1.a. Full Title: Anti-citrullinated peptide antibody (ACPA) levels may differ based on clinical periodontal disease severity in individuals without rheumatoid arthritis compared to Atherosclerosis Risk in Communities (ARIC) controls.

b. Abbreviated Title (Length 26 characters):

2. Writing Group:

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _LR_ [please confirm with your initials electronically or in writing]

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Rationale:

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized predominately by joint pain and inflammation with varying degrees of systemic involvement. It is a multifactorial disease, which appears to arise from the complex interaction between genetic and environmental factors, though its etiology remains ultimately unknown. Twin studies have estimated that genetic factors account for approximately 50-60% of the total risk for RA, and, although several environmental risk factors have been identified, they do not fully account for the remaining risk. Periodontal disease (PD) is a chronic inflammatory disease of tooth supporting tissues and shares several pathophysiological features with RA, including bony erosions, elevated cytokine levels, and local rheumatoid factor (RF) production. The incidence of RA and PD as comorbid conditions is well established in the literature, and we and others have found evidence that PD may be associated with increased risk of prevalent and incident RA.

The strongest known genetic risk factor for RA is the shared epitope (SE) in HLA-DRB1, which has also been found to be a risk factor for PD. Tobacco use is the strongest known environmental risk factor for both RA and PD. Nevertheless, these shared genetic and environmental risk factors do not fully explain the epidemiological association between RA and PD. It follows that RA may only arise in the context of PD when an additional series of environmental insults occur and/or pertinent high-risk alleles such as the SE are involved. Pathogenic microbial exposures in dysbiotic subgingival biofilms commonly observed in PD are believed to arise from the complex interaction between genetic and environmental factors, though its etiology remains ultimately unknown. Twin studies have estimated that genetic factors account for approximately 50-60% of the total risk for RA, and, although several environmental risk factors have been identified, they do not fully account for the remaining risk. Periodontal disease (PD) is a chronic inflammatory disease of tooth supporting tissues and shares several pathophysiological features with RA, including bony erosions, elevated cytokine levels, and local rheumatoid factor (RF) production. The incidence of RA and PD as comorbid conditions is well established in the literature, and we and others have found evidence that PD may be associated with increased risk of prevalent and incident RA.

The majority of patients with RA are seropositive for autoantibodies of at least two distinct types, including anti-citrullinated peptide antibodies (ACPA) and RF. Both RF and multiple distinct ACPA can be present for years prior to the onset of clinical RA in retrospective analysis of stored sera, and ACPA titers increase dramatically shortly before development of inflammatory arthritis diagnostic of RA. Although protein citrullination is a normal physiologic response to tissue damage, loss of tolerance to citrullinated proteins through the formation of antibodies (ACPA) appears to be unique to RA. Post-translational citrullination of arginine residues is mediated by peptidylarginine deiminases (PADs). Porphyromonas gingivalis, a bacterium thought causative of PD, has a novel PAD activity that enables the formation of citrullinated proteins. This fact in part generated the hypothesis that PD, through P. gingivalis, may promote the development of RA. Serologic studies demonstrate that many, but not all, individuals with RA have elevated antibody levels against PD, supporting this hypothesis. Furthermore, specific PAD found in P. gingivalis avidly citrullinates peptides, forming neo antigens that enable antibody formation, loss of tolerance and arthritis formation in mice.

While many studies have focused on analyzing PD in patients with existing RA, very few have looked at RA-free individuals with PD and assessed risk for future RA development. Assessing ACPA titers in PD populations may enhance the recruitment of high-risk populations for RA-prevention trials. Prior studies estimate that the presence of one or more ACPA in an otherwise healthy population is associated with a 5-fold increase risk of future RA. As the number of positive ACPA increase, so too does the risk for RA. Improved understanding of the early natural history of ACPA development in relation to PD could help to explain the mechanisms of pathogenesis, as well as the likelihood of RA development in this population versus the established progression rate in general populations.

