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# Interleukin-4 gene polymorphisms in Japanese and Caucasian patients with aggressive periodontitis

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***Abstract***

**Objectives:** Recently, interleukin (IL) 4 gene polymorphisms have been analyzed in association with periodontitis. Genetic differences between Caucasian and Japanese patients with periodontitis have previously been detected. The aim of the present study was to analyze IL-4 genotypes in Caucasian and Japanese patients with aggressive periodontitis (AgP).

**Material and Methods:** One hundred and twenty-four subjects were included in the study, 31 Japanese and 30 Caucasian patients with generalized AgP, plus 30 Japanese and 33 Caucasian healthy controls. IL-4 polymorphisms were determined by polymerase chain reaction. A logistic regression was used to investigate the possible association of the genotypes with the disease in both populations. Odds ratio (OR) estimates were analyzed for allele frequencies.

**Results:** No significant association of IL-4 polymorphisms with the risk of AgP was determined in either population. However, the allele frequencies showed different results between populations. The carriage of the polymorphism in intron 2 was higher in Caucasian patients compared with controls (OR: 2.0, 95% confidence interval: [1.0;4.2]. Furthermore, the frequency of the IL-4 promoter/intron 2 composite genotype (PP+/IP+) in patients and controls, respectively, was found to be approximately 25% and 60% higher in the Japanese population than in the Caucasian population.

**Conclusion:** There was no evidence of an association of IL-4 genotypes and AgP in either population, although the frequencies of the IL-4 genotypes in the Japanese and the Caucasians were different.

Key words: aggressive periodontitis; genetic polymorphism; interleukin-4; population

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Bacteria found in dental plaque are the initiators of periodontitis, but disease progression and development depends on the immune reactions of the host. The role of T cells in the pathogenesis of periodontitis has been investigated as they play a key role in the regulation of immune responses. T helper (Th) cells can be classified into at least three distinct subsets according to their cytokine production and functional properties: Th1, Th2 and Th0 cells (Salgame et al. 1991). The Th1 subset produces interleukin (IL)2, IFN-α, TNF, and lymphotoxin and facilitates cell-mediated immune responses, whereas the Th2 subset produces mainly IL-4, IL-5, IL-6, and IL-10 and assists in antibody production. Specifically, IL-4 is produced mainly by activated Th2 cells and is shown to stimulate B cell proliferation, to regulate immunoglobulin (Ig) class switching to IgG1 and IgE, and to promote T cell development (Paul 1991). It has been hypothesized that susceptibility to periodontitis may involve a Th2 response to specific types of periodontal bacteria (Gemmell & Seymour 1994), and that a localized lack of IL-4 may increase predisposition to periodontitis (Shapira et al. 1992).

It has recently been demonstrated that the susceptibility to chronic periodontitis correlates with heritability in approximately 50% of cases (Michalowicz et al. 2000) and it has been

postulated that the susceptibility to aggressive periodontitis (AgP) may also be heritable (Marazita et al. 1994). Consequently, various host risk factors involved in the pathogenesis of AgP have been analyzed at a molecular level, showing different results. While some studies have failed to detect a significant association with the disease, i.e. the genes encoding the cytokines TNF-α, IL-1 and IL-10 (Kinane et al. 1999, Walker et al. 2000, Hodge et al. 2001, Shapira et al. 2001, Yamazaki et al. 2001, Gonzales et al. 2002), others have found significant associations with AgP, i.e. certain genotypes of the Fc-receptors expressed in the surface of phagocytic cells (FcγRIIa, FcγRIIIa and FcγRIIIb) (Wilson & Kalmar 1996, Kobayashi et al. 2000) or in the human leukocyte antigens (HLA) encoded in the human major histocompatibility complex (MHC) (Shapira et al. 1994, Takashiba et al. 1994). Additionally, while polymorphisms at the vitamin D receptor (VDR) and at the *N*-formyl-1-methionyl - 1 - leucyl - 1 - phenylalanine (FMLP) receptor genes were associated with the localized form (Gwinn et al. 1999, Hennig et al. 1999), genotypes of the interleukin-1β (IL-1β) and the IL-1 receptor antagonist (IL-1RA) were found to be associated with the generalized form (Parkhill et al. 2000). Recently, two polymorphisms at the IL-4 gene were identified in Caucasian AgP patients, a C to T single nucleotide polymorphism (SNP) at position −590 in the promoter region, and a 70 bp repeat in intron 2. IL-4 levels in the sera of these patients were significantly lower compared with controls, suggesting an association of these polymorphisms with the disease (Michel et al. 2001).

