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A familial case of Aggressive Periodontitis: clinical, microbiological and genetic findings

Raquel Mantuaneli SCAREL-CAMINAGA[a], Juliana Rico PIRES[b], Patrícia Mayumi SOGUMO[a], Cristiane Ribeiro SALMON[c], Regina Célia Rocha PERES[d], Denise Madalena Palomari SPOLIDÓRIO[e]

[a]Departamento de Morfologia, Faculdade de Odontologia, Universidade Estadual Paulista – UNESP, 14801-903 Araraquara - SP, Brasil
[b]Departamento de Diagnóstico Bucal e Cirurgia, Faculdade de Odontologia, Universidade Estadual Paulista – UNESP, 14801-903 Araraquara - SP, Brasil
[c]Departamento de Morfologia, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas – UNICAMP, 13414-903 Piracicaba - SP, Brasil
[d]Departamento de Odontologia Pediátrica, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas – UNICAMP, 13414-903 Piracicaba - SP, Brasil
[e]Departamento de Fisiologia e Patologia, Faculdade de Odontologia, Universidade Estadual Paulista – UNESP, 14801-903 Araraquara - SP, Brasil

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Abstract: This study reports the clinical, microbiological and genetic findings in members of a Brazilian family with Aggressive Periodontitis (AgP). After periodontal exams in eleven members of the family, microbiological samples were collected from subgingival plaque and DNA were obtained from epithelial buccal cells. Polymerase Chain Reaction (PCR) was utilized to detect five species of periodontopathogens. PCR-RFLP (Restriction Fragment Length Polymorphism) and sequencing were used to investigate human polymorphisms in interleukin genes (*IL4, IL10*). Six members of the family showed Generalized AgP, four had Localized AgP and only one was considered unaffected by AgP. *Aggregatibacter actinomycetemcomitans* was the most prevalent pathogen (72.7%). The presence of *Porphyromonas gingivalis* was correlated with clinical findings (visible plaque, bleeding on probing and probing depth, p = 0.03). The genetic analyses revealed that 60% of the kindred affected by AgP showed specific IL4/ IL10 haplotypes combinations. The predominance of AgP in this family could be influenced by the amount of plaque and subgingival *Aggregatibacter actinomycetemcomitans*. Indeed, infection by *Porphyromonas gingivalis* was associated with clinical parameters of periodontitis. Although the majority of AgP family members presented at least one TTD/ ATA (IL4/ IL10) haplotype combination, it was unable to demonstrate an association with AgP.

Keywords: *Periodontitis; microbiology; genetics; cytokines.*

Resumo: Este estudo relata achados clínicos, microbiológicos e genéticos em indivíduos de uma família brasileira com Periodontite Agressiva (PA). Após exames periodontais realizados em onze membros da família, foram coletadas amostras microbiológicas de placa subgengival e DNA de células da mucosa oral. A Reação em Cadeia da Polimerase (do inglês, PCR) foi utilizada para detectar cinco espécies de periodontopatógenos. As técnicas do Polimorfismo por Comprimento de Fragmento de Restrição (RFLP) e sequenciamento foram usados para investigar polimorfismos humanos em genes da interleucina (*IL4, IL10*). Seis membros da família apresentaram PA Generalizada, quatro PA Localizada e apenas um indivíduo foi considerado não afetado pela PA. *Aggregatibacter actinomycetemcomitans* foi o patógeno prevalente (72,7%). A presença de *Porphyromonas gingivalis* foi relacionada com achados clínicos (placa visível, sangramento à sondagem e profundidade de sondagem, p = 0,03). A análise genética revelou que 60% dos membros da família afetados pela PA carregavam o mesmo

haplótipo formado por uma combinação de alelos dos genes IL4/ IL10. O predomínio de PA nesta família pode ter sido influenciada pela quantidade de placa bacteriana e prevalência de *Aggregatibacter actinomycetemcomitans* presente no sulco gengival. A infecção por *Porphyromonas gingivalis* pode ser associada com parâmetros clínicos relacionados a periodontite. Embora a maioria dos membros afetados pela PA tenha apresentado pelo menos uma combinação de haplótipos TTD/ ATA (IL4/ IL10), não foi possível demonstrar estatisticamente uma associação com PA.

Palavras-chave: *Periodontite; microbiologia; genética; citocinas.*

Introduction

Aggressive Periodontitis (AgP) is an inflammatory disease of the periodontal tissues caused by infection with highly virulent bacteria in subjects that may be highly susceptible to periodontal disease[1]. Generalized and Localized forms of Aggressive Periodontitis are rare types of periodontal disease that first appear at a young age, with rapid attachment and bone loss, and tend to run in the families[2]. Despite the familial aggregation of AgP, the mode of inheritance is still unclear, family linkage studies have reported X-linked dominant[3], autosomal dominant[4], autosomal recessive[5] or either X-linked dominant or autosomal dominant[6] modes of inheritance.

