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Association of interleukin-1 gene variations with moderate to severe chronic periodontitis in multiple ethnicities

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Background and Objective: Genetic markers associated with disease are often non-functional and generally tag one or more functional "causative" variants in linkage disequilibrium. Markers may not show tight linkage to the causative variants across multiple ethnicities due to evolutionary divergence, and therefore may not be informative across different population groups. Validated markers of disease suggest causative variants exist in the gene and, if the causative variants can be identified, it is reasonable to hypothesize that such variants will be informative across diverse populations. The aim of this study was to test that hypothesis using functional Interleukin-1 (IL-1) gene variations across multiple ethnic populations to replace the non-functional markers originally associated with chronic adult periodontitis in Caucasians.

Material and Methods: Adult chronic periodontitis cases and controls from four ethnic groups (Caucasians, African Americans, Hispanics and Asians) were recruited in the USA, Chile and China. Genotypes of *IL1B* gene single nucleotide polymorphisms (SNPs), including three functional SNPs (rs16944, rs1143623, rs4848306) in the promoter and one intronic SNP (rs1143633), were determined using a single base extension method or TaqMan 5′ nuclease assay. Logistic regression and other statistical analyses were used to examine the association between moderate to severe periodontitis and *IL1B* gene variations, including SNPs, haplotypes and composite genotypes. Genotype patterns associated with disease in the discovery study were then evaluated in independent validation studies.

Results: Significant associations were identified in the discovery study, consisting of Caucasians and African Americans, between moderate to severe adult chronic periodontitis and functional variations in the *IL1B* gene, including a pattern of four *IL1B* SNPs (OR = 1.87, $p < 0.0001$). The association between the disease and this *IL1B* composite genotype pattern was validated in two additional studies consisting of Hispanics (OR = 1.95, $p = 0.04$) or Asians (OR = 3.27, $p = 0.01$). A meta-analysis of the three populations supported the association

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between the IL-1 genotype pattern and moderate to severe periodontitis (OR 1.95; $p < 0.001$). Our analysis also demonstrated that *IL1B* gene variations had added value to conventional risk factors in predicting chronic periodontitis.

Conclusion: This study validated the influence of IL-1 genetic factors on the severity of chronic periodontitis in four different ethnicities.

Introduction

Chronic periodontitis in adults is highly prevalent, affecting 47% in the USA with 39% having moderate to severe disease (1). Approximately 8–13% of adults across diverse populations exhibit severe generalized disease (1–3). Although bacteria are essential for the initiation and progression of chronic periodontitis, studies in twins indicate that approximately 50–60% of the variance observed in clinical disease expression in adults is genetically determined (4).

The role of specific genetic variants in chronic periodontitis in Caucasians has been evaluated and reported for approximately 37 candidate genes, of which five have been validated by meta-analyses. Six additional loci have been implicated in a genome-wide association study (5). A small number of candidate gene studies have also been reported in Asian populations (6–8) and even fewer in other ethnic groups.

Of all the gene variations studied in chronic periodontitis, two interleukin-1 IL-1 gene variations (*IL1A* [–889; rs1800587] or the concordant *IL1A* [+4845; rs17561] and *IL1B* [+3954; rs1143634]) have been most consistently associated with severe or progressive chronic periodontitis in Caucasians with significant associations reported for 19 of 27 studies and validated in two meta-analyses (9,10). The same IL-1 variations also have been associated with higher gingival crevicular fluid levels or monocyte expression of IL-1α (11) or IL-1β in some but not all studies (12–15). However, these variants are infrequent and not informative in Chinese (16) and Japanese (17) and have uncertain value in other non-Caucasian ethnicities (18,19).

