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Identification of an Interleukin-4 Genotype in a Central American population with Aggressive Periodontitis

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Authors: Dr. José Roberto Gonzáles[1], Dr. Jörg Michel[1], Meike Hockamp[1], Jens Martin Herrmann[2], Dr. Rolf Hasso Boedeker[2], Prof. Dr. Jörg Meyle[1]
Department of Periodontology[1] and Statistics[2], Justus Liebig University, Giessen, Germany

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Abstract

Different Th-cell subsets may have a profound effect on the character of the immune response in periodontal disease. The cytokine IL-4 plays a major role in directing Th2 cell development. In a recent study, we identified two polymorphisms in the human IL-4 gene, which in combination showed a higher correlation to patients of north European heritage (EU) with aggressive periodontitis when compared to controls. The present investigation was undertaken to compare the distribution of this combined IL-4 genotype in two different populations and to determine the role of IL-4 genotypes in the immune response influenced by aggressive periodontitis. The -590 (C to T) promoter- and the 70 bp repeat (intron 2) polymorphisms were detected by specific primers and amplified by polymerase chain reaction (PCR) from dry blood samples taken in Central America (CA) from 16 patients and 14 controls. All of them were examined by a single experienced investigator and included in the study according to clinical parameters and radiographs. Man-Whitney-U-Test and Fisher's Exact Test were used in order to compare genotype frequencies between patients and controls in both populations (CA versus EU). A significant difference between both controls ($p < 0.001$) and patients ($p = 0.009$) was observed with respect to the Intron-2 polymorphism. No difference was observed regarding the promoter polymorphism. The combined genotype showed a statistical difference between the controls ($p = 0.03$), but not between the patients ($p = 0.33$). Nevertheless, we conclude that the different distribution of the genotypes in both populations is in accordance with an increased susceptibility and varying genetic-mediated pathogenesis for aggressive periodontitis in CA. Moreover, and as far as we know, this is the first report on genetic polymorphisms in patients affected by periodontitis in this part of the world.

Introduction

The contribution of genetic factors to the variance in clinical symptoms of periodontitis has been confirmed from studies with twins (Michalowicz 1994). Genetic polymorphisms of the Interleukin-1 and FcgRIIa (CD32 receptor) have been associated with different clinical forms of periodontitis (Wilson & Kalmar 1996, Kornman et al. 1997) . Several polymorphisms of the IL-4 gene are known: e.g. a 70bp repeat polymorphism in intron-2 (Mout et al. 1991) and an IL-4 promoter polymorphism (-590 C to T, see Fig. 1)(Wallney & Cookson 1996). In a previous investigation we identified these polymorphisms in 27.8% of north European patients, which in combination appeared to be associated with aggressive periodontitis (Michel et al. 2000). The aim of the present study was to identify the same genotypes in Central American patients with aggressive periodontitis and to compare the distribution of the IL-4 genotypes with individuals of north European heritage.

