ORIGINAL ARTICLE

A combination of alleles 2 of interleukin (IL)-1A^{-889} and IL-1B^{+3954} is associated with lower gingival bleeding tendency in plaque-induced gingivitis in young adults of Arabic heritage

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Abstract The aim of this study was to investigate the possible association of a distinct combination of polymorphisms in the interleukin (IL)-1 gene cluster on gingival bleeding tendency in young adult Arabs with plaque-induced gingivitis. Fifty otherwise healthy, nonsmoking volunteers, 19–28 years of age, participated. Clinical examinations included periodontal probing depth, bleeding on probing, and plaque index. Probing was done with a pressure-controlled probe at about 1.27 MPa. Examinations were repeated after 2 and 4 weeks. Polymorphisms in the IL-1 gene cluster were assessed using a reverse hybridization assay. A subject carrying alleles 2 at IL-1A^{-889} and IL-1B^{+3954} was designated genotype-positive. Twenty-six subjects were genotype-positive (52%). A repeated measures two-level (occasion, subject) model of the proportion of sites bleeding on probing, which was adjusted for gender, average plaque index, probing depth, and calculus, revealed a significantly lower proportion of bleeding sites in genotype-positive subjects (estimate −0.050, standard error 0.025, $p < 0.05$). Biserial correlations of bleeding proportions were high (0.71–0.78), confirming the steady-state plaque environment. It was concluded that inflammatory responses to dental plaque were considerably dampened in genotype-positive, nonsmoking young adults of Arabic heritage.

Keywords Interleukin-1 genotype · Gingivitis · Bleeding on probing · Steady-state · Dental plaque

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Introduction

Possible factors that may modulate the inflammatory response in gingiva to bacterial plaque residing on the adjacent tooth surface have recently been reviewed in some detail [33]. Considerable differences in clinical expression of gingivitis [28] may be explained by both intrinsic and extrinsic determinants. Among the former, genetic polymorphisms have attracted considerable attention in the study of inflammatory responses [16]. A distinct combination of alleles 2 of interleukin (IL)-1A at locus −889 and IL-1B at locus +3954 seems to be associated with more severe periodontitis in certain ethnicities [1, 17]. This composite genotype was associated with higher proportions of gingival units bleeding upon probing in nonsmoking treated periodontitis patients during supportive care [20]. In recent gingivitis experiments, however, these polymorphisms could not be related to an altered development of gingival inflammation [14, 30]. The aim of the present study was to assess the possible association of this particular genotype on plaque-associated gingivitis, a precursor of any destructive periodontitis, in a steady-state plaque environment. Because smoking has been shown to dampen the inflammatory response to plaque [31], the study was conducted in nonsmoking young adults.

Material and methods

Volunteers

To be enrolled, subjects had to meet the following inclusion criteria: (1) Arabian descent, (2) minimum age of 18 and maximum age of 28 years, (3) mild or moderate plaque-induced gingival disease as defined by slight edema and

redness in certain areas, and (4) minimal amounts of supragingival calculus. The following exclusion criteria applied: (1) any indication for antibiotic prophylaxis, (2) pregnancy or lactation, (3) any long-term medication with a possible effect on gingival inflammation, (4) any non-plaque-induced gingival disease, (5) destructive periodontal disease with a possible exception of localized gingival recession, (6) extensive tooth restoration or tooth replacement, (7) any presently undertaken orthodontic treatment, and (8) smoking. Fifty volunteers were eligible and invited to participate. The 34 female and 16 male volunteers had a mean (± standard deviation) age of 22.4±1.6 years and were systemically healthy. They had a minimum of 23 erupted teeth (mean 29.2±2.5), and, with a few exceptions (first molars due to caries), teeth had been extracted for surgical (third molars) or orthodontic reasons (premolars). After written and oral briefing on the study's objectives, procedures, risks, and benefits, volunteers gave their written consent for participation. The study design had been reviewed and approved by Kuwait University Faculty of Dentistry's Ethical Committee as conforming to Ethical Principles for Medical Research Involving Human Subjects according to the World Medical Association Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm as accessed October 12, 2006). Because time since last oral prophylaxis varied among volunteers, 1 week prior to the commencement of the clinical trial, any supragingival was removed and tooth surfaces were polished.

