Original

Association of interleukin-1 polymorphisms with periodontitis in Down syndrome

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Abstract: This study examined the association of IL1 genetic polymorphisms (IL-1A +4845, IL-1B +3954 & IL-1RN +2018) with periodontal disease status of Down syndrome (DS) individuals. Fifty-four DS patients (18-56 yr, 48.15% male, 77.78% Caucasians) were recruited from the Georgia Regional Hospital (GRH) health care system. Two comparable groups (71 mentally retarded patients and 87 control subjects) were also recruited. All subjects were nonsmokers. Periodontal evaluations (plaque index, gingival index, bleeding-on probing and clinical attachment levels (AL)), personal and professional dental care habits were recorded. Blood was collected by a venipuncture. The IL-1A +4845, IL-1B +3954 & IL-1RN +2018 loci were genotyped by the TaqMan assay. No statistically significant differences were noted in the distribution of IL-1 gene polymorphisms between the three groups. The IL-1 variant genotypes varied by race; for both IL-1A and IL-1RN, the variant gene was significantly more prevalent among whites than non-whites (ps > 0.1). ANCOVA, which also adjusted for age, showed a 3-way interaction among dental visits, gene variation and Down status [(F(1, 179) = 3.96, P = 0.048 in White subjects and F(1, 241) = 2.96, P = 0.087 in all subjects). Post-hoc t-tests confirmed lower levels of AL in IL-1RN-variant Down subjects receiving more frequent dental visits (P < 0.05). ANCOVA, which also adjusted for age, showed an interaction between IL-1A/B gene variation and Down status (F(1, 174) = 3.04, P = 0.083 in White subjects and F(1, 235) = 3.72, P = 0.055 in all subjects). Post-hoc t-tests confirmed lower levels of AL in IL-1A/B-variant Down subjects (P < 0.05). The distribution of variant IL-1 genes in DS subjects was not different from the general population. However the association between the carriage of the IL-1 rare alleles and periodontitis differed between the Down and non-Down subjects. The carriage of the IL-1 rare alleles in the Down subjects tended to confer a protective effect against loss of periodontal attachment. (J Oral Sci 53, 193-202, 2011)

Introduction

Down syndrome (DS) is a genetic disease known as trisomy 21. The condition is associated with an extra chromosome #21 in affected individuals, giving them a total chromosome count of 47. It is generally produced by an abnormal segregation of chromosomes during cell division (maternal meiotic nondisjunction). It is the most common chromosomal disorder, with an estimated prevalence of 9.2 cases per 10,000 live births in the United States (1,2). Different racial groups are equally affected (3). The condition is associated with characteristic dysmorphic features, mental retardation, congenital heart defects, and altered immune responses. In developed nations, life expectancy of individuals with DS has improved with

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average life expectancy reaching 56-60 years (4,5).

Periodontal disease is a serious and morbid oral condition among individuals with DS (6). Gingivitis and periodontitis start early and their severity increases with age (6). Fifty-eight percent of DS individuals younger than 35 years old have periodontitis (7). In addition, periodontitis is an important cause of tooth loss among individuals with DS (8).

A multitude of factors may be involved in the increased susceptibility to periodontitis among individuals with DS (9). Factors previously investigated included mental retardation (10), subgingival plaque composition, immune/inflammatory responses and others (9). Microbiological studies showed that individuals with DS have significantly higher levels of periodontopathic bacteria including *Porphyromonas gingivalis* and *Tannerella forsythensis* (11). Neutrophil chemotaxis in individuals with DS was significantly impaired and alveolar bone loss was inversely proportional to the chemotactic index (12). Other immune defects associated with periodontitis in DS included lymphocyte dysfunction (13) and altered antibody production (14). Inflammatory mediators (prostaglandin E2 and leukotriene B4) and degrading enzymes (matrix metalloproteinase-9) were also increased in gingival crevicular fluid from patients with DS (15).