Fig.1. Interleukin-4 gene and polymorphic sites

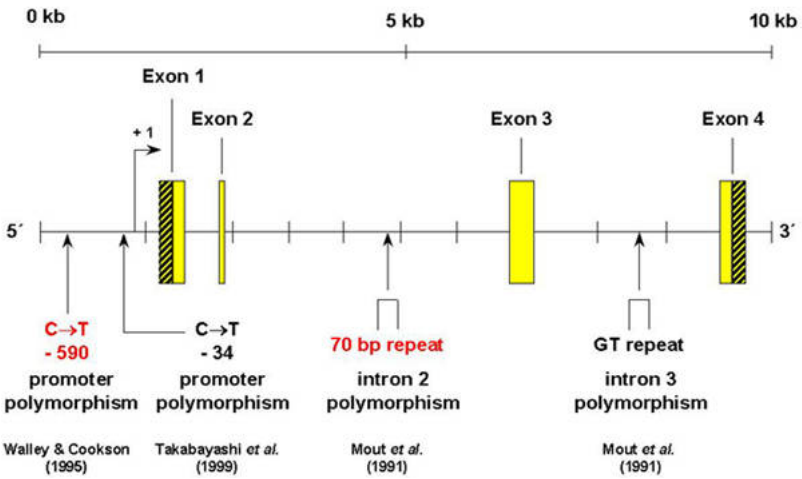

Fig.1: Interleukin-4 gene and polymorphic sites

Material and Methods

16 subjects from Central America (CA) presenting aggressive periodontitis were selected based on medical history, radiographic and clinical criteria. Additionally, 14 healthy subjects were included as a control group. Probing pocket depth (PPD), clinical attachment level (CAL), bleeding upon probing (BOP) (6 sites/tooth) and a modified plaque index (4 sites/tooth) were recorded. Dry blood samples were taken in Central America and transported to Germany for molecular analysis. DNA was isolated using the InstaGene Dry Blood Kit (Bio-Rad Laboratories GmbH, Munich, Germany) according to the instructions of the manufacturer. Two polymorphisms were analyzed: a) an IL-4 repeat (70bp) polymorphism in intron-2 was determined by a modification of the PCR described by Mout et al. (1991) and b) a -590 C to T polymorphism at the IL-4 promoter region was determined by a modification of the PCR described by Wallney & Cookson (1996). Two alleles exist at the 70bp repeat polymorphism in intron-2, being allele 1 the polymorphic marker. Homozygous individuals for this allele are classified as homozygous +. With regard to the -590 C to T polymorphism, individuals that are homozygous for the wild type allele are classified as homozygous -, individuals that are homozygous for the -590 C to T allele are classified as homozygous +, and individuals presenting both alleles are heterozygous. After identification of the IL-4 genotypes in the CA population, distributions were compared with previously detected IL-4 genotypes in patients (n = 28) and controls (n = 33) of north European heritage (EU). Mann-Whitney-U-test and Fisher's Exact test were used in order to compare genotype frequencies between CA and EU populations.

Results

Figures 2 - 5 show the genotypes distribution of both polymorphisms in patients and controls. The comparison between populations is also shown. 56.2 % of the patients and 42.8% of the controls were heterozygous in CA (Fig. 2). This result is similar to the distribution previously observed in EU, where 53.5% of the patients and 42.4% of the controls were heterozygous (Fig.3). The distribution of the intron-2 polymorphism showed a similar heterozygous pattern in both, patients (75%) and controls (78.5%) in the Central American population, however, no individuals were homozygous - (Fig. 4). This is significantly different ($p < 0.001$) with the distribution of this genotype in the north European population (Fig. 5).