Clinical examination

One week after supragingival scaling and polishing, periodontal conditions were recorded at six sites of every tooth present. Periodontal probing depth and clinical attachment level were measured with a pressure-calibrated probe (ClickProbe 1395, KerrHawe, Bioggio, Switzerland) at about 1.27 MPa to the nearest millimeter. Gingival bleeding on probing was assessed after about 20 s. Reliability of gingival bleeding after different time intervals had been assessed in a separate population [27]. Presence of calculus was recorded. Supragingival plaque was disclosed with a plaque revelator (D&C Red-28, Sultan Chemists, Englewood, NJ, USA) and its amount estimated using criteria of the plaque index (scores 0–3) system [32].

Whereas toothpaste, tooth brushing frequency, and use of dental floss were recorded in a questionnaire, no attempt was made to improve the oral hygiene of the volunteers. Instead, they were advised not to alter their oral hygiene habits. Periodontal examinations were repeated after 2 and 4 weeks.

Interleukin-1 genotype

After collecting all clinical data, a swab sample of cheek mucosa was obtained from each volunteer. Samples were immediately sent to a commercial laboratory (Hain Lifescience, Nehren, Germany) by courier mail. The samples were processed using a commercial assay (GenoType®, PST®, Hain Lifescience). DNA was extracted and sequences of the IL-1 gene cluster were amplified by polymerase chain reaction using HotStar Taq polymerase (Qiagen, Hilden, Germany) and a final $MgCl_2$ concentration of 2.5 mM. Subsequently, polymorphisms at locus −889 of the *IL-1A* gene and at locus +3954 of *IL-1B* gene were investigated by reverse hybridization of the generated amplicons to specific probes immobilized on membrane strips [3]. To be positive for the composite IL-1 genotype, a subject required at least 1 copy of allele 2 at both loci [17].

Statistical analysis

Primary outcome variables of mean periodontal probing depth, clinical attachment level, plaque index, and proportion of sites bleeding on probing and covered by calculus are given as mean ± standard deviation. Conventional repeated measures analyses of variance were applied to test the (null) hypotheses of stable periodontal conditions (percent of sites bleeding on probing, average plaque index, average periodontal probing parameters, percent of sites covered by calculus) during the 4-week experiment and no influence of the IL-1 genotype on outcomes. A repeated-measures, two-level (occasion, subject) model was then applied to assess the effect of the IL-1 genotype on the percentage of sites bleeding on probing in a steady state plaque environment [12] with covariates gender and, at each examination, average periodontal probing depth, plaque index, and calculus proportion. Because of sparseness of observations, loss of attachment was not considered. Covariates were entered after centering [18]. There is no level 1 (occasion) variation, and the proportion of sites bleeding on probing was allowed to vary among subjects (level 2). Thus, biserial correlations of bleeding proportions between subsequent occasions were calculated according to the variance/covariance matrix [12]. Special software was used (*MLwiN* 2.0, Centre for Multilevel Modeling, Bristol University, Bristol, UK).

Results

Allelic and composite IL-1 genotype distribution is shown in Table 1. In total, 26 of 50 volunteers (52%, 95% confidence interval 37%; 67%) had at least one copy of allele 2 at both loci $IL-1A^{-889}$ and $IL-1B^{+3954}$, i.e., they were diagnosed as IL-1-genotype-positive. No differences between males and females were seen. Pearson's χ^2 test revealed no deviation of genotype frequencies from Hardy–Weinberg expectations, confirming random mating (Table 1).