The role of the IL-1 family of proinflammatory cytokines in the pathogenesis of periodontitis is well documented (16). A cluster of three genes located on the long arm of chromosome 2q13 code for and regulates IL-1 production. The IL-1 gene cluster is comprised of IL-1A, IL-1B and IL-1RN genes that code for IL-1a, IL-1b and IL-1 receptor antagonist (IL-1ra) respectively. IL-1a and IL-1b are involved in initiating and propagating immune and inflammatory reactions. IL-1ra is involved in terminating the action of both IL-1a and IL-1b by blocking the IL-1 receptors. Polymorphic variations in the IL-1 genes are not uncommon and are associated with an altered/exaggerated inflammatory response (17). Single nucleotide polymorphisms at the following IL-1 gene loci IL-1A +4845, IL-1B +3954 and IL-1RN +2018 have been implicated in increased severity and susceptibility to many inflammatory diseases including periodontitis (17,18). The simultaneous occurrence of both polymorphic alleles at the IL-1A +4845 and IL-1B +3954 loci (referred to as: composite IL-1 genotype positive) has been specifically associated with increased severity of periodontitis (18).

The prevalence of IL-1 polymorphic alleles and their association with periodontitis in DS individuals has not been previously investigated. We hypothesized that DS subjects would be more likely than non-DS subjects to carry IL-1 allelic variants associated with increased susceptibility to periodontitis. The aim of the present study was to investigate the distribution of IL-1 genotypes in a Down syndrome subject population and examine the association of IL1 genetic polymorphisms at the following loci IL-1A +4845, IL-1B +3954 and IL-1RN +2018 with their periodontal disease status.

Materials and Methods

Subject recruitment and clinical evaluations were previously described (10).

Study sites

This study was done in cooperation with the Georgia Department of Human Resources/Georgia Regional Hospitals (GRH) in Atlanta, Savannah and Augusta. The study protocol and consent forms were approved by the Georgia Regional Hospital Institutional Review Board. The study included three subject groups, Down syndrome group (DS), mental retardation non-Down group (MR) and a mentally normal control group (C). Both the DS and MR subjects were recruited from the GRH healthcare systems in Atlanta, Savannah and Augusta, Georgia. All DS and MR subjects were patients of record at the three hospital locations; some were institutionalized while others were outpatients living in group-homes or with their families. All DS and MR subjects were receiving periodic dental care at one of the three GRH locations.

Inclusion criteria

The study inclusion criteria implemented for DS subjects were: confirmed diagnosis of Trisomy 21, receiving periodic dental care, age 18 years or older, a minimum of 10 teeth present, no other medical conditions known to affect periodontal status (e.g. diabetes mellitus), no antibiotic treatment in the past 3 months prior to entry in the study, no history of cigarette smoking and being able to cooperate with the study examiners. Study inclusion criteria for the MR subjects were similar to the DS subjects except for a confirmed diagnosis of mental retardation without Trisomy 21. Study inclusion criteria for the C subjects were also similar to the DS subjects except for absence of mental retardation.

Subject recruitment

The attending dentist in charge of the dental clinic at each of the three GRH sites reviewed the available records and identified dentulous DS patients who would meet the study criteria and would be able to participate in a dental examination. Then MR patients matched to the previously identified DS patients on age, race and gender were identified from the same hospital records. The matched MR

patients were selected based on their ability to cooperate and sit for the dental examination without need for sedation. Their mental retardation was secondary to head trauma at birth. The C subjects were recruited from the general population living in the vicinity of the GRH locations used. All C subjects were under care of private dentists and they also were matched on gender, race and age to the DS subjects.

Subject screening and enrollment

A total of 289 subjects were screened for the study, 26 were disqualified for medical reasons, 46 completed portions of the study evaluations and were not able to return to complete the remaining portions, and 217 completed most of the study evaluations. This report will focus on a subgroup of 212 subjects with IL-1 genotype data. Fifty-four of those were DS, 71 were MR and 87 were C.

Subject characteristics

All subjects in the three groups were adults 18 years or older, mostly Caucasian and the distribution of males to females was equal. While groups in the initial cohort were matched on age, this was no longer true after attrition. None of the subjects in all groups smoked cigarettes, had diabetes, or was on a medication known to influence periodontal status.

Ethical issues

All subjects were able to communicate and understand spoken English. Prior to commencing the study, consent was obtained and documented for all subjects. The GRH dentist in charge personally contacted the family or caretaker of each potential DS or MR subject, explained the study protocol and obtained their consent to enroll the subject in the study. In addition to obtaining the family or caretaker consent, prior to commencing the study examination, the study protocol was explained to the DS or MR subject and their personal consent was also obtained and witnessed. The C subjects consented on their own behalf.

