J Periodont Res 2008; 43: 627–634

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2007.01039.x

A familial analysis of aggressive periodontitis – clinical and genetic findings

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Nibali L, Donos N, Brett PM, Parkar M, Ellinas T, Llorente M, Griffiths GS. A familial analysis of aggressive periodontitis – clinical and genetic findings. J Periodont Res 2008; 43: 627–634. © 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard

Background and Objective: Family history is a primary diagnostic criterion for current classification of aggressive periodontitis (AgP). However, results of previous studies have shed controversy over the degree of familiarity of AgP and its possible inheritance mechanisms. The aims of this study were to estimate the percentage of affected relatives of AgP individuals, to analyse the disease phenotypes in relatives and to explore the distributions of genetic polymorphisms of interleukin-6 (IL-6) in AgP patients and in diseased and healthy relatives.

Material and Methods: Patients with AgP were clinically examined and asked to provide relatives for examination. First-degree relatives were clinically and radiographically diagnosed. Blood samples were collected, DNA was extracted and analysis of single nucleotide polymorphisms of IL-6 (at positions −174, −1363 and −1480) by polymerase chain reaction was performed in patients and relatives.

Results: Fifty-five AgP patients provided relatives for examination. A total of 100 first-degree relatives were assessed and 10 of them (10%) were found to have AgP. All relatives diagnosed with AgP had the same disease as the corresponding proband (localized AgP/localized AgP or generalized AgP/generalized AgP). The same IL-6 genotypes (−174 GG, −1480 CC) previously associated with AgP showed a tendency for association with AgP in relatives.

Conclusion: This pilot study confirmed a relatively high risk for relatives of AgP patients to have AgP (10%). Genetic polymorphisms in the IL-6 gene may have an impact in aetiopathogenesis. This study provides a sample size calculation for a novel study design using healthy relatives as control subjects.

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Key words: aggressive periodontitis; familial aggregation; diagnosis; genetics; interleukin-6

Accepted for publication July 13, 2007

The observation of a familial association of Early Onset Periodontitis dates back many decades (1). Family history is now considered a primary diagnostic criterion for current classification of AgP (2) and therefore family screening could be considered as a preventive diagnostic tool. Results of some of the studies on family history of AgP have been flawed by lenient inclusion criteria (3). However, even studies with more reliable inclusion criteria (4) show increased risk of AgP in siblings of affected probands. The family aggregation may be due to a combination of genetic and environmental factors, although the importance of genetic factors independently has been shown in twins reared apart (5). However, controversy still exists concerning the mode of transmission of the AgP trait (3,4,6–9). Common genetic variants (single nucleotide polymorphisms, SNPs) able to cause subtle differences in gene activity or protein synthesis have been implicated in disease pathogenesis. Several genetic association studies have been published in the periodontal literature, looking at the distribution of SNPs in affected individuals and unrelated healthy control subjects (10). We have recently identified an association between interleukin-6 (IL-6) SNPs and haplotypes and the presence of periodontitis and detection of periodontopathogenic bacteria in AgP individuals (11–13). From these results, IL-6 emerges as a possible genetic

predisposing factor to the periodontal tissue damage typical of AgP. Recent reports have stressed the plausibility of genetic study designs including first-degree relatives as healthy control subjects, to study genetic effects or genetic–environmental interactions (14,15). We planned to analyse first-degree relatives of AgP patients, with the following aims: (1) to estimate the percentage of affected relatives of AgP individuals; (2) to analyse the pattern of disease affecting relatives (localized AgP, LAgP, or generalized AgP, GAgP); (3) to explore the distribution of SNPs in probands, diseased relatives and healthy relatives; and (4) to propose a model for a genetic association study design using relatives as control subjects.

Material and methods

The findings on a subset of these AgP patients and relatives have already been reported in a separate paper (16). The present paper reports the findings on an extended population, and the results of a genetic analysis performed on samples collected from these subjects. Diagnosis of AgP was based on the 1999 Consensus Classification of Periodontal Diseases (2). Our diagnostic criteria took into consideration only clinical, and not laboratory, evidence (17). We classified patients as having AgP when we had evidence of the following characteristics.

