Associations of Interleukin-1 Polymorphisms with Chronic Periodontitis in Two Different Kenyan Ethnic Groups: A Case Control Study

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Abstract

Objective: The aim of this study was to investigate the association between IL-1β and IL-1α isoforms with chronic periodontitis in two Kenyan ethnic groups, Taitas and Swahilis.

Methods: A case-control study in which participants were assessed for dental plaque, gingival inflammation, pocket depth and gingival recession after informed consent. Buccal swab samples were obtained and deoxyribonucleic acid was isolated from the swabs using QIAamp DNA purification protocol followed by polymerase chain reaction amplification using specific primers to IL-1 α rs1800587 (-889) and rs17561 (+4845) and IL-1β (rs16944 (-511) and rs11443624 (+3954). Restriction fragment length polymorphisms were recorded and association with clinical data was assessed.

Results: Three hundred and ninety participants were recruited; four loci (-511, -889, +3953 and +4845) were analyzed per subject, equivalent to 1560 analysis events. No deviation from Hardy Weinberg equilibrium 1df was observed. Frequency of allele 2 at IL-1β +3954 was associated with chronic periodontitis in Taitas (OR = 1.94, 95% CI = 1.01 - 3.70, $p = 0.045$), whereas frequency of allele 1 at IL-1α -889 was associated with chronic periodontitis in Swahilis (OR = 3.16, 95% CI = 1.644 - 6.083, $p < 0.001$). Allele 1 at locus IL-1α -889 was also associated with mild, (OR = 5.2, 95% CI = 1.445 - 18.71, $p = 0.005$), moderate (OR = 4.51, 95% CI = 2.08 - 9.79, $p < 0.001$) and severe disease (OR = 2.19, 95% CI = 1.013 - 4.738, $p = 0.042$) in Swahilis. Haplotype 3 (allele 1 at all four loci) was significantly associated with chronic periodontitis in Taitas (OR = 2.4, 95% CI = 1.1 - 5.14, $p = 0.022$) and Swahilis (OR = 4.2, 95%CI = 1.35 - 13.3, $p = 0.008$).

Conclusions: This study has shown that in the African population of Bantu origin, the two polymorphisms associated with chronic periodontitis are IL-1β +3954 in Taitas and IL-1α -889 in Swahilis. Additionally, haplotype 3 was associated with chronic periodontitis in both ethnic groups.

Key words: Interleukin-1 genetic polymorphisms, chronic periodontitis, ethnic groups

Introduction

Genetic polymorphisms together with environmental factors have been reported to influence the progression of chronic periodontitis in a complex way (Kornman *et al.,* 1997). For example, in one of the early experimental studies on beagle dogs, approximately 25% of the dogs failed to develop periodontitis in spite of a uniformly sustained bacterial challenge (Lindhe *et al.*, 1973) showing that other factors, possibly genetic, contribute to the disease progression. In humans, this finding was also observed in tea plantation workers in Sri Lanka, where despite not brushing their teeth and receiving no professional care, 11% of them did not develop periodontitis, yet they all had large amounts of plaque and dental calculus as well as plaque-associated chronic gingivitis (Löe *et al.*, 1986).

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Some authors have attributed this to host susceptibility (Michalowicz *et al.*, 2000). This implies that individuals who inherit susceptibility alleles will develop the disease when exposed to risk factors of periodontal disease. A strong association has been observed between the severity of periodontitis and a specific genotype of the interleukin-1 (IL-1) gene cluster (Engebretson *et al.*, 1999). Patients carrying this periodontitis-associated genotype (PAG) were shown to demonstrate phenotypic differences, as indicated by elevated levels of the cytokine IL-1β in gingival crevicular fluid (Engebretson *et al.*, 1999).

Studies by Michalowicz *et al.* (1991, 1994 and 2000) that have examined identical twins reared together and identical twins reared apart determined that 50% of the clinical signs of disease severity were the result of genetic factors rather than environmental factors. Furthermore, these studies on twins indicated that a considerable amount of individual variability to periodontitis might be attributed to genetic rather than environmental factors (Michalowicz *et al.,* 2000). These observations and the study on Sri Lankan tea labourers by Löe *et al.* (1986) strongly suggest that genetics may have a role in chronic periodontitis.

A 15-year longitudinal study of a young population at a tea estate in Western Java Indonesia investigated, among other risk factors, the effect of sibling relationships on the periodontal condition of a group of Indonesians deprived of professional dental care (Van der Velden *et al.,* 1993). They compared data from 23 families with three or more siblings in each family and reported that loss of attachment was similar in siblings. They concluded that the results obtained supported the hypothesis that there is a genetic basis for chronic periodontitis and that chronic periodontitis aggregates in families. A longitudinal study on this same population of 23 Indonesian families, but on the married couples, showed that after 10 years of cohabitation, the periodontal condition of a spouse did not influence the partner's condition. This finding suggests that there is a genetic basis for chronic periodontitis as there were similarities in attachment loss between siblings and none between the spouses even after living together for 10 years under the same environmental conditions (Van der Velden *et al.*, 1996).

Genetic polymorphisms in the proinflammatory cytokine interleukin-1 isoforms (IL-1α and IL-1β) have been associated with chronic periodontitis in Caucasians, Asians and Arabs, but little is known about their role in Africans. Several studies done on Caucasians have shown that there were variations in the carriage of the IL-1 alleles. IL-1α -889 varies from 43% to 90% in patients and from 35% to 79% in controls, as reported by a meta-analysis study by Laine *et al.* (2010). The prevalence of IL-1 composite genotype is low among Asian populations. The prevalence reported in Chinese subjects is 2.3%, (Armitage *et al.*, 2000), 2% in Thai subjects, (Anusaksathien *et al.,* 2003), 0.2% in Japanese persons, (Kobayashi *et al.*, 1997) and 14% in Indians (Agrawal *et al.*, 2006). Other Indian studies reported no association of IL-1β (+3954) and chronic periodontitis, (Kaarthkeyan *et al.*, 2009, Gayathri *et al.,* 2011). Thus, the use of the composite genotype IL-1α (+4845) allele 2 and IL-1β (+3954) allele 2 for determining susceptibility in Asian patients is questionable (Genco, 1996).