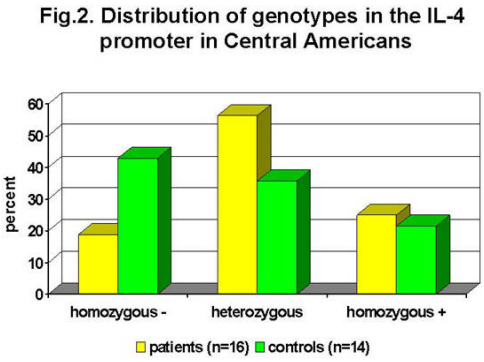

Fig.2: Distribution of genotypes in the IL-4 promoter in Central Americans

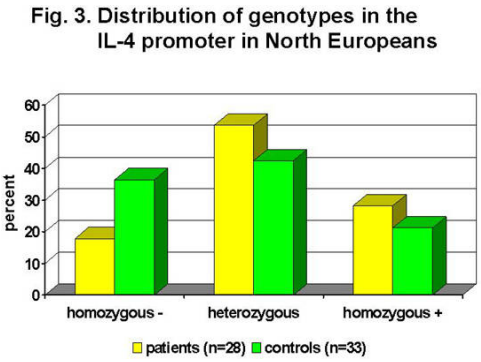

Fig.3: Distribution of genotypes in the IL-4 promoter in North Europeans

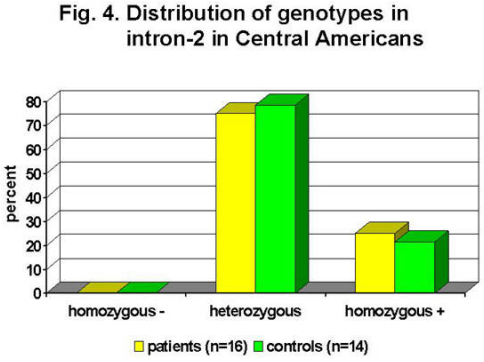

Fig.4: Distribution of genotypes in Intron-2 in Central Americans

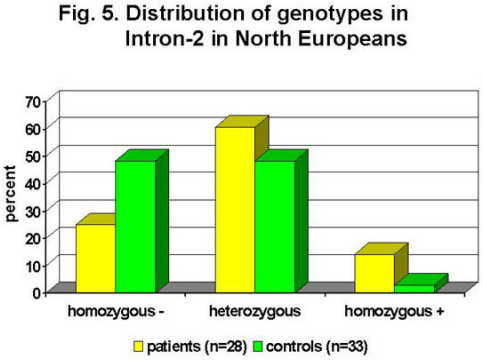

Fig.5: Distribution of genotypes in Intron-2 in North Europeans

Until now, both IL-4 polymorphisms have been mainly detected in combination in north Europeans. This result was confirmed in the Central American population. Due to this fact, we classified subjects in three different combined genotypes: PP/IP (+/+), which involves individuals who are positive for both polymorphisms; PP/IP (-/-) are those individuals who are negative for both polymorphisms and PP/IP (±), representing heterozygous subjects for both polymorphisms. Figures 6 and 7 show the distribution of these combined genotypes in patients and controls in both populations. The percentage of individuals that don't classified for this combinations are shown under rest.

Fig. 6. Distribution of the combined genotype in Central Americans

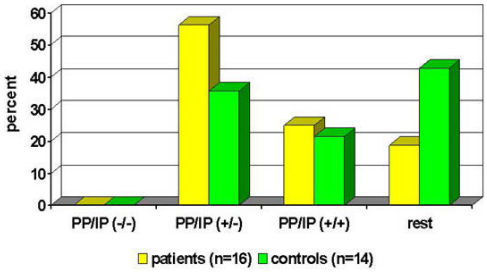

Fig.6: Distribution of the combined genotype in Central Americans

Fig. 7. Distribution of the combined genotype in North Europeans

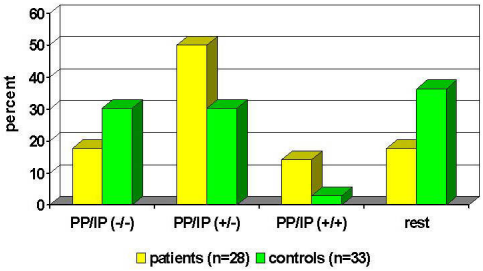

Fig.7: Distribution of the combined genotype in North Europeans

Differences in the distribution of the combined genotype were observed in both populations. In the Central American population, 25% of patients were PP/IP (+/+), compared with a 14.3% in north Europeans ($p = 0.32$). More significant was the difference between controls who were PP/IP (+/+) ($p = 0.03$). Neither patients nor controls from CA presented the combined PP/IP (-/-) genotype. This is a significant difference ($p < 0.001$) compared to the 30.3% of north European controls and 17.8% of patients. Probing pocket depth, bleeding upon probing and clinical attachment level are shown in figures 8-10. Box-plots show the median, 25th and 75th percentiles and "outliers" (*). As expected, all clinical parameters were at higher levels in patients from both populations.

Fig. 8. Probing pocket depth

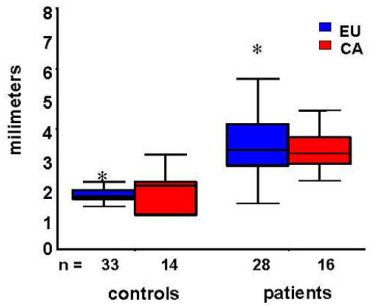

Fig. 8: Probing pocket depth

Fig. 9. Clinical attachment level

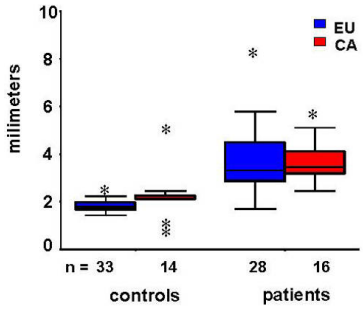

Fig. 9: Clinical attachment level

Fig. 10. Bleeding upon probing

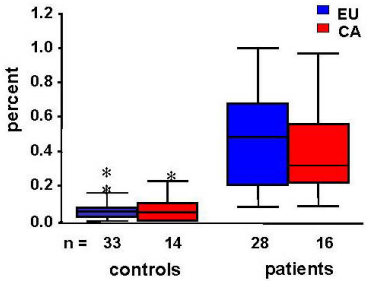

Fig. 10: Bleeding upon probing

Conclusions

A combined IL-4 genotype has been identified in patients with north European heritage. Until now, it appears that this genotype is in association with patients presenting an aggressive form of periodontitis when compared to controls ($p = 0.084$). After analyzing Central American individuals for the same genotype and comparing results with those of north Europeans, significant differences between both populations were observed. The facts that a) the negative combined genotype was not identified and b) the distribution of the positive combined genotype was detected increased in Central Americans, are a potential evidence for an increased susceptibility to the aggressive form of periodontitis in this population.