(1) *Healthy status*, except for the presence of periodontitis. All patients with possible contributory medical history, such as specific recognized genetic disease with periodontal manifestations (i.e. Papillon–Lefevre syndrome), diabetes, cardiovascular disease, or prolonged use of corticosteroids or immunosuppressive medications, were excluded.

(2) *Rapid attachment loss and bone destruction*, proven by radiographs obtained a few years apart (18). In the absence of sequential radiographs, young age was considered as a sign of rapid progression, in patients < 35 years old at the time of the initial diagnosis.

(3) *Familial aggregation*. We tried to ascertain the familial aggregation, by means of a specific questionnaire (16). However, patients showing clear clinical signs of AgP even without a reported positive family history were still included.

(4) *Clinical and radiographic diagnosis*. All the patients with a suspected diagnosis of AgP were examined by a single experienced clinician (G.S.G.). Full-mouth measures were obtained for probing pocket depth, recession (measured as distance from the cemento-enamel junction, CEJ, to the gingival margin) and lifetime cumulative attachment levels (LCAL, measured either as a direct measurement of CEJ to the base of the pocket, or as a calculation of probing pocket depth + recession). Six sites were measured for each natural tooth, one each at the mesiobuccal, midbuccal, distobuccal, distolingual, mid-lingual and mesiolingual sites encircling the tooth. Appropriate radiographic examinations were completed on each patient.

Patients were diagnosed with localized AgP (LAgP) when presenting with interproximal probing pocket depth and LCAL ≥ 5 mm and radiographic bone loss of ≥ 30% of root length on at least two permanent teeth, of which at least one was a first molar or incisor, and no more than two teeth other than first molars or incisors.

Patients were diagnosed with generalized AgP (GAgP) when presenting with generalized interproximal probing pocket depth and LCAL of ≥ 5 mm and radiographic bone loss of ≥ 30% of root length affecting at least three permanent teeth other than first molars and incisors.

All patients diagnosed with AgP in our department from December 2001 to December 2004 were given a case presentation, and the suspected genetic background of their condition was explained to them. In this context, the importance of examining their first-degree blood relatives was highlighted, and patients were given a questionnaire to complete. In this questionnaire, patients were invited to write their relatives' names and if they knew they had missing teeth, loose teeth or bleeding gums or if these relatives had been treated for gum disease. All available first-degree blood relatives were invited for a specific assessment at the Eastman Dental Hospital (EDH). The study protocol was approved by the Eastman Joint Research and Ethics Committee. The patients (defined here as probands) and the relatives who were examined provided signed informed consent.

Out of a total of 106 AgP patients examined, relatives from 55 of them were able to attend for examination at the EDH. Many patients excluded themselves because of poor family histories (no contact with relatives, family living abroad, adopted). Many other relatives refused to come for an assessment because they were not interested or did not have the possibility to attend. Therefore, no random selection was used to select this population of probands and relatives.

Examination of relatives

Relatives who attended for examination were given a medical history form to complete and were asked questions about their dental health and their previous dental treatments, with particular regard to periodontal treatment. As described previously (16), this consisted of an assessment of oral hygiene and gingival appearance, a community periodontal index of treatment needs (CPITN) examination, using a World Health Organization (WHO) probe, and assessment of mobility and furcation involvement. If the overall CPITN score was 3 or 4 (at least one site showing probing depth > 3.5 mm), a full periodontal charting was recorded (using a University of North Carolina (UNC15) probe), including probing depths, recessions and bleeding on probing at six points per tooth. Radiographic assessment included panoramic or long cone periapical radiographs. If the patients consented, a blood sample was collected and stored at −70°C.