One primary goal of genetic association studies is to localize a physical segment of the genome and identify functional gene variants that influence important phenotypic characteristics of the disease. Functional variants not only provide a target for disease modifying interventions but also should reduce the variation in genetic association across diverse populations. Based on evidence validating a role for IL-1 gene variants in chronic periodontitis in Caucasians, we sought and have previously reported the characterization of gene variations that are functional at the molecular level (20). These variants include three single nucleotide polymorphisms (SNPs) (rs16944, rs1143623 and rs4848306) in the *IL1B* promoter region that were shown to act in haplotype context to define allele-specific transcriptional differences and differences in gingival crevicular fluid levels of IL-1β and blood high-sensitivity C-reactive protein levels (21).

This study sought to determine if specific patterns of the functional *IL1B* SNPs were associated with periodontitis across multiple ethnic populations. Genotype patterns associated with disease were initially identified in a discovery study consisting of two ethnic groups followed by validation in two replication studies of additional ethnic populations.

Material and methods

Study populations

Discovery population— The subjects for the discovery phase of this study were selected from the Atherosclerosis Risk in Communities (ARIC) study (22). The ARIC is a prospective study designed primarily to investigate the causes of atherosclerosis and its clinical outcomes. The study enrolled 15,792 subjects by probability sampling from four communities in the USA. These included: Forsyth County, NC; Jackson, MS; Minneapolis, MN; and Washington County, MD. At the fourth visit, all eligible and consented subjects underwent dental examinations (23) and a random subset, including 900 Caucasians and 227 African Americans aged 53–74 years, were included in the current study for identifying genetic risk for periodontitis across multiple ethnic populations. Full mouth periodontal examinations were performed as described previously (24). Periodontal parameter data, including pocket depth, clinical attachment loss and bleeding on probing (BOP), and detailed medical history data were collected and reported previously (23–26).

Validation populations— The validation samples included both Hispanics and Asians and were recruited in two case–control studies. The Hispanic subjects were recruited in Santiago, Chile from patients receiving preventive primary care at a public healthcare center (27). The majority of the Chilean population is an admixture of European and Amerindian with varying proportions of the two lineages depending on socio-economic status. Full mouth periodontal examinations were performed to assess periodontal conditions that included measurements of pocket depth, clinical attachment loss and BOP. In addition, detailed medical history was obtained and blood samples were collected for laboratory analysis. Patients were excluded if they were younger than 35 years, had systemic diseases that influence progression of periodontitis (i.e. diabetes mellitus and rheumatoid arthritis), or had treatment with antibiotics or regular use of non-steroidal anti-inflammatory drugs in the previous 6 mo.

The Asian subjects were ethnic Han Chinese aged 35 years or older who

were recruited from dental clinics, schools and factories in Shanghai, China. All subjects underwent periodontal examinations and completed a questionnaire on demographic and general health information. Partial mouth examinations were performed according to the Community Periodontal Index of Treatment Needs (CPITN) procedures (28). Briefly, for each subject 10 index teeth (Tooth Numbers: 11, 16, 17, 26, 27, 31, 36, 37, 46, 47) were examined and periodontal pocket depth and/or CPITN index scores recorded. Other periodontal parameters, such as clinical attachment loss, alveolar bone loss, BOP and tooth mobility, were also measured and saliva samples were collected for laboratory analysis.

All subjects provided informed consent and the study protocols were reviewed and approved by the respective Institutional Review Boards at the participating institutions. Information on diagnosis of diabetes was obtained through patient interview or examination of medical records as previously described (22,27). Patients who smoked at time of enrollment or in the past were considered positive for smoking.

Disease definitions

In all three studies, patients with moderate to severe periodontitis were considered cases in case–control comparisons. Owing to differences in the methods used for examining patients across studies, slightly different criteria were used to define moderate to severe periodontitis in each population. In all cases, tooth loss information was incorporated in the case definition, as periodontitis is a major cause of tooth loss (29) and was expected to improve the accuracy.

