/jcpe.12106.

16. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. Periodontol 2000. 2007;44:127–153. doi:10.1111/j.1600-0757.2006.00193.x.

17. Casarin RCV, Barbagallo A, Meulman T, Santos VR, Sallum EA, Nociti FH, et al. Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. J Periodontal Res. 2013;48:30–36. doi:10.1111/j.1600-0765.2012.01498.x.

18. Sanz M, Ceriello A, Buysschaert M, Chapple I, Demmer RT, Graziani F, et al. TEMPORARY REMOVAL: Scientific evidence on the links between periodontal diseases and diabetes: Consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International diabetes Federation and the European Federation of Periodontology. Diabetes Res Clin Pract. 2017. doi:10.1016/j.diabres.2017.12.001.

19. Pumerantz AS, Bissett SM, Dong F, Ochoa C, Wassall RR, Davila H, et al. Standardized screening for periodontitis as an integral part of multidisciplinary management of adults with type 2 diabetes: an observational cross-sectional study of cohorts in the USA and UK. BMJ Open Diabetes Res Care. 2017;5:e000413. doi:10.1136/bmjdrc-2017-000413.

20. McGowan T, McGowan K, Ivanovski S. A Novel Evidence-Based Periodontal Prognosis Model. J Evid Based Dent Pract. 2017;17:350–360. doi:10.1016/j.jebdp.2017.05.006.

21. Offenbacher S, Divaris K, Barros SP, Moss KL, Marchesan JT, Morelli T, et al. Genome-wide association study of biologically informed periodontal complex traits offers novel insights into the genetic basis of periodontal disease. Hum Mol Genet. 2016;25:2113–2129. doi:10.1093/hmg/ddw069.

22. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2011;127:701–21.e1. doi:10.1016/j.jaci.2010.11.050.

23. Da Silva FRP, Pessoa LDS, Vasconcelos ACCG, de Aquino Lima W, Alves EHP, Vasconcelos DFP. Polymorphisms in interleukins 17A and 17F genes and periodontitis: results from a meta-analysis. Mol Biol Rep. 2017;44:443–453. doi:10.1007/s11033-017-4128-x.

24. Deng J-S, Qin P, Li X-X, Du Y-H. Association between interleukin-1β C (3953/4)T polymorphism and chronic periodontitis: evidence from a meta-analysis. Hum Immunol. 2013;74:371–378. doi:10.1016/j.humimm.2012.11.018.

25. Nibali L, Tonetti MS, Ready D, Parkar M, Brett PM, Donos N, et al. Interleukin-6 polymorphisms are associated with pathogenic bacteria in subjects with periodontitis. J Periodontol. 2008;79:677–683. doi:10.1902/jop.2008.070453.

26. Online Mendelian Inheritance in Man Entry Search - periodontitis. https://www.omim.org/search/?index=entry&sort=score+desc%2C+prefix_sort+desc&start=1&limit=10&search=periodontitis. Accessed 15 Feb 2018.

27. ClinVar Entry Search - periodontitis. https://www.ncbi.nlm.nih.gov/clinvar/?term=periodontitis. Accessed 15 Feb 2018.

28. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42 Database issue:D980–5. doi:10.1093/nar/gkt1113.

29. Haim-Munk Syndrome - NORD (National Organization for Rare Disorders). https://rarediseases.org/rare-diseases/haim-munk-syndrome/. Accessed 15 Feb 2018.

30. Cathepsin C; CTSC. Online Mendelian Inheritance in Man. https://www.omim.org/entry/602365?search=periodontitis&highlight=periodontiti#text. Accessed 15 Feb 2018.

31. Papillon-Lefevre Snydrome; PALS. Online Mendelian Inheritance in Man. https://www.omim.org/clinicalSynopsis/245000?highlight=periodontiti. Accessed 15 Feb 2018.

32. Periodontal Disease | Division of Oral Health | Centers for Disease Control and Prevention. https://www.cdc.gov/oralhealth/periodontal_disease/index.htm. Accessed 22 Nov 2017.

33. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari N-E, et al. Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. PLoS Genet. 2009;5:e1000378. doi:10.1371/journal.pgen.1000378.

34. Oral Health | Healthy People 2020. https://www.healthypeople.gov/2020/topics-objectives/topic/oral-health. Accessed 2 Oct 2017.

35. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. J Periodontol. 2015;86:611–622. doi:10.1902/jop.2015.140520.

36. Eke PI, Thornton-Evans GO, Wei L, Borgnakke WS, Dye BA. Accuracy of NHANES periodontal examination protocols. J Dent Res. 2010;89:1208–1213. doi:10.1177/0022034510377793.

37. Centers for Disease Control and Prevention - Heart Disease Home - DHDSP. https://www.cdc.gov/heartdisease/index.htm. Accessed 16 Nov 2017.

38. Coronary Artery Disease: Causes, Diagonosis & Prevention| cdc.gov. https://www.cdc.gov/heartdisease/coronary_ad.htm. Accessed 10 Dec 2017.

39. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685–1695. doi:10.1056/NEJMra043430.

40. Hanson MA, Fareed MT, Argenio SL, Agunwamba AO, Hanson TR. Coronary artery disease. Prim Care. 2013;40:1–16. doi:10.1016/j.pop.2012.12.001.

41. Hypertension - National Library of Medicine - PubMed Health. https://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0024199/. Accessed 17 Dec 2017.

42. Hypertension (Normal Vs. High Blood Pressure) | Cleveland Clinic: Health Library. https://my.clevelandclinic.org/health/articles/hypertension-high-blood-pressure. Accessed 10 Dec 2017.

43. Risk Factors for Coronary Artery Disease | Cleveland Clinic. https://my.clevelandclinic.org/health/articles/coronary-artery-disease/diagnosis-risk-factors. Accessed 10 Dec 2017.

44. Family History & Your Risk for Heart Disease | cdc.gov. https://www.cdc.gov/heartdisease/family_history.htm. Accessed 10 Dec 2017.

45. Mayer B, Erdmann J, Schunkert H. Genetics and heritability of coronary artery disease and myocardial infarction. Clin Res Cardiol. 2007;96:1–7. doi:10.1007/s00392-006-0447-y.

46. ClinVar Entry Search - coronary artery disease. https://www.ncbi.nlm.nih.gov/clinvar/?term=coronary+artery+disease. Accessed 12 Feb 2018.

47. ClinVar Entry Search - hypertension. https://www.ncbi.nlm.nih.gov/clinvar/?term=hypertension. Accessed 13 Feb 2018.

48. Online Mendelian Inheritance in Man Entry Search - hypertension. https://www.omim.org/search/?index=entry&sort=score+desc%2C+prefix_sort+desc&start=1&limit=10&search=hypertension. Accessed 13 Feb 2018.

49. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478:103–109. doi:10.1038/nature10405.

50. Waterworth DM, Ricketts SL, Song K, Chen L, Zhao JH, Ripatti S, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. Arterioscler Thromb Vasc Biol. 2010;30:2264–2276. doi:10.1161/ATVBAHA.109.201020.

51. Basso F, Lowe GDO, Rumley A, McMahon AD, Humphries SE. Interleukin-6 -174G>C polymorphism and risk of coronary heart disease in West of Scotland coronary prevention study (WOSCOPS). Arterioscler Thromb Vasc Biol. 2002;22:599–604.

52. Morrison AC, Bare LA, Chambless LE, Ellis SG, Malloy M, Kane JP, et al. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities Study. Am J Epidemiol. 2007;166:28–35. doi:10.1093/aje/kwm060.

53. Ratner R, Goldberg R, Haffner S, Marcovina S, Orchard T, Fowler S, et al. Impact of intensive lifestyle and metformin therapy on cardiovascular disease risk factors in the diabetes prevention program. Diabetes Care. 2005;28:888–894. doi:10.2337/diacare.28.4.888.