In the Arab population, a recent study reported 52% of the non-smoking healthy young adults with gingivitis to be positive for the IL-1 composite genotype polymorphisms (Muller and Barrieshi-Nusair, 2007). A study carried out on African Americans by Walker *et al.* (2000) reported on the prevalence of IL-1 polymorphisms in relation to aggressive periodontitis but not in association with chronic periodontitis. IL-1 polymorphisms in African populations are limited. The prevalence of IL-1α (+4845) allele 2 was 46.9% in cases and 22% in controls, and the prevalence of IL-1β (+3954) was 15.8% in cases and 14.3% in controls in a study on Xhosa in South Africa. This study showed that IL-1 composite polymorphism was not associated with severity of periodontitis in this South African population (Saleh, 2007). Therefore, the aim of this study was to investigate a possible association between genetic polymorphisms in IL-1α and IL-1β isoforms and chronic periodontitis in two Kenyan coastal communities of Bantu origin, the Taita and the Swahili.

Material and methods

Two local communities were identified for this study. The Taita are Bantus who occupy the southeastern part of Kenya, known as the Taita Hills, while the Swahili are found along the Kenyan coast and are of mixed heritage, with a Bantu and Arabic/European mixture. Though diverse in culture, both communities are found in the coastal area of Kenya. The reason for selecting these two groups was that they are both of Bantu origin, although the Swahili intermarried with outsiders from the Middle East and Europe (Njenga, 2010). The study of these two communities therefore allows for investigating two ethnic groups of similar origins, with diverse genetic and cultural make-up, yet occupying the same regional area.

This was a case-control study. The test was whether the marker genotypes distributed differently between two groups: one with chronic periodontitis and the other without disease. The study began with the selection of cases from the defined populations (Taita and Swahili separately), according to the definition provided by Hugoson and Norderyd (2008). This was followed by matching the cases by age and gender with healthy controls so as to evaluate the association between IL-1β and IL-1α isoforms with chronic periodontitis in both ethnic groups. The study population included cases who were individuals with chronic periodontitis selected on the basis of having at least two non-adjacent teeth with proximal attachment loss of $\geq$ 3 mm (Hugoson and Norderyd, 2008) and controls who were individuals with clinically healthy gingiva that did not bleed on probing and had no probing depths of $>$ 3 mm.

Persons who reported that they were smokers or had any history of systemic illness were excluded. Smokers were also confirmed through oral examination and systemic illness was confirmed through interview. Dichotomous scoring for presence or absence of plaque, bleeding on probing and calculus was recorded for each tooth.

There are three recognized haplotypes as reported by D'Aiuto *et al.* (2004) and they consist of the following markers: haplotype 1 - allele 2 at IL-1α +4845 and IL-1β +3954; haplotype 2 - allele 2 at IL-1β -511; haplotype 3 - allele1 at IL-1α and IL-1β. These three haplotypes were investigated and analyzed.

Subjects

The cases were selected from subjects who presented themselves to the various recreational and health centers and met the inclusion criteria. Some subjects were recruited by door-to-door screening using the pre-defined periodontal conditions, in the same geographical area and within walking distance of the recreational center. Because the inclusion criteria were pre-determined, this did not affect the method of data collection. Only individuals with pre-defined periodontal conditions were included as cases. The controls (individuals without any clinical inflammation) were recruited from the same area and were used to characterize the distribution of the genotype, as they had an equal chance of developing the disease. The study participants were all adults of ages 35 - 44 years residing in the Taita Hills and Mombasa old town. Taita participants were selected from a homogenous rural population with similar cultural and socio-economic status and level of education. The Swahili participants were also selected from the same geographical area and had similar cultural practices. The required minimum sample size for case control studies was calculated using a formula developed by Kirkwood and Sterne (2003). The minimum calculated sample size for this study was 88 for each group, (i.e., 88 cases and 88 controls for each ethnic group). The exposure rate reported in the study by Kornman *et al.* (1997) was used because there are no known Kenyan African studies on genetic polymorphism and chronic periodontitis.

$$n = \frac{\{\beta\sqrt{[\pi_0(1 - \pi_0) + \pi_1(1 - \pi_1)]} + \alpha\sqrt{(2\pi'(1 - \pi')}\}^2}{(\pi_0 - \pi_1)^2}$$

Where α is the standard normal deviation corresponding to the two-sided significance level at 5%, and β is the standard normal deviation corresponding to the one-sided power of the test at 80%; π_0 is the estimated exposure in controls, at 0.23. π_1 and π' were calculated from:

$$\pi' = (\pi_0 + \pi_1)/2$$

$$\text{and } \pi_1 = \frac{\pi_0 OR}{1 + \pi_0 (OR - 1)}$$

OR = 2.5, which is the minimum OR considered clinically significant when comparing cases and controls in this study (Tonetti *et al.,* 2005).

The specifications above yielded a sample size of 88. Anticipating a 10% loss due to inadequate DNA collection, the sample size (per group) was 88/0.9 = 98.

A minimum appropriate sample ensures proper utilization of resources, as sample size is often determined by logistic and financial considerations (Schafer *et al*., 2011).