For the discovery study (Caucasians and African Americans) the following criteria were used for identifying moderate to severe periodontitis: (i) loss of at least 10 teeth, i.e. ≤ 18 existing teeth; or (ii) at least two interproximal sites (not on same tooth) with ≥ 6 mm clinical attachment loss and at least one interproximal site with ≥ 5 mm pocket depth; or (iii) at least two interproximal sites with 4–5 mm clinical attachment loss and no interproximal sites with clinical attachment loss of ≥ 6 mm, and the percentage of sites with pocket depth ≥ 4 mm should be in the upper tertile of the study population. These criteria were developed based on the criteria described in an earlier report (30).

A similar case definition was used in the Hispanic validation study. The criteria used for identifying moderate to severe periodontitis were: (i) loss of at least 10 teeth, i.e., ≤ 18 existing teeth, or (ii) at least two interproximal sites (not on same tooth) with ≥ 6 mm clinical attachment loss and at least one interproximal site with ≥ 5 mm pocket depth, or (iii) at least two interproximal sites with ≥ 4 mm clinical attachment loss (not on same tooth) or at least two interproximal sites with ≥ 5 mm pocket depth (not on same tooth), and the percentage of sites with pocket depth ≥ 4 mm should be in the upper tertile of the study population. These criteria were modified from the definitions proposed by a Center for Disease Control/American Academy of Periodontology working group (31,32).

For the Asian validation study, the subjects were classified into four categories based on severity of periodontal disease using the following criteria: (i) healthy: pocket depth < 3.5 mm; (ii) mild: pocket depth 3.5–4 mm, clinical attachment loss 1–2 mm, radiographic bone loss $\leq \frac{1}{3}$ root length (optional), tooth mobility not detectable; (iii) moderate: 4 mm$<$pocket depth≤ 6 mm, clinical attachment loss 3–5 mm, radiographic bone loss between one-third and one-half root length (optional), mild mobility; and (iv) severe: pocket depth > 6 mm, clinical attachment loss > 5 mm, radiographic bone loss $\geq \frac{1}{2}$ root length (optional), tooth mobility II–III degree. The measurements used in the classification were based on the worst affected index tooth for each parameter. Patients who had moderate to severe periodontitis or lost at least 10 teeth were considered cases while those who were healthy with no periodontitis were considered controls. Patients with mild periodontitis were excluded in the analysis because of potential misclassification associated with the partial mouth examination method used in this study.

Genotyping

DNA was extracted from blood or saliva samples according to standard protocols. Sections of the *IL1B* gene that contain the SNPs of interest were amplified by polymerase chain reaction (PCR). Genotyping was performed using the TaqMan 5′ nuclease assay (Applied Biosystems, Foster City, CA, USA) or single base extension method as previously described (21,33,34). Briefly, for the Dental ARIC (DARIC) study, DNA was extracted from blood samples collected in EDTA-containing tubes, amplified by PCR and genotyped using the TaqMan nuclease assay according to the manufacturer's instructions. For the Chilean study, blood samples were collected from study participants and DNA was extracted from peripheral blood lymphocytes (27). DNA was amplified using multiplex PCR that simultaneously amplify multiple DNA fragments with the resulting PCR products used for SNP genotyping by the single base extension method with the SNPstream instrument and chemistry (Beckman Coulter, Brea, CA USA). Allele calls were determined by the SNPstream software and verified by a laboratory technologist. For the Shanghai study, saliva samples were collected from study participants. DNA was extracted and used for genotyping with the TaqMan nuclease assays and allele calls were made by the TaqMan Genotyper software and verified by a laboratory scientist.

Four SNPs in the *IL1B* gene were genotyped in all studies. These included *IL1B* –511 (rs16944), *IL1B* –1464 (rs1143623), *IL1B* –3737 (rs4848 306) and *IL1B* +3877 (rs1143633). All genotyping assays were validated before processing the study samples. During the assay validation step, negative controls (water), positive controls of known genotypes (Coriell) and a subset of study samples were genotyped in duplicate. Genotyping

performance was assessed by standard methods. The genotyping error rates were low (0.3–0.9%) and all SNPs were found to be in Hardy–Weinberg equilibrium with *p* values ranging from 0.13 to 0.91.