Oral/periodontal assessments

All subjects received a comprehensive oral/periodontal evaluation including probing measurements. Two experienced dental hygienists blinded to the objectives of the study performed all dental exams under supervision of investigator AK. The examiners were calibrated and standardized in the use of the clinical evaluation measures employed in the study. Standardization sessions were performed periodically to recalibrate examiners throughout the study period. The examiners recorded the Loe and Silness gingival index (GI) (19) around all teeth present. Each tooth was scored at six sites, mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual. The teeth were then disclosed with D and C Red No. 28 dye (Sunstar Americas, Inc. Chicago, IL, USA) and the Quigley Hein plaque index (20) on the same six surfaces was determined. Surfaces with large restorations and teeth with crowns were not scored.

A conventional periodontal probe with Williams markings (PQ-OW, Hu-Friedy, Chicago, IL, USA) was used for all probing measurements. Probing depth (PD) was taken on six sites per tooth, mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual. The probe was inserted parallel to the long axis of the tooth on the buccal and lingual surfaces. Interproximally, the probe was placed with slight angulation, as close to the contact area as possible. At the gingival margin the reading was taken to the nearest millimeter. The position of the gingival margin (GM) to the cementoenamel junction was recorded at the same six sites per tooth to the nearest millimeter. A (-) sign was given when the gingival margin was coronal to the cementoenamel junction and a (+) sign when it was apical. Attachment levels were calculated according to the formula AL = PD + GM. Periodontitis was defines as 5% or higher of teeth scored exhibiting attachment loss ≥5 mm. For all the aforementioned examinations, only fully erupted teeth were used, except third molars were not included. Caries and missing teeth were also recorded. The demographic and clinical data of the entire subject populations were previously presented (10).

Blood sample collection: Blood was collected by a venipuncture for IL-1 genotyping and other analysis. For IL-1 genotyping, a few drops of the collected blood sample were immediately placed on an AmpliCard (Chemicon International Inc., Temecula, CA, USA). AmpliCard was allowed to dry at room temperature then placed in an envelope and stored at -70°C for later analysis.

Analysis of genetic polymorphism

Within a three-month time-period from date of sample collection, AmpliCards were sent to Interleukin Genetics, Inc. (Boston, MA, USA) for human DNA extraction and determination of IL-1 polymorphism. Genotyping was performed without knowledge of clinical status. All samples were genotyped for the biallelic markers IL-1A(+4845), IL-1B(+3954), and IL-1RN(+2018). The wild (common) allele was designated as (1) and the variant (rare) allele was designated as (2). DNA was isolated from AmpliCards using the Whatman FTA Purification reagent (Whatman

Inc., Clifton, NJ, USA).

Genotyping was performed using the TaqMan assay (Perkin-Elmer, Foster City, CA, USA). The TaqMan assay utilizes the 5'-3' nuclease activity of Taq DNA polymerase to cleave a fluorogenic probe specific for one of two alleles at the target polymorphism site. Quantitation of fluorescence was made by comparing each sample's fluorescent activity with that of samples of known genotype, blank standards containing no DNA, and a background dye present in the reaction buffer.

Sequences and conditions used in TaqMan genotyping:

IL-1A (+4845)
Probe 1: 5'-C(•FAM)AA GCC TAG GTC ATC ACC TTT TAG CTT CTT T(•TAMRA)-3'
Probe 2: 5'-C(•TET)AA GCC TAG GTC AGC ACC TTT TAG CTT CTT T(•TAMRA)-3'
Forward: 5'-ACC CCC TCC AGA ACT ATT TTC CCT-3'
Reverse: 5'-TGT AAT GCA GCA GCC GTG AGG TAC-3'
Cycling: [95°C for 2 min] ×1; [94°C for 1 min, 65°C for 1 min, 72°C for 1 min] ×40; [94°C for 12 min, 65°C for 2 min, 72°C for 5 min] ×1

IL-1B (+3954)
Probe 1: 5'-A(•FAM)CC TAT CTT CTT TGA CAC ATG GGA TAA CGA T(•TAMRA)-3'
Probe 2: 5'-A(•TET)CC TAT CTT CTT CGA CAC ATG GGA TAA CGA T(•TAMRA)-3'
Forward: 5'-GCT CAG GTG TCC TCC AAG AAA TC-3'
Reverse: 5'-GTG ATC GTA CAG GTG CAT CGT-3'
Cycling: [95°C for 2 min, 62°C for 1 min, 72°C for 1 min] ×2; [95°C for 1 min, 62°C for 1 min, 72°C for 1 min] ×27; [94°C for 1 min, 62°C for 1 min, 72°C for 5 min] ×3