Diagnosis of relatives

Authors of previous reports investigating the family history of AgP adopted heterogeneous disease criteria,

sometimes different even within the same study between probands and relatives (3,19). We chose to apply the same diagnostic criteria to our probands and relatives, and the 1999 Consensus Classification was used (2). Based on this classification, all subjects were diagnosed as healthy or with gingivitis, chronic periodontitis or AgP, as previously described (16). The classification of AgP was based on the same criteria reported above; the only difference between the diagnosis of probands and relatives was that at the time of examination, for the relatives we already had certainty of their family history of AgP. Patients were diagnosed with chronic periodontitis when presenting at least two sites with probing pocket depth and LCAL of 5 mm, radiographic evidence of bone loss and not falling into the AgP classification.

All available fathers, mothers and siblings of our probands, above the age limit of 12 years old, were invited for examination. We are aware that patients as young as 13 years old may still be too young to manifest the disease phenotype. However, only five subjects (5.2%) were younger than 20 years old. In instances where patients had experienced multiple tooth loss, an attempt was made to understand the cause of tooth loss and to obtain old dental records. Whenever there were uncertainties, because of lack of evidence on cause of tooth loss and periodontal disease progression, a diagnosis of AgP was dismissed, in favour of a diagnosis of chronic periodontitis.

Genetic analysis

A 10 ml blood sample was collected through venipuncture in the antecubital fossa from each consenting study subject and stored at −70°C. The DNA was extracted from peripheral blood cells using the Nucleon® BACC2 kit (Nucleon Bioscience, Coatbridge, UK) as described previously (11). The DNA concentration was estimated by measuring absorbance at 260 nm using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Cambridge, UK). Ten nanograms of DNA were subsequently used for polymerase chain reaction (PCR) analysis.

Allelic discrimination assays were performed using the Applied Biosystems 7300 Real Time PCR System. Three polymorphisms in the IL-6 gene promoter region were selected, at positions −174 (CCTTTAGCAT[C-G]GCAAGAC), −1363 (CACTGTTTTATC[G-T]GATCTTG) and −1480 (ACCGTCTCT[C-G]TGTTTAG). All of these polymorphisms are single nucleotide substitutions, apart from the −1480, which is a deletion of two base pairs (CT). All of the primers and probes were designed using the Assay-by-Design service offered by Applied Biosystems (Warrington, UK). Genotyping was performed in 25 μl reactions as previously described (17). Hidden duplicates were added to each plate to test error rates. However, no detection errors were observed. All genotyping was performed blindly with respect to clinical diagnosis by a single investigator (L.N.). Whenever the results were not clear, the analysis was repeated. If, after repetition, the result was still uncertain, no result was recorded for that polymorphism. A total of five out of 402 (1.2%) genotypes were considered unclear and not scored.

Statistical analysis

The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) package was used for statistical analysis. Continuous, normally distributed variables are reported as means ± SD. The percentage of relatives affected by chronic periodontitis and AgP was calculated considering the total of relatives examined. Since we had not managed to examine all the possible relatives, another percentage was calculated to reflect all the possible relatives of the 55 probands, assuming that none of the relatives we had not examined would have AgP. Comparisons of continuous and categorical data between relatives diagnosed with periodontitis (AgP + chronic periodontitis) and healthy relatives were analysed with students unpaired *t*-test and chi-squared test, respectively. Such analyses were not performed for diagnosis of AgP, because of the small number of affected relatives. In an exploratory analysis, the demographic characteristics of each relative (gender, age, smoking and ethnicity) and the genetic polymorphisms of the correspondent probands were entered as variables, in order to detect possible risk indicators for diagnosis of AgP. Based on previous findings (13), IL-6 −174 G homozygosity, −1363 G homozygosity and −1480 C homozygosity were considered supposedly predisposing to AgP and tested vs. the other genotypes. Quanto software version 1.2 (20) was used for sample size calculations of a case-sibling genetic epidemiological study in Caucasians. The observed frequencies were entered, together with the expected prevalence of AgP in the Caucasian population (0.1%) (21). The expected power for such calculation was 0.8 in a two-sided test, with an odds ratio of 2 [(based on previous data (13)].