Statistical analysis

All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). We commenced with descriptive analyses of all variables of interest, including the demographic information, genetic markers and clinical data for study. The associations between individual SNPs, haplotypes and composite genotypes and chronic periodontal disease were determined using chi-squared tests or Fisher's exact tests. Unadjusted and multivariable logistic regressions adjusting for covariates, such as age, smoking and diabetes, were applied to estimate odds ratios (OR) and 95% confidence intervals (CI). Owing to the differences between study populations, not all analyses were adjusted for the same set of covariates. For example, as no patients with diabetes were enrolled in the Chilean study, no adjustment for diabetes was performed for analysis of the Chilean population. For the discovery study (USA) population, patients were recruited from a relatively narrow age range and age was not adjusted in some analyses. Pooled OR estimates of the three studies were calculated using DerSimonian–Laird random effects models (35), and heterogeneity was assessed with the Cochran Q test.

The incremental value of genotype beyond known risk factors is evaluated by assessing risk in non-smoking individuals without diabetes. This group, normally considered to be at low risk for periodontal disease, is stratified into genotype positive/genotype negative and significance of the OR for the former relative to the latter is calculated. The incremental value of genotype is reflected in the proportion of people whose classification changes from low to high risk and in the magnitude and significance of the elevated risk.

Results

Exploratory association of functional *IL1B* gene variations with moderate to severe chronic periodontitis in a population cohort consisting of both Caucasians and African Americans

To identify genetic variants that contribute to moderate to severe periodontitis across multiple populations, we first examined functional SNPs in the *IL1B* gene in a population cohort (DARIC) (23) that consists of two ethnic groups, Caucasians and African Americans (Table 1). The SNPs involved were previously shown to have allele-specific differences in transcription factor binding and/or rate of transcription (20) and were shown to be associated in a haplotype context with inflammatory biomarker levels (21). The functional SNPs include three SNPs in the promoter region (rs16944, rs1143623 and rs4848306) and one SNP (rs1143633) in intron 4 of the *IL1B* gene that has been associated with biological activity (36–38) but whose molecular action has not been confirmed. The three promoter SNPs produce four predominant haplotypes (B1–B4) (21), which in turn form 10 diplotypes (haplotype pairs). These diplotypes can be further stratified by the genotype of the intronic SNP (rs1143633). Combinations of diplotypes with or without stratification by the intronic SNP produce different composite genotype patterns. In some analyses, patients with diabetes were excluded due to differential rates in Caucasians and African Americans and its known influence on periodontitis.

Individual SNPs, haplotypes and diplotypes and a group of five similar composite genotype patterns (3, 15, 20, 22 and 29; Table 2) were examined in this cohort. Two SNPs (rs16944 and rs4848306), two haplotypes (B1 and B4) and five diplotypes (B1B2, B1B3, B2B4, B3B4 and B4B4) were associated with either increased or reduced risk for moderate to severe periodontitis (ORs

Table 1. Characteristics of study subjects

	DARIC study						Chilean study (Hispanic)			Shanghai study (Asian)		
	African American			Caucasian								
	Total (*n* = 222)	Case (*n* = 151)	Control (*n* = 71)	Total (*n* = 880)	Case (*n* = 435)	Control (*n* = 445)	Total (*n* = 309)	Case (*n* = 166)	Control (*n* = 143)	Total (*n* = 313)	Case (*n* = 156)	Control (*n* = 157)
	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean±SD/ frequency	Mean ± SD/ frequency	Mean±SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency	Mean ± SD/ frequency
Age	59.78 ± 4.59	60.14 ± 4.60	59.10 ± 4.56	61.68 ± 5.33	62.21 ± 5.33	61.27 ± 5.30	52.11 ± 10.72	56.45 ± 9.04	47.07 ± 10.33	52.13 ± 9.66	52.39 ± 8.66	52.01 ± 10.04
Gender (male)	0.37	0.42	0.27	0.45	0.54	0.35	0.32	0.43	0.20	0.38	0.54	0.22
Smoking	0.46	0.49	0.41	0.59	0.71	0.48	0.54	0.62	0.45	0.21	0.36	0.06
Diabetes	0.31	0.35	0.23	0.15	0.18	0.12	–	–	–	0.04	0.06	0.01