Table 1 Allele and composite genotype distribution (% in parenthesis) of $IL\text{-}1A^{-889}$ and $IL\text{-}1B^{+3954}$ polymorphisms in male and female subjects

	Male	Female	Total	Expected
$IL\text{-}1A^{-889}$				
1.1	6 (37.5)	14 (41.2)	20 (40.0)	21.1
1.2	8 (50.0)	17 (50.0)	25 (50.0)	22.8
2.2	2 (12.5)	3 (8.8)	5 (10.0)	6.1
	$\chi^2_{(2)} = 0.184,$ $p=0.912$		$\chi^2_{(1)} = 0.489,$ $p=0.484^a$	
$IL\text{-}1B^{+3954}$				
1.1	8 (50.0)	16 (47.1)	24 (48.0)	23.1
1.2	6 (37.5)	14 (41.2)	20 (40.0)	21.8
2.2	2 (12.5)	4 (11.8)	6 (12.0)	5.1
	$\chi^2_{(2)} = 0.061,$ $p=0.970$		$\chi^2_{(1)} = 0.326,$ $p=0.568^a$	
$IL\text{-}1A^{-889}\,{*}2/IL\text{-}1B^{+3954}\,{*}2$				
	8 (50.0)	18 (52.9)	26 (52.0)	
	$\chi^2_{(2)} = 0.038,$ $p=0.846$			

Expected numbers according to the Hardy–Weinberg principle are also given

[a] No deviation from Hardy–Weinberg equilibrium

Clinical periodontal conditions of the study population are presented in Table 2. Mean periodontal probing depth slightly decreased from about 1.8 to 1.7 mm ($p<0.01$). Differences between genotype-negative and -positive subjects were not significant. Any loss of clinical attachment was due to few facial areas with gingival recession with no difference between examinations or groups. The percent of sites bleeding on probing decreased during the study from 21 to 18% ($p<0.01$). Genotype-positive subjects had significantly fewer bleeding sites ($p<0.05$). This is further illustrated in quantile plots (Fig. 1) indicating distributions of proportions of sites bleeding on probing in genotype-positive and -negative subjects. In contrast, plaque scores were stable throughout the study with a mean plaque index of about 1.2 and no significant differences between groups. The mean percent of sites covered by calculus significantly increased from 3 to 6% ($p<0.001$), but differences between groups were not significant.

To further explore the association of the IL-1 genotype with gingival bleeding in gingivitis, the proportion of sites bleeding on probing was modeled in a repeated-measures, two-level model, with occasion as the lowest level. The model was adjusted for gender and, at each occasion, average periodontal probing depth, mean plaque index, and calculus proportion. In this model, the IL-1 genotype remained significant ($p<0.05$), whereas all other covariates did not significantly influence bleeding on probing proportions ($p>0.1$). Thus, in genotype-positive subjects of the same gender, with the same mean plaque index, amount of calculus, and mean periodontal probing depth, a significantly lower proportion of sites bled after probing (estimate -0.050, standard error 0.025). Biserial correlations of bleeding proportions at subsequent examinations were high (0.71–0.78), indicating stability of average bleeding tendency in a steady-state plaque environment.

Discussion

In this study, presence of a composite genotype based on polymorphisms in the *IL-1A* and *IL-1B* genes was significantly associated with decreased bleeding tendency of gingiva in young adult nonsmoking Arabs with plaque-induced gingivitis. This observation was made independent of the amount of supragingival plaque, percent of sites covered by calculus, or mean periodontal probing depth. During the menstrual cycle, subtle changes in gingival inflammation independent of the amount of plaque have recently been demonstrated [23], which might lead to an increased bleeding tendency. The two-level model did not reveal, however, a gender effect on bleeding tendency, although the majority of volunteers here (68%) were female.

