ARIC Manuscript Proposal # 1471

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1a. Full Title: Genome-Wide Association Analysis of Single Nucleotide Polymorphisms Associated with Periodontal Disease

b. Abbreviated Title: GWAS and Periodontititis

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3. Timeline:

Data arrival: February 1, 2009
Statistical analyses: February-April, 2009
Manuscript preparation: February-May, 2009
Manuscript revision: August – September 2009
Manuscript submission: September – October 2009

4. Rationale:

Periodontitis is an inflammatory response to the commensal oral bacterial flora and represents one of the most prevalent infections in man with approximately 90% of the population exhibiting some form of early disease (gingivitis), but moderate severe disease effects only 15-20% of the adult population. Despite the high prevalence of disease there are several lines of evidence that support the role of genetics in determining the severity of periodontal disease, and especially the most severe forms of disease. The earliest population studies demonstrated a pattern of familial aggregation of severe disease (1-5) suggesting a genetic component, but studies by Michalowitz in monozygotic and dizygotic twins (6) provided the first estimates of heritability that suggested that about half (48%) of the variance in disease expression in the population was attributable to genetics. Other reports by Corey et al (7) in a study among 4806 twin pairs suggested concordance rates of 0.23-0.36 for monozygotic twins and 0.08-0.16 for dizygotic twins. However, in this latter investigation environmental periodontal risk factors like smoking were not considered. Clearly, the pattern periodontal disease in the population does not follow simple Mendelian modes of inheritance and thus investigating potential genetic variants that contribute to common forms of periodontal disease will require large population-based studies to permit polygenic effects and haplotype analyses.

Linkage analyses of family-based data have identified areas of the human genome that are associated with severe forms of periodontal disease. Case-control and cross-sectional studies using candidate genes have also demonstrated gene polymorphisms associated with severe periodontitis and most of these have focused on gene affecting host response, cytokine regulation, and metabolism. These candidate genes (reviewed by Yoshie etal *Periodontology 2000*, vol **43**, 2007,102-132), include the IL-1 gene cluster (8-11 and 12 for review), Fc receptors (13-15), Interleukins 2, 4,6,10, 18, (16-20) Tumor necrosis factors (21-22), matrix metalloproteinases (23), and HLA antigens (24) to cite a few. Some of these SNP variants are shown to be more prevalent in those with disease, and in a recent review and meta-analysis (Nikolopoulus etal, *J Clinical Periodontol*, 2008;35:754-767) of the 53 of candidate-gene association studies in the literature including 4178 cases and 4590 controls six polymorphisms (two IL-1 loci) were found to be significantly associated with modest increased risk of periodontal disease. It is noteworthy that for the justification of this proposed manuscript that **to date there has been no genome-wide scan performed on any population database for periodontal**

disease. **Thus, this will be the first report of a GWAS (Genome-Wide Association Study) for periodontitis**.

Genome-wide association studies interrogate whether variation across the human genome in the form of SNPs is associated with given phenotypes. GWAS are now widely recognized as powerful data-driven tools for identifying genetic variants related to common complex diseases. At this time there are only two datasets available to interrogate GWA to our knowledge as part of the GENEVA project, the Dental ARIC, where periodontal disease was assessed by examiners, and the Physicians Health Study which was limited to self-reported history of periodontitis diagnosis or treatment. With respect to the method of phenotyping, the Dental ARIC study therefore is the more powerful and valid dataset. It has complete periodontal clinical measurements that create a continuous variable with increasing severity and extent across multiple clinical parameters. This will enable us to examine case definitions using various cut-points to define disease to associate with genotypes. In contrast the Physicians study has categorical self-reported disease definitions. Replication of findings is a key ingredient in genetic epidemiology studies and we will seek collaborations to facilitate this process. It is our intent that the Dental ARIC can serve to generate the initial GWAS candidates that can be confirmed using the Physicians Health Study dataset or others as they become available.