Table 2. IL1B alleles, haplotypes and composite genotype patterns

	rs16944	rs1143623	rs4848306	rs1143633
Allele	*IL1B* (–511)	*IL1B* (–1464)	*IL1B* (–3737)	*IL1B* (+3877)
1	C	G	C	G
2	T	C	T	A

	rs16944	rs1143623	rs4848306
Haplotype	*IL1B* (–511)	*IL1B* (–1464)	*IL1B* (–3737)
B1	1	1	2
B2	2	2	1
B3	1	1	1
B4	2	1	1

Genotype pattern	Composite genotypes
3	(B1B1 and *IL1B* (+3877) = 1.1)/B2B3/B2B4/B3B3/B3B4/B4B4/(B1B4 and *IL1B* (+3877) = 1.1)
15	B1B1/(B1B4 and *IL1B* (+3877) = 1.1)/B2B3/B2B4/B3B3/(B3B4 and *IL1B* (+3877) = 1.1)/B4B4
20	B1B1/B2B3/B2B4/B3B3/(B3B4 and *IL1B* (+3877) = 2.*)/B4B4/(B1B4 and *IL1B* (+3877) = 1.1)
22	B1B1/B2B3/B3B3/(B1B4 and *IL1B* (+3877) = 1.1)/(B2B4 and *I L1B* (+3877) = 1.1)/(B3B4 and *IL1B* (+3877) = 1.1)/(B4B4 and *IL1B* (+3877) = 1.1)
29	B3B3/B2B3/B2B4/B1B1/B3B4/B4B4/(B1B4 and *IL1B* (+3877) = 1.1)

Table 3. Association of *IL1B* genotype patterns with moderate to severe chronic periodontitis in the discovery study

IL1B genotype pattern[a]	Frequency in case	Frequency in control	OR (95% CI)[b]	*p* value[b]
3	0.42	0.28	1.87 (1.42–2.46)	< 0.0001
15	0.55	0.43	1.55 (1.20–2.00)	0.0009
20	0.53	0.43	1.54 (1.19–1.99)	0.001
22	0.53	0.42	1.48 (1.14–1.92)	0.004
29	0.57	0.44	1.66 (1.29–2.15)	< 0.0001

[a]Patterns indicate pairs of *IL1B* promoter haplotypes comprised of *IL1B* rs16944; rs1143623; and rs4848306. In some cases, *IL1B* rs1143633 further defines whether the *IL1B* promoter haplotype pair is included in the pattern. Further details of definitions of patterns appear in Table 2.
[b]Adjusted for smoking and diabetes.

ranging from 0.6 to 2.97, p values ranging from < 0.0001 to 0.02, adjusted for smoking and diabetes). All five composite genotype patterns were associated with moderate to severe periodontitis in this cohort consisting of two ethnicities (ORs ranging from 1.48 to 1.87, p values ranging from < 0.0001 to 0.004, adjusted for smoking and diabetes; Table 3). Pattern 3 demonstrated the strongest association (OR = 1.87 [95% CI: 1.42–2.46]; $p < 0.0001$) with the disease based on the OR and was selected for validation in additional ethnic populations.

When the Caucasians and African Americans from this cohort were examined separately, the *IL1B* composite genotype pattern 3 was associated with moderate to severe periodontitis in each of these two ethnicities (OR = 1.44 and $p = 0.04$ for non-diabetic Caucasians, OR = 4.28 and $p = 0.0004$ for non-diabetic African Americans, adjusted for smoking) in agreement with the findings for the combined population.