54. Tuteja S, Limdi N. Pharmacogenetics in cardiovascular medicine. Curr Genet Med Rep. 2016;4:119–129. doi:10.1007/s40142-016-0096-z.

55. K Siddiqui M, Maroteau C, Veluchamy A, Tornio A, Tavendale R, Carr F, et al. A common missense variant of LILRB5 is associated with statin intolerance and myalgia. Eur Heart J. 2017;38:3569–3575. doi:10.1093/eurheartj/ehx467.

56. Johnson JA, Cavallari LH. Pharmacogenetics and cardiovascular disease--implications for personalized medicine. Pharmacol Rev. 2013;65:987–1009. doi:10.1124/pr.112.007252.

57. Yusuf HR, Giles WH, Croft JB, Anda RF, Casper ML. Impact of multiple risk factor profiles on determining cardiovascular disease risk. Prev Med. 1998;27:1–9. doi:10.1006/pmed.1997.0268.

58. Irony TZ. The “utility” in composite outcome measures: measuring what is important to patients. JAMA. 2017;318:1820–1821. doi:10.1001/jama.2017.14001.

59. Wilson PW, D’Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837–1847. doi:10.1161/01.CIR.97.18.1837.

60. Murphy SL, Kochanek KD, Xu J, Arias E. Mortality in the united states, 2014. NCHS Data Brief. 2015;:1–8.

61. FastStats - Heart Disease. https://www.cdc.gov/nchs/fastats/heart-disease.htm. Accessed 18 Mar 2018.

62. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation. 2015;131:e29–322. doi:10.1161/CIR.0000000000000152.

63. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, et al. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation. 2011;123:933–944. doi:10.1161/CIR.0b013e31820a55f5.

64. Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, et al. The American Journal of Cardiology and Journal of Periodontology editors’ consensus: periodontitis and atherosclerotic cardiovascular disease. J Periodontol. 2009;80:1021–1032. doi:10.1902/jop.2009.097001.

65. Lockhart PB, Bolger AF, Papapanou PN, Osinbowale O, Trevisan M, Levison ME, et al. Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association?: a scientific statement from the American Heart Association. Circulation. 2012;125:2520–2544. doi:10.1161/CIR.0b013e31825719f3.

66. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. J Periodontol. 1996;67 10 Suppl:1123–1137. doi:10.1902/jop.1996.67.10s.1123.

67. Slocum C, Kramer C, Genco CA. Immune dysregulation mediated by the oral microbiome: potential link to chronic inflammation and atherosclerosis. J Intern Med. 2016;280:114–128. doi:10.1111/joim.12476.

68. D'Aiuto F, Ready D, Tonetti MS. Periodontal disease and C-reactive protein-associated cardiovascular risk. J Periodontal Res. 2004;39:236–241. doi:10.1111/j.1600-0765.2004.00731.x.

69. Bale BF, Doneen AL, Vigerust DJ. High-risk periodontal pathogens contribute to the pathogenesis of atherosclerosis. Postgrad Med J. 2017;93:215–220. doi:10.1136/postgradmedj-2016-134279.

70. Hayashi C, Papadopoulos G, Gudino CV, Weinberg EO, Barth KR, Madrigal AG, et al. Protective role for TLR4 signaling in atherosclerosis progression as revealed by infection with a common oral pathogen. J Immunol. 2012;189:3681–3688. doi:10.4049/jimmunol.1201541.

71. Beutler BA. TLRs and innate immunity. Blood. 2009;113:1399–1407. doi:10.1182/blood-2008-07-019307.

72. Li L, Messas E, Batista EL, Levine RA, Amar S. Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. Circulation. 2002;105:861–867.

73. Lindy O, Suomalainen K, Mäkelä M, Lindy S. Statin use is associated with fewer periodontal lesions: A retrospective study. BMC Oral Health. 2008;8:16. doi:10.1186/1472-6831-8-16.