Polymorphisms in genes encoding proinflammatory cytokines have been shown to be associated with systemic

Table 2 Clinical periodontal conditions in genotype-negative and -positive subjects during the experiment

	IL-1 genotype (−)			IL−1 genotype (+)		
	Baseline	2 weeks	4 weeks	Baseline	2 weeks	4 weeks
PPD/mm	1.86±0.26	1.80±0.24	1.75±0.24	1.73±0.24	1.71±0.24	1.71±0.21
CAL/mm	0.01±0.01	0.00±0.01	0.00±0.01	0.01±0.04	0.01±0.01	0.01±0.01
% BOP	24.1±12.9	21.6±11.1	21.9±13.8	**18.6±8.7**	**16.0±9.0**	**14.6±7.6**
PLI (0–3)	1.24±0.42	1.26±0.39	1.23±0.42	1.11±0.37	1.04±0.37	1.16±0.42
% CALC	2.6±2.9	5.7±4.8	7.5±8.9	3.2±4.1	4.0±3.8	4.8±4.3

Mean ± standard deviation. Data in bold are significantly lower in genotype-positive than in genotype-negative subjects at $p<0.05$ (repeated-measures ANOVA)

PPD = probing pocket depth, CAL = clinical attachment level, BOP = bleeding on probing, PLI = plaque index, CALC = calculus

Fig. 1 Quantile plots illustrating cumulative distributions of proportions of sites bleeding on probing in IL-1 genotype positive and negative subjects at examinations 1 (**a**), 2 (**b**), and 3 (**c**)
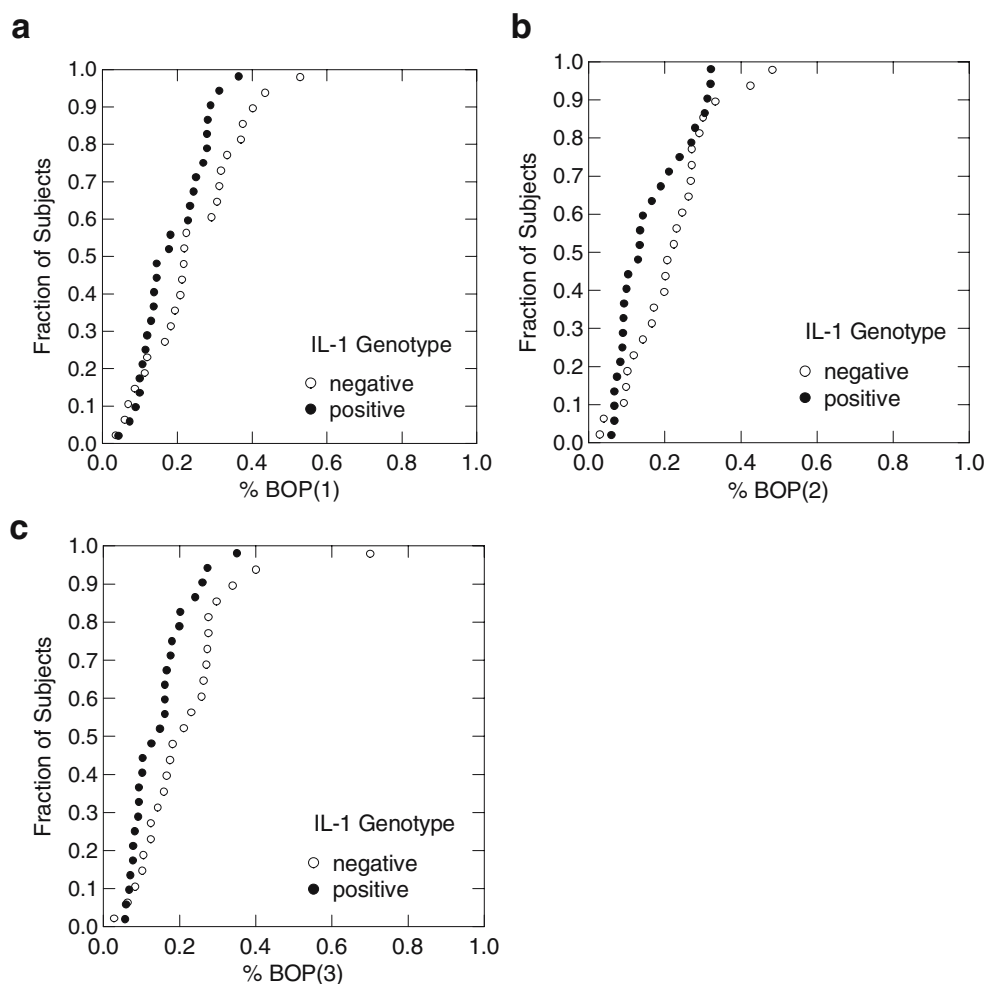
inflammation [6, 15, 21]. Here, we examined a composite IL-1 genotype that has been related in several studies to more severe destructive periodontal disease [1, 17, 19, 24]. Whereas another group could not find an association in nonsmoking adults [25], they later reported that the IL-1 genotype seems to be a strong effect modifier of the effect of smoking on periodontal structures [26]. Smoking is generally regarded the most important risk factor for destructive periodontal disease. About 50% of periodontitis in the USA may be attributed to the deleterious effects of smoking [34]. Interestingly, inflammatory responses in the gingiva were also found to be significantly suppressed [4, 9, 13]. In particular, in experiments where plaque was allowed to accumulate [5, 7, 11, 22], smoking led to a delayed and dampened gingival reaction. The present study used, for the first time, the steady-state plaque environment for the study of a possible intrinsic (genetic) influence on the association between plaque and gingival inflammation. That IL-1 genotype-positive subjects would consistently present with lower proportions of sites bleeding after probing was not expected. Once periodontitis is present, IL-1 genotype-positive patients have higher levels of proinflammatory cytokines in gingival crevice fluid and gingival tissues than their genotype-negative counterparts [10]. In a cross-sectional multiple regression analysis adjusted for the percent of tooth surfaces covered by visible plaque, IL-1 genotype-positive, treated periodontitis patients under maintenance care had a higher mean proportion of sites bleeding on probing, about 3% points [20]. The model explained only 11% of the variation in overall bleeding tendency. Genotype-positive patients had more residual periodontal pockets (mean 3.3 ± 5.2) than genotype-negative patients (2.7 ± 3.2). Age and smoking as possible confounders were not included in the model. This might explain in part the discrepancy with our present data in young adult nonsmokers without periodontal pockets. In more recent plaque accumulation experiments, being a high or low responder with regard to gingival inflammation was not related to polymorphisms in the IL-1 gene cluster, while other polymorphisms in the IL-1 gene cluster were involved [30]. No difference in the development of experimental gingivitis in IL-1 genotype-positives was observed by Jepsen et al. [14]. It should be kept in mind, however, that gingivitis might be better described in a steady-state plaque