The aim of this study is to investigate the association between periodontitis and ACPA positivity in a cohort of individuals with well characterized PD who are free of RA or other apparent autoimmune disease.
do so, we will use a developed cohort of individuals with moderate to severe PD and free of known diagnoses (including being anti-CCP negative) or symptoms of RA. A control group will be formed using healthy individuals without PD or RA from the Dental Atherosclerosis Risk in Communities (ARIC) study. If a pattern of ACPA positivity exists in RA-free individuals with PD, future RA-prevention trials could target the PD population for further analysis of risk for RA development.

5. **Main Hypothesis/Study Questions:**

**Main Hypothesis:** Among RA-free individuals, having moderate-to-severe periodontal disease significantly increases the risk of positivity for multiple distinct ACPA.

6. **Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).**

**Study Design and PD Cohort Population**

Following University of Minnesota IRB approval, participants were recruited from the University of Minnesota, School of Dentistry (SOD). Adults with moderate or severe PD as defined by the 2000 American Academy of Periodontology’s (AAP) definitions of generalized moderate to advanced disease and no history of RA or current symptoms associated with RA were recruited. Individuals with unequivocal evidence of previous generalized moderate to advanced PD were also eligible for the study, including the edentulous (n=2). Individuals were excluded if they had a history of RA, were previously hospitalized for RA, or were under the age of 18.

Based on dental records, potentially eligible participants were contacted by mail, and those who consented were given a telephone interview. At the time of the phone call, potentially eligible participants were asked: whether they had ever been diagnosed with RA, whether they had morning stiffness and pain in their hands and/or feet for > 30 minutes, and whether they had swelling in their wrists or knuckles of their hands. Individuals answering yes to these questions were excluded. During the phone interview, individuals who did not have history or symptoms of RA were asked to come in for a study visit at the University of Minnesota SOD.

Individuals who agreed to come in for a study visit were administered an electronic health assessment questionnaire (HAQ). The HAQ recorded: (1) the nature and frequency of ongoing PD care, if any; (2) family history of RA; (3) any existing diagnosis of RA or other inflammatory joint disease; (4) symptoms of stiffness or swelling suggestive of RA; (5) known history of atherosclerotic disease and associated risk factors; (6) smoking/tobacco history; (7) coffee consumption. The phone interview and HAQ assessed individuals for the prevalence of RA based on the 2010 ACR/EULAR criteria. Following the HAQ, the participants that had any features suggesting possible RA were scheduled with a board-certified rheumatologist who performed a clinical diagnostic assessment to exclude RA.

Participants confirmed to be free of signs or symptoms of RA provided informed, written consent and then underwent a blood draw, periodontal examination, and gave plaque and crevicular fluid samples. Of the 183 individuals with PD who consented to be part of the cohort, 3 will be excluded from data analysis because they had markedly elevated CCP titers consistent with established RA based on CCP specificity cutoffs. Two additional participants were excluded because they did not provide specimen samples for analysis. Participants that provided informed, written consent became part of the working PD cohort at the University of Minnesota, which comprises 178 individuals. Sera, HAQ history, smoking status, gender, age, and race from the established PD cohort will be compared to matched controls from the ARIC cohort.

**ARIC Control Cohort**

Age and gender of the U of M PD Cohort will be used to match healthy controls from the Dental ARIC (DARIC) cohort. DARIC is an ancillary study of the original ARIC study and was funded by the National Institute of Dental and Craniofacial Research. The DARIC study enrolled participants at visit 4 of the ARIC study from 1996-1998. Individuals in the DARIC study consented to have an oral examination, give
gingival crevicular fluid, oral plaque, and serum, and an interview as previously described. The oral assessment was performed by calibrated dental examiners and included probing pocket depth and gingival recession at 6 different sites on all teeth, and calculated attachment loss. Smoking history was also obtained from all DARIC participants. A total of 6017 individuals compose the working DARIC cohort.