Results

A total of 106 AgP patients were approached. Fifty-five of them (52%) had relatives available and willing to come for a periodontal examination. The demographic and clinical characteristics of these 55 subjects are reported in Table 1.

These 55 probands had a total of 232 first-degree relatives > 12 years old. A total of 100 (43%) of these relatives attended for a dental examination and their demographic characteristics are reported in Table 2. Most probands (54%) yielded just one relative each; two relatives each were examined from 14 probands, three relatives each from seven probands, four from one, five from two and seven relatives were examined from one proband. More sisters ($n = 36$) and brothers ($n = 26$) were examined, followed by mothers ($n = 24$) and fathers ($n = 14$). The majority of relatives were females, and of Caucasian origin. A total of 53 (53%) were diagnosed with periodontitis (AgP or chronic periodontitis), 10% with AgP and 43% with chronic periodontitis. The remaining 47% were divided between a diagnosis of gingivitis (27%) and 20% who were considered to be perio-

Table 1. Demographic and clinical characteristics of AgP patients who had at least one relative examined as part of the study

Parameter	All probands ($n = 55$)	AgP diagnosed in at least 1 relative ($n = 10$)	AgP not detected in any relatives ($n = 45$)
Age (mean ± SD)	28.1 ± 6.7	26.5 ± 8.1	28.5 ± 6.3
Gender (%)			
Females	39 (70.9%)	8 (20.5%)	31 (79.5%)
Males	16 (29.1%)	2 (12.5%)	14 (87.5%)
Smokers (%)			
Current heavy (≥ 20 cigarettes/day)	7 (12.7%)	2 (28.6%)	5 (71.4%)
Current light	9 (16.4%)	3 (33.3%)	6 (66.6%)
Former	14 (25.5%)	2 (14.3%)	12 (85.7%)
Never	25 (45.5%)	3 (12.0%)	22 (88.0%)
Ethnicity (percentage)			
Caucasians	33 (60.0%)	5 (15.2%)	28 (84.8%)
Blacks	12 (21.8%)	2 (16.7%)	10 (83.3%)
Asians	6 (10.9%)	2 (33.3%)	4 (66.6%)
Others	4 (7.3%)	1 (25.0%)	3 (75.0%)
Teeth at baseline (mean ± SD)	27.8 ± 2.8	28.1 ± 8.1	27.7 ± 3.0
Number of pockets ≥ 5 mm (mean ± SD)	54.3 ± 33.6	60.2 ± 45.8	52.9 ± 30.8
Probing pocket depth (mm; mean ± SD)	3.9 ± 1.1	4.1 ± 1.4	3.9 ± 1.0
LCAL (mm; mean ± SD)	4.5 ± 1.4	4.6 ± 2.1	4.5 ± 1.3

Abbreviation: LCAL, lifetime cumulative attachment loss.

Table 2. Relationships between demographic characteristics of relatives and periodontal diagnosis

Relatives ($n = 100$)	No AgP	AgP	Periodontitis (AgP + chronic periodontitis)
Gender			
Female ($n = 61$)	56 (91.8%)	5 (8.2%)	32 (52.5%)
Male ($n = 39$)	34 (87.2%)	5 (12.8%)	21 (53.8%)
Ethnicity			
Caucasian ($n = 67$)	62 (92.5%)	5 (7.5%)	35 (52.2%)
Black ($n = 15$)	13 (86.7%)	2 (13.3%)	9 (60.0%)
Asian ($n = 13$)	11 (84.6%)	2 (15.4%)	6 (46.2%)
Others ($n = 5$)	4 (80.0%)	1 (20.0%)	3 (60.0%)
Smoking			
Current heavy ($n = 5$)	4 (80.0%)	1 (20.0%)	4 (80.0%)
Current light ($n = 17$)	15 (88.2%)	2 (11.8%)	8 (47.1%)
Former ($n = 13$)	13 (100%)	0	6 (46.1%)
Never ($n = 65$)	59 (90.8%)	7 (10.6%)	35 (53.8%)
Relation to proband			
Father ($n = 14$)	13 (92.9%)	1 (7.1%)	12 (85.7%)
Mother ($n = 24$)	23 (95.8%)	1 (4.2%)	15 (62.5%)
Brother ($n = 26$)	22 (84.6%)	4 (15.4%)	10 (38.5%)
Sister ($n = 36$)	32 (88.9%)	4 (11.1%)	16 (44.4%)
Total ($n = 100$)	90 (90.0%)	10 (10.0%)	53 (53.0%)