More recently, periodontitis has been considered a complex disease (i.e., polygenic and influenced by environmental factors)[7]. Various host risk factors, such as, polymorphisms in the cytokine genes, interleukin 1 receptor antagonist (*IL1RN*)[8] and interleukin 4 (*IL4*)[9] has been positively correlated with AgP (previously referred to as early-onset periodontitis). However, other polymorphisms in the interleukin 10 (*IL10*) and tumour necrosis factor alpha (*TNFA*) genes were not associated with AgP. Different results of specific polymorphism and their relationship to a disease may be influenced by the size and ethnicity of the studied population[10].

An important factor in the pathogenesis of AgP is the infection of periodontium by pathogenic bacteria, as it acts as a primary etiologic agent in this disease[11]. *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) is strongly associated with progressing periodontitis in young patients[11,12]. Individuals with AgP are usually infected by a gram-negative anaerobic rod species[13] demonstrating that there are different microbial associations in subgingival plaques. *Tannerella forsythia* (*T. forsythia*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Treponema denticola* (*T. denticola*) seem to be related to the progression of periodontal destruction[14,15].

Diagnosis of AgP may be based on clinical, radiographic and historical data[16]. A diagnosis of AgP requires that the patient has not been affected by any systemic diseases that may severely impair host defenses and lead to premature tooth loss[16]. Moreover, laboratory findings such as microbiological and genetic data may contribute in the confirmation of the diagnosis and may help assess the relationship between specific gene polymorphisms and AgP. Therefore, the aim of this study was to report clinical, microbiological and genetic findings in members of a Brazilian family with Aggressive Periodontitis.

Materials and method

Clinical procedures

Eleven individuals of a Brazilian family were examined for the presence of AgP after one family member (II.12), the proband, was diagnosed with AgP. Although the family is composed by 14 members, siblings II.5, II.8 and II.13 did not wish to be examined because even though the periodontist had explained the familiar chance of being AgP affected, they did not convinced their selves by the importance of the periodontal exam to their oral health. Although the periodontist had offered some appointment facilities, they did not accept to be examined. Therefore, were not classified further (Figure 1). This study was approved by the Research Ethics Committee of the School of Dentistry at Araraquara, UNESP - São Paulo State University, Brazil (CEP-FOAr 41/04).

The evaluations of each family member who participated in this study consisted of a medical and dental history, oral examination and full-mouth periapical radiography survey.

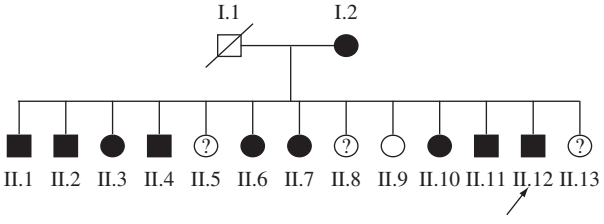

Figure 1. Heredogram of the family; circles designate women and squares men. Symbols in black represent AgP affected subjects. Arrow indicates the proband. Interrogation means uncertain diagnosis because those individuals did not accepted to be examined.

The patients did not have any systemic disease. Siblings II.1 and II.4 were current smokers and II.3 was a former smoker. The ethnicity of this family is lighter-skinned black (pardos).

One previously calibrated examiner performed complete periodontal examinations of each patient. The examiner calibration was performed evaluating probing depth in a total of 252 sites randomly selected in six subjects (42 sites per subject with a single or multirooted tooth) on two occasions, one week apart. Data were submitted to Student *t*-test. Calibration was approved because evaluation differences between the two periods were not statistically significant ($p > 0.05$).

Clinical examination included dichotomous measurements of visible plaque (VP)[17] and bleeding on probing (BoP). Measurements of probing depth (PD) and attachment level were made at six sites for each tooth using standard periodontal probe (Hu-Friedy, Chicago, IL, USA). The number of teeth present was also recorded. An individual was considered affected by AgP if there was interproximal attachment loss ≥ 5 mm on at least two permanent teeth (excluding third molars), one of which was a first molar. In order to classify the form of AgP, the number of permanent teeth other than molars and incisors with interproximal attachment loss ≥ 5 mm was taken into account (e.g., Localized AgP involves no more than two teeth, and Generalized AgP involves three or more teeth)[16]. A set of full mouth, standardized intraoral radiographs was obtained from each subject to verify the presence of alveolar bone loss.