The total number of participants included in this study was 398, of which 198 (99 cases and 99 controls) were Taitas and 200 (100 cases and 100 controls) were Swahilis. The severity of chronic periodontitis was classified into mild, moderate and severe forms according to the CDC/AAP definition (Page and Eke, 2007). The use of the CDC/AAP definition in this study was to avoid the under-estimation of disease by using both clinical attachment level and pocket depth.

The study was assessed and approved by the Kenyatta National Hospital /University of Nairobi Ethical Review Committee. All participants consented to participate in the study and signed informed consent forms after explanation of the study objectives and before buccal swab samples were collected from them. Emergency treatment was given where needed and referrals made. Facilitation to attend the nearest hospital was given in a few cases where it was felt that the patient required follow-up at the hospital, which was 50 km away from the study site. In Mombasa, referrals were directed to the Community Oral Health Officer accompanying the team and stationed at a nearby hospital.

Laboratory analysis

DNA extraction from patient samples

DNA was purified from buccal swabs using the QlAmp DNA Minikit spin protocol (Qiagen, Valencia, CA, USA). All centrifugation steps were carried out at room temperature (15 - 25^{0}C). Labeled buccal swabs were separated from the plastic stem and placed in 2 mL microcentrifuge tubes (Sigma-Aldrich Inc, St Louis, MO, USA). Four hundred µL phosphate buffering solution (PBS) was added to the sample, followed by addition of 20 µL of proteinase K (Qiagen) before 400 µL of lysis buffer AL (Qiagen) was added to the sample. These were mixed immediately by vortexing for 15 sec followed by incubation at 56^{0}C for 10 min in a water bath. After the incubation, a brief centrifuging was carried out to remove drops from inside the lid. Four hundred µL ethanol (96-100%) was added and mixed by vortexing and briefly centrifuged to remove drops from inside the lid. Six hundred µL of the mixture was then placed in a QlAamp Mini spin column (in a 2 mL collection tube) without wetting

the rim. The cap was closed and centrifugation performed at 6,000 x *g* for 1 min. The QlAamp Mini spin column was removed and placed in a clean 2 mL collection tube and the filtrate in the collection tube discarded. This process was repeated with the remaining 620 mL mixture. The QlAamp Mini spin column was carefully opened and 500 µL wash buffer AW1 added without wetting the rim. The cap was closed and centrifuging performed at 6,000 x *g* for one minute. The QlAamp Mini spin column was placed in a clean 2 mL collection tube and the filtrate in the collection tube discarded. The QlAamp Mini spin column was carefully opened and 500 µL wash buffer AW2 added without wetting the rim. Centrifugation was performed at 16,100 x *g* for 3 min. The QlAamp Mini spin column was then placed in a new clean collection tube and the old collection tube with filtrate discarded. Centrifugation at 16,100 x *g* for 1 min was done. The QlAamp Mini spin column was placed in a clean 1.5 mL microcentrifuge tube and 150 µL buffer AE added and centrifugation performed at 6,000 x *g* for 1 min. The final DNA concentrate volume was measured with the NanoDrop 2000c (Thermo Scientific, Fermentas Molecular Biology Tools, Waltham, MA, USA).

PCR and RFLP assays

The PCR (polymerase chain reaction) and RFLP (restriction fraction length polymorphism) reaction conditions were performed as previously described by Kornman *et al.* (1997) and Saiki *et al.* (1988) and are detailed below.

IL-1α [rs1800587] -889: Gene-specific primers included the forward (IL-1α -889_F: 5'-AAG CTT GTT CTA CCA CCT GAA CTA GGC-3') and reverse (IL-1α -889_R: 5'-TTA CAT ATG AGC CTT CCA TG-3') primers, both at 0.8 pmol/µL. Amplification was carried out in a total volume of 50 µL reaction incorporating 5 U of Taq polymerase (Invitrogen Corporation, Grand Island, NY, USA), 25 µL of Invitrogen 2X reaction mix, 15 µL nuclease-free water, 2 µL of the forward primer, 2 µL of the reverse primer, and 5 µL of DNA template (3-20 ng/µL). The cycling conditions consisted of an initial denaturation step at 96°C for 2 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and strand extension at 72°C for 1 min followed by a final extension step at 72°C for 5 minutes. The PCR amplicons were then digested at 37°C for 10 min, using 6 units per 30 µL reaction mixture made up of 16 µL of nuclease-free water, 3 µL of 10X FastDigest buffer, 10 µL DNA (~0.2 µg) and 1 µL FastDigest enzyme (Nco1). All the restriction enzymes and their buffers were obtained from Thermo Scientific, Waltham, MA, USA. After digestion, the restriction enzymes were inactivated by heating the mixture for 5 min at 65^{0}C. The restriction fragments were then separated by electrophoresis through a 30% polyacrylamide gel, stained with ethidium bromide (0.2 pg/mL) and visualized under ultraviolet (UV) light in an AlphaImager (Alpha Innotech Technologies, CA, USA). The results were documented by photography.

IL-1α [rs17561] +4845: The oligonucleotide primers IL-1α +4845 forward (5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA- 3') and IL-1α +4845 reverse (5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT- 3') were used at 0.8 pm/µL. Amplification was carried out in a total volume of 50 µL reaction mixture incorporating 1 µL Taq polymerase (5 U/µL) (Invitrogen Corporation), 25 µL Invitrogen 2X reaction mix, 15 µL water, 2 µL of the forward primer, 2 µL of the reverse primer and 5 µL of DNA template (3-20 ng/µL). Cycling conditions were as follows: initial denaturation at 95^{o}C for 1 min, followed by 35 cycles at 94^{o}C for 1min, 56^{o}C for 1 min, 72^{o}C for 2 min and a final extension step at 72°C for 5 min. Following amplification, the DNA was digested with Fnu4H1 enzyme (Thermo Scientific, Waltham, Mass, USA) by incubation of a mixture made up of 16 µL of nuclease-free water, 3 µL of 10X FastDigest buffer, 10 µL (~0.2 µg) DNA and 1 µL Fnu4H1 Fast Digest enzyme at 37^{0}C for 10 min. The enzyme was inactivated by heating for 5 min at 65^{0}C. The resultant fragments were separated by electrophoresis on a 30% polyacrylamide gel, and then stained by ethidium bromide (0.2 pg/mL). The fragments were then visualized under UV light in an AlphaImager and the results documented by photography.