dontally healthy. Statistical analysis (not reported in Table 2) showed that gender, ethnicity and smoking were not associated with the diagnosis of periodontitis (AgP + chronic periodontitis). The relation to the proband was associated with diagnosis of periodontitis (AgP + chronic periodontitis), respectively, 85.7, 62.5, 38.5 and 44.4% of fathers, mothers, brothers and sisters were diagnosed with chronic periodontitis (Pearson's chi-squared test, $p = 0.017$). A conservative estimate of the prevalence of AgP in these families, including the total of relatives not examined (assuming none of them to be affected by AgP), was 4.3%.

Table 3 reports the details of the 10 relatives diagnosed with AgP, and of their original proband. Aggressive periodontitis was found in one mother, one father, fur brothers and four sisters (one of whom was a dyzygous twin). The diagnosis of each AgP relative and their respective proband was always identical (GagP/GAgP or LagP/LAgP). Figures 1 and 2 show, respectively, the clinical and radiographic presentations of a 14-year-old proband of Black Caribbean origin (diagnosed with LAgP) and of her 15-year-old sister (also diagnosed with LAgP).

The Hardy–Weinberg equilibrium was satisfied for all SNPs. The distributions of genetic SNPs located in the IL-6 gene for the 55 probands, related to the diagnosis of AgP or periodontitis (AgP or chronic periodontitis) in at least one of the examined relatives, are reported in Table 4. Probands homozygous for the IL-6 −174 G and IL-6 −1480 C alleles had a higher percentage of relatives affected by AgP. For example, AgP probands (of all ethnicities) who were −1480 C homozygous presented a higher percentage of AgP among their relatives ($9/39 = 23\%$), while only one out of 16 (6%) of −1480 CG and GG probands had relatives with AgP. A similar tendency was observed among Caucasians, the largest ethnic group of the study (Table 4).

Out of the 100 relatives examined, 79 consented to have a blood sample taken for genetic analysis (Table 5). Two of the relatives who did not consent to a blood sample had been diagnosed with AgP. A similar tendency for increased diagnosis of AgP was noted for relatives homozygous for−174 allele G, −1363 allele G and −1480 allele C.

Another exploratory analysis was performed, comparing the genotype distribution of Caucasian AgP patients, who had at least one healthy

Table 3. Demographic characteristics and diagnosis of probands and correspondent relatives affected by AgP

Proband					Relative				
Gender	Age at diagnosis	Smoking	Ethnicity	Diagnosis	Relation to proband	Gender	Age at diagnosis	Smoking	Diagnosis
F	29	Former	Caucasian	GAgP	Mother	F	50	Current	GAgP
F	34	Former	mixed	GAgP	Brother	M	29	Never	GAgP
F	31	Current	Caucasian	GAgP	Twin sister	F	31	Never	GAgP
M	14	Current	Caucasian	LAgP	Brother	M	14	Never	LAgP
F	27	Never	Asian	GAgP	Sister	F	33	Never	GAgP
F	36	Current	Caucasian	GAgP	Father	M	40	Current	GAgP
F	31	Never	Asian	GAgP	Sister	F	29	Never	GAgP
F	18	Current	Black	LAgP	Brother	M	20	Never	LAgP
M	31	Current	Caucasian	GAgP	Brother	M	39	Never	GAgP
F	14	Never	Black	LAgP	Sister	F	15	Never	LAgP

