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Formylpeptide Receptor Single Nucleotide Polymorphism 348T>C and Its Relationship to Polymorphonuclear Leukocyte Chemotaxis in Aggressive Periodontitis

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Abstract

Background—Aggressive periodontitis (AgP) is associated with impaired polymorphonuclear leukocyte (PMN) chemotaxis toward bacterial N-formylpeptides. Formylpeptide receptors (FPRs) play a major role in guiding PMNs to infection sites. Previous work revealed a significant association between *FPR1* single nucleotide polymorphism (SNP) 348T>C and AgP in African Americans. We tested the hypothesis that 348T impairs PMN chemotaxis by decreasing FPR mRNA expression, thereby increasing susceptibility to AgP.

Methods—Blood samples were obtained from African American subjects (37 AgP cases and 38 controls). Chemotaxis to N-formyl-Met-Leu-Phe by freshly isolated PMNs was assayed in a modified Boyden chamber. RNA was isolated from PMNs and *FPR1* gene expression was quantified by real time PCR. To detect *FPR1* 5' SNPs, genomic DNA was isolated and four fragments spanning the *FPR1* 5' region were PCR-amplified and sequenced. Haplotype associations between SNP 348T>C and 5' SNPs were analyzed.

Results—The homozygous 348T genotype was only found in AgP cases (P=0.017, odds ratio=18.9). Subjects with this genotype exhibited a significantly lower PMN chemotactic response relative to controls and to subjects with the 348C/C or 348T/C genotypes (P<0.05). There were no significant differences in PMN *FPR1* expression between subjects with the 348C/C, 348C/T and 348 T/T genotypes. Eleven *FPR1* 5' SNPs were detected, but none of the predicted haplotypes reflected associations with AgP or with 348T.

Conclusions—Although the 348T/T genotype is relatively rare, it is associated with significantly impaired PMN chemotaxis and an increased risk of developing AgP in African Americans. These associations do not appear to be related to significant reductions in *FPR1* transcripts in subjects expressing 348T.

Keywords

aggressive periodontitis; polymorphisms; leukocytes; *FPR1* c.348T>C

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INTRODUCTION

Aggressive periodontitis (AgP) is a rapidly progressive, multifactorial periodontal disease that occurs in localized and generalized forms and has a tendency toward familial aggregation. AgP is associated with invasive bacterial pathogens like *A. actinomycetemcomitans* and *P. gingivalis*.[1,2] In addition, polymorphonuclear neutrophils (PMNs) from individuals affected by AgP exhibit reduced binding of N-formylpeptides and a significant reduction in chemotactic activity towards formylpeptides relative to PMNs from healthy control subjects.[3,4,5,6]

PMNs serve as the first line of defense against bacterial infections in the body. Upon stimulation, a complex signal transduction mechanism enables PMNs to migrate through endothelial cell walls into tissues. Bacterial formylpeptides are one of the major chemotactic stimuli that guide PMNs in their migration toward infection sites. PMNs typically express approximately 55,000 G-protein-coupled formylpeptide receptors (FPRs) per cell.[7] Binding of formylpeptides to FPRs triggers a cascade of intracellular signals that coordinate cytoskeletal reorganization, formation of pseudopodia and migration up a chemotactic gradient.[8] When PMNs arrive at an infection site, they attempt to kill bacteria by phagocytosis, production of reactive oxygen species and release of microbicidal enzymes.[9,10] The defensive capabilities of PMNs can be undermined by aberrant FPR expression.[11] FPR knockout mice exhibit an increased susceptibility to *L. monocytogenes* infection and their PMNs exhibit no chemotactic response to N-formyl-methionine-leucine-phenylalanine (fMLF).[12]

Previous analyses of *FPR1* suggest that it is highly polymorphic.[13,14,15,16,17] In some populations, AgP appears to be associated with specific single nucleotide polymorphisms (SNPs) in *FPR1* (the gene encoding the FPR).[13,14,15] *FPR1* coding region SNPs located in crucial sites can potentially affect FPR function. The FPR second intracellular loop and carboxy-terminal tail serve as major G-protein contact sites.[18,19] The amino-terminal domain can affect ligand binding to the FPR by functioning as a “lid” to the ligand-binding pocket.[20,21,22] SNPs located in the 5’ region of the *FPR1* can potentially affect gene expression. A recent study of Japanese subjects revealed that the -12915T variant of SNP -12915T>C in the FPR1 5’ region is associated with a decreased *FPR1* transcription.[15]