IL-1β [rs16944] -511: The primers used in this assay were IL-1β -511 forward (5'-TGG CAT TGA TCT GGT TCA TC-3') and IL-1β -511 reverse (5'- GTT TAG GAA TCT TCC CAC TT-3') at 1 µM concentration. Amplification was carried out in a total volume of 50 µL reaction incorporating 1 µL Taq polymerase (5 U/µL), (Invitrogen Corporation), 25 µL Invitrogen 2X reaction mix, 15 µL water, 2 µL of the forward primer, 2 µL of the reverse primer and 5 µL of DNA template (3-20 ng/µL). The cycling conditions included an initial denaturation at 96°C for 1 min followed by primer annealing at 50°C for 1 min and a strand extension at 72°C for 1 min. This cycle was repeated twice followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min and 3 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min. Digestion of the PCR products was done using a mixture composed of 16 µL nuclease-free water, 3 µL of 10X FastDigest buffer, 10 µL (~0.2 µg) DNA with 1 µL of 3 units of Ava1 (Thermo Scientific) per 30 µL reaction at 37°C for 5 min. The enzyme was inactivated by heating at 65^{0}C for 5 min. The resultant fragments were separated by electrophoresis on a 30% polyacrylamide gel and then stained using ethidium bromide (0.2 pg/mL). The fragments were then visualized under UV light using an AlphaImager and the results documented by photography.

IL-1β [rs1143634] +3954: Here, the primers included IL-1β +3954 forward (5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3') and IL-1β +3954 reverse (5'-GCT TTT TTC GTG TGA GTC CCG-3'). The concentration of the stock solution for both primers was 2 pm/µL.

Amplification was carried out in a total volume of 50 μL reaction mixture incorporating 1 μL PCR Taq polymerase, (Invitrogen), 25 μL Invitrogen 2X reaction mix, 15 μL water, 2 μL of the forward primer, 2 μL of the reverse primer and 5 μL of DNA template (3-20 ng/μL). Thermocycling was performed thus: 95°C for 1 min followed by 94°C for 1 min, 72°C for 1 min cycled twice. These were followed by 35 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 1 min, 3 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 5 min. The PCR fragments were digested at 37°C for 5min and the reaction mixture was composed of 16 μL nuclease-free water, 3 μL of 10X FastDigest buffer, 10 μL (~0.2 μg) DNA and 1 μL of 10 units per 30 μL reaction mixture of Taq 1 (Thermo Scientific). The resultant fragments were separated by electrophoresis in a 30% polyacrylamide gel and then stained using ethidium bromide (0.2 pg/mL). The products were then visualized under UV light using an AlphaImager and the results documented by photography.

Statistical analyses

Data from gel photographs were scored and tabulated prior to analyses. Analyses were performed using the Statistical Package for Social Sciences version 17 (SPSS 17 Inc., Chicago, Illinois, USA). Descriptive and bivariate analyses were carried out. Associations between exposure variables and chronic periodontitis were performed using the Pearson chi-squared test and risk assessed by odds ratio (OR) with a 95% confidence interval. The Hardy Weinberg equilibrium for the four loci (-511, +3954, -889, +4845) in Taita and Swahili participants was tested for genotype frequency by the chi-squared test, with 1 degree of freedom. The three recognized haplotypes (D'Aiuto *et al*., 2004) were also analyzed for their relationship with chronic periodontitis.

Results

No deviation from the Hardy Weinberg equilibrium was observed in either of the two ethnic groups under study.

The age range for all participants was 35 - 44 years. The Taitas had a mean age of 37.88 (SD 3.29). There were 64 (32%) males and 134 (67.7%) females: OR = 0.97, 95% CI = 0.523-1.799. Two hundred Swahili participants were examined: 108 were males and 92 were females. Of these, 54 were male cases with 54 age- and sex-matched controls, and 46 were female cases with 46 age- and sex-matched controls. There was no difference in the distribution of male and female participants: OR = 1.00 (95% CI = 0.57-1.74), $p = 1.000$.

In the Taitas, there was more plaque on the tooth surfaces of those with chronic periodontitis (cases) than in the control participants. The difference demonstrated between cases and controls was OR = 21, (95% CI = 7.8-56.4), $p < 0.001$. In the Swahilis, the mean number of teeth with plaque was 26 (SD 4.3) tooth surfaces per individual in cases and 18 (SD 5.6) tooth surfaces per individual in controls with OR = 9.2 (95% CI = 3.7-23.1), $p < 0.001$ when cases were compared to controls.

The mean number of gingival sites per individual found to bleed on probing in the Taitas was 17 (SD 10.2). Only 6 (0.2%) gingival sites were found to bleed on probing in the controls. In the Swahilis, the mean number of teeth that had bleeding on probing of the gingival tissues was 25 (SD 5.8). There was very minimal bleeding recorded in the control participants and only in 29 (1.0%) sites.

In the Taitas, there was more calculus on the tooth surfaces amongst those with chronic periodontitis (OR = 33.9, 95% CI = 13.3-86.3, $p < 0.001$). In the Swahilis, when cases were compared to controls, there was greater calculus accumulation in those with chronic periodontitis (OR = 114.6, 95% CI = 33.1-397.2, $p < 0.001$).