environment where distribution of supragingival plaque within the dentition is well defined, in particular, consistent [29]. Recently, clinical conditions and cytokine profiles in gingival exudate in a gingivitis experiment have been compared with those in persistent gingivitis in a steady state [8]. IL-1β concentrations were higher and IL-8 concentrations lower after 4 weeks of experimental gingivitis as compared to local concentrations in subjects with persistent gingivitis. It was concluded that chronic gingivitis might reflect a stage of a down-regulated proinflammatory response [8] that might be further modulated by the specific IL-1 genotype. Although a dampened inflammatory response to dental plaque may in fact lead to more periodontal destruction in heavy smokers, the analogy with the present result of lower bleeding tendency in IL-1 genotype-positive young adult Arabs with plaque-induced gingivitis has to be regarded speculatively because other pathomechanisms are certainly involved. Whether the IL-1 genotype is actually related to the development of more severe periodontitis in this Arab population needs to be further explored in future studies.

The high prevalence of the composite genotype in this Arabic population is notable. In fact, differences in prevalence have been described in the past. For example, prevalence seems to be about 36% in populations with a Northern European heritage [25], whereas in a Chinese population, this genotype was hardly detected [2]. The 52% of the present study found in healthy young adults with gingivitis may be the highest prevalence so far reported. In further studies, the effect of the IL-1 genotype on periodontal destruction in this Arabic population has to be addressed.

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