In addition to current advances in the identification of genetic risk factors associated with inflammatory diseases, actual knowledge of molecular variability between populations is also increasing. Recent data suggest that this variability may exist in patients with AgP. Significant differences in the distribution of IL-1 genotypes between Caucasians and African Americans (Walker et al. 2000), Caucasians and Japanese (Tai et al. 2002), and of FcγRIIIa genotypes and haplotypes within the IL-10 promoter between Caucasians and Japanese are some examples (Turner et al. 1997, Sugita et al. 1999, Meisel et al. 2001, Yamazaki et al. 2001).

The present study was thus performed to evaluate the prevalence and association of the IL-4 polymorphisms in the promoter (−590 C to T) and in the intron 2 (70 bp repeat) in Japanese patients with AgP compared with unrelated controls. A comparison with the genotype distribution between Japanese and Caucasian patients with AgP was a further aim.

## Material and Methods

### Selection of subjects

Sixty-one unrelated patients were recruited to this study. Thirty-one Japanese patients with AgP referred to the Periodontal Clinic of the Niigata University Dental Hospital. Informed consent was obtained from all participants with the signed form that was previously reviewed and approved by the Ethical Committee for the Use of Human Subjects in Research, Niigata University Faculty of Dentistry. Thirty white Caucasians of European origin were selected from patients presenting at the Dental School Department of Periodontology, University of Giessen. The selection of patients was made according to the clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions (Armitage 1999), using five clinical parameters and full mouth or panoramic radiographs of diagnostic quality. All patients presented the generalized form of AgP, with interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors. All patients were under 35 years of age at the time of inclusion, with the exception of two patients in the Caucasian population, who were over this age but had a clear history of symptoms of the disease prior to that age. Subjects completed personal and familial medical and dental history questionnaires; exclusion criteria were a history of diabetes, current pregnancy or lactation, chronic usage of anti-inflammatory drugs and a history of hepatitis or HIV infection.

The control group included race-matched healthy subjects of either population (30 Japanese and 33 Caucasian) with no evidence of periodontitis. The following clinical parameters were assessed in patients and controls using a manual probe (PCP-UNC 15): probing pocket depths (PPDs), clinical attachment level (CAL), and bleeding upon probing (BOP) at six sites per tooth. Additionally, a modified plaque index (PLI) and a modified papillary bleeding index (PBI) were recorded at four sites per tooth (O'Leary et al. 1972).

### Isolation of genomic DNA

In the Japanese subjects, genomic DNA was isolated from peripheral blood using a DNA isolation kit (Easy-DNA® Kit; Invitrogen, San Diego, CA, USA). In Germany, whole blood samples were obtained (EDTA-blood: 1 mg/ml), and genomic DNA was isolated according to the instructions of the manufacturer of a different kit (InstaGene Whole Blood Kit, Bio-Rad Laboratories GmbH, Munich, Germany). This typically yielded 5 ng DNA/μl.

### Genotyping

Japanese samples were sent to Germany, where genotyping was performed employing polymerase chain reaction (PCR) and restriction fragment length product (RFLP) techniques. A Mastercycler Gradient was used (Eppendorf–Netheler–Hinz, Hamburg, Germany). Details of the PCR and RFLP methods have previously been reported (Michel et al. 2001) and are briefly described here: the IL-4 70 bp repeat polymorphism in intron 2 was determined using the following oligonucleotide primers: 5′-TAG GCT GAA AGG GGG AAA GC-3′ and 5′-CTG TTC ACC TCA ACT GCT CC-3′. PCR was carried out using hot start Taq polymerase (HotStarTaq™, Qiagen, Hilden, Germany) in a volume of 100 μl containing 25 ng of genomic DNA under standard buffer conditions. Cycling was performed as follows: (95°C, 15 min) 1×, (94°C 1 min; 55°C, 1 min; 72°C 1 min) 40×, (72°C 10 min) 1×. The allele 1 (183 bp) and allele 2 (253 bp) were visualized on a 2% agarose gel after electrophoresis and ethidium bromide staining.