Microbiological analysis

Microbiological samples of each patient were obtained from subgingival plaque of two affected sites (the two deepest sites with probing pocket depths ≥ 5 mm) and two healthy sites (with probing depth ≤ 3 mm). The sample sites were isolated with cotton rolls, and the supragingival plaque was carefully removed with a curette. The sites were then air-dried. A subgingival plaque sample was taken by two sterile paper points that were inserted into the periodontal pocket and removed after 30 seconds. Paper points were transferred to a vial containing 0.5 mL of Ringer solution. The samples were boiled for 10 minutes and centrifuged. The supernatant was subsequently used for PCR analysis[18].

Microbiological analyses by Polymerase Chain Reaction (PCR) were performed on a Mastercycler Gradient (Eppendorf, Hamburg, Germany) using primers (Invitrogen, Frederick, MD, USA) and PCR conditions (Platinum Taq DNA Polymerase, Invitrogen, São Paulo, SP, Brazil) previously described.

The following bacterial strains were used as positive and/ or negative controls: *P. gingivalis* – ATCC 33277, *T. forsythia* – ATCC 43037, *A. actinomycetemcomitans* – ATCC 29522, *P. nigrescens* – NCTC 9336, *T. denticola* and Leukotoxin *A. actinomycetemcomitans* (JP2). Microbiological analyses were performed by Polymerase Chain Reaction (PCR) using primers (Invitrogen, Frederick, MD, USA) and PCR conditions (Platinum Taq DNA Polymerase, Invitrogen, São Paulo, SP, Brazil) using a Mastercycler Gradient (Eppendorf, Hamburg, Germany) as previously described: *P. gingivalis*[19], *T. denticola*[20], *P. Nigrescens*[21], *T. forsythia*[20], *A. actinomycetemcomitans*[22] and Leukotoxin *A. actinomycetemcomitans*[23]. PCR amplification products were analysed by 1.5% agarose (BioAgency, São Paulo, SP, Brazil) gel electrophoresis. Each gel was stained with ethidium bromide and photographed on an ultraviolet light transilluminator (GDS 8000 System, UVP, Upland, CA, USA).

Genetic polymorphisms analysis

DNA was extracted from epithelial buccal cells with sequential phenol/chlorophorm/isoamilic alcohol solution (25:24:1) and precipitated with salt/ethanol solution[24].

Single nucleotide polymorphisms (SNPs) were investigated in the genes: *IL10* (-1087 G/A, -819 C/T, -592 C/A) (25) and *IL4* (-590 C/T (26), +33 C/T (27), Insertion/Deletion of a 70 bp in intron 3)[9]. Those polymorphisms in the *IL4* and *IL10* genes are in linkage disequilibria composing haplotypes[27,28].

The PCR-Restriction Fragment Length Polymorphism (RFLP) method was used to genotype subjects for the polymorphisms, except the -1087 G/A polymorphism in the *IL10* gene, which was genotyped by sequencing[25]. PCR reagents were similar to those used in detecting periodontopathogens, and the assay conditions were similar to those described by the references[9,25-27]. PCR-RFLP products were analysed in 10% polyacrylamide (Amersham Biosciences, Uppsala, Sweden) gel electrophoresis after silver staining[29].

Statistical analysis

Statistical analyses were performed using BioEstat 4.0 software (UFPA, MCT, CNPq, Belém, PA, Brazil). The mean values and standard deviations for all clinical parameters were calculated for each subject. To detect differences in clinical parameters between Generalized AgP and Localized AgP a Mann-Whitney's test was performed. The frequencies of periodontopathogens detected in the kindred were determined. Associations between each clinical parameter and the presence of a specific microorganism were assessed by Mann-Whitney's test. The differences were considered significant when $p < 0.05$.

In order to calculate gene heterozygosity, Hardy-Weinberg expectations and linkage disequilibrium, the computer program package ARLEQUIN 2.0 was used (ARLEQUIN v. 2000, Geneva, Switzerland).

Result

A total of 11 members of a family with Aggressive Periodontitis participated in this study. Table 1 shows the clinical, radiographic, microbiological and genetic findings in family members with Generalized AgP, Localized AgP and in one patient considered unaffected by AgP (II.9). Although the ages in the Generalized AgP and Localized AgP groups were similar (29.2 ± 13.4 and 27.5 ± 7.3 years old, respectively), all of the periodontal parameters in Generalized AgP were higher than those observed in Localized AgP. The comparison of periodontal parameters between both AgP groups showed statistically significant higher values of CAL ≥ 5mm ($p < 0.05$), probing depth ($p < 0.05$), visible plaque and bleeding on probing ($p < 0.01$) in Generalized AgP group. The Generalized AgP group showed more bone loss, but these values were not statistically different from those of the Localized AgP group. The proband (subject II.12) showed alveolar bone loss and clinical attachment loss in four teeth, aside the first molars and incisors, at a young age.