Our previous work has shown that African Americans who are homozygous for the 348T variant of the synonymous SNP 348T>C have a significantly increased risk of developing AgP. The homozygous 348T genotype appears to occur only in individuals with AgP.[23] Although 348T>C does not code for a change in the amino acid structure, synonymous coding region SNPs are occasionally associated with 5’ SNPs in promoter or enhancer regions that can alter gene expression. This phenomenon has been observed in the human β_2 adrenergic receptor and the CC chemokine receptor.[24,25] It is reasonable to hypothesize that the 348T FPR variant is associated with PMN FPR expression defects, PMN chemotaxis defects and increased susceptibility to AgP. It is also feasible that 348T is linked to one or more SNPs in the 5’ region of *FPR1* that contribute to impaired FPR expression and increased susceptibility to AgP. To test these hypotheses, we assayed the chemotactic activity of PMNs obtained from African-Americans with AgP and from healthy ethnically matched controls to determine whether 348T is associated with reduced PMN chemotaxis. To ascertain the mechanism by which 348T could influence PMN chemotaxis, we examined the expression *FPR1* transcripts and screened our subjects for *FPR1* 5’ SNPs that could potentially be associated with 348T.

MATERIALS AND METHODS

Subject recruitment

African American AgP cases and healthy control subjects were enrolled between January 2003 and July 2008 under a protocol approved by the Ohio State University Institutional Review

Board. Written informed consent was obtained from each subject. The criteria for diagnosis of AgP were consistent with the 1999 International Workshop for Classification of Periodontal Diseases and Conditions.[26] Individuals diagnosed with either localized aggressive periodontitis (LAgP, n=18) or generalized aggressive periodontitis (GAgP, n=19), and who were otherwise healthy, were included in the AgP case group. Cases with attachment loss involving no more than two permanent teeth other than the first molars and incisors were diagnosed with LAgP.[26] These subjects had ≥4mm attachment loss involving at least two permanent first molars and incisors, including at least one first molar.[26] Cases involving at least three teeth other than the first molars and incisors, with an attachment loss of ≥4mm were diagnosed with GAgP.[26, 27] The control subjects (n=38) were periodontally healthy based on clinical and radiographic examinations. None of the controls exhibited clinical attachment loss of >3 mm or probing depths >4 mm.

PMN isolation and chemotaxis assays

Peripheral blood was drawn from cases and controls by venipuncture. PMNs were isolated by Ficoll-Hypaque density gradient centrifugation and dextran sedimentation as previously described.[28] Freshly isolated PMNs were resuspended at a density of 10^6/ml in RPMI 1640 medium containing 0.3 % bovine serum albumin. Chemotaxis was assayed in a 48-well modified Boyden chamber.‡ Suspended PMNs were added to the upper wells and fMLF§ was added to the lower wells at concentrations of 0, 10 and 30 nM in RPMI 1640 medium containing 0.3% bovine serum albumin. Upper and lower wells were separated by a polycarbonate filter‖ (5 μm pore size). The chamber was incubated at 37° C for 30 minutes. Following incubation, the chamber was disassembled and the filter was carefully removed and washed with sterile PBS. PMNs that failed to migrate through the filter were wiped off the top surface of the filter. PMNs that had migrated through the filter were stained with a commercially available kit¶ and counted with an oil immersion microscope objective (400X, 5 fields per well). Chemotaxis was assayed with PMNs isolated from a total of 26 cases and 32 controls.

RNA isolation and cDNA synthesis

Total RNA was isolated from PMNs obtained from 26 AgP subjects and 37 healthy controls with TRIzol.# Freshly isolated PMN pellets were suspended in TRIzol (1ml per 10^7cells) and vortexed for 15 seconds. After addition of chloroform (0.2ml per 10^7 cells), the samples were vortexed for 15 seconds and placed on ice for 10 minutes. The samples were then centrifuged at 4900 × g and the aqueous phase containing RNA was aspirated and placed in new centrifuge tubes. Isopropanol (0.5 ml per ml of TRIzol) was added to the new tubes, which were then vortexed for 15 seconds and placed on ice for 10 minutes. The samples were centrifuged for 10 minutes at 4900 × g and the supernatant was discarded. The RNA pellets were washed in 70% isopropanol (1 ml per ml of TRIzol). The resultant RNA pellets were then air-dried for 5 minutes and resuspended in RNase free water. RNA concentrations were measured by spectrophotometry at 260 nm and 280 nm.