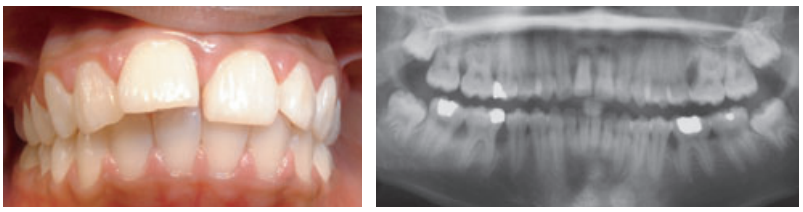

Fig. 1. Frontal intraoral picture and orthopantomograph of a 14-year-old Black-Caribbean female patient. Localized vertical bone defects are present around all first molars and 11 (UR1). A diagnosis of LAgP was made.

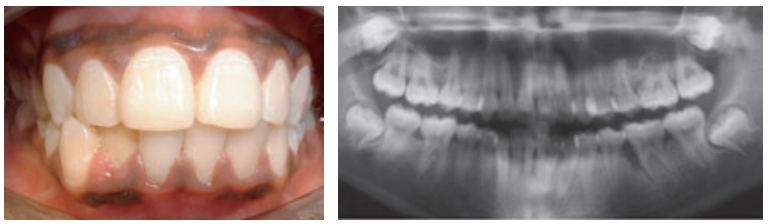

Fig. 2. Frontal intraoral picture and orthopantomograph of the 15-year-old Black-Caribbean female patient, sister of the patient shown in Fig. 1. Localized vertical bone defects are present around 26 (UL6) and 36 (LL6). A diagnosis of LAgP was made.

relative, with one of their respective healthy relatives (Table 6). Despite their clear genetic similarities (being siblings), higher percentages of IL-6 −174 GG, IL-6 −1363 GG and IL-6 −1480 CC were detected in the AgP group. For example, 76% of this group of AgP patients were −1480 CC, compared with 53% of their periodontally healthy relatives. These data were used to perform a power calculation with Quanto software, which revealed that 286 discordant sib-pairs of affected–healthy siblings would be needed to confirm such an association.

Discussion

A subset of AgP patients examined in our department provided relatives who lived in the UK and were willing to volunteer for a specific periodontal examination. Eighteen per cent (10/55) of the families of AgP patients included in the study had at least one additional individual affected with AgP. The prevalence of AgP in first-degree blood relatives of AgP patients that we managed to examine was 10%. This prevalence is considerably lower than has been reported in most previous studies (3,22). This discrepancy is likely to be due to ascertainment bias, since most studies focused on one family with multiple subjects affected (22), or to the excessively lenient definition often adopted for the diagnosis of AgP in relatives (3). However, our findings are in agreement with the report from Saxen (4), who examined 127 subjects from 33 Finnish families of AgP patients and found AgP in 11 siblings (9%) from eight families (24%).

In keeping with the reported difficulty of examining relatives (15), we managed to see 100 out of 232 (43%) possible relatives, which carries the risk of possible self-selection bias. Out of the 232 first-degree blood relatives, some were deceased, some lived abroad and some of them were odontophobic or simply did not wish to have a dental examination. This led to an increased female/male ratio in relatives, as expected from previous similar studies (9) and might also have led to over- or underestimation of AgP prevalence in these families. Even if all the non-examined probands were periodontally healthy, at least 10 out of 232 (4.3%) would be affected by AgP. This conservative prevalence would be higher than the prevalence of AgP, considered to be around 0.1–1% in different populations (21). Moreover, 53% of the examined relatives were found to be affected by periodontitis (AgP or chronic periodontitis). This percentage is just slightly higher than reported in a random UK population, where 43% of all subjects were found to have ≥4 mm LCAL (23). Therefore, this study supports the concept of familial aggregation in AgP and suggests the