The IL-4 C to T promoter polymorphism at position −590 was determined using the oligonucleotide primers: 5′-ACT AGG CCT CAC CTG ATA CG-3′ and 5′-GTT GTA ATG CAG TCC TCC TG-3′. PCR was carried out using hot start Taq (HotStarTaq®, Qiagen) in a volume of 100 μl containing 50 ng of genomic DNA under standard buffer conditions. Cycling conditions were (95°C, 15 min) 1×, (94°C 1 min; 57°C, 1 min; 72°C 1 min) 40×, (72°C 10 min) 1×. This resulted in a PCR product of 252 bp

spanning positions −522 to −774 in the IL-4 promoter region, verified by using 1/5th of the volume of the PCR reaction on a 2% agarose gel after electrophoresis and ethidium bromide staining. The remaining PCR product was purified using the QIAquick PCR Purification Kit (Qiagen) according to the instructions of the manufacturer, and subsequently digested with 6 U *Bsm*FI (NEB, Schwalbach, Taunus, Germany) in 1 × NEBuffer 4 in a total volume of 40 µl for 6 h at 65°C. Cleavage by *Bsm*FI results in two fragments of 192 and 60 bp, whereas the −590 C to T polymorphism abolishes this site. The resulting products were visualized on a 3% agarose gel after electrophoresis and ethidium bromide staining.

#### Data analysis

There are three allele expressions for the genotypes in the –590 promoter region (IL-4 promoter) and for the genotypes in intron 2 (IL-4 intron): those subjects who are homozygous for allele 1 (1,1), homozygous for allele 2 (2,2) and heterozygous (1,2). The homozygous individuals for the polymorphic alleles have been previously detected in higher numbers in Caucasian patients with AgP (Michel et al. 2001). Therefore, a composite analysis consisting of carriage of the −590 C to T polymorphism in the promoter region and a 70 bp repeat polymorphism in intron 2, has been proposed. That means, all those individuals who are homozygous for allele 2 in the IL-4 promoter and homozygous for allele 1 in the IL-4 intron 2 are carrying the positive genotype (PP+/IP+). Conversely, all subjects who are neither homozygous for allele 2 in the IL-4 promoter nor homozygous for allele 1 in the IL-4 intron 2, are carrying the negative genotype (PP−/IP−). In the present study, these polymorphisms were therefore analyzed separately and in combination. The Fisher's exact test was used to analyze whether the distributions of the genotypes and alleles in patients and controls were different within populations (SAS Institute Inc., Cary, NC, USA). Significance was set at 5%. A logistic regression (Wald test) was used in order to analyze whether the different genotypes of the two populations were associated with AgP (Kleinbaum 1994). The disease was included as the dependent variable, whereas the different genotypes and the different alleles were estimated as explanatory variables. Additionally, odds ratios (ORs) and confidence intervals (CIs) were calculated separately for the IL-4 promoter and IL-4 intron 2 alleles for both populations.

Deviation from Hardy–Weinberg equilibrium was assessed for both loci in both populations, also separated for cases and controls, by goodness-of-fit between the observed and expected numbers using $\chi^2$ test with 1 degree of freedom.

## Results

Table 1 provides a summary of the clinical characteristics in cases and controls in the two populations. Thirty-one Japanese subjects (mean age: 31 ± 4 years) and 30 Caucasians of European heritage (mean age: 29 ± 6 years) presented an aggressive form of periodontitis. Additionally, 30 Japanese (mean age: 24 ± 1 years) and 33 Caucasian (mean age: 25 ± 3 years) healthy subjects were included as controls. Radiographic bone loss analysis indicated that all patients presented generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors (data not shown).

All genotype frequencies were in Hardy–Weinberg equilibrium. The distribution of genotypes and alleles is described in Table 2. The ORs and CIs for the alleles are included in the legend to this table.

#### −590 C to T promoter polymorphism

In the Japanese population, the distribution of homozygous individuals for allele 2 was higher in both groups. No significant difference between patients and controls was found for IL-4 promoter alleles ($p = 0.2$, Fisher's exact). The logistic regression, however, showed an indication for the explanatory contribution of IL-4 promoter genotypes 1,2 versus 2,2 ($p = 0.09$; OR: 2.6, 95% CI: [0.8;8.3]). In Caucasians, a higher distribution of heterozygous individuals was estimated for both groups. No significant difference between patients and controls was found for IL-4 promoter alleles ($p = 0.3$, Fisher's exact). The logistic regression analysis showed no indication for an explanatory contribution of IL-4 genotypes 1,2 versus 2,2 or 1,1 versus 2,2 in this population.