5. Main Hypotheses/Study Questions:

To complete analyses on the genome-wide SNP data available (~1,000,000 genotyped SNPs + approximately 2.5 million imputed SNPs) on the ARIC sample through its collaboration with the Broad Institute. Assessed phenotypes will include the following (in order of priority):

- Periodontal disease using a five-level disease definition. This is based upon the BGI classification* (Biofilm-Gingival Interface, reference Offenbacher 2008). This classification defines health, gingivitis, and mild (P1), moderate (P2) and severe periodontitis (P3).
- Periodontal disease using the three-level CDC classification** (Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol. 2007; 78(7 Suppl): 1387-99.). This creates a health, entry and severe definition of disease.
- Number of teeth missing (ie. extracted) for treatment of decay or periodontitis.

The key distinction between the two periodontal case-classification systems in that the CDC classification requires evidence of destructive periodontitis on at least two teeth, and hence it can be biased by the person's extent of tooth loss. In contrast, the BGI classification is based upon only two clinical signs that have been demonstrated to create more homogenous disease classifications with regards to the underlying biological phenotype that includes biofilm composition, serum antibody response and local inflammatory response. When a tooth is extracted because of disease, the underlying pathology is usually periodontitis or dental decay or both, but there are equally important contributing factors that cause patients and their dentists to chose extraction in favor of alternatives (eg. surgical treatment of periodontitis, root canal treatment and a subsequent

crown). Those contributing causes, in turn, are influenced by attitudes, money, and access to dental care. Like obesity, diabetes and other conditions that have a substantial component of "lifetstyle" and health care in their etiology, tooth loss is not solely a marker of the biology and pathology underlying periodontitis and dental decay. The Dental ARIC study has numerous measures of those lifestyle and health care factors as they relate to oral health. Hence, in addition to clarifying distinctions between the two periodontitis case definitions, the proposed search for genome wide associations with tooth loss seeks to identify the biological (ie. genetic) endowment that underlies the more complex phenotype of tooth loss.

*BGI Definition: [Offenbacher etal *J Periodontol*, 2007, 78:1911-1925.]
BGI-Health: Subjects with all probing depths (PD) at 3mm or less with ≤10% bleeding on probing (BOP).
BGI-Gingivitis: Subjects will all probing depths (PD) at 3mm or less with >10% bleeding on probing (BOP).
BGI-P1: One or more sites with PD≥4mm and BOP≤ 10%.
BGI-P2: One or more sites with PD≥4mm and BOP >10<50%.
BGI-P3: One or more sites with PD≥4mm and BOP≥50% .

**CDC definition: (Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol. 2007; 78(7 Suppl): 1387-99.)

The periodontitis case definition was developed by the American Academy of Periodontology in collaboration of the U.S. Centers of Disease Control and Prevention in 2003. Cases have moderate or severe periodontitis and non-cases have mild levels or no signs of the disease. Moderate periodontitis was defined as the presence of two sites between adjacent teeth where gum has lost 4+ mm of attachment to the tooth, OR at least two sites with probing pocket depth of 5+ mm. Severe periodontitis was characterized by the presence of at least two sites between adjacent teeth where gum has lost 6+ mm of attachment, and where there was at least one PPD of 5+ mm.

Tooth loss definition: count of teeth that, in the examiner's judgment, have been extracted due to decay or periodontitis.

Summary Table showing cross-tabulation of subjects using the two disease classifications

	BGI classifications		**BGI classifications**		
	Health	Gingivitis	P1	P2	P3
N (%)	971 (16.6%)	1023 (17.5%)	1217 (20.8)	2875 (32.0%)	872 (14.9%)
CDC Definition [n(%)]	796 (82.0%)	832 (81.3%)	412 (33.9%)	701 (26.1%)	62 (7.1%)
Health	175 (18.0%)	191 (18.7%)	654 (53.7%)	1403 (52.3%)	361 (41.4%)
Entry	0 (0.0%)	0 (0.0%)	151 (12.4%)	581 (21.6%)	449 (51.5%)
Severe					

6. Design and Analysis:

Subjects and Sample size:

The subject characteristics regarding age, race, sex and major periodontal disease risk factors are shown.