IL-1RN (+2018)
Probe 1: 5'-C(•FAM)AA CCA ACT AGT TGC TGG ATA CTT GCA AG(•TAMRA)-3'
Probe 2: 5'-C(•TET)AA CCA ACT AGT TGC CGG ATA CTT GCA AG(•TAMRA)-3'
Forward: 5'-AAG TTC TGG GGG ACA CAG GAA G-3'
Reverse: 5'-ACG GGC AAA GTG ACG TGA TG-3'
Cycling: [96°C for 1 min] ×1; [94°C for 1 min, 63°C for 1 min, 70°C for 1 min] ×35; [63°C for 5 min, 70°C for 5 min] ×1.

Statistical analysis
Analysis of variance (parametric data) and chi-square analysis (non-parametric data) were used to examine the differences between the groups. Analysis of covariance (ANCOVA) was used to examine the effect of IL-1 variant genes on periodontal disease measures after adjusting for age, gender, plaque levels and dental visits.

Results
Demographic and clinical data of this subset of subjects with IL-1 genotyping data are summarized in table 1. In

Table 1 Summary of demographic and clinical data of a subset of subjects with IL-1 genotype data presented in this report

Variable	DS $n = 54$	C $n = 87$	MR $n = 71$	P value
Demographics:				
Average age range (years)	35.96 (1.64) 18-56	40.77 (1.29) 18-73	46.00 (1.43) 22-84	0.0001
White percent	77.78%	70.11%	80.28%	NS
Male percent	48.15%	58.62%	42.25%	NS
Institution	42.59%	NA	81.69%	0.001
Clinical:				
Plaque index	1.56 (0.16)	1.25 (0.08)	1.77 (0.09)	0.0002
Gingival index	0.92 (0.04)	0.68 (0.03)	0.99 (0.03)	0.0001
Attachment level average (mm)	2.66 (0.10)	2.25 (0.07)	2.24 (0.08)	0.002
Percentage of sites with AL ≥5 mm	9.32 (1.49)	4.37 (1.17)	3.64 (1.30)	0.001
Missing teeth	4.57 (0.53)	1.78 (0.42)	4.54 (0.46)	0.0001
Dental visits (Once or more/yr)	98.11%	93.10%	100%	0.0002

Data presented as mean (SE) or percentage. NA: non-applicable. NS: $P > 0.10$.

summary all three groups were matched on race and gender. The MR group was older than the DS and C group ($P = 0.0001$). Number of institutionalized subjects in the MR group was higher than the DS group (0.001). Clinically, subjects in both DS and MR groups showed higher levels of GI ($P = 0.0001$), PI ($P = 0.0002$) and missing teeth ($P = 0.0001$) than the control group. The DS subjects showed greater loss of clinical periodontal attachment ($P = 0.002$) and higher percentage of teeth with AL $\geq$5mm ($P = 0.001$) than both the MR and the C groups. Percentage of subjects with periodontitis was similarly distributed among the three groups, DS 72%, C 71% and MR 80%.

The IL-1A, IL-1B and IL-1RN genotype and allele frequencies were essentially similar between the three groups. Also no statistical differences were found between the three groups in the distribution of IL-1 composite genotype (simultaneous carriage of variant alleles in both the IL-1A and IL-1B genes. Thus the carriage of the IL-1 gene polymorphisms in the Down subjects was similar to the non-Down subjects. Table 2 summarizes the distribution of the alleles for all subjects combined.

On the other hand significant differences were found in the distribution of IL-1 genotypes between Whites and non-Whites. Table 3 shows that the IL-1 variant (rare) genotypes varied by race. For both IL-1A and IL-1RN, the variant gene was significantly more prevalent among Whites than non-Whites. For IL-1B and composite genotype IL-1A/B, the variant gene also trended higher in Whites, but did not reach statistical significance. Thus, data generally suggest that variant forms of the IL-1 genes are more likely to be found among White than Non-white individuals.