A total of 44 subgingival plaque samples were analyzed. The affected sites chosen for subgingival plaque collection had a mean probing depth of 6.8 mm. The mean probing depth of healthy sites was 2.5 mm. The most frequent periodontal pathogens found in the subgingival microbiota in this family were *A. actinomycetemcomitans* (72.7%), followed by *P. nigrescens* (51.5%), *T. forsythia* (45.5%), *P. gingivalis* (36.3%) and *T. denticola* (9.0%). The proband (subject II.12) was colonized by A. actinomycetemcomitans, *P. gingivalis*, *P. nigrescens* and *T. denticola*.

Table 1 shows the microorganisms that were detected in the subgingival plaque of each family member. *A. actinomycetemcomitans* was detected in all family members except the I.2, II.1 and II.3 individuals. The samples from the family members who were positive for *A. actinomycetemcomitans* were examined for the presence of highly leukotoxic strains of this microorganism by PCR. Only one affected site from sibling II.6 (Generalized AgP) was infected by a highly leukotoxic *A. actinomycetemcomitans* strain.

Descriptive results of the genetic analyses are shown in Table 1. The genotype distributions of the *IL4* and *IL10* polymorphisms were consistent with the assumption of Hardy-Weinberg equilibrium and revealed linkage disequilibria between all pairs of loci ($P < 0.001$), justifying the analyses of polymorphisms as haplotypes. Sixty percent of the family members affected by both types of AgP showed at least one TTD (*IL4*) and one ATA (*IL10*) haplotype. Although this family presented a high number of individuals with AgP (10 members), this number is too low to permit accurate statistical analyses. Moreover, because of the existence of one unaffected sibling, it was not possible to perform any statistical analyses in order to determine whether genetic data could be associated with susceptibility to AgP. Therefore, we were unable to demonstrate any associations between genetic findings and AgP, considering each polymorphism separately or in combination as haplotypes.

The relationships between the clinical findings and the presence of the periodontopathogens were examined using Mann-Whitney's test (Table 2). Pair-wise comparisons revealed that individuals infected by *P. gingivalis* had significantly higher percentages of sites with visible plaques, bleeding on probing and probing depth ($P = 0.03$) than individuals who were not infected by this microorganism. Furthermore, although not statistically significant, we observed an association between *P. nigrescens* and bleeding on probing and probing depth ($P = 0.06$).

Discussion

Because periodontitis is a complex disease, it is worth to discussing some of the genetic and environmental factors related to AgP in the studied family. Considering environmental factors, the high prevalence of AgP in the present Brazilian family seemed to be related to their poor socio-economic condition and oral hygiene habits, as a large amount of visible plaque was observed in the majority of family members (Table 1). Indeed, 30% of the kindred were current smokers (siblings II.1 and II.4) and former smokers (sibling II.3). These environmental factors must be taken into account along with the genetic constitutions of these kindred. Recently, it was reported that Brazilian, lighter-skinned black people presented with higher levels of periodontal disease when compared with white people (Odds Ratio = 1.5; 95% Confidence Interval = 1.2-1.8), even after controling for age, gender, schooling, per capita income and geographic region[30]. In a study of 642 young army recruits, the prevalence of AgP was correlated with genetic factors such as ethnic origin (North Africa, $P < 0.0001$) and environmental factors such as smoking habits ($P < 0.03$)[31].

Cortelli et al.[32] studied the prevalence of *A. actinomycetemcomitans* in subgingival plaque of patients with severe periodontitis. They detected *A. actinomycetemcomitans* in 63% of the pocket samples. In the present study, analysis of the subgingival microbiota revealed that *A. actinomycetemcomitans* is the most prevalent microorganism (72.7%). This result is in agreement with other studies of Brazilian AgP patients[33] and other ethnic populations[34,35]. It is worth noting that *A. actinomycetemcomitans* was also detected in the one sibling not affected by AgP (II.9). Similar results were reported by Slots, Ting[35], who observed *A. actinomycetemcomitans* in healthy sites and/or subjects. This fact could be explained by the presence of non-virulent pathogens in healthy sites[36]. In contrast, *A. actinomycetemcomitans* is also thought to be closely associated with AgP[1]. Interestingly, this mi-

Table 1. Clinical, radiographic, microbiological and genetic findings of family members.