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This Poster was submitted by Dr. José Roberto Gonzáles.

Correspondence address:
Dr. José Roberto Gonzáles
Department of Periodontology
Justus-Liebig-University
Schlangenzahl 14
35392 Giessen
Germany

Poster Faksimile:

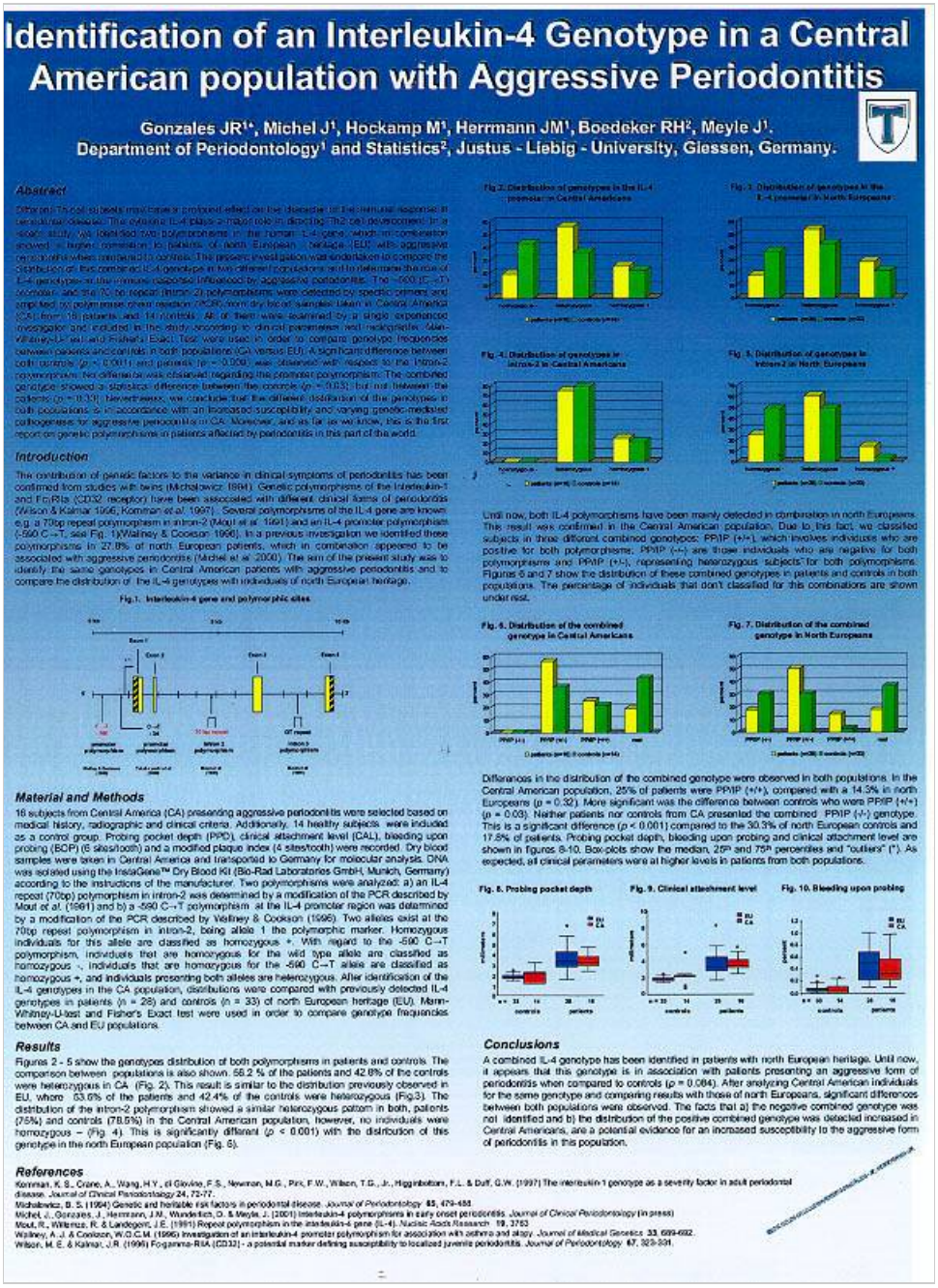

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Fig. 2. Distribution of genotypes in the IL-4 promoter in Central Americans

Fig. 3. Distribution of genotypes in the IL-4 promoter in North Europeans

Fig. 4. Distribution of genotypes in Intron-2 in Central Americans

Fig. 5. Distribution of genotypes in Intron-2 in North Europeans

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References

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