#### 70bp repeat polymorphism in intron 2

In the Japanese population, more individuals were homozygous for allele 1. No significant differences were found for the distribution of genotypes between patients and controls ($p = 0.2$, Fisher's exact). The logistic regression showed no tendency for the explanatory contribution of IL-4 intron genotypes 1,2 versus 1,1 or for the genotypes 2,2 versus 1,1. In the Caucasian population, no significant differences between patients and controls were estimated ($p = 0.1$, Fisher's exact), and no tendency for an explanatory contribution was detected for the genotypes 1,2 versus 1,1. In this case, however, the logistic regression showed an indication for an explanatory contribution of genotypes 2,2 versus 1,1 ($p = 0.08$; OR: 0.1, 95% CI: [0.01;1.3]).

*Table 1.* Clinical parameters of patients and controls in Japanese and Caucasian populations

| Clinical parameters | Japanese | | Caucasian | |
|---|---|---|---|---|
| | patients ($n = 31$) | controls ($n = 30$) | patients ($n = 30$) | controls ($n = 33$) |
| PPD (mm)* | 4.1 (3.3;5.1) | 1.8 (1.6;1.8) | 3.4 (2.8;4.2) | 1.8 (1.6;2.0) |
| PPD (mm)† | 4.2 ± 1.1 | 1.7 ± 0.2 | 3.6 ± 1.1 | 1.8 ± 0.2 |
| CAL (mm)* | 4.5 (3.8;5.9) | 1.8 (1.6;1.8) | 3.4 (2.8;4.8) | 1.8 (1.6;2.0) |
| CAL (mm)† | 4.7 ± 1.4 | 1.8 ± 0.2 | 3.8 ± 1.3 | 1.9 ± 0.3 |
| BOP (%)* | 33.3 (13.9;75.0) | 5.9 (3.3;10.0) | 52.3 (20.3;68.6) | 5.7 (1.6;9.7) |
| BOP (%)† | 44.8 ± 35.1 | 6.9 ± 4.7 | 47.6 ± 28.5 | 6.8 ± 6.4 |
| PLI (%)* | 59.8 (35.2;79.4) | 26.7 (22.5;33.3) | 55.2 (20.8;73.5) | 37.0 (24.5;47.1) |
| PLI (%)† | 56.0 ± 22.9 | 27.8 ± 7.7 | 50 ± 31.8 | 37.2 ± 16.2 |
| PBI (%)* | ND | ND | 23 (7.7;47.8) | 5.0 (1.0;8.5) |
| PBI (%)† | ND | ND | 29.7 ± 26.4 | 7.6 ± 9.3 |

PPD, probing pocket depth; CAL, clinical attachment level (6 × /tooth); BOP, bleeding on probing; PLI, plaque index; PBI, papillary bleeding index (percentage of total sites 6 × /tooth); ND, not determined; SD, standard deviation.

*Median and interquartile range.

†Mean values ± SD (in order to be comparable with literature).

*Table 2*. Frequency of IL-4 genotypes and alleles in patients and controls in each population

| Genotypes | Japanese | | Caucasian | |
|---|---|---|---|---|
| | Patients ($n = 31$) *n* (%) | Controls ($n = 30$) *n* (%) | Patients ($n = 30$) *n* (%) | Controls ($n = 33$) *n* (%) |
| IL-4 promoter | | | | |
| 1,1 | 4 (12.9) | 3 (10) | 6 (20) | 12 (36.3) |
| 1,2 | 13 (41.9) | 7 (23.3) | 16 (53.4) | 14 (42.4) |
| 2,2 | 14 (45.2) | 20 (66.7) | 8 (26.6) | 7 (21.3) |
| Allele 1 (wild type) | 21 (33.9) | 13 (21.7) | 28 (46.6) | 38 (57.5) |
| Allele 2 (C to T) | 41 (66.1) | 47 (78.3) | 32 (53.3) | 28 (42.5) |
| IL-4 intron | | | | |
| 1,1 | 13 (42) | 19 (63.3) | 4 (13.3) | 1 (3) |
| 1,2 | 14 (45.1) | 8 (26.7) | 18 (60) | 16 (48.5) |
| 2,2 | 4 (12.9) | 3 (10) | 8 (26.7) | 16 (48.5) |
| Allele 1 (polymorph.) | 40 (64.5) | 46 (76.7) | 26 (43.3) | 18 (27.3) |
| Allele 2 (wild type) | 22 (35.5) | 14 (23.3) | 34 (56.7) | 48 (72.7) |
| Total *n* of alleles | 62 | 60 | 60 | 66 |
| PP−/IP− | 19 (61.3) | 11 (36.7) | 26 (86.7) | 32 (97) |
| PP+/IP+ | 12 (38.7) | 19 (63.3) | 4 (13.3) | 1 (3) |