This study was approved as an ancillary ARIC study, which allowed consenting individuals from the DARIC cohort to be used as matched controls for U of M PD Cohort. A +/- 5-year window will be allowed for age matching. Effort will be made to match smoking status between the PD cohort and the DARIC controls; however, the strength of the association between PD and smoking may make matching controls based on gender, age, and smoking status impractical, despite the size of the DARIC cohort. DARIC participants will be excluded if they were either hospitalized for RA during follow-up or had a positive CCP measurement at the initial blood draw.

**Clinical Measurement and Definition of PD**

At the study visit to establish a working cohort, individuals with PD were assessed for disease severity. A trained examiner inspected all sites on all teeth. Measurements included number of missing teeth, gingival index (GI), plaque index, probing depths, attachment loss measurements and bleeding upon probing. Individuals were then assessed for PD severity; according to the 2000 AAP definitions, consisting of 2 or more teeth in at least 2 quadrants with ≥ 2 mm of clinical attachment loss, ≥ 5 mm of probing pocket depth and ≥ 30% sites with bleeding on probing. DARIC Controls will be considered periodontally healthy if they had a GI score of 0 or 1 on ≥ 90% of their tooth sites and 95% of their probing depths were ≤ 3 mm with no probing depths > 4 mm.

**Blood Collection, ACPA titers, and CCP-2 analysis**

Sera and plasma collected from participants at the study visit were aliquoted and stored in a -80° freezer until analysis. Sera will be sent to the University of Washington, a CLIA-certified center, for analysis of anti-CCP antibodies as previously described. ACPA analysis – for both the PD cohort and matched DARIC controls – will be performed at Stanford University using the BioPlex multiplex assay platform and the Luminex 200 System as previously described.

**Positive ACPA Titer Cutoff Values**

An RA sera sample is usually considered to be ACPA ‘positive’ (contain ACPA that bind to a specific auto-antigen on the array), if the mean corresponding fluorescence value is three times that of the mean value for the same antigen in healthy controls, a number produced based on receiver operating characteristic (ROC) curves; however, there are no population standard cut points defining positive or high levels for specific ACPA. Cutoff values for ACPA positivity in RA-free individuals with PD have not been established in literature; therefore, this study will compare linear ACPA titer trends and ACPA titers above pre-determined cutoff values based on RA analyses in the PD cohort to the ARIC control cohort.

**Statistical Analysis**

Baseline characteristics of the patients in each cohort will be examined. Categorical variables reported as n (percentage) while continuous variables presented as mean ± standard deviation and median (interquartile range). Categorical variables will be compared between cohorts using a chi-square test or Fisher’s exact test. Continuous variables will be compared between cohorts with a t-test for means and the Kruskal-Wallis test for medians. All statistical analyses will be performed using SAS v 9.4 (SAS Inc., Cary, North Carolina) and STATA 15 (STATA Corp, College Station, Texas).

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 ICTDER03 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
8.a. Will the DNA data be used in this manuscript? ___ 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 ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ___ 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

___ 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)?


Periodontal Disease Increases Risk of Incident Rheumatoid Arthritis, and is Associated with Elevated Number of ACPA Titers: The ARIC Study: Manuscript proposal # 1859: not submitted.


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

___ A. primarily the result of an ancillary study (list number*: 1996.01 (Dental)

__X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* Study #1996.01 (Ref. 1 above) was a related published study on which Dr. Offenbacher was an author. MP 929 (Ref. 3 above) developed the ACPA data used in the D-ARIC cohort used in accepted MP #2840 (Ref. 7 above) and used in the current manuscript proposal MP #3225 which is a supplemental manuscript proposal to #2840; all authors of MP #2840 are also authors on this MP #3225.

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/ (← this link is broken)

12a. 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.
A manuscript will be prepared and submitted within 3 years after ARIC manuscript approval.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PubMed Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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