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Association of interleukin-1 gene polymorphisms with early-onset periodontitis

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Abstract

Background, aims: Early onset periodontal diseases (EOP) are a group of inflammatory disorders characterised by a rapid rate of periodontal tissue destruction, in young individuals who are otherwise healthy. There is now substantial evidence to suggest that genetic factors play a rôle in the pathogenesis of EOP but the precise nature of these factors remains unclear. Polymorphisms in cytokine genes which may underpin inter-individual differences in cytokine synthesis and secretion have been associated with other diseases which have an inflammatory pathogenesis, including chronic adult periodontal disease (CAPD).

Method: We therefore investigated the frequency of polymorphisms in the genes encoding interleukin-1β (IL-1β) and its receptor antagonist (IL-1RA) in 70 EOP patients, including a subgroup of 21 localised EOP (L-EOP) patients and 72 periodontally healthy controls. All subjects were of Caucasian heritage and systemically healthy. A single nucleotide polymorphism (SNP) in exon 5 of the IL-1β gene (IL-1β^{+3953}) was analysed by amplifying the polymorphic region using PCR, followed by restriction digestion with Taq1 and gel electrophoresis.

Results: The frequency of IL-1β genotypes homozygous for allele 1 (corresponding to the presence of a restriction site) of the IL-1β^{+3953} SNP was found to be significantly increased in EOP patients (χ^2 test, $p=0.025$). Upon stratification for smoking status a significant difference was found in the IL-1β genotype distribution between EOP smokers compared to control smokers (F-exact test, $p=0.02$), but not between EOP non-smokers and control non-smokers. The IL-1β 1/1 genotype occurred at a higher frequency in EOP smokers (odds ratio=4.9) compared to control smokers. A variable number tandem repeat polymorphism (VNTR) in intron 2 of the IL-1RA gene was analysed by amplifying the polymorphic region using PCR and fragment size analysis by gel electrophoresis. There was no evidence for an association of an IL-1RA genotype with EOP. However the combination of IL-1β allele 1 and IL-1RA allele 1 (corresponding to 4 repeats) was associated with EOP (Clump, $p=0.01$).

Conclusions: These findings suggest that an IL-1β genotype in combination with smoking, and a combined IL-1β and IL-1RA genotype are risk factors for EOP and support a role for genetic and environmental factors in susceptibility to EOP.

Key words: interleukin-1β; interleukin-1 receptor antagonist; early-onset periodontitis; gene polymorphisms

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Early onset periodontal diseases (EOP) comprise a sub-group of periodontal diseases with a particularly aggressive clinical course characterised by localised or generalised loss of alveolar bone early in life (Novak & Novak 1996). The results of population and family studies indicate that genetic factors seem to have a strong influence on susceptibility to EOP (Schenkein 1994, Hart 1996). The genetic influence on chronic adult periodontal disease (CAPD) is less apparent, since the incidence of this disorder is strongly affected by environmental variables such as oral hygiene and smoking. At the tissue level, EOP shares many pathogenic features with the more common CAPD. Therefore, although EOP is comparatively rare in the general population, there is considerable inter-

est in studies of this disorder which may reveal factors and in particular genetic variants which may also influence CAPD and other disorders with an inflammatory pathogenesis (Van Dyke & Schenkein 1996).

The results of segregation analyses investigating the modes of inheritance of EOP in families support the hypothesis that EOP is inherited as a genetic trait in an autosomal dominant fashion in many families (Boughman et al. 1990, Marazita et al.1994). However, attempts to identify a gene of major effect through linkage analysis studies have not produced consistent results. These studies are undoubtedly hampered by difficulty in applying uniform diagnostic criteria to families of several generations. The fact that a single gene of major effect in EOP has not been identified may indicate that an alternative hypothesis will be needed to explain the genetic influence and that other experimental approaches may need to be adopted.

The work of Duff (1998) has revealed that many cytokine genes harbour genetic polymorphisms. These common genetic variants may influence cytokine secretion levels and thereby explain the observation that stable inter-individual differences in cytokine responses to standard stimuli exist in humans (Molvig et al. 1988, Endres et al. 1989, Duff 1998). There is strong evidence for a key rôle for pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) in the pathogenesis of periodontal diseases where they are considered to indirectly contribute to connective tissue destruction and bone resorption (Page 1991, Alexander & Damoulis 1994, Okada & Murakami 1998). Furthermore, a number of studies have reported that increased levels of both IL-1α and IL-1β in gingival crevicular fluid correlate with the severity of periodontal disease (Wilton et al. 1992, Hou et al. 1995) and a model for severe periodontal disease, emphasising the importance of individual differences in inflammatory mediators, has been proposed (Offenbacher et al. 1993).