74. Jockel-Schneider Y, Bechtold M, Haubitz I, Störk S, Fickl S, Harks I, et al. Impact of antiinfective periodontal therapy on parameters of vascular health. J Clin Periodontol. 2017. doi:10.1111/jcpe.12849.

75. Hamilton JA, Hasturk H, Kantarci A, Serhan CN, Van Dyke T. Atherosclerosis, Periodontal Disease, and Treatment with Resolvins. Curr Atheroscler Rep. 2017;19:57. doi:10.1007/s11883-017-0696-4.

76. Yu Y-H, Chasman DI, Buring JE, Rose L, Ridker PM. Cardiovascular risks associated with incident and prevalent periodontal disease. J Clin Periodontol. 2015;42:21–28. doi:10.1111/jcpe.12335.

77. Mucci LA, Hsieh C-C, Williams PL, Arora M, Adami H-O, de Faire U, et al. Do genetic factors explain the association between poor oral health and cardiovascular disease? A prospective study among Swedish twins. Am J Epidemiol. 2009;170:615–621. doi:10.1093/aje/kwp177.

78. Schaefer AS, Bochenek G, Jochens A, Ellinghaus D, Dommisch H, Güzeldemir-Akçakanat E, et al. Genetic evidence for PLASMINOGEN as a shared genetic risk factor of coronary artery disease and periodontitis. Circ Cardiovasc Genet. 2015;8:159–167. doi:10.1161/CIRCGENETICS.114.000554.

79. Oral Health Studies | School of Dental Medicine | University of Pittsburgh. http://www.dental.pitt.edu/oral-health-studies. Accessed 30 Nov 2017.

80. Appalachian Region of US.png - Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Appalachian_Region_of_US.png. Accessed 19 Mar 2018.

81. Polk DE, Weyant RJ, Crout RJ, McNeil DW, Tarter RE, Thomas JG, et al. Study protocol of the Center for Oral Health Research in Appalachia (COHRA) etiology study. BMC Oral Health. 2008;8:18. doi:10.1186/1472-6831-8-18.

82. C O H R A Research Procedures. Research Protocol Manual.

83. Shaffer JR, Polk DE, Wang X, Feingold E, Weeks DE, Lee M-K, et al. Genome-wide association study of periodontal health measured by probing depth in adults ages 18-49 years. G3 (Bethesda). 2014;4:307–314. doi:10.1534/g3.113.008755.

84. Shungin D, Cornelis MC, Divaris K, Holtfreter B, Shaffer JR, Yu Y-H, et al. Using genetics to test the causal relationship of total adiposity and periodontitis: Mendelian randomization analyses in the Gene-Lifestyle Interactions and Dental Endpoints (GLIDE) Consortium. Int J Epidemiol. 2015;44:638–650. doi:10.1093/ije/dyv075.

85. Wiener RC. Relationship of Edentulism, Sleep Disordered Breathing and Cardiovascular Disease: NHANES, 2007-2008. Cardiol Angiol. 2015;3:167–174. doi:10.9734/CA/2015/17944.

86. Illumina, Inc. Illumina. Computer software. San Diego, CA: Illumina; 2018.

87. PubMed. PubMed. https://www.ncbi.nlm.nih.gov/pubmed. Accessed 17 Feb 2018.

88. Reference SNP (refSNP) Cluster Report: rs10864294 . https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10864294. Accessed 19 Feb 2018.

89. Reference SNP (refSNP) Cluster Report: rs4252120 . https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4252120. Accessed 19 Feb 2018.

90. Reference SNP (refSNP) Cluster Report: rs1800795 **Clinical Channel**. https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1800795. Accessed 19 Feb 2018.

91. Reference SNP (refSNP) Cluster Report: rs10965215 . https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10965215. Accessed 19 Feb 2018.