Validation of the association between an *IL1B* composite genotype pattern and moderate to severe chronic periodontitis in Hispanic and Asian populations

To validate the association between *IL1B* gene variation and moderate to severe chronic periodontitis in Hispanics, patients selected from Santiago, Chile were examined (27); these included 166 patients with moderate to severe periodontitis and 143 individuals with mild or no periodontitis. *IL1B* genotype pattern 3 was significantly associated with moderate to severe periodontitis in this population (OR = 1.95, $p = 0.04$, adjusted for age and smoking).

To validate the association between *IL1B* gene variation and moderate and severe chronic periodontitis in Asians, a population selected from Shanghai, China was examined. All study subjects were selected from a pre-existing patient database in Shanghai where periodontal data were collected for 10 index teeth for each patient according to WHO's CPITN method (39). In this study, patients with mild periodontitis were excluded due to potential misclassification associated with the partial mouth examination method used in the population to assess periodontal disease status. Compared to individuals without periodontitis, patients with moderate to severe periodontitis were more likely to carry *IL1B* genotype pattern 3 (17% vs. 6%). The OR for moderate to severe periodontitis for individuals positive for *IL1B* genotype pattern 3 compared to individuals who were negative for this genotype pattern (OR = 3.27, $p = 0.01$, adjusted for age, smoking and diabetes) showed a significant association.

Meta-analysis

To assess the overall effect of *IL1B* gene variation on periodontitis across multiple ethnic populations, a meta-analysis was conducted with data obtained from the three studies above which included four ethnicities. The meta-analysis showed significant association between the *IL1B* composite

Table 4. Meta-analysis of *IL1B* genotype pattern 3 with the pooled ethnic populations

	DARIC		Chilean		Shanghai		Pooled		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*p* for Heterogeneity[a]
Model 1	1.86(1.42,2.45)	< 0.001	1.97(1.12,3.45)	0.019	3.02(1.24,7.39)	0.015	1.95(1.54,2.46)	< 0.001	0.596
Model 2	1.88(1.43,2.48)	< 0.001	2.17(1.22,3.87)	0.009	3.42(1.36,8.63)	0.009	2.01(1.58,2.55)	< 0.001	0.459
Model 3	1.91(1.45,2.52)	< 0.001	1.95(1.03,3.69)	0.041	3.38(1.33,8.56)	0.011	1.99(1.56,2.55)	< 0.001	0.516
Model 4	1.87(1.42,2.46)	< 0.001	NA[b]	NA	3.29(1.29,8.36)	0.012	2.06(1.35,3.15)	0.001	0.255
Model 5	1.90(1.43,2.50)	< 0.001	NA[b]	NA	3.27(1.28,8.35)	0.014	2.05(1.41,2.99)	< 0.001	0.277

Model 1: unadjusted.
Model 2: adjusting for smoking.
Model 3: adjusting for smoking and age.
Model 4: adjusting for smoking and diabetes.
Model 5: adjusting for smoking, age and diabetes.
[a]*p* for *Q*-test for heterogeneity.
[b]Patients with diabetes were excluded in this study.

Table 5. The IL-1 genotype has added value to conventional risk factors, smoking and diabetes, in predicting moderate to severe periodontitis[a,*]

		Smoking or diabetes[b]		IL-1 genotype[c,d]	
		(All subjects, $n = 1100$)		(Non-smoking and non-diabetic: $n = 343$[e])	
		Negative	Positive	Negative	Positive
Control	Count	237	277	154	51
	%	60	39	66	46
Case[f]	Count	156	430	79	59
	%	40	61	34	54

[a]DARIC population.
[b]OR = 2.4, $p < 0.0001$.
[c]*IL1B* composite genotype pattern 3.
[d]OR = 2.3, $p = 0.0005$.
[e]Out of 393 individuals who were non-smoker and non-diabetic 343 had data for IL-1 genotype.
[f]Moderate to severe periodontitis.
*[Corrections added on 17th September 2014, after first online publication: errors in Table 5 were rectified.].

genotype pattern 3 and moderate to severe periodontitis unadjusted for risk factors (OR = 1.95 [95% CI: 1.54–2.46], $p < 0.001$; Table 4). In fact, meta-analysis was significant in all models that adjusted for risk factors. Heterogeneity was not detected between these studies and a random effects model was used to combine the data.