There is now a considerable literature relating the results of genetic association studies which identify particular alleles of polymorphic cytokine genes as factors that influence common diseases with an inflammatory pathogenesis. It has been hypothesised that dysregulation of cytokine gene expression may be responsible for the repeated cycles of tissue inflammation observed in these disorders (Duff 1998). Significantly, there have been several recent reports indicating that cytokine gene polymorphisms may influence severity of CAPD (Kornman et al. 1997, Galbraith et al. 1998, Gore et al. 1998). Thus the genetic basis for many common disorders with multifactorial pathogenesis such as periodontal disease may not be related to single or limited highly penetrant genetic variants, but may be influenced by subtle phenotypic variations caused by common alleles of a number of polymorphic genes relevant to disease pathogenesis (Hart & Kornman 1997). This may explain the inconsistent results obtained with traditional genetic studies of periodontal disease, their variable clinical features and the fact they share many pathogenic features with other inflammatory diseases. We hypothesise that studies of EOP are more likely to reveal a genetic basis for periodontal disease and may inform studies of the more common adult forms of the disease. In the present study we explore the possible association of IL-1 gene polymorphisms with EOP.

Material and Methods

Subjects

EOP patients (n=70) attending the Periodontology clinics at Newcastle and Birmingham Dental Hospitals (UK) were recruited over a period of 3 years according to diagnostic criteria for EOP specifically defined in accordance with the clinical criteria for EOP agreed by consensus at the World Workshop in Periodontics in 1989 (Ferris et al. 1989). EOP patients were subdivided according to the extent of the disease at the time of the study to allow stratification in the data analysis. A subgroup of localised disease (n=21) was characterised using radiographic criteria according to Genco et al. (1986); Localised EOP was classified as periodontal destruction ($\geqslant$ 3mm bone loss) confined only to incisors and first molars and juxtaposed tooth surfaces. Control subjects (n=72) with no clinical evidence of periodontal diseases (probing attachment distances $\leqslant$1mm from the cemento-enamel junction) were recruited from staff and other patients in the Dental Hospitals. The patient data and diagnostic criteria for the control and patients groups are outlined in Table 1.

All subjects were of Caucasian heritage and free of systemic disease. Information on smoking status was collected in a structured interview. The nature of the study was explained to all subjects and informed consent was obtained. The study protocol was approved by the Joint Ethics Committee of the University of Newcastle and the Newcastle and North Tyneside Health Authority.

Sample collection and extraction of DNA

20 ml of peripheral blood was obtained from each subject by venepuncture and collected into heparinised tubes. Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation (Lymphoprep^{TM}, Nycomed Pharma AS, Oslo, Norway) and stored in foetal calf serum containing 10% (v/v) dimethylsulphoxide. DNA was extracted from whole blood or PBMC using phenol/chloroform extraction and ethanol precipitation procedures.

Analysis of the IL-1^{+3953} SNP

DNA samples were subjected to polymerase chain reaction (PCR) to amplify the 194bp region containing the SNP. The sequence of the primers designed to flank the region of exon 5 of the IL-1β gene were synthesised as follows: 5′ CTC AGG TGT CCT CGA AGA AAT CAA A 3′ and 5′ GCT TTT TTG CTG TGA GTC CCG 3′ Kornman et al. 1997. The IL-1β^{+3953} SNP has 2 allelic variants termed allele 1 and allele 2 based on their relative frequency in the population. Allele 1, by convention, is the most common variant. The SNP results from a C→T base transition at position +3953 which destroys a Taq 1 restriction site (Pociot et al. 1992).

The DNA samples were amplified in 50 μl volume of a reaction mixture containing 10X reaction buffer, 1.5mmol/l $MgCl_2$, 0.2mmol/l dNTP, 0.75μmol/l of each primer, 100–500 ng of sample DNA and 1.25U Taq Polymerase (Thermoprime^{+}, Advanced Biotechnologies Ltd, Epson, UK).