*n* = number of individuals in each group. Percentages in parentheses. In Japanese, for total number of patients (62 alleles) and total number of controls (60 alleles) OR = 0.5 (95% CI: [0.2;1.2]) for the promoter polymorphism, and OR = 0.5 (95% CI: [0.2;1.2]) for the polymorphism in intron 2. In Caucasians, for total number of patients (60 alleles) and total number of controls (66 alleles) OR = 1.5 (95% CI: [0.8;3.1]) for the promoter polymorphism, and OR = 2.0 (95% CI: [1.0;4.2]) for the polymorphism in intron 2. Frequency of the positive (PP+/IP+) and negative (PP−/IP−) composite analysis of the IL-4 genotypes in patients and controls in each population. IL, interleukin; OR, odds ratio; CI, confidence interval.

#### Composite analysis

In the Japanese population, the logistic regression showed an explanatory contribution of the negative (PP−/IP−) genotype versus the positive (PP+/IP+) genotype ($p = 0.05$; OR: 0.4, 95% CI: [0.1;1.0]).

In the Caucasian population, the logistic regression model showed no tendency for an explanatory contribution of the negative (PP−/IP−) genotype versus the positive (PP+/IP+) genotype ($p = 0.1$; OR: 4.9, 95% CI: [0.5;46.8]).

## Discussion

The cellular mechanisms involved in periodontitis and the T cytokine profiles at the local and peripheral levels have been described by Gemmell & Seymour (1994, 1998). According to the hypothesis of these authors, susceptible subjects for the progression of periodontitis present a Th2-dominated response, whereas non-susceptible subjects respond predominantly with a Th1-dominated response. The cytokine profiles of cells extracted from humans with periodontal disease have been analyzed by several investigators. Specifically, IL-4 has been analyzed alone or in combination with other cytokines at the local and peripheral levels. Levels of IL-4 were found to be elevated in the sera of patients with periodontal disease (McFarlane & Meikle 1991) and the percentage of IL-4 producing cells isolated from biopsies obtained from patients with periodontitis has been significantly higher in comparison with those biopsies of patients with gingivitis ($p < 0.01$) (Yamazaki et al. 1994, Wassenaar et al. 1995). Similar results have been obtained by investigating the mRNA levels of resident MØs in inflamed gingival tissues (Yamamoto et al. 1997, Yamazaki et al. 1997). A higher percentage of IL-4 production was detected in the cells obtained from the AgP lesions in comparison with those obtained from gingivitis lesions ($p < 0.01$) (Manhart et al. 1994).

In recent years, the role of genetic risk factors in the pathogenesis of peridontitis has been investigated, showing associations of different genetic polymorphisms with periodontal disease (Shapira et al. 1994, Kornman et al. 1997, Hennig et al. 1999, Parkhill et al. 2000). To date, most of these studies have been conducted in the Caucasian and Japanese populations. In Caucasians, a genetic association with AgP has been reported for the VDR (Hennig et al. 1999), FMLP (Gwinn et al. 1999) genes and a combination of genotypes of the IL-1$\beta$ and the IL-1RA (Parkhill et al. 2000).