Subject Characteristic	BGI-H	BGI-G	BGI-P1	BGI-P2	BGI-P3	p-value
Number of Subjects (% Total)	971 (14.3%)	1023 (15.1%)	1217 (18.0%)	2685 (39.7%)	872 (12.9%)	
Age	62.2 (0.2)	62.4 (0.2)	62.4 (0.2)	62.4 (0.1)	62.8 (0.2)	0.20
Race: African-American	314 (32.3%)	257 (25.1%)	140 (11.5%)	314 (11.7%)	275 (31.5%)	
Caucasian	657 (67.7%)	766 (74.9%)	1077 (88.5)	2371 (88.3%)	597 (68.5%)	<0.0001
Sex : Male	261 (26.9%)	378 (37.0%)	586 (48.2%)	1352 (50.4%)	516 (59.2%)	
Female	710 (73.1%)	645 (63.1%)	631 (51.9%)	1333 (49.7%)	356 (40.8%)	<0.0001
Diabetes: Yes	105 (10.9%)	164 (16.1%)	128 (10.6%)	357 (13.4%)	180 (20.9%)	
No	855 (89.1%)	853 (83.9%)	1081 (89.4%)	2316 (86.6%)	680 (79.1%)	<0.0001
Education : *Basic*	141 (14.5%)	170 (16.7%)	86 (7.1%)	305 (11.4%)	215 (24.7%)	
Intermediate	401 (41.3%)	440 (43.1%)	489 (40.2%)	1239 (46.2%)	344 (39.5%)	
Advanced	428 (44.1%)	410 (40.2%)	641 (52.7%)	1139 (42.5%)	311 (35.8%)	<0.0001
Smoke : *Current*	102 (10.6%)	88 (8.6%)	179 (14.7%)	324 (12.1%)	147 (17.0%)	
Former	355 (36.8%)	342 (33.5%)	560 (46.1%)	1142 (42.6%)	345 (39.9%)	
Never	509 (52.7%)	590 (57.8%)	476 (39.2%)	1215 (45.3%)	372 (43.1%)	<0.0001
Pack Year	11.3 (0.7)	9.2 (0.6)	16.2 (0.6)	13.7 (0.4)	17.2 (0.7)	<0.0001
BMI (kg/m^2)	28.5 (0.2)	28.8 (0.2)	27.7 (0.2)	28.6 (0.1)	29.3 (0.2)	<0.0001

Quality control of genotyping data

The following quality control analyses of genotype data have already taken place prior to our receiving it: analyses of blind duplicates, individuals analyzed for excessive missing data, SNPs analyzed for excessive missing data, exclusion of SNPs without chromosomal location, exclusion of monomorphic SNPs, analysis of Hardy-Weinberg equilibrium and exclusion of autosomal SNPs with $p<10^{-6}$, and exclusion of individuals with outlying heterozygosity.

Publication strategy

This study will represent the first major GWAS publication in periodontal disease. We will collaborate with the Physicians Health Study investigators to attempt to replicate our findings within the restraints of the disease definitions available. We have extensive biological phenotype data available in the gene-environment domain and in the host-response domain. These will be secondary subgroup analyses. Once the primary GWAS analyses are completed we will revisit this possibility.

Definitions and treatment of variables

Genotype: In our large-scale analysis we will use an additive model to estimate the association between SNP and periodontal disease. In an additive model the SNP is coded as a continuous variable (0=major homozygote, 1=heterozygote, 2=minor homozygote), thus the heterozygote is forced to have an effect midway between the two homozygotes. While less flexible than the codominant (or general) model, the additive model is often seen as biologically plausible. Nonetheless, it was selected primarily for its usefulness in meta-analytic procedures. The additive model, while not as flexible as the co-dominant (general) model, has been shown to perform well when the underlying mode of inheritance is additive or dominant, but less well when recessive (Lettre, Lange et al. 2007).