	Subjects	Age	CAL ≥ 5 mm (%)	VP (%)	BoP (%)	PD ≥ 4mm (%)	Alveolar bone loss (% per teeth)		Microorganisms	Genetic Haplotypes	
							Anterior	Posterior		*IL4*	*IL10*
Generalized AgP	I. 2	54	35.2	72.2	88.9	27.7	44.4	22.2	P.n	CCI/ CCI	GCC/ ATC
	II. 3	31	28.6	77.4	65.9	20.6	4.7	28.6	P.g, P.n, T.d, T.f	CCI/ TTD	GCC/ ATA
	II. 4	29	54.2	82.8	95.8	30.2	6.3	56.3	A.a, P.g, P.n	CCI/ TTD	GCC/ ATA
	II. 6	25	15.9	86.9	63.8	11.6	8.7	34.8	A.a	CCI/ CCI	GCC/ ATA
	II. 10	20	4.8	76.8	56.5	11.3	0.0	3.6	A.a, P.g, P.n, T.f	CCI/ TTD	GCC/ ATA
	II. 12	18	19.0	90.2	91.1	32.7	21.4	39.3	A.a, P.g, P.n, T.f	CCI/ TTD	GCC/ ATA
	Mean ± SD	29.2 ± 13.4	26.3 ± 17.3*	81 ± 6.8**	77 ± 16.8**	22.4 ± 9.4*	14 ± 16.4	30.8 ± 17.6			
Localized AgP	II. 1	35	7.3	65.0	33.3	7.3	0.0	16.0	T.f	CCI/ TTD	ATA/ ATA
	II. 2	32	7.0	75.0	44.6	10.2	9.7	16.2	A.a	CCI/ CCI	ATA/ ATA
	II. 7	24	2.2	47.5	31.6	5.0	0.0	13.3	A.a, P.n, T.f	CCI/ CCI	GCC/ ATA
	II. 11	19	1.7	41.4	28.7	6.3	0.0	0.0	A.a	CCI/ TTD	GCC/ ATA
	Mean ± SD	27.5 ± 7.3	4.5 ± 3.0	57.2 ± 15	34.5 ± 6.9	7.2 ± 2.2	2.4 ± 4.8	11.4 ± 7.1			
Unaffected	II. 9	22	0.0	35.9	38.5	3.6	0.0	0.0	A.a	CCI/ CCI	GCC/ ATA

CAL = Clinical Attachment Loss; VP = Visible Plaque; BoP = Bleeding on Probing; PD = Probing Depth; ± SD = Standard Deviation.

*Difference statistically significant between means of groups with Generalized AgP and Localized AgP in the parameter analysed (Mann-Whitney's test), $P < 0.05$.

**Difference statistically significant between means of groups with Generalized AgP and Localized AgP in the parameter analysed (Mann-Whitney' test), $P < 0.01$.

Table 2. Clinical findings of the kindred by presence of periodontopathogens

Periodontal parameters (%)	Pathogens - Mean values (± SD)				
	A. a.	*P. g.*	*P. n.*	*T. d.*	*T. f.*
CAL ≥ 5 mm	13.1 (± 17.9)	26.6 (± 20.8)	24 (19.6)	NA	12.3 (11.1)
VP	67.1 (± 21.8)	81.8 (± 6.2)*	74.4 (14.5)	NA	71.3 (16.0)
BoP	56.3 (± 25.7)	77.3 (± 19.1)*	71.6 (25.0)	NA	55.7 (24.7)
PD ≥ 4 mm	13.8 (± 11.3)	23.7 (± 9.8)*	21.2 (11.1)	NA	15.4 (11.4)
Anterior alveolar bone loss	5.7 (± 7.6)	8.1 (± 9.3)	12.8 (17.4)	NA	5.2 (9.3)
Posterior alveolar bone loss	20.4 (± 20.8)	31.9 (± 22.1)	27.2 (18.8)	NA	20.2 (13.9)

CAL = Clinical Attachment Loss; VP = Visible Plaque; BoP = Bleeding on Probing; PD = Probing Depth; ± SD = Standard Deviation; NA = not; ± SD = standard deviations; * P ≤ 0.05 by Mann-Whitney's test.

croorganism was detected in all family members except sibling II.1. Therefore, the presence of this pathogen may be an important factor in the diagnosis of AgP, as suggested by Schacher et al.[37]. Indeed, intrafamilial transmission of *A. actinomycetemcomitans*, where parents and siblings of an individual with *A. actinomycetemcomitans*-positive AgP may have an increased susceptibility to periodontitis, was previously demonstrated[38].

Some studies[11,15] have shown the existence of different microbial associations in subgingival plaque. Several authors[14,32,39,40] have reported an association between *P. gingivalis*, *T. denticola* and *T. forsythensis* with periodontal disease. This microbial association, combined with clinical signs of periodontitis, was found in the subgingival plaque of sibling II.3, while the plaques of siblings II.10 and II.12 contained *P. gingivalis* and *T. forsythia* (Table 1).