Table 4. Genotype distributions in AgP probands for IL-6 SNPs

	All subjects ($n = 55$)		Caucasians ($n = 33$)	
Probands ($n = 55$) Parameter	AgP not diagnosed in any relatives ($n = 45$)	AgP diagnosed in at least 1 relative ($n = 10$)	AgP not diagnosed in any relatives ($n = 28$)	AgP diagnosed in at least 1 relative ($n = 5$)
IL-6 −174				
CC/CG ($n = 17$)	16 (94.1%)	1 (5.9%)	13 (92.9%)	1 (7.1%)
GG ($n = 37$)	28 (75.7%)	9 (24.3%)	14 (77.8%)	4 (22.2%)
IL-6 −1363				
TT/TG ($n = 5$)	4 (80.0%)	1 (20.0%)	4 (100%)	0 (0%)
GG ($n = 50$)	41 (82.0%)	9 (18.0%)	24 (82.8%)	5 (17.2%)
IL-6 −1480				
GG/CG ($n = 16$)	15 (93.7%)	1 (6.3%)	12 (92.3%)	1 (7.7%)
CC ($n = 39$)	30 (76.9%)	9 (23.1%)	16 (80.0%)	4 (20.0%)

The percentages of probands with relatives affected are reported in the columns. The first two columns report the results in subjects of all ethnicities, while the last two describe the results in Caucasians. One of the IL-6 −174 genotypes gave no clear result and was not scored.

Table 5. Genotype distributions in relatives of AgP probands for IL-6 SNPs

	All subjects ($n = 79$)		Caucasians ($n = 50$)	
Relatives ($n = 79$)	No AgP ($n = 71$)	AgP ($n = 8$)	No AgP ($n = 45$)	AgP ($n = 4$)
IL-6 −174				
CC/CG ($n = 31$)	29 (93.5%)	2 (6.5%)	26 (92.9%)	2 (7.1%)
GG ($n = 47$)	41 (87.2%)	6 (12.8%)	19 (90.5%)	2 (9.5%)
IL-6 −1363				
TT/TG ($n = 7$)	7 (100%)	0 (0%)	6 (100%)	0 (0%)
GG ($n = 72$)	64 (88.9%)	8 (11.1%)	40 (90.9%)	4 (9.1%)
IL-6 −1480				
GG/CG ($n = 28$)	27 (96.4%)	1 (3.6%)	24 (96.0%)	1 (4.0%)
CC ($n = 48$)	42 (90.8%)	6 (9.2%)	20 (90.9%)	2 (9.1%)

The percentages of probands with relatives affected are reported in the columns. Results relative to all subjects (mixed ethnicities) and Caucasians are reported.

Table 6. Genotype distributions in AgP Caucasian probands who had at least one healthy relative examined, and in their respective healthy relatives

Caucasians	AgP patients with examined healthy relatives ($n = 17$)	Healthy relatives ($n = 17$)
IL-6 −174		
CC/CG	5 (29.4%)	8 (50.0%)
GG	12 (70.6%)	8 (50.0%)
IL-6 −1363		
TT/TG	0	2 (11.7%)
GG	17 (100.0%)	15 (88.3%)
IL-6 −1480		
GG/CG	4 (23.5%)	7 (46.7%)
CC	13 (76.5%)	8 (53.3%)

employment of routine examination of first-degree blood relatives of AgP patients as a tool for secondary prevention. We have already reported how the proband report about their relatives' periodontal condition was reasonably reliable, and effective in the selection of risk families to examine (16). Nonetheless, the extent of this familial aggregation is probably not large enough to justify its inclusion as one of the principal features of the diagnosis.

A large part of the familial aggregation of periodontitis can be explained through genetic mechanisms (5). We recently reported an association between IL-6 polymorphisms and presence of periodontitis (11), detection of periodontopathogenic bacteria (12) and response to periodontal treatment (24). In particular, the IL-6 −174 GG genotype, which is associated with increased IL-6 promoter activity (25), has been associated with increased risk of periodontitis (11,26). We recently identified two other polymorphisms in the IL-6 promoter region (−1363 and −1480), which might have an impact on IL-6 production and therefore disease pathogenesis (13). Therefore, we performed an exploratory analysis on these three IL-6 genotypes (−174, −1363, −1480) on our probands (subjects with AgP) and their relatives (periodontally healthy and diseased). Nine out of 39 (23%) of the probands who were IL-6 −1480 CC homozygous had relatives examined who also had AgP, compared with one out of 16 (6%) of the relatives of C/G and GG probands (Table 4). Therefore, the same genotype that predisposes the probands to AgP (13) may also be present in the relatives of these probands and might predispose them to AgP also. The mechanisms of disease predisposition linked with IL-6 production may involve a modulation of the inflammatory response to periodontally pathogenic bacteria (12).