Reverse transcription of total RNA was performed with a kit** according to manufacturer's instructions. For each sample, 2 μg of total RNA was added to a reaction mixture containing 5 mM $MgCl_2$, 4 μl of 10 X reverse transcription buffer, 1 mM of each deoxynucleoside triphosphate, 1 μg of random primers, 40 U of recombinant ribonuclease inhibitor and 26.4 U of avian myeloblastosis virus reverse transcriptase (40 μl total volume). The samples were

‡AP-48, Neuroprobe Inc, Gaithersburg, MD, USA
§Sigma-Aldrich, St. Louis, MO
‖Neuroprobe Inc, Gaithersburg, MD, USA
¶Protocol Hema3® stain kit, Fisher Scientific, Pittsburgh, PA,USA
#TRIzol® Reagent, Invitrogen, Carlsbad, CA, USA
**Reverse Transcription System, Promega, Madison, WI., USA

incubated at room temperature for 10 minutes, heated to 42° C for 1 hour and then placed in boiling water for 5 minutes. Immediately afterward, samples were cooled on ice for 5 minutes and 60 µl of RNase free water was added to bring the final volume of each sample to 100 µl. The resultant cDNA samples were stored at −80° C until the time of assay by real-time polymerase chain reaction (PCR).

Quantitative real-time PCR

All cDNA samples were subjected to real time PCR to quantify *FPR1* expression. A known positive control sample expressing *FPR1* was included in each plate at 1, 1:10, 1:100 and 1:1000 dilutions for obtaining standard curves and a baseline value. Human *β-actin* was used as the internal control. For each sample, reactions were run in duplicates on 96-well plates. To quantify *FPR1* and *β-actin*, commercially available gene expression assays[††] containing primer-probe combinations were used. To each reaction well, 1.25µl of primer-probe mix, 12.5 µl universal PCR master mix [‡‡], 2.5 µl of cDNA and RNase free water were added to give a final volume of 25 µl. Samples were subjected to 40 cycles of amplification, each consisting of 2 minutes at 50° C, 10 minutes at 95°, 15 seconds at 95°, and 1 minute at 60°. Real-time PCR analysis was performed with a dedicated software package. [§§]

Real time PCR data was analyzed by the comparative Ct (threshold cycle) method as previously described.[29] The delta Ct (ΔCt = *FPR1* Ct minus *β-actin* Ct) values calculated for each sample were used to make comparisons between groups. The ΔCt values are normally distributed and amenable to analysis by one way ANOVA, but are not inherently suitable for descriptive purposes. For presentation (Figure 1, Table 3 and Table 4), they were standardized and transformed to ΔΔCt (which represents the fold difference in gene expression relative to baseline). The transformation process results in positive skewing of the variability within samples. Since this skewing misrepresents the actual variability, standard errors were not included in the data presentation.

PCR and sequencing of the *FPR1* coding and 5' regions

Genomic DNA was isolated from whole blood obtained from 37 cases and 38 controls with a commercially available kit[‖‖]. *FPR1* SNP 348T>C and other coding region SNPs were detected by PCR amplification and sequencing of a 439 bp fragment of *FPR1* as previously described.[23] To detect 5' SNPs, validated primer sets were used to amplify four fragments spanning approximately 10 kb of the *FPR1* 5' region, of sizes 500 bp, 264 bp, 188 bp and 783 bp.[15] PCR was carried out for 37 cycles in a reaction mixture containing 45 µl PCR supermix [¶¶], 1 µl of each of the two primers, 1.5 µl (18–30 pg) of the DNA sample and 1.5 µl water. Prior to initiating PCR, the reaction mixture was heated at 94° C for 5 minutes. The parameters for one PCR cycle included denaturation at 94° C for 30 seconds, annealing at 60° for 30 seconds and extension at 72° for 30 seconds. The cycles were followed by a final 7 minute extension step at 72°. PCR products were purified and sequenced with an automated DNA analyzer.[##] Differences in allele frequencies for each SNP were analyzed by the Fisher exact test.