The present study reveals a statistically significant association between clinical findings (VP, BoP and PD ≥ 4 mm) and the presence of *P. gingivalis* (Table 2). Papapanou et al.[14] showed evidence that suggested a relationship between the amount of *P. gingivalis* and the level of periodontitis progression in an untreated Chinese population. Some studies have demonstrated higher levels of periodontal inflammation with a concomitant increase in the prevalence of pathogens such as *A. actinomycetemcomitans*, *P. gingivalis*, *P. nigrescens*, *T. denticola* and *T. forsythensis*[13,41].

It is important to note that the Brazilian family studied here was also studied by Trevilatto et al.[42] However, comparisons between our data and the previous data were not possible due to differences in study design. While periodontal examination in the current study was performed in 2004 and considered six points around each tooth, periodontal examination in the previous study was performed in 1998 and considered four points[42]. In the first report of this family, there was no information about visual plaques, bleeding on probing or probing depth. In the current study, four sites of each individual (two affected and two healthy) were chosen for collecting gingival fluid. Fluid was obtained by inserting paper points into the sites for 30 seconds and then placing the samples into a vial of Ringer solution. Trevilatto et al.[42] examined only affected sites (the number was not mentioned), and the samples were placed into vials of sterile water. PCR was used to detect *T. denticola* and *A. actinomycetemcomitans*. Moreover, we have tested for the presence of toxic strains of *A. actinomycetemcomitans* in the family members infected by this microorganism and analyzed the relationship between clinical data and the presence of periodontopathogens. Finally, different genes and haplotypes were used for genetic analysis in the present study.

Host mechanisms, such as genetic factors, are important in determining one's susceptibility to periodontitis[43,44]. Although some groups have investigated SNPs in the *IL4*[9] and *IL10*[45] genes in individuals with early-onset periodontitis, this is the first investigation of these specific *IL4* and *IL10* haplotypes.

Sixty percent of the family members with AgP showed a haplotype formed by at least one TTD (-590; +33; 70 bp of *IL4* gene) and one ATA (-1087; -819; -592 of *IL10* gene). Although we were unable to demonstrate a statistically significant association between these haplotypes and AgP (there is only one sibling unaffected by AgP), we speculate that this combination may be biologically relevant in the context of AgP. It has been reported that a homozygous composite genotype formed by -590T and 70 bp (Del) *IL4* polymorphisms was found in 27.8% of individuals with early-onset periodontitis and that they had IL-4 serum levels below the detection limit[9]. Moreover, the homozygous ATA haplotype in the *IL10* gene was associated with lower IL-10 production following microbial lipopolysaccharide (LPS) stimulation[46]. Although Yamazaki et al.[45] and Gonzales et al.[47] did not find a relationship between *IL10* SNPs and AgP (likely due to the ethnicity and size of those populations), Brazilian women with the ATA haplotype were 2.57 times more susceptible to developing chronic periodontitis (OR = 2.57; 95% CI = 1.10 – 5.95)[25].

Our results suggest that the combination of TTD and ATA may confer a synergistic, depleted anti-inflammatory response due to a lower production of IL-4 and IL-10 anti-inflammatory cytokines. Therefore, this may contribute to the development of periodontitis in this studied family. Moreover, the following studies support this hypothesis: (i) lack of IL-4 contributes to the continuous accumulation of macrophages and the high production of inflammatory mediators, such as IL1-β, in periodontal tissues[48], (ii) IL-10, another anti-inflammatory cytokine, acts by inhibiting the synthesis of pro-inflammatory cytokines and bone resorption in vitro[49] and (iii) lower levels of IL-4 and IL-10 were found in the gingival crevicular fluid of individuals with chronic periodontitis, suggesting that the initiation and progression of periodontal inflammation might be due to a lack or inappropriate response of anti-inflammatory cytokines[50]. Interestingly, the conclusion of Bozkurt et al.[50] is in agreement with the idea that the absence of IL-4 triggers periodontal disease, as suggested by Shapira et al.[48]. In contrast, to confirm the hypothesis presented here of the biological and synergistic relevance of the TTD and ATA haplotypes in the *IL4* and *IL10* genes in host susceptibility to AgP, segregation analysis of these polymorphisms in other families with AgP is required.

In conclusion, the results presented here revealed a large number of family members affected by AgP. *A. actinomycetemcomitans* was the most prevalent pathogen detected, and *P. gingivalis* was associated with the clinical parameters of periodontitis. Although the majority of AgP family members had at least one TTD/ATA (*IL4/IL10*) haplotype combination, we were unable to demonstrate an association between this haplotype and AgP.