Reactions were performed in a DNA thermocycler (model Techne PHC-3, Techne Ltd, Cambridge, UK) and consisted of 95°C for 4 min, 35 cycles at 95°C for 1 min, 59°C for 1 min and 74°C for 1 min, and finally 74°C for 5 min.

The PCR product was digested with 1 unit of Taq 1 (New England Biolabs, Hitchin, UK) and the restriction frag-

Table 1. Subject details and diagnostic criteria

Subject group	Diagnostic criteria	*n*	Age (years) * mean ±sd	(range)	Gender male	female
EOP	Clinical and radiographic evidence of a rapid rate of tissue destruction; age of onset prior to 35 years (Ferris et al., 1989)	70	36.1±7.3	(16–50)	30	40
L-EOP	Vertical bone defects (>3 mm) localized to incisors, first molars and juxtaposed tooth surfaces. Symmetrical pattern of destruction on at least 1 tooth type (Genco et al. 1986)	21	32.2±8.1	(16–50)	6	15
Controls	No clinical evidence of periodontal disease; probing attachment distance <1 mm from CEJ	72	41.1±9.5	(23–62)	37	35

* This denotes the age at sampling for each group. The age at diagnosis for the EOP group was considerably younger in most cases and below 35 years in all cases.

ments were determined on a 3% high resolution agarose gel (Anachem, Luton, UK) stained with 0.1% ethidium bromide. The resulting products of 12bp+85bp+97bp (allele 1; presence of restriction site) and 12bp + 182bp (allele 2; absence of restriction site) are diagnostic.

Analysis of the IL-1RA VNTR

Oligonucleotide primers immediately flanking the polymorphic region within the second intron of the IL-1RA gene, which contains variable numbers of a tandem repeat of 86bp, were used (Tarlow et al. 1993): 5′ CTC AGC AAC ACT CCT AT 3′ and 5′ TCC TGG TCT GCA GGT AA 3′.

The polymorphic region was amplified by PCR. The reaction conditions used were as described for IL-1β using 1μmol/l primer concentrations. The PCR conditions consisted of 95°C for 5min, 35 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min and finally 72°C for 5 min. Genotype was determined by molecular size analysis of PCR products by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide. The polymorphic sequence in intron 2 of the IL-1RA gene gives rise to 5 possible alleles corresponding to 2, 3, 4, 5 and 6 repeats of the 86bp sequence (Tarlow et al. 1993).

Statistical analysis

The associations of allele and genotype frequencies in the patient and control groups were determined using the χ^2 test and, in the case of test groups 5 or less in number, the Fexact test (Mehta & Patel 1986). Statistical significance was set at the 95% level. The strength of the associations were determined using an odds ratio (OR) calculation and 95% confidence intervals (CI).

The distribution of the combined IL-1β and IL-1RA genotypes was compared in EOP patients and controls using the "CLUMP" software package (Sham & Curtis 1995). The package uses a Monte Carlo approach which generates multiple simulated datasets to measure the differences in allele frequencies between the two groups, by evaluating the number of times the observed difference might be simulated if the frequencies were actually the same. Thus, the 'T4' test statistic which measures the overall difference in all allele frequencies simultaneously is generated. This test statistic is derived in the same way as the conventional χ^2 values, but the use of the Monte Carlo approach to assess significance avoids the need for a Bonferroni correction used previously in association studies (Kornman et al. 1997) and the problems associated with small numbers of allele combinations.

The linkage disequilibrium coefficient (Δ) was calculated according to Mattiuz et al. (1970) as PAB= PA×PB+ΔAB, where PA and PB are the allelic frequencies, Δ AB= $\sqrt{F4-\sqrt{(F2-F4)(F3+F4)}}$, and $F2$, $F3$ and $F4$ are the frequencies of +/−, −/+, −/−, respectively [+/+=presence of two considered alleles (allele 1 in both cases), +/−=presence of the IL-1β allele 1 alone, −/+=presence of the IL-1RA allele 1 alone, −/−=absence of both alleles].