We next investigated the effect of the IL-1 gene polymorphisms on gingival inflammation. ANCOVA, which also adjusted for age, plaque and group differences, showed that in individuals positive for IL-1B (+3954) gene polymorphism had less GI scores (F(1, 242) = 5.70,

Table 2 Distribution of the IL-1 alleles for all subjects combined

Genotype	Percentage of subjects
IL-1A	
1/1	53.2
1/2	40
2/2	6.8
IL-1B	
1/1	57
1/2	34.7
2/2	8.3
Composite Genotype	
1-2/1-2	22.4
1-2/2-2 or 2-2/1-2	7.2
2-2/2-2	2.4
Total	32

1 = wild allele, 2 = variant allele
1/1 = both alleles are wild, 1/2 = one allele is wild and the other allele is variant, 2/2 = both alleles are variant.

Table 3 IL-1 variant genotype distribution by race

Genotype	Whites $n = 160$	Non-Whites $n = 52$	P value
IL-1A			
1/2	44.09%	28.13%	
2/2	6.99%	6.25%	
Total	51.08%	34.38%	0.01
IL-1B			
1/2	35.60%	32.31%	
2/2	9.42%	4.62%	
Total	45.03%	36.92%	NS
IL-1 A/B composite			
1-2/1-2	25.27%	14.06%	
1-2/2-2 or 2-2/1-2	6.45%	9.38	
2-2/2-2	3.23%	0	
Total	34.95%	23.44%	0.08
IL-1RN			
1/2	41.67%	21.54%	
2/2	7.29%	1.54%	
Total	48.96%	23.08%	0.0002

NS = $P > 0.1$, 1 = wild allele, 2 = variant allele, 1/1 = both alleles are wild, 1/2 = one allele is wild and the other allele is variant, 2/2 = both alleles are variant.

$P = 0.01$). GI for IL-1 +3954 positive individuals averaged 0.75, while IL-1 +3954 negative individuals averaged 0.88. The model showed no differences in effect on GI between Down and non-Down subjects. This analysis suggests that the variant form of IL-1B genotype appears to lessen gingival inflammation scores. No effects were found for IL-1A or IL-1RN or IL-1 composite positive genotype on gingival inflammation scores.

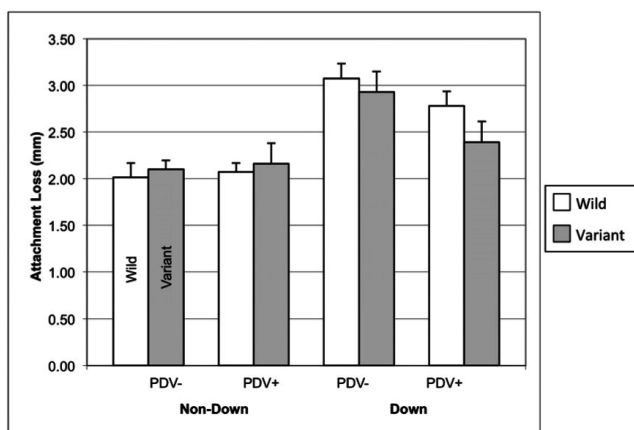We next investigated whether professional dental visits

Fig. 1 Bar chart of adjusted AL mean (SEM) and IL1-RN polymorphism by group and frequency of dental visits. The bars represent the AL mean after adjusting for age. The whiskers indicate the standard error of the mean. PDV- = professional dental visits once or less per year. PDV+ = professional dental visits more than once per year. Significance of differences among groups was sought using ANCOVA.
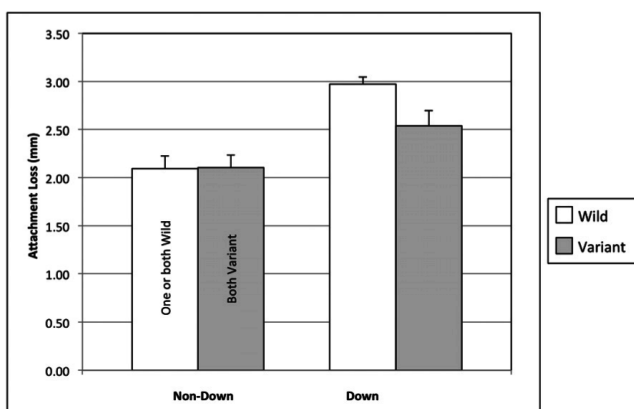