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References

1. Tonetti MS, Mombelli A. Early-onset periodontitis. Ann Periodontol. 1999;4:39-52.
2. Llorente MA, Griffiths GS. Periodontal status among relatives of aggressive periodontitis patients and reliability of family history report. J Clin Periodontol. 2006;33:121-5.
3. Page RC, Vandersteen GE, Ebersole JL, Williams BL, Dixon IL, Altman LC. Clinical and laboratory studies of a family with high prevalence of juvenile periodontitis. J Periodontol. 1985;66:602-10.
4. Marazita ML, Burmeister LC, Gunsolley JC, Koertge TE, Lake K, Schenkein HA. Evidence for autosomal dominant inheritance and race-specific heterogeneity in early-onset periodontitis. J Periodontol. 1994;65:623-30.
5. Beaty TH, Boughman JA, Yang P, Astemborski JA, Suzuki JB. Genetic analysis of juvenile periodontitis in families ascertained through an affected proband. Am J Hum Genet. 1987;40:443-52.
6. Hodge PJ, Teaque PW, Wright AF, Kiane DF. Clinical and genetic analysis of a large north European Caucasian family affected by early-onset periodontitis. J Dent Res. 2000;79:857-63.
7. Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol. 2005;32:159-79.
8. Tai H, Endo M, Shimada Y, Gou E, Kobayashi T, Yamazaki K, et al. Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. J Clin Periodontol. 2002;29:882-8.
9. Michel J, Gonzáles JR, Wunderlich D, Herrmann JM, Meyle J. Interlekin-4 polymorphisms in early-onset periodontitis. J Clin Periodontol. 2001;28:483-8.
10. Kinane DF, Hart TC. Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med. 2003;14:430-49.
11. Kamma JJ, Nakou M, Gmür R, Baehni PC. Microbiological profile of early onset/ aggressive periodontitis patients. Oral Microbiol Immunol. 2004;19:314-21.
12. Timmerman MF, van der Weijden GA, Arief EM, Armand S, Abbas F, Winkel EG, et al. Untreated periodontal disease in Indonesian adolescents. Subgingival microbiota in relation to experienced progression of periodontits. J Clin Periodontol. 2001;28:617-27.
13. Mullaly BH, Dace B, Schelburne CE, Wolff LF, Coulter WA. Prevalence of periodontal pathogens in localized and generalized forms of early onset periodontitis. J Periodontal Res. 2000;35:232-41.
14. Papapanou PN, Baelum V, Luan WM, Madianos PN, Chen X, Fejerskov O, et al. Subgingival microbiota in adult Chinese: prevalence and relation to periodontal disease progression. J Periodontol. 1997;68:651-66.
15. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent Jr RL. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25:134-44.
16. Lang NP, Bartold PM, Cullinam M, Jeffcoat M, Mombelli A, Murakami S, et al.. Consensus Report: aggressive periodontitis. Ann Periodontol. 1999;4:53.
17. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol. 1967;38:610-6.
18. Savitt ED, Strzempko MN, Vaccaro KK, Peros WJ, French CK. Comparison of cultural methods and DNA probe analyses for the detection of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and