[††]Taqman® Gene Expression Assays, Applied Biosystems, Foster City, CA, USA
[‡‡]Taqman Universal PCR Master Mix Applied Biosystems, Foster City, CA, USA
[§§]Prism 7000 Sequence Detection System Software, Applied Biosystems, Foster City, CA, USA
[‖‖]QIAamp DNA Blood Mini Kit, Qiagen, Valencia, CA, USA
[¶¶]PCR SuperMix, Invitrogen, Carlsbad, CA, USA
[##]3730 automated DNA analyzer, Applied Biosystems, Foster City, CA, USA

Stability of mRNA structure

Genetic association software*** was used to predict the predominant *FPR1* haplotypes and analyze potential associations of SNP 348T>C with 5' SNPs. The secondary mRNA structures derived from *FPR1* haplotypes were predicted with the mfold web server.[30]

RESULTS

Clinical Characteristics

The case group comprised 18 subjects diagnosed with LAgP and 19 subjects diagnosed with GAgP. No differences were observed between LAgP and GAgP subjects with respect to mean PMN chemotaxis and 348T allele association, so data presentation in the following sections does not distinguish between these clinical diagnoses. The clinical and demographic characteristics of the case and control groups are presented in Table 1. Clinical attachment loss in the control group was noted only at isolated sites and did not exceed 3 mm at any site. None of the control subjects exhibited radiographic evidence of bone loss.

Association of 348T with Aggressive Periodontitis

Apart from SNP 348T>C, four other previously identified *FPR1* SNPs (301G>C, 546C>A, 568A>T and 576T>C>G) were detected in the fragment analyzed. None of these were associated with AgP (data not shown). Three different 348T>C genotypes (C/C, T/C and T/T) were detected in the AgP case group, while only two genotypes (C/C and C/T) were detected in controls. The 348T/T genotype was expressed by 7 of the 37 case subjects. There was a significant relationship between 348T/T and case/control status ($P = 0.017$; odds ratio = 18.9, Table 2).

PMN Chemotaxis and Relative *FPR1* Expression

To examine the relationship between 348T, the PMN chemotactic response and chemotactic receptor expression, chemotaxis activity was assayed with PMNs obtained from a subset of 26 cases and 32 controls and relative *FPR1* gene expression assayed in a subset of 26 AgP cases and 37 controls. In the presence of 10 nM fMLF, the mean chemotaxis of PMNs obtained from AgP cases was approximately 22% lower than in PMNs obtained from controls ($P = 0.001$, Mann-Whitney rank sum test, data not shown). The mean chemotactic response to 30 nM fMLF was 19% lower in cases than in control subjects ($P < 0.001$, t-test, Figure 1A). There was no significant difference between cases and controls with respect to random migration (data not shown). The overall level of PMN *FPR1* expression in cases was significantly lower than in controls ($P < 0.001$, t-test, Figure 1B).

The mean chemotactic response to 30 nM fMLF was 34% lower in cases with the 348T/T genotype than the average response by controls ($P < 0.05$, Holm-Sidak test, Table 3), and chemotaxis in cases with the 348C/C genotype was 20% lower than in controls ($P < 0.05$, Holm-Sidak test). The level of *FPR1* gene expression by PMNs from cases with the 348T/T genotype was not significantly different from the levels observed in controls or cases with the 348C/C or 348T/C genotypes ($P = 0.26$, Table 3). However, PMNs from cases with the 348T/C genotype expressed significantly lower levels of *FPR1* transcripts than the mean level expressed by controls ($P = 0.006$, Holm-Sidak test).

Table 4 portrays the relationship between PMN chemotaxis activity, *FPR1* expression levels and 348T>C genotype in a pool of case and control subjects. There were significant differences in chemotaxis activity between subjects with the 348C/C, 348T/C and 348T/T genotypes ($P <$

***Golden Helix Inc., Bozeman, MT, USA. HelixTree® Software

0.001, ANOVA). In particular, the mean chemotaxis activity observed with subjects with the 348T/T genotype was 26% lower than in subjects with the 348T/C genotype and 33% lower than those with the 348C/C genotype ($P < 0.05$, Holm-Sidak test). Although subjects with the 348T/T genotype exhibited the lowest observed level of *FPR1* expression, no statistically significant differences in relative *FPR1* expression were found between the three 348 genotype groups ($P = 0.26$, Kruskal-Wallis one way ANOVA).

Figure 1 compares chemotaxis activity and *FPR1* gene expression between cases and controls with the 348C/C, 348T/C and 348T/T genotypes. The mean chemotaxis activity of PMNs from 348C/C cases was 16% lower than controls with the same genotype ($P = 0.008$, t-test, Figure 1A), while the mean chemotaxis activity in 348T/C cases was 17% lower than in 348T/C controls ($P = 0.02$). Cases with the 348T/T genotype exhibited the lowest chemotactic response. The level of *FPR1* expression was not significantly different between cases and controls with the 348C/C genotype ($P = 0.63$, Figure 1B), but expression by 348T/C controls was significantly higher than observed in 348T/C cases ($P < 0.001$, t-test).

