1.a. Full Title: Haplotypes based on functional Interleukin-1 gene variations define risk for chronic periodontitis severity in multiple ethnic populations

b. Abbreviated Title (Length 26 characters): Haplotypes, .3Interleukin-1 gene

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3. Timeline: Submission: May 31, 2005

4. Rationale: One of the primary goals of studies of gene variations is to identify the sequences that contribute in some manner to the observed genetic variance of specific traits. Efforts to identify genetic influence in complex diseases have been aided by recent advances in detection and analysis of genetic variations, new understandings of the structure of single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) blocks, and innovations in the selection of SNPs that assure more coverage of the
information in a physical region of the genome. However, since linkage disequilibrium may vary greatly among different ethnic populations, markers of haplotypes or LD that are informative and show association with a phenotype in one population often prove to be of limited value in other populations. We therefore sought to determine a method that allows the efficient analysis of association between multiple genetic markers and phenotypes across multiple ethnic populations. We report below that identification of IL-1 haplotypes that predominate in multiple ethnic groups can be used to define a small set of composite genotypes that were shown to differentially influence tissue levels of IL-1β protein and clinical expression of chronic periodontitis in both Japanese and Caucasian populations.

5. **Main Hypothesis/Study Questions**: To determine if IL-1 SNPs shown to be functional in haplotype context would influence risk for clinical severity of periodontitis in both Japanese and Caucasian populations.

6. **Data (variables, time window, source, inclusions/exclusions)**: The two populations that were studied were from Japan (Asian) and the United States of America (Caucasian).

**Japanese Subjects**

One hundred and ninety-six unrelated subjects (age 40-65 years) with different levels of chronic periodontitis (CP) were recruited for this study, out of a total of 431 patients who had been referred to the Periodontal Clinic of Niigata University Dental Hospital and agreed to participate in this research. All participants were of Japanese descent and non-smokers as determined by a standard questionnaire. None of them had a history or current manifestation of systemic disease. The study was approved by the Institutional Review Board of the Niigata University Faculty of Dentistry, and a written informed consent was obtained from every participant before inclusion in the study in accordance with the Helsinki declaration.

All subjects were evaluated clinically and radiographically at the first visit by several periodontists to assess the following periodontal measurements: number of teeth, probing pocket depth (PPD), clinical attachment level (CAL), supragingival plaque accumulation, bleeding on probing (BOP) and alveolar bone loss (BL) as previously described and performed by Kobayashi et al. (2000).

The subjects were classified into the following 3 groups according to the partially modified criteria of Kornman et al. (1997) and Greenstein et al. (2002).

1. **Severe CP**: subjects having more than 7 interproximal sites with \( \geq 50\% \) BL and total mean BL of \( \geq 34\% \).
2. **Moderate CP**: subjects having less than 3 interproximal sites with \( \geq 50\% \) BL and total mean BL of 16\% to 34\%.
3. **Controls**: subjects having no PPDs \( >3 \) mm and no sites with BL\( >15\% \).

**USA Subjects (ARIC STUDY POPULATION)**

The subjects from the USA were part of the Dental ARIC, an ancillary study funded by the National Institute of Dental and Craniofacial Research, was conducted at ARIC visit 4 in 1996 to 1998. The Dental ARIC consisted of an oral examination; collection of
gingival crevicular fluid (GCF), oral plaque, and serum; and interviews. Persons requiring antibiotic prophylaxis for periodontal probing were excluded. Clinical measures collected included probing pocket depth and gingival recession on 6 sites for all teeth. Attachment level (AL) was calculated from the sum of pocket depth and gingival recession scores. The dental examiners were calibrated against a standard examiner, as well as each other. Attachment level, a valid measure of historical periodontal destruction was derived from percentage of sites with AL >=3 mm from the periodontal examination. Participants were defined as never smokers, former smokers, or current smokers by interview.

Although there are differences in the way the periodontal disease was measured and classified in each study, the current study is not merging the data together for data analysis. The focus remains to look at Interleukin-1B haplotypes and "chronic periodontitis" (as defined by one measure that is common to both datasets: number of pockets probing >= 4 mm). This will allow readers to compare across studies. We will then use other endpoints to round out the analysis of each individual study.

Genotyping

The IL-1 SNPs shown in Table 1 were selected for this study based on a) extensive literature on disease association with IL-1A(+4845), or the perfectly concordant marker IL-1A(-889), IL-1B(+3954), and the IL-1 receptor antagonist markers, IL-1RN(+2018) and/or the variable number tandem repeat in IL-1RN and b) functional data (Chen et al. “Linkage Disequilibrium in the Interleukin-1 Chromosomal Region and Haplotypic Context of Gene Regulation” manuscript in preparation May 2005).

<table>
<thead>
<tr>
<th>SNP#</th>
<th>Gene</th>
<th>Relative to +1</th>
<th>rs##1</th>
<th>Location</th>
<th>Nucleotide 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL-1A</td>
<td>+4845</td>
<td>17561</td>
<td>Exon</td>
<td>G/T</td>
</tr>
<tr>
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<td>4848306</td>
<td>Promoter</td>
<td>C/T</td>
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<tr>
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<td>G/C</td>
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</tr>
<tr>
<td>7</td>
<td>IL-1RN</td>
<td>+2018</td>
<td>419598</td>
<td>Exon 2</td>
<td>C/T</td>
</tr>
</tbody>
</table>

2 reference sequence # 11143627 also refers to the same SNP
3 has also been described as sequence location -3751
4 has also been described as sequence location -1464 and -1468
5 first nucleotide listed is the most common allele in Caucasians

SNPs:

IL-1A(+4845), IL-1B(+3954), IL-1B(-511) and IL-1RN(+2018) have been previously
genotyped as part of earlier work with the group from UNC (Offenbacher and Beck). The other SNPs will be genotyped in Sheffield using DNA that remains on the samples ---i.e. no additional DNA is required. The new SNPs were selected based on results of our research on functional haplotypes that are well represented in all major ethnic populations. IL-1B(-511), IL-1B(-1468), IL-1B(-3737) meet the criteria of being functional and prevalent in all populations we have studied.

**Statistical Analysis**

To determine the independent association between IL-1 polymorphisms and the presence of generalized periodontitis, we performed multivariate adjusted logistic analyses and reported with 95% confidence intervals (CI). The odds ratio for periodontitis was calculated using major allele genotype as the reference group. We considered variables for inclusion in multivariate models if they were related to either periodontitis or the distribution of polymorphisms. We used the expectation-maximization (EM) algorithm implemented in SAS/Genetics to infer haplotypes for each individual (28-30). Then we repeated all logistic regression models assessing association of vertebral fracture for each genotype constituted by inferred haplotype separately.

Data analysis has been completed the in the Japanese population with periodontal endpoints, and the data are highly significant.

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.csec.unc.edu/ARIC/search.php](http://www.csec.unc.edu/ARIC/search.php)
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)?

There is an unpublished manuscript proposal # 698 on IL-1 genotype and periodontal disease. We are asking modify that proposal to include these data.

Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study.
Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

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* _15________)  
   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.