The frequency of allele 1 at IL-1α -889 (i.e., a combination of '1 - 1' or '1 - 2' at locus -889) amongst the Swahili participants was associated with chronic periodontitis (OR = 3.16, 95% CI = 1.644 - 6.083, $p < 0.001$; *Table 1*). Amongst the Swahili participants, allele 1 at locus IL-1α -889 was associated with mild, (OR = 5.2, 95% CI = 1.445 - 18.71, $p = 0.005$), moderate (OR = 4.51, 95% CI = 2.08 - 9.79, $p < 0.001$) and severe disease (OR = 2.19, 95% CI = 1.013 - 4.738, $p = 0.042$; *Table 2*). This was not the case in Taita participants. The frequency of allele 2 at IL-1β +3954 (i.e., a combination of '2 - 2' or'1 - 2' at locus +3954) was associated with chronic periodontitis in the Taita participants (OR = 1.94, 95% CI = 1.01 - 3.70, $p = 0.045$; *Table 3*). There were no associations of any the alleles with the severity of chronic periodontitis in Taita participants. The severity of chronic periodontitis was categorized into three groups according to the CDC/AAP consensus on case definitions of periodontal diseases (Page and Eke, 2007).

In the Swahilis, the frequency distribution of allele 2 at -511 was higher than allele 1. The frequencies of allele 2 were, 117 (60.9%) and 112 (58.2%) in cases and controls, respectively, and 75 (39.1%) and 78 (41%) in cases and controls, respectively, for allele 1, $p = 0.983$. For IL-1β +3954, the total frequency of allele 1 was not significantly higher in cases (115, 65.5%) than in controls (99, 58.2%), with a p value of 0.174. The frequency of allele 2 polymorphism at -889 in cases was 148 (80.4%) and in controls was 182 (92.9%), whereas the frequency of allele 1 was 36 (19.6%) and 14 (7.1%) in cases and controls, respectively ($p < 0.001$, OR = 3.16, 95% CI = 1.644 - 6.083). The frequency for allele 2 at loci +4845 was 107 (60.8%) and 112 (62.2%) for cases and controls, respectively ($p = 0.782$).

When the significant variables at the bivariate analysis were explored for effect modification or confounding of the relationship between genotype and chronic periodontitis, none of the variables were found to be significant in the Taitas. Amongst the Swahili participants, there was effect modification by plaque levels.

The odds of having chronic periodontitis were less in subjects without allele 2 at position -511 compared to subjects with the genotype when the plaque levels were low (≤ 15 tooth surfaces), as reported previously by Wagaiyu *et al.* (2014).

In the Taitas, allele 1 polymorphism at position -511 was carried by 57 (38.5%) cases and 49 (38.3%) controls (p = 0.968). Of these, only 5 (6.8%) cases and 6 (9.4%) controls were homozygous (p = 0.784). On the other hand, allele 2 at position -511 was carried by 91 (61.5%) and 79 (61.7%) cases and controls, respectively, with homozygous distribution of allele 2 at 22 (29.7%) and 21 (32.8%) in cases and controls (p =0.801). The frequency for allele 1 polymorphism at position +3954 was 89 (70.6%) in cases and 79 (82.3%) in controls (OR = 0.52 95% CI = 0.27 - 0.99, p = 0.045). Allele 2 was carried by 37 (29.4%) and 17 (17.7%) in cases and controls, respectively (OR = 1.936 95% CI = 1.009 - 3.698, p = 0.44). The frequencies of allele 1 and allele 2 at position -889 were 106 (60.9%) and 92 (56.1%) for allele 1 and 68 (39.1%) and 72 (43.9%) for allele 2 in cases and controls (p = 0.368). The frequency for allele 1 polymorphism at position +4845 was 60 (40.8%) and 66 (47.8%) in cases and controls, respectively, whereas the carriers of allele 2 polymorphism at +4845 in cases and controls were 88 (59.5%) and 72 (52.2%), respectively (p = 0.215).

Multivariate analysis between plaque levels and the significant genotypes in the Taitas showed that plaque level continued to be significantly associated with chronic periodontitis (OR = 18.97, 95% CI = 7.01 - 51.31, p < 0.001). However, genotype +4845 (1 - 1) and +3954 (1 - 2) were no longer associated with chronic periodontitis in this multivariate model, as shown in *Table 4*.

Multivariate analysis in the Swahilis to test the plaque levels and genotype data in the development of chronic periodontitis showed that only plaque was significantly associated with chronic periodontitis (OR = 7.99, 95% CI = 3.16 - 20.24, p < 0.001). The initially significant genotype IL-1α -889 was no longer significantly associated with chronic periodontitis (*Table 5*).

There are three recognized haplotypes, as reported by D'Aiuto *et al.* (2004). The first haplotype was not associated with chronic periodontitis in the two ethnic groups. The second haplotype (allele 2 at IL-1β -511) was found to be associated with chronic periodontitis after stratifying for plaque levels amongst the Swahili participants only, as reported by Wagaiyu *et al.* (2014).

The third haplotype, requiring allele 1 in all four genotypes, was significantly associated with chronic periodontitis in the Taita participants (OR = 2.4, 95% CI = 1.12-5.14, p = 0.022) and the Swahili participants (OR = 4.2, 95% CI = 1.35-13.3, p = 0.008).

Table 1. Odds ratios of IL-1β and IL-1α genotype and allele frequencies amongst the Swahili participants.