FPR1 5' SNPs

Eleven 5' SNPs were identified, including seven (-12915C>T, -10107A>G, -10056T>C, -8430A>G, -8318A>G, -5426G>A and -4949C>T) that have been previously described[15] and four newly identified SNPs (-13045C>T>A, -12888T>A, -9997C>T and -8375G>T). None of the SNPs exhibited significant differences in allele frequencies between cases and controls (Table 5), but -13045C>T>A, -12915C>T and -8430A>G most closely approached statistical significance. Haplotype association of 348T>C with each of these three SNPs was analyzed. However, none of the predicted haplotypes containing 348T in combination with one or more of these three 5' SNPs exhibited associations with AgP that were any more significant than found with 348T alone (data not shown).

Stability of Predicted mRNA Secondary Structures associated with 348T and 348C

A total of thirteen haplotypes with frequencies ≤ 0.01 were predicted among AgP cases. Five pairs of haplotypes that differed only at 348T>C were identified. The free energy (dG) values of mRNA structures predicted for the 348T-containing haplotypes 301G.348T.546A.568A.576T, 301C.348T.546C.568A.576G, 301C.348T.546C.568A.576T, 301G.348T.546C.568A.576G and 301G.348T.546C.568A.576T were, on average, 2.73 ± 0.26 kcal/mole higher than those predicted for the 348C-containing haplotypes 301G.348C.546A.568A.576T, 301C.348C.546C.568A.576G, 301C.348C.546C.568A.576T, 301G.348C.546C.568A.576G and 301G.348C.546C.568A.576T ($P < 0.001$, paired t test). The mRNA associated with haplotypes containing 348T consistently exhibited a different secondary structure and higher free energy (lower stability) than those containing 348C (data not shown).

DISCUSSION

The present study extends our previous observation that *FPR1* SNP 348T>C is associated with AgP to a larger population of African Americans. Subjects with the 348T/T genotype appear to have a significantly increased risk of developing AgP compared to those with the 348T/C or 348C/C genotypes (odds ratio = 18.9). Consistent with our hypothesis, subjects with the 348T/T genotype exhibit a significantly lower PMN chemotactic response to formylpeptides than observed with subjects with the 348T/C or 348C/C genotypes. However, our studies refute the hypothesis that 348T contributes to impaired chemotaxis through a mechanism involving reduction of *FPR1* transcripts and provide no evidence that 348T is associated with SNPs in the 5' region of *FPR1*.

Consistent with previous studies,[3,4,5,6] PMNs from our AgP cases exhibited significantly decreased chemotaxis toward fMLF and reduced *FPR1* expression relative to PMNs from controls. We observed a 19–22% decrease in chemotaxis activity, while previous investigations reported a decrease of approximately 20–50%. This discrepancy may be related to differences in chemotaxis assay techniques. Many past studies used blind-well Boyden chambers and measured average PMN migration at various levels of a thick filter. The present study used a modified Boyden chamber and assayed PMN migration though a thin filter, an approach that is perhaps less sensitive. Subjects with the 348T/T genotype, who were all AgP cases, exhibited the lowest level of PMN chemotactic activity observed in this study. This finding supports the hypothesis that 348T/T increases the risk of developing AgP through a mechanism that involves impairment of PMN chemotaxis.

Although 348T is a synonymous SNP that does not alter the FPR amino acid sequence, it is reasonable to hypothesize that it could be associated with other SNPs in the *FPR1* promoter or enhancer regions. Linkages of this type have been observed in the human β_2 adrenergic receptor[24] and the CC chemokine receptor,[25] in which specific haplotypes containing variants of synonymous coding region SNPs and non-synonymous promoter SNPs were associated with altered receptor expression. In the present study, subjects with the 348T/C genotype exhibited the highest level of relative *FPR1* expression, while those with the 348T/T genotype presented the lowest level of expression. Interestingly, *FPR1* expression was significantly higher in 348T/C controls than in cases with the same genotype. It is not unusual for genes to exhibit individual differences in allelic expression.[31] While there were no statistically significant differences in *FPR1* expression between the three 348T>C genotypes (Table 4), the relatively small number of subjects with the 348T/T genotype limited the power of statistical analysis.