Results

IL-1^{+3953} SNP and EOP

A significant difference was found in the IL-1β genotype distribution (Table 2) between EOP patients and controls (p=0.025). The IL-1β^{+3953} 1/1 genotype was associated with EOP; $OR_{(1/1 \text{ vs } 1/2+2/2)}$ =2.22, 95% CI=1.1–4.4. No significant difference was found in the IL-1β allele frequency (p=0.119). There was a significant difference in the IL-1β genotype (p=0.02) and the IL-1β allele (p=0.034) distribution between EOP smokers and control smokers (Table 3). The 1/1 genotype was associated with EOP smokers; $OR_{(1/1 \text{ vs } 1/2+2/2)}$=4.9, 95% CI=1.4–16.9. Allele 1 alone was also associated with EOP smokers; $OR_{(1 \text{ vs } 2)}$=2.63, 95% CI=1.1–6.5. There was no difference in the frequency of IL-1β genotypes (p=0.172) and IL-1β alleles (p=0.82) in EOP non-smokers and control non-smokers (data not shown).

There was a significant difference in the genotype distribution between L-EOP patients and controls (p=0.041), but there was no difference in the allele frequency (p=0.275) (Table 4). The 1/1 genotype was associated with L-EOP patients; $OR_{(1/1 \text{ vs } 1/2+2/2)}$=2.55, 95%CI=1.5–4.2. However, upon stratification for smoking there was no difference in the genotype (p=0.249) and allele (p=0.606) distributions between L-EOP non-smokers and control non-smokers (data not shown). We did not carry out statistical tests on L-EOP smokers due to the small number in the group.

IL-1RA polymorphism and EOP

In this study only the most common alleles, corresponding to 4 and 2 repeats respectively, were identified; these alleles are designated 1 and 2, respectively (Tarlow et al. 1993). There was no significant difference in the IL-1RA genotype distributions between EOP patients and controls (p=0.110) (Table 2). There was a significant difference in the allele frequency (p=0.028). The OR for allele 1 versus allele 2 in EOP was 1.9,

Table 2. IL-1β^{+3953} SNP and IL-1RA VNTR genotype frequencies in EOP patients and controls

	Genotype	EOP patients (%)	Controls (%)	Statistical analysis*
IL-1β	1/1	41 (58.6)	28 (38.9)	p=0.025
	1/2	24 (34.3)	41 (56.9)	$OR_{(1/1 \text{ vs } 1/2+2/2)}$=2.22
	2/2	5 (7.1)	3 (4.2)	CI=1.1–4.4
IL-1RA	1/1	48 (68.6)	37 (51.4)	p=0.110 (ns)
	1/2	17 (24.3)	26 (36.1)	
	2/2	5 (7.1)	9 (12.5)	

* See text for details on the statistical tests that were used.

Table 3. IL-1β^{+3953} SNP and IL-1RA VNTR genotype frequencies in EOP smokers and control smokers

	Genotype	EOP smokers (%)	Control smokers (%)	Statistical analysis*
IL-1β	1/1	21 (63.6)	5 (26.3)	p=0.02
	1/2	12 (36.4)	14 (73.7)	$OR_{(1/1 \text{ vs } 1/2+2/2)}$=4.9
	2/2	0	0	CI=1.4–16.9
IL-1RA	1/1	23 (69.7)	10 (52.6)	p=0.338 (ns)
	1/2	8 (24.2)	6 (31.6)	
	2/2	2 (6.1)	3 (15.8)	

* See text for details on the statistical tests that were used.

Table 4. IL-1β^{+3953} SNP and IL-1RA VNTR genotype frequencies in L-EOP patients and controls

	Genotype	L-EOP (%)	Controls (%)	Statistical analysis*
IL-1β	1/1	13 (61.9)	28 (38.9)	p=0.041
	1/2	6 (28.6)	41 (56.9)	$OR_{(1/1 \text{ vs } 1/2+2/2)}$=2.55
	2/2	2 (9.5)	3 (4.2)	CI=1.5–4.2
IL-1RA	1/1	15 (71.43)	37 (51.4)	p=0.337 (ns)
	1/2	5 (23.81)	26 (36.1)	
	2/2	1 (4.76)	9 (12.5)	

* See text for details on the statistical tests that were used.

95% CI=1.4, 2.5. There was no difference in the frequencies of the IL-1RA genotype (p=0.338) and allele (p=0.118) distributions between EOP smokers and control smokers (Table 3). Additionally there was no difference in the IL-1RA genotype (p=0.276) and allele (p=0.157) distributions between EOP non-smokers and control non-smokers (data not shown).

No significant difference was found in the distribution of IL-1RA genotypes (p=0.337) and alleles (p=0.076) between L-EOP patients and controls (Table 4). There was no significant difference in the IL-1RA genotype (p=0.463) and allele (p=0.152) frequencies between L-EOP non-smokers and control non-smokers (data not shown).