Fig. 2 Bar chart of adjusted AL mean (SEM) and IL1 A/B composite genotype by group. The bars represent the AL mean after adjusting for age. The whiskers indicate the standard error of the mean. Significance of differences among groups was sought using ANCOVA.

and variation in the IL-1 genotypes might mediate the association between AL and Down status. Figure 1 shows, for IL-RN, AL of just over 2 mm for non-Down subjects, regardless of visits or gene variation. On the other hand, Down subjects with lower levels of dental care or higher levels of dental care and the wild genotype averaged near 3 mm of AL. Interestingly, however, Down subjects with higher levels of dental care and the variant IL-RN genotype, averaged near 2 mm of AL, similar to non-Down subjects. ANCOVA, which also adjusted for age, showed a 3-way interaction among dental visits, gene variation and Down status [($F(1, 179) = 3.96$, $P = 0.048$ in White subjects and $F(1, 241) = 2.96$, $P = 0.087$ in all subjects). While this interaction was stronger among White subjects, race did not produce a statistically significant difference in this interaction]. Post-hoc t-tests confirmed lower levels of AL in IL-1RN-variant Down subjects getting higher than lower levels of dental care ($P < 0.05$). Thus, AL appears preventable in one subgroup of Down subjects, those that receive more professional care and also evidence the variant form of the IL-1RN genotype. Stated differently, this analysis suggests that the wild form of this gene appears to put Down individuals at increased risk for periodontal disease.

We next investigated whether professional dental visits also interacted with the IL-1A and IL-1B genotypes to mediate the association between AL and Down status. However, while no effects were found for either IL-1A or IL-1B, a simpler effect was noted for the IL-1A/B composite measure. Figure 2 shows, for IL-1 composite, AL of just over 2 mm for non-Down subjects, regardless of visits or gene variation. On the other hand, Down subjects showing the wild genotype averaged near 3 mm of AL., while Down subjects with the variant IL-RN genotype, averaged near 2.5 mm of AL. ANCOVA, which also adjusted for age, showed an interaction between IL-1A/B gene variation and Down status ($F(1, 174) = 3.04$, $P = 0.083$ in White subjects and $F(1, 235) = 3.72$, $P = 0.055$ in all subjects). Post-hoc t-tests confirmed lower levels of AL in IL-1A/B-variant than IL-1A/B-wild Down subjects ($P < 0.05$). Thus, AL appears less severe in the subgroup of Down subjects that evidence the variant form of the IL-1A/B genotype. As above, this analysis suggests that the wild form of the IL1-A/B genotype appears to put Down individuals at increased risk for periodontal disease.

Discussion

The objectives of this study were to compare the distribution of IL-1 genotypes between Down and non-Down subjects and examine the association between presence of IL-1 variant (rare) alleles with AL in Down

and non-Down subjects. Our data showed no difference in the distribution of IL-1 genotypes between Down and non-Down subjects, however the association between IL-1 polymorphic genotypes and periodontitis differed between the Down and non-Down subjects. Presence of the variant alleles of the IL-1 gene family in the Down subjects tended to be protective against loss of periodontal attachment.

The distribution and frequency of IL-1 alleles and positive IL-1 composite genotype is known to vary by race and ethnic groups. In European populations the frequency of a positive IL-1 composite genotype ranges from 29% to 46% (21-23). In the United States the frequency of a positive IL-1 composite genotype in Whites ranges from 29% to 38% (18,24-26) and in African Americans 14% (27). Frequency of the positive IL-1 composite genotype in Whites in our study (34.95%) falls within the range previously reported in White populations. However the positive IL-1 composite genotype frequency (23.08%) in our non-White population (predominantly African Americans) was higher than previously reported by Walker et al. in African Americans (27). The higher frequency of the IL-1 variant alleles in the non-Whites in our study may be due to the diverse ethnic backgrounds of non-White subjects.

There is mounting evidence linking the presence of IL-1 gene polymorphisms with various inflammatory diseases including periodontitis (17,18). The premise behind this association may be attributed to increased IL-1 cytokines production and delayed termination of their action thus resulting in an exaggerated and uncontrolled inflammatory response. Research (28) showed that monocytes from polymorphic IL-1B positive individuals produce higher amounts of IL-1b than monocytes from individuals with the wild IL-1B genotype. Engebertson et al. (29) reported increased IL-1b cytokine levels in gingival fluid in IL-1 composite genotype positive subjects. Shirodaria et al. (30) reported that polymorphisms in the IL-1A gene are associated with increased levels of IL-1a protein in gingival fluid. On the other hand, IL-1RA genotype 2 is associated with reduced mucosal concentrations of IL1ra cytokine proteins (31); thus in affected individuals the action of both IL-1a and IL-1b may be prolonged.