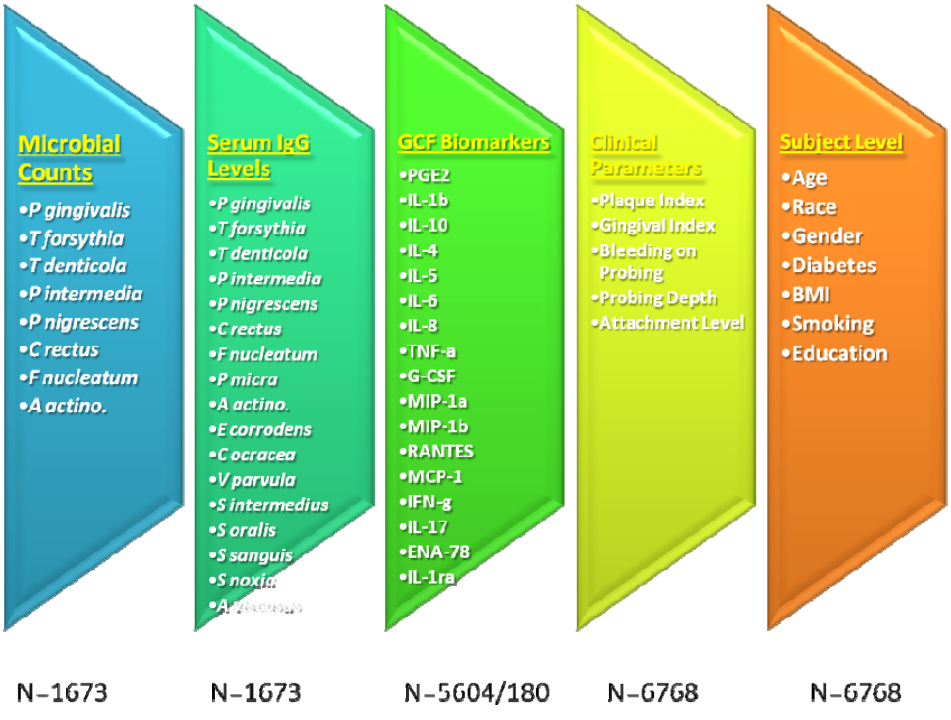
Phenotype measures:

Clinical Measures used in phenotype characterization in Dental ARIC are continuous measures as summarized for individual clinical signs as tabulated under the BGI classifications in the following Table.

	BGI		**BGI**			
	Health	Gingivitis	P1	P2	P3	
Periodontal Measures						P-value
Number of Teeth	19.7 (0.2)	18.5 (0.2)	23.9 (0.2)	23.5 (0.1)	19.4 (0.2)	<0.0001
Mean Plaque Score	0.34 (0.02)	0.54 (0.02)	0.29 (0.01)	0.48 (0.01)	0.99 (0.02)	<0.0001
Mean GI Score	0.33 (0.02)	0.56 (0.02)	0.16 (0.01)	0.35 (0.01)	1.15 (0.02)	<0.0001
Mean Extent BOP	3.3 (0.38)	30.1 (0.36)	4.6 (0.34)	26.1 (0.23)	71.3 (0.40)	<0.0001
Mean PD	1.47 (0.01)	1.52 (0.01)	1.84 (0.01)	2.00 (0.01)	2.69 (0.02)	<0.0001
Extent PD ≥4mm	-	-	5.69 (0.27)	8.65 (0.18)	22.61 (0.32)	<0.0001
Extent PD ≥5mm	-	-	1.61 (0.20)	3.38 (0.13)	12.97 (0.23)	<0.0001

Mean AL	1.38 (0.03)	1.41 (0.03)	1.67 (0.03)	1.76 (0.02)	2.93 (0.03)	<0.0001
Extent AL ≥3mm	12.5 (0.66)	13.6 (0.64)	21.41 (0.59)	23.4 (0.40)	48.7 (0.70)	<0.0001
Extent AL≥4mm	4.2 (0.53)	4.9 (0.52)	9.6 (0.47)	11.0 (0.32)	30.9 (0.56)	<0.0001
Extent of teeth w/ ≥3mm Interproximal	23.9 (0.84)	27.1 (0.81)	39.7 (0.75)	44.9 (0.50)	73.0 (0.88)	<0.0001
Extent of teeth with ≥3mm Interproximal & PD≥5mm	-	-	6.5 (0.4)	11.7 (0.3)	31.7 (0.5)	<0.0001
CDC Definition [n(%)]* Health Entry Severe	796 (82.0%) 175 (18.0%) 0 (0.0%)	832 (81.3%) 191 (18.7%) 0 (0.0%)	412 (33.9%) 654 (53.7%) 151 (12.4%)	701 (26.1%) 1403 (52.3%) 581 (21.6%)	62 (7.1%) 361 (41.4%) 449 (51.5%)	<0.0001