IL-1β and IL-1RA in EOP

A significant difference was observed in the combined genotype frequencies between EOP patients and controls (CLUMP, p=0.01). Allele 1 of the IL-1β and IL-1RA polymorphisms was associated with EOP patients (smokers and non-smokers); OR=2.6, 95%CI=1.7, 3.9. Analysis of the combined genotype for L-EOP was not carried out due to the small sample size.

Linkage disequilibrium

A negative linkage disequilibrium coefficient (Δ) was found between these alleles in the control group (Δ=-423.76) and in the patient group (Δ=-994.3), indicating that although the genes lie on the same chromosome 2, they are not in linkage disequilibrium nor are they harboured on the same haplotype.

Discussion

The periodontal diseases are believed to have a multi-factorial aetiology and genetically inherited factors, which may contribute by conferring disease susceptibility, are likely to be expressed through variations in the host's inflammatory-immune response to periodontal pathogens. Where environmental factors play an important role, such as in CAPD, the varying degrees of penetrance or phenotypic expression of individual genetic variants are likely to result in obscuring their true impact. However, it is accepted that the clinical diagnostic criteria for assigning a label of EOP are better defined than for CAPD and the range of environmental factors involved seems to be narrower. We therefore hypothesise that studies of EOP are more likely to reveal a genetic basis for periodontal disease and may inform studies of the more common adult forms of the disease.

There is now substantial evidence to support the existence of an association between certain cytokine gene polymorphisms and human diseases which involve an inflammatory pathogenesis (Duff 1998). Several reports have described the application of this approach to reveal the genetic influence on chronic adult periodontal diseases (CAPD) (Kornman et al. 1997, Galbraith et al. 1998, Gore et al. 1998). Although the results of previous studies are somewhat inconsistent, a common finding is that allele 2 of the IL-1β^{+3953} SNP, either alone or in combination with the IL-1α^{-889} SNP, is associated with certain groups of patients with severe periodontitis (Kornman et al. 1997, Gore et al. 1998). Our results indicate that those individuals homozygous for allele 1 of the IL-1β^{+3953} SNP have an increased susceptibility to early-onset periodontitis (EOP) and localised EOP (L-EOP) odds ratio (OR=2.22 and 2.55, respectively). Given that there is no association between carriage of allele 1 of IL-1β^{+3953} SNP and either EOP or L-EOP, we conclude that there may be a dosage effect for the influence of this allele on disease susceptibility in these patients.

One popular interpretation for these data is that particular cytokine genotypes influence directly the disease pathogenesis via an effect on cytokine synthesis. Data supporting the existence of stable inter-individual differences in cytokine synthesis and secretion to standard stimuli are well established (Molvig et al. 1988, Endres et al. 1989). Furthermore, it has been proposed that there is a dichotomy in par-

ticular populations with respect to IL-1 cytokine secretion; some individuals are 'high responders' and some 'low responders' (Endres et al. 1989). Significantly, Pociot et al. (1992) reported that allelic variation as the result of a Taq1 restriction fragment length polymorphism (SNP) in the IL-1β gene (later found to correspond to the IL-1β^{+3953} SNP) correlated with monocyte IL-1β secretion *in vitro*. Interestingly, there appears to be a dosage effect of allele 2 on IL-1β secretion, which is associated with progressively increasing levels of IL-1β in individuals heterozygous and homozygous for that allele (Pociot et al. 1992). These findings have been cited extensively to explain the association of allele 2 of the IL-1β^{+3953} SNP with several chronic inflammatory diseases (including CAPD), in which IL-1β is considered to have a rôle in the pathogenesis (Kornman et al. 1997, Duff 1998, Gore et al. 1998). Extrapolating this notion to the present study, EOP in our cohort would seem to be associated with a low responder genotype that is the homozygote for the IL-1β^{+3953} allele 1. Similar conclusions have been drawn from a recently published study, which investigated inheritance of IL-1 alleles using transmission disequilibrium analysis (Diehl et al. 1999). These findings may reflect differences in the rôle of IL-1β in the pathogenesis of EOP and CAPD. Thus IL-1β may have a key rôle in preventing the initiation of early-onset, rapidly progressing inflammatory diseases such as EOP and carriage of allele 2 of the IL-1β^{+3953} gene polymorphism giving rise to increased IL-1β levels may be protective for EOP (Diehl et al. 1999).