Bacteroides intermedius in subgingival plaque samples. J Periodontol. 1988;59:431-8.
19. Benkirane RR, Guillot E, Mouton C. Immunonagnetic PCR and DNA probe for detection and identification of *Porphyromonas gingivalis*. J Clin Microbiol. 1995;33:2908-12.
20. Ashimoto A, Chen, C, Bakker I, Slots J. Polymerase chain reaction 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol. 1996;11:266-73.
21. Guillot E, Mouton C. PCR-DNA probe assays for identification and detection of *Prevotella intermedia* sensu strictu and *Prevotella nigrescens*. J Clin Microbiol. 1997;35:1876-82.
22. Ávila-Campos MJ, Velásquez-Meléndez G. Prevalence of putative periodontopathogens from periodontal patients and healthy subjects in São Paulo, SP, Brasil. Rev Inst Med Trop Sao Paulo. 2002;44:1-5.
23. Rosalem Jr W, de Souza RC, de Andrade AFB, Colombo APV. Analysis of leukotoxin gene types of *Actinobacillus actinomycetemcomitans* in Brazilians with aggressive periodontitis. Braz J Microbiol. 2006;7:127-34.
24. Sambrook J, Russel DW. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2001.
25. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito Jr RB, Camargo LEA, Line SRP. *Interleukin 10* gene promoter polymorphisms are associated with chronic periodontitis. J Clin Periodontol. 2004;31:443-8.
26. Scarel-Caminaga RM, Trevilatto PC, Souza AP, Brito Jr RB, Line SRP. Investigation of *IL4* gene polymorphism in individuals with different levels of chronic periodontitis in a Brazilian population. J Clin Periodontol. 2003;30:587-91.
27. Noguchi E, Nukaga-Nishio Y, Jian Z, Yokuchi Y, Kamioka M, Yamakawa-Kobayashi K, et al. Haplotypes of the 5' region of the IL-4 gene SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. Hum Immunol. 2001;62:1251-7.
28. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnot PJ, Hutchinson I. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet. 1997;24:1-8.
29. Sanguinetti CJ, Neto ED, Simpson AJG. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. BioTechniques. 1994;17:915-9.
30. Peres MA, Antunes JLF, Boing AF, Peres KG, Bastos JLD. Skin colour is associated with periodontal disease in Brazilian adults: a population-based oral health survey. J Clin Periodontol. 2007;34:196-201.
31. Levin L, Baev V, Lev R, Stabholz A, Ashkenazi M. Aggressive periodontitis among young Israeli army personnel. J Periodontol. 2006;77:1392-6.
32. Cortelli JR, Cortelli SC, Jordan SF, Haraszthy VI, Zambon JJ. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol. 2005;32:860-6.
33. Tinoco EMB, Beldi MI, Loureiro CA, Lana M, Campedelli F, Tinoco NM, et al. Localized juvenile periodontitis and *Actinobacillus actinomycetemcomitans* in a Brazilian population. Eur J Oral Sci. 1997;105:9-14.
34. Zambon JJ, Christersson LA, Slots J. *Actinobacillus actinomycetemcomitans* in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. J Periodontol. 1983;54:707-11.
35. Slots J, Ting M. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. Periodontol 2000. 1999;20:82-121.
36. Socranky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol. 1992;63:322-31.
37. Schacher B, Baron F, Roβberg M, Wohlfeil M, Arndt R, Eickholz P. *Aggregatibacter actinomycetemcomitanns* as indicator for aggressive periodontitis by two analysing strategies. J Clin Periodontol. 2007;34:566-73.
38. Dogan B, Kipalev AS, Okte E, Sultan N, Asikainen SE. Consistent intrafamilial transmission of *Actinobacillus actinomycetemcomitans* despite clonal diversity. J Periodontol. 2008;79:307-15.
39. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000. 1994;5:78-111.
40. Haffajee AD, Cugini MA, Dibart S, Smith C, Kent Jr RL, Socransky SS. Clinical and microbiological features of subjects with adult periodontitis who responded poorly to scaling and root planning. J Clin Periodontol. 1997;24:767-76.
41. Darby IB, Hodge PJ, Riggio MP, Kinane DF. Microbial comparison of smoker and non-smoker adult and early-onset periodontitis patients by polymerase chain reaction. J Clin Periodontol. 2000;27:417-24.
42. Trevilatto PC, Tramontina VA, Machado MAN, Gonçalves RB, Sallum AW, Line SRP. Clinical, genetic and microbiological findings in a Brazilian family with aggressive periodontitis. J Clin Periodontol. 2002;29:233-9.
43. Kornman KS, Crane A, Wang HY, Di Giovine FS, Newman MG, Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol. 1997;24:72-7.

44. Shapira L, Wilensky A, Kinane DF. Effect of genetic variability on the inflammatory response to periodontal infection. J Clin Periodontol. 2005;32:72-86.
45. Yamazaki K, Tabeta K, Nakajima T, Ohsawa Y, Ueki K, Itoh H, et al. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. J Clin Periodontol. 2001;28:828-32.
46. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum. 1999;42:1101-8.
47. Gonzales JR, Michel J, Diete A, Herrmann JM, Böedeker RH, Meyle J. Analysis of genetic polymorphisms at the interleukin-10 loci in aggressive and chronic periodontitis. J Clin Periodontol. 2002;29:816-22.
48. Shapira L, van Dyke TE, Hart TC. A localized absence of interleukin-4 triggers Periodontal disease activity: a novel hypothesis. Med Hypoth. 1992;39:319-22.
49. Alaeddine N, DiBattista JA, Pelletier JP, Kiansa K, Clouter JM, Martel-Pelletier J. Inhibition of TNF- induced PGE2 production by anti-inflammatory cytokines IL-4, IL-10 and IL-13 in osteoarthritic synovial fibroblasts: distinct targeting in signaling pathways. Arthritis Rheum. 1999;42:710-20.
50. Bozkurt FY, Ay ZY, Berker E, Tepe E, Akkus S. Anti-inflammatory cytokines in gingival crevicular fluid in patients with periodontitis and rheumatoid arthritis: a preliminary report. Cytokine. 2006;35:180-5.

Corresponding author:
Profa. Dra. Raquel Mantuaneli Scarel-Caminaga
raquel@foar.unesp.br

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