Sequencing analysis of the *FPR1* 5' region in our subjects revealed eleven SNPs, including the -4949C>T SNP that is located in the putative *FPR1* promoter region. A previous study of Japanese AgP cases and controls reported a total of eight SNPs in this region.[15] Seven of these SNPs were detected in the present study, along with four SNPs that had not been previously described. None of these 5' SNPs occurred at a significantly different frequency in cases than in controls, although there were differences in the frequencies of -13045C>T>A, -12915C>T, and -8430A>G that approached statistical significance. In a previous study of Japanese subjects, -12915C>T and -8430A>G were associated with AgP, while -12915C>T was associated with decreased *FPR1* expression and decreased transcriptional efficiency.[15] Haplotype analysis was conducted with -13045C>T>A, -12915C>T, and -8430A>G to determine whether any of these 5' SNPs are linked with 348T, but no association was found. Collectively, our findings suggest that the 348T allele is not associated with significantly decreased *FPR1* expression.

While it is unclear why individuals with the 348T/T genotype exhibit a lower PMN chemotactic response to fMLF, it is possible that 348T could reduce the number of FPRs through a post-transcriptional regulatory mechanism. Consistent with this possibility, we recently reported that the mRNA associated with the haplotype 348T.568A exhibited higher free energy (lower stability) and a different secondary structure than that predicted for 348C.568A, which could potentially lower mRNA stability and decrease translational efficiency.[23] Moreover, within five haplotype pairs differing only at 348T>C, the predicted secondary mRNA structures of haplotypes containing 348T were all associated with higher free energy (and lower stability) than those containing 348C. There is increasing evidence that synonymous SNPs that are associated with disease can affect mRNA stability. As an example, a single synonymous SNP 971T>C in the gene encoding corneodesmosin is associated with psoriasis and contributes to a 2-fold increase in mRNA stability. Transcripts associated with the 971T variant of this SNP have a decreased affinity for a cytoplasmic protein, which affects the rate of mRNA decay.[32] Furthermore, the synonymous SNP 3435C>T in the gene encoding the multidrug resistance 1

protein contributes to a 2-fold decrease in mRNA levels. The 3435T variant of this SNP reportedly changes mRNA folding structure.[33]

AgP is a multifactorial disorder that involves a complex interplay of environmental, genetic and behavioral factors that could potentially affect individual susceptibility to disease. It often results in early tooth loss and significant functional and esthetic problems. The findings of this study suggest that African Americans who have a homozygous 348T genotype have an increased risk of developing AgP. This susceptibility may, in part, be related to impaired PMN chemotaxis to formylpeptides. Contrary to our hypothesis, 348T does not appear to be linked to *FPR1* 5'SNPs that could potentially regulate the level of *FPR1* expression at the level of transcription. However, evidence from molecular modeling suggests that 348T could alter the folding of FPR mRNA in a manner that decreases its stability. It is uncertain whether these changes in folding decrease the half-life of *FPR1* mRNA and exert a clinically significant post-transcriptional influence on the number of FPR expressed by PMNs. Further investigations are needed to fully understand the mechanism by which 348T is associated with decreased PMN chemotaxis.

Key finding

The homozygous *FPR1* 348T genotype is associated with an increased risk of Aggressive Periodontitis and significant impairment of PMN chemotaxis, but is not associated with a significant reduction in the level of *FPR1* transcripts in PMNs.