IL1B gene variations have added value to conventional risk factors in predicting moderate to severe periodontitis

Major conventional risk factors for periodontitis include smoking and diabetes, and strong interactions between the *IL-1* gene variations and smoking have been reported for periodontitis (40–44). To address the question whether the functional *IL1B* gene variations have added value to conventional risk factors across ethnic populations in predicting moderate to severe periodontitis, the DARIC data set (see Material and methods) was used as it had data on all three risk factors. In this data set ($n = 1100$), the frequency of moderate to severe periodontitis cases was 53%. Among the 707 individuals who either smoked or had diabetes or both, the rate of cases was 61% vs. 40% for those with neither risk factor (OR = 2.4, $p < 0.0001$). Among the remaining 393 individuals who had neither risk factor and would otherwise be classified as having low risk, 343 had data for IL-1 genotype, and 54% of the 110 patients with *IL1B* genotype pattern 3 had moderate to severe periodontitis versus 34% of those without this pattern (OR = 2.3, $p = 0.0005$). Thus, 32% of non-smoking patients without diabetes can be classified as having higher risk based on *IL1B* gene variations (Table 5) [Corrections added on 17th September 2014, after first online publication: errors were rectified in this paragraph describing Table 5. The conclusions arrived at from the data remain unchanged.].

Discussion

We report that patterns of functional SNPs in the regulatory region of the *IL1B* gene were associated with moderate to severe periodontitis in Caucasians, African Americans, Hispanics and Chinese. We believe that these data add further support for the clinical validity of IL-1 genetic variants as a predictor of more severe or progressive chronic periodontitis. In addition, the study supports the concept that functional genetic variants should be inherently more informative than non-functional genetic variants and may be a key element of a common allele effect in certain common diseases (45,46).

The clinical validity of previously reported non-functional markers in

the *IL1A* (rs17561) and *IL1B* (rs1143634) genes for severe periodontitis in Caucasians is supported by 19 studies to date and two meta-analyses (9,10). However, the minor allele frequencies of those markers vary greatly among different ethnic populations, with *IL1A* (+4845) reported at 26–33% in Caucasians and 0.5–14% in Asians and the minor allele frequencies of *IL1B* (+3954) reported at 21–29% in Caucasians and 1–7% in Asians (47). The composite genotype of the two was therefore uncommon and uninformative in Asians (16). In addition, contradicting findings have been reported regarding the association between these IL-1 markers and periodontitis (10) or between these markers and the production of IL-1 proteins (13,48); although in some studies, the small sample size might have contributed to negative or inconclusive findings. The IL-1 functional patterns used in this study were found commonly in all the populations studied, and as expected, the carriage rate differed among the ethnic groups, with for example 72% of African Americans testing positive compared to 27% of Caucasians and 11% of Asians. Of Hispanics without periodontitis 17% tested positive. The influence of other genetic factors in chronic periodontitis has not yet been validated across multiple ethnicities, but one may expect that the relative contributions of different genetic factors may differ among ethnicities. Similarly, although one may speculate that some populations will have gene–gene interactions that may mitigate the influence of a functional genetic variant we saw no evidence of anything that differentially modified the association between the disease and the IL-1 variants by ethnicity.

The association of severe periodontitis with gene variants that increase the expression of IL-1 protein levels in gingival fluid is consistent with a previously established critical role for IL-1 in the pathogenesis of chronic periodontitis. IL-1 protein level in gingival fluid is one of the strongest and most consistent predictors of periodontitis severity and progression (15,24,49–51). IL-1β is a downstream regulator of osteoclastic bone resorption and matrix metalloproteinases 8, 9 and 13, which are implicated in the tissue destruction characteristic of periodontitis in humans (50,52–56). Perhaps most importantly, in a primate model of periodontitis with an aggressive bacterial induction of disease, recombinant soluble IL-1 receptor type 1 protein that specifically blocks IL-1 biological activity reduced bone loss by 60% in spite of a continuing bacterial challenge (57). The clinical utility of IL-1 genetic variants in periodontitis has recently been demonstrated by showing that, in dental patients with no history of smoking or diabetes and not carrying IL-1 gene variations, two preventive dental cleanings annually had no benefits beyond one annually in terms of long-term tooth loss. Patients with one or more of the risk factors, of which the IL-1 genetic risk factor was the most frequent, had significant benefit from additional preventive care (58).