Other genetic polymorphisms that have been found in association with AgP are the Fc$\gamma$RIIa, Fc$\gamma$RIIIa and Fc$\gamma$RIIIb receptors expressed in the surface of phagocytic cells (Wilson & Kalmar 1996, Kobayashi et al. 1997, 2000). The Fc$\gamma$RIIa exhibits either an arginine or a histidine at amino acid position 131 in the ligand binding site (Fc$\gamma$RIIa-R131/Fc$\gamma$RIIa-H131). In the Fc$\gamma$RIIIa gene, a G to T polymorphism results in a valine (V) phenylalanine (F) substitution at amino acid position 158 in the EC2 domain, and in vitro studies have shown that the Fc$\gamma$RIIIa-158V allotype exhibits a higher affinity for immune complexed IgG1, IgG3 and IgG4 in comparison with the Fc$\gamma$RIIIa-158F (Koene et al. 1997). The Fc$\gamma$RIIIb bears the NA1-NA2 polymorphism (Van Dyke et al. 1987), whereby the Fc$\gamma$RIIIb-NA1 displays a more efficient interaction with IgG1- and IgG3-opsonized bacteria, compared with the Fc$\gamma$RIIIb-NA2. The association of these genotypes with periodontitis has been demonstrated to be different for the Caucasian and Japanese populations. Japanese individuals having the Fc$\gamma$RIIa-R/R131 exhibited higher recurrence rates of periodontitis (Kobayashi et al. 1997). The Fc$\gamma$RIIIa-F/F genotype was found to be associated with recurrence of adult periodontitis in the Japanese population (Sugita et al. 1999); conversely, the Fc$\gamma$RIIIa-V/V genotype was associated with increased severe bone destruction in Caucasians with adult periodontitis (Meisel et al. 2001). In Japanese patients, the recurrence of periodontitis was associated with a significant overrepresentation of the Fc$\gamma$RIIIb-NA2/NA2 genotype ($p < 0.05$; OR: 4.29, 95% CI: [1.19;16.24]) but to date, these genotypes have not been analyzed in the Caucasian population. Further genetic differences between these two populations have been described, i.e. an association of the DQB1 0401 HLA class II alleles with increased susceptibility to AgP was detected in Japanese, but not in Caucasians (Takashiba et al. 1994, Ohyama et al. 1996, Hodge et al. 1999).

Recently, many polymorphisms in cytokine genes have been analyzed in our laboratory in patients with AgP and chronic periodontitis, as well as in healthy subjects. Two polymorphisms in the IL-4 gene, a C to T polymorphism at position –590 in the promoter region

and a 70 bp repeat polymorphism in intron 2 were detected in 27.8% in Caucasian patients with AgP (Michel et al. 2001). Since IL-4 levels in the sera of these patients were not detected, being significantly different compared with the other groups ($p < 0.01$), an association of the composite consisting of carriage of these two polymorphisms with the disease was postulated.

In the present study, the IL-4 promoter and IL-4 intron 2 genotypes were analyzed separately and in combination in the two populations. For this purpose, methods of explorative data analysis were used. The logistic regression model, in which the disease was analyzed as a dependent variable among the different genotypes for the disease within both populations was applied. The total number of alleles analyzed for patients and controls was similar in both populations. However, no significant differences between patients and controls were detected within either population with regard to the distribution of both alleles. The reason may be the lack of the necessary sample size in order to avoid type II errors. Nevertheless, the results of the present study showed evident differences between the two populations with regard to the distribution of alleles and genotypes. Whereas more Japanese individuals presented the polymorphic alleles of both the IL-4 promoter and the IL-4 intron 2, irrespective of the group, the Caucasian population showed another distribution. In this population, the difference between patients and controls for the intron 2 alleles ($p = 0.06$) was higher than for the promoter alleles ($p = 0.3$) and the carriage of the polymorphic allele in intron 2 showed an OR (patients versus controls) of 2.0 with a 95% CI: [1.0;4.2]), as described in Table 2. A larger sample is necessary in order to confirm the association of the investigated genotypes with AgP in the Caucasian population. In the Japanese sample, there was an indication for an explanatory contribution for heterozygous individuals versus homozygous individuals for allele 2 in the IL-4 promoter region ($p = 0.09$). In the Caucasian population, there was an indication for an explanatory contribution for homozygous individuals for allele 2 versus homozygous individuals for allele 1 in the intron 2 ($p = 0.08$). Furthermore, the differences between populations were more evident in the distribution of the composite analysis consisting of carriage of the IL-4 promoter and the IL-4 intron 2 polymorphisms (PP+/IP+). Whereas in the Japanese population, the distribution of the PP+/IP+ genotype was higher in controls than in patients ($p = 0.05$), in Caucasians, the distribution was higher in patients than in controls.

In conclusion, no associations could be demonstrated between the IL-4 genotypes and AgP in any of the populations studied. According to the results of the present study, IL-4 itself might not be a biologic factor that is strongly contributing to the aethiopathogenesis of AgP. These polymorphisms, however, are some of many that may be involved in this complex genetic condition, as previously shown by other investigators. The different distributions of the IL-4 polymorphisms in the two populations indicates that the relative risk for having AgP might involve particular haplotypes associated with particular races. Many of these polymorphisms may either be non-functional or be dependent on the presence of multiple other gene polymorphisms or environmental factors in order to manifest themselves as risk contributors to the disease.

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