This is the first study, to our knowledge, to report genetic polymorphism analysis in AgP patients and their relatives. Preliminary data from this pilot study point towards the possible importance of genetic predisposition to AgP in families. Recent reports have stressed the plausibility of genetic study designs including first-degree relatives as healthy control subjects, to study genetic–environmental interactions (15). This would, however, be a novel approach in relation to periodontitis. This study presents pilot data on the feasibility to perform such studies in a population with AgP. Our analysis revealed that 286 sib-pairs would be needed in such a study. The advantage of such a study design, compared with a traditional case-control design, is the elimination of control subjects whose genetic background differs systematically from those of cases (15). This type of design would be particularly advantageous in cases of gene–environmental interaction. For example, it could be used for discordant sib-pairs studies looking at genetic–microbiological interactions in AgP. Furthermore, this study design may overcome the recognised difficulty of examining a large number of

relatives of affected probands in family studies (15).

It is interesting to observe the exact correspondence of diagnosis between probands and relatives affected by AgP. A diagnosis of LAgP was made in three relatives of LAgP patients, and GAgP was diagnosed in seven relatives of GAgP patients. A similar observation was noted by Saxen (4) and suggests that LAgP and GAgP might have different pathogenetic factors (13).

We have not tested any hypothesis for the mode of inheritance in our sample. However, according to our female/male ratios and to the observation of the absence of periodontitis in some of the daughters of an affected father, the likelihood of an X-linked transmission (27) seems to be small. In autosomal dominant mode of transmission (3), the disease will tend to appear in successive generations. Although we managed to examine only nine complete couples of parents in this study, none of them was affected by AgP. Furthermore, only two out of the other 20 parents were affected. This does not seem to support an autosomal dominant mode of transmission. In an autosomal recessive mode of inheritance, the phenotype is present only in subjects homozygous for the mutation. The parents are usually carriers (heterozygous) and there is 25% chance for the offspring to be affected. However, AgP may behave like many common diseases, such as diabetes, asthma, Parkinson's disease and rheumatoid arthritis, which do not conform to any recognized pattern of Mendelian inheritance and show multifactorial inheritance (28). This means that several genes contribute to disease susceptibility, each exerting additive effects, which interact with environmental factors to determine disease onset. One of these genetic factors might be located in the IL-6 gene. Considering the importance of environmental factors, such as bacteria and smoking, and the supposed importance of multiple genes as risk factors for periodontitis, this polygenic model seems a more likely mode of inheritance for AgP. Nonetheless, heterogeneity in genetic pathogenesis may mean that even a single gene defect may give rise to AgP in some individuals and families (8).

In conclusion, this study suggests the employment of routine examination of first-degree blood relatives of AgP patients as a useful preventive tool and supports the use of family history as an easily accessible tool for individual disease prevention in AgP (29). Furthermore, with the limitation of a sample of subjects with mixed ethnicities, this study supports the importance of IL-6 genetic factors in AgP pathogenesis and proposes a novel model for genetic analysis. If the associations we observed with presence of periodontopathogens, familial aggregation and treatment outcome are confirmed, IL-6 SNPs and haplotypes might represent possible new criteria for diagnosis of AgP. Larger studies should investigate the role of IL-6 in aggressive periodontitis.

Acknowledgements

This work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. This study was supported by the Periodontal Research Fund of the Eastman Dental Institute. The kind assistance of the clinical staff of the Periodontology Unit at University College London is gratefully acknowledged.

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