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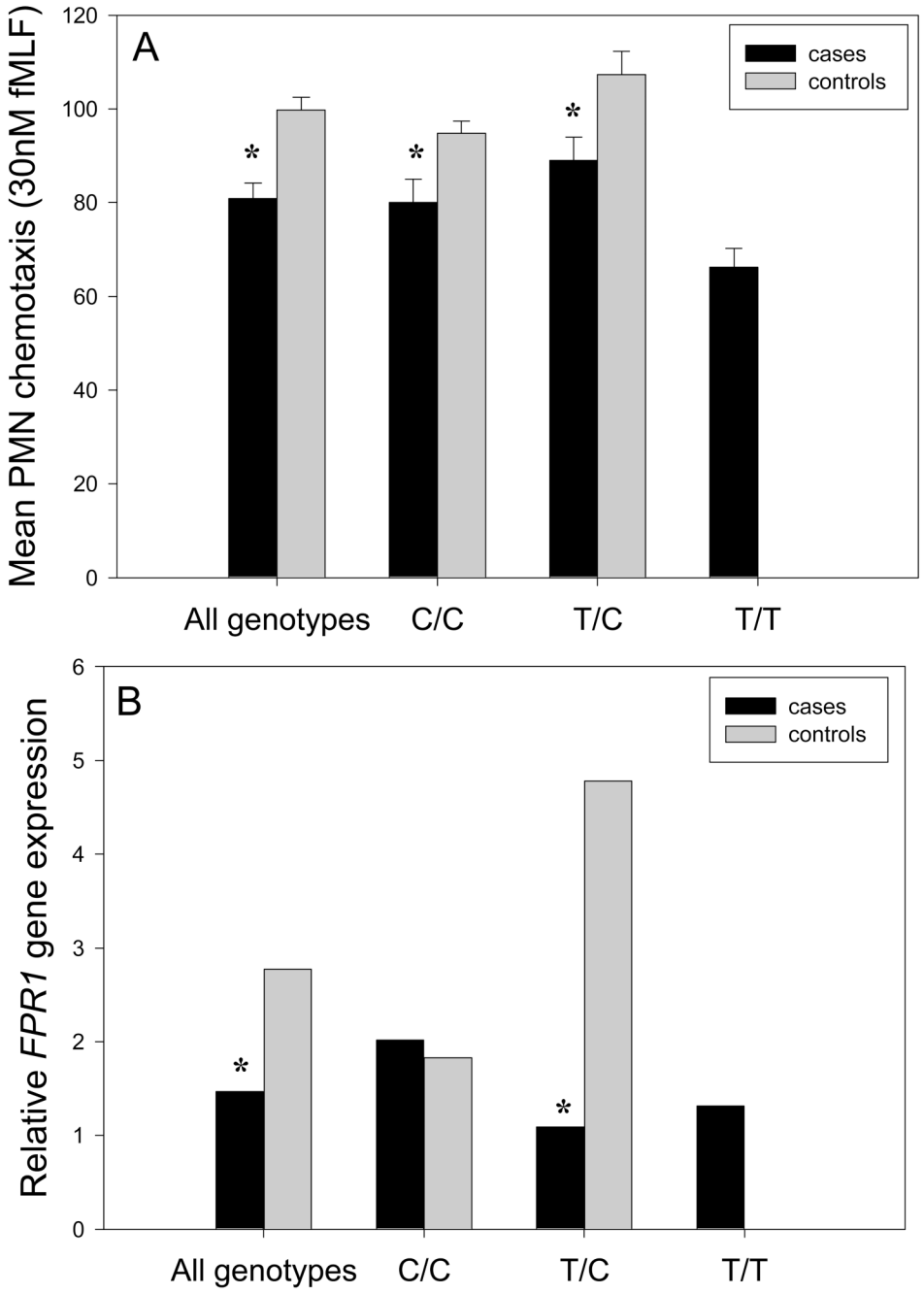

Figure 1.

Relationship of 348T>C genotype to PMN chemotaxis activity and PMN *FPR1* expression. Statistically significant differences between cases and controls are denoted by * ($P < 0.05$, t test). Upper panel: Chemotaxis activity was assayed in the presence of 30 nM fMLF with PMNs obtained from 26 case subjects and 32 controls. Data are presented as mean ± SEM. Lower panel: *FPR1* expression was assayed with RNA isolated from 26 case and 37 control subjects. Transformed values ($\Delta\Delta Ct$) represent n-fold change in *FPR1* expression relative to baseline. Since the transformation skews the variation within samples, error bars were not included.

Table 1

Clinical and Demographic Characteristics of the Study Population*

	Cases † (n = 37)	Controls‡ (n = 38)
Age	24.1 ± 1.31	27.7 ± 1
Gender composition	62.2% Female	63.2% Female
Number of teeth with Probing Depth ≥ 5 mm	15.6 ± 1.14	0
Depth of sites with Probing Depth ≥ 5 mm	6.24 ± 0.11 mm	N/A
No. of teeth with Clinical Attachment Loss ≥ 4 mm	16.5 ± 1.3	0
AL of sites with Clinical Attachment Loss ≥ 4 mm	5.38 ± 0.15 mm	N/A

* Data are presented as mean ± SEM

† *FPR1* 5' SNPs and 348T>C were analyzed in all 37 cases, but some subjects were lost to follow-up studies. Chemotaxis and *FPR1* gene expression were assayed in a subset of 26 subjects. The characteristics of the subset were not significantly different from the full case population.