		Cases (n = 94)		Controls (n = 96)					
							OR	95% CI for OR	
Genotype		n	%	n	%	*p*		Lower	Upper
IL-1β -511	1 - 1	4	4.2	6	6.3	0.516			
	1 - 2	67	69.8	66	69.5	0.881			
	2 - 2	25	26	23	24.2	0.741			
Allele	1	75	39.1	78	41.1	0.691	0.92	0.611	1.386
	2	117	60.9	112	58.9				
IL-1β +3954	1 - 1	43	48.9	35	41.2	0.246			
	1 - 2	29	33	29	34.1	1.000			
	2 - 2	16	18.2	21	24.7	0.363			
Allele	1	115	65.3	99	58.2	0.174	1.35	0.875	2.089
	2	61	34.7	71	41.8				
IL-1α -889	1 - 1	15	16.3	5	5.1	0.018*			
	1 - 2	6	6.5	4	4.1	0.516			
	2 - 2	71	77.2	89	90.8	0.001*			
Allele	1	36	19.6	14	7.1	< 0.001*	3.16	1.644	6.083
	2	148	80.4	182	92.9				
IL-1α +4845	1 - 1	5	5.7	6	6.7	0.756			
	1 - 2	59	67	56	62.2	0.668			
	2 - 2	24	27.3	28	31.1	0.519			
Allele	1	69	39.2	68	37.8	0.782	1.062	0.693	1.628
	2	107	60.8	112	62.2				

*p < 0.05; OR, odds ratio; CI, confidence interval

Table 2. Odds ratios of IL-1α and IL-1β genotype and allele frequencies in a) mild, b) moderate and c) severe chronic periodontitis amongst Swahili participants.

a) Mild chronic periodontitis

		Cases		Controls				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	***p***	**OR**	**Lower**	**Upper**
IL-1α -889 Allele	1	4	28.57	14	7.1	0.005*	5.2	1.445	18.71
	2	10	71.43	182	92.9				
IL-1α +4845 Allele	1	6	42.86	68	37.8	0.706	1.24	0.411	3.713
	2	8	57.14	112	62.2				
IL-1β -511 Allele	1	7	43.75	78	41.1	0.83	1.11	0.399	3.125
	2	9	56.25	112	58.9				
IL-1β +3954 Allele	1	9	56.25	99	58.2	0.88	0.92	0.328	2.592
	2	7	43.75	71	41.8				

b) Moderate chronic periodontitis

		Cases		Controls				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	***p***	**OR**	**Lower**	**Upper**
IL-1α -889 Allele	1	17	25.76	14	7.1	< 0.001*	4.51	2.08	9.79
	2	49	74.24	182	92.9				
IL-1α +4845 Allele	1	24	41.38	68	37.8	0.624	1.16	0.64	2.13
	2	34	58.62	112	62.2				
IL-1β -511 Allele	1	26	39.39	78	41.1	0.813	0.93	0.527	1.654
	2	40	60.61	112	58.9				
IL-1β +3954 Allele	1	40	68.97	99	58.2	0.148	1.6	0.845	3.01
	2	18	31.03	71	41.8				

c) Severe chronic periodontitis

		Cases		Controls				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	***p***	**OR**	**Lower**	**Upper**
IL-1α -889 Allele	1	15	14.42	14	7.1	0.042*	2.19	1.013	4.738
	2	89	85.58	182	92.9				
IL-1α +4845 Allele	1	39	37.50	68	37.8	0.962	0.988	0.6	1.627
	2	65	62.50	112	62.2				
IL-1β -511 Allele	1	42	38.18	78	41.1	0.625	0.887	0.548	1.435
	2	68	61.82	112	58.9				
IL-1β +3954 Allele	1	66	64.71	99	58.2	0.292	1.32	0.79	2.18
	2	36	35.29	71	41.8				

*$p < 0.05$; OR, odds ratio; CI, confidence interval

Table 3. Odds ratios of IL-1β and IL-1α genotype and allele frequencies amongst the Taita participants.

		Cases (n = 94)		Controls (n = 96)				95% CI for OR	
Genotype		**n**	**%**	**n**	**%**	***p***	**OR**	**Lower**	**Upper**
IL-1β -511	1 - 1	5	6.8	6	9.4	0.784			
	1 - 2	47	63.5	37	57.8	0.112			
	2 - 2	22	29.7	21	32.8	0.801			
Allele	1	57	38.5	49	38.3	0.968	1.01	0.62	1.64
	2	91	61.5	79	61.7				
IL-1β +3954	1 - 1	34	54	34	70.8	0.914			
	1 - 2	21	33.3	11	22.9	0.045*			
	2 - 2	8	12.7	3	6.3	0.112			
Allele	1	89	70.6	79	82.3				
	2	37	29.4	17	17.7	0.045*	1.94	1.01	3.70
IL-1α -889	1 - 1	36	41.4	28	34.1	0.183			
	1 - 2	34	39.1	36	43.9	0.849			
	2 - 2	17	19.5	18	22	0.906			
Allele	1	106	60.9	92	56.1	0.368	1.22	0.79	1.88
	2	68	39.1	72	43.9				
IL-1α +4845	1 - 1	1	1.4	8	11.6	0.018*			
	1 - 2	58	78.4	50	72.5	0.181			
	2 - 2	15	20.3	11	15.9	0.367			
Allele	1	60	40.5	66	47.8	0.215	0.74	0.46	1.19
	2	88	59.5	72	52.2				

*$p < 0.05$; OR, odds ratio; CI, confidence interval

Table 4. Multivariate analysis of Taita participants' plaque levels and significant genotype data.