It is not yet clear whether the altered IL-1β secretion by monocytes observed in in vitro experiments is the direct consequence of IL-1β genotype or an indirect effect. It is known for instance that IL-1β levels are co-ordinately regulated with other cytokines of the IL-1 family and may be influenced by polymorphism outside the IL-1β gene rather than by the IL-1β^{+3953} SNP itself (Hurme et al. 1998, Santilla et al. 1998). Furthermore, we do not know the effect of altered levels of IL-1β on the pathogenesis and clinical course of periodontal diseases. Given the diversity and redundancy of cytokine networks in immune responses and disease processes, determining the solution to this question represents a considerable challenge (Balkwill 1993).

IL-1β genotype may exert its effect on the pathogenesis of periodontal diseases through linkage to other disease-causing alleles. The IL-1β gene is located on chromosome 2 juxtaposed to the genes which encode the other cytokines of the IL-1 family and their receptors (Nicklin et al.1994). Therefore, it is not surprising that linkage disequilibrium for the IL-1 genes has been identified and common haplotypes involving multiple polymorphic loci have been defined (Cox et al. 1998). Our data indicate that allele 1 of the IL-1β^{+3953} SNP and allele 1 of the IL-1RA VNTR in combination are significantly elevated in EOP as compared to controls but that this finding is not explained by linkage disequilibrium. One interpretation is that these genes are acting independently, having a cumulative effect on susceptibility to EOP. The combination of Il-1β and IL-1RA alleles is not part of a common haplotype (Cox et al. 1998).

Our data also indicate that, in addition to differences in the allelic association of the IL-1β^{+3953} SNP between CAPD and EOP, there are also differences when the patient groups are stratified with respect to smoking habit. Furthermore, both the 1/1 genotype of the IL-1β^{+3953} SNP as well as carriage of allele 1 itself are significantly elevated in EOP smokers. We found no significant differences between IL-1β^{+3953} genotype or allele frequency in EOP non-smokers and non-smoking controls. This provides evidence for interaction between smoking and genotype in conferring susceptibility to EOP. If having a 'low responder' genotype for IL-1β increases susceptibility to EOP, it is reasonable to hypothesise that a decrease in local IL-1β levels due to a combination of smoking and the genotype may be important in the initiation of the disease.

The rôle of smoking in the pathogenesis of periodontal disease is uncertain, but one potential mechanism is that it exacerbates disease by altering the immune response to periodontal pathogens. In vitro studies have addressed the potential alteration of cytokine production in smoke-exposed macrophages. There are variable reports of suppressed or unaltered IL-1β secretion from macrophages in smokers (Soliman & Twigg 1992, Sauty et al. 1994). Smoking may not actually affect the synthesis of IL-1β and may suppress its release from cells (Soliman & Twigg 1992). Furthermore, Bernzweig et al. (1998) observed that nicotine significantly downregulated IL-1β secretion by gingival mononuclear cells. Allele 1 of the IL-1β^{+3953} SNP may have a synergistic immunosuppressive effect with smoking, thereby increasing susceptibility to EOP.

In contrast to our findings, it was found that a combined IL-1β/IL-1α genotype was significantly increased in non-smokers with severe CAPD but not in non-smokers (Kornman et al. 1997). These findings may reflect differences in the pathogenesis and/or clinical course of EOP and CAPD. It is nevertheless clear that stratification of sample populations with respect to smoking habit influences the results of association studies and this observation is worthy of further investigation.

We have previously described an association of a VDR gene polymorphism with L-EOP but, in contrast to the IL-1β^{3953} SNP, not the overall EOP group (Hennig et al. 1999). Studies of adult periodontal disease have also revealed that various genetic polymorphisms have different association patterns in particular clinical subgroups (Kobayashi 1997, Kornman et al. 1997, Gore et al. 1998). It is possible that the variable findings for genetic association with different polymorphic loci reflect the heterogeneity of this disease entity. Thus, the VDR may specifically affect the development of the L-EOP clinical phenotype whereas IL-1β has likely a more general rôle in periodontal inflammation associated with EOP irrespective of clinical characteristics. Further genetic association studies should consider the dynamic nature of this disease rather than the cross-sectional approach taken in this and other studies. For instance, it has been suggested that EOP should be classified according to clinical parameters relating to the severity and rapidity of attachment loss (Albandar et al. 1997). The use of this classification system in genetic association studies may give rise to more meaningful data.