‡ *FPR1* 5' SNPs and 348T>C were analyzed in all 38 controls, but some subjects were lost to follow-up studies. Chemotaxis was assayed with 32 subjects and *FPR1* gene expression was assessed with 37 subjects. The characteristics of these two subsets were not significantly different from the full control population.

Table 2

Allele association of *FPR1* SNP 348T>C with AgP cases in African-Americans*

Genotype	AgP		Controls	
	n	%	n	%
348 C/C	17	(45.9)	22	(57.9)
348 T/C	13	(35.1)	16	(42.1)
348 T/T	7	(19)	0	(0)

* The relationship between genotype and AgP case/control status is significant (P=0.017, Fisher Exact test). Individuals with the 348T/T genotype are at greater risk for AgP than those with the 348T/C and 348C/C genotypes combined (odds ratio=18.9, 95% confidence interval=1.04–333).

Table 3

PMN Chemotactic Activity and Relative *FPR1* Expression in Healthy Controls and AgP Cases of Various Genotypes[*]

Group	Chemotaxis activity to 30 nM fMLF[†]	Fold increase in *FPR1* expression[¶]
All controls	99.7 ± 2.8[‡ §] (n = 32)	2.77[#] (n = 37)
348 C/C Cases	80.0 ± 5.0[§] (n = 11)	2.02 (n = 11)
348 T/C Cases	89.0 ± 5.0[‖] (n = 10)	1.09[#] (n = 10)
348 T/T Cases	66.2 ± 4.0[‡‖] (n = 5)	1.32 (n = 5)

[*] Differences among the groups in the columns are statistically significant ($P \leq 0.006$, ANOVA). Within columns, like superscripts indicate values that are significant different ($P<0.05$, Holm-Sidak test)

[†] Data are reported as mean ± SEM

[¶] Transformed values (ΔΔCt) represent n-fold change in *FPR1* expression relative to baseline. Variability is not shown since the transformed data is positively skewed.

Table 4

Relationship of 348T>C Genotype to PMN Chemotactic Activity and Relative *FPR1* Expression in a Pool of All Case and Control Subjects

Genotype Group	Chemotaxis activity to 30 nM fMLF*	Fold increase in *FPR1* expression‡
348 C/C	89.3 ± 2.8† (n = 30)	1.92 (n = 32)
348 T/C	99.3 ± 4.0† (n = 23)	2.93 (n = 26)
348 T/T	66.2 ± 4.0† (n = 5)	1.32 (n = 5)

*Data are reported as mean ± SEM. Differences among the groups in this column are statistically significant ($P < 0.001$, ANOVA). Within this column, like superscripts indicate values that are significantly different ($P < 0.05$, Holm-Sidak test)

‡Transformed values (ΔΔCt) represent n-fold change in *FPR1* expression relative to baseline. Variability is not shown since the transformed data is positively skewed and can be misleading. Differences among the groups in this column are not statistically significant ($P = 0.26$, Kruskal-Wallis ANOVA on ranks).

Table 5

Allele Frequencies of *FPR1* 5' SNPs in Cases and Controls

SNP		AgP Cases		Controls		P value*
		n	%	n	%	
−13045C>T>A	C	58	(78.4)	68	(89.5)	0.11
	T	3	(4)	3	(3.9)	
	A	13	(17.6)	5	(6.6)	
−12915C>T	C	2	(2.7)	8	(10.5)	0.098
	T	72	(97.3)	68	(89.5)	
−12888 T>A	T	73	(98.6)	72	(94.7)	0.37
	A	1	(1.4)	4	(5.3)	
−10107A>G	A	61	(82.4)	63	(82.9)	1
	G	13	(17.6)	13	(17.1)	
−10056T>C	T	2	(2.7)	5	(6.6)	0.44
	C	72	(97.3)	71	(93.4)	
−9997C>T	C	72	(97.3)	72	(94.7)	0.68
	T	2	(2.7)	4	(5.3)	
−8430A>G	A	70	(94.6)	66	(86.8)	0.16
	G	4	(5.4)	10	(13.2)	
−8318A>G	A	55	(74.3)	56	(73.7)	1
	G	19	(25.7)	20	(26.3)	
−8375G>T	G	60	(81.1)	65	(85.5)	0.51
	T	14	(18.9)	11	(14.5)	
−5426G>A	G	62	(83.8)	67	(88.2)	0.49
	A	12	(16.2)	9	(11.8)	
−4949C>T	C	49	(66.2)	50	(65.8)	1
	T	25	(33.8)	26	(34.2)	

* Determined by Fisher exact test