This study has clear strengths, including the use of multiple independently derived populations from diverse locations and backgrounds, and the use of SNPs and haplotypes with well-defined molecular function that are associated with differential biomarker expression at the clinical level. In addition, the genotyping for two of the three populations was performed in a USA Clinical Laboratory Improvement Act-certified molecular genetics laboratory, and samples from the third population (Shanghai) were genotyped in a laboratory in China that had been validated against the US laboratory providing validation of the genotyping results. Although many genetic associations with complex diseases are reported, few are shown to have clinical validity and clinical utility, which may be due in part to the challenges of identifying functional causative variants in non-coding regulatory regions of the genome (46). The genetic patterns used in this study include functional gene variations in the *IL1B* promoter region (20) that act in the haplotype context to define significant differences in gingival crevicular fluid levels of IL-1β and blood high-sensitivity C-reactive protein levels (21).

Some limitations should also be mentioned with respect to the study. First, there are limited databases available for this type of study that include adequate numbers of non-Caucasians with appropriate disease characteristics, and consent and availability of DNA. Secondly, as the databases were independently collected, the clinical parameters differed among the databases, which required that we establish a broader definition of moderate to severe generalized disease that could be used across all of the populations. Thirdly, essentially all accepted definitions of periodontitis implicitly describe currently measurable signs of previous exposure to periodontitis, but do not include missing teeth in the disease definition. Clinically, one may extract six to eight teeth in a patient with severe generalized periodontitis and convert that patient to a clinical definition of mild to moderate localized disease. As genetics represents a life-long exposure, we defined disease criteria that automatically defaulted to a severe disease classification if a certain number of teeth were missing. We believe that this is a reasonable approach to reduce misclassification, as we are not aware of other approaches to handle this issue and acknowledge that this approach is a compromise. Fourthly, not all SNPs included in the disease-associated genotype patterns have a well-established function. *IL1B* (+3877) rs1143633 is included in the patterns although its function has not yet been validated. Previous studies, and our own experience, suggest *IL1B* (+3877) itself, or a locus in strong linkage disequilibrium, is important in differentiating multiple inflammatory phenotypes in Asian populations (59–61).

Chronic periodontitis is a complex disease and several risk factors have been validated as predictive of disease severity and treatment response. Multiple studies have reported that combinations of IL-1 genotype and smoking are additive predictors of patient responses to periodontitis therapy (40,41,43,44,62,63), as well as complications following placement of

dental implants (64,65). A small number of additional genetic variants have been validated in multiple studies of chronic periodontitis (66–70), and one genome-wide association study has implicated markers such as *NIN*, *NPY*, *WNT5A*, *NCR2*, *EMR1* and 10p15 (5). The field is currently constrained by the limited availability of adequate databases to fully explore and validate gene–gene interactions in periodontitis. Those interactions may be different in different ethnic populations and should be considered.

Conclusion

In conclusion, this study used functional genetic variants in the promoter region of the *IL1B* gene to validate the influence of IL-1 genetic factors on the severity of chronic periodontitis across four different ethnicities.

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Conflict of interest

Drs. Xiaodong Wu, Leon Wilkins, Lynn Doucette-Stamm and Kenneth Kornman are employees and shareholders of Interleukin Genetics, Inc. Dr. Kenneth Kornman is also an officer of Interleukin Genetics, Inc. Drs. Hwa-Ying Wang, John Rogus and Jing Zhou are consultants of Interleukin Genetics, Inc.

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