It is important to recognise the intrinsic limitations of genetic association studies both in terms of their use as a model for genetic disease and in relation to their application to the study of complex diseases such as periodontal disease. Although this approach has the advantage of being sensitive enough to detect genes with a small or modest effect, a major weakness in genetic associ-

ation studies is their sensitivity to differences between populations and to population stratification or 'admixtures' (Lander & Schork 1994). This underlines the importance of identifying control groups from the same population as the disease group. The use of family-based genetic approaches such as sib-pair analyses and transmission disequilibrium tests not only circumvent the problem of control groups, but also overcomes any difficulties associated with low-disease frequencies in the population under study (Lander & Schork 1994, Weeks & Lathrop 1995).

Another difficulty with genetic association studies is that if the gene under investigation only has a weak effect on phenotype or is in weak linkage disequilibrium with disease genes, this is likely to reduce the power of detection of association and lead to variable results in different studies (Risch & Merikangas 1996). This may apply to studies of genetic polymorphism and periodontal disease since these variants probably influence disease susceptibility and clinical course via the accumulated effect of multiple polymorphisms (Hart & Kornman 1997, Hennig et al. 1999).

If the genetic basis of a complex disorder such as periodontal disease is to be more fully understood genetic association studies using the candidate gene approach should not be carried out in isolation but in the context of other family-based genetic approaches as outlined above (Sobell et al. 1992, Weeks & Lathrop 1995).

Therefore, the accumulating evidence for an association between different genetic polymorphisms with periodontal diseases constitutes only preliminary evidence in support of a rôle for these genetic variants in the aetiology and/or pathogenesis of these common disorders.

A stated aim of the human genome project is to establish a database for SNPs and to investigate their rôle in genetic variation associated with human disease (Collins et al. 1998). This initiative, coupled with the development of high throughput DNA detection methodologies, may enable genome wide association studies to be performed. This approach may provide a more powerful means than genetic models based on linkage analysis to detect multiple genes (or gene variants) of modest effect which contribute to complex human disease (Risch & Merikangas 1996, Collins et al. 1997). Furthermore, the results of the present study and others emphasize the importance of assessing both environmental and genetic risk factors in studies of disease association. We believe data from genetic studies of periodontal disease will only be put in meaningful context when more holistic models of disease pathogenesis are developed.

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Zusammenfassung

Assoziation des Interleukin-1-Gen-Polymorphismus mit früh beginnender Parodontitis

Einleitung: Früh beginnende Parodontitiden ("early onset periodontitis": EOP) sind eine Gruppe von Erkrankungen, die durch rasche parodontale Zerstörung bei sonst gesunden jungen Patienten gekennzeichnet ist. Es existieren nun hinreichende Beweise dafür, daß genetische Faktoren eine Rolle in der Pathogenese der EOP spielen. Die genaue Natur dieser Faktoren bleibt aber unklar. Polymorphismen von Zytokingenen, die interindividuelle Unterschiede in der Zytokinsynthese und -sekretion untermauern könnten, wurden mit anderen Erkrankungen von entzündlicher Pathogenese wie der chronischen Erwachsenenparodontitis (CEP) in Zusammenhang gebracht. Deshalb wurde in dieser Studie bei 70 Patienten mit EOP, davon 21 Patienten mit lokalisierter EOP (1-EOP), und 72 parodontal gesunden Kontrollprobanden die Häufigkeit von Polymorphismen der Gene untersucht, die Interleukin-Iβ (IL-1β) und seinen Rezeptorantagonisten (IL-1RA) kodieren.

Methoden: Alle Personen waren europoiden Ursprungs und allgemein gesund. Ein einzelner Nukleotidpolymorphismus (SNP) in Exon 5 des IL-1β-Gens (IL-1β^{+3953}) wurde untersucht, indem die polymorphe Region mittels PCR amplifiziert wurde. Anschließend erfolgten Verarbeitung mit dem Restriktionsenzym Taq1 und Gelelektrophorese. Eine variable Zahl von Tandem-Wiederholungs-Polymophismen (VNTR) des Intron 2 auf dem IL-1RA-Gen wurden durch Amplifikation mittels PCR und Analyse der Fragmentgrößen durch Gelelektrophorese untersucht.