The finding that IL-1B (+3954) positive individuals had less GI scores than IL-1B (+3954) negative individuals contradicts with the presumed exaggerated inflammatory response associated with presence of IL-1 polymorphic genes. Muller et al. (32) reported similar findings in young adults. The type of gingivitis being investigated may explain this contradiction. Steady-state gingivitis (a longstanding gingival inflammation, as investigate in this study) is characterized by a dampened inflammatory

response associated with low IL-1b levels in gingival crevicular fluid (33). Thus other late-stage intervening mechanisms involved in controlling the gingival inflammatory responses may have masked the hyper-inflammatory effect of the IL-1 gene polymorphisms.

The initial study (18) that investigated the relation between presence of IL-1 polymorphic genes and periodontitis reported that in White non-smokers, the simultaneous presence of variant IL-1 alleles at the IL-1A-889 (currently +4845) and IL-1B+3953 (currently +3954) loci are associated with increased severity of periodontitis. When both IL-1 polymorphic alleles were present, the affected individual was referred to as "composite genotype-positive". Presence of IL-1 gene polymorphisms at a single locus did not associate with periodontal disease. Other studies in White populations confirmed similar associations between the IL-1 composite genotype and severity of chronic periodontitis (26,34) and aggressive periodontitis (22). However other studies reported contradictory findings disputing the association between the presence of the IL-1 composite genotype and periodontitis (23,27,35). In our study, in the non-Down subjects there was no association between the presence of the variant alleles of IL-1A, IL-1B, IL-1RN alone or in-combination (when simultaneously present) and loss of periodontal attachment. However in the Down subjects, the presence of the IL-1 composite genotype or the presence of IL-1RN variant allele inversely associated with loss of periodontal attachment. It is of interest to note that presence of IL-1RN variant allele needed the added benefit of more frequent preventive dental visits to exert its beneficial effect on the periodontal status of Down subjects.

The lack of association between the IL-1 variant alleles and periodontitis in the non-Down subjects in our study may be explained by the fact that all subjects were receiving periodic preventive dental care. Perhaps in the general population periodic preventive dental care can circumvent and negate the adverse genetic influence of the IL-1 variant alleles on the periodontium. These findings agree with other studies that reported successful outcome of both non-surgical and surgical periodontal therapy in IL-1 composite genotype positive individuals (36-39).

The inverse association noted in this study between the presence of the IL-1 variant alleles and loss of periodontal attachment in Down subjects is contradictory to previous reports in the general population. It suggests that the presence of the IL-1 variant alleles in Down individuals bestows protection against periodontitis. This was a surprising and unexpected finding that refuted our hypothesis for increased susceptibility to periodontitis in Down subjects. Perhaps the altered/exaggerated inflam-

matory reaction associated with the presence of the variant IL-1 alleles compensates for other immune deficiencies in the Down individuals and helps them cope better with their periodontal problems.

Even though the initial report associating IL-1 genotyping and periodontal disease excluded cigarette smokers from the analysis (18), other studies showed that both IL-1 genotyping and smoking are relevant independent risk factors for periodontal disease and they also represent a gene-environmental interaction in periodontitis (24,25,40). In the present study the interaction of smoking with IL-1 genotyping was not investigated because none of our Down subjects smoked. Both our controls and MR subjects were selected to match the Down group and thus none of the subjects smoked.

In conclusion, it is well documented that Down syndrome individuals are more susceptible to periodontal loss of clinical attachment than non-Down individuals. Our study showed that even though the IL-1 genotype distribution in the Down subjects is similar to the non-Down subjects, the association between the carriage of the IL-1 rare alleles and periodontitis differed between the Down and non-Down subjects. The carriage of the IL-1 rare alleles in the Down subjects tended to bestow a protective effect against loss of periodontal attachment. This suggests that the pathogenisis of periodontitis in Down subjects is different from the general population.

Acknowledgements

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