Biological Phenotype Data: Biological phenotyping data are available for a random subset of the Dental ARIC population. The following table summarizes the biological phenotype data available for the periodontal status that reflects the oral biofilm composition (1673 subjects), the serum IgG titer to specific biofilm organisms (n=1673), the local production of inflammatory mediators within the periodontal lesion [Il-1 and PGE2 (n=5604), others (n=180)], as well as the clinical and subject level variables.



Analysis strategy / statistical analysis

Modeling strategy: Prior to running genetic models, sex- and race-specific residuals will be calculated for each phenotype controlling for age, smoking, education, BMI, ARIC field center, and diabetes (yes/no). Next, as mentioned above, we will run additive models to estimate the association between the SNP and the sex- and race-specific residual. Additive models were selected as the genetic model of choice to facilitate meta-analyses with our collaborators with whom we will replicate results, such as the Physicians Health study. Linear regression models will be used to assess the relationship between quantitative traits and the genetic factors and logistic regression models will be used for assessing the relationship with qualitative traits.

Meta-analytic strategy: There is currently no Meta-analysis plans as this will be the first study that has full-mouth data available on periodontal status to permit a GWAS analysis.

Population stratification: Because systematic differences in ancestry can produce spurious associations, all analyses will be stratified by race to account for systematic allele frequency differences between racial groups. Initially, the analyses will be performed on the Caucasian ARIC dataset, as the genotyping data for the African Americans are a component of the CARE project. A description of this study is available at the following URL: http://www.broad.mit.edu/gen_analysis/care/index.php/Main_Page. It is our intent to simultaneously apply using a manuscript proposal for access to these data. However, we will also need to account for population substructure within racial groups. While there are a number of methods available to the analyst, we can take advantage of GWAS data most effectively using the principal components analysis method developed by Price and colleagues (Price, Patterson et al. 2006), implemented in the software EIGENSOFT. This method explicitly models ancestry and has higher power to detect true associations than other methods. Principal components have been generated for the ARIC study and will be incorporated into genetic models to account for population stratification in each of the samples.

Multiple testing: The large number of statistical tests these analyses entail will yield false positive results unless appropriate corrections are made for multiple testing. We will control for this using the Bonferroni correction on an overall α=0.05, a standard approach in GWA analyses, resulting in a significant p-value of approximately 0.05×10^{-6}. Secondarily, we will use permit an appropriate false discovery rate (FDR) (Benjamini, et al. ref 25; van den Oord and Sullivan ref 26) and permutation-based procedures (Nichols and Holmes ref 27). Because the false discovery rate is data dependent, we cannot specify its threshold in advance, although experience suggests that it is likely to be around 10%. This means that probability of a type I error will be greater than the Bonferroni correction (0.05×10^{-6}) which permits no false discoveries, but less than the

unadjusted conventional P=0.05 (Strimmer K. A unified approach to false discovery rate estimation. BMC Bioinformatics 2008, 9:303 doi:10.1186/1471-2105-9-303).

7.a. Will the data be used for non-CVD analysis in this manuscript?

__X_ Yes
__ No

b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used?

_X_Yes
___ No

(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?

_x__ Yes
____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"?

__x_ Yes
____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

__x_ Yes
____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

We are the only group addressing periodontal disease among ARIC investigators.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?

__X__ Yes
__ ___ No

11.b. If yes, is the proposal
_X_ A. primarily the result of an ancillary study (list number __AS#1996.01_)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

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