						95% CI for OR	
		B	**SE**	***p***	**OR**	**Lower**	**Upper**
Plaque	≤ 15	Reference			1		
	> 15	2.943	0.508	< 0.001	18.971	7.014	51.309
Genotype +4845 1-1	Absent	Reference			1		
	Present	-1.108	1.209	0.359	0.330	0.031	3.530
Genotype +3954 1-2	Absent	Reference			1		
	Present	0.630	0.476	0.186	1.878	0.738	4.777

B, gradients in multivariate equation; SE, standard error; OR, odds ratio; CI, confidence interval

Table 5. Multivariate analysis of Swahili participants' plaque levels and significant genotype data.

						95% CI for OR	
		B	**SE**	***p***	**OR**	**Lower**	**Upper**
Plaque	≤ 15	Reference			1		
	> 15	2.079	0.474	< 0.001	7.993	3.156	20.243
Genotype -889 1-1	Absent	Reference			1		
	Present	0.266	0.731	0.716	1.304	0.312	5.462
Genotype -889 2-2	Absent	Reference			1		
	Present	-0.760	0.531	0.153	0.468	0.165	1.325

B, gradients in multivariate equation; SE, standard error; OR, odds ratio; CI, confidence interval

Discussion

In this study, proximal sites on non-adjacent teeth were selected in order to minimize the likelihood of including lesions that were a result of abrasion on the buccal surfaces or habits in the interdental areas, rather than due to chronic periodontal disease. The 3 mm threshold was based upon studies that examined incremental attachment loss measurement, where the error of the recording method was calculated at 2.5 mm (Tonetti and Claffey, 2005). Recording >3 mm attachment loss ensures that all cases actually have chronic periodontitis of a moderate or severe form, as recommended by Schafer *et al.* (2011).

In the present study, the association between interleukin-1 and chronic periodontitis amongst Taita participants was seen in heterozygous (C/T) IL-1β at position [rs1143634] +3954, $p = 0.045$ and allele 2 (T) at position +3954. Heterozygotes interleukin-1β +3954 C/T carriers were found to be at an increased risk of having chronic periodontitis amongst the Taita participants ($p = 0.045$). This is similar to a case control study done on Chilean subjects where heterozygotes of the IL-1β +3954 C/T were significantly higher in cases than in controls and were associated with periodontitis ($p = 0.001$; Lopez *et al.,* 2005). Other studies on Caucasians also found a significant association between IL-1β +3954 C/T and periodontitis, as reviewed by Laine *et al.* (2010).

The association of heterozygotes for IL-1β +3954 (C/T) with chronic periodontitis in the Taita participants contrasts with a study by Parkhill *et al.* (2000), who reported heterozygotes at this locus as being the most frequent genotype in controls. The difference is attributed to the populations under study. The current study was on an African population of Bantu origin, whereas Parkhill *et al.* (2000) studied a Caucasian group. The literature shows that there are differences between racial groups (Zhang *et al.,* 2011; Laine *et al.,* 2010). A study by Lopez *et al.* (2005) found heterozygous IL-1β +3954 to be strongly associated with periodontitis. This finding is similar to the outcome in the current study. Lopez *et al.* (2005) also reported homozygous allele 1 of IL-1β to be protective, which was also in agreement with the findings of this study, where allele 1 of IL-1β +3954 was more prevalent amongst the healthy controls, signifying a protective effect. A study performed in India also reported that the homozygous genotype allele 1 (C/C) of IL-1β +3954 was the most frequent genotype in control subjects, whereas the heterozygous (C/T) genotype dominated in case subjects (Gayathri *et al.,* 2011).

Interleukin-1α, with the SNP at position [rs1800587] -889 C/T is in the transcriptional regulatory region and [rs17561] +4845 G/T is in the coding region. IL-1α -889 T (allele 2), which is in linkage disequilibrium with +4845, alters the transcriptional ability of IL-1α and thus results in an increased production of cytokine IL-1α (Hulkkonen *et al.,* 2000). Amongst the Taita participants, allele 2 at [rs17561] +4845, which is associated with increased production of cytokine IL-1α, was evenly distributed in cases and controls. It was therefore not associated with disease.

The only polymorphism that was found to be significantly associated with chronic periodontitis in the Swahilis was IL-1α -889. Homozygous allele 1 (C/C) ($p = 0.018$) and carriage of total allele 1 (OR = 3.16, 95% CI = 1.644-6.083, $p < 0.001$) were both associated with chronic periodontitis amongst Swahili participants. Interleukin-1α -889 has been associated with chronic periodontitis in some populations and not others (Kornman *et al.*, 1997; Engebretson *et al.,* 1999; Van der Velden *et al.*, 1996; Papapanou *et al.,* 2001; Tai *et al.*, 2002). Hence, amongst the Swahili participants, the association between [rs1800587] IL-1α -889 and chronic periodontitis was similar to other studies on Caucasians. The distribution of allele 2 in Swahili participants was 80.4% in cases and 92.9% in controls (OR = 0.32, 95% CI = 0.16 – 1.61, $p < 0.0010$. This is in contrast to other studies where allele 2 IL-1α -889 was associated with severe periodontitis. It has been shown by Hulkkonen *et al.* (2000) that homozygous allele 2 IL-1α was associated with high IL-1α transcription, and these high levels then induced IL-1β production only in the presence of IL-1β -511. In the Swahili participants, allele 2 of IL-1β -511 was associated with chronic periodontitis but this effect was modified by the presence of plaque. In the presence of low plaque levels, the association between [rs16944] IL-1β -511 was observed, but this association was lost when the plaque levels were high (> 15 teeth with plaque). Thus the effect of allele 2 of IL-1β -511 is expressed when there is less plaque. When there is more plaque, the genotype effect is masked by the strong association between plaque and chronic periodontitis. The explanation could be that when plaque levels are low, the gene expression is observed in that there is more disease (chronic periodontitis) in those with the allele, but when there is more plaque, the effect of plaque on disease overrides the genetic effect.