Ergebnisse: Die Häufigkeit des homozygoten IL-1β-Genotyps für Allel 1 (korrespondierend zum Vorhandensein einer Restriktionsstelle) für IL-1β^{+3953} SNP war bei EOP-Patienten signifikant erhöht (χ^2-Test; $p=0.025$). Nach Stratifizierung hinsichtlich Zigarettenkonsum zeigte sich ein signifikanter Unterschied der Verteilung des IL-1β-Genotyps zwischen EOP-Rauchern im Vergleich zu Rauchern aus der Kontrollgruppe (Fexact-Test; $p=0.02$), während sich keine Unterschiede zwischen den nichtrauchern aus beiden Gruppen zeigten. Der IL-1β-1/1-Genotyp fand sich häufiger bei EOP-Rauchern als bei EOP-Nichtrauchern (relatives Risiko= 4.9). Es konnten keine Hinweise für eine Assoziation zwischen dem IL-1RA-Genotyp und EOP gefunden werden. Allerdings war die Kombination des Allels 1 von IL-1β und des Allels 1 von IL-1RA (entsprechend 4 Wiederholungen) mit EOP assoziiert (Clump; $p=0.01$).

Schlußfolgerungen: Diese Beobachtungen deuten darauf hin, daß ein IL-1β-Genotyp in Kombination mit Zigarettenrauchen und ein kombinierter IL-1β- und IL-1RA-Genotyp Risikofaktoren für EOP darstellen und unterstützen die Bedeutung genetischer wie äußerer Einflüsse auf die Empfänglichkeit für EOP.

Résumé

Association des polymorphismes du gène de l'interleukine1 avec la parodontite d'apparition précoce

Les maladies parodontales à début précoce font partie d'un groupe de désordres inflammatoires caractérisés par un taux rapide de destruction tissuelaire, chez des individus jeunes, par ailleurs en bonne santé. Il y a désormais des preuves substantielles qui suggèrent que des facteurs génétiques jouent un rôle dans la pathogénie de ces maladies mais la nature précise de ces facteurs reste floue. Des polymorphismes des gènes codant des cytokines, qui pourraient entrainer des différences entre les individus pour la synthèse et la sécrétion de ces cytokines, ont été associés à d'autres maladies à pathogénie inflammatoire, dont la parodontite chronique de l'adulte. Nous avons donc recheché la fréquence des polymorphismes pour les gènes codant l'interleukine-1β (IL-1β) et son récepteur antagoniste (IL-1RA) chez 70 patients atteints de parodontite à début précoce, dont un sous-groupe de 21 patients présentant des lésions localisées et 72 patients contrôles présentant un parodonte sain. Tous les sujets étaient d'origine caucasienne et en bonne santé systémique. Un polymorphisme ponctuel de nucléotide sur l'exon 5 du gène de IL-1β (IL-1β^{+3953}) fut analysé par amplification PCR de la région polymorphique, digestion par restriction avec Taq1 et gel d'electrophorèse. La fréquence des génotypes homozygotes d' IL-1β pour l'allèle1 (correspondant à la présence d'un site de restriction) du polymorphisme ponctuel de nucléotide IL-1β^{+3953} était significativement augmentée chez les patients atteints de paradontite à dé-

but précoce (test χ^2, $p=0.025$). Après ajustment pour le tabagisme, une différence significative était trouvée pour la distribution du génotype IL-1β entre les patients atteints de parodontite à début précoce et les fumeurs du groupe controle, mais pas entre les patients ayant la maladie, qui ne fumaient pas, et les fumeurs du groupe contrôle. Le génotype IL-1β 1/1 était retrouvé avec une plus grande fréquence chez les fumeurs atteints de la maladie que chez les fumeurs du groupe contrôle (odds ratio=4.9). Un polymorphisme de répétition d'un nombre variable de tandem sur l'intron 2 du gène de IL-1RA fut analysé par amplification PCR de la région polymorphique et la taille du fragment fut determinée par gel d'électrophorèse. Aucune association entre un génotype IL-1RA et une parodontite à début précoce ne fut mise en évidence. Cependant, la combinaison IL-1β et IL-1RA allèle1 (correspondant a une répétition) était associée avec une parotontite à début précoce (Clump, $p=0.01$) Ces résultats suggèrent qu'un génotype IL-1β chez un fumeur, et un génotype combiné IL-1β et IL-1RA, sont des facteurs de risque pour une parodontite à début précoce et confirme le rôle des facteurs génétiques et environnementaux concernant la susceptibilité vis a vis des parodontites à début précoce.

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