92. StataCorp. STATA. Computer software. College Station, TX: StataCorp, LLC; 2017.

93. USC. Quanto. Computer software. California: University of Southern California; 2009.

94. Detail view for rs1800795. HaploReg. http://archive.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs1800795. Accessed 19 Mar 2018.

95. Interleukin 6; IL6. OMIM. https://www.omim.org/entry/147620#0001. Accessed 17 Feb 2018.

96. 173350: Plasminogen; PLG. Online Mendelian Inheritance in Man. https://www.omim.org/entry/173350?search=plg&highlight=plg. Accessed 3 Mar 2018.

97. Detail view for rs4252120. HaploReg. http://archive.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs4252120. Accessed 19 Mar 2018.

98. Congrains A, Kamide K, Ohishi M, Rakugi H. ANRIL: molecular mechanisms and implications in human health. Int J Mol Sci. 2013;14:1278–1292. doi:10.3390/ijms14011278.

99. 600431: Cyclin-Dependent Kinase Inhibitory 2B; CDKN2A. Online Mendelian Inheritance in Man. https://www.omim.org/entry/600431?search=cdkn2b&highlight=cdkn2b. Accessed 3 Mar 2018.

100. 600160: Cyclin-Dependent Kinase Inhibitor 2A; CDKN2A. Online Mendelian Inheritance in Man. https://www.omim.org/entry/600160?search=cdkn2a&highlight=cdkn2a. Accessed 3 Mar 2018.

101. 613149: CDKN2B Antisense RNA: CDKN2BAS. Online Mendelian Inheritance in Man. https://www.omim.org/entry/613149?search=anril&highlight=anril. Accessed 3 Mar 2018.

102. Detail view for rs10965215. HaploReg. http://archive.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs10965215. Accessed 19 Mar 2018.

103. 603657: Vesicle-Associated Membrane Protein 3; VAMP3. Online Mendelian Inheritance in Man. https://www.omim.org/entry/603657?search=vamp3&highlight=vamp3. Accessed 3 Mar 2018.

104. 611501: Calmodulin-Binding Transcription Activator 1; CAMTA1. Online Mendelian Inheritance in Man. https://www.omim.org/entry/611501?search=camta1&highlight=camta1. Accessed 3 Mar 2018.

105. Wey E, Schäfer BW. Identification of novel DNA binding sites recognized by the transcription factor mPOU (POU6F1). Biochem Biophys Res Commun. 1996;220:274–279. doi:10.1006/bbrc.1996.0395.

106. Detail view for rs10864294. HaploReg. http://archive.broadinstitute.org/mammals/haploreg/detail_v4.1.php?query=&id=rs10864294. Accessed 19 Mar 2018.

107. Divaris K, Monda KL, North KE, Olshan AF, Lange EM, Moss K, et al. Genome-wide association study of periodontal pathogen colonization. J Dent Res. 2012;91 7 Suppl:21S–28S. doi:10.1177/0022034512447951.

108. National Research Council (US) and Institute of Medicine (US) Roundtable on Translating Genomic-Based Research for Health. Currently Available Direct-to-Consumer Genetic Tests. 2010.

109. Access to Health Services | Healthy People 2020. https://www.healthypeople.gov/2020/topics-objectives/topic/Access-to-Health-Services. Accessed 13 Apr 2018.

110. Reports C, Reports C. Health Insurance Coverage in the United States: 2015.

111. Rai R, Naveen Kumar PG, Hirekalmath S, Sunil LA. Genetics and oral health. Dent Med Res. 2016;4:9. doi:10.4103/2348-1471.171918.

112. Centers for Disease Control and Prevention - Public Health System and the 10 Essential Public Health Services - OSTLTS. https://www.cdc.gov/stltpublichealth/publichealthservices/essentialhealthservices.html. Accessed 28 Feb 2018.

113. Fantasia JE. Syndromes with unusual dental findings or gingival components. Atlas Oral Maxillofac Surg Clin North Am. 2014;22:211–219. doi:10.1016/j.cxom.2014.05.006.