Although there were observed differences between alleles 1 and 2 of IL-1β +3954, amongst the Swahili participants, IL-1β +3954 was not associated with chronic periodontitis. It also differed in the results from the Taita participants. The frequency rate for the rare allele 2 IL-1β +3954 amongst the Swahili participants was exactly as that reported for a Caucasian population by Thomson *et al.* (2001). Drozdzik *et al.* (2006) reported a frequency rate of 34% in patients and 40% in controls in a Caucasian group. Swahili participants, being of mixed heritage, had a similar carriage rate as the Caucasian population.

In the Arab population, reports show homozygous allele 1 (C/C) at IL-1α -889 to be 37.5% in cases and 57.1% in controls in Syrians (Shibani *et al.*, 2011). Yemeni Arabs, on the other hand, have been reported to have a frequency of 20% and 32.5% in cases and controls, respectively (Al-hebshi *et al.*, 2012). These results show that there was a higher frequency in controls. In this study, the frequency of homozygous allele 1 in the Taitas was higher in those with chronic periodontitis. However, amongst the Swahili participants, homozygous allele 1 of IL-1α -889 was found to be more frequent in those with chronic periodontitis. Although the frequency was low (16.3% in cases and 5.1% in controls), this allele was associated with chronic periodontitis. In the Swahili participants the association of allele 1 at IL-1α -889 with chronic periodontitis is unique. Amongst the Swahilis, there was a 3-fold chance of developing chronic periodontitis in the presence of allele 1 at IL-1α -889. It is also unique when compared with the Taita participants. This uniqueness may be attributed to the Swahili participants being of mixed heritage. They are Bantu in origin but have over the years intermarried with Arabs and Europeans, especially of Persian heritage (Hubbard *et al.*, 2012). Thus, this may affect their genetic makeup. This finding requires further investigation, with a mapping of their genome to find out how their genes have changed, if at all, over the years.

The two ethnic groups are different despite having a common Bantu origin. Intermarrying appeared to bring about differences. In spite of the Taita participants having a higher prevalence of the genotype IL-1α -889, it was not associated with chronic periodontitis. IL-1α -889 was instead associated with chronic periodontitis in the Swahili participants. IL-1β +3954 was independently associated with chronic periodontitis in the Taita participants and not the Swahilis.

Haplotypes are a set of closely linked alleles at adjacent loci on the chromosome that are inherited together (D'Aiuto *et al.*, 2004). In this study, only the third haplotype (allele 1 at the IL-1α and IL-1β markers) was significantly associated with chronic periodontitis in both the Swahili and Taita participants. This means that having allele 1 simultaneously at -511, +3954, -889 and +4845 is likely to make these individuals more susceptible to chronic periodontitis.

The significant association of allele 2 at IL-1β +3954 with chronic periodontitis in the Taita participants confirmed the importance of this genotype in disease pathogenesis, as monocytes and polymorphonuclear leukocytes in those homozygous for this allele have been shown to produce 4-fold more IL-1β (Engrebretson *et al.*, 1999), a proinflammatory cytokine that promotes chronic periodontitis. A weakness of the design of our study is that we did not assay for the levels of IL-1β protein in the Taita participants. Instead, our study applied a qualitative molecular analytical approach (RFLP) instead of quantitative measurement of the cytokine levels. It would have been more informative to carry out quantification of protein levels of the cytokine. However, the lack of this quantification of IL-1β levels does not alter our conclusions about the importance of allele 2 at IL-1β +3954 with chronic periodontitis in the Taita participants.

Increased susceptibility to chronic periodontitis in the Swahili participants with allele 1 at IL-1α -889 was also not surprising. This genotype is associated with significantly lower transcriptional activity of the IL-1α gene and lower levels of IL-1α. Variations in IL-1α may affect production of IL-1β. This may lead to increased destruction of the periodontium (Engrebretson *et al.*, 1999) in carriers of this genotype. The association of haplotype 3 with chronic periodontitis in the two African tribes suggests that this haplotype is an important risk factor for chronic periodontitis in Africans of Bantu origin.

Clinical implications

Genetic testing for chronic periodontitis is available for the composite genotype of allele 2 of IL-1α at -889 and allele 2 of IL-1β at +3954. This test would not be applicable in those of Bantu origin because the findings do not support the association between the composite genotype and chronic periodontitis. This study has shown that in the African population of Bantu origin, the two polymorphisms that produced significant single risk factors in each ethnic group in this study were IL-1β +3954 amongst the Taita and IL-1α-889 amongst the Swahili. Additionally, haplotype 3, with the wild type allele at loci -511, +3954, -889 and +4845, was associated with chronic periodontitis in both ethnic groups. Hence the development of genetic testing for susceptible individuals would need to include the testing of the various loci singly as well as testing for haplotype 3 in the African population. However, the cost implication may not allow the testing of a majority of individuals in this country because dental treatment is largely financed by out-of-pocket expenses. Finally, plaque control, as a well tested mode of prevention of chronic periodontitis, has been proven in this study.

Conclusion

The two ethnic groups are genotypically different in the IL-1 gene cluster despite having a common Bantu origin. Intermarrying appears to have engendered differences in the frequency rates of the genes and associations of the genes with chronic periodontitis. The IL-1α -889 locus was associated with chronic periodontitis in the Swahili participants. IL-1β +3954 was independently associated with chronic periodontitis in the Taita participants. To overcome some of the limitations in this study, there is a need to carry out a similar study in different Bantu groups, in Cushites and in Nilotes resident in Kenya to assess whether the same gene polymorphisms are associated with chronic periodontitis and if haplotype 3 will remain significant in other African groups. A similar study with a larger sample size is needed to assess whether the genetic polymorphisms associated with chronic periodontitis remains significant.

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